

## Quantitative Trait Loci Associated with Moisture, Protein, and Oil Content in Soybean [*Glycine max* (L.) Merr.]

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### Abstract

The objectives of this study were to detect quantitative trait loci (QTL) for moisture, protein, and oil content in soybean grown in two plant density environments. Soybean recombinant inbred lines (RIL), obtained from a cross between cultivars PI 438489B and Hamilton, were used. Thirty one linkage groups were obtained from high density genetic linkage maps constructed by 1,536 Universal Soy Linkage Panel 1.0 of single nucleotide polymorphisms (SNP) markers. For the lines cultivated in higher plant density (25 cm row space), ten QTLs were mapped in A2, B2, C2, D1a, M and O linkage groups for moisture, protein, and oil. Two QTLs for moisture, two QTLs for protein and six QTLs for oil explained 0.09, 15.36 and 3.54% of the phenotypic variation of moisture, protein, and oil respectively. For the lines cultivated in lower density (50 cm row space), three QTLs for each of moisture and protein were mapped in linkage groups A2, C1, D1b, L and O; QTLs for moisture and protein explained 0.96 and 10.63% of the phenotypic variation respectively. These QTL will facilitate the implementation of MAS for moisture, protein and oil content in soybean-breeding programs.

**Keywords:** Soybean seed, SNP, QTL, moisture, protein, oil, PI 438489B and Hamilton

### 1. Introduction

Soybean (*Glycine max* L. Merr.) is one of the world's major crops grown mainly for protein and oil. On average, seed of current USA leading soybean cultivars contains approximately 41% protein and 21% oil on a zero percent dry weight basis (Hartwig & Kilen, 1991). Seed moisture also is an important factor affecting qualities of soybeans. However, the precise composition of soybean seed depends on genotype, planting date, soil, seasonal weather factors, and growing condition (Chen, Lai, Cheng, & Shanmugasundaram, 1991). Growing conditions, especially the density of plants' effects on soybean oil and protein contents (Rahman, Mwakangwale, Hampton, & Hill, 2005; Yin & Vyn, 2005). Soybean in the former is primarily used as feed, with some food applications, while the latter is more broadly incorporated into food, feed, and biodiesel (Clemente & Cahoon, 2009). An increase in seed protein and oil concentrations in commonly grown cultivars is needed to keep soybean competitive as a human food and livestock feed.

In soybean, the majority of agronomic traits, such as abiotic stress, biological stress, protein concentration, oil content and yield, are quantitative traits controlled by multiple genes (Guohua et al., 2011). Polygenic quantitative traits result from interactions between multiple genes and the environment. Detailed genetic analysis of these traits has become possible by the availability of a large number of molecular markers to which quantitative trait loci (QTL) can be linked (Dudley, 1993). In addition, estimation of trait values can be greatly improved using recombinant inbred (RI) populations (Carrillo, Rousset, Qualset, & Kasarda, 1990), with use of such a population. Trait values can be measured repeatedly in different environments (Lark, Chase, Adler, Mansur, & Orf, 1995).

Most of the QTLs in soybean were detected from the genetic linkage maps constructed with RFLPs, AFLPs, RAPDs, and SSRs; however, the genetic maps in soybean have not been exclusively SNP-based but contain

SNPs anchored with other markers such as RFLPs, AFLPs, RAPDs, and SSRs except of a recent high density soybean SNP-based map containing 1,790 SNPs (Hyten et al., 2010a). The ideal marker for anchoring and orienting the soybean genome is the single nucleotide polymorphism (SNP), primarily because SNPs are the most abundant marker available. Cultivated soybean (*Glycine max* L. Merr.) has nucleotide diversity ( $\theta$ ) of about 0.001 (Choi et al., 2007; Zhu et al., 2003), which translates into an average SNP frequency of one SNP per 1000 bp of contiguous sequence. Another advantage of SNPs is the wide array of currently available technologies for performing multiplex assays that can range from genotyping a few SNPs at a time to over 1 million SNPs in parallel (Fan, Chee, & Gunderson, 2006). The reliability and rapidity of this assay were recently documented with soybean SNPs (Choi et al., 2007; Hyten et al., 2008).

