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Genetic Mapping and Confirmation of Quantitative Trait Loci for Seed Protein and Oil Contents and Seed Weight in Soybean

Safiullah M. Pathan

University of Missouri Delta Research Center

Tri Vuong

University of Missouri, vuongt@missouri.edu

Kerry Clark

University of Missouri, clarkk@missouri.edu

Jeong-Dong Lee

Kyungpook National University

J. Grover Shannon

Univ. of Missouri Delta Research Center, shannong@missouri.edu

See next page for additional authors

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Authors

Safiullah M. Pathan, Tri Vuong, Kerry Clark, Jeong-Dong Lee, J. Grover Shannon, Craig A. Roberts, Mark R. Ellersieck, Joseph W. Burton, P. B. Cregan, David L. Hyten, Henry T. Nguyen, and David A. Sleper

Genetic Mapping and Confirmation of Quantitative Trait Loci for Seed Protein and Oil Contents and Seed Weight in Soybean

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ABSTRACT

Demand for soybean [*Glycine max* (L.) Merr.] meal has increased worldwide and soybean importers often offer premiums for soybean containing higher contents of protein and oil. Objectives were to detect quantitative trait loci (QTL) associated with soybean seed protein, oil, and seed weight in a soybean mapping population and confirm detected QTL across genetic backgrounds and environments. Two populations of 216 and 156 recombinant inbred lines were developed from Magellan × PI 438489B and Magellan × PI 567516C crosses, respectively, and grown in two Missouri environments in 2008. More than 900 simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers were used for mapping in each population. Across environments and genetic backgrounds, we have identified seven QTL for protein, six for oil, and four for seed weight. Two QTL were detected in common for protein and oil, one on chromosome (Chr.) 5 and another on Chr. 6. Additionally, we have detected one new seed weight QTL on Chr. 6, in the same region of protein and oil QTL. Confirmed protein and oil QTL on Chrs. 5 and 6 may be important targets to find candidate genes involved in modification of protein and oil contents and seed weight using genetic and genomic approaches. Also, SSR and SNP markers closely associated with these QTL can be useful for marker-assisted selection.

S.M. Pathan and J.G. Shannon, National Center for Soybean Biotechnology (NCSB) and Division of Plant Sciences, Univ. of Missouri Delta Research Center, Portageville, MO 63873; T. Vuong, K. Clark, H.T. Nguyen, and D.A. Sleper, NCSB and Division of Plant Sciences, Univ. of Missouri, Columbia, MO 65211; C.A. Roberts, Division of Plant Sciences, Univ. of Missouri, Columbia, MO 65211; M.R. Ellersieck, Division of Statistics, Univ. of Missouri, Columbia, MO 65211; J.W. Burton, Soybean and Nitrogen Fixation Research Unit, USDA-ARS, Raleigh, NC 27607; D.L. Hyten, Pioneer Hi-Bred International Inc., Johnston, IA 50131; P.B. Cregan, Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD 20705; J.-D. Lee, School of Applied Biosciences, Kyungpook National Univ., Daegu 702-701, Republic of Korea. S.M. Pathan and T. Vuong contributed equally to the article. Received 5 Mar. 2012. *Corresponding author (sleperd@missouri.edu).

Abbreviations: BREC, Bradford Research and Extension Center; Chr., chromosome; DRC, Delta Research Center; H^2 , broad-sense heritability; LOD, logarithm of the odds; MPB, Magellan × PI 438489B; MPC, Magellan × PI 567516C; MQM, multiple-QTL method; MU, University of Missouri–Columbia; NIR, near-infrared reflectance; QTL, quantitative trait loci; RIL, recombinant inbred line; SBM, soybean meal; SCN, soybean cyst nematode; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

THE UNITED STATES is the leading world soybean producer and exporter of soybean as whole bean, oil, and soybean meal (SBM). Important uses of soybean are protein rich SBM for livestock feed and oil for human consumption. Recently, demand for SBM has increased worldwide and the current standard for high-protein SBM is 480 g kg⁻¹ crude protein. Soybean prices are determined primarily by protein and oil contents (Brumm and Hurburgh, 1990; Hurburgh,

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1994; Orf and Helms, 1994) and soybean importers often offer premiums for soybean containing higher amounts of protein and oil. Soybean provides 68% of the edible consumption of fats and oils in the United States (SOYSTATS, 2011). Also, soybean is used as an industrial and pharmaceutical raw material for production of biodiesel and soy foods. Seed protein and seed size play significant roles in determining quality of soy food products (Clarke and Wiseman, 2000; Friedman and Brandon, 2001). Small seeded soybean is generally used for soy sprouts, miso, and natto while large seeded soybean with higher protein content is desirable for tofu and edamame. Other than economic and nutritional importance, different clinical studies also suggested that soybean has numerous human health benefits such as a reduction in cancer, a decrease in blood cholesterol, and reduction of osteoporosis and heart disease. It also serves as a good source of minerals, vitamin B, folic acid, and isoflavones (Birt et al., 2004; Wilson, 2004).

