

Crops and Soils Research Paper

Cite this article: Li X *et al.* (2019). Mapping QTLs for protein and oil content in soybean by removing the influence of related traits in a four-way recombinant inbred line population. *The Journal of Agricultural Science* **157**, 659–675. <https://doi.org/10.1017/S0021859620000040>

Received: 4 June 2019

Revised: 17 December 2019

Accepted: 29 January 2020

Key words:


Conditional QTL; four-way recombinant inbred lines; protein and oil content; soybean

Author for correspondence:

Hailong Ning,

E-mail: ninghailongneau@126.com

Mapping QTLs for protein and oil content in soybean by removing the influence of related traits in a four-way recombinant inbred line population

Xiyu Li¹, Hong Xue^{1,2}, Kaixin Zhang¹, Wenbin Li¹, Yanlong Fang¹, Zhongying Qi¹, Yue Wang¹, Xiaocui Tian¹, Jie Song¹, Wenxia Li¹ and Hailong Ning¹ 

¹Key Laboratory of Soybean Biology, Ministry of Education/Key Laboratory of Soybean Biology and Breeding/Genetics, Ministry of Agriculture, Northeast Agricultural University, Harbin, Heilongjiang province, China and

²Keshan Branch of Heilongjiang Academy of Agricultural Sciences, Keshan, Heilongjiang, China

Abstract

Protein content (PC) and oil content (OC) are important breeding traits of soybean [*Glycine max* (L.) Merr.]. Quantitative trait locus (QTL) mapping for PC and OC is important for molecular breeding in soybean; however, the negative correlation between PC and OC influences the accuracy of QTL mapping. In the current study, a four-way recombinant inbred lines (FW-RILs) population comprising 160 lines derived from the cross (Kenfeng14 × Kenfeng15) × (Heinong48 × Kenfeng19) was planted in eight different environments and PC and OC measured. Conditional and unconditional QTL analyses were carried out by interval mapping (IM) and inclusive complete IM based on linkage maps of 275 simple sequences repeat markers in a FW-RILs population. This analysis revealed 59 unconditional QTLs and 52 conditional QTLs among the FW-RILs. An analysis of additive effects indicated that the effects of 13 protein QTLs were not related to OC, whereas OC affected the expression of 13 and eight QTLs either partially or completely, respectively. Eight QTLs affecting OC were not influenced by PC, whereas six and 26 QTLs were partially and fully affected by PC, respectively. Among the QTLs detected in the current study, two protein QTLs and five oil QTLs had not been previously reported. These findings will facilitate marker-assisted selection and molecular breeding of soybean.

Introduction

Soybean is an economically important crop worldwide and a major source of edible oil and protein. Improving protein content (PC) and oil content (OC) is an important objective in soybean breeding programs. These quantitative traits are controlled by multiple genes and affected by the environment. Detecting quantitative trait loci (QTLs) underlying PC and OC in soybean is helpful to improve the efficiency of soybean breeding.

Diers *et al.* (1992) identified eight QTLs PC and nine QTLs for OC by mapping a population derived from a cross between a *G. max* experimental line (A81-356022) and the wild soybean (*G. soja*) line PI468916. Since then a growing number of QTLs controlling protein and OC have been identified. As of December 2018, 241 QTLs for PC and 339 QTLs for OC have been detected according to SoyBase (<http://www.soybase.org>). These QTLs were detected using F₂ and recombinant inbred line (RIL) populations derived from crosses between two parents (Liang *et al.*, 2010; Qi *et al.*, 2011a; Lu *et al.*, 2013). However, this technique fails to identify some QTLs due to the relatively small differences in the additive effects of putative QTLs and the low degree of coverage of the genome, because molecular markers often exhibit little polymorphism between two parents.

To increase the power of QTL detection, Xu (1996, 1998) developed a QTL mapping technique based on a four-way (FW) hybrid design. Furthermore, Ning *et al.* (2015, 2016) established a FW-RILs population in soybean that was subsequently used for linkage map construction and QTL detection for PC and OC (Ning *et al.*, 2016) and pod number-related traits (Liu *et al.*, 2019). Because of highly polymorphic molecular markers, FW-RILs populations show more variation than common mapping populations. The construction of FW-RILs populations involves four parents, and each QTL has a maximum of four alleles. As long as there is a substantial difference in genetic effects between two alleles in a QTL, the QTL can be detected.

There is a significantly negative correlation between PC and OC in soybean (Zhang *et al.*, 2004). The mutual interference among these traits can affect the results of QTL mapping. Zhu (1995) proposed a conditional variable method to analyse the related traits that could eliminate

© The Author(s) 2020. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

CAMBRIDGE
UNIVERSITY PRESS

the mutual influence of these traits and improve the accuracy of estimating the genetic effects of QTLs. This conditional method has been used for QTL mapping in rice (*Oryza sativa*) (Liu et al., 2012), oilseed rape (*Brassica napus*) (Jiao et al., 2015) and wheat (*Triticum aestivum*) (Tian et al., 2011).

The current study aimed to enhance the detection of QTLs for PC and OC and improve the accuracy of QTL mapping. The PC and OC data obtained from an FW-RIL soybean population (Ning et al., 2016) were reanalysed by both unconditional and conditional methods. The QTLs controlling PC and OC identified in the current study will facilitate the molecular breeding of soybean.

Materials and methods

Plant materials

Four parental soybean lines that significantly differ in terms of PC and OC, i.e. Kenfeng14 (PC 39.69%, OC 20.34%), Kenfeng15 (PC 38.68%, OC 22.76%), Heinong48 (PC 44.71%, OC 19.05%) and Kenfeng19 (PC 42.52%, OC 19.26%), were mated in a four-way cross. In 2008, two single crosses, Kenfeng14 × Kenfeng15 and Heinong48 × Kenfeng19, were performed and in 2009, the progeny were crossed as (Kenfeng14 × Kenfeng15) × (Heinong48 × Kenfeng19) in Harbin (44°05'N, 125°42'E, 127.95 m a.s.l), Heilongjiang Province, China. From 2010 to 2012, the progeny were planted in Harbin in the summer and Hainan (17°50'N, 109°00'E, 130.12 m a.s.l) in the winter, and each individual was selected by the single-seed descent method. Ultimately, 160 FW-RILs were obtained, which were subjected to phenotypic and genotypic analyses.

Field experiment and evaluation of phenotypic traits

The four parental and 160 FW-RIL lines were planted in Harbin and Keshan (48°03'N, 125°53'E, 236 m a.s.l), as follows: in 2013, plants were sown on 10 May at planting densities of 2.22×105 plants/ha and 2.80×105 plants/ha, in Harbin (E1) and Keshan (E2), respectively. In 2014, parental lines and FW-RILs were both planted at 2.22×105 plants/ha, in Harbin only, on 10 May (E3) and 20 May (E4). Finally, in 2015, all sowing was done on 10 May but at four planting densities: 2.2×105 plants/ha (E5) and 3.08×105 plants/ha (E6) in Harbin, and 2.58×105 plants/ha (E7) and 3.51×105 plants/ha (E8) in Keshan for parental and FW-RILs, respectively (the planting and management plan is shown in Table S1). The parents (parental traits are shown in Table S2) and FW-RILs were planted in a randomized complete block design with three replications. A plot containing three rows (3 m long × 0.65 m wide) was planted for each line. The seeds of ten mature plants from the middle row of each plot were harvested, and the PC and OC of the dry matter of seed determined using an Infratec 1241 NIR Grain Analyser (FOSS, Sweden).

Simple-sequence repeat analysis and construction of a genetic linkage map

The third tender trifoliate leaf was collected from each FW-RILs line and stored in liquid nitrogen: DNA was extracted from the samples using the cetyl trimethylammonium bromide method. A total of 638 simple sequence repeat (SSR) markers evenly distributed across the soybean genome (Cregan et al., 1999) were selected to screen for polymorphisms among the four parents. Of these, 275 primer pairs showed polymorphism in the FW-RILs and were used

for SSR genotyping. A genetic map of soybean containing 275 SSR primers on 20 linkage groups was constructed using GAPL 1.0 software (<https://www.gapl-system.org/Releases/4.10.1.html>). Two adjacent markers in the genetic map with a genetic distance ≥ 40 cM were uncoupled, reducing the length of the genetic map. The map covers a genome length of 3636.26 cM with an average length between markers of 15.47 cM. The length of each linkage group ranges from 49.36 to 319.02 cM and the number of markers varies from 6 to 20 (Ning et al., 2015).

Conditional variable

The conditional effect value was calculated through the method described by Zhu (1995). Specifically, conditional variables were estimated using the following formula:

$$y_{pro|oil} = y_{pro} - C_{pro,oil}(y_{oil} - \bar{y}_{oil}) / V_{oil} \quad (1)$$

$$y_{oil|pro} = y_{oil} - C_{oil,pro}(y_{pro} - \bar{y}_{pro}) / V_{pro} \quad (2)$$

where $y_{pro|oil}$ is the conditional variable PC (dependent on OC), calculated by subtracting the influence of OC from PC. Similarly, $y_{oil|pro}$ is the conditional variable OC (dependent on PC). y_{pro} and y_{oil} are the PC and OC of each individual, respectively, while \bar{y}_{pro} and \bar{y}_{oil} are the mean values of these contents. $C_{pro,oil}$ and $C_{oil,pro}$ are the covariance between PC and OC, and V_{pro} and V_{oil} are the variance of PC and OC, respectively. The conditional variables were calculated using Microsoft Excel 2010.

Phenotypic data analysis

Based on the mean of unconditional and conditional variables of the three replicates per line in each environment, descriptive and correlation analyses were conducted, respectively. An analysis of variance (ANOVA) was carried out depending on the unconditional and conditional variables of the three replicates per line for each environment. All analyses were conducted using SAS 9.2 software (SAS Institute Inc., USA).

Heritability was estimated using the following equations:

For a single environment

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_e^2} \quad (3)$$

For multiple environments

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_{GE}^2 / r) + (\sigma_e^2 / re)} \quad (4)$$

where h^2 indicates the broad sense heritability, σ_G^2 is the genetic variance, σ_{GE}^2 is the variance due to the genotype × environment interaction, σ_e^2 is the error variance, r is the number of blocks and e is the number of environments in the study. σ_G^2 , σ_e^2 and σ_{GE}^2 were estimated using a mixed method implemented using Proc Mixed in SAS 9.2.

