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RECOGNITION OF QTL FOR SEED PROTEIN AND OIL CONTENT IN TWO SOYBEAN RECOMBINAT INBRED LINES POPULATIONS

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ABSTRACT

Enhancing protein and oil amounts in soybean seed is one of the main objectives of breeding programs. The aim of this study was to identify quantitative trait loci (QTLs) responsible for protein and oil contents in two recombinant inbred lines (RILs) of soybean. Genotyping of the RIL3613 (Dongnong L13×Heihe 36) and RIL6013 (Dongnong L13×Henong 60) populations was performed using simple sequence repeat markers (SSRs), respectively. Phenotypic data for protein and oil contents in both populations were collected in three different environments. The results indicated wide variations across the lines and among different environments in protein and oil contents, with statistical significance. Nine and five QTLs responsible for protein and oil contents, respectively, explained 6.4515%-13.0368% and 5.7236%-17.7396% of the overall phenotypic variations. Of the detected QTLs, one was previously unknown. These QTLs would be very valuable for breeding soybean with improved nutritional quality.

Key words: Soybean, Protein content, Oil content, Association mapping population, QTL
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INTRODUCTION

Improvements of protein and oil contents in soybean [*Glycine Max.* (L.) Merry] would increase the nutritional value of this crop. Meanwhile, extensive phenotypic variations of protein (34.1% to 56.8%) and oil (8.1% to 27.9%) contents in soybean suggest a huge improvement potential (Wilson, 2004). Detection of quantitative trait loci (QTLs) controlling protein and oil contents would help genetically improves the amounts of these nutrients in soybean.

Soybean seed protein and oil contents follow the inheritance model as quantitative traits controlled by multiple genes whose expression levels have small or large effects, federatively or severally (Boerma and Specht, 2004). Multiple QTLs for seed protein and oil amounts are distributed on 20 chromosomes in soybean (Diers *et al*, 1992; Lee, *et al*, 1996; Brummer, *et al*,1997; Orf, *et al*, 1999; Qiu, *et al*, 1999; Sebolt, *et al*, 2000; Specht, *et al*, 2001; Chung, *et al*,2003; Tajuddin, *et al*, 2003; Hyten *et al*, 2004; Kabelka, *et al*, 2004; Jun, *et al*, 2008; Panthee, *et al*, 2006; Reinprecht *et al*, 2006; Gai *et al*, 2007; Shibata, *et al*, 2008; Kim *et al*, 2010; Liang, *et al*, 2010; Li, *et al*, 2010; Qi *et al*, 2011; Pandurangan, *et al*, 2012; Wang, *et al*, 2012; Eskandari, *et al*, 2013; Lu, *et al*, 2013; Mao, *et al*, 2013; Rossi, *et al*,2013; Han, *et al*, 2015; Warrington, *et al*, 2015). Most studies searched QTLs responsible for protein and oil contents in single populations under the same environmental conditions. Such QTLs cannot be validated for different genetic backgrounds or environments, and are not useful for molecular selection in order to improve the quality traits of soybean.

Because of the complex effects of genetic background and environmental conditions on protein and oil contents, different QTLs have been reported by various researches, which limited the application of QTLs in soybean breeding programs. To increase the accuracy and reliability of the detected QTLs, studies have included populations of different genetic backgrounds, with multiple germplasms planted in multiple environments (years and/or locations). For instance, Lee *et al* (1996) identified thirteen and eleven QTLs responsible for protein and oil contents, respectively, using two recombinant inbred line (RIL) populations. Meanwhile, Brummer *et al* (1997) analyzed protein and oil amounts in eight populations across multiple

environments, and identified twelve and eleven QTLs responsible for protein and oil contents, respectively. Orf *et al* (1999) found five and six QTLs that control protein and oil contents, respectively, using three RIL populations. Sebolt *et al* (2000) reported two proteins and two oil QTLs in two RIL populations; Mao *et al* (2013) identified forty and thirty-five QTLs associated with protein and oil contents, respectively, in three RIL populations across eight environments. The remaining studies were based on single populations. For example, Diers *et al* (1992) reported eight and nine QTLs responsible for protein and oil contents, respectively, while Hyten *et al* (2004) mapped four and six QTLs associated with protein and oil contents, respectively, in a RIL population of 131 lines. Association mapping populations are important entities involving multiple backgrounds, constructed by a common parent crossing with other parents; this has the advantage of constructing high density linkage maps and enhancing QTL detection (Simon *et al*, 2008; O'Neill *et al*, 2008). Meanwhile, since there are common markers among different populations, the mapped QTLs could be compared and validated (Wang *et al*, 2014). Thirteen and eleven QTLs were identified for protein and oil content, respectively using two recombinant inbred lines (RIL) populations (Lee *et al.,* 2019). The analyzed data of oil and protein content in eight populations across multiple environments revealed twelve QTLs for protein and eleven QTLs for oil content (Tian *et al.,* 2020). Five QTLs controlling protein content and six OTLs controlling oil content using three recombinant inbred line populations were used (Zhang *et al.,* 2020). Two protein QTLs and two oil QTLs in two recombinant inbred line populations were observed (Zhu *et al.,* 2020).

Past studies conducted on a single population identified with eight QTLs for protein content and nine QTLs for oil content (Xue *et al.,* 2019) and mapped four QTLs for protein content and six QTLs for oil content in RIL population of 131 lines (Zhang *et al.,* 2019).Association mapping populations were a type of population involved with multiple backgrounds, which were constructed by one common parent and by crossing with other parents with the advantage of constructing high-density linkage maps and enhancement of QTL detecting power (Scott *et al.,*2020). Also, there are common markers among the different populations, and the mapped QTLs could be compared and validated with each other (Kaler *et al.,* 2020).

In the present work, two association recombinant inbred line populations of soybean were used as genetic materials and planted in three environments. Then, phenotypic (protein and oil contents) and genotypic data were assessed for each line by the inclusive composite interval mapping method (ICIM) to detect QTLs. The objective of this research was to identify stable QTLs associated with protein and oil contents, by validation of repeated findings in different populations and environments. Our findings would be of great importance in improving soybean quality.

MATERIALS AND METHODS

Genetic materials: The current study involved two F2:6-derived RIL populations, which were developed by the single-seed descent method at Northeast Agricultural University in Harbin, China. The RIL3613 population was comprised of 134 RILs, derived from Dongnong L13 (45.50% protein, 18.74% oil)×Heihe36 (39.80% protein, 19.28% oil) crosses; RIL6013 encompassed 156 RILs, obtained by DongnongL13×Henong 60 (38.47% protein, 22.25% oil) crosses. The seeds of all RILs were planted in Keshan (E125.64 °, N48.25 °) in May 2013 and Harbin (E 126.63 °, N 45.75 °) in May 2014 and 2015, respectively. The two RIL populations were planted by the randomized complete block design, with three replications in the same field. In all experiments, seeds were planted in rows of 5m, 0.65 m apart, with a 6 cm space between two plants. Exactly 10 plants of a single genotype from each plot were harvested for seed collection. Three seed samples totaling about 20–25 grams were assessed per plot for protein and oil content son a FOSS Infracted 1241 NIR grain analyzer (FOSS, Sweden).

Genotyping: The CTAB method was used for genomic DNA extraction from three parents and RILs (Doyle and Doyle, 1990). A total of 652 pairs of simple sequence repeat (SSR) primers selected from Soybase (http://www.soybase.org) were screened according to polymorphisms between the two parents of each cross. Polymerase chain reaction (PCR) consisted of 11.5µl of ddH2O, 3µl of genomic DNA (25 ng/µl), 3µl of SSR primers (2µM) and 0.2µl of Taq polymerase (10 units/µl). The amplification program consisted of 5 min at 94°C, followed by 38 cycles of 30 sec at 94°C, 30 sec at 47°C and 30 sec at 72°C, with a final extension for 5 min at 72°C. The resulting PCR products were mixed with loading buffer (2.5 mg/ml diphenylamine blue, 10mM EDTA, and 95% (v/v) formamide), denatured for 10 min at 94°C and placed on ice for 5 min. The denatured PCR products were separated by 6% (w/v) denaturing polyacrylamide gel and visualized by silver staining (Mao *et al*., 2013).

QTL mapping: Based on the simple sequence repeat (SSR) linkage map constructed in previous research (Ning *et al*, 2018), QTL IciMapping version 4.0 (Wang *et al*., 2014) was used for QTL analysis for each environment in the mapping populations. QTL analysis was performed by Inclusive Composite Interval Mapping (ICIM) methods implemented via IM-ADD and ICIM-ADD modules to evaluate QTL effects; the LOD score

threshold was set at 2.5. The estimated QTL position was the point of maximum LOD score in the region under consideration. Genotype predictions for each line were also based on posterior probability of the genotype for each line.

Statistical analysis: Statistical analyses for mean, standard deviation, minimum, and maximum were based on the average of each trait for every line in different environments. Analysis of variance (AVOVA) was carried out, including genotypic, environmental and error effects.

Broad-sense heritability of protein and oil contents was computed as

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/e + \sigma^2/(ge)}$$

Where σ_g^2 and σ_e^2 are genetic and residual variances obtained from the expected mean squares of AVOVA (Nyquist 1991). All analyses were performed with the SAS software version 9.2 (SAS Institute, Cary); PROC MEANS, PROC GLM, and PROC VARCOMP were used for statistical analysis, ANOVA, and estimation of variance components, respectively.

