

QTL Mapping of Kernel Oil Content of Chromosome 6 in a High Oil Maize Mutant (*Zea mays* L.)

Han Jing^{1,3}, Wang Hongwu^{1,2} and Chen Shaojiang^{1*}

¹National Maize Improvement Center of China, China Agriculture University, Beijing 100094, China

²Beijing Key Laboratory of Crop Genetic Improvement, China Agriculture University, Beijing 100094, China

³Maize Research Institute, Shandong Academy of Agricultural Science, Jinan, Shandong Province 250100, China

Received May 6, 2008; accepted June 20, 2008

ABSTRACT

An infrequent high oil maize mutant CE03005 (white seed color and high oil content) from ethyl methane sulphonate pollen mutagenesis was investigated in this research. A F₂ population derived from CE03005 × B73 was used to analysis the genetic effects influencing kernel oil content and to map kernel oil content QTL. Ears from three generations were harvested and the individual kernel oil content of each generation was measured with nuclear magnetic resonance. Genetic analysis showed that the average oil content of white kernels was significantly higher than that of yellow kernels. Genotypic and phenotypic analyses were compared using composite interval mapping. A QTL (*oilc6-m1*) associated with high kernel oil content was detected in the three generations at bin 6.01 of chromosome 6 near the phenotypic marker SC (seed color). The mapping results were congruent with the genetic analysis. The results explained the reason why the oil content of the white colored kernels was higher than that of the yellow ones because the oil content QTL linked tightly with the seed color gene in chromosome 6 in the mutant. The way of QTL mapping was credible. Molecular and phenotypic markers identified could be used for breeding purpose and would lend convenience in cloning QTL.

Key words: high oil maize mutant, kernel oil content, phenotypic marker, quantitative trait loci (QTL), SSR markers.

INTRODUCTION

High oil maize (*Zea mays* L.) is a unique type of maize in which the oil content in the grains is over 6% (Lambert, 2001). The use of high-oil maize kernels not only improves livestock feed conversions but also reduces the amount of supplemental fats needed in

livestock feed (Goldman, 1994). Its use as a dietary ingredient in livestock feed has also been shown to increase the growing and productive potential of livestock (Benitez et al., 1999; O'Quinn et al., 2000) and has also led to daily weight increases in both poultry and swine (Han et al., 1987; Goss and Kerr, 1992).

The traditional means to develop high oil germplasm was through long term repeated selection. The development of high oil maize is an important

*To whom correspondence should be addressed.
Email: shaoj@cau.edu.cn.

contribution to maize breeding, supporting the arduous selective breeding regime to generate the germplasm (Song et al., 2004). For example, the Illions High Oil (IHO) long term selection (over 90 generations) experiment in Burr's White maize was initiated in 1896 (Alretai et al., 1995). Song et al. (2004) developed BHO (Beijing High Oil) from synthetic variety Zhongzong No. 2 through a succession of 18 recurrent selections and increased the mean kernel oil content from 4.71% to 15.55%. The widespread use of molecular markers to develop genetic maps and map chromosomal regions affecting quantitative traits (QTLs) have provided more insight into the genetic basis of quantitatively inherited traits in maize as well as in other crops (Lee, 1995; Khavkin and Coe, 1997; Kraja and Dudley, 2000; Jiang and Zeng, 1995; Stuber and Sisco, 1991). In addition, the results from marker assisted selection (MAS) and introgression studies encourage the use of this technology in transferring desired genes between breeding lines (Stuber and Sisco, 1994). In recent years, a number of QTLs for oil content were detected with RFLP and simple sequence repeat markers in maize. Many QTLs associated with oil content were identified (Goldman et al., 1994; Berke and Rocheford, 1995; Song et al., 2004) in numerous populations.

Chemical mutagenesis has the advantage of generating new germplasm quickly through breeding programs. The chemical mutagen EMS (ethyl methane sulphonate), a kind of alkylating agent, often induces single site mutations. A special high oil mutant CE03005 (mean oil content = 85.61 g/kg), which has white kernels and a purple stem, was developed with EMS treated pollen from a normal yellow hybrid ND108 (oil content = 43.45 g/kg). The normal hybrid ND108 was chosen because it was a widely planted and high-yield hybrid in China at that time. Based on the fact that the kernel oil content of white kernels in an F₂ population of CE03005 × B73 (The seed color of B73 was yellow with oil content of 36.10 g/kg) was higher than that of yellow kernels, we propose that an important QTL that controls maize kernel oil content may be linked with the gene that controls seed color. The maize *y1* gene in chromosome 6 has undergone recent selection for an endosperm color phenotype (Kelly et al., 2004). Maybe *y1* gene mutation resulted in the white colored kernels in the mutant.

The objective of this study was to analyze the genetic effects of the kernel oil content gene and map the kernel oil content QTLs in chromosome 6 with SSR markers. This approach provides the opportunity to diagnose the relationship between seed color and kernel oil content.