Quantitative trait loci analysis

The phenotypes of unconditional and conditional means of PC and OC for each line in every environment and the linkage

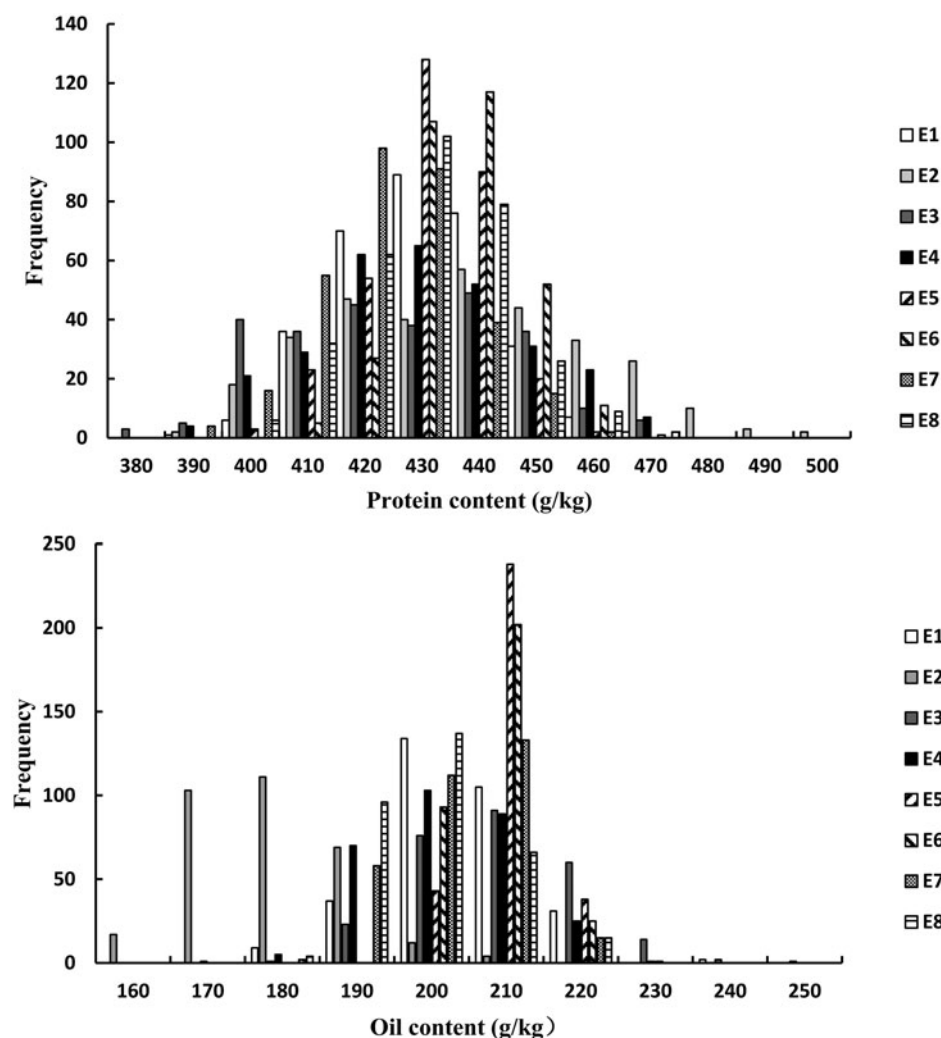


Fig. 1. Frequency distribution of PC and OC QTLs in FW-RILs. E, environment; E1, Harbin in 2013; E2, Keshan in 2013; E3, the first sowing date in Harbin in 2014; E4, the second sowing date in Harbin in 2014; E5, 2.22×10^5 plants/ha in Harbin; E6, 3.08×10^5 plants/ha in Harbin; E7, 2.58×10^5 plants/ha in Keshan; E8, 3.51×10^5 plants/ha in Keshan.

map were used for QTL analysis via interval mapping (IM) (Lander and Botstein, 1989) and inclusive complete interval mapping (ICIM) (Li *et al.*, 2007). The QTLs were identified using logarithm of the odds (LOD) thresholds of the empirical value generated based on 1000 permutations at 0.05 probability of type I error and the least LOD was 2.5. A QTL that was detected by two methods simultaneously was considered to be real.

Naming conventions for quantitative trait loci

QTLs were named as follows: q + trait name – chromosome name – sequence number, where q represents QTL, Pro represents PC and oil represents OC. QTLs that were mapped to the same marker region were given the same sequence number. Unified numbering was applied for both methods (IM and ICIM).

The linkage maps were exhibited using MapChart 2.1 (<https://www.wur.nl/en>).

Results

Phenotypic variation

For the FW-RILs, the frequency of individuals for PC and OC followed a normal distribution (Fig. 1, Table 1). For a single environment, the PC and OC for the FW-RILs (Table 2) exhibited a

significant variation in all eight environments. For multiple environments, the variation among genotypes, environments and genotype \times environments was significant at $P < 0.01$, indicating that the population was suitable for QTL mapping studies and could potentially reveal a variety of QTLs in different environments.

Correlation and heritability analysis

The correlation between PC and OC based on unconditional and conditional variables is shown in Table 1. There was a significant negative correlation between the unconditional variables of PC and OC. The heritability of the two traits, which was estimated based on the conditional variables, was different from that of the unconditional variable, indicating that the negative correlation between PC and OC has a greater impact on the variation of each trait. The coefficient of variation, skewness and kurtosis of unconditional variables were higher than those of the conditional variables, indicating that this negative correlation influences the degree of variation of the phenotypic values of these two traits.

Quantitative trait loci associated with protein content

Among the eight environments, 34 QTLs associated with PC were identified on 16 chromosomes (A1, A2, B1, B2, C1, C2, D1b, D2, F,

Table 1. Descriptive analysis of PC and OC under different environments

Env.	Method	PC (g/kg)								OC (g/kg)								Correlation
		Min.	Max.	Mean	CV	Skew.	Kurt.	<i>F</i>	BSH	Min.	Max.	Mean	CV	Skew.	Kurt.	<i>F</i>	BSH	
E1	Uncon	381.4	460.3	425.2	3.29	-0.05	-0.18	124.27 (<i>P</i> < 0.01)	0.98	173.5	232.9	198.6	4.8	0.01	0.76	49.15 (<i>P</i> < 0.01)	0.94	-0.47 (<i>P</i> < 0.01)
	Con	392.4	462	425	2.91	0.1	0.18	79.46 (<i>P</i> < 0.01)	0.96	175.7	236.7	198.8	4.25	0.33	2.03	39.27 (<i>P</i> < 0.01)	0.93	
E2	Uncon	384.8	490.8	432.9	5.29	0.22	-0.58	279.18 (<i>P</i> < 0.01)	0.99	152.1	206	174.2	5.49	0.36	0.09	55.15 (<i>P</i> < 0.01)	0.95	-0.30 (<i>P</i> < 0.01)
	Con	386.1	495.4	433.2	5.05	0.16	-0.57	269.15 (<i>P</i> < 0.01)	0.99	153.5	201.9	174.1	5.26	0.42	-0.1	49.17 (<i>P</i> < 0.01)	0.94	
E3	Uncon	377.4	466.5	421.5	4.91	0.01	-0.67	246.75 (<i>P</i> < 0.01)	0.99	179.3	241.1	204	5.49	0.34	0.43	66.84 (<i>P</i> < 0.01)	0.96	-0.46 (<i>P</i> < 0.01)
	Con	388.9	458.3	421.7	4.36	0	-1.14	177.97 (<i>P</i> < 0.01)	0.98	183.1	233.1	203.8	4.87	0.16	-0.34	61.18 (<i>P</i> < 0.01)	0.95	
E4	Uncon	388.3	467.8	425.7	4.34	0.04	-0.52	165.16 (<i>P</i> < 0.01)	0.98	167.1	224	197.1	4.96	-0.09	-0.28	45.72 (<i>P</i> < 0.01)	0.94	-0.42 (<i>P</i> < 0.01)
	Con	389.1	469.3	425.7	3.95	0.18	-0.43	153.17 (<i>P</i> < 0.01)	0.98	170.1	217.9	197.1	4.52	0.12	-0.13	43.54 (<i>P</i> < 0.01)	0.93	
E5	Uncon	398	457	426.2	2.72	-0.01	-0.12	71.72 (<i>P</i> < 0.01)	0.96	190.3	223	205	2.52	0.25	0.58	13.21 (<i>P</i> < 0.01)	0.80	-0.70 (<i>P</i> < 0.01)
	Con	401.9	447.1	426.2	1.95	-0.53	0.48	41.36 (<i>P</i> < 0.01)	0.93	195.7	218	205	1.8	0.26	0.38	8.49 (<i>P</i> < 0.01)	0.71	
E6	Uncon	409	466	432.3	2.47	0.16	0.17	60.28 (<i>P</i> < 0.01)	0.95	190.4	218.4	202.8	2.46	0.46	0.51	15.00 (<i>P</i> < 0.01)	0.82	-0.59 (<i>P</i> < 0.01)
	Con	405.5	456.3	432.3	2	-0.17	0.35	40.05 (<i>P</i> < 0.01)	0.93	191.2	215	202.8	2	0.4	0.37	10.88 (<i>P</i> < 0.01)	0.77	
E7	Uncon	381.5	452.2	418.9	3.06	-0.09	0.12	97.57 (<i>P</i> < 0.01)	0.97	179.3	216.5	198	4.2	-0.16	-0.68	36.97 (<i>P</i> < 0.01)	0.92	-0.42 (<i>P</i> < 0.01)
	Con	382.6	450.8	418.9	2.77	-0.01	0.32	75.69 (<i>P</i> < 0.01)	0.96	183.2	215.4	198	3.8	-0.12	-0.83	36.68 (<i>P</i> < 0.01)	0.92	
E8	Uncon	394	468.4	425.6	3	0.35	0.3	87.58 (<i>P</i> < 0.01)	0.97	176.9	216.3	194.9	4.2	0.26	-0.38	34.54 (<i>P</i> < 0.01)	0.94	-0.26 (<i>P</i> < 0.01)
	Con	394.9	467	425.5	2.91	0.24	0.24	82.27 (<i>P</i> < 0.01)	0.96	179.2	215.6	194.9	4.05	0.28	-0.54	3.59 (<i>P</i> < 0.01)	0.46	

Env, environment; Uncon, unconditional variables; Con, conditional variables; Min., minimum; Max., maximum; Skew., skewness; Kurt., kurtosis; CV, coefficient of variation and the unit is %; BSH, broad-sense heritability.

