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Abstract Soybean [*Glycine max* (L.) Merr.] is an important crop which contributes approximately 58% of the world's oilseed production. Palmitic and stearic acids are the two main saturated fatty acids in soybean oil. Different levels of saturated fatty acids are desired depending on the uses of the soybean oil. Vegetable oil low in saturated fatty acids is preferred for human consumption, while for industrial applications, soybean oil with higher levels of saturated fatty acids is more suitable. The objectives of this study were to identify quantitative trait loci (QTL) for saturated fatty acids, analyze the genetic effects of single QTL and QTL combinations, and discuss the potential of marker-assisted selection in soybean breeding for modified saturated fatty acid profiles. A population of recombinant inbred lines derived from the cross of SD02-4-59 × A02-381100 was grown in five environments and the seed samples from each environment were evaluated for fatty acid content. Genotyping of the population was performed with 516 polymorphic single nucleotide polymorphism markers and 298 polymorphic simple sequence repeat markers. Eight QTL for palmitic acid, five QTL for stearic acid and nine QTL for total saturated fatty acids were detected by composite interval mapping and/or interval mapping, with a high level of consistency or repeatability in multiple environments. Most of these QTL have not been reported previously, with the exception of *qPAL-A1* which confirmed the result of a previous study. Significant QTL × QTL interactions were not detected. However, significant QTL × environment interactions were detected in most cases. Comparisons of two-locus and three-locus combinations indicated that cumulative effects of QTL were significant for both palmitic and stearic acids. QTL pyramiding by molecular marker-assisted selection would be an appropriate strategy for improvement of saturated fatty acids in soybean.

Keywords Soybean · Palmitic acid · Stearic acid · QTL · Marker-assisted selection

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Introduction

Soybean [*Glycine max* (L.) Merr.] is an important crop which contributes 57.7% of the world's oilseed

production (Cardinal et al. 2007). Soybean oil consists mainly of five fatty acids: saturated palmitic (16:0) and stearic (18:0) acids, and unsaturated oleic (18:1), linoleic (18:2) and linolenic (18:3) acids (Wilson 2004). Soybean oil is extensively used in a wide range of products for human consumption and industrial applications, such as cooking oil, margarine, lubricants, inks and biodiesel (Panthee et al. 2006). The relative composition of saturated and unsaturated fatty acids in the seed determines the quality of soybean oil (Cardinal et al. 2007). Depending on the application of soybean oil, different concentrations of a particular fatty acid are desirable (Panthee et al. 2006). For human food consumption, vegetable oils with reduced saturated fatty acid levels are usually desirable to reduce the health risk of coronary diseases and breast, colon and prostate cancer associated with saturated fatty acids (Hu et al. 1997; Henderson 1991). However, for certain food and industrial applications, e.g., for the production of various 'baking fats' such as margarines or shortenings, increased levels of saturated fatty acids could be an advantage (Spencer et al. 2003). The solid-fat content of a vegetable oil determines its suitability for use in the production of 'baking fats'. A soybean oil with approximately 30% total saturates can make a suitable *trans*-free margarine through the process of interestification (List et al. 2000). Since approximately 52% of USA soybean oil is utilized for the production of various 'baking fats' (Spencer et al. 2003), increasing the saturated fatty acid content to meet the needs of the oil-processing industry will be an important breeding objective as well for soybean oil and oil quality improvement.

Oil and oil fatty acid components in soybean are quantitatively inherited (Burton et al. 1983; White et al. 1961). Phenotypic selection for quantitative traits is time-consuming, labor-intensive, and low in efficiency. Molecular marker-assisted selection (MAS) based on genotypic information can be performed at the early stages of plant growth and in the early generations of segregating populations. Thus, MAS may improve the accuracy and efficiency of selection, and speed up the progress of plant breeding. However, application of MAS depends on the availability of markers associated with the target traits. Identification of quantitative trait loci (QTL) associated with fatty acids would be helpful to improve oil quality by combining or pyramiding different favorable alleles of QTL or genes derived from diverse genetic resources. QTL analysis of oil content and fatty acids in soybean has been conducted (Diers and Shoemaker 1992; Li et al. 2002; Spencer et al. 2003; Fasoula et al. 2004; Hyten et al. 2004a, b; Panthee et al. 2006; Cardinal et al. 2007; Xie et al. 2011). Li et al. (2002) reported two QTL on linkage groups A1 and M for reduced palmitic acid content in the F_2 and $F_{2:3}$ population of Cook × N87-2122-4. These QTL were associated with simple sequence repeat (SSR) markers Satt684 and Satt175, respectively, and accounted for 51% of the total phenotypic variation in the F_2 population and 43% of the variation in the $F_{2:3}$ population. Hyten et al. (2004b) detected 12 QTL on six linkage groups for the fatty acids in an F_6-derived recombinant inbred line (RIL) population and identified a single marker interval on linkage group L associated with palmitic, oleic, linoleic and linolenic acids. Panthee et al. (2006) reported that the SSR marker Satt537 on linkage group D1b was associated with palmitic acid; and Satt168 and Satt429 on linkage groups B2 and J, respectively, were associated with stearic acid.

Although progress in QTL analysis of fatty acids has been made previously, QTL for saturated fatty acids are still very limited (Li et al. 2002; Spencer et al. 2003; Panthee et al. 2006; Hyten et al. 2004b; Reinprecht et al. 2006). Moreover, of the QTL identified for saturated fatty acids, there are only a few that could be consistently detected across multiple environments and different genetic backgrounds. Therefore, it is necessary to map and validate fatty acid QTL in different environments and different genetic backgrounds to better understand the genetic basis of differences in fatty acids and to effectively use soybean germplasm with genetic variation in fatty acid levels. The objectives of this study were: (1) to map QTL for the saturated fatty acids (palmitate and stearate) and detect the interactions between the QTL and environments, (2) to compare the effects of the QTL for individual loci and locus combinations, and (3) to provide suggestions for QTL selection and pyramiding in practical soybean breeding programs. The data related to unsaturated fatty acids are presented in another paper.

Materials and methods

Plant materials and fatty acid analysis

The cross of SD02-4-59 × A02-381100 was made in 2004, and subsequently a total of 87 F_5 RILs were

developed by single seed descent with additional off-season generation advances in the winter. SD02-4-59 is an advanced line derived from A2242 × IA2021, developed by the South Dakota State University Soybean Breeding Program. A02-381100, derived from the cross of IA2064 × XB27U01, is a breeding line with a modified fatty acid profile developed by the Iowa State University Soybean Breeding Program. All the RILs were planted in single-row plots with one replication at Aurora, SD, USA in 2007 and 2008; and two-row plots with two replications in a randomized complete block design at Aurora and Beresford, SD in 2009 and at Aurora, SD in 2010. The rows were 4.42 m long with a space of 0.76 m between rows and were planted at 26 seeds per meter. Correspondingly, the experiments/environments were designated as E07, E08, E09AU, E09BF and E10AU, respectively.

The seed fatty acid content was determined using gas chromatography. The analysis was performed in the USDA/ARS laboratory in Peoria, IL, USA for the E07 and E08 samples; and in the Iowa State University DNA Facility in Ames, IA, USA for the E09AU, E09BF and E10AU samples.

Molecular markers and linkage map construction

Young trifoliate leaf tissues were collected from five plants per line at the V1–V2 growth stage, and then stored in a freezer for DNA extraction. Total genomic DNA was extracted using the CTAB method (Keim et al. 1998) with slight modifications. A total of 516 polymorphic single nucleotide polymorphism (SNP) markers and 298 polymorphic SSR markers were used to genotype the mapping population. The SNP marker screening was performed at the USDA/ARS Soybean Genomics and Improvement Lab, Beltsville, MD, USA (Hyten et al. 2010), and the SSR markers were screened in the South Dakota State University Soybean Breeding and Genetics Lab. In the case of the SSR markers, the polymerase chain reactions were performed by following the protocol described on the SoyBase website (http://soybase.org/). The amplified products were separated by a 6% non-denaturing polyacrylamide gel electrophoresis and stained with ethidium bromide (Wang et al. 2003).

