Theor Appl Genet (2011) 122:1481–1488
DOI 10.1007/s00122-011-1547-2

ORIGINAL PAPER

Dynamic QTL analysis of linolenic acid content in different developmental stages of soybean seed

Yingpeng Han · Dongwei Xie · Weili Teng · Shuzheng Zhang · Wei Chang · Wenbin Li

Received: 18 October 2010 / Accepted: 2 February 2011 / Published online: 23 February 2011

Abstract Linolenic acid (LN) in soybean (*Glycine max* L. Merr.) seed mainly contributes to the undesirable odors and flavors commonly associated with poor oil quality. LN deposition at various stages of soybean seed development had not been reported by 2010. The objects of this study were (1) to identify and measure quantitative trait loci (QTL) underlying LN content and (2) to estimate the QTL effects expressed from earlier seed developmental stages to drying seed of soybean. One hundred and twenty-five $F_{5:8}$ and $F_{5:9}$ recombinant inbred lines derived from the cross of soybean cultivars 'Hefeng 25' and 'Dongnong L5' were used for the identification of QTL underlying LN content from the 37 day (D) to 86D stages after flowering, at Harbin in 2008 and 2009. QTL × Environment interactions (QE) effects were evaluated using a mixed genetic model (Zhu in J Zhejiang Univ (Natural Science) 33:327–335, 1999). Twelve unconditional QTL and 12 conditional QTL associated with LN content were identified at different developmental stages. Most of the QTL explained <10% of phenotypic variation of LN content. Unconditional QTL QLNF-1, QLNC2-1, QLND1b-1, QLNA2-1 and QLNH-1 influenced LN content across different development stages and environments. Conditional QTL QLNF-1, QLNC2-1 and QLNH-1 were identified in multiple developmental stages and environments. Conditional and unconditional QTL clustered in neighboring intervals on linkage groups A2, C2 and D1b. Ten QTL with conditional additive main effects (*a*) and/or conditional additive × environment interaction effects (*ae*) at specific developmental stage were identified on nine linkage groups. Of them, six QTL only possessed additive main effects and seven QTL had significant *ae* effects in different developmental stages. A total of 13 epistatic pairwise QTL were identified by conditional mapping in different developmental stages. Two pairs of QTL only showed *aa* effects and five pairs of QTL only showed *aae* effects at different developmental stages. QTL with *aa* effects, as well as their environmental interaction effects, appeared to vary at different developmental stages.

Communicated by D. Lightfoot.

Y. Han and D. Xie have equal contributions to the paper.

Y. Han · D. Xie · W. Teng · S. Zhang · W. Chang · W. Li (✉)
Soybean Research Institute (Key Laboratory of Soybean Biology in Chinese Ministry of Education), Northeast Agricultural University, Harbin 150030, China
e-mail: wenbinli@neau.edu.cn

Introduction

Fatty acid composition is an important determinant of soybean oil quality. Soybean contains five predominant fatty acid including palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic acids (18:3, LN; Wilson 2004). LN has been identified as an unstable component of soybean oil that was mainly responsible for the undesirable odors and shorter self life associated with poor oil quality (Dutton et al. 1951; Smouse 1979). As a result, attempts have been made to develop soybean lines with linolenic acid concentration substantially below those found in commercial varieties (Takagi et al. 1990). Traditional plant improvement has relied on phenotypic selection of populations from crosses between commercial cultivars or experimental lines (Stuber et al. 1992). However, LN content of soybean seeds is controlled by multiple genes with small or large genetic effects and interaction effects between genes and environments (Thorne and Fehr 1970). Hence, selections for soybean cultivars with lower LN content required evaluation in multiple environments over several years,

which has been expensive, time-consuming and labor-intensive.

Molecular markers offer a faster and more accurate approach to breeding, since selection can be based on genotype rather than solely on phenotype. The use of marker-assisted selection (MAS) can improve the efficiency of traditional plant breeding. MAS could potentially improve selection of traits that have low heritability by using markers with high heritability. Song et al. (2004) developed an integrated genetic linkage map of soybean including 1,015 SSR markers in one or more of five different populations, and aligned the molecular linkage groups (LGs) into a consensus map of 20 LGs that correspond to the 20 pairs of soybean chromosomes (Zou et al. 2003). A genetic transcript map of the soybean genome was developed by Choi et al. (2007) through mapping one SNP in each of 1,141 genes in one or more of the three recombinant inbred line (RIL) mapping populations, which provided a picture of the distribution of genic sequences across the mapped portion of the genome. This information has greatly facilitated MAS in soybean breeding.