Table 2. Analysis of variance and heritability of PC and OC across multiple environments in FW-RILs

Trait	Min	Max	Mean	CV (%)	F_E	F_G	$F_{G \times E}$	BSH
PC (g/kg)	377.4	490.8	426	3.96	1914.38 ($P < 0.01$)	304.86 ($P < 0.01$)	114.01 ($P < 0.01$)	0.39
PC OC (g/kg)	382.6	495.4	426	3.56	1922.02 ($P < 0.01$)	209.59 ($P < 0.01$)	97.87 ($P < 0.01$)	0.31
OC (g/kg)	152.1	241.1	196.8	6.55	8056.56 ($P < 0.01$)	82.29 ($P < 0.01$)	30.99 ($P < 0.01$)	0.40
OC PC (g/kg)	153.5	236.7	196.8	6.24	5917.45 ($P < 0.01$)	42.03 ($P < 0.01$)	19.06 ($P < 0.01$)	0.33

PC|OC, protein content condition on oil content in eight environments; OC|PC, oil content condition on protein content in eight environments; Min, minimum; Max, maximum; CV, coefficient of variation; F_E , F value for environment effects; F_G , F value for genetic effects; $F_{G \times E}$, F value for genotype \times environment interaction effects; BSH, broad-sense heritability.

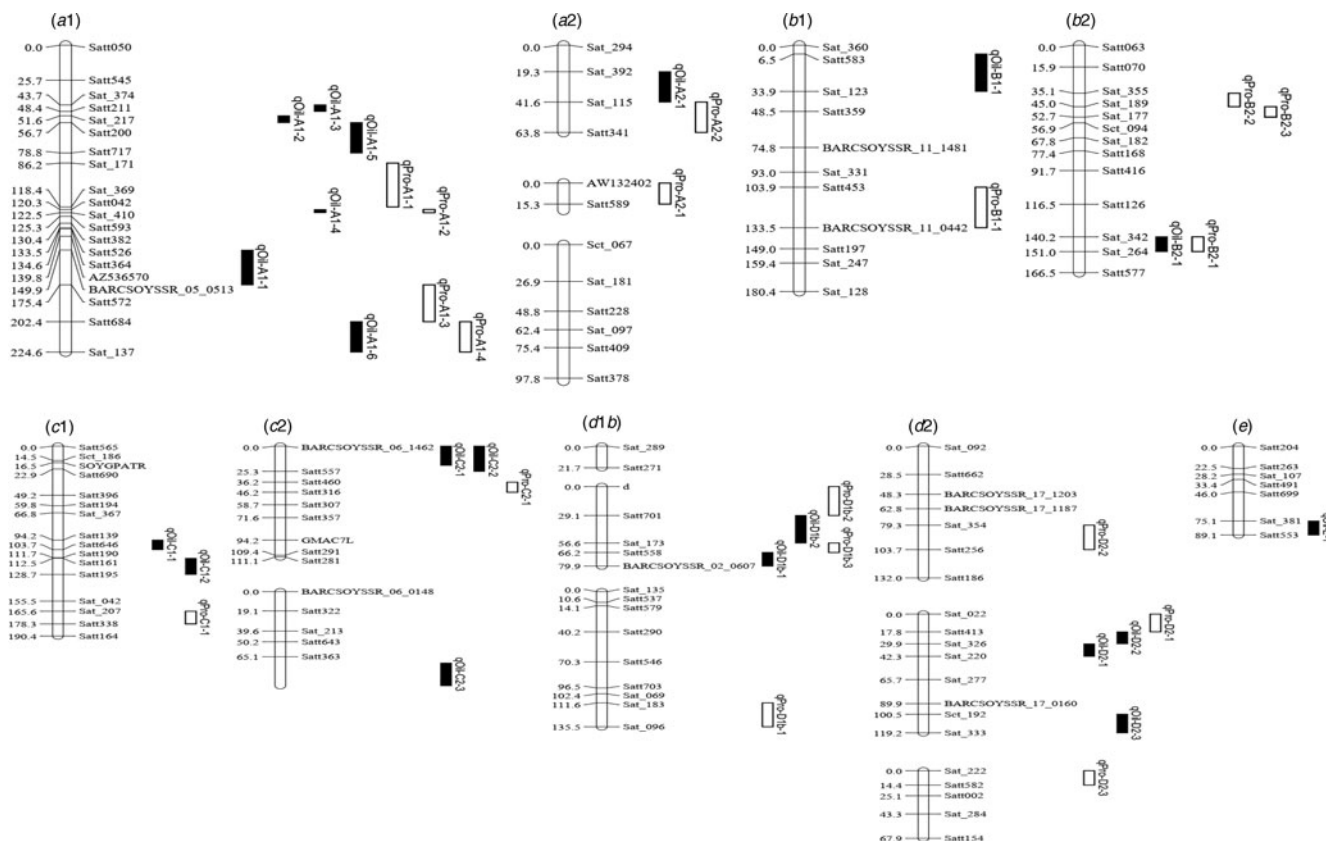


Fig. 2. Integrated linkage map of QTLs for PC and OC detected in the present study based on the positions of markers in the FW-RILs map. These QTL are distributed on left chromosome, and the size of the QTL interval is represented by the length of the QTL. Bars filled with white and black colour represent PC and OC QTL detected in FW-RILs, respectively. The chromosome unit is cM.

G, H, J, K, L, M and N) (Fig. 2, Fig. S1) in the FW-RILs, which explained 2.85–15.40% of the total variation (Table 3). Of these QTLs, 13 (qPro-A1-1, qPro-A1-2, qPro-A1-3, qPro-B2-1, qPro-C2-1, qPro-D2-2, qPro-D2-3, qPro-F-1, qPro-F-2, qPro-H-1, qPro-H-2, qPro-N-2 and qPro-N-4) were only identified by unconditional variable analysis, indicating that the expression of these QTLs was not affected by OC. Eight QTLs (qPro-A1-4, qPro-B1-1, qPro-B2-3, qPro-D1b-1, qPro-G-1, qPro-G-2, qPro-M-1 and qPro-N-1) were only identified by conditional variable analysis, indicating that the expressions of these QTLs were completely affected by OC. The 13 remaining QTLs (qPro-K-2, qPro-N-3, qPro-A2-1, qPro-A2-2, qPro-B2-2, qPro-C1-1, qPro-D1b-2, qPro-D1b-3, qPro-D2-1, qPro-J-1, qPro-K-1, qPro-K-3 and qPro-L-1) were detected by both conditional and unconditional variable analysis, indicating that the expression of these QTLs

was partially affected by OC. Comparing phenotypic variation explanation (PVE) ratio and additive effects, two QTLs (qPro-D1b-2 and qPro-D1b-3) were slightly affected by OC, and 11 of the 13 QTLs (qPro-A2-1, qPro-A2-2, qPro-B2-2, qPro-C1-1, qPro-D2-1, qPro-J-1, qPro-K-1, qPro-K-2, qPro-K-3, qPro-L-1 and qPro-N-3) were partially affected by OC.

Among all QTLs, the parent Kenfeng14 carried positive additive effect alleles for 11 QTLs, Kenfeng15 carried positive additive effect alleles for 12 QTLs, Heinong48 carried positive additive effect alleles for 16 QTLs and Kenfeng19 carried positive additive effect alleles for 22 QTLs (Table S3). There were 28, 27, 34 and 31 synergistic allelic genotypes with additive effects ranging from 0.02–1.20, 0.01–1.50, 0.01–1.49 and 0.01–1.63 in Kenfeng14, Kenfeng15, Heinong48 and Kenfeng19, respectively, which could increase PC.

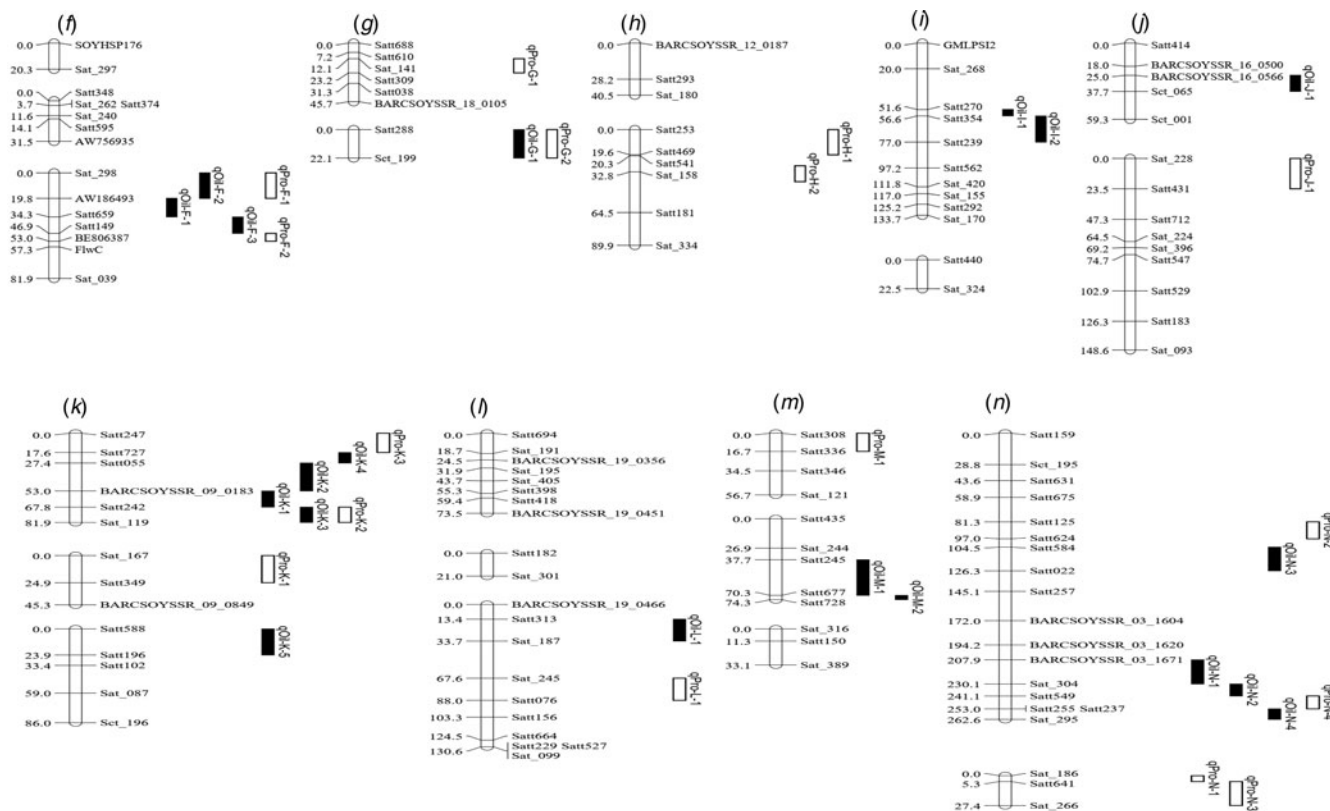


Fig. 2. Continued.