There exist a wide range of genetic variation in protein and oil contents among accessions of the USDA Soybean Germplasm Collection but it is extremely rare to find an accession with higher contents of both protein and oil (Wilson, 2004). Protein and oil are genetically negatively correlated (Burton, 1987; Wilcox, 1998), which makes it difficult to improve both traits simultaneously. Yield is genetically negatively correlated with protein content but positively correlated with oil (Burton, 1987; Wilcox and Goudong, 1997). Heritability of soybean seed protein and oil contents are moderately high to high (Brim, 1973; Burton, 1987) and primarily governed by additive genetic effects (Thorne and Fehr, 1970). In the last 20 yr (1986 to 2004), soybean yield has increased significantly at a rate of 10.9 kg ha⁻¹ yr⁻¹ (USDA, 2005). Brumm and Hurburgh (2006) on their survey (1994 to 2004) reported that U.S. average oil content did not change, but average protein content dropped by 0.1% relative to the previous survey because soybean breeders have maintained protein levels while increasing yield. Mahmoud et al. (2006) also reported a decline in protein content after six decades of selection and breeding. It is difficult to simultaneously improve protein and oil content; however, it is possible to improve either seed trait consecutively (Burton, 1987; Thorne and Fehr, 1970). Current seed protein content generally meets industrial standards (Brumm and Hurburgh, 2006), but fluctuations in environmental conditions may lower protein content below the standard (Rotundo et al., 2009). Under water stress, seed protein tends to decrease and oil tends to increase (Specht et al., 2001) and seed oil also increases at high temperatures during seed development (Brumm and Hurburgh, 2006). Significant variations in seed composition at different regions within the United States have been reported (Brumm and Hurburgh, 2006).

Protein, oil, and seed size are quantitative traits controlled by multiple genes having small or large effects

(Hyten et al., 2004). Therefore, molecular markers may be helpful to identify quantitative trait loci (QTL) controlling these traits. Ultimately, these markers when confirmed may be used in marker-assisted breeding. For last two decades, several studies have identified QTL for soybean seed protein and oil contents and seed weight (Diers et al., 1992; Hyten et al., 2004; Mansur et al., 1996; Orf et al., 1999; Panthee et al., 2005; Specht et al., 2001). To discover new QTL, scientists have developed and used mapping populations from an interspecific cross between *Glycine max* (cultivated soybean) and *Glycine soja* Siebold & Zucc. (wild soybean) (Diers et al., 1992; Sebolt et al., 2000). They also used soybean Plant Introductions as both parents for the mapping populations (Mansur et al., 1996; Specht et al., 2001), Plant Introductions as one of the parents (Orf et al., 1999; Fasoula et al., 2004), and soybean cultivars and/or advanced breeding lines as both parents (Hyten et al., 2004; Panthee et al., 2005; Reinprecht et al., 2006). Researchers also used a single large mapping population to detect QTL with minor effects (Mansur et al., 1996; Orf et al., 1999; Specht et al., 2001) or used numerous small populations with different parental combinations to detect major QTL and to validate identified QTL across different genetic backgrounds (Brummer et al., 1997). Hyten et al. (2004) summarized reported QTL for protein and oil contents and seed weight with their genetic locations, effects, significance, and mapping parents. Currently, SoyBase (<http://soybase.org>, accessed 12 Feb. 2012) reported a large number of QTL for different seed traits, including 115 for protein, 113 for oil, five for protein and oil, and 120 for seed weight. Genetic positions of some of these reported QTL are very close (<10 cM) so they may not be truly different QTL. Among these, only a few have been confirmed so far (Fasoula et al., 2004). To verify stable QTL, it is important to identify QTL from many populations developed from diverse parents and to evaluate in multiple locations. For example, one of the most consistent protein and oil QTL on chromosome (Chr.) 20 was identified in different environments and genetic backgrounds and mapped between map positions of 32.4 to 39.4 cM (Brummer et al., 1997; Chung et al., 2003; Diers et al., 1992; Nichols et al., 2006; Sebolt et al., 2000; Specht et al., 2001). Some of this high protein QTL have pleiotrophic effects or are associated with reduced oil (Diers et al., 1992; Chung et al., 2003; Sebolt et al., 2000). A protein and oil QTL was mapped on Chr. 5 between map positions 93.6 to 94.9 cM in three independent studies (Mansur et al., 1996; Orf et al., 1999; Specht et al., 2001). Also on Chr. 7, a protein QTL was mapped between map position 33.5 and 41.9 cM in four studies (Csanadi et al., 2001; Hyten et al., 2004; Orf et al., 1999; Specht et al., 2001). Some of these QTL were detected in a single population, for example seed weight QTL on Chr. 10 (Csanadi et al., 2001).