In the past decade, few studies have focused on the mapping of quantitative trait loci (QTL) associated with soybean LN content (Hyten et al. 2004; Spencer et al. 2004; Panthee et al. 2006). The phenotypic values of LN were usually measured post-harvest and used for QTL analysis in most of those reports. Those studies, however, had ignored the distinct gene expression at different developmental stages, which were important factors affecting the development of quantitative traits such as seed composition, yield and plant height (Yan et al. 1998a; Sun et al. 2006; Li et al. 2007; Teng et al. 2009). The statistical analysis showed that the development of some quantitative traits like morphology occurred through the actions and interactions of many genes that might behave differentially during different growth stages, and that gene expression was modified by interactions with other genes and by the interaction with environments (Atchley and Zhu 1997). During soybean development, sets of genes are expressed selectively at different growth stages and the genes expressed were influenced by both genotype and environment (Vodkin et al. 2004). QTL analysis can be adapted to include the effects of developmental stages (Zhu 1995). Therein, and hereafter, the net genetic effect that is referred to as conditional genetic effects (the QTL as defined by Sen and Churchill 2001) are the loci detected at a specific growth stages. In contrast, unconditional genetic effects (the QTL as defined by Sen and Churchill 2001) are effective throughout seed development. Identification of both conditional and unconditional QTL will be desirable for MAS. It will be essential, therefore, to include the dynamics of gene expression and interactions with environments when analyzing developmental quantitative traits. Detailed analyses of conditional QTL will provide the basis for map-based cloning of genes underlying the loci. DNA markers derived from those genes will improve the efficiency of MAS (Xu 1997).

The association of developmental behavior of quantitative traits with molecular markers had been reported in rice and cotton (Yan et al. 1998a, b; Wu et al. 1999; Ye et al. 2003) and in morphological traits and seed quality traits of soybean (Sun et al. 2006; Li et al. 2007; Teng et al. 2009). The objectives of the present study were to investigate the developmental behavior of LN content in soybean seed, to identify both conditional and unconditional QTL, and to measure the GE interaction and epistasis effects of LN content during seed development.

Materials and methods

Plant materials

The mapping population of 125 $F_{5:8}$ and $F_{5:9}$ RILs were derived from the cross between 'Hefeng 25' (average LN 6.2±1.5% (w/w)) and 'Dongnong L5' (average LN 2.5 ±0.5% (w/w)) through single-seed-descent.

Field experiment

The RILs and their parents were grown in a randomized complete block design with three replications at Harbin of China (45°N, fine-mesic chernozen soil) in 2008 and 2009, with rows 3 m long, 70 cm apart and 6 cm between plants within rows. The seeds of 10 plants per genotype were collected from each plot and later were used to analyze LN content. Pods (from 10 plants per RIL) were picked off. Partial harvests were made eight times, from nodes 5 to 7 on the main stem, every 7 days since 37 days (initial stage 37D) after flowering until maturity (final stage 86D). Seeds were dried for 30 min in an oven at 105°C and then continuously dried at 60–70°C until the seed mass was stable.

LN extraction and gas chromatograph analysis

LN content was determined by gas chromatography (GC-14C, Shimadzu Company, Japan). About 0.4–0.5 g of soybean flour was transferred to a test tube. Five milliliters of anesthetic grade ether was added to the test tube containing the soybean flour and closed with a stopper. The test tubes were incubated at room temperature for at least 6 h. After the extraction, the transparent liquid was decanted into a new test tube and dried over night in an aerator. When ether volatilization was completed, 100 µl of each oil sample was pippeted into a clean test tube with 2 ml of solvent (anesthetic ether:*n*-hexane, 2:1 (v/v)) and mixed. Two milliliters of methanol was added and mixed followed

by mixing 2 ml of potassium hydroxide in methanol (0.8 mol/L), which is then held at room temperature for 10–20 min. Finally, 2 ml of dihydrogen oxide was put into the test tube, mixed and incubated for 10 min. This process caused the LN to be solubilized. Exactly 1 µl of the top liquid was used to detect the LN content by gas chromatography. The test columns all had nominal dimensions of 30 m × 0.125 m × 0.13 µm film thickness. Operation conditions were as follows: carrier, hydric (40 ml/min) split injection, injection temperature 250°C, detector temperature 250°C and column temperature 210°C (a method modified by Panthee et al. 2006).

