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Characterization of QTL for oil content in maize kernel

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Abstract Kernel oil content in maize is a complex quantitative trait. Phenotypic variation in kernel oil content can be dissected into its component traits such as oil metabolism and physical characteristics of the kernel, including embryo size and embryo-to-endosperm weight ratio (EEWR). To characterize quantitative trait loci (QTL) for kernel oil content, a recombinant inbred population derived from a cross between normal line B73 and high-oil line By804 was genotyped using 228 molecular markers and phenotyped for kernel oil content and its component traits [embryo oil content, embryo oil concentration, EEWR, embryo volume, embryo width, embryo length, and embryo width-to-length ratio (EWLR)]. A total of 58 QTL were identified for kernel oil content and its component traits in 26 genomic regions across all chromosomes. Eight main-effect QTL were identified for kernel oil content, embryo oil content, embryo oil concentration, EEWR, embryo weight, and EWLR, each accounting for over 10 % of the phenotypic variation in six genomic regions. Over 90 % of QTL identified for kernel oil content co-localized with QTL for component traits, validating their molecular contribution to kernel oil content. On chromosome 1, the QTL that had the largest effect on kernel oil content (*qKO1-1*) was associated with embryo width; on chromosome 9, the QTL for kernel oil content (*qKO9*) was related to EEWR (*qEEWR9*). Embryo oil concentration and embryo width were identified as the most important component traits controlling the second largest QTL for kernel oil content on chromosome 6 (*qKO6*) and a minor QTL for kernel oil content on chromosome 5 (*qKO5-2*), respectively. The dissection of kernel oil QTL will facilitate future cloning and/or functional validation of kernel oil content, and help to elucidate the genetic basis of kernel oil content in maize.

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Introduction

Maize oil is highly valued for both animal feed and human food. The growth rate, feed efficiency, and productivity of livestock improve with increasing oil content within the maize kernel as a result of the increased metabolizable energy and improved protein quality (Han et al. 1987; Benitez et al. 1999; O'Quinn et al. 2000; Lambert et al. 2004). Maize oil is high in polyunsaturated fatty acids and low in linolenic acid, making it a desirable vegetable oil (Lambert 2001). Improving the quantity and quality of maize kernel oil content is therefore an important target for breeding.

Understanding the genetic basis of oil synthesis and accumulation will provide useful insight for developing new approaches to improve oil quantity and quality using

marker-assisted selection or genetic engineering. Many studies have been conducted to identify quantitative trait loci (QTL) associated with oil content in maize kernels (Goldman et al. 1994; Berke and Rocheford 1995; Mangolin et al. 2004; Laurie et al. 2004; Song et al. 2004; Wassom et al. 2008a; Zhang et al. 2008; Han et al. 2008; Yang et al. 2010). Approximately 50 QTL for oil content, accounting for over 50 % of phenotypic variation, were identified in a large randomly mated population [IHO (70) × ILO (70); Laurie et al. 2004], which agreed well with earlier predictions of many minor genetic factors controlling oil content (Dudley 1977). These results suggested that oil content is controlled by a large number of genes with small but additive effects. In contrast, using segregating or recombinant inbred line populations, a relatively small number of QTL were detected, accounting for a large percentage of the total phenotypic variation in oil content (Goldman et al. 1994; Berke and Rocheford 1995; Mangolin et al. 2004; Song et al. 2004; Wassom et al. 2008a; Zhang et al. 2008; Han et al. 2008; Yang et al. 2010). Interestingly, a major QTL on chromosome 6 was identified across all mapping, segregating, and recombinant inbred line populations and was later cloned by Zheng et al. (2008). These results indicate that oil content is controlled by a few QTL with very large effects. Recent studies by Wassom et al. (2008b) and Yang et al. (2010) indicate that epistasis is also important in the genetic basis of oil content in maize kernel.

Maize oil is composed largely of triacylglycerol and is stored within the kernels. The quantity of triacylglycerol and the capacity of the triacylglycerol storage organ are the two key factors affecting oil accumulation in kernels. In maize, the quantity of triacylglycerol is determined by the amount of five fatty acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids (Lambert 2001). A considerable portion of QTL for fatty acid composition is co-localized with QTL for oil content, suggesting that some loci increase oil content by increasing fatty acid composition (Alrefai et al. 1995; Wassom et al. 2008b; Yang et al. 2010). Maize kernels consist of endosperm and embryo. The endosperm, which is composed mainly of starch components, accounts for 80 % of kernel mass, whereas the embryo accounts on average for only 10 % of kernel mass (Val et al. 2009). However, over 80 % of total kernel oil is located in the embryo, compared to only 5 % in the endosperm (Lambert 2001). Thus, the ratio of embryo-to-endosperm weight can partly determine the accumulation of oil, because oil content is negatively correlated with starch content. A long-term selection experiment for Illinois High Oil (IHO) demonstrated that embryo size was also associated with oil content in maize kernels (Dudley and Lambert 2004). Therefore, the identification of QTL for traits involved in physical characteristics of the kernel, including the ratio of embryo-to-endosperm weight and embryo size, will allow causal variants of oil content to be identified. This will provide valuable information for isolating and validating the function of genes associated with kernel oil content by map-based cloning or candidate gene association mapping.

In this study, we used the B73 × By804 recombinant inbred line mapping population to investigate the genetic basis of oil content in maize kernels by dissecting the kernels into endosperms and embryos to consider the roles and identify QTL for various traits associated with kernel oil content.