The SNP and SSR genotypic data were used to construct the genetic linkage map by using MapMaker/EXP 3.0 (Stephen et al. 1993), JoinMap version 3.0 (Van Ooijen and Voorrips 2001) and MapChart 2.1 (Voorrips 2002). Seventy-six markers which exhibited significant segregation distortion (i.e. significant at $P = 0.01$) and 206 markers for which data were missing in more than 10 lines were excluded from the map construction. Finally, 294 SNP and 238 SSR markers were used to construct the linkage map. A logarithm of odds (LOD) score of 3.0 was set as the threshold value for linkage grouping.

QTL mapping and statistical analysis

ANOVA was performed to determine the genotypic differences between the RILs with experiments/environments E09AU, E09BF and E10AU, except for E07AU and E08AU with only one replication. Broad-sense heritability was estimated on the line mean basis as $h^2 = \sigma_g^2 \Big/ \left[\sigma_g^2 + \left(\sigma_{ge}^2/e\right) + (\sigma^2/re)\right]$, where h^2 represents the broad-sense heritability, σ_g^2 is genotypic variance, σ_{ge}^2 is genotype × environment interaction variance, σ^2 is error variance, r is number of replications, and e is number of environments (Fehr 1987).

QTL analysis was conducted in WinQTLCart version 2.5 (Wang et al. 2005) for each environment and the average data over five environments. Single marker analysis (SMA), interval mapping (IM), and composite interval mapping (CIM) were performed. Multiple interval mapping (MIM) was performed to detect the QTL × QTL interactions (epistasis). By treating the data from individual experiments as separate traits, multiple-trait IM or CIM analysis was performed to detect the QTL × environment interaction. A LOD value of 2.5 was set as the threshold value, but all the QTL with a LOD value above 2.0 that were also detected by SMA and IM were claimed. If a QTL was detected by CIM as well as SMA and/or IM, only the result of CIM was presented. If a QTL was not detected by CIM but detected by IM and SMA, the result of IM was presented. If a QTL was not detected by CIM or IM but detected by SMA only, it was not claimed and presented. Therefore, only the results of CIM and the QTL which were not detected by CIM but detected by IM in at least two individual environments and in the combined data (overall averages) are presented in this paper. To validate or confirm the results by QTL Cartographer (Wang et al. 2005) and detect epistatic QTL or bigenic interacting QTL between those intervals which were not significant

alone, inclusive composite interval mapping (ICIM) (Wang et al. 2011) was also performed.

A comparison between two groups of RILs carrying different marker alleles from the parents was conducted based on ANOVA to verify the validation of QTL and to provide information for MAS. Likewise, a comparison of different QTL/marker combinations for multiple loci was also computed based on the results of ANOVA (Jiang et al. 2007).

Results

ANOVA results showed that both the genotypic and environmental differences were highly significant for both palmitic and stearic acids and for total saturated fatty acid content as well ($P < 0.01$) (Table 1). The genotype × environment interactions were not significant for palmitic acid and total saturated fatty acids, but highly significant for stearic acid. The broad-sense heritability estimates were high for palmitic acid and total saturated fatty acids, and moderate for stearic acid (Table 1). The population exhibited a lower average level of saturated acids than those for common soybean cultivars (Schnebly and Fehr 1993), and a considerable range of values for both palmitic and stearic acids. The wide range of variation and the highly significant differences among RILs indicated that this population was appropriate for QTL analysis.

QTL mapping and analysis

A linkage map, consisting of all the 20 linkage groups in soybean, was constructed with the genotypic data of 532 DNA markers. The linkage map spanned 1977.07 cM in total, and the interval length averaged 3.95 cM. Due to lack of polymorphism in the related regions or without enough markers mapped on, a gap >20 cM existed in a few cases, and four of the linkage groups involved two unlinked sub-groups and four involved three unlinked sub-groups. In spite of this, the linkage map still served the QTL mapping and analysis well, because these gaps did not prevent detection of QTL outside of the gap regions.

QTL mapping and analysis were performed in QTL Cartographer (Wang et al. 2005) and ICIM software (Wang et al. 2011) separately. The results from these two methods exhibited a high consistency in detection of QTL in most cases, except for some differences in the magnitude of LOD, genetic effects and phenotypic variance explained. No more QTL were detected by ICIM than by QTL Cartographer. Therefore, only the results from QTL Cartographer are presented here.

Eight QTL for palmitic acid on linkage groups A1, C2, E, F, G and K were detected in the individual experiments and the combined data by CIM and/or IM analysis (Table 2). The linkage groups and locations of these QTL are presented in Fig. 1. A major QTL on linkage group A1 (*qPAL-A1*) was consistently detected in all the individual environments and in the combined data, explaining 23.01–32.88% of the phenotypic variation. On linkage group F, two QTL (*qPAL-F-1* and *qPAL-F-2*) were repeatedly detected in three or all five individual environments and the average data, accounting for 13.81–21.68% and 4.79–9.42% of the phenotypic variation, respectively. On linkage group K, two QTL (*qPAL-K*-1 and *qPAL-K*-2) were detected in four or all five individual environments and the average data, explaining 7.55–17.84% and 12.44–15.69% of the variation, respectively. All the QTL described above exhibited negative genetic effects. In contrast, a QTL on linkage group E (*qPAL-E*) exhibited positive genetic effects. This QTL was detected in three individual environments and the combined data, accounting for 13.38–17.24% of the total variation.

For stearic acid, five QTL were detected on linkage groups A1, F, G and N in single environments and/or

Table 1 Descriptive statistics for saturated fatty acids (% oil content) in the RIL population of SD02-4-59 × A02-381100 over three environments E09AU, E09BF and E10AU

Fatty acid	Mean	Range	F_g	F_e	$F_{g\times e}$	$LSD_{0.05}$	h^2
Palmitic	6.70 ± 1.63	3.65–9.93	45.20**	39.55**	1.14	0.67	0.97
Stearic	3.92 ± 0.35	2.93–4.83	7.85**	356.42**	2.43**	0.34	0.69
Total saturated	10.62 ± 1.74	6.58–14.21	36.70**	150.06**	1.00	0.80	0.97

** Significant at $P < 0.01$

Table 2 QTLs for saturated fatty acids detected by CIM (or IM) in the RIL population of SD02-4-59 × A02-0381100