DNA extraction and polymerase chain reaction

Total DNA of the parents and each RIL were isolated from leaf tissue by the CTAB method (Doyle and Doyle 1990). Polymerase chain reaction (PCR) was performed in a 20 µl volume containing 2 µl genomic DNA (25 ng/µl), 2 µl $MgCl_2$ (25 mM), 0.3 µl dNTP mixtures (10 mM), 2 µl 10 × PCR buffer, 3 µl SSR primer (2 µM), 0.2 µl *Taq* polymerase (10 units/µl), 11.5 µl double-distilled water. PCR conditions were 94°C for 5 min, following by 38 cycles of 30 s at 94°C, 30 s at 47°C, 30 s at 72°C, then 5 min at 72°C. After amplification, the PCR products were mixed with loading buffer (2.5 mg/ml bromophenol blue, 2.5 mg/ml diphenylamine blue, 10 mM EDTA, 95% (v/v) formamide), and denatured for 10 min at 94°C, then kept at 4°C. The denatured PCR products were separated on a 6% (w/v) denatured polyacrylamide gel and visualized by silver staining (Trigizano and Caetano-Anolles 1998).

The analysis of unconditional and conditional QTL at different developmental stages

Unconditional QTL were detected at each growth stage using mean phenotypic values and composite intervals (Zeng 1993, 1994). The genetic effect was the net accumulation of several gene sets from the initial time of plant growth to the time point *t*. Conditional phenotypic values at time *t* were given by subtracting the phenotypic means measured at time $t - 1$ from the mean at time *t* (Zhu 1995). The derived genetic effect reflected changes accumulating in the days prior to the measurement rather than the net genetic effect of accumulation in the unconditional QTL. Conditional QTL analysis relied on the composite interval method (Zeng 1993, 1994) and analysis of time-independent genetic effects (Zhu 1995). Unconditional QTL, conditional QTL, and the genetic effects of conditional QTL (including additive and additive × additive epistatic effects, as well as their environmental interaction effects) were analyzed according to method by Yan et al. (1998a, b) and Wang et al. (1999), respectively.

Results

Gas chromatograph analysis of LN content in soybean seed

Phenotypic values of LN content in soybean seed at different developmental periods across two environments were shown in Table 1. The differences of LN content between the two parents were significant at all developmental stages measured across diverse environments. However, LN content was higher in 2009 than in 2008. In contrast, the LN variation of 125 RILs across both environments was not significant. Both skew and kurtosis values of LN were <1.0 at all growth stages measured in diverse environments, suggesting that the segregation of this trait fit a normal distribution model.

Linkage analysis

A total of 600 SSR markers were used to detect polymorphisms between the two parents. One hundred and twelve (18.67%) of them were polymorphic and were mapped onto 18 LGs, which encompassed about 2,718 cM with mean distance of 24.26 cM between markers (data not shown).

Unconditional and conditional QTL associated with LN content at different developmental stages

A total of 12 unconditional QTL underlying LN content at different developmental stages were identified based on 2 years data at Harbin (Table 2). Among them, six QTL (QLN F-1 at 44D,51D, 58D and 75D stages, QLNC2-1 at 37D and 51D stages, QLNJ-1 at 58D stage, QLNA2-1 at 65D stage, QLNA2-2 at 58D stage and QLNH-1 at 65D stage) were consistently detected in both years. Of them, QLN F-1 was detected at 37D–65D stages in 2008, and at 44D–72D stages in 2009, respectively. QLNC2-1 was detected at 37D and 51D stages in 2008 and 2009, and at 72D stage in 2009, respectively. QLND1b-1 was detected at 58D and 72D stages in 2008, and at 37D and 72D stage in 2009, respectively. QLNA2-1 was detected at 65D stage in 2008, and at 44D, 65D, 72D stages in 2009, respectively. QLNH-1 was detected at 51D and 65D stages in 2008, and at 65D and 79D stages in 2009, respectively. QLNB2-1 accounted for the largest amount of phenotypic variation (29.98%) at the 72D stage (Table 2).

Twelve conditional QTL underlying LN content at different developmental stages were identified based on 2 years data at Harbin (Table 2). Of them, QLN F-1 was identified at 37D–58D, 72D and 86D stages, respectively. QLNC2-1 was identified at 51D stage in 2008, but at 58D and 72D stages in 2008 and 2009, respectively. QLNH-1 was detected at 51D and 65D stages in 2008, but at 51D and 79D stages in 2009, respectively.