Although a large number of QTL have been identified for protein and oil contents and seed weight, only a few QTL have been confirmed across genetic backgrounds and/or

environments. Population size (small to moderate mapping population sizes), genetic background of parental lines, environments, phenotyping methods, and use of different molecular markers (variation in polymorphisms level) are possible reasons for inconsistency with previously detected QTL. The objectives of the current study were (i) to detect QTL associated with soybean seed protein and oil contents and seed weight in a recombinant inbred line (RIL) population derived from a Magellan × PI 438489B cross, (ii) to confirm detected QTL in a different RIL population derived from a Magellan × PI 567516C cross, and (iii) to identify possible genotype × environment interactions in seed traits.

MATERIALS AND METHODS

Plant Materials

Two mapping populations were developed from a cross of Magellan × PI 438489B (henceforth designated as MPB) and Magellan × PI 567516C (henceforth designated as MPC). Magellan (PI 595362) (Schapaugh et al., 1998) is a high-yielding cultivar with lower seed protein and higher oil contents than standard cultivars. Plant Introduction 438489B and PI 567516C are soybean cyst nematode (SCN) resistant germplasm with black and greenish-brown seed coat colors, respectively, and higher seed protein and lower oil content than standard cultivars. Both PI 438489B and PI 567516C originated from China (Yue et al., 2000; USDA-ARS-GRIN, 2012). Crosses were made at the Bradford Research and Extension Center (BREC) of the University of Missouri–Columbia (MU) in the summer of 2005. The F₁'s were harvested in October and then sent to Costa Rica for generation advancement. Generations were advanced until the F₅ stage via single-seed descent method (Brim, 1966). The F₅ single plants were harvested and threshed separately and were grown in rows, harvested row in bulk, and then advanced to the next generation. Initially, these two populations were developed and used for mapping of SCN (Vuong et al., 2010, 2011). The F_{5:7} seeds of 216 RILs of the MPB and 156 RILs of the MPC populations and parents were planted in 2.7 m long plots with 0.75 m spacing between rows. Three replications in a randomized complete block design were grown at BREC, Columbia, MO, and the MU Delta Research Center (DRC) in Portageville, MO, in the summer of 2008. All three parents (Magellan, PI 438489B, and PI 567516C) are classified as maturity group IV.

Phenotypic Analysis

Approximately 5 to 6 g of whole soybean seed was used to quantify seed protein and oil contents using near-infrared reflectance (NIR) spectroscopy according to procedures of Duckworth (1998) and Shenk and Westerhaus (1991). Lines with cracked seed coat of both populations were excluded from analysis and all other seeds (black, brown, green, and yellow) were included for analysis. The spectrophotometer was a FOSS NIRSystems 6500; the software used to store spectra and develop calibrations was WinISI version 1.02 (InfraSoft, 1999). Two individual samples from each replication of each location were analyzed at regular intervals to confirm repeatability of measurements. Samples were double scanned and the

Table 1. Calibration statistics for near-infrared spectroscopic determination of soybean seed protein and oil contents.

Trait	No. of RILs [†]	Mean	SEC [‡]	SECV [§]	R ²	1 – VR [¶]
—g kg ⁻¹ DM [#] —						
Protein	63	394.4	5.8	8.9	0.93	0.84
Oil	63	170.8	2.2	4.7	0.97	0.85

[†]RIL, recombinant inbred line.

[‡]Standard error of calibration (SEC) calculated in modified partial least squares regression.

[§]Standard error of cross validation (SECV) calculated in modified partial least squares regression.

[¶]One minus the variance ratio (VR) calculated in cross validation in modified partial least squares regression.

[#]DM, dry matter.

spectra averaged. Chemical analysis of 63 RILs of different seed colors were also performed for cross-validation. Protein was determined by combustion (Eurofins test ID QD052 [AOAC Int., 2005]), and oil was determined by ethyl ether extraction (Eurofins test ID QD061 [AOAC Int., 2005]). A calibration was developed by regressing chemical data against spectral data using partial least squares regression over the entire spectra (400 to 2500 nm) with six validation groups. Math treatment used the second derivative of log 1 – reflectance (R) with a standard normal variance pretreatment (Barnes et al., 1989). The optimum calibrations were selected on the basis of low standard errors and high squared correlation coefficients (Table 1).