Quantitative trait loci associated with oil content

Among the eight environments, 39 QTLs associated with OC were identified on 17 chromosomes (A1, A2, B1, B2, C1, C2, D1, D2, E, F, G, I, J, K, L, M and N) (Fig. 1, Fig. S1) with a PVE of 1.98–13.04% (Table 4). Among these QTLs, seven (qOil-A1-4, qOil-C2-2, qOil-E-1, qOil-F-2, qOil-I-2, qOil-M-2 and qOil-N-2) were only identified by unconditional variable analysis, indicating that the expression of these QTLs was not affected by OC. Six of the 39 OC QTLs (qOil-A1-2, qOil-C2-3, qOil-K-3, qOil-K-4, qOil-K-5 and qOil-L-1) were only identified by conditional variable analysis, indicating that the expression of these QTLs was completely affected by PC. The 26 remaining QTLs were detected simultaneously by both conditional and unconditional variable analysis, indicating that the expression of these QTLs was partially affected by PC. Comparing PVE and additive effects, ten QTLs (qOil-B2-1, qOil-C1-1, qOil-C1-2, qOil-D2-2, qOil-D2-3, qOil-F-3, qOil-I-1, qOil-J-1, qOil-N-3 and qOil-N-4) were slightly affected by PC, while the 16 remaining QTLs (qOil-A1-1, qOil-A1-3, qOil-A1-5, qOil-A1-6, qOil-A2-1, qOil-B1-1, qOil-C2-1, qOil-D1b-1, qOil-D1b-2, qOil-D2-1, qOil-F-1, qOil-G-1, qOil-K-1, qOil-K-2, qOil-M-1 and qOil-N-1) were partially affected by PC.

Among these QTLs, the parent Kenfeng14 carried positive additive effect alleles for 24 QTLs, Kenfeng15 carried positive additive effect alleles for 15 QTLs, Heinong48 carried positive additive effect alleles for nine QTLs and Kenfeng19 carried positive additive effect alleles for seven QTLs (Table S3). There were 41, 39, 30 and 35 synergistic allelic genotypes with additive effects ranging from 0.02–1.20, 0.01–1.50, 0.01–1.49 and 0.01–1.63 in

Kenfeng14, Kenfeng15, Heinong48 and Kenfeng19, respectively, which could increase OC.

Quantitative trait loci with multiple effects

Six intervals controlled both PC and OC (Table 5). Among the QTLs, three regions (Sat_342–Sat_264, Satt288–Sct_199 and Satt242–Sat_119) affected only one trait in one environment, while the three remaining regions (Satt042–Sat_410, Satt684–Sat_137 and Sat_298–AW186493) controlled two traits in one environment.

Perhaps QTLs whose allelic effects were detrimental to both PC and OC could be used to improve a single trait.

Discussion

The use of four-way recombinant inbred lines populations enhances quantitative trait loci detection

QTLs for PC and OC were previously identified using separate populations derived from biparental cross populations (e.g. F₂, BC and RIL). In these populations, a single QTL contained only two alleles, its polymorphic information content was low and the mapping results could not be extended to other combinations, resulting in a narrow statistical inference space. Compared to separate populations derived from biparental crosses, the use of FW-RILs populations could increase the statistical inference space and their polymorphic information content is high, thereby increasing the density and coverage of the resulting four-way

Table 3. QTLs underlying PC in soybean detected in eight environments

QTL	Marker interval	Method	LOD	PVE (%)	PC				PC OC				Env.	Reference (QTL located in previous studies)
					Add.1	Add.2	Add.3	Add.4	Add.1	Add.2	Add.3	Add.4		
qPro-A1-1	Sat_171-Sat_369	ICIM/IM	3.94/3.94	6.37/6.37	-0.35/-0.35	-0.09/-0.09	-0.15/-0.15	0.60/0.60					E5	Mansur <i>et al.</i> (1996), Mao <i>et al.</i> (2013)
		ICIM/IM	3.68/3.68	4.61/6.82	-0.38/-0.38	0.01/0.01	-0.26/-0.26	0.62/0.62					E7	
qPro-A1-2	Satt042-Sat_410	ICIM/IM	4.02/4.02	7.44/7.44	-0.08/-0.08	-0.04/-0.04	-0.53/-0.53	0.65/0.65					E5	Mansur <i>et al.</i> (1996), Mao <i>et al.</i> (2013)
qPro-A1-3	Satt572-Satt684	ICIM/IM	2.53/2.53	6.43/6.43	-0.61/-0.61	-0.60/-0.60	1.49/1.49	-0.28/-0.28					E3	
qPro-A1-4	Satt684-Sat_137	ICIM/IM	3.26/3.42	8.85/8.00					-0.13/-0.15	-1.05/-0.97	-0.19/-0.51	1.36/1.63	E4	
qPro-A2-1	AW132402-Satt589	ICIM/IM	4.05/4.00	8.12/6.93					-0.31/-0.27	-0.30/-0.35	-0.03/-0.07	0.64/0.69	E1	Lu <i>et al.</i> (2013), Pathan <i>et al.</i> (2013), Reinprecht <i>et al.</i> (2006), Tajuddin <i>et al.</i> (2003)
		ICIM/IM	3.51/3.51	7.55/7.55	-0.23/-0.23	-0.46/-0.46	-0.12/-0.12	0.81/0.81					E1	
qPro-A2-2	Sat_115-Satt341	IM	2.67	7.26					1.00	-0.71	0.72	-1.00	E2	Lu <i>et al.</i> (2013)
		ICIM/IM	3.24/3.15	4.66/4.56					0.64/0.69	-0.62/-0.67	0.40/0.52	-0.42/-0.54	E4	
		ICIM/IM	2.77/3.48	8.24/9.37	1.09/1.20	-0.95/-1.07	0.72/0.89	-0.86/-1.03					E2	
		ICIM/IM	3.84/3.98	10.31/10.13	0.84/0.83	-0.85/-0.85	0.69/0.85	-0.69/-0.83					E4	
qPro-B1-1	Satt453-BARCSOYSSR_11_0442	ICIM/IM	2.91/2.91	11.58/11.58					0.60/0.60	0.06/0.06	-0.53/-0.53	-0.13/-0.13	E6	Gai <i>et al.</i> (2007), Reinprecht <i>et al.</i> (2006)
qPro-B2-1	Sat_342-Sat_264	ICIM/IM	3.06/3.06	8.39/8.39	-0.83/-0.83	0.17/0.17	0.45/0.45	0.21/0.21					E1	Akond <i>et al.</i> (2014)
qPro-B2-2	Sat_355-Sat_189	ICIM/IM	4.63/4.63	8.78/7.77					-0.38/-0.38	0.51/0.51	-1.29/-1.29	1.16/1.16	E2	Mao <i>et al.</i> (2013), Warrington <i>et al.</i> (2015)
		ICIM/IM	4.07/4.07	7.82/6.69	-0.36/-0.36	0.35/0.35	-1.22/-1.22	1.23/1.23					E2	
qPro-B2-3	Sat_189-Sat_177	ICIM/IM	2.56/2.80	5.65/5.69					-0.15/-0.16	-0.21/-0.23	0.19/0.22	0.17/0.17	AVE	Akond <i>et al.</i> (2014), Diers <i>et al.</i> (1992), Kabelka <i>et al.</i> (2004), Lee <i>et al.</i> (1996), Mao <i>et al.</i> (2013), Warrington <i>et al.</i> (2015)
qPro-C1-1	Sat_207-Satt338	ICIM/IM	2.51/2.51	4.27/4.27					0.19/0.19	-0.04/-0.04	0.20/0.20	-0.35/-0.35	E6	Brummer <i>et al.</i> (1997); Kabelka <i>et al.</i> (2004), Lee <i>et al.</i> (1996), Mao <i>et al.</i> (2013), Wang <i>et al.</i> (2014b)
		ICIM/IM	2.89/2.89	7.08/7.08	-0.70/-0.70	0.55/0.55	-0.07/-0.07	0.22/0.22					E1	
		ICIM/IM	2.90/2.90	6.41/5.13	-0.35/-0.35	-0.11/-0.11	0.10/0.10	0.35/0.35					AVE	

(Continued)

Table 3. (Continued.)