QTL[a]	LG	Closest marker	Int-L[b]	Int-R[b]	LOD	A.E.[c]	R^2	Env.[d]
Palmitic								
qPAL-A1	A1	BARC-031311-07043	Sat_137	BARC-017935-02459	8.28 to 10.74	−0.90 to −1.17	23.01 to 32.88	1–5,0
qPAL-C2	C2	AW734043	AW734043	Sat_130	2.24 to 3.95	−0.49 to −0.51	5.79 to 10.18	1,2,5 0
qPAL-E[e]	E	Satt452	BARC-060905-16966	Satt045	2.67 to 3.44	0.58 to 0.81	13.38 to 17.24	1,4–5,0
qPAL-F-1[e]	F	Sat_240	Sat_298	Sat_240	2.32 to 3.64	−0.66 to −0.97	13.81 to 21.68	1–5,0
qPAL-F-2	F	Sat_297	Sat_297	BARC-025599-06528	2.11 to 3.63	−0.39 to −0.57	4.79 to 9.42	2,4,5,0
qPAL-G	G	Satt472	BARC-018441-03188	Satt472	3.34 to 3.90	−0.53 to −0.57	7.55 to 10.13	1,3,0
qPAL-K-1	K	Satt544	Satt349	Satt381	3.41 to 6.76	−0.46 to −0.88	7.55 to 17.84	1,3–5,0
qPAL-K-2[e]	K	Sat_293	BARC-053193-11760	Sat_293	2.54 to 3.26	−0.60 to −0.74	12.44 to 15.69	1–5,0
Stearic								
qSTE-A1-1[e]	A1	BARC-041257-07953	BARC-041257-07953	BARC-050849-09931	3.79 to 5.10	0.16 to 0.26	19.41 to 25.33	3,4,0
qSTE-A1-2[e]	A1	BARC-029787-06340	Sat_374	BARC-029787-06340	2.55 to 4.46	−0.16 to −0.22	13.66 to 24.19	3,4,0
qSTE-F	F	Satt252	Satt146	Satt252	2.36 to 3.87	−0.10 to −0.15	6.50 to 10.08	3,0
qSTE-G	G	Sat_064	Sct_199	Sat_064	3.18 to 4.33	0.14 to 0.16	10.57 to 14.19	1,2
qSTE-N	N	BARC-014699-01621	BARC-900569-00953	Satt022	2.23 to 6.67	−0.08 to −0.17	6.78 to 20.13	4,5
Total saturated								
qSAT-A1	A1	BARC-017935-02459	Sat_137	BARC-017935-02459	2.93 to 10.73	−0.50 to −1.25	7.68 to 32.59	1–5,0
qSAT-C2	C2	AW734043	AW734043	BARC-015973-02029	2.50 to 5.65	−0.52 to −0.79	6.28 to 16.50	2,4,5
qSAT-E[e]	E	Satt452	BARC-060905-16966	Satt045	2.34 to 2.79	0.64 to 0.79	11.90 to 13.14	1,4,5,0
qSAT-F-1[e]	F	Sat_240	Sat_298	Sat_240	2.30 to 3.65	−0.70 to −1.04	13.60 to 21.68	1–5,0
qSAT-F-2	F	Sat_297	Sat_297	BARC-063863-18477	2.06 to 2.67	−0.41 to −0.53	6.15 to 6.79	2,5,0
qSAT-G	G	Satt472	BARC-018441-03188	Satt472	3.39	−0.69	9.08	1
qSAT-K-1	K	Satt544	Satt349	Satt381	3.26 to 5.66	−0.55 to −0.90	8.06 to 16.40	1,4,0

Table 2 continued

QTL[a]	LG	Closest marker	Int-L[b]	Int-R[b]	LOD	A.E.[c]	R^2	Env.[d]
qSAT-K-2	K	Sat_293	Sat_352	Sat_293	2.92 to 6.12	−0.51 to −0.72	7.01 to 16.80	3–5
qSAT-N	N	Satt022	BARC-016535-02085	Satt022	3.04 to 5.79	−0.55 to −0.71	8.21 to 16.05	2,3,5

[a] QTL for the same trait detected in different environments with the same or overlap marker interval were designated as one QTL

[b] Int-L and Int-R indicate the left and right side marker of the interval, respectively

[c] A.E., additive effect. The positive value indicates that the A02-381100 homozygous alleles increase the values of traits, or the SD02-4-59 alleles decrease the values of traits, and vice versa

[d] Env, environments in which QTL were detected. The numbers 1–5 and 0 indicate environment E07, E08, E09AU, E09BF and E10AU, and the average data over five environments, respectively

[e] Interval mapping (IM) results for the QTL that were not detected by CIM

in the combined average data by CIM or IM (Table 2, Fig. 1). On linkage group A1, two QTL (*qSTE-A1*-1 and *qSTE-A1-2*) with different sources of alleles (i.e., exhibiting positive or negative genetic effects) were detected by IM and SMA in two individual environments and the combined data, explaining 19.41–25.33 and 13.66–24.19% of the total variation, respectively, although they were not detected by CIM. The QTL on linkage group G was detected in two environments, explaining 10.57–14.19% of the phenotypic variation. The remaining two QTL exhibited lower effects.

For the content of total saturated fatty acids, nine QTL were detected on linkage groups A1, C2, E, F, G, K and N by CIM and/or IM analysis (Table 2, Fig. 1). Eight of these QTL were consistent with the ones for palmitic acid and one was consistent with *qSTE-N* for stearic acid, except for slight differences in the size of marker intervals, in the magnitude of the genetic effects and in the phenotypic variation explained. Among the QTL for total saturated fatty acids, two major QTL (*qSAT-A1* and *qSAT-F-1*) were repeatedly detected in all the individual environments and in the average data. They were mapped on linkage groups A1 and F, explaining up to 32.59 and 21.68% of the phenotypic variation, respectively. Three QTL on linkage groups C2 (*qSAT-C2*), K (*qSAT-K-2*) and N (*qSAT-N*) were consistently detected in three individual environments, but none of them was detected in the average data (Table 2). On linkage group K, another QTL (*qSAT-K-1*) was detected by CIM analysis in two individual environments and the average data, accounting for 8.06–16.40% of the phenotypic variation. On linkage group E, a QTL with moderate effects was detected by IM analysis and SMA in three individual environments and the average data, explaining 11.90–13.14% of the phenotypic variation, though it was not detected by CIM. Except for the QTL on linkage group E (*qSAT-E*), all the other QTL had the same source of alleles, exhibiting negative genetic effects on the total saturated fatty acids.

QTL × QTL (epistasis) and QTL × E interactions

For the QTL for saturated fatty acids described above, no significant QTL × QTL interactions or epistatic effects were detected by either MIM or ICIM analysis. However, significant QTL × environment interactions were detected in most cases. By using multiple trait CIM analysis, significant QTL × environment interactions were detected for the QTLs *qPAL-A1*, *qPAL-C2*, *qPAL-E*, *qPAL-F-1* and *qPAL-K-1* for palmitic acid, *qSTE-A1*-1, *qSTE-A1-2* and *qSTE-N* for stearic acid, and *qSAT-A1*, *qSAT-E*, *qSAT-K-1* and *qSAT-N* for total saturated fatty acids. No significant QTL × environment interactions were detected for the other QTL. The results of ICIM analysis were highly consistent with multiple-trait CIM analysis, except for QTL *qSAT-C2* and *qSAT-E*. In the ICIM analysis, QTL × environment interactions were significant for *qSAT-C2* but not significant for *qSAT-E*.

By using the ICIM analysis, epistatic QTL or bigenic interacting QTL were detected for palmitic acid and the total fatty acids (Table 3). However, most of these epistatic QTL were not repeatedly detected in multiple environments. No epistatic QTL was detected for stearic acid.