RESULTS

Phenotypic variation analysis: Results further showed larger variation in both RIL populations for oil and protein content. Both populations were significantly influenced by the environment (Table 1).

However, on average both populations exhibited similar performance, minimum and maximum values for the two traits within the three environments. The means of oil and protein content (42.81 and 19.28%) for recombinant inbred line 3613 were slightly lower than those observed in the recombinant inbred line 6013 (43.10 and19.57%proteinand oil content, respectively) (Table 2).

Table 1. Variation and hereditability of protein and oil content for RIL3613 and RIL6013

Traits	Mean (%)	Standard deviation	minimum	maximum	F_{env}[A]	F_{gen}[B]	h^{2D}
RIL3613							
Protein content	42.81	1.93	36.74	47.37	14.07**[C]	1.98**	0.90
Oil content	19.28	1.81	15.19	22.37	1053.8**	2.05**	0.12
RIL6013							
pro	43.10	1.66	37.18	47.49	50.63**	1.33**	0.29
oil	19.57	0.92	16.40	22.63	88.96**	1.36**	0.39

AF_{env} means F value for environment effect. B means F value for genotypic effects. C **mean significant at 0.01 level. D h^2 means heritability.

Table 2. Statistical analysis on oil and protein content in three environments for parents, RIL3613 and RIL6013 (%).

Environment[A]	Parents			RIL3613				RIL6013			
	Dongnong L13	Heihe 36	Henong 60	Mean	Standard Deviation	Mini-mum	Maxi-mum	Mean	Standard deviation	Mini-Mum	Maxi-mum
					Protein content						
2013KS	44.50	40.52	39.60	43.39	2.07	38.00	47.37	42.13	1.90	37.18	46.30
2014HRB	44.13	39.66	40.24	42.35	2.23	36.74	46.14	43.63	1.55	39.63	47.49
2015HRB	43.82	38.86	39.76	42.62	1.26	38.6	44.80	43.58	0.92	39.90	46.00
					Oil content						
2013KS	17.86	18.02	21.42	17.07	0.86	15.19	18.89	19.03	0.90	16.40	22.13
2014HRB	18.43	20.44	21.67	20.51	1.01	17.89	22.37	19.52	0.93	16.73	22.39
2015HRB	18.88	20.21	21.88	20.43	0.51	18.54	21.92	20.16	0.50	18.45	22.63

A: 2013KS mean Keshan in 2013; 2014HRB means Harbin in2014, 2015HRB means Harbin in2015

Significant effects of environments were observed on oil and protein content, indicating its liability in the detection of different QTLs in various environments (Table 3). In both populations, the heritability was extremely varied. The heritability for protein content was higher in recombinant inbred line 3613 as compared to recombinant inbred line 6013, while cross-current was observed for the oil content. This showed the existence of differences between the two crosses on the power to discover QTL for oil and protein content.

QTL analysis: We identified six QTLs for protein content in RIL3613 and two in RIL6013 on the linkage groups A1, B1, D2, F, I, J, L, and O (Figure 1, Figure 2, Table 3). In RIL3613, qpro-B1-1 (satt359-satt197) explained 8.73% of the phenotypic variation, with synergic alleles from Dongnong L13; qpro-D2-1

(sat_001-sat_326) accounted for 18.38% of the phenotypic variation, with positive additive effect alleles from Heihe 36. The positive additive effects were from the qpro-I-1 (satt367-satt270) alleles in Dongnong L13, which explained 22.94% of the phenotypic variation. The qpro-J-1 alleles (sat_350-satt414) in Heihe 36 could increase protein content and contributed to 13.75% of the phenotypic variation. For qpro-L-1, positive additive effect alleles were carried by Dongnong L13, and accounted for 15.62% of the phenotypic variation. Meanwhile, qpro-O-1 (Satt358-sat-303) could enhance protein content via Dongnong L13 alleles, defining 27.96% of the total variation. In RIL6013, qpro-F-1 could enhance protein content through alleles carried by Henong 60 parents, with an explanation ratio of 20.86% for the phenotypic variation. Dongnong L13 carried positive synergic alleles of qpro-O-2 (Satt633-Sat_303), which explained 14.08% of the total variation.

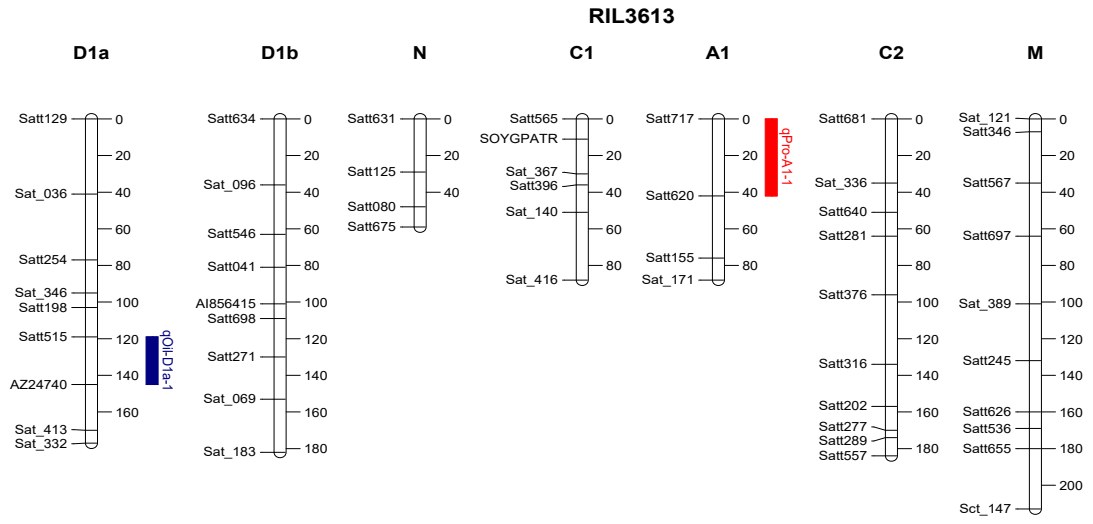
Table 3. QTL for protein and oil content in two populations across three environments.

QTL	Interval	LOD[A]	PVE[B] (%)	Additive effect	Popula-tion	Environ-ment	reference[C]
Protein content							
*qpro-A*1-1	Satt717~Satt620	2.6612	30.4078	-1.1460	RIL3613	2013KS	[1,2,3,4]
*qpro-B*1-1	Satt359~Satt197	2.6149	8.7317	0.3787	RIL3613	2015HRB	[5,6,7,8,9,10]
*qpro-D*2-1	Sat_001~Sat_326	4.0585	18.3751	-0.9058	RIL3613	2013KS	[2,3,11]
qpro-F-1	Sat_417~sat_039	3.0418	20.8565	-0.7073	RIL6013	2014HRB	[1,2,10-20]
qpro-I-1	Satt367~Satt270	3.4880	22.9465	1.0136	RIL3613	2013KS	[1-3,7,9,10,14,18-27]
qpro-J-1	Sat_350~Satt414	2.5638	13.7492	-0.8266	RIL3613	2013KS	[5,12,14,28]
qpro-L-1	Sat_134~Sat_191	3.0405	15.7262	0.8169	RIL3613	2013KS	[5,9,14,22,24,28,29]
qpro-O-1	Satt358~Sat_303	3.1040	27.9655	1.3115	RIL3613	2014HRB	[8,9,10,13,14,28,30,31]
qpro-O-2	Satt633~Sat_303	2.6445	14.0824	0.7576	RIL6013	2013KS	[8,9,10,13,14,28,30,31]
Oil content							
*qoil-A*2-1	Sct_067~Satt589	5.5640	18.9927	-0.3737	RIL3613	2013KS	[9]
		4.3329	16.0570	-0.4035	RIL3613	2014HRB	
*qoil-C*1-1	satt565~satt396	3.6129	14.3395	-0.3992	RIL6013	2013KS	[9,14,19,32,33]
*qoil-D*1*a*-1	Satt515~AZ24740	3.4940	22.0383	-0.2550	RIL3613	2015HRB	[2,3,11]
*qoil-D*2-1	sat_333~sat_194	5.3279	16.4686	-0.4271	RIL6013	2013KS	[1,4,10,11,12,14,17,19,28,29,34,35]
qoil-H-1	Satt181~satt434	2.7528	7.1346	-0.2724	RIL6013	2013KS	

A LOD means log of odd.

B PVE means phenotypic variation explanation ratio.