Materials and methods

Development and genetic characterization of mapping population

A mapping population (F_7) composed of 245 recombinant inbred lines (RILs) was developed from a single cross between an inbred line with average oil content (B73) and another with high oil content (By804) (Table 1). The population was genotyped using 228 molecular markers including 202 microsatellites, 6 InDels, and 20 molecular markers that were developed from 18 candidate genes involved in the lipid metabolism pathway in maize. An improved linkage map of 1,655 cM, with an average interval of 7.3 cM between adjacent markers, was constructed using MAPMAKER/EXP 3.0 (Lincoln et al. 1993). Details were previously described by Yang et al. (2010).

Phenotypic data collection

The mapping population and parental inbred lines were grown in a complete randomized block design with three replications at the Agronomy Farm, China Agricultural University, Beijing, in 2005 and 2006. Each genotype was grown in a single-row plot (5-m rows with 0.67 m between rows) with a plant density of 45,000 plants/ha. Over six plants in each row were pollinated by bulked pollen from within the row to avoid xenia effects. Three hundred kernels were bulked for each row with equal amounts from the harvested ears. Two out of three field replications were used for phenotyping.

Twenty representative kernels from each plot were selected from the 300 bulked kernels. Kernels were dried for 60 h at 45 °C and then weighed, and oil content was measured by pulsed nuclear magnetic resonance (NMR) on a Minispec PC 20 NMR (Bruker, US). Kernels were then soaked in deionized water for 60 h at 45 °C and dissected into embryo and endosperm. Embryos and endosperms were dried to the same moisture level of kernels before

Table 1 Descriptive statistics of component traits of kernel oil content for parental lines (By804 and B73) and a population of 245 RILs

Traits	Parent (mean ± SD)		RIL						
	By804	B73	Mean ± SD	Range	R (E)[c]	E[a]	G[a]	G × E[a]	H^2 ± SE (%)
KO (%)	12.10 ± 1.33	3.53 ± 0.68	6.83 ± 1.54	2.78–11.21	2.67	197.22**	25.08**	1.87**	90.2 ± 1.2
EO (%)	63.69 ± 4.42	39.10 ± 2.77	49.79 ± 5.87	30.35–67.41	2.66	24.82**	16.16**	2.15**	83.5 ± 2.0
EOD (mg/mL)	599.17 ± 31.65	395.60 ± 32.40	484.25 ± 55.71	323.08–666.64	2.14	20.28**	7.99**	1.41**	76.0 ± 2.8
EEWR (%)	24.87 ± 1.78	11.73 ± 1.20	17.33 ± 2.85	9.53–27.37	26.58**	569.49**	30.66**	2.17**	91.1 ± 1.1
EV (μL)	39.00 ± 7.95	13.17 ± 4.53	28.26 ± 8.90	8.00–58.67	2.94	329.67**	5.07**	1.79**	57.4 ± 5.2
EW (mm)	7.25 ± 0.34	3.53 ± 0.36	5.16 ± 0.65	3.49–7.23	2.74	558.81**	11.66**	1.75**	80.4 ± 2.4
EL (mm)	6.93 ± 0.38	5.95 ± 0.47	7.01 ± 0.71	4.98–9.05	9.73**	159.87**	5.83**	2.28**	55.5 ± 5.4
EWLR (%)	104.67 ± 2.76	59.21 ± 3.01	73.9 ± 8.80	49.67–107.88	4.08*	116.66**	11.8**	1.77**	80.2 ± 2.3

KO kernel oil content, *EO* embryo oil content, *EOD* embryo oil density, *EEWR* embryo-to-endosperm weight ratio, *EV* embryo volume, *EL* embryo length, *EW* embryo width, *EWLR* embryo width-to-length ratio, *SD* standard deviation, H^2 ± *SE* broad-sense heritability ± standard error

* Significant at $p < 0.05$

** Significant at $p < 0.01$

[a] *F* values and significance tests of four effects from ANOVA analysis for traits over two years

dissection and then weighed, and the ratio of embryo-to-endosperm weight was calculated. Embryo oil content was measured using the Minispec PC 20 NMR. Embryos were then photographed and the length and width of 20 kernels were determined using ImageJ software for image analysis (http://rsb.info.nih.gov/ij). Embryo shape was derived from the ratio of embryo width-to-length. Embryo volume (EV) was estimated by the displacement method using a burette (precision 0.1 mL). Embryo oil density (EOD) was calculated by the amount of absolute oil in the embryo and the embryo volume. In summary, traits used in this study were: kernel oil content (KO) expressed as the percentage of total kernel weight, embryo oil content (EO) expressed as the percentage of total embryo weight, EOD, embryo-to-endosperm weight ratio (EEWR), EV, embryo width (EW), embryo length (EL), and embryo width-to-length ratio (EWLR).

Phenotypic data analysis

Phenotypic data were analyzed using SAS version 8.02 (SAS Institute 1999). Variance components of genotype, replication (environment), environment, and genotype × environment (G × E) were estimated by PROC MIXED. These variance components were then used to calculate broad-sense heritability (H^2) on a family mean basis (Holland et al. 2003). The significance of each variance component was tested by PROC GLM. PROC CORR was conducted to analyze phenotypic correlations among measured traits using the mean phenotypic values of RILs. The contributions of the seven traits (EO, EOD, EEWR, EV, EW, EL, and EWLR) to kernel oil content were calculated by PROC GLM.