In this study SNPs based genetic linkage maps was used with aimed to detect QTLs for moisture, protein, and oil contents using the soybean cultivars PI 438489B, 'Hamilton' and their recombinant inbred lines (RILs) cultivated under two different plant densities.

## 2. Materials and Methods

### 2.1 Plant Material

In this study, a recombinant inbred line (RIL) populations (n=50) derived from a cross between PI 438489B and Hamilton, were used. The cross was performed at the University of Missouri Agronomy Research Center (Yue, Arelli, & Sleper, 2001) and advanced to the F<sub>6:13</sub> generation by Dr. Silvia Cianzio at the Iowa State University research site at the Isabela Sub-station of University of Puerto Rico. Hamilton was developed at the Illinois Agricultural Experiment Station and released for its high-yield performance (Nickell, Thomas, & Stephens, 1990).

### 2.2 Plants Cultivation Conditions and Traits Measurements

Seeds of the parental cultivars (PI 438489B and 'Hamilton') and RILs were sown in potting soil in pots (15x14 cm) and kept in the greenhouse at 25±10°C under natural daylight for 3 weeks. After that, the plants were divided into 2 groups, Group I and II were planted maintained at 25 cm and 50 cm row space respectively in a field in St Pauls, NC (Bladen County). Cultivation condition at the 25 cm row space generated a density of 16 seeds/plants m<sup>-2</sup> (160,000 plants ha<sup>-1</sup>) and the 50 cm row space generated 9 seeds/plant m<sup>-2</sup> (90,000 plants ha<sup>-1</sup>). Plants were allowed to grow under irrigated conditions until maturity, after which seed samples were collected from single plants of two plant densities plots. Seed moisture, protein and oil contents were determined at the Identity Preserved Grain (IPG) Laboratory of the Illinois Crop Improvement Association (IL Crop). Measurements were taken on a 'as is' for moisture and dry basis (0% moisture) for protein and oil using near-infrared reflectance spectroscopy. Correlation and analysis of variance were performed from row both 25 and 50 cm spacing data. All statistical analyses were performed on JMP 9.0 (SAS Institute Inc., Cary, NC, USA).

### 2.3 SNP Genotyping, Genetic Map Construction and QTL Mapping

The 1,536 Universal Soy Linkage Panel 1.0 (Hyten et al., 2010b) were used to screen the 50 RILs. The GoldenGate assay was performed as per the manufactures protocols and as described previously by Fan et al. (2003) and Hyten et al. (2008). The Illumina BeadStation 500G (Illumina Inc., San Diego, CA) was used for genotyping the GoldenGate assay. The automatic allele calling for each locus was accomplished with the BeadStudio version 3.2 software (Illumina Inc., San Diego, CA). All BeadStudio data for the 1,536 SNPs were visually inspected and rescored, if any errors in calling the homozygous or heterozygous clusters were evident. The genetic map was constructed using Join-Map 4.0 software (Feltus et al., 2010). The SNP markers were initially grouped and assigned to the soybean chromosomes based on their mapped position on the soybean Consensus 4.0 map (Hyten et al., 2010b). We used the regression mapping algorithm with the default parameters and Kosambi's mapping function to determine map order and genetic distances. For mapping QTL and estimating their effects, com-posite interval mapping (CIM) was performed using the software, Win-QTL Cartographer, version 2.5 (Wang, Basten, & Zeng, 2005). The Model 6 and its default settings were adopted. Experimental-wise LOD cutoff values for declaring QTL significance at  $P \leq 0.05$  were established by performing 1,000 permutations.

## 3. Results

Average values of moisture, protein, and oil contents of the parents and RILs cultivated in the two different plant densities (160,000 plants ha<sup>-1</sup> / 25 cm row space vs. 90,000 plants ha<sup>-1</sup> / 50 cm row space) is presented in Table 1. At higher plant density (50 cm row space), the mean values of the RILs were lower from the mid-parental values for moisture, protein and higher for oil, but in lower plant density (50 cm row space), the mean values of the

RILs were higher from the mid-parental values for moisture, protein and lower for oil. Among RILs of soybeans cultivated in lower density had 0.09%, 15.36% and 3.54% coefficient of variation (CV) for moisture protein and oil content, whereas the variations for the traits were at higher density 0.12%, 10.63 and 5.60% (Table 1) respectively. Protein content of RIL differs significantly ( $p < 0.001$ ) between two plant densities and differences for moisture and oil content were not significant (Figure 1). Interestingly protein content was highly negatively correlated ( $r = -0.996$ ,  $P < 0.0001$ ; data not shown) with oil content at higher plant density, but it was not negative at lower plant density.