MATERIALS AND METHODS

Material and oil assay

A cross between CE03005 × B73 was completed in 2001. The F₁ progeny were planted the following year and three self-pollinated ears were harvested for kernel oil assays. The methodology of mean separation was used in the kernel oil content among different generations and different gene types. One of the three self-pollinated ears was chosen to develop the F₂ population. The kernel oil content of all individual kernels was measured with a Mini spec PC 20 pulsed NMR (Nuclear Magnetic Resonance) made by Bruker company of Germany. The original unit of the oil content was in percent style (g/100 g). And the unit was transformed to g/kg following method of Mangolin et al (2004). The kernels can be divided into three seed color genotypes including homozygous dominant yellow (*YY*), heterozygous yellow (*Yy*) and homozygous recessive white (*yy*). The different genotypes can be distinguished by performing test crosses to observe the phenotypes exhibited in the next generation. The offspring of the heterozygous yellow genotype segregated into both seed colors (yellow and white) while crosses of the homozygous yellow remained yellow and homozygous white remained white.

A F₂ population comprised of 214 individuals was derived from a single cross between a high-oil line (CE03005) and a normal line (B73). The kernel oil content of CE03005 was 85.60 g/kg and the seed color white. The kernel oil content of B73 was 36.10 g/kg and the seed color yellow. The B73 line is both currently and historically an important normal line. 600 kernels resulting from the F₂ population as well as two parents and F₁ from B73 and CE03005 were planted (60,000 plants/ha) in 2004 in the field at China Agricultural University's (CAU) Experiment Station in Beijing. First approximately 430 plants of

F₂ populations were randomly self-pollinated to generate F₃ kernels. Ultimately, 214 F₂ ears with enough (more than 50 kernels) F₃ kernels were harvested for kernel oil assays. Fifty kernels from the central part of each F₂ ear were used to measure kernel oil content with a Mini spec PC 20 pulsed NMR. The mean oil content of the 50 kernels was used for analysis. This procedure was used because this trait presents a higher narrow-sense heritability coefficient; i.e., higher than 70.0% (Mangolin et al., 2004). Young leaves from the F₂ plants were harvested, freeze dried with liquid N₂ and ground to a powder. DNA was extracted following the CTAB procedure (see below). In 2005, the 214 F_{2:3} seeds (ear lines), as well as the parents B73, CE03005 and their F₁ were planted in three replications, randomized blocks design, in a stand of 60,000 plants/ha at the Experiment Station of CAU. Each plant was cross-pollinated within the same ear line. Five ears of the same ear line were harvested. The middle kernels from each ear were threshed and mixed together to measure oil content. The mean oil content of the three replications was used as the kernel oil content of the F₄ generation.

Broad-sense heritability and other variance analyses

Broad-sense heritability of the measured traits was computed according to methodology of Knapp et al. (1985) and Song et al. (2004). The heritability (H_B^2) was calculated as: $H_B^2 = V_G / V_P$, where V_G is the genetic variance and V_P is the phenotypic variance. The genetic variance $V_G = V_P - V_E$, where V_E is the variance due to environmental effects, was calculated as: $V_E = 1/3 (V_{P1} + V_{P2} + V_{F1})$. V_{P1} is the phenotypic variance of parent B73 and V_{P2} the phenotypic variance of parent CE03005, as a result $H_B^2 = [V_P - 1/3 (V_{P1} + V_{P2} + V_{F1})] / V_P$.

The segregation ratio for seed color was tested using the Chi-square tests and the test of normality for kernel oil content was tested using Kolmogorov-Smirnov test and Shapiro-Wilk test. Single marker analysis was used to test the Linkage between phenotypic marker SC and oil content in F₂ population. Analysis and all the tests including t-tests and the method of Bonferroni (LSDMOD) for mean separation were

computed with the statistical software package SPSS 12.0 and software SAS.

Molecular marker selection and analysis

The genomic DNA extraction followed the procedures (CTAB procedure) outlined by Hoisington et al. (1994). SSR analysis was conducted as reported in Senior and Manfred (1993) with publicly available primers from maizeGDB (Maize Genetics and Genomics Database) (2004). The *y1* gene that controls seed color in maize is located in chromosome 6 (Buckner et al., 1990). Therefore, 150 primers that possess at least one marker per bin in the maize map of chromosome 6 were chosen. The microsatellites were screened against the inbred parents, and the F₁ plants, to assess the genetic identity of the F₁'s used to generate the F₂ reference population. Thirty-three markers demonstrating the clearest polymorphisms among the parents were then chosen for genotyping the 214 F₂ plants. A Chi-square test was applied to identify any distorted segregation of markers ($p < 0.01$) from the expected ratios of 1:2:1. Thirty SSR markers and one phenotypic marker SC (seed color) exhibiting co-dominant segregation based on the Chi-square value were used to construct the primary genetic linkage map with Mapmaker/Exp v.3.0 at a *LOD* threshold > 3.0 (Lincoln et al., 1992). The recombination frequency between linked loci was transformed into centimorgan (cM) distances using Kosambi's mapping function. This procedure was also used by Mangolin et al. (2004) and Sibov et al. (2003).