QTL mapping

A mixed linear model (Yang et al. 2007), presented in QTLNetwork version 2.0 (Yang et al. 2008), was employed to identify QTL associated with traits at 1-cM intervals with a window size of 10 cM. The 10-cM windows were defined to distinguish two adjacent test statistic peaks, whether or not they represented two QTL. *F*-statistic ($\alpha = 0.01$) was conducted to declare the presence of a QTL with 10,000 random permutations (Doerge and Churchill 1996). Additive effects were estimated using a Bayesian method with Gibbs sampling (Wang et al. 1994). Marker intervals from QTLNetwork version 2.0 were modified if the position of adjacent QTL for different traits was <10 cM. For all traits, the sum of individual phenotypic variances explained by each QTL was calculated as the total phenotypic variance explained by all QTL for each trait.

Results

Phenotypic variation

Significant differences were observed between the parents, By804 and B73, for all traits (Table 1). The high-oil parent By804 had over twofold greater values for KO, EEWR, EV, and EW compared to B73. Although differences were not pronounced, By804 had significantly greater EWLR, EO, EOD, and EL compared to B73. Highly significant differences were observed within the RIL population for all traits (Table 1). Within the RIL population, a normal distribution was observed for all traits with transgressive

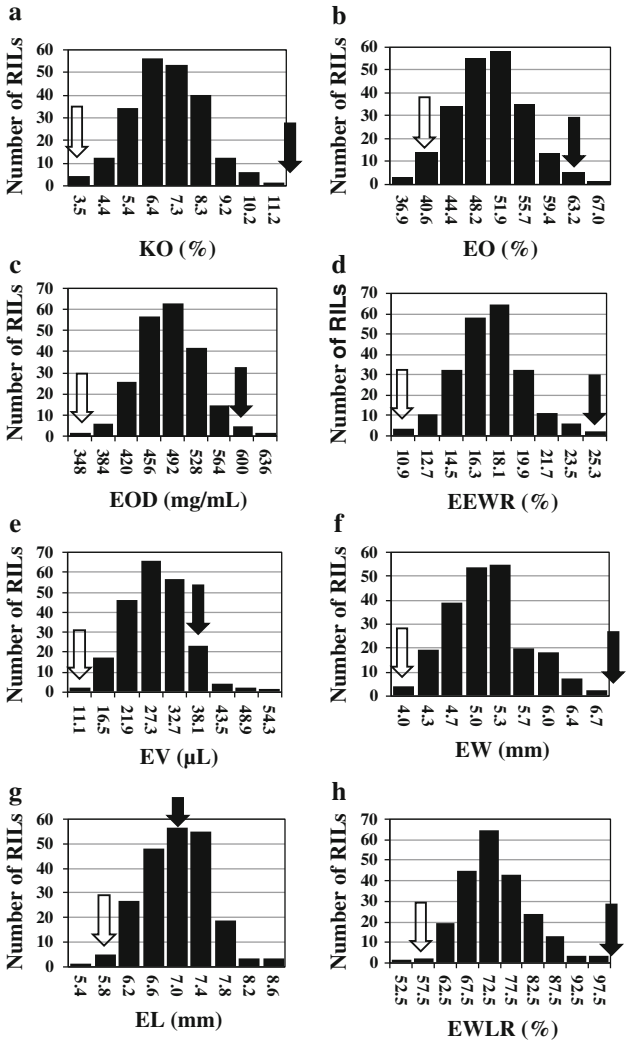

Fig. 1 Frequency distribution of RILs for KO and its component traits combined over years. Parental values are indicated with *arrows* (B73, *black*; By804, *white*). *KO* kernel oil content, *EO* embryo oil content, *EOD* embryo oil density, *EEWR* embryo-to-endosperm weight ratio, *EV* embryo volume, *EL* embryo length, *EW* embryo width, *EWLR* embryo width-to-length ratio

segregation, indicating quantitative genetic control (Fig. 1). Combined means over years of the RIL population were smaller than the corresponding mid-parent value for all traits, with the exception of EV and EL. Highly significant genotype, environment, and G × E interactions were observed for each trait (Table 1). Broad-sense heritability (H^2) was high for KO, EO, EEWR, EW, and EWLR (ranging from 80.2 to 91.1 %), and moderate for EOD, EV, and EL (ranging from 55.5 to 76.0 %).

Phenotypic correlations for all traits associated with kernel oil content revealed moderate but highly significant correlations (Table 2). There were no significant correlations between EL and EO, and between EL and EOD. Kernel oil content was positively correlated with all measured traits. The highest correlation between kernel oil content and associated traits was EEWR ($r = 0.81$, $p < 0.01$), followed by EO, EOD, EW, EV, EWLR, and EL. Regression analysis revealed that EO, EOD, and EEWR contributed moderately to kernel oil content; EV, EW, and EWLR contributed less to kernel oil content; EL contributed very little to kernel oil content.

QTL analysis

A total of 58 QTL were identified for the eight traits (Table 3). QTL were distributed across all ten chromosomes, clustering in 26 chromosomal regions (Fig. 2). The phenotypic variation explained by each QTL ranged from 1.1 % (*qEO3-1* and *qEWLR7*) to 20.5 % (*qEO6*). Eight main-effect QTL, accounting for over 10 % of phenotypic variance, were identified for six traits (KO, EO, EOD, EEWR, EW, and EWLR) in six chromosomal regions. Alleles from the high-oil parent By804 contributed positively to over 90 % of all QTL identified. The total number of QTL detected for each trait ranged from three (EV) to twelve (KO), while the percentage of the total phenotypic variation was accounted for by each trait ranging from 15.5 % (EV) to 60.2 % (KO).