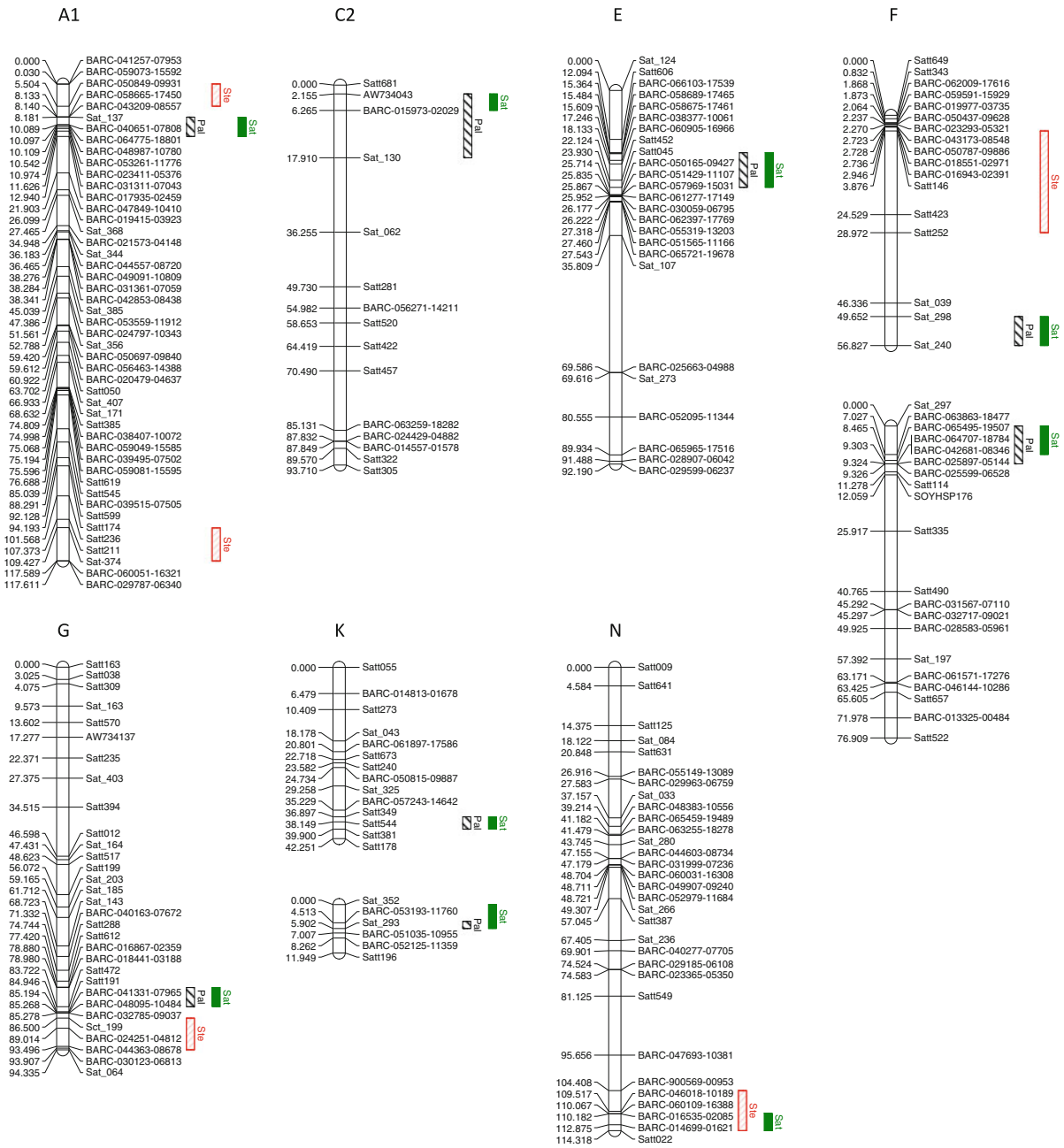

Fig. 1 Map locations of QTL for unsaturated fatty acids detected in the RIL population of SD02-4-59 × A02-0381100

Comparison of single QTL alleles

ANOVA for group comparison of QTL/markers indicated that the differences between two alternative alleles in overall averages of traits were significant or highly significant for all the QTL. The averages of alternative alleles of QTL associated with saturated fatty acids and the differences between them are listed in Table 4. Except for the QTL on linkage group E (*qPAL-E*), the QTL for palmitic acid had the same sources of alleles and showed negative effects. The alleles of these QTL from the parent A02-381100 exhibited highly significant effects resulting in decreased palmitic acid content ($P < 0.01$). In

Table 3 Epistatic QTLs for palmitic acid and total saturated fatty acids detected by ICIM in the RIL population of SD02-4-59 × A02-381100

Trait and environment	Interval 1			Interval 2			LOD	R^2 (%)	Add1	Add2	Add1 × Add2
	LG	Left-marker	Right-marker	LG	Left-marker	Right-marker					
PAL-07AU	D1b	BARC-042881-08448	Sat_069	I	BARC-055173-13105	BARC-042619-08314	5.42	24.70	−0.23	0.04	1.01
PAL-08AU	A2	BARC-065759-19720	BARC-039797-07587	B1	BARC-900336-00920	BARC-054037-12260	8.12	26.43	−0.23	0.20	−1.03
PAL-09AU	G	Satt235	Sat_403	I	BARC-029301-06148	Satt330	5.08	20.49	−0.56	−0.35	0.90
PAL-10AU	A2	BARC-065759-19720	BARC-039797-07587	B1	BARC-042299-08241	BARC-900336-00920	9.00	19.95	−0.08	−0.02	−0.73
SAT-07AU	C1	Satt690	Sat_140	C2	Satt681	AW734043	5.44	9.47	−0.07	−0.57	−0.71
SAT-08AU	A2	BARC-065759-19720	BARC-039797-07587	B1	BARC-900336-00920	BARC-054037-12260	7.23	26.79	−0.12	0.28	−1.09
SAT-09AU	D2	BARC-051885-11289	BARC-060181-16456	M	Satt677	BARC-028385-05858	8.94	17.05	−0.08	−0.04	−0.74
SAT-09AU	F	SOYHSP176	Satt335	J	BARC-028159-05778	BARC-059919-16214	5.62	7.95	0.00	0.11	−0.49
SAT-09BF	A2	BARC-065759-19720	BARC-039797-07587	B1	BARC-042299-08241	BARC-900336-00920	5.31	11.93	0.12	0.22	−0.66
SAT-10AU	A2	BARC-065759-19720	BARC-039797-07587	N	Satt549	BARC-047693-10381	6.11	23.97	−0.47	0.22	−1.09
SAT-10AU	B2	BARC-039667-07536	BARC-012703-00380	K	BARC-014813-01678	Satt273	5.30	10.98	0.26	0.34	0.60

contrast, the effects of the alleles of *qPAL-E* from A02-381100 very significantly increased palmitic acid content. The major QTL on linkage group A1 (*qPAL-A1*) exhibited the largest effects, the second was the QTL on linkage groups K (*qPAL-K-1* and *qPAL-K-2*), F (*qPAL-F1*), E (*qPAL-E*) and G (*qPAL-G*). The QTL on linkage group C (*qPAL-C2*) and another QTL on F (*qPAL-F2*) exhibited the smallest effects (Table 4).

For stearic acid, the two QTL (*qSTE-A1-1* and *qSTE-A1-2*) on linkage group A1 which were detected in multiple environments had different sources of alleles that increased or decreased the fatty acid, and both showed the largest genetic effects among all the QTL detected (Tables 2, 4). The QTL on linkage group G (*qSTE-G*) also exhibited large genetic effects and had the same source of alleles as the QTL *qSTE-A1-1*. The QTL on linkage groups F (*qSTE-F*) and N (*qSTE-N*) with the same source of alleles as *qSTE-A1-2* showed significant but lower genetic effects compared with the QTL on linkage group A1.

For the total saturated fatty acid content, the major QTL on linkage group A1 (*qSAT-A1*) exhibited the largest genetic effect and the largest difference in the overall performance between the two groups of RILs carrying alternative alleles (Table 2 and 4). The two QTL on linkage group F (*qSAT-F-1* and *qSAT-F-2*) both had negative effects associated with the allele from the A02-381100 parent, but the magnitudes of

Table 4 Means of RILs carrying different alleles of the QTLs for saturated fatty acids in the RIL population of SD02-4-59 × A02-381100 over five environments

