RESEARCH ARTICLE

Mapping of quantitative trait loci for oil content in cottonseed kernel

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Abstract

Oil content in cottonseed is a major quality trait which when improved through breeding could enhance the competitiveness of cottonseed oil among other vegetable oils. Cottonseed oil content is a quantitative trait controlled by genes in the tetraploid embryo and tetraploid maternal plant genomes, and the knowledge of quantitative trait loci (QTLs) and the genetic effects related to oil content in both genomes could facilitate the improvement in its quality and quantity. However, till date, QTL mapping and genetic analysis related to this trait in cotton have only been conducted in the tetraploid embryo genome. In the current experiment, an IF_2 population of cottonseed kernels from the random crossing of 188 intraspecific recombinant inbred lines which were derived from the hybrid of two parents, HS46 and MARCABUCAG8US-1-88, were used to simultaneously locate QTLs for oil content in the embryo and maternal plant genomes. The four QTLs found to be associated with oil content in cottonseed were: *qOC-18-1* on chromosome 18; *qOC-LG-11* on linkage group 11; *qOC-18-2* on chromosome 18; and *qOC-22* on chromosome 22. At a high selection threshold of 0.05, there was strong evidence linking the QTLs above the oil content in cottonseed. Embryo additive and dominant effects from the tetraploid embryo genome, as well as maternal additive effects from the tetraploid maternal plant genome were found to be significant contributors to genetic variation in cottonseed oil content.

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Introduction

Cottonseed is the second major product from the cotton plant (next to fiber) which serves as raw material for oil extraction or animal feed production (Ashokkumar and Ravikesavan 2008; Hamilton *et al.* 2004). It belongs to the group of unsaturated vegetable oil seeds that include safflower, corn, soybean, rapeseed and sunflower (Bert *et al.* 2003). The five largest cottonseed producing countries (China, 29%; United States, 19.9%; India, 14.2%; Pakistan, 9.5% and Brazil, 5%) currently acount for 73.1% of global output (Song and Zhang 2007). Its fatty acid profile generally consists of 70% unsaturated and 30% saturated fatty acids. Because of its low-cost and flavor stability compared to olive oil or canola, it has become increasingly acceptable in a wide range of processed foods, including bread and snack such as potato chips (Dowd *et al.* 2010). Cottonseed oil is however under scrutiny by some nutritionists for having too high-saturated fat and too low mono-unsaturated fat (Weil 2007). To address these concerns and to improve the quantity and quality of cottonseed oil content, various breeding procedures have been employed with different levels of success (Cherry *et al.* 1981; Dani 1990). Breeding cottonseed for oil content has depended mainly on phenotypic information that is used to select varieties with high seed oil content (Azhar and Ahmad 2000; Ash and Dohlman 2006; Pahlavni *et al.* 2008). These methods have proven to be unfeasible for the effective genetic improvement of this trait. The genetic basis controlling oil content has received little attention (Khan *et al.* 2007; Wu *et al.* 2010), although a few studies have shown that oil content in cottonseed is determined by genetic effects from the embryo and maternal plant genomes (Van Heerden 1969; Kohel 1980; Singh *et al.* 1985; Ramos 1985; Dani and Kohel 1989). Understanding the distribution of quantitative trait loci (QTL) on chromosomes and the genetic systems that control the performance of these traits could facilitate the application of this information for cottonseed improvement that will help ensure better oil quality (Zheng *et al.* 2008; Shi *et al.* 2009). Cottonseed is considered a different generation from its maternal plant although the