QTL	Marker interval	Method	LOD	PVE (%)	PC				PC OC				Env.	Reference (QTL located in previous studies)
					Add.1	Add.2	Add.3	Add.4	Add.1	Add.2	Add.3	Add.4		
qPro-C2-1	Satt460-Satt316	ICIM/IM	2.57/2.57	3.62/3.62	-0.42/-0.42	-0.29/-0.29	-0.56/-0.56	1.27/1.27					E3	Csanádi <i>et al.</i> (2001), Hyten <i>et al.</i> (2004), Mao <i>et al.</i> (2013), Pathan <i>et al.</i> (2013)
qPro-D1b-1	Sat_183-Sat_096	ICIM/IM	3.17/2.67	7.35/4.93					-0.40/-0.44	0.43/0.38	0.25/0.27	-0.28/-0.21	E1	Chen <i>et al.</i> (2007), Gai <i>et al.</i> (2007), Kabelka <i>et al.</i> (2004), Mao <i>et al.</i> (2013), Qi <i>et al.</i> (2014b)
qPro-D1b-2	Sat_373-Satt701	ICIM/IM	3.01/3.01	5.05/5.05					-0.64/-0.64	0.37/0.37	0.16/0.16	0.11/0.11	E8	Kabelka <i>et al.</i> (2004), Mao <i>et al.</i> (2013)
		ICIM/IM	2.97/2.97	7.08/7.08	-0.65/-0.65	0.38/0.38	0.18/0.18	0.10/0.10					E8	
qPro-D1b-3	Sat_173-Satt558	ICIM/IM	2.52/2.58	5.39/5.41					0.03/0.05	0.00/-0.02	0.21/0.23	-0.25/-0.26	AVE	Mao <i>et al.</i> (2013)
		ICIM/IM	2.76/2.65	5.90/5.36	0.08/-0.01	-0.07/-0.08	0.31/0.37	-0.32/-0.28					AVE	
qPro-D2-1	Sat_022-Satt413	ICIM/IM	3.29/3.16	4.27/3.35					0.53/0.51	-0.26/-0.27	-0.74/-0.75	0.47/0.51	E4	Tajuddin <i>et al.</i> (2003), Warrington <i>et al.</i> (2015)
		ICIM/IM	3.44/3.35	6.80/6.19	0.54/0.53	-0.17/-0.17	-1.00/-1.05	0.63/0.70					E4	
qPro-D2-2	Sat_354-Satt256	ICIM/IM	3.00/3.00	9.09/9.09	0.78/0.78	-0.86/-0.86	0.07/0.07	0.01/0.01					E1	Mao <i>et al.</i> (2013), Tajuddin <i>et al.</i> (2003), Warrington <i>et al.</i> (2015)
qPro-D2-3	Sat_222-Satt582	ICIM/IM	2.58/2.84	7.89/7.09	0.17/0.17	-0.5/-0.52	0.15/0.10	0.18/0.25					AVE	Mao <i>et al.</i> (2013), Wang <i>et al.</i> (2014b)
qPro-F-1	Sat_298-AW186493	ICIM/IM	2.66/2.66	5.92/5.92	0.64/0.64	-0.09/-0.09	-0.16/-0.16	-0.39/-0.39					E5	Mao <i>et al.</i> (2013)
		ICIM/IM	3.65/2.84	6.36/7.39	0.78/0.69	-0.26/-0.16	0.04/0.01	-0.56/-0.53					E7	
qPro-F-2	Satt149-BE806387	ICIM/IM	2.80/2.80	4.49/4.49	0.32/0.32	-0.54/-0.54	0.07/0.07	0.16/0.16					E5	Mao <i>et al.</i> (2013)
		IM	2.67	4.91	0.35	-0.58	0.07	0.16					E7	
qPro-G-1	Sat_141-Satt309	ICIM/IM	3.58/3.11	8.51/6.49					-0.52/-0.49	-0.13/-0.15	0.16/0.08	0.49/0.56	E1	Liang <i>et al.</i> (2010), Mao <i>et al.</i> (2013), Reinprecht <i>et al.</i> (2006)
qPro-G-2	Satt288-Sct_199	ICIM/IM	2.60/2.85	5.82/5.83					-0.19/-0.19	-0.20/-0.23	0.18/0.19	0.20/0.23	AVE	Diers <i>et al.</i> (1992), Mao <i>et al.</i> (2013)
qPro-H-1	Satt253-Satt469	ICIM/IM	2.92/2.92	7.20/7.20	0.13/0.13	0.26/0.26	0.42/0.42	-0.80/-0.80					E1	Lu <i>et al.</i> (2013)
qPro-H-2	Satt293-Sat_180	ICIM/IM	2.71/2.71	10.14/10.14	-0.58/-0.58	-0.10/-0.10	0.47/0.47	0.22/0.22					E6	Eskandari <i>et al.</i> (2013b), Qiu <i>et al.</i> (1999)
qPro-J-1	Sat_228-Satt431	ICIM/IM	2.51/2.51	4.05/4.05					-0.15/-0.15	0.08/0.08	0.35/0.35	-0.29/-0.29	E6	Jun <i>et al.</i> (2008), Lee <i>et al.</i> (1996)
		ICIM/IM	2.60/2.60	4.16/4.16	-0.21/-0.21	-0.42/-0.42	0.52/0.52	0.10/0.10					E5	
		ICIM/IM	3.10/3.10	6.37/6.37	-0.20/-0.20	0.03/0.03	0.52/0.52	-0.35/-0.35					E6	
		IM	2.63	4.82	-0.12	-0.53	0.63	0.02					E7	

qPro-K-1	Sat_167-Satt349	ICIM/IM	2.64/3.52	5.40/7.71					-0.13/-0.32	0.82/1.25	0.08/-0.06	-0.77/-0.86	E4	Eskandari <i>et al.</i> (2013b), Lee <i>et al.</i> (1996), Lu <i>et al.</i> (2013), Mao <i>et al.</i> (2013), Rossi <i>et al.</i> (2013), Tajuddin <i>et al.</i> (2003), Wang <i>et al.</i> (2014b)
		ICIM/IM	2.65/2.83	4.68/7.12	0.02/-0.17	0.61/1.03	0.21/0.09	-0.85/-0.95						
qPro-K-2	Satt242-Sat_119	IM	2.61	2.85					-0.41	0.75	-0.04	-0.29	E4	Hyten <i>et al.</i> (2004), Jun <i>et al.</i> (2008), Mao <i>et al.</i> (2013), Qi <i>et al.</i> (2014b)
		ICIM/IM	3.19/4.21	4.85/6.16	-0.3/-0.39	0.84/1.05	-0.03/-0.03	-0.52/-0.63						
qPro-K-3	Satt247-Satt727	ICIM/IM	5.25/4.78	10.09/6.42					0.14/0.28	1.04/0.66	-0.01/0.21	-1.16/-1.14	E4	Mao <i>et al.</i> (2013)
		ICIM/IM	4.7/4.38	10.43/7.25	0.20/0.35	0.86/0.52	0.16/0.26	-1.21/-1.13					E4	
		per(IM)	4.78	15.40					0.28	0.66	0.21	-1.14	E4	
		per(ICIM)	5.25	15.40					0.14	1.04	-0.01	-1.16	E4	
qPro-L-1	Sat_245-Satt076	ICIM/IM	4.36/4.36	13.83/13.83					-1.64/-1.64	0.82/0.82	-0.03/-0.03	0.85/0.85	E3	Chapman <i>et al.</i> (2003), Mansur <i>et al.</i> (1996), Mao <i>et al.</i> (2013), Orf <i>et al.</i> (1999)
		ICIM/IM	3.54/3.54	11.28/11.28	-1.84/-1.84	0.57/0.57	0.25/0.25	1.03/1.03					E3	
		IM	2.63	5.37	0.30	-1.07	0.08	0.69					E4	
qPro-M-1	Satt308-Satt336	ICIM/IM	2.57/2.57	7.73/7.73				0.67/0.67	-0.13/-0.13	-0.54/-0.54	0.01/0.01	E8	Kabelka <i>et al.</i> (2004), Lu <i>et al.</i> (2013)	
qPro-N-1	Sat_186-Satt641	ICIM/IM	3.11/3.11	5.62/5.62				0.05/0.05	0.10/0.10	-0.70/-0.70	0.55/0.55	E8	Lee <i>et al.</i> (1996)	
qPro-N-2	Satt125-Satt624	ICIM/IM	2.87/2.87	6.54/6.54	-0.25/-0.25	-0.34/-0.34	-0.03/-0.03	0.63/0.63					E5	Lee <i>et al.</i> (1996), Wang <i>et al.</i> (2014b)
qPro-N-3	Satt641-Sat_266	IM	3.16	12.61					-0.74	1.50	-1.06	0.30	E4	Lee <i>et al.</i> (1996), Mao <i>et al.</i> (2013), Pathan <i>et al.</i> (2013), Wang <i>et al.</i> (2014b)
		IM	3.18	10.36	-0.59	1.20	-0.95	0.34					E4	
		ICIM/IM	3.43/3.43	7.70/7.70	-0.02/-0.02	0.21/0.21	-0.63/-0.63	0.44/0.44					E6	
		ICIM/IM	3.25/3.25	8.52/8.52	0.03/0.03	0.13/0.13	-0.73/-0.73	0.57/0.57					E8	
qPro-N-4	Satt549-Satt255	ICIM/IM	2.87/2.98	6.12/4.98	-0.44/-0.45	0.10/0.06	0.12/0.17	0.21/0.22					AVE	Kabelka <i>et al.</i> (2004), Mao <i>et al.</i> (2013)

LOD, logarithm of the odds; OC, oil content; PC|OC, protein content effect on oil content; PVE, phenotypic variation explanation ratio; ICIM, inclusive complete interval mapping; IM, interval mapping; per(ICIM), permutations of ICIM; per(IM), permutations of IM; Env., environment, i.e. which environment identified QTL; AVE, mean; E1, Harbin in 2013; E2, Keshan in 2013; E3, the first sowing date in Harbin in 2014; E4, the second sowing date in Harbin in 2014; E5, 2.22×10^5 plants/ha in Harbin; E6, 3.08×10^5 plants/ha in Harbin; E7, 2.58×10^5 plants/ha in Keshan; E8, 3.51×10^5 plants/ha in Keshan; Add.1, the additive effect of Kenfeng14; Add.2, the additive effect of Kenfeng15; Add.3, the additive effect of Heinnong48; Add.4, the additive effect of Kenfeng19.