Kernel oil content

A total of twelve QTL were detected for KO and together explained 60.2 % of the phenotypic variation for KO. One main-effect QTL, *qKO1-1*, was identified for KO on chromosome 1, between markers umc1598 and umc1884, contributing to 14.3 % of phenotypic variance for KO. The additive effect of the By804 allele at this locus increased KO by 0.59 %. On chromosome 6 (between markers Q8 and umc1979), QTL *qKO6* explained 9.6 % of the phenotypic variation for KO. The By804 allele at this QTL increased KO by 0.49 %. Two QTL on chromosome 2 (*qKO2-1*) and chromosome 10 (*qKO10*) were also identified with moderate additive effects on KO. The phenotypic variation explained by QTL *qKO2-1* and *qKO10* was >5.0 %, and the increased effect by By804 alleles was 0.41 % for *qKO2-1* and 0.36 % for *qKO10*, respectively. An additional eight QTL were identified on chromosomes 1, 2, 4, 5, and 7, explaining between 1.3 and 4.4 % of the phenotypic variation. By804 contributed positively to all QTL, with the increased effects ranging from 0.18 to 0.33 %.

Embryo oil content

Nine QTL were identified for EO, accounting for over 52.8 % of the total phenotypic variation. The By804 allele had a positive effect on EO for eight QTL, contributing up

Table 2 Phenotypic correlation coefficients (r) among kernel oil content and its component traits, and the partial R^2 of each trait to total variation in kernel oil

Traits	KO	EO	EOD	EEWR	EV	EW	EL	EWLR	R^2 (%)
KO	1								
EO	0.76**	1							60.9
EOD	0.70**	0.91**	1						55.3
EEWR	0.81**	0.28**	0.26**	1					65.9
EV	0.53**	0.26**	0.24**	0.55**	1				23.7
EW	0.63**	0.32**	0.31**	0.66**	0.78**	1			37.8
EL	0.21**	−0.01	0.01	0.33**	0.75**	0.47**	1		0.8
EWLR	0.48**	0.36**	0.33**	0.41**	0.19**	0.66**	−0.35**	1	31.5

KO kernel oil content, *EO* embryo oil content, *EOD* embryo oil density, *EEWR* embryo-to-endosperm weight ratio, *EV* embryo volume, *EL* embryo length, *EW* embryo width, *EWLR* embryo width-to-length ratio

** Significant at $p < 0.01$

to 2.6 %. The largest QTL, *qEO6*, co-located with *qKO6* and accounted for 20.5 % of the phenotypic variation. The allele from high-oil parent By804 increased EO at this locus. Additional QTL were located on chromosomes 1, 2, 3, and 8, and explained between 1.1 and 7.7 % of the phenotypic variation. Their additive effects ranged from 0.61 to 1.63 %, with alleles from both parents positively increasing EO.

Embryo oil density

A total of seven QTL were identified for EOD, explaining a total of 50.1 % of the phenotypic variation. Two highly significant QTL (*qEOD6* and *qEOD8*) were detected on chromosomes 6 and 8, respectively. QTL *qEOD6* was located in the same genomic region as QTL *qKO6* and *qEO6*, and accounted for 18.5 % of the phenotypic variation in EOD. The allele from By804 at this locus increased EOD by 24.31 mg/mL. QTL *qEOD8* (flanked by umc1130 and umc1562) explained 10.7 % of the phenotypic variance, and the By804 allele positively contributed to this locus, increasing EOD by 18.48 mg/mL. The additional five QTL explained 1.5–6.1 % of the phenotypic variation for EOD. By804 had a positive effect on four QTL, contributing to an increase in EOD of 3.68–13.74 mg/mL.

Embryo-to-endosperm weight ratio

A total of ten QTL were identified for EEWR, distributed across all chromosomes with the exception of chromosomes 3, 6, and 8, and explaining a total of 52.9 % of the phenotypic variation. By804 increased EEWR at all loci with the exception of *qEEWR2-2*. The QTL *qEEWR9*, between markers dupssr6 and bnlg244 on chromosome 9, explained 11.7 % of phenotypic variation. At this locus, the By804 allele positively contributed to EEWR, increasing EEWR by 0.96 %. The remaining nine QTL explained between 2.7 and 6.2 % of phenotypic variation, with the changed effects ranging from 0.46 to 0.70 %.

Embryo volume

Three QTL with small effects were identified for EV, located on chromosomes 2, 4, and 5. These QTL (*qEV2*, *qEV4*, and *qEV5*) explained 4.4, 4.2, and 6.9 % of the phenotypic variation, respectively. The By804 allele increased EV at these loci by 1.74, 1.71, and 2.18 μL, respectively.

Embryo width

Six QTL were detected for EW on chromosomes 1, 2, 4, 5, and 7. Together these QTL accounted for 43.1 % of the phenotypic variation, with the By804 allele increasing EW at all loci. The QTL *qEW5* had the largest effect on EW, accounting for 15.1 % of the phenotypic variation, and was located on chromosome 5 (flanked by markers umc2373 and umc2026). The By804 allele at this locus had an additive effect, increasing EW by 0.24 mm. The remaining QTL for EW explained between 3.3 and 9.2 % of the phenotypic variation, with the additive effect of the By804 allele ranging from 0.11 to 0.24 mm.