Palmitic			Stearic			Total saturated		
QTL (marker)	Allele[a]	Mean	QTL (marker)	Allele[a]	Mean	QTL (marker)	Allele[a]	Mean
qPAL-A1	A	6.10 ± 1.68	*qSTE-A1-1*	A	4.05 ± 0.27	*qSAT-A1*	A	9.08 ± 1.24
(Sat_137)	S	7.82 ± 1.42	(BARC-041257-07953)	S	3.74 ± 0.28	(BARC-017935-02459)	S	11.70 ± 1.40
	Diff[b]	−1.72****		Diff	0.31****		Diff	−2.61****
qPAL-C2	A	6.60 ± 1.69	*qSTE-A1-2*	A	3.44 ± 0.28	*qSAT-C2*	A	10.48 ± 1.84
(AW734043)	S	7.63 ± 1.66	(BARC-029787-06340)	S	4.08 ± 0.26	(AW734043)	S	11.48 ± 1.74
	Diff	−1.03**		Diff	−0.32****		Diff	−1.00*
qPAL-E	A	7.68 ± 1.64	*qSTE-F*	A	3.76 ± 0.29	*qSAT-E*	A	11.54 ± 1.79
(Satt452)	S	6.45 ± 1.52	(Satt252)	S	3.96 ± 0.31	(Satt452)	S	10.34 ± 1.62
	Diff	1.24****		Diff	−0.20***		Diff	1.20***
qPAL-F-1	A	6.42 ± 1.74	*qSTE-G*	A	3.98 ± 0.30	*qSAT-F-1*	A	10.21 ± 1.84
(Sat_240)	S	7.72 ± 1.59	(Sat_064)	S	3.75 ± 0.30	(Sat_240)	S	11.65 ± 1.68
	Diff	−1.30****		Diff	0.24****		Diff	−1.45****
qPAL-F-2	A	6.57 ± 1.52	*qSTE-N*	A	3.79 ± 0.27	*qSAT-F-2*	A	10.45 ± 1.64
(Sat_297)	S	7.61 ± 1.83	(BARC-014699-01621)	S	3.97 ± 0.31	(Sat_297)	S	11.46 ± 1.95
	Diff	−1.04**		Diff	−0.18*		Diff	−1.01*
qPAL-G	A	6.51 ± 1.72				*qSAT-G*	A	10.42 ± 1.88
(Satt472)	S	7.77 ± 1.59				(Satt472)	S	11.58 ± 1.69
	Diff	−1.26****					Diff	−1.17***
qPAL-K-1	A	6.55 ± 1.50				*qSAT-K-1*	A	10.40 ± 1.64
(Satt544)	S	7.90 ± 1.82				(Satt544)	S	11.81 ± 1.89
	Diff	−1.35****					Diff	−1.41****
qPAL-K-2	A	6.38 ± 1.46				*qSAT-K-2*	A	10.20 ± 1.55
(Sat_293)	S	7.73 ± 1.77				(Sat_293)	S	11.65 ± 1.89
	Diff	−1.36****					Diff	−1.44****
						qSAT-N	A	10.24 ± 1.76
						(Satt022)	S	11.59 ± 1.74
							Diff	−1.35****

[a] A = Homozygous alleles for A02-381100, and S = Homozygous alleles for SD02-4-59, respectively

[b] *Diff* difference, the positive value indicates that the A02-381100 homozygous alleles increase the value of the trait, or the SD02-4-59 homozygous alleles decrease the value of the traits, and vice versa

*, **, ***, **** Significant at $P < 0.05$, 0.01, 0.005 and 0.001, respectively

the effects differed. The two QTL on linkage group K (*qSAT-K-1* and *qSAT-K-2*) also showed negative effects associated with the A02-381100 parent and the magnitudes of the effects were quite similar. The QTL on linkage group E (*qSAT-E*) was the only QTL which had a different source of alleles from the others. The QTL on linkage group C2 (*qSAT-C2*) and N (*qSAT-N*) significantly ($P < 0.05$) or highly significantly ($P < 0.01$) altered the content of total saturated fatty acids as well.

Cumulative effects of QTL combinations for multiple loci

As described above, the mapping results of the total saturated fatty acids were highly consistent with the

results of palmitic acids. Therefore, only palmitic and stearic acids are discussed here for the cumulative effects of QTL combinations at multiple loci (Tables 5, 6). For palmitic acid, the two-locus comparison results showed that the mean values of the RILs carrying the alleles with negative effects which decreased the fatty acid content at both loci were significantly ($P < 0.05$) lower than those of the reciprocal genotypes and those of RILs with negative alleles at only one locus in all cases (Table 5). For three-locus combinations, the RILs with negative alleles at all three loci exhibited lower values in most cases than those carrying negative alleles at two loci only. For increasing the palmitic acid content, on the contrary, in most cases the mean values of the RILs carrying the positive alleles at both loci were significantly ($P < 0.05$) higher than those of reciprocal genotypes and those of RILs in which only one locus carried positive alleles. Likewise, the results of three-locus comparisons indicated that the RILs carrying positive alleles at all three loci generally exhibited higher palmitic acid than the RILs carrying positive alleles at two loci only, though the differences were not significant in some cases (Table 5). Comparisons of four QTL combinations (data not shown) showed a tendency similar to those for three-locus combinations. For the desired three-locus combinations, the fourth QTL allowed additional alteration (increasing or decreasing) of the fatty acid content in most cases, with a few exceptions in which the fatty acid content was altered slightly in the opposite direction.

For stearic acid, the two-locus and three-locus comparison results showed a trend similar to those of palmitic acid. In most cases, the RILs carrying negative alleles that decreased the fatty acids at both loci exhibited significantly lower values of the trait than those of the reciprocal genotypes and those of RILs in which only one locus carried negative alleles (Table 6). The RILs carrying negative alleles at all three loci exhibited lower values of the trait than the RILs carrying negative alleles at only two loci, with insignificant differences in a few cases (Table 6). Comparisons of four QTL combinations indicated that no consistent alteration of stearic acid occurred with the addition of the fourth QTL.

Discussion

Soybean is the leading oilseed crop in the United States in terms of gross vegetable oil production and economic importance (Wilcox 2004). The quality of soybean oil is determined by its fatty acid composition. Different concentrations of a particular fatty acid may play a decisive role in the end use or application of soybean oil. Polyunsaturated fatty acids (linoleic and linolenic acids) are susceptible to the oxidation process, which affects the stability and flavor of soybean oil (Wilson 1987). Human nutritional studies have proved that too high an intake of saturated fatty acids may lead to health problems such as coronary diseases and breast, colon and prostate cancer (Hu et al. 1997; Henderson 1991). Therefore, soybean oils with decreased polyunsaturated fatty acids, decreased saturated fatty acids and increased monounsaturated oleic acid are more suitable for human consumption (Mounts et al. 1988; Oliva et al. 2006). For certain food and industrial applications, however, increased levels of saturated fatty acids might be an advantage for the production of various 'baking fats' such as margarines or shortenings (Spencer et al. 2003). Therefore, the preferred fatty acid composition varies with the intended use of the soybean oil.

Previous studies on fatty acids were mainly aimed at improving the quality of soybean oil for human consumption, i.e., decreasing saturated and polyunsaturated fatty acids and increasing monounsaturated acid (Erickson et al. 1988; Nickell et al. 1991; Li et al. 2002; Oliva et al. 2006; Panthee et al. 2006; Primomo et al. 2002; Hyten et al. 2004b; Cardinal et al. 2007). Soybean lines with reduced palmitic acid or stearic acid content have been developed through mutagenesis, recurrent selection and hybridization (Erickson et al. 1988; Bubeck et al. 1989; Wilcox and Cavins 1990; Burton et al. 1994). Research regarding elevated palmitic and stearic acids is still very limited (Pantalone et al. 2002; Spencer et al. 2003). However, approximately 52% of USA soybean oil is utilized for the production of various 'baking fats', and the requirements of soybean oil for industrial applications have increased recently (Spencer et al. 2003). Therefore, QTL analyses of saturated fatty acids will be helpful to obtain a better understanding of the genetic basis and the breeding of soybean varieties for high or low saturated fatty acid content. In this study, we tried to identify the QTL and combinations of QTL for decreased or increased saturated fatty acids in soybean oil.

Comparison of QTL for saturated fatty acids

A major QTL on linkage group A1 with the SSR marker Satt684 for reduced palmitic acid content was

Table 5 Means of RILs carrying different alleles of QTL combinations for palmitic acid in the RIL population of SD02-4-59 × A02-381100 over five environments