Table 1 Statistical analyses of mean linolenic acid content for two soybean parents and an RIL population at different growth stages

	DS	Parents				RIL population											
		'Hefeng 25'		'Dongnong L5'		Mean		Range		SD		CV		Skew		Kurtosis	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
LN % (w/v)	37D	15.82	9.88	4.11	3.66	8.94	8.68	5.31–12.02	4.08–12.67	2.75	3.78	0.31	0.44	0.19	−0.19	−1.01	−0.94
	44D	8.92	12.71	2.82	2.85	6.41	6.68	3.86–9.45	3.03–11.22	2.17	2.81	0.34	0.42	0.28	0.32	−0.46	−0.74
	51D	6.39	6.45	3.55	2.47	5.18	5.18	3.19–6.92	2.79–7.97	1.74	2.24	0.34	0.43	−0.16	0.08	−0.82	−1.14
	58D	4.71	5.89	3.82	2.66	4.93	4.55	3.22–7.18	2.25–7.59	1.36	1.78	0.28	0.39	0.39	0.26	−0.66	−1.12
	65D	5.01	6.50	4.33	2.52	4.89	4.64	3.09–7.21	2.30–7.48	1.37	1.75	0.28	0.38	0.24	0.14	−0.99	−1.06
	72D	6.05	6.56	3.22	3.14	4.79	4.79	2.91–7.01	2.42–7.77	1.34	1.77	0.28	0.37	0.15	0.26	−0.98	−0.95
	79D	6.18	6.33	3.56	2.62	5.13	5.03	3.36–7.89	2.52–7.99	1.36	1.77	0.27	0.35	0.43	0.12	−0.61	−1.02
	86D	5.74	7.25	2.81	2.56	5.01	5.34	2.64–7.86	2.58–8.55	2.92	1.81	0.58	0.34	0.34	0.23	−0.66	−0.91

DS developmental stage, *SD* standard deviation, *CV* coefficient of variation

The analysis of conditional QTL × environment interactions at different developmental stages

Ten QTL with conditional additive main effects (*a*) and/or conditional additive × environment interaction effects (*ae*) at certain specific stages were identified in nine linkage groups (Table 3). Of them, seven had significant additive main effects ($P < 0.01$ or 0.005), three QTL (QLN F-1 at 86D stage, QLNC2-1 at 37D stage, QLND1b-1 at 58D and 72D stages) had positive effects on LN accumulation and seven QTL (QLN F-1 at 37D, 44D and 65D stages, QLNC2-1 at 51D stage, QLND1b-1 at 37D stage, QLNB2-1 at 72D stage, QLNC1-1 at 51D stage, QLNH-1 at 51D and 79D stages, QLNO-1 at 86D stage) showed negative effects for seed LN accumulation. The remainder was inconsistent as they had positive and negative effects at different growth stages. Six QTL (QLN F-1 at 44D and 65D stages, QLNC2-1 at 51D stage, QLND1b-1 at 58D and 72D stages, QLNC1-1 at 51D stage, QLNH-1 at 51D stage, QLNO-1 at 86D stage) had significant additive main effect (*a*), rather than significant *ae* effects. The lower LN content parent, 'Dongnong L5', contributed alleles for decreased LN content at QTL QLNB2-1 (at 72D stage), QLNC1-1 (at 51D stage) and QLNH-1 (at 51D and 79D stages). However, the allele of QTL QLNO-1 from 'Dongnong L5' increased LN content (at 86D stage). This suggested that alleles controlling low LN content were dispersed within the two parents. The impact of some QTL varied at different development stages. For example, the allele of QTL QLNF-1 decreased LN content at 37D, 44D and 65D stages, but increased LN content at the 86D stage.

Eight QTL had significant *ae* interaction effects at different developmental stages (Table 3). Of them, five QTL (QLN F-1 at 51D and 58D stages, QLNC2-1 at 37D stage, QLND1b-1 at 37D stage, QLNB2-1 at 72D stage, QLNH-1 at 65D stage) had significant *ae* effects at different stages in each environment. Seven QTL (QLN F-1 at 51D, 58D and 72D stages, QLNC2-1 at 58D and 72D stages, QLNB2-1 at 37D stage, QLNB1-1 at 79D stage, QLNA2-1 at 44D, 65D and 72D stages, QLNA2-2 at 58D stage, QLNH-1 at 65D stage) had significant *ae* effects, but no significant additive main effects (*a*). There were two QTL (QLNC2-1 at the 37D and 72D stages, QLND1b-1 at 37D stage) that increased LN content, four QTL (QLNB2-1 at 72D stage, QLNB1-1 at 79D stage, QLNA2-1 at 65D stage, QLNA2-2 at 58D stage) that decreased LN content in 2008 through *ae* effects. There was one QTL (QLNA2-1 at 44D and 72D stages) that increased LN content, three QTL (QLNC2-1 at 37D and 58D stage, QLND1b-1 at 37D stage, QLNH-1 at 65D stage) that decreased LN content and two QTL (QLN F-1, QLNB2-1) that both increased and decreased LN content at different developmental stages in 2009 by *ae* effects. Four QTL (QLN F-1 at 37D and 86D stages, QLNC2-1 at 37D stage, QLND1b-1 at 37D stage, QLNB2-1 at 72D stage, QLNH-1 at 79D stage) had both additive main effects (*a*) and additive QTL × environment interaction effects (*ae*).