QTL analysis

Composite interval mapping (CIM) was used to map QTL and estimate the effects (Zeng, 1994). Model 6 of the Zmapqtl procedure in QTL Cartographer Version 2.0 was employed (Zeng, 1994). Empirical threshold levels for ascertaining QTL significance were determined by performing 1000 permutations of the data (Zeng, 1994; Mangolin et al., 2004). QTL position was assigned at the point of the maximum *LOD* score within the considered regions. Two peaks generated for the same trait on the same chromosome were determined as two different QTLs, exhibiting a minimum distance of 20 cM

(Hackett, 2002; Song et al., 2004). The minimum distance might be lower depending on whether the signs of additive and/or dominant effects at the two QTLs were different. QTL confidence/support intervals were calculated at the point along the significance peak at which the *LOD* score was 1.0 unit less than the peak's *LOD* score (Mickelson, 2002). Overlapping confidence intervals (less than 20 cM) were used to determine whether any QTLs were common among the F_2 , F_3 and F_4 kernel evaluations (Flint-Garcia et al., 2003).

Levels of dominance were estimated as $DR = \frac{\text{dominant effects (d)}}{\text{additive effects (a)}}$ ratio for each QTL, and the average level of dominance was calculated weighing each DR ratio by its respective partial R^2 value. Gene action was determined using the criteria of Bohn et al. (1996) as additive (A): $0.1 \leq DR < 0.2$, partially dominant (PD): $0.2 \leq DR < 0.8$, dominant (D): $0.8 \leq DR < 1.2$, and over dominant (OD): $1.2 \leq DR$ (Song et al., 2004).

RESULTS

Variation of kernel oil content

Mean and variations:

The phenotypic variations (oil content) of the F_2 population, both parents and the hybrid F_1 over three generations are listed in **Table 1**. Kernel oil content varied widely in the different three generations. The overall means of the F_2 , F_3 and F_4 kernel oil content were 67.91 g/kg, 68.31 g/kg and 66.55 g/kg. The kernel oil content varied from 38.4 g/kg to 96.5 g/kg in F_2 kernels, from 33.9 g/kg to 92.5 g/kg in F_3 kernels

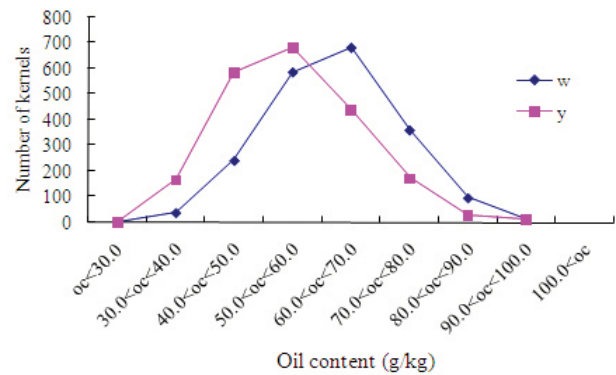


Figure 1. Distribution of kernel oil content in yellow (y) and white kernels (w) in F_3 show that the oil content of white colored kernels is higher than that of the yellow colored kernels

and from 37.6 g/kg to 93.4 g/kg in F_4 kernels. No significant differences in the kernel oil content among different generations were observed using the method of mean separation (**Table 1**). For the parents, the kernel oil content of the high-oil mutant CE03005 (mean oil content = 85.60 g/kg) was significantly higher than that of the normal parent B73 (mean oil content = 36.10 g/kg) (**Table 1**).

Different color segregations of the F_2 kernels (on F_1 ears) were obvious. The Chi-square value was 0.42 ($\chi^2 = 0.42 < \chi^2_{0.05,1} = 3.84$), so the segregation ratio of seed color was in agreement with the expected ratio of 3:1 (phenotypic yellow: white) in F_2 kernels. SC (seed color) could be used as a perfect phenotypic marker based on the Chi-square test. The mean kernel oil content of white and yellow colored kernels were 70.21 g/kg and 65.56 g/kg in three F_1 ears (F_2 kernels), respectively. The oil content of white kernels

Table 1. Descriptive statistics of oil content in the F_2 population, the two parents and the F_1 from three generations.

Years and generations	Kernel oil content (g/kg)									
	F ₂ population			MC*	CE03005		F ₁		B73	
	Mean ± SD	Range	SE	P= 0.05	Mean ± SD	SE	Mean ± SD	SE	Mean ± SD	SE
2003(F ₂)	67.91 ± 9.77	38.4 - 96.5	0.68	a	85.61 ± 3.99	1.11	68.03 ± 2.79	1.10	36.51 ± 2.52	0.51
2004(F ₃)	68.31 ± 12.07	33.9 - 92.5	0.85	a	85.32 ± 5.01	0.99	66.31 ± 5.52	0.51	36.09 ± 2.10	0.40
2005(F ₄)	65.50 ± 9.53	37.6 - 93.4	0.67	a	85.18 ± 2.91	1.11	67.92 ± 4.78	1.19	35.12 ± 2.50	0.29
mean	67.10 ± 10.56	33.9 - 96.5	0.43	-	85.60 ± 3.12	0.96	67.42 ± 2.36	0.92	36.10 ± 2.15	0.21

SD, standard deviation.