Table 1. Mean values and co-efficient of variance for moisture, protein and oil contents of PI 438489B by Hamilton mid parent (MP) and recombinant inbred lines (RILs) from the plant cultivated in two plant densities

Traits	Higher plant density/ 25 cm Row Space					Lower plant density /50 cm Row Space				
	MP	RILs				MP	RILs			
		Mean	Std Dev	Range	CV%		Mean	Std Dev	Range	CV%
Moisture	7.25	6.91	0.30	1.03	0.09	6.20	6.80	0.35	1.78	0.12
Protein	46.66	44.98	3.92	14.9	15.36	41.25	49.08	2.65	16.43	10.63
Oil	16.32	17.41	1.88	9.28	3.54	20.57	16.96	2.20	10.3	5.60

The parents PI 438489B and Hamilton were screened with the 1536 SNP markers of which 679 SNPs were characterized as polymorphic between the two lines. Those markers were used to construct the genetic linkage map. It was found that the map contained 31 linkage groups (LG) with 648 linked SNP markers (Kassem et al. 2012a) and 31 unlinked. The map coverage was 1,524.7 cM. The average distance between markers was 2.35 cM (Kassem et al., 2012a). Data of SNP genetic linkage map was analyzed with composite interval mapping (CIM) using WinQTLCart (Version 2.5\_009) for identify QTL of moisture, protein and oil contents. A total of 16 QTL for moisture, protein and oil on nine different chromosomes or LGs were identified and are presented in Table 2 & Figure 2.

Table 2. The sixteen QTLs that underlie moisture, protein and oil detected in the soybean PI 438489B by Hamilton RIL populations. CIM QTL analysis was performed on average trait value from two plant densities (higher-25 cm and lower – 50 cm row space) using the WinQTL Cartographer, and reported with LOD scores equal or greater than 2.5. QTL were named according to the Soybean Genetics Committee recommendations as revised in March 2007 (<http://soybase.org/resources/QTL.php>)

Trait	No.	Chr./LG	QTL	Marker / Interval	Position (cM)	LOD	R <sup>2</sup> (%)
Moisture 25 cm	1	Chr_8/A2	qMOIST001	SS107919504-SS107925080	92.0-97.6	2.87	0.15
	2	Chr_10/O	qMOIST002	SS107912744-SS107920438	41.7-45.4	2.71	0.33
Moisture 50 cm	3	Chr_4/C1	qMOIST003	SS107929551-SS107919464	56.4-63.3	3.75	0.26
	4	Chr_8/A2	qMOIST004	SS107912848-SS107923737	27.1-35.7	2.78	0.15
	5	Chr_19/L	qMOIST005	SS107912667-SS107920213	10.8-19.2	2.99	0.19
Protein 25 cm	6	Chr_6/C2	qPRO001	SS107924435-SS107925078	39.9-43.7	3.31	0.17
	7	Chr_10/O	qPRO002	SS4969617-SS107912744	18.4-41.4	3.43	0.18
Protein 50 cm	8	Chr_2/D1b	qPRO003	SS107913858-SS107929550	21.8-25.9	3.07	0.23
	9	Chr_2/D1b	qPRO004	SS107913056-SS107913946	42.4-49.7	3.92	0.23
	10	Chr_19/L	qPRO005	SS107912667-SS107913748	11.4-20.1	2.62	0.16
Oil 25 cm	11	Chr_1/D1a	qOIL001	SS107915391-SS107917465	00.0-2.00	2.61	0.07
	12	Chr_7/M	qOIL002	SS107918251-SS107913778	24.5-33.4	6.20	0.20
	13	Chr_7/M	qOIL003	SS107928971-SS107912841	61.7-73.0	4.52	0.20
	14	Chr_8/A2	qOIL004	SS107919504-SS107925080	93.4-98.6	3.75	0.13
	15	Chr_8/A2	qOIL005	SS107927037-SS107923735	99.5-105.8	3.54	0.13
	16	Chr_14/B2	qOIL006	SS107913527-SS107930533	2.50-8.90	3.13	0.09