Two-locus combination						Three-locus combination					
	Allele[a]	Mean		Allele[a]	Mean		Allele[a]	Mean		Allele[a]	Mean
A1 + K-2	A + A	5.49 ± 1.08	K-2 + E	A + A	6.85 ± 1.16	A1 + K-2 + K-1	A + A+A	5.20 ± 1.09	A1 + F-1 + G	A + A+A	4.87 ± 0.91
	A + S	6.99 ± 2.13		A + S	5.92 ± 1.31		A + A+S	6.24 ± 0.64		A + A+S	6.15 ± 0.59
	S + A	7.35 ± 1.13		S + A	8.37 ± 1.72		S + S+A	7.78 ± 1.09		S + S+A	7.74 ± 1.45
	S + S	8.12 ± 1.51		S + S	6.96 ± 1.57		S + S+S	8.47 ± 1.76		S + S+S	8.17 ± 1.42
	LSD[b]	0.43		LSD	0.43		LSD	0.59		LSD	0.60
A1 + K-1	A + A	5.66 ± 1.42	K-1 + F-1	A + A	6.18 ± 1.60	A1 + K-2 + F-1	A + A+A	5.14 ± 1.09	A1 + F-1 + E	A + A+A	6.52 ± 1.57
	A + S	7.11 ± 1.88		A + S	7.13 ± 1.25		A + A+S	6.02 ± 0.94		A + A+S	5.17 ± 1.06
	S + A	7.50 ± 0.94		S + A	7.22 ± 2.19		S + S+A	8.14 ± 1.87		S + S+A	8.73 ± 1.37
	S + S	8.17 ± 1.75		S + S	8.17 ± 1.72		S + S+S	8.12 ± 1.40		S + S+S	7.21 ± 1.02
	LSD	0.43		LSD	0.48		LSD	0.60		LSD	0.58
A1 + F-1	A + A	5.53 ± 1.31	K-1 + G	A + A	5.83 ± 1.41	A1 + K-2 + G	A + A+A	5.42 ± 1.07	K-2 + K-1 + F-1	A + A+A	5.73 ± 1.43
	A + S	7.17 ± 1.92		A + S	7.21 ± 1.25		A + A+S	5.25 ± 1.24		A + A+S	6.80 ± 1.30
	S + A	7.67 ± 1.52		S + A	7.34 ± 1.78		S + S+A	7.75 ± 1.72		S + S+A	7.57 ± 2.46
	S + S	7.96 ± 1.41		S + S	8.68 ± 1.70		S + S+S	8.44 ± 1.34		S + S+S	8.74 ± 1.53
	LSD	0.45		LSD	0.43		LSD	0.62		LSD	0.66
A1 + G	A + A	5.66 ± 1.59	K-1 + E	A + A	7.29 ± 1.23	A1 + K-2 + E	A + A+A	6.00 ± 0.81	K-2 + K-1 + G	A + A+A	5.65 ± 1.37
	A + S	6.61 ± 1.66		A + S	6.08 ± 1.44		A + A+S	5.23 ± 1.14		A + A+S	6.83 ± 1.20
	S + A	7.35 ± 1.45		S + A	8.07 ± 1.93		S + S+A	8.52 ± 1.67		S + S+A	8.16 ± 2.02
	S + S	8.23 ± 1.35		S + S	7.27 ± 1.51		S + S+S	7.63 ± 1.25		S + S+S	8.68 ± 1.70
	LSD	0.44		LSD	0.44		LSD	0.54		LSD	0.62
A1 + E	A + A	7.21 ± 1.75	F-1 + G	A + A	5.54 ± 1.44	A1 + K-2 + F-2	A + A+A	5.33 ± 0.96	K-2 + K-1 + E	A + A+A	6.85 ± 1.04
	A + S	5.25 ± 1.03		A + S	7.44 ± 1.53		A + A+S	5.69 ± 1.25		A + A+S	5.68 ± 1.35
	S + A	8.03 ± 1.55		S + A	7.46 ± 1.58		S + S+A	7.79 ± 1.59		S + S+A	8.68 ± 1.89
	S + S	7.39 ± 1.14		S + S	7.99 ± 1.63		S + S+S	8.43 ± 1.45		S + S+S	7.71 ± 1.76
	LSD	0.41		LSD	0.45		LSD	0.58		LSD	0.61
K-2 + K-1	A + A	6.06 ± 1.39	F-1 + E	A + A	7.12 ± 1.53	A1 + K-1 + F-1	A + A+A	5.46 ± 1.42	K-1 + F-1 + G	A + A+A	5.47 ± 1.51
	A + S	7.01 ± 1.45		A + S	5.82 ± 1.69		A + A+S	6.36 ± 1.55		A + A+S	7.04 ± 1.14
	S + A	7.10 ± 1.53		S + A	8.42 ± 1.56		S + S+A	8.01 ± 2.51		S + S+A	7.74 ± 1.70
	S + S	8.42 ± 1.84		S + S	6.89 ± 1.20		S + S+S	8.32 ± 1.63		S + S+S	8.93 ± 1.58
	LSD	0.45		LSD	0.43		LSD	0.63		LSD	0.62

Table 5 continued

Two-locus combination						Three-locus combination					
	Allele[a]	Mean		Allele[a]	Mean		Allele[a]	Mean		Allele[a]	Mean
K-2 + F-1	A + A	5.78 ± 1.37	F-1 + F-2	A + A	5.91 ± 1.44	A1 + K-1 + G	A + A+A	5.06 ± 1.06	K-1 + F-1 + E	A + A+A	7.20 ± 1.23
	A + S	7.00 ± 1.46		A + S	7.15 ± 1.95		A + A+S	6.21 ± 1.29		A + A+S	5.45 ± 1.40
	S + A	7.08 ± 1.99		S + A	7.51 ± 1.31		S + S+A	7.51 ± 1.79		S + S+A	8.67 ± 1.64
	S + S	8.12 ± 1.53		S + S	7.84 ± 1.77		S + S+S	9.03 ± 1.43		S + S+S	7.01 ± 1.35
	LSD	0.46		LSD	0.47		LSD	0.58		LSD	0.62
K-2 + G	A + A	5.89 ± 1.19	G + E	A + A	6.99 ± 1.57	A1 + K-1 + E	A + A+A	7.00 ± 1.43	F-1 + G+E	A + A+A	6.03 ± 1.29
	A + S	7.34 ± 1.63		A + S	6.03 ± 1.61		A + A+S	5.13 ± 1.04		A + A+S	5.39 ± 1.49
	S + A	7.35 ± 2.03		S + A	8.34 ± 1.48		S + S+A	8.42 ± 1.82		S + S+A	9.20 ± 1.23
	S + S	7.98 ± 1.59		S + S	6.91 ± 1.31		S + S+S	7.49 ± 1.57		S + S+S	6.91 ± 1.06
	LSD	0.46		LSD	0.44		LSD	0.58		LSD	0.59

[a] A = Homozygous alleles for A02-381100, and S = Homozygous alleles for SD02-4-59, respectively. For two-locus combinations, all the possible allele groups are listed. For three-locus combinations, only allele groups which combine the third favorable alleles for further decreasing or increasing the value of the trait are listed, based on the corresponding combinations at the first two loci

[b] LSD at $P < 0.05$

previously identified in the F_2 and $F_{2:3}$ population of Cook × N87-2122-4, accounting for 31–38% of phenotypic variation (Li et al. 2002). In our study, a major QTL on linkage group A1 (*qPAL-A1*) in the marker interval Sat_137–BARC-017935-02459 was also detected in all the environments and in the combined data. Referring to the genetic map (Song et al. 2010, Supplementary Table 1), Sat_137 is at 3.63 cM, Satt684 at 5.85 cM, and BARC-017935-02459 at 17.41 cM. Thus Satt684 is in the marker interval of Sat_137–BARC-017935-02459. Therefore, we conclude that *qPAL-A1* confirmed the QTL reported by Li et al. (2002). The QTL on linkage group C2 (*qPAL-C2*) in the marker interval AW734043–Sat_130 was detected in three environments and the combined data in this study. Reinprecht et al. (2006) also detected a minor QTL on linkage group C2 with the marker Satt489 associated with palmitic acid content. The genetic map (Song et al. 2004, 2010) indicates that the marker interval AW734043–Sat_130 is far (>90 cM) away from the marker Satt489. It is clear that *qPAL-C2* is a new QTL for palmitic acid, different from the QTL previously reported (Reinprecht et al. 2006). On linkage group G, a QTL with the marker Satt288 or Sat_164 was reported (Reinprecht et al. 2006; Xie et al. 2011). In our study, *qPAL-G* in the marker interval BARC-018441-03188–Satt472 was detected in two individual environments and the average data. Although Satt288 was only 2.23 cM away from the marker interval of *qPAL-G*, no significant association of Satt288 with palmitic acid content was detected by any method in this study. Referring to the genetic map (Song et al. 2010), Sat_164 was 5.70 cM upstream of the marker Satt288, and thus was further away from *qPAL-G*. Therefore, we suppose that *qPAL-G* is different from the QTL reported by Reinprecht et al. (2006) and Xie et al. (2011). On linkage group K, we identified two QTL for palmitic acid in this population. Hyten et al. (2004b) reported a QTL at the position of 78.6 cM on linkage group K on the integrated soybean genetic map. However, without comparable markers and/or intervals presented, it cannot be concluded that this QTL is the same as or similar to either of the QTL (*qPAL-K1* or *qPAL*-K2) detected in our study. No QTL associated with palmitic acid on linkage groups E and F have been reported previously, indicating that the QTL on these linkage groups identified in this study might also be new ones for palmitic acid.