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maternal plant supplies assimilates for seed development. This situation further complicates the genetic mechanism of seed traits, requiring a more detailed study that could help facilitate the selection for oil content with more precision. The development of molecular markers in cotton (Yu *et al.* 1996; Connell *et al.* 1998; Yu and Kohel 1999; Ulloa *et al.* 2000; Reddy *et al.* 2001; Kumpatla *et al.* 2002; Saha *et al.* 2003; Nguyen *et al.* 2004; Han *et al.* 2004; Park *et al.* 2005), generated from the cotton genome or EST sequences, have demonstrated that they could serve as powerful selection tools for enhancing selection efficiency and curtailing time and resources involved in traditional selection procedures. Studies on QTLs related to oil content have been conducted in other oilseed crops including soybean (Brummer *et al.* 1997), oilseed rape (Burns *et al.* 2003; Zhao *et al.* 2008), jatropha (Liu *et al.* 2011), maize (Alrefai *et al.* 1995) and sunflower (Leon *et al.* 2003). Song and Zhang (2007) evaluated a population of 140 cotton BC_1S_1 lines developed from a cross between TM-1 and Hai 7124 cultivars on a linkage map consisting of 918 markers from this population and identified them among other seed-quality traits, one significant QTL for kernel oil percentage in (*qOP-D8-1*) between SSR markers BNL2860_190 and NAU1369_400 in the region of 9.2 cM. Yu *et al.* (2012) also mapped 17 QTLs on 12 chromosomes for oil content in cottonseed. However the above studies were conducted based only on embryo genome in cotton. In rice, Zheng *et al.* (2008) and Shi *et al.* (2009) successfully mapped QTLs related to seed-quality traits in the endosperm and maternal plant genomes, however till date there is no report on the mapping of QTLs based on different genomes in cottonseed. In this study QTLs for oil content in cottonseed kernels of 376 IF_2 lines, obtained from the random crossing of 188 intraspecific recombinant inbred lines (RILs) that were derived from the hybrid of two parents HS46 and MARCABUCAG8US-1-88, were simultaneously mapped on the tetraploid embryo and tetraploid maternal plant genomes, and the impact of the genetic effects from the QTLs on oil content were analysed.

Materials and methods

Planting materials

The experimental materials used in this study comprise of a set of 188 intraspecific RILs, derived from the hybrid of the cross between two upland cotton parents, HS46 and MARCABUCAG8US-1-88, that were kindly supplied by USDA-ARS, Starkville, Mississippi, USA. These two parents have wide genetic differences in different traits including oil seed content. The HS46 is adapted for high-yield with excellent fiber qualities, and MARCABUCAG8US-1-88 is adapted for resistance to stress. The RIL population was constructed through the modified single-hill (bulked progeny row) method. The seeds of 188 RILs and two parents were provided in 1992 and have been conserved by selfing over years. Their yield, fiber qualities as well as related traits have a normal distribution, which showed that this intraspecific RIL population is an excellent population for genetic research and QTL mapping.

Field experiments

Seeds of RILs and two parents were sown on the experimental farm in Hainan, China, on 10 October 2009, in a randomized block design with two replications at plating distances of 0.3 m between rows and 0.25 m between plants. At flowering stage, the 188 RILs were randomly crossed 376 times according to a partial diallel design. The F_1 seeds obtained from the crosses between the RILs formed the immortal F_2 (IF_2) population which had the same genetic structure as the F_2 seeds derived of the hybrid from the cross between HS46 and MARCABUCAG8US-1-88. All management on the field followed standard agronomic practices. At maturity, seeds of both parents and the IF_2 population were harvested for further analyses (Chao 2009).

Sample preparation

Normally open bolls were harvested ginned, acid-delinted and dried at 38°C in a forced-air oven to reduce moisture content and to prevent germination. From each sample, 200 kernels were selected for preparation. Kernels were removed from the cottonseed shells and then separated from all foreign materials before grinding into powder with the Universal high-speed grinder DFT-50 (Linda Machinery, Wenlin, Zhejiang, China). Each sample was ground for four times at 10 s intervals for each time to obtain particle sizes between 0.5 and 0.8 mm. After grinding, samples were dried to equilibrium at 25°C and their moisture content was balanced to 7%.