Embryo length

A total of four QTL were identified for EL, accounting for a total of 19.2 % of the phenotypic variation. Two QTL, *qEL1-1* and *qEL1-2*, were detected on chromosome 1, with the low-oil parent B73 positively contributing to EL. *qEL1-1* accounted for 3.9 % of phenotypic variation, with an additive effect of 0.14 mm, while *qEL1-2* accounted for 6.9 % of phenotypic variation, with an additive effect of 0.18 mm. A further two QTL were detected on chromosomes 5 and 7, accounting for 4.9 and 3.5 % of the

Table 3 Summary of significant QTL for kernel oil content and associated component traits

Traits	QTL[a]	Chr	Marker interval	p[b]	Range[c]	A[d]	r^2(a) (%)[e]
KO (%)	*qKO1-1*	1	umc1598–umc1884	84.7	82.7–85.7	0.59	14.3
	qKO1-2	1	ols1–phi308707	196.6	194.0–199.6	0.33	4.3
	qKO2-1	2	phi96100–kt2	19.2	17.2–22.2	0.41	7.0
	qKO2-2	2	umc2372–C9-3	135.3	132.3–142.2	0.19	1.5
	qKO4	4	bnlg1189–umc1466	101.0	95.0–108.8	0.18	1.3
	qKO5-1	5	umc1097–phi024	6.4	3.7–11.4	0.23	2.2
	qKO5-2	5	umc2373–umc2026	95.8	88.3–98.8	0.33	4.4
	qKO6	6	Q8–umc1979	44.5	41.5–47.5	0.49	9.6
	qKO7	7	phi034–bnlg1792	46.4	45.8–55.5	0.26	2.7
	qKO8	8	umc1130–umc1562	65.9	64.2–66.7	0.31	3.8
	qKO9-1	9	dupssr6–bnlg244	13.7	9.7–18.7	0.31	3.9
	qKO10	10	umc1367–umc2016	40.2	39.3–40.9	0.36	5.3
Subtotal[f]							60.2
EO (%)	*qEO1*	1	fad83–mcat81	66.3	60.3–67.3	1.19	4.2
	qEO2-1	2	phi96100–kt2	11.0	6.0–16.0	1.12	3.7
	qEO2-2	2	umc2372–C9-3	134.3	132.3–136.2	1.34	5.3
	qEO2-3	2	kass2–umc1551	173.0	168.3–177.0	0.75	1.6
	qEO3-1	3	umc2101–umc2256	1.0	0.0–5.0	−0.61	1.1
	qEO3-2	3	sad7004–dupssr23	112.5	104.1–121.6	0.85	2.1
	qEO6	6	Q8–umc1979	43.5	41.5–45.5	2.64	20.5
	qEO8-1	8	umc1075–umc1304	22.0	17.0–29.0	1.63	7.7
	qEO8-2	8	umc1130–umc1562	65.9	65.2–66.7	1.51	6.7
Subtotal[f]							52.8
EOD (mg/mL)	*qEOD1*	1	umc1598–umc1884	90.0	88.0–93.1	13.74	5.9
	qEOD2-1	2	phi96100–kt2	11.0	6.0–21.2	13.92	6.1
	qEOD2-2	2	umc2372–C9-3	134.3	130.6–140.2	11.19	3.9
	qEOD3	3	sad7004–dupssr23	111.5	108.1–114.5	10.54	3.5
	qEOD4	4	bnlg1755–bnlg2291	65.3	62.1–70.3	−6.84	1.5
	qEOD6	6	Q8–umc1979	43.5	41.5–45.5	24.31	18.5
	qEOD8	8	umc1130–umc1562	65.9	64.2–66.7	18.48	10.7
Subtotal[f]							50.1
EEWR (%)	*qEEWR1-1*	1	umc1598–umc1884	74.8	72.6–76.8	0.55	3.8
	qEEWR1-2	1	umc2232–umc1988	113.9	110.8–115.9	0.58	4.3
	qEEWR1-3	1	ols1–phi308707	196.6	194.0–200.6	0.57	4.1
	qEEWR2-1	2	phi96100–kt2	11.0	7.0–15.0	0.69	6.0
	qEEWR2-2	2	bnlg1520–apat5	189.2	186.9–192.2	−0.55	3.8
	qEEWR4	4	bnlg1755–bnlg2291	77.4	76.3–82.4	0.61	4.8
	qEEWR5	5	umc1447–umc1692	70.9	68.9–72.9	0. 70	6.2
	qEEWR7	7	phi034–bnlg1792	44.9	36.0–45.8	0.46	2.7
	qEEWR9	9	dupssr6–bnlg244	13.7	10.7–16.7	0.96	11.7
	qEEWR10	10	umc1367–umc2016	40.2	39.3–40.9	0.66	5.5
Subtotal[f]							52.9
EV (μL)	*qEV1*	2	phi96100–kt2	18.2	7.0–22.2	1.74	4.4
	qEV4	4	bnlg1189–umc1466	103.0	98.0–108.8	1.71	4.2
	qEV5	5	umc2373–umc2026	90.3	86.3–97.8	2.18	6.9