SE, standard error.

MC*, mean separation result of the oil content of F_2 population by the method of Bonferroni (LSDMOD) show the kernel oil content is not significantly different within three generations.

Table 2. Comparison of the mean kernel oil content of white and yellow kernels in F₂, F₃, F₄ and parents.

Seed Color	Kernel oil content (g/kg)			
	F ₂	F ₃	F ₄	parents
\bar{Y}^a	65.45	65.04	63.84	36.10 (B73)
\bar{W}^b	69.80	72.75	70.04	85.61 (CE03005)
W-Y ^c	4.35	7.71	6.20	49.51
(W-Y) ^d %	6.65%	11.85%	9.71%	137.15%
T value ^e	$t = 5.5866, p = 0.005$			

^aThe mean oil content of yellow kernels.

^bThe mean oil content of white kernels.

^cAbsolute value of kernel oil content in white kernels higher than in yellow kernels.

^dPercentage of kernel oil content in white kernels higher than in yellow kernels.

^eThe t-value of the t-test using the mean oil content between white kernels and yellow kernels among three generations.

was significantly higher than that of yellow kernels ($t = 24.49 > t_{0.01} = 9.955$; results not provided in Tables). The oil content of white colored F₃ and F₄ kernels was significantly higher than that of the yellow colored kernels ($t = 5.5866, p = 0.005$) (Fig 1 and Table 2). The mean oil content of F₂ yellow and white colored kernels was 65.45 g/kg and 69.80 g/kg, 65.04 g/kg and 72.75 g/kg in F₃ kernels, and 63.84 g/kg and 70.04 g/kg in F₄ kernels. The mean oil content of white kernels was 6.65% (4.35 g/kg), 11.85% (7.71 g/kg) and 9.71% (6.20 g/kg) higher than that of F₂, F₃ and F₄ yellow kernels. The mean oil content of parent CE03005 was 137.15% (49.51 g/kg) higher than the normal line B73 (Table 2). Single marker analysis result show that the phenotypic marker SC was linked tightly with the kernel oil content QTL [The F value was 16.17 ($p = 0.0001$)].

The kernel oil content revealed in each of the three genotypes and their mean separation values are provided in Table 3. The oil content of homozygous recessive white (*yy*) colored F₂, F₃, and F₄ kernels were 70.49 g/kg, 71.82 g/kg, 71.62 g/kg and mean 70.58 g/kg, were 15.60% (9.51 g/kg), 21.09% (12.51 g/kg), 15.70% (9.72 g/kg) and mean 16.28 % (9.88 g/kg) higher than homozygous yellow (*YY*) kernels, which exhibited oil contents of 60.98 g/kg, 59.31 g/kg and 61.90 g/kg in the F₂, F₃, and F₄ generations and mean 60.70 g/kg, respectively (Table 3). The oil content of heterozygous yellow (*Yy*) F₂, F₃, and F₄ kernels was 70.15 g/kg, 69.25 g/kg and 67.21 g/kg, mean 68.67 g/kg, comparable to the oil content

values of homozygous white (*yy*) kernels which listed above. The heterozygous yellow seed oil content was significantly higher ($p = 0.01$) than the mid-value of *YY* and *yy* types. The oil content of white kernels (*yy*) was 0.48% (0.34 g/kg), 3.71% (2.57 g/kg), 6.56% (4.41 g/kg) and mean 2.78% (1.91 g/kg) higher than that of the heterozygous yellow (*Yy*) kernels and the oil content of *Yy* were 15.04% (9.17 g/kg), 16.76% (9.94 g/kg), 8.58% (5.31 g/kg) and mean 13.13% (7.97 g/kg) more than that of the *YY* genotypes in F₂, F₃, and F₄ kernels. Anova showed there were significant differences among genotypes ($p = 0.0029$) and no significant differences among generations ($p = 0.2584$). The method of mean separation was used to determine the oil content of *Yy* and *yy*. The results yielded no significant difference between the two genotypes. However, the oil content of both *Yy* and *yy* were significantly higher than that of the *YY* genotype. QTL data revealed a linkage between oil content and seed color suggesting that the mean oil content of the homozygous genotype (*OO*) (For the convenience to explain we chose *OO/Oo/oo* as acronyms for the genotype of oil content.) was not significantly higher than that of the heterozygous genotype (*Oo*). The mean oil content of both *Oo* and *OO* were significantly higher than that of the *oo* genotype. Generally, the mean oil content of *Oo* should be equal to the mean value of *OO* and *oo*. However, its mean oil content was significantly higher than the mid-parent value and was lower than that of *OO*. These results indicate that the dominant and partial dominant

Table 3. Contrast between the mean oil content of the heterozygous yellow genotype, homozygous yellow genotype and homozygous recessive white in F₂, F₃, and F₄ kernels.