Table 4. QTLs underlying OC in soybean detected in eight environments

QTL	Marker interval	Method	LOD	PVE (%)	OC				OC PC				Env.	Reference (QTL located in previous studies)
					Add.1	Add.2	Add.3	Add.4	Add.1	Add.2	Add.3	Add.4		
qOil-A1-1	BARCSOYSSR_05_0513-Satt572	ICIM/IM	2.66/2.66	5.65/5.65					0.23/0.23	0.02/0.02	-0.18/-0.18	-0.06/-0.06	AVE	Han <i>et al.</i> (2015)
		IM	2.61	3.79	0.25	0.08	-0.66	0.33					E3	
		ICIM/IM	3.53/3.14	4.89/3.88	0.25/0.25	-0.15/-0.18	0.20/0.22	-0.30/-0.29					E7	
		IM	3.18	6.69	0.28	0.05	-0.23	-0.10					AVE	
qOil-A1-2	Sat_217-Satt200	ICIM/IM	3.72/3.14	6.81/4.38					0.30/0.34	0.32/0.29	-0.38/-0.43	-0.24/-0.21	E3	Akond <i>et al.</i> (2014), Brummer <i>et al.</i> (1997), Han <i>et al.</i> (2015), Liang <i>et al.</i> (2010), Mansur <i>et al.</i> (1996), Moongkanna <i>et al.</i> (2011), Orf <i>et al.</i> (1999), Pathan <i>et al.</i> (2013), Qi <i>et al.</i> (2011b), Qi <i>et al.</i> (2011a), Specht <i>et al.</i> (2001), Wang <i>et al.</i> (2014b)
qOil-A1-3	Sat_374-Satt211	ICIM/IM	3.09/3.09	4.93/4.58					0.32/0.32	-0.30/-0.30	-0.21/-0.21	0.19/0.19	E4	Liang <i>et al.</i> (2010), Moongkanna <i>et al.</i> (2011), Specht <i>et al.</i> (2001), Wang <i>et al.</i> (2014b)
		ICIM/IM	2.57/2.83	2.62/3.53	0.32/0.49	-0.15/-0.10	-0.38/-0.53	0.21/0.14					E3	
		ICIM/IM	3.15/2.52	4.87/3.22	0.34/0.34	-0.31/-0.25	-0.19/-0.28	0.17/0.19					E4	
qOil-A1-4	Satt042-Sat_410	ICIM/IM	4.43/4.43	9.11/10.87	0.09/0.09	-0.06/-0.06	0.26/0.26	-0.28/-0.28					E5	Han <i>et al.</i> (2015), Mansur <i>et al.</i> (1996), Qi <i>et al.</i> (2011b), Qi <i>et al.</i> (2011a), Rossi <i>et al.</i> (2013)
qOil-A1-5	Satt200-Satt717	ICIM/IM	2.74/2.74	2.07/2.07					0.36/0.36	0.11/0.11	-0.17/-0.17	-0.30/-0.30	E2	Brummer <i>et al.</i> (1997), Han <i>et al.</i> (2015), Liang <i>et al.</i> (2010), Mansur <i>et al.</i> (1996), Moongkanna <i>et al.</i> (2011), Orf <i>et al.</i> (1999), Qi <i>et al.</i> (2011b), Qi <i>et al.</i> (2011a)
		ICIM/IM	3.43/3.43	6.67/3.37	0.39/0.39	0.18/0.18	-0.18/-0.18	-0.39/-0.39					E2	
		IM	2.78	6.78	0.53	-0.19	-0.52	0.18					E4	
		ICIM/IM	5.58/3.47	9.62/6.00	0.24/0.23	0.10/0.09	-0.18/-0.23	-0.16/-0.09					AVE	
qOil-A1-6	Satt684-Sat_137	ICIM/IM	3.03/3.31	8.05/9.90					-0.01/-0.01	-0.3/-0.28	-0.40/-0.50	0.71/0.79	E4	Han <i>et al.</i> (2015), Wang <i>et al.</i> (2012)
		IM	2.64	5.62	0.08	0.54	0.19	-0.81					E1	
qOil-A2-1	Sat_392-Sat_115	ICIM/IM	3.44/3.30	8.05/9.19					-0.28/-0.30	0.59/0.66	-0.25/-0.25	-0.05/-0.11	E4	
		IM	2.53	3.16	-0.28	0.26	-0.38	0.39					E3	
		IM	4.01	6.46	-0.33	0.59	-0.35	0.09					E4	

Table 4. (Continued.)

QTL	Marker interval	Method	LOD	PVE (%)	OC				OC PC				Env.	Reference (QTL located in previous studies)	
					Add.1	Add.2	Add.3	Add.4	Add.1	Add.2	Add.3	Add.4			
qOil-D1b-2	Satt701-Sat_173	ICIM/IM	2.67/3.39	3.97/5.21	0.13/0.02	0.01/0.19	-0.18/-0.24	0.04/0.03						AVE	
		ICIM/IM	2.67/2.67	6.07/6.07						-0.41/-0.41	0.71/0.71	-0.30/-0.30	0.00/0.00	E2	Mao et al. (2013)
		IM	2.90	5.62						0.54	0.19	-0.50	-0.22	E3	
qOil-D2-1	Sat_326-Sat_220	IM	3.43	10.21	-0.48	0.86	-0.35	-0.03						E2	
		ICIM	2.65	2.65	0.14	0.26	0.09	-0.49						E3	Han et al. (2015), Liang et al. (2010)
		ICIM/IM	4.84/3.4	6.91/9.15						0.17/0.14	0.09/0.12	-0.12/-0.12	-0.14/-0.14	E6	
qOil-D2-2	Satt413-Sat_326	ICIM/IM	3.42/3.42	5.05/6.20	0.19/0.19	0.07/0.07	-0.20/-0.20	-0.06/-0.06						E6	
		per (ICIM)	4.84	9.15						0.17	0.09	-0.12	-0.14	E6	
		ICIM/IM	6.31/5.89	12.97/9.7						0.26/0.28	0.39/0.41	-0.24/-0.23	-0.41/-0.46	E8	Han et al. (2015), Liang et al. (2010)
qOil-D2-3	Sct_192-Sat_333	ICIM/IM	2.98/2.98	3.91/3.91					0.06/0.06	0.17/0.17	-0.02/-0.02	-0.21/-0.21	AVE		
		IM	5.02	9.33	0.28	0.40	-0.24	-0.43						E8	
		per (ICIM)	5.37	9.88	0.25	0.39	-0.25	-0.40						E8	
qOil-E-1	Sat_381-Satt553	per (ICIM)	6.31	11.15					0.26	0.39	-0.24	-0.41	E8		
		per(IM)	5.02	9.88	0.28	0.40	-0.24	-0.43					E8		
		per(IM)	5.89	11.15					0.28	0.41	-0.23	-0.46	E8		
qOil-F-1	AW186493-Satt659	ICIM/IM	2.59/2.59	1.98/1.98					0.14/0.14	0.34/0.34	-0.15/-0.15	-0.33/-0.33	E2		
		IM	2.85	2.83	0.24	0.31	-0.21	-0.34					E2		
qOil-F-2	Sat_298-AW186493	ICIM/IM	2.52/3.68	4.7/3.28	-0.11/-0.13	0.17/0.23	-0.34/-0.44	0.28/0.34					E1		
		ICIM/IM	4.14/3.31	6.91/4.15						-0.30/-0.29	-0.34/-0.36	0.27/0.23	0.37/0.41	E3	Wang et al. (2012)
		ICIM/IM	3.30/2.55	5.78/6.09	0.22/0.19	-0.23/-0.22	-0.03/-0.04	0.04/0.06					E6		
qOil-F-3	Satt659-Satt149	ICIM/IM	6.50/2.98	8.04/3.69	-0.39/-0.22	-0.55/-0.48	0.43/0.32	0.51/0.39					E3	Wang et al. (2012)	
		ICIM/IM	4.43/3.62	7.15/7.69	-0.3/-0.29	0.07/0.04	0.09/0.11	0.15/0.14					E5		
		per (ICIM)	6.50	9.48	-0.39	-0.55	0.43	0.51					E3		
qOil-F-3	Satt659-Satt149	ICIM/IM	2.76/2.75	6.08/5.48					0.25/0.28	-0.30/-0.25	-0.11/-0.26	0.15/0.22	E8	Qi et al. (2011a), Wang et al. (2012)	
		ICIM/IM	2.77/2.51	5.29/5.44	0.21/0.29	-0.32/-0.27	-0.07/-0.22	0.17/0.21					E8		

Table 4. (Continued.)

QTL	Marker interval	Method	LOD	PVE (%)	OC				OC PC				Reference (QTL located in previous studies)	
					Add.1	Add.2	Add.3	Add.4	Add.1	Add.2	Add.3	Add.4		Env.
qOil-N-2	Sat_304-Satt549	IM	2.76	4.32	0.58	-0.18	-0.11	-0.29						Han et al. (2015), Mao et al. (2013), Qi et al. (2011a)
		ICIM	3.39	5.24	0.37	-0.25	0.11	-0.24						
qOil-N-3	Satt584-Satt022	ICIM/IM	3.00/3.00	4.48/4.48				0.46/0.46	-0.35/-0.35	-0.40/-0.40	0.29/0.29			Akond et al. (2014), Chen et al. (2007), Han et al. (2015), Mao et al. (2013), Pathan et al. (2013), Qi et al. (2011b), Qi et al. (2011a), Wang et al. (2012) Wang et al. (2014b)
		IM	3.02	5.76	0.51	-0.35	-0.42	0.26						
qOil-N-4	Satt237-Sat_295	ICIM/IM	3.37/3.37	5.39/5.39				0.16/0.16	0.06/0.06	-0.05/-0.05	-0.18/-0.18			Chen et al. (2007), Han et al. (2015), Mao et al. (2013), Qi et al. (2011b), Qi et al. (2011a)
		ICIM/IM	5.12/4.52	8.10/7.09	0.22/0.25	0.07/0.05	-0.12/-0.10	-0.18/-0.21						

LOD, logarithm of the odds; OC, oil content; PVE, phenotypic variation explanation ratio and the unit is %; OC|PC, oil content effect on protein content. Env., environment; AVE, mean; E1, Harbin in 2013; E2, Keshan in 2013; E3, the first sowing date in Harbin in 2014; E4, the second sowing date in Harbin in 2014; E5, 2.22×10^5 plants/ha in Harbin; E6, 3.08×10^5 plants/ha in Harbin; E7, 2.58×10^5 plants/ha in Keshan. Add.1, the additive effect of Kenfeng14; Add.2, the additive effect of Kenfeng15; Add.3, the additive effect of Heinong68; Add.4, the additive effect of Kenfeng19. ICIM, inclusive complete interval mapping; IM, interval mapping; per(ICIM), permutations of ICIM; per(IM), permutations of IM.

population linkage map (Ao and Xu, 2006). Therefore, FW-RILs populations could be used to improve QTL detection.

The number of QTLs for PC and OC in soybean previously identified in biparental populations ranged from 2 to 23 (Brummer et al., 1997; Chapman et al., 2003; Akond et al., 2014), whereas many more QTLs can be detected using FW-RILs populations. In the current study, 73 QTLs for PC and OC in soybean were identified. The similar additive effect values between two parents also affected QTL positioning. By contrast, four-way populations contain four parents, allowing QTLs to be detected as long as the allele effects of two parents are different. For example, for qPro-A1-3 (Satt572-Satt684), the additive effects of alleles from the four parents were -0.61, -0.60, 1.49 and -0.28. Since there was little difference in additive effects between the first and second parents, this QTL would not be identified using a biparental population from the Kenfeng14 × Kenfeng15 cross; since the difference in additive effect values between other parents was large, the QTL was identified using four-way populations.