Analysis of epistasis in different developmental stages

A total of 13 epistatic pairwise QTL were identified by conditional mapping in different developmental stages (Table 4). Of them, epistatic effects of two pairs of QTL (QLN F-1-QLNA2-1 at 44D and 65D stages, QLNC2-1-QLND1b-1 at 37D and 65D stages) were detected at two developmental stages. Other QTL were identified only at one stage. This indicated that epistatic effects existed mostly for a short time period, so that they were hardly observed during multiple developmental stages. This was inferred from the fact that epistatic effects were mostly found among conditional QTL but additive effects were mostly found among unconditional QTL.

Table 2 Unconditional and conditional QTL for seed linolenic acid content measured at different measuring stages

Trait	Interval	DS	2008						2009					
			t[a]			$t/t-1$[b]			t			$t/t-1$		
			LOD	A	R^2	LOD	A	R^2	LOD	A	R^2	LOD	A	R^2
QLN F-1	Satt149–Satt656	37D	3.89	−0.78	5.56	2.45	−0.32	8.75				2.25	−0.85	8.23
		44D	4.45	0.60	9.39	5.57	−0.45	5.40	2.30	−0.39	16.67	3.59	−0.79	10.45
		51D	2.37	−0.48	12.20	4.76	−0.27	12.96	7.78	−0.80	5.03	6.59	−0.80	8.89
		58D	4.40	−0.67	9.98	3.40	−0.83	10.02	2.02	3.78	11.2			
		65D	5.59	−0.90	7.73				2.01	2.1	6.06	2.81	0.64	14.66
		72D				5.50	−0.98	7.45	4.51	0.26	13.96	3.41	0.49	7.80
		86D				3.76	−0.30	4.40				4.50	−0.80	10.08
QLNC2-1	Sat251–Satt134	37D	4.08	−0.69	10.20				2.81	−0.17	8.75			
		51D	3.30	−0.92	13.35	4.45	−0.32	11.21	6.75	−0.49	5.56			
		58D				9.75	−0.76	4.45				3.27	−0.67	8.95
		72D				3.81	−0.80	5.57	2.74	−0.54	9.69	6.86	−0.89	7.65
QLNC2-2	Satt457–Sat_251	72D				5.65	−0.87	3.29	2.10	−0.96	3.96	3.31	−0.67	4.56
QLND1b-1	Satt546–Sat_274	37D				2.69	0.64	20.75	3.98	−0.80	4.75			
		58D	4.35	−0.41	6.70	5.68	−0.32	8.89						
		72D	2.05	0.72	16.68				2.43	−0.76	5.67			
QLND1b-2	Satt141–Satt546	37D				2.52	1.03	10.33				3.78	−0.71	7.70
QLNJ-1	Sat_339–Sat_151	58D	2.56	0.19	8.37				4.75	0.89	13.69			
QLNB2-1	Satt126–Satt726	37D				2.49	0.88	11.21	4.54	0.88	3.46			
		72D	4.90	0.89	29.28	3.05	−0.55	8.27						
QLNB1-1	Sat270–Satt426	79D	2.47	−0.23	11.50	4.86	0.64	7.88	4.97	−0.67	4.66			
QLNC1-1	Satt476–AW277661	51D							2.09	0.49	6.74	2.09	−1.00	11.00
QLNA2-1	Sat_347–Satt424	44D				5.90	−0.87	4.86	2.42	−0.65	8.36			
		65D	3.78	−0.87	7.83	5.43	−0.70	6.43	6.98	−0.80	5.52			
		72D				3.76	−0.47	7.86	16.96	−0.87	2.34			
QLNA2-2	Satt424-Sat_162	58D	7.02	−0.80	6.65				2.72	−2.95	9.82	4.86	−1.06	7.76
QLNH-1	Satt629–Satt142	51D				5.09	−0.65	3.87	6.65	−3.00	10.96	2.25	−0.27	7.11
		65D	4.09	−0.76	8.65	4.76	−0.84	2.99	2.97	−2.96	5.39			
		79D	3.87	−0.55	7.78							3.76	−0.58	3.20
QLNO-1	Sat109–Sat282	86D							2.96	0.71	10.92			
QLNO-2	Sat282–Satt608	86D				3.23	0.73	13.96						