Genotype	Mean oil content (g/kg)			Mean	MC ^g ($p=0.01$)
	F ₂	F ₃	F ₄		
White ^a (yy)	70.49	71.82	71.62	70.58	a
Heterozygous yellow ^b (Yy)	70.15	69.25	67.21	68.67	a
Homozygous yellow ^c (YY)	60.98	59.31	61.90	60.70	b
Mean	67.22	67.49	65.23	—	—
MC ^h	F = 1.9340 P = 0.2584			—	—
(Yy-YY)% ^d	9.17 (15.04%)	9.94 (16.76%)	5.31 (8.58%)	7.97(13.13%)	—
(yy-Yy)% ^e	0.34 (0.48%)	2.57 (3.71%)	4.41 (6.56%)	1.91(2.78%)	—
(yy-YY)% ^f	9.51 (15.60%)	12.51 (21.09%)	9.72 (15.70%)	9.88(16.28%)	—

^a White kernels which had two recessive allele *yy*.

^b Heterozygous yellow kernels which had one dominant allele *Y* and one recessive allele *y*.

^c Homozygous yellow kernels which had two dominant alleles *YY*.

^d The absolute value and the percentage that the oil content of heterozygous yellow kernels is higher than that of the homozygous yellow kernels.

^e The absolute value and the percentage that the oil content of white kernels is higher than that of the heterozygous yellow kernels.

^f The absolute value and the percentage that the oil content of white kernels is higher than that of the homozygous yellow kernels.

^g The mean separation (multiple comparisons) of the three type of genotypes by the method of Bonferroni (LSDMOD) show the oil content of *YY* are significantly lower than *Yy* and *yy*.

^h The mean separation of the oil content of different genotype within generations by the method of Bonferroni (LSDMOD) show no significantly different across F₂, F₃ and F₄.

effects of gene action at high oil mutant gene loci are located at this position.

Heritability:

The broad-sense heritability estimated for oil content was high in the three generations. The H_B^2 was 89.5%, 86.4% and 86.0% respectively in F₂, F₃ and F₄ kernels. Continuous variations, exhibiting a normal distribution for kernel oil content, were observed among the 214 F₂ individuals, as well as in F₃ and F₄ kernels (Fig. 2). The Kolmogorov-Smirnov test results showed that the p value were all 0.200* ($p > 0.05$) in the three generations. And the p values of Shapiro-Wilk test were 0.995, 0.999 and 0.981 in F₂, F₃ and F₄ kernels respectively. The distribution of kernel oil content in the F₂, F₃ and F₄ kernels was tested normally according to the results of both tests.