Table 6 Means of RILs carrying different alleles of QTL combinations for stearic acid in the RIL population of SD02-4-59 × A02-381100 over five environments

Two-locus combination						Three-locus combination					
	Allele[a]	Mean		Allele[a]	Mean		Allele[a]	Mean		Allele[a]	Mean
A1-2 + A1-1	A + A	3.76 ± 0.19	A1-1 + F	S + A	3.63 ± 0.27	A1-2 + A1-1 + G	A + S+A	3.84 ± 0.26	A1-2 + F+N	A + A+A	3.67 ± 0.27
	A + S	3.74 ± 0.28		S + S	3.85 ± 0.24		A + S+S	3.64 ± 0.28		A + A+S	3.68 ± 0.26
	S + A	4.09 ± 0.26		A + A	3.94 ± 0.22		S + A+A	4.17 ± 0.26		S + S+A	4.02 ± 0.09
	S + S	–		A + S	4.17 ± 0.28		S + A+S	3.95 ± 0.22		S + S+S	4.28 ± 0.28
	LSD[b]	0.11		LSD	0.11		LSD	0.14		LSD	0.18
A1-2 + G	A + A	3.84 ± 0.25	A1-1 + N	S + A	3.71 ± 0.29	A1-2 + A1-1 + F	A + S+A	3.63 ± 0.28	A1-1 + G+F	S + S+A	3.58 ± 0.24
	A + S	3.70 ± 0.28		S + S	3.80 ± 0.26		A + S+S	3.85 ± 0.24		S + S+S	3.85 ± 0.31
	S + A	4.15 ± 0.26		A + A	3.92 ± 0.21		S + A+A	3.98 ± 0.21		A + A+A	4.00 ± 0.16
	S + S	3.95 ± 0.22		A + S	4.13 ± 0.30		S + A+S	4.20 ± 0.28		A + A+S	4.23 ± 0.29
	LSD	0.11		LSD	0.12		LSD	0.14		LSD	0.16
A1-2 + F	A + A	3.65 ± 0.26	G + F	S + A	3.70 ± 0.29	A1-2 + A1-1 + N	A + S+A	3.71 ± 0.30	A1-1 + G+N	S + S+A	3.65 ± 0.24
	A + S	3.87 ± 0.24		S + S	3.88 ± 0.30		A + S+S	3.80 ± 0.26		S + S+S	3.75 ± 0.38
	S + A	3.98 ± 0.21		A + A	3.91 ± 0.27		S + A+A	3.99 ± 0.15		A + A+A	4.00 ± 0.18
	S + S	4.18 ± 0.28		A + S	4.00 ± 0.31		S + A+S	4.17 ± 0.30		A + A+S	4.26 ± 0.26
	LSD	0.11		LSD	0.12		LSD	0.16		LSD	0.17
A1-2 + N	A + A	3.72 ± 0.28	G + N	S + A	3.72 ± 0.24	A1-2 + G+F	A + S+A	3.60 ± 0.24	A1-1 + F+N	S + A+A	3.68 ± 0.27
	A + S	3.82 ± 0.24		S + S	3.89 ± 0.29		A + S+S	3.90 ± 0.27		S + A+S	3.41
	S + A	3.99 ± 0.15		A + A	3.92 ± 0.27		S + A+A	4.04 ± 0.15		A + S+A	4.02 ± 0.09
	S + S	4.17 ± 0.30		A + S	4.01 ± 0.33		S + A+S	4.20 ± 0.29		A + S+S	4.23 ± 0.30
	LSD	0.12		LSD	0.12		LSD	0.16		LSD	0.18
A1-1 + G	S + A	3.84 ± 0.26	F + N	A + A	3.75 ± 0.27	A1-2 + G+N	A + S+A	3.66 ± 0.26	G + F+N	S + A+A	3.65 ± 0.23
	S + S	3.64 ± 0.27		A + S	3.86 ± 0.30		A + S+S	3.83 ± 0.29		S + A+S	3.84 ± 0.32
	A + A	4.15 ± 0.26		S + A	3.97 ± 0.16		S + A+A	4.04 ± 0.16		A + S+A	4.07 ± 0.23
	A + S	3.91 ± 0.23		S + S	3.99 ± 0.32		S + A+S	4.26 ± 0.26		A + S+S	4.00 ± 0.34
	LSD	0.11		LSD	0.13		LSD	0.17		LSD	0.18

[a] A = Homozygous alleles for A02-381100, and S = Homozygous alleles for SD02-4-59, respectively. For two-locus combinations, all the possible allele groups are listed. For three-locus combinations, only allele groups which combine the third favorable alleles for further decreasing or increasing the value of the trait are listed, based on the corresponding combinations at the first two loci

[b] LSD at $P < 0.05$

Eight QTL for stearic acid in soybean have been reported and located on linkage groups B2, C2, D1b, F, G, J and L (Diers and Shoemaker 1992; Hyten et al. 2004b; Panthee et al. 2006; Reinprecht et al. 2006; Xie et al. 2011). However, these QTL were not confirmed in subsequent studies. In our study, five QTL for stearic acid were identified in total. Two QTL with different sources of alleles for increased or decreased stearic acid were mapped on linkage group A1. They were detected in two individual environments and the average data, and exhibited the largest genetic effects (R^2 ranging from 13.66 to 25.33%). On linkage group N, a QTL was detected in two environments E09BF and E10AU. No previous studies suggested QTL on linkage groups A1 and N for stearic acid, and thus we suppose that the QTL on linkage groups A1 and N are novel ones. Reinprecht et al. (2006) detected one QTL on linkage group F with the marker Sat_090 and two QTL on linkage group G with the markers UBC517-$_{1500}$ and Satt288. According to the marker order and distances and referring to the soybean genetic map (Song et al. 2010), the QTL on linkage group F should be located around 102.95 cM, and both of the two QTL on linkage group G should be located within the interval of 43.27–71.6 cM. In this study, a QTL on linkage group F and one QTL on linkage group G were also detected in multiple environments or in the combined data. Referring to the genetic map (Song et al. 2010), however, they should be located in the intervals of 22.62–37.14 cM on linkage group F and 85.66–101.82 cM on linkage group G, respectively, which were far (65.8 or 14.1 cM) away from the QTL reported by Reinprecht et al. (2006). Therefore, we suppose that the QTL on linkage groups F and G (*qSTE-F* and *qSTE-G*) detected in this study are also new for stearic acid in soybean.

Total saturated fatty acids in soybean are the sum of palmitic and stearic acids. No QTL for total saturated fatty acids have previously been reported. In this study, we tried to perform QTL analysis for total saturated fatty acids directly. The content of total saturated fatty acids was mainly attributable to palmitic acid, as indicated by the average correlations between total saturated acids and palmitic or stearic acid which were 0.98 ($P > 0.01$) and 0.42 ($P > 0.01$), respectively. Moreover, the QTL for total saturated fatty acid content were in close agreement with those for palmitc acid, except for *qSAT-N* which was consistent with the stearic QTL on linkage group N (*qSTE-N*). Therefore, palmitic acid could be first considered for alteration in saturated fatty acid content, unless specific concerns are placed on stearic acid or high-stearic germplasm is used, such as A6 (Hammond and Fehr 1983).