Spectroscopic analysis

The NIRSystem mode 5000 monochromator (NIRSystemL Silver Spring, USA) was used to scan a cup full of each powdered sample four times, after which the reflectance spectra (log1/R) from 1100 to 2498 nm, were recorded at 2 nm intervals (Wu *et al.* 2002). The average scan values were used to develop calibration models for oil content. Reflectance spectra meant for the calibration set was first processed using the second derivative mathematical treatment (2,8,8,1) to correct baseline shifts, and inverse multiple scatter correction (I-MSC) algorithm to correct scattering of spectra due to particle size (in '(2,8,8,1)', it is a derivative treatment of background noise where the first number indicates the order of derivative (one is first derivative of log 1/R), the second number is the gap in nm over which the derivative is calculated, the third number is the number of nm used in the first smoothing and the fourth number refers to the number of nm over which the second smoothing is applied). Using the modified partial least squares (MPLS), a

robust calibration model for oil content was developed. The results from calibration were then used in the study of the corresponding QTLs for oil content on the linkage map as well as the genetic effects from different genomes on the trait (Qin *et al.* 2010).

Linkage map for QTL mapping

In the present study a relatively higher density genetic linkage map was constructed based on the RIL population, with three kinds of molecular markers, SSR, SRAP and RAPD. It contained 388 markers, covering 30 linkage groups with a total genome size of 1946.22 cM and at an average distance of 5.03 cM between the adjacent markers and spanned 42% of the cotton genome. Currently, this map is considered as the highest density map for markers with a wide coverage in cotton intraspecific RIL population (Chao 2009). In the present experiment only segments of linkage groups associated with QTLs detected are shown.

Statistical analysis

Descriptive statistics including mean, standard deviation, minimum and maximum values and skewness of the cottonseed oil phenotype, were calculated with the SPSS 16.0 Data Editor (IBM Corporation, Somers, USA). QTL analyses were carried out on cottonseed oil content with the QTLNetwork-CL-2.0-Seed program (Zhejiang University, China) to simultaneously map QTLs based on different genetic systems (the tetraploid embryo and the tetraploid maternal plant genomes), using the mixed-model based composite interval mapping (MCIM) method with a 10 cM window size and a 1 cM walking speed. The procedure of mixed linear model-based interval mapping was conducted according to the mapping strategy proposed by Yang *et al.* (2007). A map function (Kosambi 1944) was utilized to translate from recombination frequency to distance or vice versa which was used to calculate an LOD score at each increment (walking step). The likelihood test was performed over all the markers constituting a systematic strategy of searching for QTLs throughout the genomes. The QTLs identified in this population were tested for acceptance using the likelihood ratio test. Likelihood ratio values of 6.63, 7.88 and 10.82 represent significant values with probabilities of 0.01, 0.05 and 0.01, respectively with one degree of freedom (Shappley *et al.* 1998). The genetic effects of QTL in the model included embryo additive effect and embryo dominance effect from tetraploid embryo nuclear genes, and maternal additive effect from tetraploid maternal plant nuclear genes. These genetic effects and their corresponding *P* values were estimated using the Markov Chain Monte Carlo (MCMC) algorithm for Gaussian mixed linear model via Gibbs sampling in a single environment (Zhu and Weir 1998; Wang 2000). Estimated genetic additive and dominance effects were tested for significance by using the standard normal distribution (Shappley *et al.* 1998). QTLs were named following the standard nomenclature suggested by McCouch *et al.* (1997). QTLs were labelled with 'q' for QTL, followed by an abbreviation of trait name and the name of chromosome or linkage group, followed by the QTL number affecting the trait on the chromosome or linkage group. QTL numbers are indicated only when there are more than one QTL on a chromosome or a linkage group (Yang *et al.* 2007). In this report, 'C' for 'chromosome' is omitted but 'LG' for 'linkage group' is applied to the nomenclature. For example, *qOC-LG-11* represents the QTL (*q*) for oil content (*OC*) on linkage group (*LG*) number 11.

Results

Phenotypic analysis of oil content in the parents and IF_2 populations

Phenotypic data for oil content from samples of the parents and the IF_2 population are summarized in table 1. There was a slight variation in the oil content among parents and IF_2 generation as indicated by the mean values. Variability in the sample population according to the standard deviation values was high. Parents and IF_2 were normally distributed making the population suitable for QTL determination, although skewing for oil content in HS46 slightly exceeded 1. The distribution of phenotypic values for oil content in HS46, MARCABUCAG8US-1-88 and the IF_2 populations are shown in figure 1. The mean values for parents were less than that of the IF_2 suggesting a possible occurrence of heterosis (Karademir *et al.* 2007).