C [1] Bachlava *et al*, 2009; [2] Brummer, *et al*,1991;[3] Han, *et al*, 2015;[4] Ha *et al*, 2014[5] Kim *et al*, 2010; [6] Gai *et al*, 2007; [7] Diers *et al*, 1992; [8] Li, *et al*, 2011; [9] Qi *et al*, 2011; [10] Reinprecht*et al*, 2006; [11] Hyten *et al*, 2004; [12] Eskandari, *et al*, 2013; [13] Kabelka, *et al*, 2004; [14] Mao, *et al*, 2013; [15]Qiu, *et al*, 1999; [16] Rossi, *et al*, 2013; [17] Sams, *et al*, 2004; [18] Specht, *et al*, 2001; [19] Sun, *et al*, 2011; [20] Wang, *et al*, 2011; [21] Chung, *et al*, 2003;[22] Lu, *et al*, 2013; [23] Pandurangan, *et al*, 2012; [24]Pathan, *et al*, 2013; [25] Shibata, *et al*, 2008; [26] Tajuddin, *et al*, 2003; [27] Warrington, *et al*, 2015; [28] Panthee, *et al*, 2005; [29] Kan, *et al*, 2016; [30] Jun, *et al*, 2006; [31] Li *et al*, 2010; [32] Orf, *et al*, 1999; [33]Lee, 1996; [34] Wang, *et al*, 2012; [35] Xie, *et al*, 2012 [36] Panthee *et al*. 2005



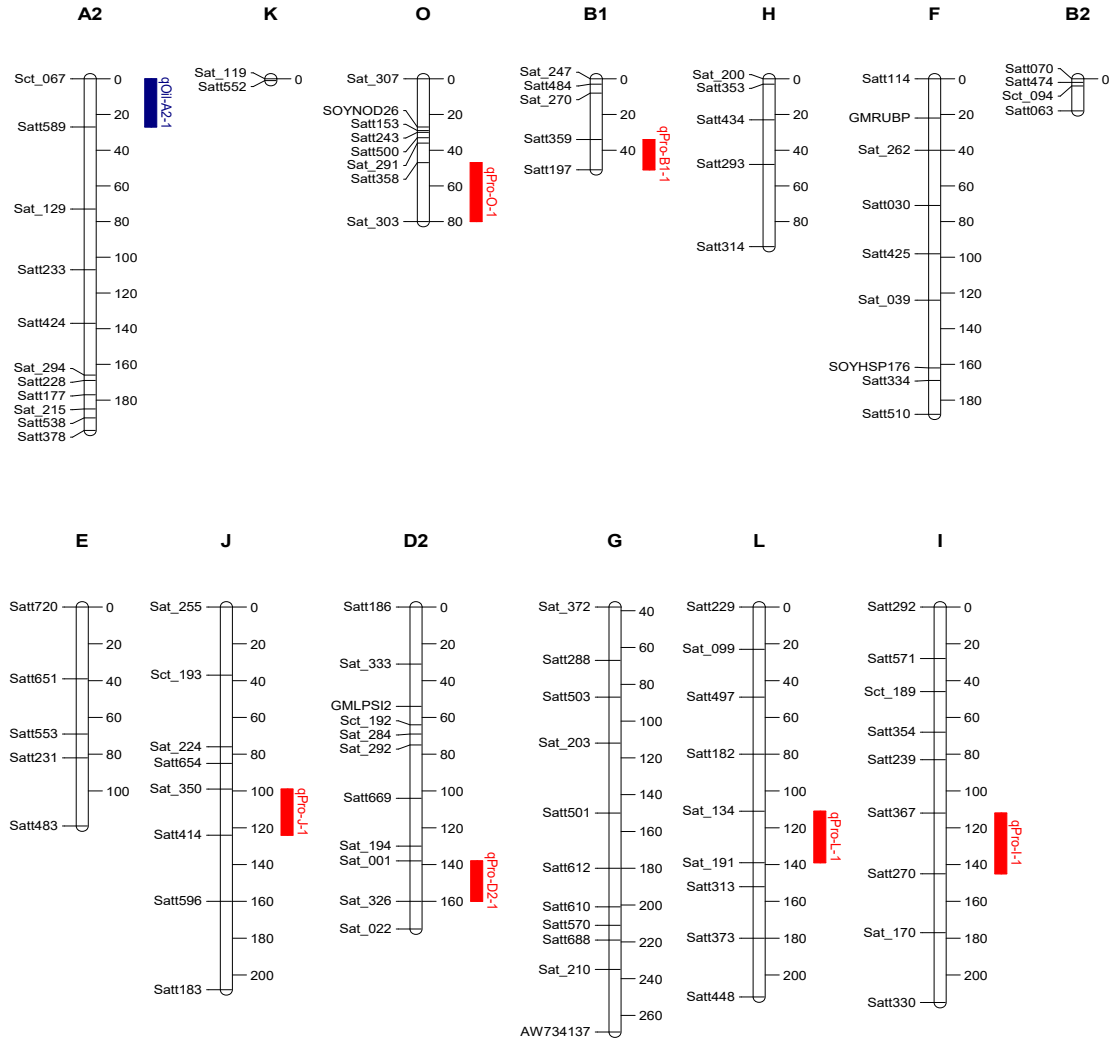
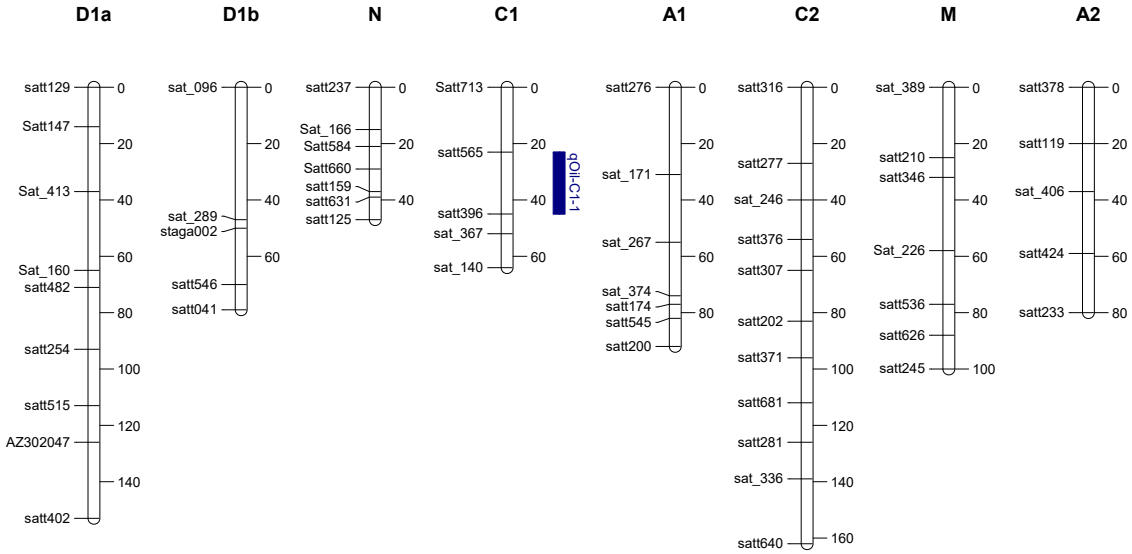


Figure 1 QTL controlling protein (red color) and oil (blue color) content identified in RIL3613



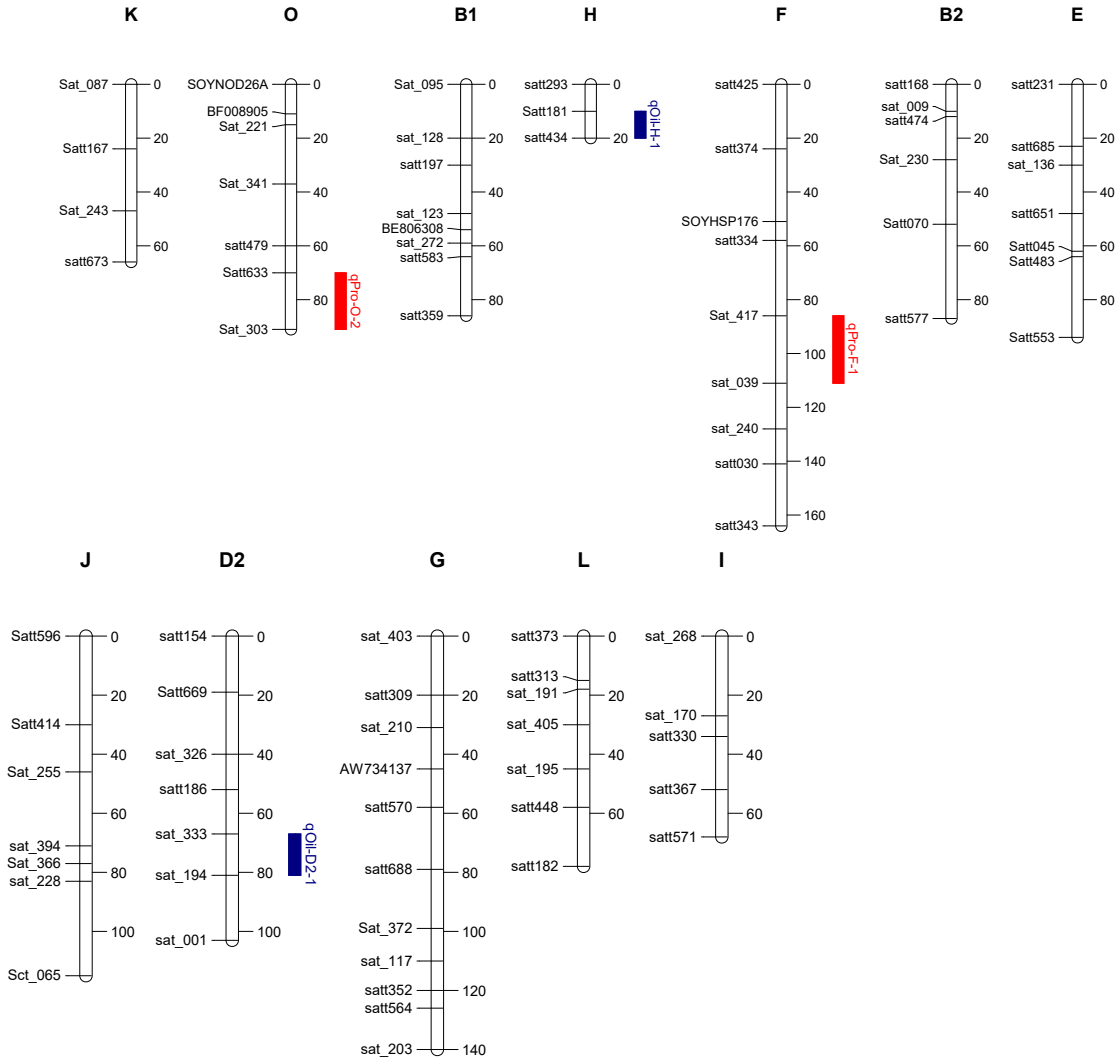


Figure 2 QTL controlling protein (red color) and oil (blue color) content identified in RIL6013