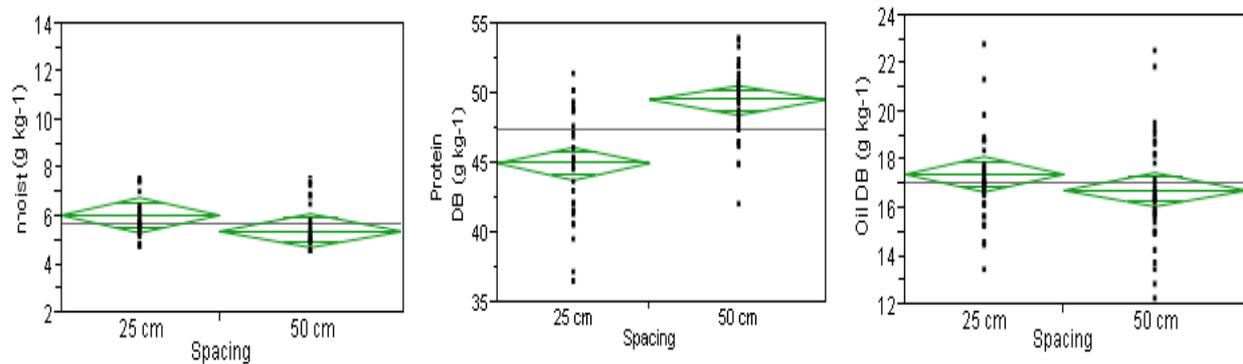


Figure 1. Average moisture, protein and oil content ( $\text{g kg}^{-1}$ ) of a soybean recombinant inbred line population, PI438489B x Hamilton cultivated under two different plant densities spacing systems (higher-25 cm and lower-50 cm row space). Difference is significant at  $P < 0.001$  for protein but differences are not significant for moisture and oil