DS developmental stages

[a] Unconditional QTL

[b] Conditional QTL

The epistasis × environment interaction effect (*aae*) was an important component of the total QTL × Environment (QE) interaction effects. Two pairs of QTL(QLN F-1-QLNC2-1 at 51D stage, QLN F-1-QLNO-1 at 86D stage) were detected with conditional epistatic effects (*aa*), five pairs (QLN F-1-QLND1b-1 at 37D stage, QLN F-1-QLNA2-1 at 44D and 65D stage, QLNC2-1-QLND1b-1 at 58D stage, QLNB2-1-QLNA2-1 at 72D stage, QLNA2-1-QLNH-1 at 65D stage) had only *aae* effects, two pairs of epistatic QTL (QLNC2-1-QLND1b-1 at 37D stage, QLND1b-1-QLNA2-2 at 58D stage) had both *aa* and *aae* effects. These results indicated that environments could greatly affect the expression of the QTL with epistatic effects during quantitative trait development.

Discussion

Conditional QTL may have controlled gene expressions that occurred in a specific period of plant growth. Unconditional QTL reflected cumulative genetic effects. Some unconditional QTL can also be detected as conditional

Table 3 Additive (*a*) and additive × environment interaction (*ae*) effect of QTL for LN content at eight different stages for 2008 and 2009 in Harbin

QTL	Marker interval	DS	*a* effect	*ae* effect in 2008	*ae* effect in 2009
QLN F-1	Satt149–Satt656	37D	−0.43	−0.16	
		44D	−0.31		
		51D		−0.19	0.21
		58D		0.53	−0.54
		65D	−0.87		
		72D		0.21	
		86D	0.50	0.93	
QLNC2-1	Sat251–Satt134	37D	0.75	0.98	−1.00
		51D	−0.67		
		58D			−0.19
		72D		0.21	
QLND1b-1	Satt546–Sat_274	37D	−0.85	0.65	−0.70
		58D	0.67		
		72D	0.88		
QLNB2-1	Satt126–Satt726	37D			−0.80
		72D	−0.20	−0.56	0.60
QLNB1-1	Sat270–Satt426	79D		−0.39	
QLNC1-1	Satt476–AW277661	51D	−0.11		
QLNA2-1	Sat_347–Satt424	44D			0.48
		65D		−0.50	
		72D			0.30
QLNA2-2	Satt424–Sat_162	58D		−0.28	
QLNH-1	Satt629–Satt142	51D	−0.23		
		65D		0.67	−0.70
		79D	−0.56	−0.03	
QLNO-1	Sat109–Sat282	86D	0.79		

Table 4 Epistatic (*aa*) and epistasis × environment interaction (*aae*) effect of QTL for LN content at eight different stages for 2008 and 2009 in Harbin

QTL	Marker interval	QTL	Marker interval	DS	*aa* effect	*aae* effect in 2008	*aae* effect in 2009
QLN F-1	Satt149–Satt656	QLND1b-1	Satt546–Sat_274	37D		0.06	0.12
		QLNB2-1	Satt126–Satt726	37D	0.09	0.80	
		QLNA2-1	Sat_347–Satt424	44D		−0.17	0.65
				65D		−0.45	0.05
		QLNC2-1	Sat251–Satt134	51D	−0.12		
		QLNO-1	Sat109–Sat282	86D	−0.54		
QLNC2-1	Sat251–Satt134	QLND1b-1	Satt546–Sat_274	37D	1.00	0.04	
				58D			0.18
QLND1b-1	Satt546–Sat_274	QLNA2-2	Satt424–Sat_162	58D	−0.86	0.10	0.17
		QLNB2-1	Satt126–Satt726	72D	0.47		0.06
QLNB2-1	Satt126–Satt726	QLNA2-1	Sat_347–Satt424	72D		0.05	
QLNB1-1	Sat270–Satt426	QLNH-1	Satt629–Satt142	79D	0.13		0.03
QLNA2-1	Sat_347–Satt424	QLNH-1	Satt629–Satt142	65D		0.19	0.31

QTL. For example, the unconditional QTL QLNF-1 that significantly affected LN content in seed was detected continuously from 37D to 65D stages. However, other unconditional QTL were not found by the conditional analysis or were placed in the wrong interval by the summed effects of two linked loci.