A total of 5 QTLs were identified for oil content (Table 3). In RIL3613, qoil-A2-1 (Sct_067-Satt589) and qoil-D1a-1 were identified with PVE ranging from 16.06% to 22.04%, respectively. In RIL6013, three QTLs (qoil-C1-1, qoil-D2-1, and qoil-H-1) explained 7.13%-22.04% of the phenotypic variation. Heihe 36 enhanced oil contents in RIL3613 progenies based on qoil-A2-1 and qoil-D1a-1, while Henong 60 improved oil content of RIL6013 progenies through qoil-C1-1, qoil-D2-1, and qoil-H-1.

DISCUSSION

Advantages of association recombinant inbred line populations: Although QTLs for protein and oil contents were distributed across all twenty chromosomes (https://www.soybase.org/), a limited number of detected QTLsis still obtained by a single mapping population. Discovering QTLs by multiple mapping populations attracts increasing attention (Lee *et al*, 1996; Brummer *et al*, 1997; Orf *et al*, 1999; Sebolt *et al*, 2000; Mao *et al*, 2013; Diers *et al*, 1992; Hyten *et al*, 2004). In this study, two RIL populations were assessed, and 14 QTLs were found, including nine and five responsible for protein and oil contents, respectively. Among the fourteen QTLs, only qpro-O-1 in RIL 3613 and qpro-O-2 of RIL6013 were located in the same region; the genomic regions of eight QTLs (qpro-A1-1, qpro-B1-1, qpro-D2-1, qpro-I-1, qpro-J-1, qpro-L-1, qoil-A2-1, qoil-D1a-1) derived from RIL3613 were different from those of the four QTLs (qpro-F-1, qoil-C1-1, qoil-D2-1, qoil-H-1) in RIL6013. Summarizing previous and present researches, it is obvious that multiple populations could mutually complement in detecting genomic regions that carry QTLs.

Authenticity verification of the QTL: To assess the authenticity of the QTLs detected in this study, we compared the genomic regions on the integrated map

(Song *et al*, 2010) of the QTLs discovered in the present study with the previously reported. For QTLs controlling protein content, all nine regions have been detected in previous studies. qpro-A1-1 located in the region ranging from 51.95 cM (Satt717) to 69.20 cM (Satt619) overlapped with one QTL for seed oleic content, and contained one oil QTL (Bachlava *et al*, 2009; Brummer, *et al*,1991; Han, *et al*, 2015; Ha *et al*, 2014). There are four QTLs responsible for oleic acid, linoleic acid, palmitic acid, and oil contents that are anchored in the qpro-B1-1 region (46.38 cM to 102.55 cM) (Kim *et al*, 2010; Gai *et al*, 2007; Diers *et al*, 1992; Li, *et al*, 2011; Qi *et al*, 2011; Reinprecht *et al*, 2006) . The interval between Sat_001 and Sat_326 contains qpro-D2-1 that overlapped with two detected QTLs associated with oleic acid and oil contents (Hyten *et al*, 2004; Brummer, *et al*,1991; Han, *et al*, 2015). In the region from 27.87 cM (Sat_417) to 135.94 cM (sat_039) of qpro-F-1, 25 QTLs associated with linoleic acid, oil, oleic acid, palmitic acid, stearic acid, and protein contents have been reported previously (Bachlava *et al*, 2009; Brummer, *et al*,1991; Eskandari, *et al*, 2013; Hyten *et al*, 2004; Kabelka, *et al*, 2004; Mao, *et al*, 2015; Qiu, *et al*, 1999; Reinprecht *et al*, 2006; Rossi, *et al*,2013; Sams, *et al*,2004; Specht, *et al*,2001; Sun, *et al*,2011). The qpro-I-1 region has been reported thirty-two times in previous studies, in association with oil and protein contents (Bachlava *et al*, 2009; Brummer, *et al*,1991; Chung, *et al*,2003; Diers *et al*, 1992; Han, *et al*, 2015; Lu, *et al*, 2013; Mao, *et al*, 2015; Pandurangan, *et al*, 2012; Pathan, *et al*, 2013; Qi *et al*, 2011; Reinprecht *et al*, 2006; Sebolt, *et al*, 2000; Shibata, *et al*, 2008; Specht, *et al*, 2001; Sun, *et al*,2011; Tajuddin, *et al*, 2003; Warrington, *et al*, 2015). The qpro-J-1 interval includes four identified QTLs responsible for linoleic acid, oil and oleic acid contents (Mao, *et al*, 2015; Kim *et al*, 2010; Eskandari, *et al*, 2013; Panthee, *et al*, 2006). The genomic fragment of qpro-L-1 was found to control oil, palmitic acid, stearic acid, protein contents in different genetic backgrounds (Mao, *et al*, 2015; Panthee, *et al*, 2006; Lu, *et al*, 2013; Kim *et al*, 2010; Pathan, *et al*, 2013; Qi, *et al*, 2011; Kan, *et al*, 2016). The genomic regions of qpro-O-1 (Satt358~Sat_303) (5.44cM-20.93cM) and qpro-O-2 (Sat_303~Satt633) (20.93 cM -56.93 cM) are flanked adjacently, with four QTLs associated with oil, plasmatic acid and linoleic acid contents (Li, *et al*, 2011; Qi, *et al*, 2011; Reinprecht, *et al*, 2006; Panthee *et al*. 2005).

There were four oil QTLs among the five identified in this study that had overlapping regions in previous reports. For instance, qoil-A2-1 located in the region from 14.99 cM to 33.95 cM, overlaps partly with an identified QTL responsible for oil content (Qi, *et al*, 2011). Meanwhile, the genomic interval between satt565 and satt396 of qoil-C1-1 contains QTLs associated with protein and oil contents (Mao, *et al*, 2013; Sun, *et al*, 2011; Qi, *et al*, 2011; Orf, *et al*, 1999; Lee, 1996).The qoil-D1a-1 region (Satt515~AZ254740) overlaps with those of two adjacent QTL intervals associated with oil content (Hyten, *et al*, 2004; Brummer, *et al*, 1997; Han, *et al*, 2015). In the genomic region of qoil-D2-1 between sat_333(5.83 cM) and sat_194 (86.69 cM), fourteen QTLs responsible for palmitic, oil, linoleic acid, palmitic plus stearic acid, palmitic acid, and oleic acid contents are known (Bachlava, *et al*, 2009; Eskandari, *et al*, 2013; Ha, *et al*, 2014; Hyten, *et al*, 2004; Kan, *et al*, 2016; Mao, *et al*, 2013; Panthee, *et al*, 2006; Reinprecht, *et al*,2006; Sams, *et al*, 2004; Sun, *et al*, 2011; Wang, *et al*, 2012; Xie, *et al*, 2012).

Based on repeated mapping across multiple populations, we could consider the above thirteen genomic regions detected in this study to actually carry QTLs for protein or oil content. No QTL in the qoil-H-1 region (Satt181~satt434) has been previously reported. Therefore, qoil-H-1 may represent a new QTL responsible for oil content. This should be confirmed in future studies.

Selection of parents for molecular pyramiding breeding: A pyramiding breeding program should assemble all synergic alleles of all QTLs from the two parents into an individual (variety). In this research, six QTLs responsible for protein content were identified. To achieve highest protein amounts, the desired genotype should include qpro-D2-1 (qq), qpro-I-1 (QQ), qpro-J-1 (qq), qpro-L-1 (QQ), qpro-O-1 (QQ), and qpro-B1-1 (QQ). In RIL3613, two lines, i.e. RIL3613-50 and RIL3613-83, contained these six excellent allele genotypes. Two QTLs associated with oil content were detected in single environments; for highest oil levels, the desired allelic genotype should include qoil-A2-1 (qq) and qoil-D1a-1 (qq). There were 16 lines with these two allelic genotypes in RIL3613 (Table S1). In the RIL6013 population, two QTLs responsible for protein content were found, with ideal synergic allelic genotype combination being qpro-F-1 (qq) and qpro-O-2 (QQ). Meanwhile, 50 lines carried these two excellent allelic genotypes. For oil content, three QTLs were detected; the optimal allelic genotype combination was qoil-C1-1 (qq), qoil-D2-1 (qq), and qoil-H-1 (qq), and three lines contained these three allelic genotypes simultaneously (Table S2). These lines could be used as parents to improve protein or oil amounts in soybean.