QTL analysis for oil content

A high selection threshold, such as 0.05 or 0.01, provides strong evidence that the reported QTLs are actually associated with the oil content (Zhu and Weir 1994). QTL analysis based on the likelihood ratio values suggested a total of

Table 1. Statistical analysis of oil content in parents and IF_2 populations.

Generation	Minimum	Maximum	Mean	SD	Skew
MARCABUCAG8US-1-88	29.1088	33.3258	31.4900	1.2554	–
HS46	29.6358	34.6105	31.0100	1.2636	–
IF_2	28.6800	38.4400	33.0650	1.5100	0.2900

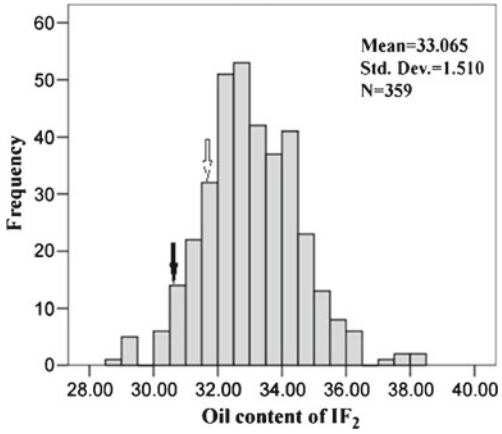

Figure 1. Frequency distributions for cottonseed kernel oil content within the parents' and IF_2 populations. White and black arrows indicate mean values for HS46 and MARCABUCAG8US-1-88, respectively.

four significant QTLs associated with oil content in cottonseed (figure 2). The QTLs identified were *qOC-18-1* between markers JESPR204 (c18) and CIR099 (c18) in the region of 2.91 cM on chromosome 18; *qOC-LG-11* between markers NAU979 and NAU1162 in the region of 4.56 cM on linkage group 11; *qOC-18-2* located between markers NAU1184b and BNL3479 (c18) in the region of 11.50 cM on chromosome 18; and *qOC-22* between markers CIR253 and JESPR65 in the region of 6.10 cM on chromosome 22.

Genetic effects on QTLs controlling cottonseed oil content

The genetic effects controlling oil content in cottonseed oil are given in table 2. For each QTL, the level of significance additive and dominance genetic effects for oil content can be observed. Significant ($P < 0.01$) embryo additive effect (a^{em}) for *qOC-18-1* and *qOC-22*, as well as significant ($P < 0.01$) embryo dominance effect (d^{em}) for *qOC-LG-11* and *qOC-18-2* were observed. Maternal additive effect (a^{m}) was significant ($P < 0.01$) for *qOC-18-2* and less significant ($P < 0.05$) for *qOC-LG-11*. *qOC-18-2* had the largest embryo additive effect ($a^{em} = 0.4572$**) followed by *qOC-18-1* ($a^{em} = -0.2499$**) and *qOC-22* ($a^{em} = 0.2022$**). Embryo additive effect for *qOC-LG-11* ($a^{em} = 0.0656$) was not significant, although it had the largest and only significant embryo dominance effect ($d^{em} = -0.3660$**). The largest maternal additive effect was recorded for *qOC-18-2* ($a^{m} = -0.307$**) followed by *qOC-LG-11* ($a^{m} = 0.1579$*). Maternal additive effects for *qOC-18-1* ($a^{m} = -0.0320$) and *qOC-22* ($a^{m} = 0.0112$) were not significant. It was observed that the embryo could significantly influence a decrease in oil content at *qOC-18-1* and *qOC-LG-11* or an increase at *qOC-18-2* and *qOC-22*. At *qOC-LG-11*, the possible decreasing effects from the embryo in oil content were evident. Similar observations were made in the maternal plant genome from where genetic effects could significantly increase oil content at *qOC-LG-11* or decrease it at *qOC-18-2*. All QTLs collectively accounted for 18.4% of heritability, with the embryo additive effects contributing 15%, embryo dominant effects