The efficiency of QTL detection is attributed to the algorithms, mapping methods, number of polymorphic markers, and population type and size. Major QTL or main-effect QTL can be easily detected by different methods and with various populations. However, a large population size should be required to detect minor-effect QTL. In this study, the population size of 87 RILs seemed to be small for detection of minor-effect QTL. However, eight QTL for palmitic acid, five QTL for stearic acid, and nine QTL for total saturated fatty acids were repeatedly detected in multiple environmental conditions and years, and were confirmed by two methods (QTL Cartographer and ICIM). Therefore, the results presented in this paper showed a high level of confidence for QTL in saturated fatty acids and would be useful to the soybean breeding programs. Of course, additional QTL, especially minor-effect QTL, might be identified in further studies with more germplasms and larger mapping populations.

In general, the magnitude of phenotypic variation explained by a QTL is affected by several factors, such as the number of QTL detected, the degree of association of the DNA marker or markers with the genes controlling the trait, the range of variation within the population, and the population size. It also varies with genetic backgrounds and phenotyping environments. In this study, the proportions of total variation explained by single QTL were close to or above 10% in many cases. It might be possible that the genetic effects of these QTL were overestimated due to the population size (Beavis 1994). The major QTL for palmitic acid on linkage group A1 (*qPAL-A1*) was detected in multiple environments and validated in different studies (Li et al. 2002). Therefore, we would recommend use of this QTL in practical breeding for the improvement of fatty acids. For the QTL which were detected in multiple environments in this study but have not been validated yet, they may also be used on a trial basis in practical breeding. Further confirmation and validation of these QTL is needed.

QTL × E and QTL × QTL (epistasis) interactions

It is known that fatty acid composition is a quantitative trait which is easily affected by environment (Wolf

et al. 1982; Diers and Shoemaker 1992; Wilson et al. 2002; Panthee et al. 2006). However, only a very few investigations have reported QTL × E interactions for fatty acids, especially for saturated fatty acids in soybean. In our study, significant environmental differences ($P < 0.01$) were detected for palmitic and stearic acids as well as the total saturated fatty acids, but significant genotype × environment interactions ($P < 0.01$) were detected only for stearic acid by ANOVA. The multiple-trait CIM analysis results indicated that QTL × environment interactions were significant for most QTL, including those QTL which could be detected in all the environments and in the combined data. For more accurate mapping and more effective use of the QTL/genes, it would be helpful to validate them in additional environments. However, most of the QTL associated with saturated fatty acids were consistently detected in multiple or even all the environments, indicating high levels of repeatability and potential for use in marker-assisted breeding.

Genetic variation may also be attributed to epistasis or QTL × QTL interaction. Significant epistatic interactions have been reported in soybean for plant height (Lark et al. 1995), seed yield (Orf et al. 1999) and isoflavone content (Primomo et al. 2005). However, there is very little knowledge of epistasis for fatty acids in soybean at present. In this study, no significant QTL × QTL interaction was detected for the saturated fatty acids by either MIM or ICIM. This might be attributable in part to the limited population size and the limited number of individuals or lines in certain genotypic classes (Tanksley 1993). Epistatic QTL or bigenic interacting QTL were detected by ICIM analysis for palmitic acid and the total fatty acids. However, most of these epistatic QTL were not repeatedly detected in multiple environments. It might be possible that there were epistatic QTL × environment interactions, which needs further investigation. No epistatic QTL was detected for stearic acid. This indicates that the fatty acids in this population were predominantly inherited in an additive mode, with a small contribution from epistasis in some cases.

Marker-assisted selection for saturated fatty acids in breeding practice

To enhance or improve a quantitatively inherited trait in plant breeding, pyramiding of multiple genes or QTL is recommended as a potential strategy (Richardson et al. 2006). The cumulative effects of QTL pyramiding have been proved previously in soybean (Li et al. 2010; Njiti et al. 2001, 2002) and other crops (Huang et al. 1997; Jiang et al. 2007; Richardson et al. 2006). In this study, we analyzed the cumulative effects of QTL for the saturated fatty acids by a group comparison of different QTL combinations for multiple loci based on the results of ANOVA (Jiang et al. 2007). Two-locus QTL comparison results showed that the RILs carrying negative alleles at both loci that decreased saturated fatty acid content exhibited significantly lower values of saturated fatty acids than those of the reciprocal genotypes in all cases and than those of RILs with negative alleles at only one locus in most cases. Three-locus QTL pyramiding analysis also indicated that additional negative alleles at the third locus would further decrease the values of the traits to some extent, compared with the RILs with negative alleles at two loci only, although the differences were not significant in some cases. On the other hand, the averages of saturated fatty acids were increased along with an increase of loci with positive alleles in the RILs. Comparisons of four QTL combinations (data not shown) indicated that the fourth QTL allowed additional alternation (increasing or decreasing) for palmitic acid in most cases, though no consistent alternations were observed for stearic acid. In addition, the extreme lines in the population carried the desired alleles (either positive or negative) for all or most of the QTL detected, regardless of whether one wanted to increase or decrease the saturated fatty acids.

Based on the results of QTL pyramiding analysis, the following QTL combinations are suggested for consideration in practical marker-assisted breeding. For low palmitic acid, the two-locus combinations of *qPAL-A1* + *qPAL-K-2*, *qPAL-A1* + *qPAL-F-1*, *qPAL-A1* + *qPAL-E* and *qPAL-F-1* + *qPAL-G*, and three-locus combinations of *qPAL-A1* + *qPAL-K-2* + *qPAL-F-1*, *qPAL-A1* + *qPAL-K-1* + *qPAL-G*, *qPAL-A1* + *qPAL-F-1* + *qPAL-E* and *qPAL-A1* + *qPAL-F-1* + *qPAL-G*; for high palmitic acid, the two-locus combinations of *qPAL-K-2* + *qPAL-K-1*, *qPAL-K-2* + *qPAL-E*, *qPAL-K-1* + *qPAL-G* and *qPAL-F-1* + *qPAL-E*, and three-locus combinations of *qPAL-A1* + *qPAL-K-1* + *qPAL-G*, *qPAL-K-1* + *qPAL-F-1* + *qPAL-G* and *qPAL-F-1* + *qPAL-G* + *qPAL-E*; for low stearic acid, the two-locus combinations of *qSTE-A1-2* + *qSTE-F*, *qSTE-A1-1* + *qSTE-G* and *qSTE-*

A1-1 + *qSTE-F*, and three-locus combinations of *qSTE-A1-2* + *qSTE-G* + *qSTE-F* and *qSTE-A1-1* + *qSTE-G* + *qSTE-F*; and for high stearic acid, the two-locus combinations of *qSTE-A1-2* + *qSTE-F*, *qSTE-A1-2* + *qSTE-N* and *qSTE-A1-1* + *qSTE-F*, and three-locus combinations of *qSTE-A1-2* + *qSTE-G* + *qSTE-N*, *qSTE-A1-2* + *qSTE-F* + *qSTE-N* and *qSTE-A1-1* + *qSTE-G* + *qSTE-N* should be the appropriate options for altering fatty acid profiles in soybeans.

In practical breeding, the complexity and/or difficulty of marker-assisted selection increases with an increase in QTL or markers involved. There is no general idea or extensively applicable standard about how many QTL or markers have to be used or are best for breeding programs. It depends on the magnitude of the genetic effects of the individual QTL, the relationship between the QTL involved, the genetic distance between the two parents, and the sources available. In this study, a comparison of more than five QTL combinations seemed to be less applicable or informative because of the limited population size. However, analysis of QTL alleles for the extreme lines in the population might provide suggestions of interest for practical marker-assisted selection. According to such an analysis and discussion above, therefore, we would suggest that 5–6 QTLs have to be used for palmitic acid, and particularly the desired alleles for 4–5 loci of the six QTL *qPAL-A1*, *qPAL-K-1*, *qPAL-K-2*, *qPAL-F-1*, *qPAL-G* and *qPAL-E* should be selected. For stearic acid, desired alleles of 4–5 loci might be required. For the total saturated fatty acids, five of the six QTL *qSAP-A1*, *qSAT-F-1*, *qSAT-K-1*, *qSAT-K-2*, *qSAT-N* and *qSAT-E* are needed.

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