Conclusion: Nine protein QTL and five oil contents QTL were identified in this study, which included thirteen QTLs confirmed for authenticity by previous reports, and one newly discovered QTL. Our findings are useful for future research especially for researchers working on soybean genetics. One new QTL can be utilized for marker assisted breeding of soybean.

Author contribution statement: W.X. Li and H. Ning conceived and designed the experiments. A. A. Kaleri, S.

Xu, L. Li, Y. Zhang, W. Liu, C Jiang, Y. Zhang, C. Liu performed the field experiments and quality analysis. A. A. Kaleri and H. Ning analyzed and interpreted the results. A. A. Kaleri and H. Ning drafted and revised the manuscript. All authors edited the manuscript.

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REFERENCES

Bachlava, E., Dewey, R., Burton, J., Cardinal, A (2009). Mapping and comparison of quantitative trait loci for oleic acid seed content in two segregating soybean populations. Crop Sci. 49(2):433-442.

Boerma, H.R., Specht, J.E (2004). (Eds). SOYBEANS:Improvement, Production, and Uses. Third Edition.American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, ISBN 0-89118-154-7.

Brummer, E.C., Graef, G.L., Orf, J.H., Wilcox, J.R., Shoemaker, R.C (1997). Mapping QTL for seed protein and oil content in eight soybean populations. Crop Sci. 37(2):370-378.

Brummer, E. C., Bouton, J. H (1991). Plant traits associated with grazing-tolerant alfalfa. Agron. J., 83 (6): 996-1000.

Chung, J., Babka, H.L., Graef, G.L., Staswick, P.E., Lee, D.J., Cregan, P.B., Shoemaker, R.C., Specht, J.E (2003). The seed protein, oil, and yield QTL on soybean linkage group I. Crop Sci. 43(3):1053-1067.

Diers, B.W., Shoemaker, R.C (1992). Restriction fragment length polymorphism analysis of soybean fatty acid content. J. Am. Oil Chemists Soc. 69(12):1242-1247.

Doyle, J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12, 13-15.

Eskandari, M., Cober, E., Rajcan, I (2013). 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. Theor. Appl. Genet. 126(2):403-495.

Gai, J., Wang, Y., Wu, X., Chen, S (2007). A comparative study on segregation analysis and QTL mapping of quantitative traits in plants-with a case in soybean. Front. of Ag. in China. 1(1):1-7.

Ha, B., Kim, H., Velusamy, V., Vuong, T., Nguyen, H., Shannon, J., Lee, J (2014). Identification of quantitative trait loci controlling linolenic acid concentration in PI483463 (Glycine soja).Theor. Appl. Genet. 127(7):1501-1512.

Han, Y., Teng, W., Wang, Y., Zhao, X., Wu, L., Li, D., Li, W (2015). Unconditional and conditional QTL underlying the genetic interrelationships between soybean seed isoflavone, and protein or oil contents. Plant Breed. 134(3):300-309.

Hyten, D.L., Pantalone, V.R., Sams, C.E., Saxton, A.M., Landau-Ellis, D., Stefaniak, T.R., Schmidt, M.E (2004). Seed quality QTL in a prominent soybean population.Theor. Appl. Genet. 109(3):552-561.

Jun, T., Van, K, Kim, M, Lee, S, Walker, D (2008). Association analysis using SSR markers to find QTL for seed protein content in soybean. Euphytica. 162(2):179-191.

Kabelka, E.A., Diers, B.W., Fehr, W.R., Le, Roy., A.R., Baianu, I.C., You,T, Neece, D.J., Nelson, R.L (2004). Putative alleles for increased yield from soybean plant introductions. Crop Sci. 44(3):784-791.

Kaler, A.S., J.D. Gillman, T. Beissinger and L.C. Purcell (2020). Comparing different statistical models and multiple testing corrections for association mapping in soybean and maize. Front. Plant Sci. 10: 1794.

Kan, G., Zhang, X., Wang, Y., Zhang, W., Du, H., Yu, D (2016). Quantitative trait loci (QTL) Mapping for Glycinin and beta-Conglycinin contents in soybean (*Glycine max* L. Merr.). J. Agric. Food Chem. 64(17):3473-3483.

Kim, H., Kim, Y., Kim, S., Son, B., Choi, Y., Kang, J., Park, Y., Cho, Y., Choi, I (2010). Analysis of Quantitative Trait Loci (QTLs) for Seed Size and Fatty Acid Composition Using Recombinant Inbred Lines in Soybean. J. Life Sci. 20(8):1186-1192.

Lee, S., K. Van, M.Sung, R. Nelson, J. LaMantia, L.K, McHale and M.A.R. Mian (2019). Genome-wide association study of seed protein, oil and amino acid contents in soybean from maturity groups I to IV. Theor. Appl. Genet.123:1639–1659.

Lee, S.H., Bailey, M.A., Mian, M.A.R., Carter, T.E. Jr., Shipe, E.R., Ashley, D.A., Parrott, W.A., Hussey, R.S., Boerma, H.R (1996). RFLP loci associated with soybean seed protein and oil content across populations and locations. Theor. Appl. Genet. 93(5-6):649-657.

Li, D., Sun, M, Han, Y, Teng, W, Li, W (2010). Identification of QTL underlying soluble pigment content in soybean stems related to

resistance to soybean white mold (Sclerotinia sclerotiorum). Euphytica. 172(1):49-57.

Li, H., Zhao, T., Wang, Y., Yu, D., Chen, S., Zhou, R., 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. Euph. 182(1):117-132.

Liang, H., Yu, Y., Wang, S., Lian, Y., Wang, T., Wei, Y., Gong, P., Liu, X., Fang, X., Zhang, M (2010). QTL mapping of isoflavone, oil and protein contents in soybean (*Glycine max* L. Merr.). Ag. Sci. China 9(8):1108-1116.

Lu, W., Wen, Z., Li, H., Yuan, D., Li, J., Zhang, H., Huang, Z., Cui, S., Du, W (2013). Identification of the quantitative trait loci (QTL) underlying water soluble protein content in soybean.Theor. Appl. Gen. 126 (2) :425-33.

Mao, T., Jiang, Z, Han, Y, Teng, W, Zhao, X, Li, W (2013). Identification of quantitative trait loci underlying seed protein and oil contents of soybean across multi-genetic backgrounds and environments. Plant Breed , 132(6):630-641.

Ning, H., Yuan, J., Dong, Q., Li, W., Xue, H., Wang, Y., Tian, Y., Li, W. X (2018). Identification of QTLs relatedto the vertical distribution and seed-set of podnumber in soybean [*Glycine max* (L.) Merri]. PLoSONE 13(4) :1-15.

Nyquist, W. E., (1991). Estimation of heritability and prediction of selection response in plant populations Crit. Rev. Plant Sci. 10: 235–322.

O'Neill, C., M, Morgan., C, Kirby., J, Tscheop., H, Deng., PX, Brennan., M, Rosas., U, Fraser., F, Hall., C, Gill., S, Bancroft., I (2008). Six new recombinant inbred populations for the study of quantitative traits in Arabidopsis thaliana. Theor Appl Genet. 116: 623−634.

Orf, J.H., Chase, K., Jarvik, T., Mansur, L.M., Cregan, P.B., Adler, F.R., Lark, K.G (1999). Genetics of soybean agronomic traits: I. Comparison of three related recombinant inbred populations. Crop Sci. 39(6):1642-1651.

Pandurangan, S., Pajak, A., Molnar, S., Cober, E., Dhaubhadel, S., Hernandez-Sebastia, C., Kaiser, W., Nelson, R., Huber, S (2012). Marsolais, F. Relationship between asparagine metabolism and protein concentration in soybean seed. J. Exp. Bot. 63 (8):3173.

Panthee, D., Pantalone, V., Saxton, A., West, D., Sams, C (2006). Genomic regions associated with amino acid composition in soybean. Mol. Breed. 17(1):79-89.

Panthee, D.R., Pantalone, V.R., West, D.R., Saxton, A.M., Sams, C.E (2005). Quantitative trait loci for seed protein and oil concentration, and seed size in soybean. Crop Sci. 45(5):2015-2022.

Pathan, S., Vuong, T, Clark, K, Lee, J, Shannon, J, Roberts, C, Ellersieck, M, Burton, J, Cregan, P, Hyten, D, Nguyen, H, Sleper, D (2013). Genetic mapping and confirmation of quantitative trait loci for seed protein and oil contents and seed weight in soybean. Crop Sci. 53:765-777.

Qi, Z., Wu, Q., Han, X., Sun, Y., Du, X., Liu, C., Jiang, H., Hu, G., Chen, Q (2011). Soybean oil content QTL mapping and integrating with meta-analysis method for mining genes. Euphytica. 179:499-514.