Table 3 continued

Traits	QTL[a]	Chr	Marker interval	p[b]	Range[c]	A[d]	r^2(a) (%)[e]
Subtotal[f]							15.5
EW (mm)	*qEW1*	1	umc1598–umc1884	70.6	68.3–73.6	0.19	9.2
	qEW2-1	2	phi96100–kt2	17.2	9.0–20.2	0.14	5.2
	qEW2-2	2	umc2032–umc1065	111.5	106.8–114.6	0.11	3.3
	qEW4	4	bnlg1189–umc1466	98.0	92.6–103.0	0.16	6.6
	qEW5	5	umc2373–umc2026	96.8	92.8–98.8	0.24	15.1
	qEW7	7	atf2–umc2332	77.6	74.2–85.6	0.12	3.7
Subtotal[f]							43.1
EL (mm)	*qEL1-1*	1	umc1598–umc1884	91.1	89.0–94.1	−0.14	3.9
	qEL1-2	1	bnlg1556–f6a	146.7	143.7–157.5	−0.18	6.9
	qEL5	5	umc2373–umc2026	91.3	86.3–98.8	0.15	4.9
	qEL7	7	atf2–umc2332	76.2	70.2–80.6	0.13	3.5
Subtotal[f]							19.2
EWLR (%)	*qEWLR1-1*	1	umc1598–umc1884	71.6	69.6–73.6	3.22	13.0
	qEWLR1-2	1	ols1–phi308707	196.6	193.0–200.6	2.18	6.0
	qEWLR2-1	2	phi96100–kt2	8.0	2.0–14.0	1.75	3.9
	qEWLR2-2	2	umc2032–umc1065	125.0	123.0–126.9	2.30	6.7
	qEWLR3	3	umc2101–umc2256	3.0	0.0–7.0	−1.43	2.6
	qEWLR4	4	phi213984–phi096	40.6	35.6–46.6	2.86	10.3
	qEWLR7	7	phi328175–dupssr13	101.5	100.7–103.4	0.95	1.1
Subtotal[f]							43.5

KO kernel oil content, *EO* embryo oil content, *EOD* embryo oil density, *EEWR* embryo-to-endosperm weight ratio, *EV* embryo volume, *EL* embryo length, *EW* embryo width, *EWLR* embryo width-to-length ratio

[a] The number following each letter represents the chromosome location of the QTL. Different numbers following the dash indicate putatively different QTL located on the same chromosome

[b] The peak position of QTL on chromosome was estimated by QTLNetwork

[c] Supporting interval of QTL on chromosome was estimated by QTLNetwork

[d] Additive effects estimated by QTLNetwork. Positive (+) value indicates that the By804 allele increased the trait; negative (−) value indicates that the B73 allele increased the trait

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

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

phenotypic variation, respectively. Alleles from By804 increased EL at both loci (0.15 mm for QTL location on chromosome 5 and 0.13 mm for the QTL located on chromosome 7).

Embryo width-to-length ratio

Seven regions with QTL associated with EWLR were identified, and explained 43.5 % of the total phenotypic variation in EWLR. The high-oil parent By804 was positively associated with increased EWLR at all loci with the exception of *qEWLR3*. QTL *qEWLR1-1* had the largest effect and was located in the same genomic region as *qKO1-1*. This QTL explained 13.0 % of the phenotypic variance, and the allele from By804 increased EWLR by 3.22 %. QTL *qEWLR4* was detected on chromosome 4 (between markers phi213984 and phi096), contributing 10.3 % to the phenotypic variation. At this locus, the By804 allele increased EWLR by 2.86 %. Five more QTL were identified on chromosomes 1, 2, 3, and 7, and accounted for 1.1 to 6.7 % of the phenotypic variation in EWLR.

Discussion

The identification of loci controlling oil-related traits should contribute to a better understanding of oil synthesis and storage in maize kernels. Among 26 identified genomic regions associated with KO and its component traits, 25 regions contained previously identified QTL for oil content or fatty acid composition in the maize kernel (Goldman et al. 1994; Berke and Rocheford 1995; Mangolin et al. 2004; Song et al. 2004; Wassom et al. 2008a, 2008b; Zhang et al. 2008; Han et al. 2008; Yang et al. 2010). The newly detected genomic regions contained QTL controlling physical characteristics of the kernel (*qEEWR2-2*). Comparing the