Distinctive and stable quantitative trait loci in various environments

PC and OC are quantitative traits controlled by genotype and the environment. Since these traits are affected by environments and genotypes, the expression of QTLs controlling these traits differs. Comparing to QTLs detected in the single environment, QTL positioning using data from multiple environments increases the detection ability and accuracy of estimating the location and the effect of a QTL. Wang et al. (2014a) identified two stable QTLs based on 3 years of data in three environments. Qi et al. (2014a) determined that among the 56 OC QTLs detected in 17 environments, eight were stable in multiple environments. Mao et al. (2013) identified 40 PC QTLs, with nine that exist stably in multiple environments, as well as 35 OC QTLs detected simultaneously in eight environments with four that exist stably in over two environments.

For the detection of QTLs expressed in multiple environments, in the current study QTL mapping was conducted in eight environments. Of the 73 QTLs detected, 23 were detected in two environments and four were detected in three environments. The PVE ranged from 2.65 to 13.83% in multiple environments, indicating that QTLs for PC and OC varied in different environments and were affected by non-genetic factors. QTLs located in a single environment have poor environmental stability and are susceptible to non-genetic factors, as a specific QTL might be influenced only by environmental factors.

Advantages of conditional variable analysis of quantitative trait loci

In previous studies, unconditional QTL mapping methods were used to locate QTLs for protein and oil in soybean (Mao et al., 2013; Eskandari et al., 2013a; Wang et al., 2014b). However, this method relies on final phenotypic values being affected by related traits, which would lead to a bias in estimating LOD scores and the additive effects of putative QTLs. The conditional variable method proposed by Zhu (1995), which is based on a net-effect analysis, makes up for the deficiencies in the unconditional variable method.

The combination of unconditional and conditional variable analysis for mapping QTLs for PC and OC has two advantages.

Table 5. QTLs with pleiotropic effects

QTL	Left marker	Right marker	Environment	
			Protein	Oil
qPro-A1-2	qOil-A1-4	Satt042	E5	E5
qPro-A1-4	qOil-A1-6	Satt684	E4	E4,E1
qPro-B2-1	qOil-B2-1	Sat_342	E1	E2
qPro-F-1	qOil-F-2	Sat_298	E5,E7	E3,E5
qPro-G-2	qOil-G-1	Satt288	AVG	E3
qPro-K-2	qOil-K-3	Satt242	E4	E5,E8

AVE, mean; E1, Harbin in 2013; E2, Keshan in 2013; E3, the first sowing date in Harbin in 2014; E4, the second sowing date in Harbin in 2014; E5, 2.22×10^5 plants/ha in Harbin; E7, 2.58×10^5 plants/ha in Keshan; E8, 3.51×10^5 plants/ha in Keshan.

Firstly, conditional variable analysis corrects the bias in estimating genetic effects. The PC of soybean is negatively correlated with OC (Zhang *et al.*, 2004). Therefore, when the variation of the target trait is evaluated, the influence of the related trait on the target traits would lead to a deviation in the calculation of heritability. The conditional variable analysis method analysed the heritability from a single-trait perspective and avoids the effects of interaction between PC and OC. In the current study, the heritability values of PC and OC were determined to be 0.39 and 0.40, respectively, using unconditional variables. After using the conditional variable analysis method, the heritability values of PC and OC were 0.31 and 0.33, respectively.

Secondly, conditional analysis could be used to explore the interaction mechanism of related traits in a FW-RILs population. That is to say, the conditional traits enhance or decrease the allelic additive effect values. In the current study, for qOil-M-1, the absolute additive effect values of the four-way population parental lines were determined to be 0.60, 1.17, 0.23 and 0.34 in the unconditional analysis and 0.12, 0.45, 0.30 and 0.03 in the conditional analysis. Comparing the absolute additive effect values obtained from the two analytical methods, PC decreased the first, second and fourth allelic additive effect values and enhanced the third value.

Conditional variable analysis and strategies for molecular breeding

Conditional variable analysis methods have been used to analyse the molecular genetic relationships of related traits, and the results have been applied to molecular marker-assisted breeding (Zhang *et al.*, 2016). Of the 73 QTLs detected in the current study, only 20 functioned independently, whereas the expression of 53 QTLs was fully or partially affected by related traits. Therefore, when designing molecular breeding strategies, the 20 QTLs that function independently could be used directly to affect the target traits, whereas when using the 53 remaining QTLs, the influence of related traits should be considered.

Comparing the quantitative trait loci identified in the current study with those reported previously

To compare QTLs detected in the present study with previously identified QTLs, the newly identified QTLs were integrated into an available publicly soybean genome map. Of the 73 QTLs examined, 66 partially or completely overlapped with previously reported

QTLs and might therefore represent the same QTLs, including 14 QTLs detected only by conditional analysis. Of the seven remaining QTLs, qPro-A1-3, qPro-A1-4 and qOil-A2-1 were only identified by conditional analysis. Among them, qPro-A1-3 and qPro-A1-4 appear to be present in the same locus (Satt684), and qOil-A2-1 is located in region 81.01 cM (Sat_115)–106.29 cM (Sat_392), which is adjacent to two gene regions underlying OC, i.e. 67.33 cM (A111_1)–77.7 cM (Satt341) (Qi *et al.*, 2011b) and 108.83–146.83 cM (Satt327) (Chen *et al.*, 2007).

In addition, qOil-C1-1 is localized in region 70.51 cM (Satt646)–74.45 cM and qOil-C1-2 is localized in region 73.38 cM (Satt161)–84.8 cM (Satt161–Satt195), which partially overlap. Two QTLs were previously detected in adjacent regions (61.88–65.08 cM) (Li *et al.*, 2011) and (85.37–120.12 cM) (Han *et al.*, 2015), but whether these two QTLs are identical remains to be confirmed. Moreover, qOil-D2-3 is located in region 5.83 cM (Sct_192)–11.17 cM (Sat_333) and a QTL region near this site, at 24.52 cM (Satt458)–57.07 cM (Satt154), was previously detected (Hyten *et al.*, 2004). Finally, qOil-E-1 is located in region 64.18 cM (Sat_381)–67.91 cM (Satt553), which is adjacent to two regions underlying OC, i.e. 53.10–57.70 cM (Li *et al.*, 2011) and 84.62–85.15 cM (Mao *et al.*, 2013), each harbouring a QTL site. These QTLs located by conditional analysis are present in the QTL enrichment region, which verifies the necessity of detecting QTLs using the conditional variable method.

Conclusions

QTLs for PC and OC were identified by both conditional and unconditional variable analysis. Among the 34 protein and 39 oil QTLs identified, 13 only affect PC and seven only affect OC. The eight remaining PC QTLs were completely affected by the oil and six OC QTLs were fully influenced by PC. The 13 PC QTLs and 26 OC QTLs were detected by both unconditional and conditional variable methods. Finally, seven QTLs (qPro-A1-3, qPro-A1-4, qOil-A2-1, qOil-C1-1, qOil-C1-2, qOil-D2-3 and qOil-E-1) were identified for the first time.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0021859620000040>.

Financial support. This research was financially supported by the National Key Research and development Program of China (2017YFD0101303) to Wen-Xia Li.

Conflict of interest. None.

Ethical standards. Not applicable.