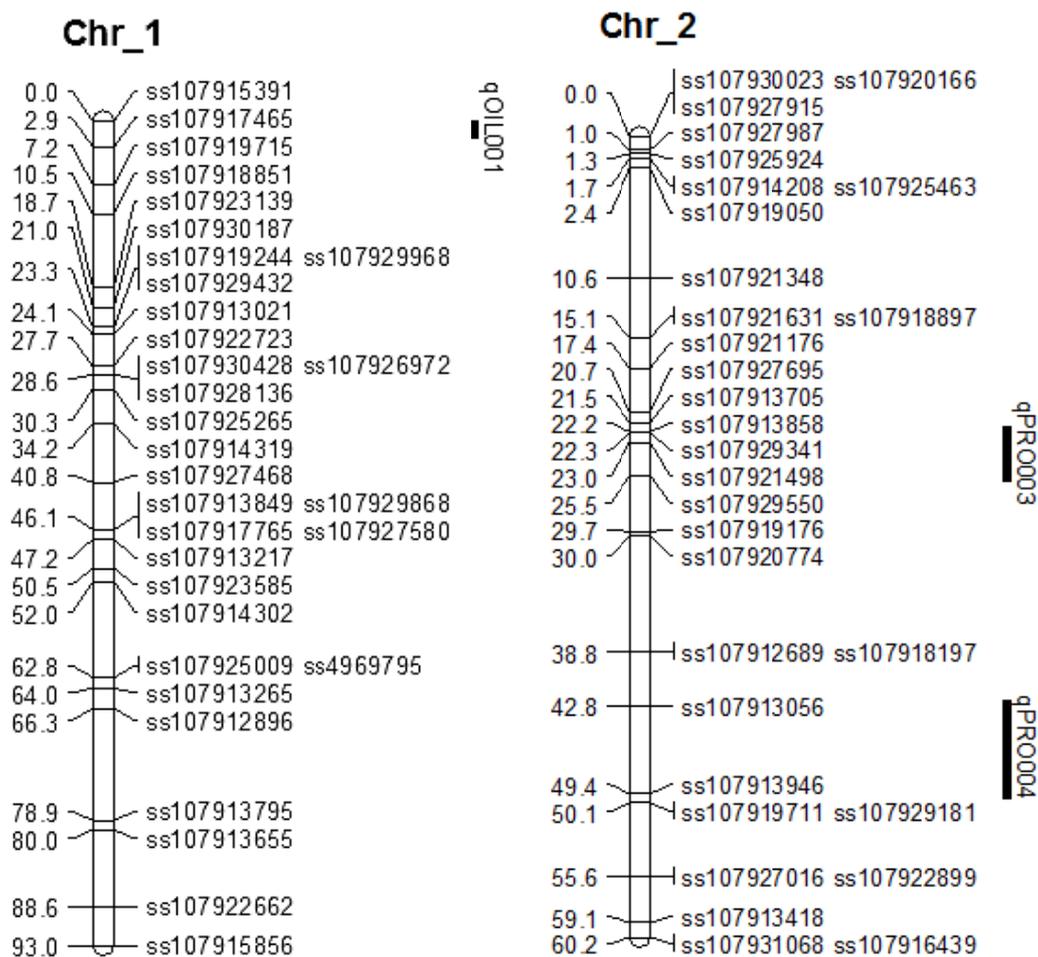
Five significant QTL for moisture were identified through composite interval mapping in the RILs population. Two QTLs (qMOIST001- qMOIST002) for moisture in higher plant density (25 cm row spacing) were identified on chromosomes 8 (Chr\_8/ LG A2) and Chr\_10 (LG O) and three QTL (qMOIST003- qMOIST005) for moisture in lower plant density (50 cm row spacing) were identified on Chr\_4 (LG C1), Chr\_8 (LG A2) and Chr\_19 (LG L). There is also a total of five seed protein concentration QTLs that were identified in the RIL population. Two protein QTLs (qPRO001-qPRO002) were identified on Chr\_6 (LG C2) and Chr\_10 (LG O) at higher plant density, whereas there QTLs (qPRO003-qPRO005) were identified on Chr\_2 (LG D1b) and Chr\_19 (LG L) in lower plant density. In total six QTLs oil contents (*qOIL001- qOIL006*) were identified in higher plant density on Chr\_1 (LG D1a), Chr\_7 (LG M), Chr\_8 (LG A2) and Chr\_14 ((LG B2) but no QTL for oil content was detected for planting soybeans at lower plant density. Among 31 linkage groups, 9 contained QTLs for moisture, protein and oil. LG A2 (Chr\_8) contained four QTLs (2 for moisture and 2 for oil); LG D1b (Chr\_2), LG M (Chr\_7), LG O (Chr\_10), and LG L (Chr\_19) each contained two of QTLs and rest LGs contained one QTL each.