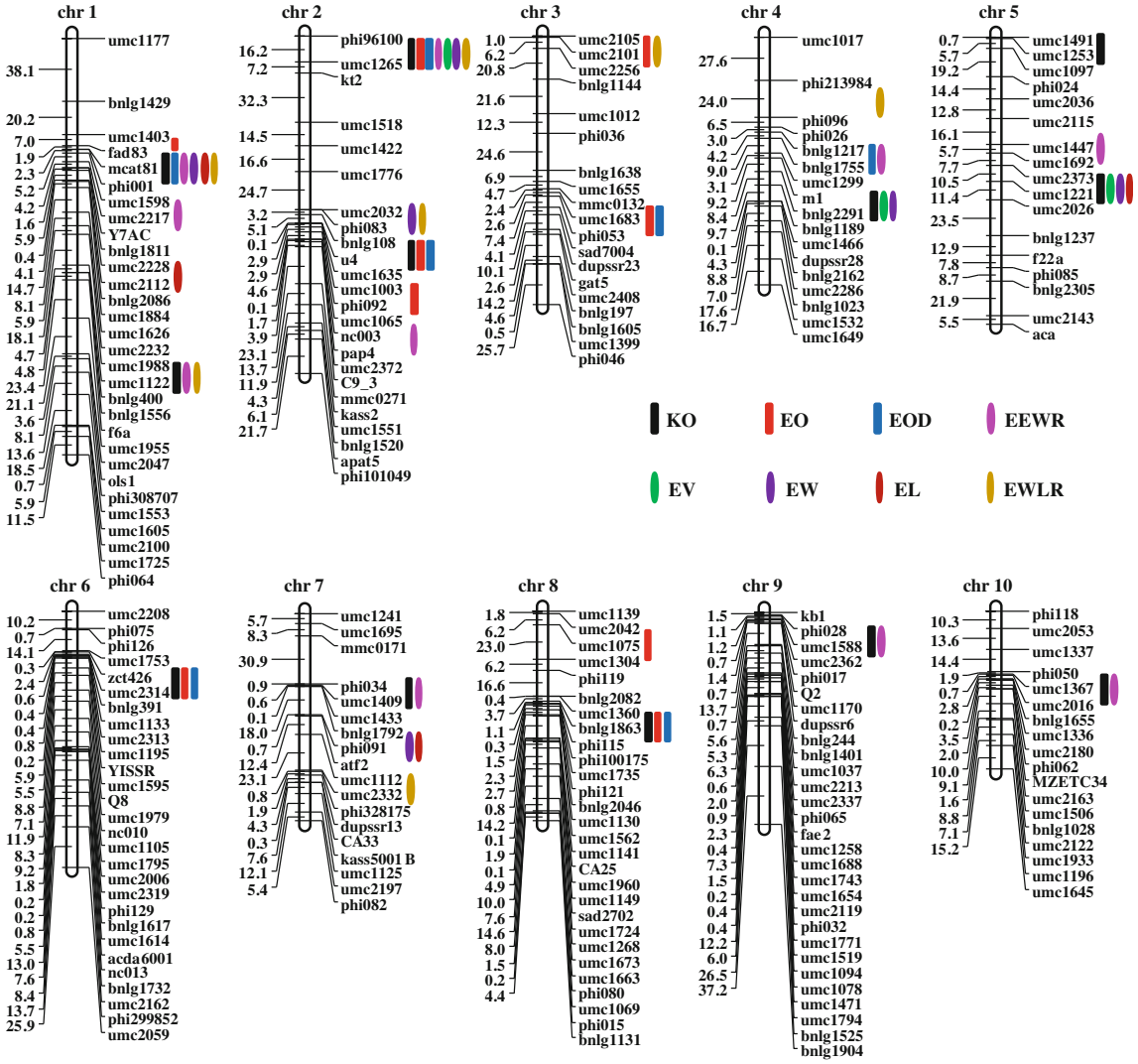

Fig. 2 Distributions of putative QTL for KO and its component traits. *KO* kernel oil content, *EO* embryo oil content, *EOD* embryo oil density, *EEWR* embryo-to-endosperm weight ratio, *EV* embryo volume, *EL* embryo length, *EW* embryo width, *EWLR* embryo width-to-length ratio

locations of mapped loci for KO-related traits with lipid-related candidate genes (Li et al. 2010; Yang et al. 2010), 20 genomic regions were found to fall near or within lipid-related genes mapped in silico by Li et al. (2010), and nine genomic regions were found to fall near or within orthologs of known lipid metabolic enzymes mapped by Yang et al. (2010). These results suggest that most QTL for oil-related traits are controlled by lipid-related genes. However, further characterization of the QTL for KO is needed, especially for QTL co-located with QTL for physical characteristics of kernels.

Among twelve QTL identified for KO, nine were previously reported using the same population and mapping method but different phenotyping methodology (Yang et al. 2010). The direction of parental contribution and additive effect variations were consistent between two studies. However, higher estimated additive effects were identified in this study. The additional three minor QTL (*qKO2-2*, *qKO5-1*, and *qKO7*) were identified only in this study, explaining <3 % of phenotypic variation. This result may be caused by some minor differences in KO values measured by NMR and gas chromatography, although the KO values measured by both methods were highly correlated. The presence of common QTL identified across phenotyping systems allows the integration of QTL for fatty acid composition from Yang et al. (2010) for characterizing KO QTL.

Over 90 % of QTL associated with KO co-localized with QTL for EO, EOD, EEWR, EV, EW, EL, and EWLR. The number of QTL for other traits co-localizing with QTL associated with KO ranged from two to six, with the relationship and co-localization of traits with KO following a similar pattern, as identified through correlations between KO and associated traits. The By804 alleles contributed

positively to all loci with the exception of *qEL1-1*. Furthermore, six of the seven identified large-effect QTL explaining over 10 % of the phenotypic variation were located in the same genomic region as QTL for KO. These results indicated that QTL for KO are strongly associated with the other traits measured in this study. Interestingly, all QTL for EV were identified within loci containing QTL for KO. Nevertheless, QTL identified for EV explained <7 % of the total phenotypic variation for EV, and regression analysis showed EV contributed very little to KO.