References

- Akond M, Liu S, Boney M, Kantartzi SK, Meksem K, Bellaloui N, Lightfoot DA and Kassem MA (2014) Identification of quantitative trait loci (QTL) underlying protein, oil, and five major fatty acids' contents in soybean. *American Journal of Plant Sciences* 5, 158–167.
- Ao Y and Xu C (2006) Maximum likelihood method for mapping QTL in four-way cross design. *Acta Agronomica Sinica* 32, 51–56.
- Brunner E, Graef G, Orf J, Wilcox J and Shoemaker R (1997) Mapping QTL for seed protein and oil content in eight soybean populations. *Crop Science* 37, 370–378.
- Chapman A, Pantalone VR, Ustun A, Allen FL, Landau-Ellis D, Triganio RN and Gresshoff PM (2003) Quantitative trait loci for agronomic and seed quality traits in an F₂ and F_{4:6} soybean population. *Euphytica* 129, 387–393.
- Chen QS, Zhang ZC, Liu CY, Xin DW, Qiu HM, Shan DP, Shan CY and Hu GH (2007) QTL analysis of major agronomic traits in soybean. *Agricultural Sciences in China* 6, 399–405.
- Chung J, Babka H, Graef G, Staswick P, Lee D, Cregan P, Shoemaker R and Specht J (2003) The seed protein, oil, and yield QTL on soybean linkage group I. *Crop Science* 43, 1053–1067.
- Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, Vantoai TT, Lohnes D, Chung J and Specht JE (1999) An integrated genetic linkage map of the soybean genome. *Crop Science* 39, 1464–1490.
- Csanádi G, Vollmann J, Stift G and Lelley T (2001) Seed quality QTLs identified in a molecular map of early maturing soybean. *Theoretical and Applied Genetics* 103, 912–919.
- Diers BW, Keim P, Fehr WR and Shoemaker RC (1992) RFLP analysis of soybean seed protein and oil content. *Theoretical and Applied Genetics* 83, 608–612.
- Eskandari M, Cober ER and Rajcan I (2013a) Genetic control of soybean seed oil: I. QTL and genes associated with seed oil concentration in RIL populations derived from crossing moderately high-oil parents. *Theoretical and Applied Genetics* 126, 483–495.
- Eskandari M, Cober ER and Rajcan I (2013b) Genetic control of soybean seed oil: II. QTL and genes that increase oil concentration without decreasing protein or with increased seed yield. *Theoretical and Applied Genetics* 126, 1677–1687.
- Fasoula VA, Harris DK and Boerma HR (2004) Validation and designation of quantitative trait loci for seed protein, seed oil, and seed weight from two soybean populations. *Crop Science* 44, 1218–1225.
- Gai J, Wang Y, Wu X and Chen S (2007) A comparative study on segregation analysis and QTL mapping of quantitative traits in plants-with a case in soybean. *Frontiers of Agriculture in China* 1, 1–7.
- Han Y, Teng W, Wang Y, Zhao X, Wu L, Li D and Li W (2015) Unconditional and conditional QTL underlying the genetic interrelationships between soybean seed isoflavone, and protein or oil contents. *Plant Breeding* 134, 300–309.
- Hyten DL, Pantalone VR, Sams CE, Saxton AM, Landau-Ellis D, Stefaniak TR and Schmidt ME (2004) Seed quality QTL in a prominent soybean population. *Theoretical and Applied Genetics* 109, 552–561.
- Jiao CC, Huang JX, Wang YL, Zhang XY, Xiong HX, Ni XY and Zhao JY (2015) Genetic analysis of yield-associated traits by unconditional and conditional QTL in *Brassica napus*. *Acta Agronomica Sinica* 41, 1481–1489.
- Jun TH, Van K, Kim MY, Lee SH and Walker DR (2008) Association analysis using SSR markers to find QTL for seed protein content in soybean. *Euphytica* 162, 179–191.
- Kabelka EA, Diers BW, Fehr WR, Leroy AR, Baianu IC, You T, Neece DJ and Nelson RL (2004) Putative alleles for increased yield from soybean plant introductions. *Crop Science* 44, 784–791.
- Kim HK, Kim YC, Kim ST, Son BG, Choi YW, Kang JS, Park YH, Cho YS and Choi IS (2010) Analysis of quantitative trait loci (QTLs) for seed size and fatty acid composition using recombinant inbred lines in soybean. *Journal of Life Science* 20, 1186–1192.
- Lander ES and Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121, 185–199.
- Lee SH, Bailey MA, Mian MAR, Carter TE, Shipe ER, Ashley DA, Parrott WA, Hussey RS and Boerma HR (1996) RFLP loci associated with soybean seed protein and oil content across populations and locations. *Theoretical and Applied Genetics* 93, 649–657.
- Li H, Ye G and Wang J (2007) A modified algorithm for the improvement of composite interval mapping. *Genetics* 175, 361–374.
- Li H, Zhao T, Wang Y, Yu D, Chen S, Zhou R and Gai J (2011) Genetic structure composed of additive QTL, epistatic QTL pairs and collective unmapped minor QTL conferring oil content and fatty acid components of soybeans. *Euphytica* 182, 117. doi: 10.1007/s10681-011-0524-9.
- Liang HZ, Yu YL, Wang SF, Lian Y, Wang TF, Wei YL, Gong PT, Liu XY, Fang XJ and Zhang MC (2010) QTL mapping of isoflavone, oil and protein contents in soybean (*Glycine max* L. Merr.). *Agricultural Sciences in China* 9, 1108–1116.
- Liu J, Niu FA, Jiang JH, Sun C, Chen L, Guo Y, Fu SH and Hong DL (2012) Unconditional and conditional QTL mapping for yield and yield related traits in japonica rice in multi-environments. *Chinese Journal of Rice Science* 26, 144–154.
- Liu S, Xue H, Zhang K, Wang P, Su D, Li W, Xu S, Zhang J, Qi Z, Fang Y, Li X, Wang Y, Tian X, Song J, Wang J, Yang C, Jiang S, Li WX and Ning H (2019) Mapping QTL affecting the vertical distribution and seed set of soybean [*Glycine max* (L.) Merr.] pods. *The Crop Journal* 7, 694–706.
- Lu W, Wen Z, Li H, Yuan D, Li J, Zhang H, Huang Z, Cui S and Du W (2013) Identification of the quantitative trait loci (QTL) underlying water soluble protein content in soybean. *Theoretical and Applied Genetics* 126, 425–433.
- Mansur LM, Lark KG, Kross H and Oliveira A (1993) Interval mapping of quantitative trait loci for reproductive, morphological, and seed traits of soybean (*Glycine max* L.). *Theoretical and Applied Genetics* 86, 907–913.
- Mansur LM, Orf JH, Chase K, Jarvik T, Cregan PB and Lark KG (1996) Genetic mapping of agronomic traits using recombinant inbred lines of soybean. *Crop Science* 36, 1327–1336.
- Mao T, Jiang Z, Han Y, Teng W, Zhao X and Li W (2013) Identification of quantitative trait loci underlying seed protein and oil contents of soybean across multi-genetic backgrounds and environments. *Plant Breeding* 132, 630–641.
- Moongkanna J, Nakasathien S, Novitzky W, Kwanyuen P, Sinchaisri P and Srinives P (2011) SSR markers linking to seed traits and total oil content in soybean. *Thai Journal of Agricultural Science* 44, 233–241.
- Ning HL, Li Q, Li WB, Xue H, Li BY, Bai XL, Zhuang X and Li WX (2015) Construction of linkage map based on a four-way recombinant inbred lines population. *Soybean Science* 34, 776–781.
- Ning HL, Bai XL, Li WB, Xue H, Zhuang X, Li WX and Liu CY (2016) Mapping QTL protein and oil contents using population from four-way recombinant inbred lines for soybean (*Glycine max* L. Merr.). *Acta Agronomica Sinica* 42, 1620–1628.
- Orf JH, Chase K, Jarvik T, Mansur LM, Cregan PB, Adler FR and Lark KG (1999) Genetics of soybean agronomic traits: I. Comparison of three related recombinant inbred populations. *Crop Science* 39, 1642–1651.
- Palomeque L, Liu LJ, Li W, Hedges B, Cober ER and Rajcan I (2009) QTL in mega-environments: II. Agronomic trait QTL co-localized with seed yield QTL detected in a population derived from a cross of high-yielding adapted × high-yielding exotic soybean lines. *Theoretical and Applied Genetics* 119, 429–436.
- Pathan SM, Vuong T, Clark K, Lee JD, Shannon JG, Roberts CA, Ellersieck MR, Burton JW, Cregan PB, Hyten DL, Nguyen HT and Sleper DA (2013) Genetic mapping and confirmation of quantitative trait loci for seed protein and oil contents and seed weight in soybean. *Crop Science* 53, 765–774.
- Qi ZM, Han X, Sun YN, Wu Q, Shan DP, Du XY, Liu CY, Jiang HW, Hu GH and Chen QS (2011a) An integrated quantitative trait locus map of oil content in soybean, *Glycine max* (L.) Merr., generated using a meta-analysis method for mining genes. *Agricultural Sciences in China* 10, 1681–1692.
- Qi ZM, Wu Q, Han X, Sun YN, Du XY, Liu CY, Jiang HW, Hu GH and Chen QS (2011b) Soybean oil content QTL mapping and integrating with meta-analysis method for mining genes. *Euphytica* 179, 499–514.
- Qi Z, Han X, Hou M, Xin D, Wang Z, Zhu R, Hu Z, Jiang H, Li C, Liu C, Hu G and Chen Q (2014a) QTL analysis of soybean oil content under 17 environments. *Canadian Journal of Plant Science* 94, 245–261.

- Qi Z, Hou M, Han X, Liu C, Jiang H, Xin D, Hu G and Chen Q (2014b) Identification of quantitative trait loci (QTLs) for seed protein concentration in soybean and analysis for additive effects and epistatic effects of QTLs under multiple environments. *Plant Breeding* **133**, 499–507.
- Qiu BX, Arelli PR and Sleper DA (1999) RFLP markers associated with soybean cyst nematode resistance and seed composition in a 'Peking' × 'Essex' population. *Theoretical and Applied Genetics* **98**, 356–364.
- Reinprecht Y, Poysa VW, Yu K, Rajcan I, Ablett GR and Pauls KP (2006) Seed and agronomic QTL in low linolenic acid, lipoxygenase-free soybean (*Glycine max* (L.) Merrill) germplasm. *Genome* **49**, 1510–1527.
- Rossi ME, Orf JH, Liu LJ, Dong Z and Rajcan I (2013) Genetic basis of soybean adaptation to North American vs. Asian mega-environments in two independent populations from Canadian × Chinese crosses. *Theoretical and Applied Genetics* **126**, 1809–1823.
- Shibata M, Takayama K, Ujiie A, Yamada T, Abe J and Kitamura K (2008) Genetic relationship between lipid content and linolenic acid concentration in soybean seeds. *Breeding Science* **58**, 361–366.
- Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Germann M, Orf JH and Lark KG (2001) Soybean response to water. *Crop Science* **41**, 493–509.
- Tajuddin T, Watanabe S, Yamanaka N and Harada K (2003) Analysis of quantitative trait loci for protein and lipid contents in soybean seeds using recombinant inbred lines. *Breeding Science* **53**, 133–140.
- Tian B, Liu B, Zhu ZL, Xie QG and Tian JC (2011) Conditional and unconditional QTL mapping of grain starch accumulation in wheat. *Scientia Agricultura Sinica* **44**, 4551–4559.
- Wang X, Jiang GL, Green M, Scott RA, Hyten DL and Cregan PB (2012) Quantitative trait locus analysis of saturated fatty acids in a population of recombinant inbred lines of soybean. *Molecular Breeding* **30**, 1163–1179.
- Wang LL, Liu CY, Jiang ZF, Yao BC, Shen YR, Ma ZZ, Xin DW, Han X, Tao Li T, Hu GH and Chen QS (2014a) Analysis of QTL underlying protein content of soybean in multi-environments. *Chinese Journal of Oil Crop Sciences* **36**, 443–449.
- Wang X, Jiang GL, Green M, Scott RA, Song Q, Hyten DL and Cregan PB (2014b) Identification and validation of quantitative trait loci for seed yield, oil and protein contents in two recombinant inbred line populations of soybean. *Molecular Genetics and Genomics* **289**, 935–949.
- Warrington CV, Abdel-Haleem H, Hyten DL, Cregan PB, Orf JH, Killam AS, Bajjalieh N, Li Z and Boerma HR (2015) QTL for seed protein and amino acids in the Benning × Danbaekkong soybean population. *Theoretical and Applied Genetics* **128**, 839–850.
- Xu S (1996) Mapping quantitative trait loci using four-way crosses. *Genetics Research* **68**, 175–181.
- Xu S (1998) Iteratively reweighted least squares mapping of quantitative trait loci. *Behavior Genetics* **28**, 341–355.
- Zhang L, Huang Z, Li J, Hu C and Dai O (2004) Preliminary study of protein and oil contents of M type hybrid soybean. *Chinese Journal of Oil Crop Sciences* **26**, 17–21.
- Zhang H, Chen J, Li R, Deng Z, Zhang K, Liu B and Tian J (2016) Conditional QTL mapping of three yield components in common wheat (*Triticum aestivum* L.). *The Crop Journal* **4**, 220–228.
- Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. *Genetics* **141**, 1633–1639.