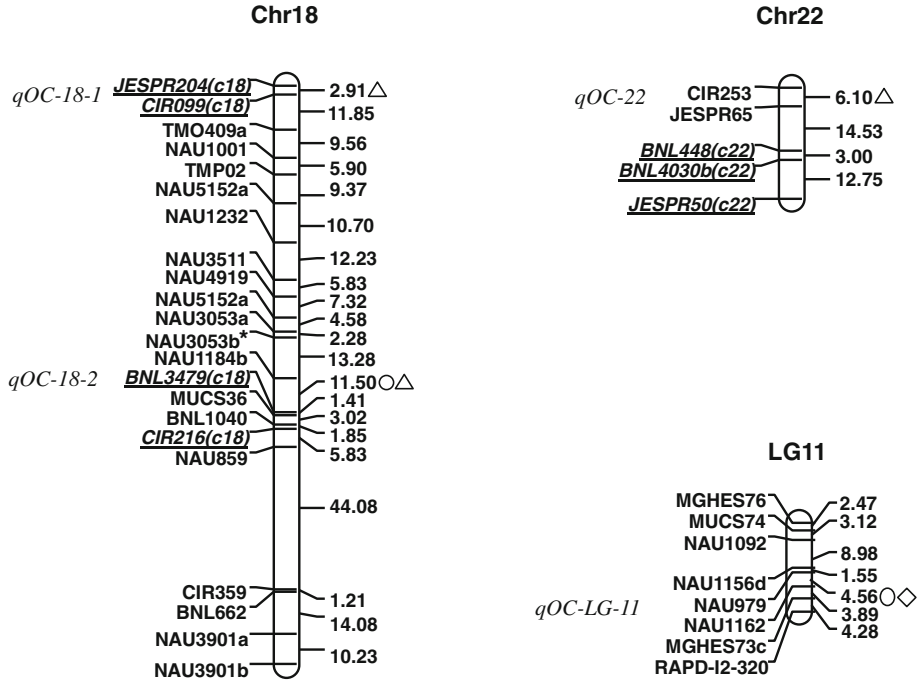

Figure 2. Distribution of QTLs and their genetic effects in embryo and maternal plant nuclear genomes for oil content in cottonseed. △, Embryo additive effect of QTL from tetraploid embryo genome; ◇, embryo dominance effect of QTL from tetraploid embryo genome; ○, maternal additive effect of QTL from tetraploid maternal plant genome.

Table 2. The genetic effects and heritability of QTLs for oil content in cottonseed.

QTL	*qOC-18-1*	*qOC-18-2*	*qOC-22*	*qOC-LG-11*
Interval	JESPR204(c18)-CIR099(c18)	NAU1184b-BNL3479(c18)	CIR253-JESPR65	NAU979-NAU1162
Position	0.0	101.8	0.0	20.1
Range	0.0–2.0	92.5–108.3	0.0–3.0	18.1–22.7
a^{em}	−0.2499**	0.4572**	0.2022**	0.0656
d^{em}	0.0682	0.1007	−0.0124	−0.3660**
a^{m}	−0.0320	−0.307**	0.0112	0.1579*
$H^2(a^{em})$	0.0446	0.0878	0.0172	0.0000
$H^2(d^{em})$	0.0000	0.0000	0.0000	0.0146
$H^2(a^{m})$	0.0000	0.0158	0.0000	0.0035
H^2	0.0446	0.1036	0.0172	0.0181

a^{em} embryo additive effect of QTL from tetraploid embryo genome; d^{em} embryo dominance effect of QTL from tetraploid embryo genome; a^{m} maternal additive effect of QTL from tetraploid maternal plant genome; $H^2(a^{em})$ ratio of variance caused by a^{em}, to phenotypic variance; $H^2(d^{em})$ ratio of variance caused by, d^{em} to phenotypic variance; $H^2(a^{m})$ ratio of variance caused by a^{m} to phenotypic variance; H^2 total heritability of a single QTL; $*P < 0.05$; $**P < 0.01$; negative sign (–) represents the allele from MARCABUCAG8US-1-88 increasing the value of the trait.