To further unravel the genetic basis of QTL associated with KO, we dissected KO QTL into QTL for KO-related traits based on the co-localization of QTL for KO and other traits measured in this study. The largest QTL for KO, *qKO1-1*, was identified on chromosome 1 between markers umc1598 and umc1884. Six QTL for EO, EOD, EEWR, EW, EL, and EWLR were detected within or close to this genomic region (Fig. 2), suggesting that the additive effects of *qKO1-1* were associated with the QTL identified for the other measured traits in this study. The high percentage of phenotypic variation explained by QTL for EW (*qEW1*, 9.2 %) and EWLR (*qEWLR1-1*, 13.0 %) suggested that traits related with embryo size were key factors for increasing KO at this locus, although no QTL for EV were detected at this locus. For EL, the direction of effect did not agree with the decrease in EL by the By804 allele, indicating that the EL QTL at this locus was not the causal QTL. In addition, EL is a major component of EV, and no QTL affecting EV were identified at this locus. Therefore, it is likely that the QTL for EW was the major factor associated with *qKO1-1*. However, QTL for EO, EOD, and EEWR identified at this locus had only small additive effects and may also have small contributions to *qKO1-1*, as revealed through low or moderate phenotypic correlations with EW or KO. Similar QTL determinants were observed for *qKO2-1*, although the percentage of phenotypic variation explained by EW QTL at this locus was not significantly higher than that explained by QTL for EO, EOD, and EEWR.

A different pattern of correlations between QTL for KO and for the other traits measured in this study was investigated for *qKO6*, the second-most significant QTL controlling KO (chromosome 6, flanked by markers Q8 and umc1979). Two large-effect QTL for EO (*qEO6*) and EOD (*qEOD6*) co-localized in this region with the By804 allele, contributing positively to both traits. It is hypothesized that the QTL identified for KO at this locus was dependant on the QTL for EOD. Previously, three QTL with large effects for fatty acid compositions were identified at this locus (Yang et al. 2010). Furthermore, this locus was cloned, validating that a gene encoding the acyl-CoA:diacylglycerol acyltransferase in the metabolism pathway of oil synthesis and accumulation (*DGAT1-2*) is located within this region (Zheng et al. 2008). Similarly, the underlying genetic basis of QTL *qKO8* (chromosome 8, flanked by markers umc1130 and umc1562) may be related to oil synthesis and accumulation, because it is located in the same genomic region as a QTL for EOD (*qEOD8*), which explains a considerable percentage of phenotypic variation for EOD (10.7 %). In addition, the relatively low phenotypic variance explained by QTL for oleic acid composition at this locus (Yang et al. 2010) supports the existence of QTL *qKO8* with small effects. QTL co-localization at the genomic region between markers umc2373 and C9-3 on chromosome 2 demonstrated a similar genetic basis for dissecting KO QTL, *qKO2-2*, although it had a small effect that was not stable using different phenotyping methodology. A gene coding phosphatidic acid phosphatase, which acts downstream of oil synthesis and storage, has been identified near this locus, strengthening the hypothesis that the EOD QTL was the causal variant for KO at this locus.

As the embryo is the main storage organ of maize oil, embryo size is an important factor associated with KO (Dudley and Lambert 2004). In this study, QTL for EW were located in the same region as QTL for KO (*qKO1-1*). However, it is not possible to confirm that the EW QTL were the main QTL resulting in increased KO, because co-localization of QTL may result from pleiotropic effects of a single gene or by close physical linkage of genes controlling different traits. This is different from QTL co-localization at the genomic regions harboring *qKO4* and *qKO5-2*, particularly for *qKO5-2*. These two genomic regions indicated that the QTL for embryo size was the actual QTL for KO QTL, as was further validated by the presence of EV QTL. *qKO5-2* was identified on chromosome 5 between markers umc2373 and umc2026; an additional three QTL for traits related to embryo size were identified in this genomic region, with agreement in the direction of effect of parental contribution. Among these QTL, *qEW5* accounted for 15.1 % of phenotypic variation in EW, strongly supporting the EW QTL as an important determinant of the KO QTL identified in this locus. The proportion of phenotypic variation explained by QTL for EV, EW, and EL was consistent with the estimated contribution of each trait to KO.

Over 60 % of QTL for EEWR were identified within genomic regions containing QTL for KO, indicating that EEWR was an important contributor to the identification of QTL associated with KO at these loci. EEWR is usually treated as the best representation of embryo size, because other measurements of embryo size (volume, length, width, etc.) are challenging to measure precisely for many samples. Nevertheless, the correlation between EEWR and embryo size was mediate ($r = 0.55$, 0.66 and 0.33 for EV, EW and EL, respectively). Furthermore, EEWR may be an

independent factor influencing KO, as the expression of embryo and endosperm weight was based on the metabolic network, and the ratio between them directly reflects the negative correlation between oil and starch. Among these loci, it is very likely that EEWR QTL (*qEEWR9*) was a major factor associated with QTL *qKO9* (chromosome 9, between markers dupssr6 and bnlg244). The EEWR QTL at this locus (*qEEWR9*) contributed to 11.7 % of the phenotypic variation. In addition, the inclusion of additional markers in this region to a fine map of major QTL controlling palmitic acid composition revealed three separate QTL peaks (Lin Li, unpublished).

In conclusion, the majority of QTL associated with KO were co-localized with QTL related to the oil metabolism pathway, embryo size, and EEWR. This information provides further insight that will help to unravel the genetic basis of oil content in maize kernels by identifying causal variants underlying KO QTL. Maize kernel oil content is a complex quantitative trait, controlled by a few large-effect QTL and numerous minor QTL (Yang et al. 2010). The dissection of oil content into its component traits, including fatty acid composition, embryo size, and EEWR, allowed the identification of QTL associated with these causal traits and increased the genetic effect. Increasing the phenotypic variation explained by each locus will improve the detection of variations between the parent lines used in advanced fine mapping populations and lines containing homologous alleles at a given locus, and will thus enhance the feasibility of cloning QTL associated with kernel oil content.

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