Twelve unconditional QTL and twelve conditional QTL associated with LN content were identified in different developmental stages. Most of them explained <10% of phenotypic variation for LN content in different environments or individual developmental stage. The low level of phenotypic variation evaluated by these unconditional or conditional QTL indicated the accumulation of LN in soybean seeds were controlled by many QTL with small effects and may explain the difficulty of genetic improvement by phenotype selection. Unconditional QTL QLN F-1, QLNC2-1, QLND1b-1, QLNA2-1 and QLNH-1 influenced LN content of soybean seed at different developmental stages and environments. Conditional QTL QLNF-1, QLNC2-1 and QLNH-1 were identified across many growth stages and environments. These stable QTL identified in the present study may be due to one or a combination of the following factors: (1) The stable QTL were responsible for major effects and were associated with high LOD scores. As suggested by Tanksley (1993) and Zhuang et al. (1997), QTL with major effects were more likely to be stable across multiple environments; (2) Highly heritable traits tend to be more repeatable and stable across multiple environments (Paterson et al. 1991).

Usually, QTL × environment effects were treated as random effects, especially in different years. This implied that QTL would be affected by different environments. QTL × environment interaction has been reported by comparing QTL detected in specific environments (Paterson et al. 1991; Stuber et al. 1992; Lu et al. 1997). However, QTL detected separately in each environment were not the real QTL × environment interactions (Jansen et al. 1995). The mixed model approach for QTL mapping could provide unbiased prediction of QTL × environment interactions when the experiment was conducted in multiple environments (Zhu 1999). In the present study, six QTL had significant additive main effect (*a*), seven QTL had significant *ae* effects. Other QTL had both additive main effects and significant *ae* effects in different developmental stages. QTL with only QTL × environment effects were mainly contributed by the variation among environments, which cannot be used in MAS. Therefore, the QTL with additive main effects should be used in MAS.

It has been proved that epistasis between different loci is common among plant species (Lynch 1991; Orr 1995; Hutter 1997; Chase et al. 1997). Recent QTL mapping suggested that epistasis was main genetic basis underlying complex traits (Li et al. 1997; Chase et al. 1997; Kulwal et al. 2005). In our study, a total of 13 epistatic pairs of QTL were detected. Two pairs of QTL (QLN F-1-QLNC2-1 at 51D stage, QLN F-1-QLNO-1 at 86D stage) showed only *aa* effects. Five pairs of QTL (QLN F-1-QLND1b-1 at 37D stage, QLN F-1-QLNA2-1 at 44D and 65D stage, QLNC2-1-QLND1b-1 at 58D stage, QLNB2-1-QLNA2-1 at 72D stage, QLNA2-1-QLNH-1 at 65D stage) showed *aae* effects. Therefore, the inheritance of LN content was shown to be governed primarily by additive effects rather than dominant or epistatic genetic effects. This finding was in agreement with earlier reports of LN content (Thorne and Fehr 1970).

Acknowledgments This study was conducted in the Key Laboratory of Soybean Biology of Chinese Education Ministry and Soybean Development Centre of Agricultural Ministry, and financially supported by National High Technology Project (2006AA10Z1F1 and 2006AA100104-4), 948 project of Agricultural ministry of China (2006-G5), National Nature Science Foundation projects (30671318, 30971810, 60932008), National 973 Project (2009CB118400), Provincial Education Ministry for the team of soybean molecular design.

References

Atchley WR, Zhu J (1997) Developmental quantitative genetics, conditional epigenetic variability and growth in mice. Genetics 147:765–776

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

Choi IY, Hyten DL, Matukumalli KL, Song Qj, Chaky MJL, Chase K, Quigley VC, Lark KG, Reiter RS, Yoon MS, Hwang EY, Yi SI, Young ND, Randy C, van Tassell PC, Specht EJ, Cregan PB (2007) A soybean transcript map: gene distribution, haplotype and single-nucleotide polymorphism analysis. Genetics 176:685–696

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dutton HJ, Lancaster CR, Evans CD, Cowan CI (1951) The flavor problem of soybean oil. VIII. Linolenic acid. J Am Oil Chem Soc 28:115–118

Hutter P (1997) Genetics of hybrid inviability in *Drosophila*. Adv Genet 36:157–185

Hyten DL, Pantalone VR, Saxton AM, Schmidt ME, Sams CE (2004) Molecular mapping and identification of soybean fatty acid modifier quantitative trait loci. J Am Oil Chem Soc 81(12):1115–1118

Jansen RC, Van Ooijen JW, Stam P, Lister C, Dean C (1995) Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. Theor Appl Genet 91:33–37