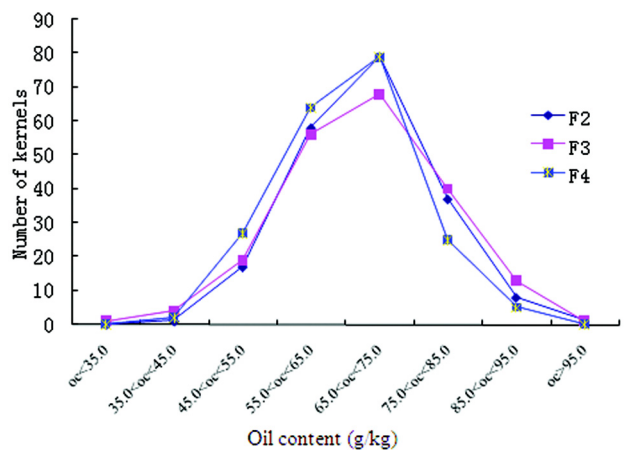


Figure 2. Distribution of kernel oil content in F₂, F₃ and F₄ kernels

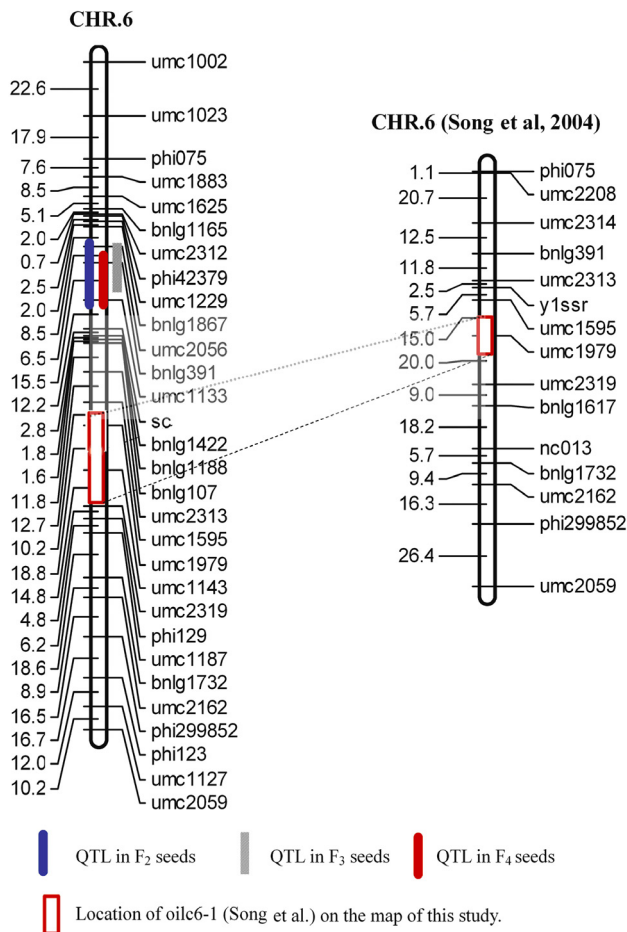


Figure 3. Linkage map of the F₂ population in chromosome 6 constructed with 30 SSR markers and one phenotypic marker. The markers are indicated on the right of the map and the map distances, in centimorgans (cM), are indicated on the left of the chromosomes. Illustrational bars indicate oil content QTL detected in this study and previous studies in chromosome 6.

Genetic linkage map

One hundred and fifty SSR markers in chromosome 6 were used to screen for polymorphisms between the two parents B73 and CE03005. Thirty SSR markers and one phenotypic marker were co-dominantly segregated and used to construct the linkage map (Fig. 3). SC denotes seed color, when F₂ kernels are yellow and the self-pollinated ear has no segregation, the marker is 1, when F₂ kernels are yellow and the self-pollinated ear exhibits segregation the marker is 3, and if the kernels are white, the marker is 2. The resulting linkage map of chromosome 6 was 280 cM long, with an average interval of 9.03 cM between two markers. All of the markers, except p-umc1143

were located in regions similar to those in MaizeGDB (2004) maps.

QTL mapping

In order to map the QTLs, the mean oil content of each line (F₃ and F₄ kernels) or kernel oil content (F₂ kernels) was generated. Additionally the genetic map and the composite interval mapping (CIM) were employed (Zeng, 1994). A 3.0 LOD value was set as the significant threshold ($p=0.05$) after running 1000 permutations (Doerge and Churchill, 1996; Mangolin et al., 2004; Song et al 2004). A QTL significantly ($LOD > 3$ suggested by the permutation test) associated with oil content was detected in F₂, F₃ and F₄ kernels at bin 6.01 near the phenotypic marker SC. In addition, SSR markers p-bnlgl1422, p-bnlgl107 and p-umc2313 in chromosome 6 supported the QTL association with oil content in F₂, F₃ and F₄ kernels (Table 4 and Fig. 3). We determined whether the QTLs were common among the F₂, F₃ and F₄ kernels evaluations using the method of Flint-Garcia et al (2003). The overlapping confidence intervals of the F₂, F₃ and F₄ generations were less than 20 cM. Therefore, this analysis reveals that the three QTLs for kernel oil content were identical among the F₂, F₃ and F₄ kernels (Flint-Garcia et al., 2003; Song et al., 2004).

The additive and dominant effects of QTLs for oil content, estimated by QTL Cartographer Version 2.0, showed that the DR values (calculated by the expressions $DR = | \text{dominant effects (d)} | / | \text{additive effects (a)} |$) were 1.03, 0.36 and 0.42 in F₂, F₃ and F₄ kernels. These data suggest that QTLs in F₂ kernels have dominant effects (D) and QTLs in F₃ and F₄ kernels elicit incomplete or partial dominance (PD) at both loci (Table 4). Berke and Rocheford (1995) and Song et al. (2004) indicate that the high-oil parent (CE03005) contributed all of the positive alleles at each QTL for kernel oil content (the additive value was negative) (Table 3). The proportion of phenotypic variation (partial R²) explained by a single QTL was 17.1 % in the F₂ kernels, 8.1% in the F₃ kernels and 9.3% in the F₄ kernels. The maximum LOD scores were 8.47 in F₂ kernels, 3.82 in F₃ kernels and 3.62 in the F₄ kernels.

Table 4. Position, genetic effects and coefficients of determination of the QTLs in chromosome 6 associated to the kernel oil content.

Generation	Flanking markers	Position ^a (cM)	Genetic effect		Gene action ^d	LOD	Partial R ^{2e}
			A ^b	D ^c			
F ₂ kernels	bnlg107-umc2313	127.8	-5.62	5.81	D (1.03)	8.47	17.1
F ₃ kernels	Sc-blg1422-bnlg107	113.6	-7.80	2.80	PD (0.36)	3.82	8.1
F ₄ kernels	bnlg107-mc2313-umc1257	135.6	-7.91	3.31	PD (0.42)	3.62	9.3

^a Genetic map position of the QTL peak by cM.

^b Additive effects estimated by QTL Cartographer.

^c Dominant effects estimated by QTL Cartographer.