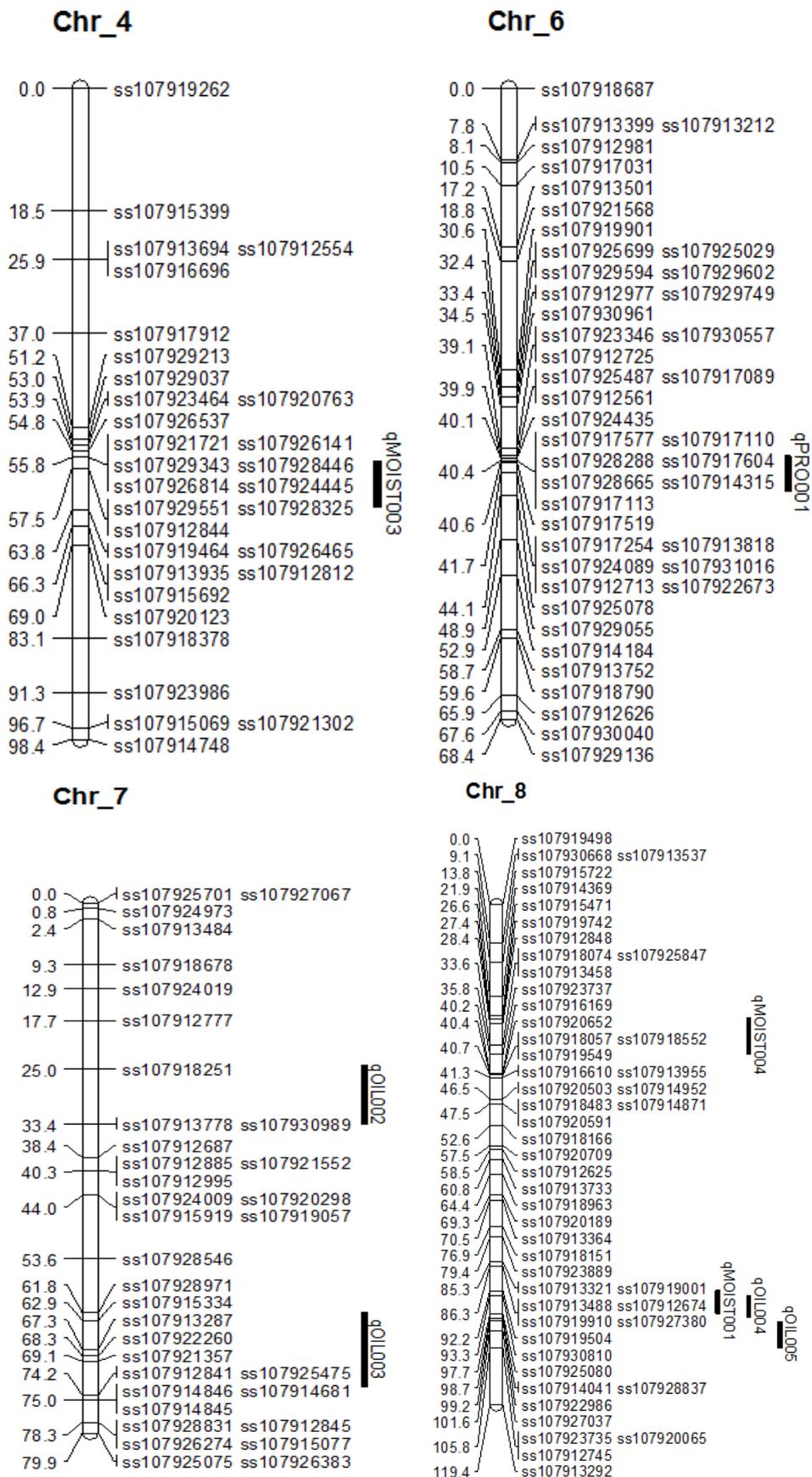
#### 4. Discussion

The observed variation for moisture, protein and oil contents in the RIL population can be beneficial for soybean improvement programs. The RIL population and the parents presented genetic variability for moisture, protein and oil contents within a plant density. Significant variation was found for protein in higher vs. lower plant density environment while for moisture and oil contents between plant densities were not significant. For the lines cultivated in higher plant density (25 cm row space), ten QTLs were mapped in LG A2, LG B2, LG C2, LG D1a, LG M, and LG O linkage groups for moisture, protein, and oil. Two QTLs for moisture, two QTLs for protein and six QTLs for oil explained 0.09%, 15.36%, and 3.54% of the phenotypic variation of moisture, protein and oil, respectively. For the lines cultivated in higher density (50 cm row space), three QTLs for each moisture and protein were mapped in linkage groups LG A2, LG C1, LG D1b, LG L, and LG O; QTLs for moisture and protein explained 0.96% and 10.63% of the phenotypic variation respectively. Soybean seed protein and oil content were identified as negatively correlated, which is similar to findings of Burton (1987). The negative correlation between soybean protein and oil content is may due: (i) to a single pleiotropic QTL with two alleles, wherein one allele simultaneously causes high oil and low-protein, and the other allele simultaneously causes low oil and high protein, or (ii) to two tightly linked QTLs, with the high oil allele at the oil QTL and the low protein allele of the protein QTL currently locked into a repulsion phase not yet reversed by recombination event (Sun, 2011).