Qiu, B.X., Arelli, P.R., Sleper, D.A (1999). RFLP markers associated with soybean cyst nematode resistance and seed composition in a 'Peking' x 'Essex' population. Theor. Appl. Genet. 98(3-4):356-364.

Reinprecht, Y., Poysa, V., Yu, K., Rajcan, I., Ablett, G., Pauls, K (2006). Seed and agronomic QTL in low linolenic acid, lipoxygenase-free soybean (Glycine max (L.) Merrill) germplasm. Genome. 49(12):1510-1527.

Rossi, M., Orf, J., Liu, L., Dong, Z., Rajcan, I (2013). Genetic basis of soybean adaptation to North American vs. Asian mega-environments in two independent populations from Canadian x Chinese crosses.Theor. Appl. Genet. 126(7):1809-1823.

Sams, C., Schmidt, M., Saxton, A., Pantalone, V., Hyten, D (2004). Molecular mapping and identification of soybean fatty acid modifier quantitative trait loci. J. Am Oil Chem. Soc. 81(12):1115-1118.

Scott, M.F., O. Ladejobi, S. Amer, A.R. Bentley, J. Biernaskie, S.A Boden and N. Fradgley (2020). Multi-parent populations in crops: a toolbox integrating genomics and genetic mapping with breeding. Heredity.1-21.

Sebolt, A.M., Shoemaker, R.C., Diers, B.W (2000). Analysis of a quantitative trait locus allele from wild soybean that increases seed protein concentration in soybean. Crop Sci. 40(5):1438-1444.

Shibata, M., Takayama, K., Ujiie, A., Yamada, T., Abe, J., Kitamura, K (2008). Genetic relationship between lipid content and linolenic acid concentration in soybean seeds. Breed. Sci. 58(4):361-366.

Simon, M., Loudet., O, Durand., S, Berard., A, Brunel., D, Sennesal., FX, Durand-Tardif., M, Pelletier., G, Camilleri., C (2008). Quantitative trait loci mapping in five new large recombinant inbred line populations of Arabidopsis thaliana genotyped with consensus single-nucleotide polymorphism markers. Genet Soc Am, 178: 2253−2264.

Song, Q., Jia G, Zhu Y, Grant D, Nelson RT, Hwang E-Y, Hyten DL,Cregan PB (2010). Abundance of

SSR motifs and development of candidate polymorphic markers (BARCSOYSSR_1.0) in soybean. Crop Sci 50:1950–1960.

Specht, J.E., Chase, K., Macrander, M., Graef, G.L., Chung, J., Markwell, J.P., Germann, M., Orf, J.H., Lark, K.G (2001). Soybean Response to Water:A QTL Analysis of Drought Tolerance. Crop Sci. 41(2):493-509.

Sun, Y., Luan, H, Qi, Z, Shan, D, Liu, C, Hu, G, Chen, Q, Qi, Z, Wu, Q, Han, X, Sun, Y, Du, X, Liu, C, Jiang, H, Hu, G, Chen, Q, Shan, D (2011). An Integrated Quantitative Trait Locus Map of Oil Content in Soybean, *Glycine max* (L.) Merr., Generated Using a Meta-Analysis Method for Mining Genes. Euphytica 2011, 179(3): 499-527.

Tajuddin, T., Watanabe, S., Yamanaka, N., Harada, K (2003). Analysis of quantitative trait loci for protein and lipid contents in soybean seeds using recombinant inbred lines. Breed. Sci. 53(2):133-140.

Tian, X., K. Zhang, S. Liu, X. Sun, X. Li, J. Song and S. Jiang (2020). Quantitative trait locus analysis of protein and oil content in response to planting density in soybean (Glycine max [L.]Merri.)seeds based on SNP linkage mapping. Front. Genet. 11.

Wang, JK., Li, HH., Zhang. Y and Meng, L (2014). Users' Manual of QTL IciMapping v4.0. Institute of Crop Science, CAAS, Beijing.

Wang, X., Jiang G-L, Green M, Scott R A, Song Q, Hyten D L,Cregan P B. (2014) . Identification and validation of quantitative trait loci for seed yield, oil and protein contents in two recombinant inbred line populations of soybean. Mol Genet Genomics, 289:935–949.

Wang, X., Jiang, G., Green, M., Scott, R., Hyten, D., Cregan, P (2012). Quantitative trait locus analysis of saturated fatty acids in a population of recombinant inbred lines of soybean. Mol. Breed. 30(2):1163-1179.

Wang, K., Yang, YW., Liu, B, Sanyal Zimmerman, R C., Yong, C., Bryan, RL., Angeline., P, Ryan A., Rajnish, AG., Wysocka, J., Ming, L., Dekker, J., Helms, JA and Howard, YC.(2011) . A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression.[J] Nature. (472):7341.

Warrington, C., Abdel-Haleem, H., Hyten, D., Cregan, P., Orf, J., Killam, A., Bajjalieh, N., Li, Z., Boerma, H (2015). QTL for seed protein and amino acids in the Benning _ Danbaekkong soybean population. Theor Appl Genet. 128(5):839-850.

Wilson, RF., Seed composition. In: Boerma RH, Specht JE (2004). (eds) Soybeans: improvement, production, and uses, 3rd edn. American Society of Agronomy, Madison. pp 621–669.

Xie, D., Han, Y., Zeng, Y., Cheng, W., Teng, W., Li, W (2012). SSR- and SNP-related QTL underlying linolenic acid and other fatty acid contents in soybean seeds across multiple environments. Mol. Breed. 30(1):169-179.

Xue, H., Tian, X., Zhang, K., Li, W., Qi, Z., Fang Y., Ning, H (2019). Mapping developmental QTL for plant height in soybean [Glycine max (L.) Merr.]using a four-way recombinant inbred line population. PloS one. 14:11.

Zhang, S., D. Hao, S. Zhang, D. Zhang, H. Wang, H. Du and D. Yu (2020). Genome-wide association mapping for protein, oil and water-soluble protein contents in soybean. Mol. Genet. Genom.1-12.

Zhang, X., A. Hina, S. Song, J.Kong, J.A Bhat and T. Zhao (2019). Whole-genome mapping identified novel "QTL hotspots regions" for seed storability in soybean (Glycine max L.). BMC genomics. 20(1): 499.

Zhu, X., W.L. Leiser, V. Hahn and T. Wurschum (2020). Identification of seed protein and oil related QTL in 944 RILs from a diallel of early-maturing European soybean. J. Crop. 1-10.

SUPPLEMENTARY INFORMATION

Table S1 Allelic genotype prediction of QTLs for oil and protein content in RIL3613.

Individual ID	*qpro-J*-1	*qpro-D*2-1	*qpro-L*-1	*qpro-I*-1	*qpro-O*-1	*qpro-B*1-1	*qoil-A*2-1	*qoil-A*2-1	*qoil-D*1*a*-1
RIL3613-001	qq	QQ	qq	QQ	qq	QQ	qq	QQ	QQ
RIL3613-002	qq	qq	QQ	qq	QQ	Qq	qq	qq	QQ
RIL3613-003	QQ	QQ	qq	qq	qq	Qq	QQ	QQ	QQ
RIL3613-004	QQ	QQ	qq	qq	QQ	QQ	qq	qq	qq
RIL3613-005	qq	QQ	qq	QQ	qq	Qq	qq	qq	qq
RIL3613-006	qq	QQ	qq	qq	qq	Qq	qq	qq	qq

RIL3613-007	qq	qq	qq	qq	QQ	QQ	qq	qq	qq
RIL3613-008	qq	QQ	qq	qq	QQ	QQ	QQ	QQ	qq
RIL3613-009	qq	qq	qq	qq	QQ	QQ	qq	qq	qq
RIL3613-010	qq	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-011	QQ	QQ	qq	qq	qq	QQ	qq	qq	QQ
RIL3613-012	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-013	qq	qq	QQ	qq	QQ	QQ	qq	qq	qq
RIL3613-014	qq	QQ	QQ	qq	qq	QQ	QQ	QQ	qq
RIL3613-015	qq	qq	qq	qq	qq	QQ	qq	qq	qq
RIL3613-016	QQ	QQ	QQ	QQ	qq	QQ	QQ	QQ	QQ
RIL3613-017	QQ	QQ	QQ	qq	QQ	QQ	qq	qq	QQ
RIL3613-018	qq	QQ	QQ	QQ	qq	Qq	qq	qq	QQ
RIL3613-019	QQ	QQ	QQ	qq	qq	Qq	qq	qq	QQ
RIL3613-020	QQ	QQ	qq	qq	QQ	Qq	qq	QQ	QQ
RIL3613-021	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-022	QQ	QQ	QQ	QQ	qq	Qq	QQ	QQ	qq
RIL3613-023	qq	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-024	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ	qq
RIL3613-025	QQ	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ
RIL3613-026	QQ	qq	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-027	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-028	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-029	qq	QQ	qq	QQ	QQ	QQ	qq	QQ	QQ
RIL3613-030	qq	QQ	qq	QQ	qq	QQ	qq	qq	QQ
RIL3613-031	qq	QQ	QQ	QQ	qq	Qq	QQ	QQ	qq
RIL3613-032	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-033	QQ	qq	QQ	qq	QQ	QQ	qq	qq	QQ
RIL3613-034	QQ	QQ	QQ	qq	QQ	Qq	qq	qq	qq
RIL3613-035	qq	QQ	QQ	qq	qq	QQ	qq	qq	QQ
RIL3613-036	qq	QQ	QQ	QQ		QQ	qq		QQ
RIL3613-037	qq	QQ	qq	qq	QQ	QQ	QQ	qq	QQ
RIL3613-038	qq	QQ	qq	qq	QQ	QQ	qq	qq	QQ
RIL3613-039	QQ	QQ	qq	QQ	qq	QQ	QQ	qq	qq
RIL3613-040	QQ	QQ	qq	QQ	qq	QQ	QQ	QQ	QQ
RIL3613-041	QQ	QQ	qq	QQ	QQ	QQ	qq	QQ	qq
RIL3613-042	QQ	QQ	qq	QQ	QQ	Qq	qq	QQ	QQ
RIL3613-043	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-044	QQ	qq	QQ	QQ	QQ	QQ	qq	QQ	QQ
RIL3613-045	qq	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-046	qq	qq	qq	QQ	QQ	QQ	QQ	qq	QQ
RIL3613-047	qq	QQ	qq	qq	QQ	QQ	qq	QQ	QQ
RIL3613-048	qq	QQ	qq	qq	QQ	QQ	QQ	qq	QQ
RIL3613-049	qq	QQ	qq	qq		QQ	qq		qq
RIL3613-050	qq	qq	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-051	qq	qq	QQ	QQ	QQ	Qq	QQ	QQ	QQ
RIL3613-052	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-053	qq	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ

RIL3613-054	QQ	qq	qq	qq	QQ	Qq	qq	qq	QQ
RIL3613-055	QQ	qq	QQ	qq		QQ	QQ		qq
RIL3613-056	qq	qq	qq	qq	QQ	Qq	qq	QQ	qq
RIL3613-057	QQ	qq	QQ	qq	QQ	Qq	qq	qq	QQ
RIL3613-058	QQ	qq	qq	qq	QQ	QQ	QQ	qq	QQ
RIL3613-059	QQ	qq	QQ	QQ	QQ	Qq	QQ	QQ	QQ
RIL3613-060	qq	qq	qq	qq	QQ	QQ	qq	qq	qq
RIL3613-061	QQ	qq	QQ	QQ		QQ	qq		QQ
RIL3613-062	QQ	qq	qq	qq	QQ	qq	QQ	qq	QQ
RIL3613-063	QQ	qq	qq	qq		qq	QQ		qq
RIL3613-064	QQ	qq	qq	QQ		qq	QQ		QQ
RIL3613-065	QQ	qq	qq	qq		qq	QQ		QQ
RIL3613-066	qq	QQ	QQ	QQ	QQ	qq	QQ		QQ
RIL3613-067	QQ	QQ	QQ	qq	QQ	qq	QQ	qq	QQ
RIL3613-068	QQ	QQ	QQ	QQ	QQ	qq	QQ	QQ	QQ
RIL3613-069	qq	QQ	QQ	QQ	QQ	qq	QQ	qq	qq
RIL3613-070	QQ	QQ	qq	QQ	QQ	qq	qq	QQ	QQ
RIL3613-071	QQ	qq	qq	QQ		qq	qq	qq	qq
RIL3613-072	QQ	qq	QQ	QQ	QQ	qq	qq	QQ	qq
RIL3613-073	QQ	QQ	qq	QQ	QQ	QQ	qq	QQ	QQ
RIL3613-074	QQ	QQ	qq	qq	qq	qq	QQ	qq	QQ
RIL3613-075	QQ	qq	QQ	QQ	QQ	QQ	qq	qq	qq
RIL3613-076	QQ	QQ	qq	QQ	QQ	qq	qq	qq	qq
RIL3613-077	qq	QQ	QQ	QQ	QQ	qq	QQ	qq	qq
RIL3613-078	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-079	QQ	QQ	qq	QQ	QQ	QQ	QQ	qq	qq
RIL3613-080	QQ	QQ	QQ	QQ	QQ	QQ	QQ	qq	QQ
RIL3613-081	QQ	qq	QQ	QQ	QQ	qq	QQ	qq	qq
RIL3613-082	QQ	QQ	qq	qq	QQ	QQ	qq	QQ	QQ
RIL3613-083	qq	qq	QQ	QQ	QQ	QQ	qq	qq	QQ
RIL3613-084	QQ	QQ	QQ	qq	QQ	QQ	QQ	QQ	qq
RIL3613-085	qq	QQ	qq	QQ	qq	qq	qq	QQ	qq
RIL3613-086	QQ	qq	qq	QQ	QQ	QQ	QQ	QQ	qq
RIL3613-087	QQ	qq	qq	QQ	QQ	QQ	qq	qq	qq
RIL3613-088	QQ	qq	QQ	QQ	qq	qq	qq	QQ	QQ
RIL3613-089	QQ	QQ	QQ	QQ	qq	QQ	QQ	qq	QQ
RIL3613-090	QQ	qq	qq	QQ	QQ	QQ	qq	QQ	qq
RIL3613-091	QQ	QQ	QQ	QQ	QQ	QQ	qq	QQ	QQ
RIL3613-092	QQ	QQ	qq	qq	QQ	QQ	QQ	QQ	QQ
RIL3613-093	qq	qq	QQ	QQ	QQ	qq	QQ	QQ	QQ
RIL3613-094	QQ	QQ	QQ	QQ	qq	QQ	QQ	qq	QQ
RIL3613-095	QQ	QQ	qq	qq	QQ	QQ	qq	qq	qq
RIL3613-096	QQ	qq	qq	QQ	QQ	qq	QQ	QQ	QQ
RIL3613-097	QQ	qq	qq	qq	QQ	QQ	QQ	QQ	QQ
RIL3613-098	QQ	QQ	qq	QQ	QQ	QQ	QQ	qq	QQ
RIL3613-099	QQ	QQ	qq	QQ	QQ	QQ	qq	qq	QQ
RIL3613-100	QQ	QQ	qq	qq	QQ	QQ	qq	qq	QQ

RIL3613-101	QQ	QQ	qq	QQ	QQ	QQ	qq	qq	QQ
RIL3613-102	QQ	QQ	qq	QQ		qq	qq		QQ
RIL3613-103	QQ	QQ	QQ	QQ		QQ	qq		QQ
RIL3613-104	QQ	QQ	QQ	qq	QQ	qq	qq	QQ	QQ
RIL3613-105	qq	QQ	qq	QQ		QQ	qq		QQ
RIL3613-106	QQ	qq	QQ	QQ		QQ	qq		QQ
RIL3613-107	QQ	QQ	QQ	QQ	qq	QQ	qq	qq	QQ
RIL3613-108	QQ	qq	QQ	qq	QQ	QQ	qq	QQ	QQ
RIL3613-109	QQ	qq	qq	QQ	QQ	qq	qq	qq	qq
RIL3613-110	QQ	qq	qq	QQ	qq	qq	QQ	QQ	QQ
RIL3613-111	QQ	qq	qq	qq	QQ	qq	QQ	qq	QQ
RIL3613-112	QQ	QQ	qq	QQ	QQ	qq	QQ	QQ	qq
RIL3613-113	QQ	qq	qq	QQ	qq	QQ	QQ	qq	QQ
RIL3613-114	QQ	qq	QQ	QQ	qq	qq	QQ	QQ	qq
RIL3613-115	QQ	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-116	qq	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-117	QQ	QQ	QQ	qq		QQ	qq		qq
RIL3613-118	QQ	qq	QQ	qq		qq	QQ		qq
RIL3613-119	QQ	qq	qq	qq		qq	QQ		QQ
RIL3613-120	QQ	qq	QQ	QQ		qq	QQ		QQ
RIL3613-121	QQ	QQ	QQ	QQ		qq	QQ		QQ
RIL3613-122	QQ	QQ	QQ	QQ		qq	QQ		qq
RIL3613-123	QQ	qq	QQ	QQ		qq	QQ		qq
RIL3613-124	QQ	QQ	QQ	QQ		QQ	QQ		QQ
RIL3613-125	QQ	QQ	QQ	qq	QQ	qq	QQ	QQ	QQ
RIL3613-126	QQ	QQ	QQ	QQ	qq	qq	qq	qq	qq
RIL3613-127	QQ	QQ	QQ	QQ	qq	QQ	QQ	QQ	qq
RIL3613-128	QQ	QQ	QQ	QQ	QQ	QQ	QQ	QQ	qq
RIL3613-129	QQ	QQ	QQ	QQ	QQ	qq	Qq	qq	QQ
RIL3613-130	QQ	QQ	QQ	QQ	QQ	QQ	Qq	qq	QQ
RIL3613-131	QQ	qq	qq	qq	QQ	QQ	Qq	qq	QQ
RIL3613-132	QQ	qq	QQ	QQ	QQ	QQ	QQ	QQ	QQ
RIL3613-133	QQ	qq	qq	QQ	QQ	qq	QQ	QQ	QQ
RIL3613-134	QQ	qq	qq	QQ	QQ	qq	QQ	QQ	QQ

Note: Boldface indicates the individuals carrying optimal allelic genotypes. QQ indicate allelic genotypes from female parents; qq indicate allelic genotypes from male parents.

Table S2 Allelic genotype prediction of QTLs for oil and protein content in RIL6013.