^d D and PD represent dominant, partial dominant effects respectively (DR = |d|/|a|; additive (A): 0.1 ≤ DR < 0.2; partially dominant (PD): 0.2 ≤ DR < 0.8 dominant (D): 0.8 ≤ DR < 1.2; over dominant (OD): 1.2 ≤ DR).

^e Percentage of the phenotypic variation explained by the QTL.

DISCUSSION

A QTL influencing kernel oil content in maize was clearly identified using the high oil maize mutant. Overlapping confidence intervals determined that the three QTLs were common among the F₂, F₃ and F₄ kernel evaluations (Flint-Garcia et al., 2003; Song et al., 2004). The gene actions are D (dominant) in F₂ kernels and PD (partially dominant) in F₃ and F₄ kernels (Table 4). Transgressive segregation due to mutation parent had been observed (Many kernels oil content are more than 85.61 g/kg.), which proved the gene action estimated here was true. The high oil mutant was found to be dominant via the phenotypic analysis. The results demonstrate that QTL Cartographer and a traditional Mendelian genetic analysis are congruent. The F₃ kernels were from self-pollinated F₂ plants and F₄ kernels were cross-pollinated from the same F_{2:3} ear lines. Through subsequent generations homozygous gene loci increased, raising the A (additive) value and decreasing the DR = |d|/|a| value. As a result, the differences observed in the gene actions among F₂ (D), F₃ (PD) and F₄ (PD) generations followed expected Mendelian inheritance patterns. The minimal differences in QTLs among the three generations could be due to environmental effects, or because the phenotypic value of the F₂ kernels was produced from a single kernel. The peak of the QTL (113.6) is distributed close to the phenotypic marker SC (seed color, 111.6, about 2 cM away) and SSR markers p-bnlg1422, p-bnlg107 and p-umc2313 on the linkage map. This supported our prediction

that a QTL controlling the oil content of the high-oil mutant exists near the gene that controls seed color in chromosome 6.

Oil content QTL identified in this study (oilc6-m1) was compared with QTL (oilc6-1) from mapping populations derived from BHO × B73 (Song et al., 2004) (Fig. 3). The flanking markers of this study were at bin 6.01 and at position 127.8 and 113.6 on the linkage map. The flanking markers identified by Song et al. (2004) (umc1595-umc2319) were at bin 6.02-6.04. The two QTLs have no overlapping confidence intervals and the distance between them is approximately 30-50 cM. According to the overlapping interval (< 20 cM), the two QTLs should be different from the QTL oilc6-1 reported by Song et al. (2004). It was reported independently by Goldman et al. (1994), Berke and Rocheford (1995), Alrefai et al. (1995) and Margolin et al. (2004) that a QTL control oil content had been mapped in chromosome 6. This suggests it is an important locus for kernel oil content in maize. The difference between the position of QTLs mapped in our study and other reports could be due to the use of a different germplasm. The germplasm in this study was a chemically induced high oil mutant while in previous studies it was bred from high oil population.

The QTL in chromosome 6 explains a fraction of the inherent phenotypic variation however broad-sense heritability is high, the reason of which is that broad-sense heritability is the behavior of all QTLs in the genome, that is, may be other QTLs are present in other chromosomes in addition to chromosome 6.

In order to find QTLs which are present in other chromosomes, studies of constructing genetic linkage map of all chromosomes and whole genomic scanning for QTLs are needed in the future. However, though the phenotypic variation explained by the QTL in this study was not high, all data support the existence of QTL in chromosome 6 of the special mutant CE03005 and the QTL effects could be detected by genetic analysis easier than with other loci. Therefore, the identified molecular and phenotypic markers could also be used relatively easier for breeding purpose, the advantages over traditional long term selection by NMR is that it could be used to select oil content QTL in a special chromosome, while NMR could only be used to select a kernel's total oil content. More importantly, the successful methodology achieved in this study would lend convenience in cloning QTLs.

Genotype by environment interaction is accepted as another important component of cultivar performance, and has received considerable attention in crop breeding programs (Dudley et al., 1996). The existence of QTL \times E interactions can lead to the differences of positions and effects in QTLs among generations (or different environments). In practice, QTL \times E interaction will have some influence in determining the adaptation and fitness of QTLs to the environment. QTL with minor effects is influenced easily by the environment interactions. To date, how the QTLs with minor effects worked in the forming of phenotypes is not yet very clear. Although the effects are not very high, the role of QTL with minor effects can't be ignored in maize breeding programs. Most of the main effect QTLs can be found through different populations and with different QTL analysis methods. There are really some QTL that are not stable across populations, which may lead to a wider confidence interval and bring difficulty to the marker-assisted-selection. In this study, the result is relatively stable in the three generations, since the primary population of F₂ was used, which influenced the accurate of QTL mapping in some degree. The method of fine mapping should be used if a more accuracy result is expected.