Using composite interval mapping (CIM), a total of 16 QTL for moisture, protein and oil were identified on nine different chromosomes (Table 2, Figure 2). On chromosome 1 (LG D1a) 'qOIL001' was identified to be associated with the oil trait by the marker interval ss107915391-ss107917465. On the same chromosome, QTL for soybean sudden death syndrome (SDS) (Kassem et al., 2012) and soybean cyst nematode (SCN) resistance were previously identified using the same soybean population. QTL for several other agronomic traits such as Japanese root-knot nematode resistance, protein concentration, oil content, and leaf length, were also identified in the same chromosome 1 (LG D1a; SoyBase, 2012). On LG C1 (Chr\_4) 'qMOIST003' was identified

associated with the seed moisture trait by the marker interval ss107929551–ss107919464 on 56.4-63.3 cM with LOD 3.75. On same LG (Chr\_4), QTLs for root rot severity (qRRS001-02 and qFDS004-03) was also mapped, along with other QTL for several morphological traits such as seed weight, pod maturity, and seed yield have been mapped at this chromosome or LG (SoyBase, 2012). On LG C2 (Chr\_6) QTL for protein ‘qPRO001’ was detected at 39.9-43.7cM. Using the same RIL population (PI 438489B x Hamilton), QTL for SCN resistance were mapped the same LG by Yue, Arelli, & Sleper (2001). Recently, QTL associated with the foliar diseases severity (FDS) ‘qFDS003-03’ was also identified (Kassem et al., 2012). LG C2 (Chr\_6) also contained QTL for SDS resistance (cqRfs4; leaf DX) and several FDS resistance QTLs ‘qFDS003-03’, ‘qFDS003-05’, ‘qFDS004-03’, and ‘qFDS004-04’ (Kazi et al., 2008). LG A2 (Chr\_8), a cluster of moisture (qMOIST001 and qMOIST004) and oil (qOIL004 and qOIL005) QTL was identified. Interestingly, ‘qMOIST001’ (92.0-97.6 cM) and ‘qOIL004’ (93.4-98.6 cM) were overlapped in the same region and ‘qOIL005’ was identified just after that region (99.5-105.8 cM). On the same LG or chromosome, QTLs for soybean cyst nematode resistance (SCN 1-1; Concibido, Diers, & Arelli, 2004), SCN 13-2, sudden-death syndrome (SDS 5-2) resistance (Prabhu et al., 1999), SCN 19-1 (Yue, Arelli, & Sleper, 2001), and SCN 27-2 (Meksem et al., 2001) were identified previously. In LG L (Chr\_19) QTL for moisture (qMOIST005) and protein (PRO005) were overlapped in the region of 10.8-20.1 cM.