Individual ID	*qpro-O*-2	*qpro-F*-1	*qoil-C*1-1	*qoil-H*-1	*qoil-D*2-1
RIL6013-001	QQ	QQ	QQ	QQ	QQ
RIL6013-002	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-003	Qq	qq	QQ	QQ	Qq
RIL6013-004	QQ	QQ	QQ	QQ	Qq
RIL6013-005	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-006	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-007	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-008	Qq	qq	QQ	QQ	QQ

RIL6013-009	Qq	qq	QQ	Qq	QQ
RIL6013-010	Qq	QQ	QQ	QQ	Qq
RIL6013-011	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-012	QQ		QQ	qq	QQ
RIL6013-013	Qq	qq	qq	QQ	QQ
RIL6013-014	Qq	qq	QQ	QQ	QQ
RIL6013-015	Qq	qq	QQ	QQ	Qq
RIL6013-016	Qq	qq	qq	QQ	QQ
RIL6013-017	Qq	qq	QQ	QQ	QQ
RIL6013-018	Qq	QQ	QQ	QQ	Qq
RIL6013-019	Qq	qq	QQ	QQ	QQ
RIL6013-020	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-021	QQ	QQ	QQ	QQ	QQ
RIL6013-022	Qq	QQ	QQ	QQ	QQ
RIL6013-023	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-024	Qq	QQ	QQ	QQ	QQ
RIL6013-025	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-026	QQ	QQ	QQ	QQ	QQ
RIL6013-027	QQ	QQ	qq	QQ	QQ
RIL6013-028	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-029	QQ	QQ	QQ	QQ	QQ
RIL6013-030	QQ	QQ	QQ	QQ	QQ
RIL6013-031	QQ	QQ	QQ	qq	Qq
RIL6013-032	QQ	QQ	**qq**	**qq**	**Qq**
RIL6013-033	**QQ**	**qq**	qq	qq	QQ
RIL6013-034	QQ	QQ	QQ	QQ	QQ
RIL6013-035	QQ	QQ	QQ	QQ	QQ
RIL6013-036	**QQ**	**qq**	qq	QQ	Qq
RIL6013-037	QQ	QQ	QQ	QQ	Qq
RIL6013-038	QQ		QQ	QQ	QQ
RIL6013-039	QQ	QQ	qq	QQ	QQ
RIL6013-040	**QQ**	**qq**	QQ	qq	QQ
RIL6013-041	QQ		QQ	QQ	QQ
RIL6013-042	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-043	**QQ**	**qq**	QQ	QQ	Qq
RIL6013-044	QQ		QQ	qq	Qq
RIL6013-045	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-046	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-047	QQ	QQ	QQ	QQ	QQ
RIL6013-048	QQ		QQ	QQ	QQ
RIL6013-049	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-050	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-051	QQ	QQ	QQ	qq	Qq
RIL6013-052	QQ	QQ	QQ	QQ	QQ
RIL6013-053	QQ	QQ	QQ	QQ	QQ
RIL6013-054	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-055	Qq	QQ	QQ	QQ	QQ

RIL6013-056	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-057	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-058	QQ	QQ	QQ	QQ	QQ
RIL6013-059	Qq	qq	QQ	QQ	QQ
RIL6013-060	QQ	QQ	QQ	qq	QQ
RIL6013-061	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-062	**QQ**	**qq**	QQ	qq	QQ
RIL6013-063	QQ	QQ	QQ	QQ	QQ
RIL6013-064	Qq	qq	qq	qq	QQ
RIL6013-065	QQ	QQ	qq	qq	QQ
RIL6013-066	Qq	QQ	qq	qq	QQ
RIL6013-067	QQ	QQ	QQ	QQ	QQ
RIL6013-068	**QQ**	**qq**	QQ	qq	QQ
RIL6013-069	QQ	QQ	qq	QQ	QQ
RIL6013-070	QQ		QQ	QQ	QQ
RIL6013-071	QQ		QQ	QQ	QQ
RIL6013-072	Qq	QQ	QQ	QQ	QQ
RIL6013-073	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-074	QQ	QQ	QQ	qq	QQ
RIL6013-075	Qq	QQ	QQ	qq	Qq
RIL6013-076	Qq	qq	QQ	qq	QQ
RIL6013-077	QQ	QQ	QQ	QQ	QQ
RIL6013-078	Qq		QQ	QQ	QQ
RIL6013-079	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-080	QQ	QQ	QQ	qq	Qq
RIL6013-081	QQ	QQ	QQ	QQ	QQ
RIL6013-082	Qq		qq	qq	QQ
RIL6013-083	Qq	QQ	QQ	qq	Qq
RIL6013-084	Qq	qq	QQ	QQ	QQ
RIL6013-085	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-086	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-087	Qq	QQ	QQ	qq	QQ
RIL6013-088	QQ	QQ	qq	QQ	QQ
RIL6013-089	**QQ**	**qq**	QQ	qq	QQ
RIL6013-090	QQ	QQ	qq	QQ	Qq
RIL6013-091	QQ	QQ	qq	qq	QQ
RIL6013-092	QQ	QQ	QQ	QQ	QQ
RIL6013-093	Qq	qq	QQ	QQ	QQ
RIL6013-094	Qq	QQ	QQ	QQ	QQ
RIL6013-095	Qq	QQ	qq	QQ	QQ
RIL6013-096	**QQ**	**qq**	QQ	QQ	Qq
RIL6013-097	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-098	QQ		QQ	qq	QQ
RIL6013-099	**QQ**	**qq**	qq	QQ	QQ
RIL6013-100	**QQ**	**qq**	QQ	QQ	Qq
RIL6013-101	**QQ**	**qq**	QQ	QQ	Qq
RIL6013-102	QQ		QQ	QQ	Qq

RIL6013-103	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-104	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-105	Qq	QQ	QQ	qq	Qq
RIL6013-106	Qq	qq	QQ	QQ	QQ
RIL6013-107	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-108	**QQ**	**qq**	QQ	qq	QQ
RIL6013-109	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-110	Qq	QQ	QQ	QQ	Qq
RIL6013-111	Qq	qq	QQ	QQ	Qq
RIL6013-112	Qq		QQ	QQ	QQ
RIL6013-113	**QQ**	**qq**	qq	QQ	QQ
RIL6013-114	QQ	QQ	**qq**	**qq**	**Qq**
RIL6013-115	QQ	QQ	QQ	QQ	QQ
RIL6013-116	QQ	QQ	qq	qq	QQ
RIL6013-117	Qq	qq	qq	qq	QQ
RIL6013-118	QQ	QQ	QQ	qq	Qq
RIL6013-119	QQ	QQ	QQ	QQ	Qq
RIL6013-120	QQ	QQ	QQ	QQ	QQ
RIL6013-121	**QQ**	**qq**	QQ	QQ	Qq
RIL6013-122	QQ	QQ	QQ	QQ	QQ
RIL6013-123	QQ	QQ	QQ	QQ	QQ
RIL6013-124	Qq	QQ	QQ	QQ	QQ
RIL6013-125	QQ	QQ	QQ	QQ	Qq
RIL6013-126	QQ	QQ	QQ	qq	QQ
RIL6013-127	QQ	QQ	qq	QQ	QQ
RIL6013-128	Qq	qq	QQ	QQ	Qq
RIL6013-129	Qq	qq	qq	QQ	QQ
RIL6013-130	Qq		QQ	QQ	Qq
RIL6013-131	QQ	QQ	QQ	qq	QQ
RIL6013-132	**QQ**	**qq**	qq	QQ	QQ
RIL6013-133	QQ	QQ	QQ	qq	QQ
RIL6013-134	QQ	QQ	qq	QQ	QQ
RIL6013-135	**QQ**	**qq**	qq	qq	QQ
RIL6013-136	Qq	QQ	qq	QQ	QQ
RIL6013-137	**QQ**	**qq**	qq	QQ	Qq
RIL6013-138	Qq		qq	QQ	QQ
RIL6013-139	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-140	QQ	QQ	QQ	QQ	Qq
RIL6013-141	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-142	Qq	QQ	qq	qq	QQ
RIL6013-143	QQ	QQ	QQ	QQ	QQ
RIL6013-144	**QQ**	**qq**	QQ	QQ	QQ
RIL6013-145	QQ		QQ	QQ	QQ
RIL6013-146	**QQ**	**qq**	QQ	QQ	Qq
RIL6013-147	Qq	qq	**qq**	**qq**	**Qq**
RIL6013-148	Qq	qq	QQ	QQ	QQ
RIL6013-149	**QQ**	**qq**	QQ	qq	Qq

RIL6013-150	Qq	QQ	QQ	QQ	QQ
RIL6013-151	QQ	QQ	QQ	QQ	QQ
RIL6013-152	Qq	qq	QQ	QQ	Qq
RIL6013-153	Qq	qq	QQ	qq	QQ
RIL6013-154	**QQ**	**qq**	QQ	qq	QQ
RIL6013-155	Qq	qq	QQ	qq	QQ
RIL6013-156	QQ	QQ	qq	QQ	QQ

Note: Boldface indicates the individuals carrying optimal allelic genotypes. QQ indicate allelic genotypes from female parents; qq indicate allelic genotypes from male parents.