REFERENCES

Alrefai R, Berke TG and Rocheford TR (1995) Quanti-

tative trait locus analysis of fatty acid concentrations in maize. *Genome* 38: 894-901.

Benitez JA, Gernat AG, Murillo JG et al. (1999) The use of high oil corn in broiler diets. *Poultry Sci.* 78: 861-865

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

Bohn M, Khairallah MM, Gonzalez-de-Leon DHF, Utz JA, Deutsch DC, Jewell JA, Mihn and Melchinger AE (1996) QTL mapping in tropical maize-I. Genomic regions affecting resistance to sugar-cane borer and other traits. *Crop Sci.* 37: 1892-1902.

Buckner B, Todd L, Kelson and Robertson DS (1990) Cloning of the y1 locus of Maize, a Gene Involved in the Biosynthesis of Carotenoids. *The Plant Cell* 2: 867-876.

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

Dudley JW, Lamkey KR and Geadelmann JL (1996) Evaluation of populations for their potential to improve three maize hybrids. *Crop Sci.* 36: 1553-1559.

Flint-Garcia SA, McMullen MD and Darrah LL (2003) Genetic relationship of stalk strength and ear height in maize. *Crop Sci.* 43: 23-31.

Goldman I, Rocheford TR and Dudley JW (1994) Molecular markers associated with maize kernel oil concentration in an Illinois high protein \times Illinois low protein cross. *Crop Sci.* 34: 908-915.

Goss J and Kerr PS (1992) Challenges and opportunities for identity preserved varieties. In: Proc 47th Annual Corn and Sorghum Research Conference, Am. Seed Trade Assoc., Washington, DC, USA. pp. 1-31.

Hackett CA (2002) Statistical methods for QTL mapping in cereals. *Plant Mol. Biol.* 48: 585-599.

Han Y, Parsons CM and Alexander DE (1987) The nutritive value of high-oil corn for poultry. *J. Poult. Sci.* 66: 103-111.

Hoisington D, Khairallah M and Gonzalez-de-leon D (1994) *Laboratory Protocols*, CIMMYT Applied Molecular Genetics Laboratory, 2nd ed, Mexico.

Jiang CJ and Zeng ZB (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* 140: 1111-1127.

- Kelly P, Michele MST and Antoni R (2004) Long-range patterns of diversity and linkage disequilibrium surrounding the maize *Y1* gene are indicative of an asymmetric selective sweep. *Proc. Natl. Acad. Sci.* 101(26): 9885-9890.
- Khavkin E and Coe E (1997) Mapped genomic locations for developmental functions and QTLs reflect concerted groups in maize (*Zea mays* L.). *Theor. Appl. Genet.* 95: 343-352.
- Knapp SJ, Stroup VW and Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. *Crop Sci.* 25: 192-194.
- Kong FL (ed) (2005) *Quantitative Genetics in Plants*. BeiJing.
- Kraja AT and Dudley JW (2000) QTL analysis of two maize inbred line crosses. *Maydica* 45: 1-12.
- Lambert RJ (2001) High-oil corn Hybrids. In *Spical Corns*, A.R. Hallauer, ed, Boca Raton, Florida, pp131-154.
- Lee M (1995) DNA markers and plant breeding programs. *Adv. Agron.* 55: 265-344.
- Lincoln S, Daly M and Lander E (1992) Constructing genetics maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report. Whitehead Institute, Cambridge, Massachusetts, USA.
- Maize Genetics and Genomics Database (2004) <http://www.maizegdb.org> Cited 18 Sept 2004.
- Mangolin CA, Souza JCLD, Garcia AAF et al. (2004) Mapping QTLs for kernel oil content in a tropical maize population. *Euphytica* 137: 251-259.
- 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.
- Mickelson SM, Stuber CS, Senior L et al. (2002) Quantitative trait loci controlling leaf and tassel traits in a B73 × Mo17 population. *Crop Sci.* 42: 1902-1909.
- O'Quinn PR, Nelssen JL, Goldman FD et al. (2000) Nutritional value of a genetically improved high-lysine, high-oil corn for young pigs. *J. Theor. Biol.* 117: 1-10.
- Senior ML and Manfred H (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. *Genome* 36: 884-889.
- Sibov TT, Souza JrCL, Garcia AAF et al. (2003) Molecular mapping in tropical maize (*Zea mays* L.) using microsatellites markers. *Hereditas* 139: 96-106.
- Song TM and Chen SJ (2004) Long Term Selection for Oil Concentration in Five Maize Populations. *Maydica* 49: 9-14.
- Song XF, Song TM, Dai JR et al. (2004) QTL mapping of kernel oil concentration with high-oil maize by SSR markers. *Maydica* 49: 41-48.
- Stuber CW and Sisco P (1991) Marker-facilitated transfer of QTL alleles between elite inbred lines and responses in hybrids, Paper presented at the 46th Annual Corn and Sorghum Research, Washington DC, USA, pp. 104-113.
- Zeng AB (1994) Precision mapping of quantitative trait loci. *Genetics* 136: 1457-1468.