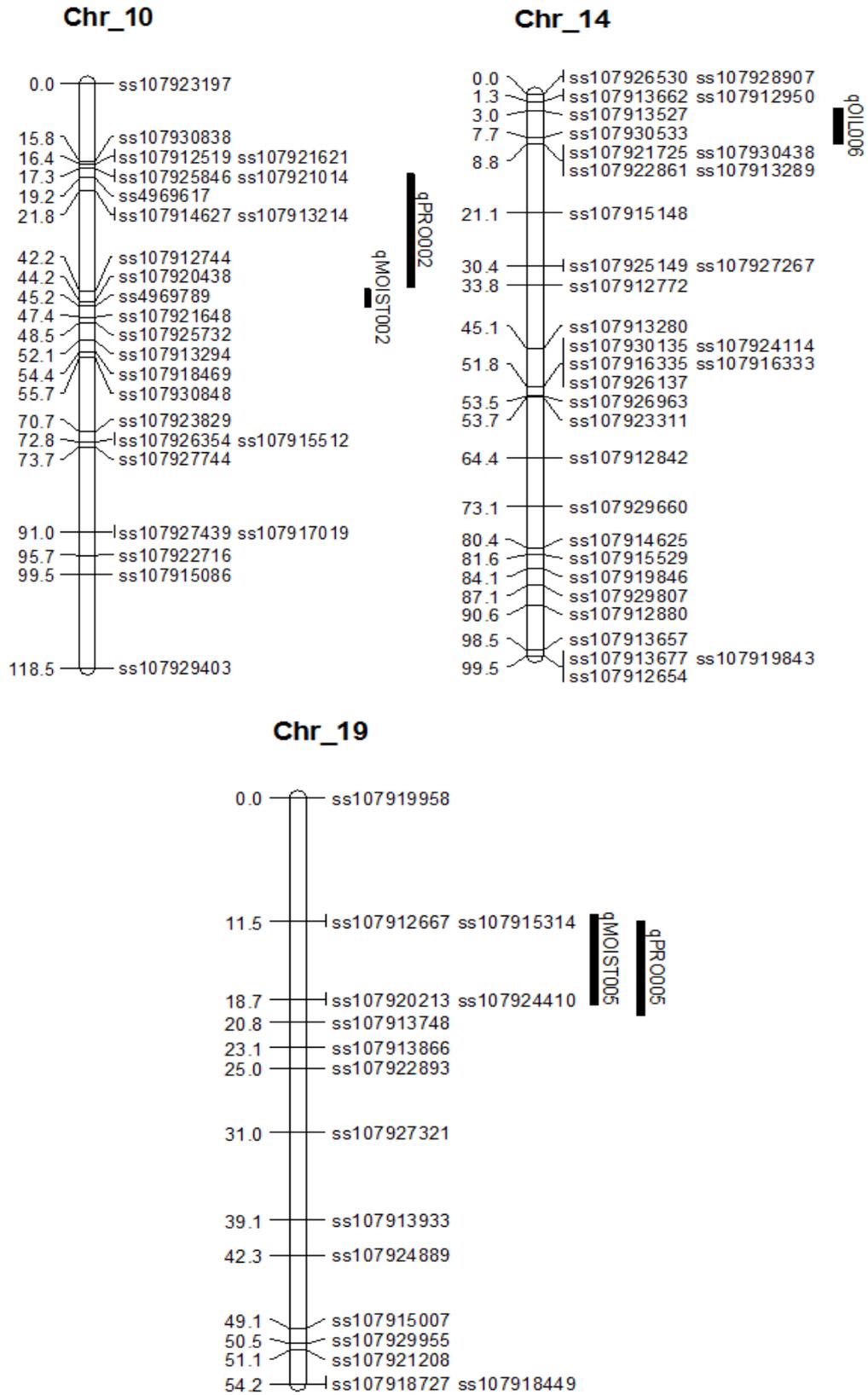


Figure 2. Locations of SNP markers and the QTL underlying moisture (MOIST), protein (PRO), and oil (OIL) contents in the recombinant inbred lines (RIL) of soybean derived from a cross between PI 438489B by ‘Hamilton’

Currently, SoyBase (2012) contains 128 QTLs for seed oil, 109 QTLs for protein and two QTLs for oil/protein and all they have been mapped in many different populations and environments. A few identical QTLs were identified in same experimental population but different cultivation environment by different researchers or in different years (Orf et al., 1999; Mansur, Lark, Kross, & Oliveira, 1993; Mansur et al., 1996). Many of the oil QTL in SoyBase (2012) were discovered through simple linear regression methods and have not been detected through interval mapping. Among the commonly used models in QTL mapping, simple interval mapping (SIM) and composite interval mapping (CIM) are known to be widely used models. The advantage of CIM methods is that they accommodate multiple QTL. It gives more power and precision than SIM because the effects of other QTL are not present as residual variance, and it can eliminate the bias that would normally be caused by QTL that is linked with the position being tested (Nagabhushana, Mane, & Hittalmani, 2006). Therefore, the QTLs presented here will have significant contribution in future gene discovery in soybean. All of these interfered in QTLs to improve protein and oil content of soybean by marker-assisted selection (MAS). On the other hand, several studies reported SNP markers associated with QTL for several traits in soybean (Vuong, Wu, Pathan, Valliodan, & Nguyen, 2007), but there are only two studies reported an exclusively SNP-based genetic map (Kassem, 2012; Hyten et al., 2010a).

The genetic relationship between moisture, protein, oil and other traits could be explored by comparing QTL mapping results when available and fine mapping of each trait would provide accurate genetic information. The QTLs found in this study can be considered as preliminary work on MAS for the protein and oil content in soybean breeding. Research is under way to confirm identified QTL for these traits in two additional populations with different genetic background.

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