contributing 1.4% and maternal additive effects contributing 2%. QTLs with significant genetic effects located on the tetraploid embryo genome (*qOC-18-1*, *qOC-18-2, qOC-22* and *qOC-LG-11)* were considered to be expressed in tissues of the seed. On the other hand, the significant genetic effect from the tetraploid maternal plant genome on *qOC-18-2* indicated that this QTL was simultaneously expressed in both the tetraploid embryo and the tetraploid maternal plant genomes constituting a possible link between the parent and the next generation. These results suggest that there could be significant genetic effects expressed by QTLs located on the chromosomes of both the embryo and the maternal plant for oil content in cottonseed. The heritability of the QTLs (table 2) could be an indicator of their effectiveness in marker-assisted selection (MAS). It can be observed that, *qOC-18-2* has a high total heritability which indicates that it is a major QTL. With such a high heritability from the embryo additive effect, it could be effective when used for MAS. Although the heritability of *qOC-18-1* is relatively low, it was mainly controlled by embryo additive effects making it also suitable for MAS.

Discussion

Cottonseed oil is a major vegetable oil used mainly in industrial food processing due to the stability of its flavor. An improvement in its quality through breeding could enhance its consumer acceptability and reduce its health-related risks. In the present study, the use of near infrared reflectance spectroscopy (NIRS) for measuring the oil content in cottonseed kernel allows for larger quantities of cottonseed samples to be evaluated while increasing the variability in the specific traits as well as precision in the determinations. Seed quantitative traits are genetically complex and this makes the improvement of oil content in cottonseed more difficult. At the moment, the nature of interactions between QTLs and the different (i.e. maternal and embryo) genomes on cottonseed are still not very clear. To understand these interactions there is a need to map the target traits and study the genetic effects based on these genomes on the traits. However, genetic effects from different genomes on QTLs located on their respective chromosomes can not be clearly distinguished using conventional QTL mapping methods and softwares (Zheng *et al.* 2008; Shi *et al.* 2009). In this study, the simultaneous mapping of QTLs for oil content based on the tetraploid embryo and tetraploid maternal plant genomes was possible due to the establishment of the Network-CL-2.0-Seed software. It revealed that QTLs controlling oil content in cotton were distributed on several chromosomes in two genomes. It also allowed us to further understand more genetic mechanisms from the seed trait phenotype to gene identification. The results in the present experiment indicate that QTL could be simultaneously expressed in different genome-specific tissues as can be observed in the expression of *qOC-18-2* in both the tetraploid embryo and tetraploid maternal plant genomes. The additive effects on *qOC-18-2* in the embryo and maternal plant genomes have opposite directions, suggesting that the expression of this QTL is different in these two genomes. In the embryo genome, the allele at this locus from HS46 could increase oil content by 0.4572% while the one from MARCABUCAG8US-1-88 could decrease oil content by 0.4572%. In the maternal plant genome, the allele at this locus from MARCABUCAG8US-1-88 could increase oil content by 0.307% while that from HS46 could decrease oil content by 0.307%. In this case, either the embryo or maternal plant could be considered during selection at *qOC-18-2* depending on other considerations from the breeder. In oilseed crops, when the quality traits are mainly controlled by the genetic effects from the QTLs located on chromosomes in the maternal plant genome, they can be improved according to the performance of the maternal plant. However, for those traits controlled mainly by the QTLs located

on chromosomes in the embryo genome, it is applicable to select a single seed of good quality because of the differences among seeds (Shi *et al.* 2009). The results from this study also showed that, there were more additive than dominant effects from both genomes on the QTLs, suggesting their potential suitability for MAS even with a relatively low heritability as it was in the case of *qOC-18-1*.

The genetic mechanisms controlling oil content in cotton are closely related to QTLs located on the chromosomes. Although QTLs for oil content in cotton has been mapped in two previous studies (Song and Zhang 2007; Yu *et al.* 2012), they fell short of providing a detailed information on the genetic effects based on different genetic systems. In the present study, the four new QTLs (*qOC-18-1, qOC-18-2, qOC-22* and *qOC-LG-11*) identified in oil content based on genetic effects from the tetraploid embryo and tetraploid maternal plant genomes, thus constitute a major landmark setting the pace for a more accurate selection for the genetic improvement of cottonseed oil.

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