Kulwal P, Kumar N, Kumar A, Gupta RK, Balyan HS, Gupta PK (2005) Gene networks in hexaploid wheat: interacting quantitative trait loci for grain protein content. Funct Integr Genomics 5:254–259

Li Z, Pinson S, Park W, Paterson A, Stansel J (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145:453–465

Li WB, Sun DS, Du YP, Chen QS, Zhang ZC, Qiu LJ, Sun GL (2007) Quantitative trait loci underlying the development of seed composition in soybean (*Glycine max* L. Merr.). Genome 50:1067–1077

Lu C, Shen L, Tan Z, Xu Y, He P, Chen Y, Zhu L (1997) Comparative mapping of QTLs for agronomic traits of rice across environments by using a doubled-haploid population. Theor Appl Genet 94:145–150

Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45:622–629

Orr H (1995) The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics 139:1805–1813

Panthee DR, Pantalone VR, Saxton AM (2006) Modifier QTL for fatty acid composition in soybean oil. Euphytica 152:67–73

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127:181–197

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

Smouse TH (1979) A review of soybean oil reversion flavor. J Am Oil Chem Soc 56:747A–750A

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Spencer MM, Landau-Ellis D, Meyer EJ, Pantalone VR (2004) Molecular markers associated with linolenic acid content in soybean. J Am Oil Chem Soc 81(6):559–562

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:832–839

Sun DS, Li WB, Zhang ZC, Chen QS, Ning HL, Qiu LJ, Sun GL (2006) Quantitative trait loci analysis for the developmental behavior of Soybean (*Glycine max* L. Merr.). Theor Appl Genet 112:665–673

Takagi Y, Hossain ABMM, Yanagita T, Matsueda T, Murayama A (1990) Linolenic acid content in soybean improved by X-ray irradiation. Agric Biol Chem 54:1735–1738

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Teng W, Han Y, Du Y, Sun D, Zhang Z, Qiu L, Sun G, Li W (2009) QTL analyses of seed weight during the development of soybean (*Glycine max* L. Merr.). Heredity 102:372–380

Thorne JC, Fehr WR (1970) Incorporation of high-protein, exotic germplasm into soybean populations by 2- and 3-way crosses. Crop Sci 10:652–655

Trigizano RN, Caetano-Anolles G (1998) Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. Hortic Technol 8:413–423

Vodkin LO, Khanna A, Shealy R, Clough SJ, Gonzalez DO, Philip R, Zabala G, Thibaud-Nissen F, Sidarous M, Stromvik MV, Shoop E, Schmidt C, Retzel E, Erpelding J, Shoemaker RC, Rodriguez-Huete AM, Polacco JC, Coryell V, Keim P, Gong G, Liu L, Pardinas J, Schweitzer P (2004) Microarrays for global expression constructed with a low redundancy set of 27, 500 sequenced cDNAs representing an array of developmental stages and physiological conditions of the soybean plant. BMC Genomics 5:73

Wang DL, Zhu J, Li ZK, Paterson AH (1999) Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. Theor Appl Genet 99:1255–1264

Wilson RF (2004) Seed composition. In: Boerma HR, Specht JE (eds) Soybean: improvement, production and users, 3rd edn. ASA, CSSA, SSA, Madison

Wu W, Li W, Tang D, Lu H, Worland AJ (1999) Time-related mapping of quantitative trait loci underlying tiller number in rice. Genetics 151:297–303

Xu YB (1997) Quantitative trait loci: separating, pyramiding, and cloning. Plant Breed Rev 15:85–139

Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1998a) Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza stativa* L.). Theor Appl Genet 97:267–274

Yan J, Zhu J, He CX, Benmoussa M, Wu P (1998b) Molecular dissection of developmental behavior of plant height in rice (*Oryza sativa* L.). Genetics 150:1257–1265

Ye Z, Lu Z, Zhu J (2003) Genetic analysis for developmental behavior of some seed quality traits in upland cotton. Euphytica 129: 183–191

Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 140:745–754

Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. Genetics 141:1633–1639

Zhu J (1999) Mixed model approaches of mapping genes for complex quantitative traits. J Zhejiang Univ (Natural Science) 33:327–335

Zhuang JY, Lin HX, Lu J, Qian HR, Hittalmani S, Huang N, Zheng KL (1997) Analysis of QTL × environment interaction for yield components and plant height in rice. Theor Appl Genet 95: 799–808

Zou J, Lee J, Singh R, Xu SS, Cregan PB, Hymowitz T (2003) Assignment of molecular linkage groups to the soybean chromosomes by primary trisomics. Theor Appl Genet 107:745–750