Seed weight was determined by weighing 100 random seeds per plot. Genomic DNA extraction and molecular marker analysis (simple sequence repeat [SSR] and single nucleotide polymorphism [SNP]) were performed as previously described (Vuong et al., 2010).

Data Analysis

Phenotypic data for seed protein and oil contents and seed weight were subjected to an ANOVA using the PROC GLM (general linear model) mixed model of SAS version 9.2 (SAS Institute, 2002). The linear statistical model contains the effects of environment, replication within environment, genotype, and environment × genotype interaction. Environment, genotype, and environment × genotype interaction were considered as fixed effects. Replication within environment was used as the denominator of the *F*-value of environment. The residual mean square was used to test genotype and genotype × environment interaction. Broad-sense heritability (*H*²) of each trait was estimated on an entry mean basis following Nyquist (1991):

$$H^2 = (\sigma_g^2) / [\sigma_g^2 + (\sigma_{ge}^2 / e) + (\sigma_{re}^2 / r)],$$

in which σ_g^2 is genetic variance, σ_{ge}^2 is genotype × environment variance, σ^2 is error variance, *r* is number of replications, and *e* is the number of environments. Phenotypic correlations were determined using the PROC CORR procedure of SAS. Genetic correlations were calculated using the following formula (Falconer and Mackay, 1996):

$$r_G = \text{Cov}_{xy} / (\sigma_x^2 \sigma_y^2)^{1/2},$$

in which r_G represents genetic correlation, Cov represents genetic covariance, *x* represents the first trait, *y* represents the second trait, and σ^2 is genetic variance. Cross-products between traits were generated using PROC GLM procedure of SAS with MANOVA (multivariate analysis of variance) option.

Table 2. Mean, range, standard deviation, and heritability for seed protein and oil contents and seed weight in $F_{5:7}$ derived two soybean populations, Magellan $\times$ PI 438489B (216 recombinant inbred lines [RILs]) and Magellan $\times$ PI 567516C (156 RILs) averaged over two environments in Missouri in summer 2008.

Traits	Magellan $\times$ PI 438489B					Magellan $\times$ PI 567516C					Parents		
	Mean	Range	SD	P-value	H^2 [†]	Mean	Range	SD	P-value	H^2 [†]	P1 [‡]	P2 [§]	P3 [¶]
Protein, g kg ⁻¹	413	371–452	21	<0.0001	0.63	412	347–466	26	<0.0001	0.76	380	412	405
Oil, g kg ⁻¹	180	163–203	11	<0.0001	0.62	181	156–206	13	<0.0001	0.66	202	160	170
Seed weight, g per 100 seed	13	9–18	1.6	<0.0001	0.75	10	6–16	1.5	<0.0001	0.86	15	11	5

[†] H^2 , broad-sense heritability, calculated on an entry mean basis.

[‡]P1, Magellan.

[§]P2, PI 438489B.

[¶]P3, PI 567516C.

Linkage Mapping and Quantitative Trait Loci Analysis

Two genetic linkage maps constructed earlier for the MPB and MPC populations (Vuong et al., 2010, 2011) were used in this study. Phenotypic data for seed protein and oil contents obtained through NIR and seed weight of each location were used for QTL analysis. The QTL for protein and oil contents and seed weight were initially predicted using the interval mapping method. Subsequently, the multiple-QTL method (MQM) with the program MapQTL 5.0 (van Ooijen, 2004) was used to declare a significant QTL based on appropriate cofactors (van Ooijen and Voorrips, 2001) and a logarithm of the odds (LOD) score generated by the genomewide permutation tests (Churchill and Doerge, 1994). The confidence interval for QTL location was estimated using 1 – LOD support interval value as described by van Ooijen (1992). The proportion of the phenotypic variance explained by the QTL effects was estimated by MQM at QTL peaks. The molecular genetic maps with QTL intervals and LOD plots were subsequently created using the MapChart 2.2 program (Voorrips, 2002).

RESULTS

Two genetic linkage maps were constructed using RILs of the MPB and MPC populations with more than 900 polymorphic SSR and SNP markers (Vuong et al., 2010, 2011). Overall, marker order between loci of our newly constructed genetic maps was in good agreement with the soybean consensus genetic map (Hyten et al., 2010). Approximately 95% of the soybean genome was covered by both of the new MPB and MPC genetic maps. More than 700 polymorphic SNP markers of the USLP 1.0 array (Hyten et al., 2010) and 200 SSR markers were used to detect QTL for soybean seed protein and oil contents and seed weight.

Analyses of Seed Protein and Oil Contents and Seed Weight

There were significant differences ($P < 0.0001$) among RILs for seed protein content in both MPB and MPC populations (Table 2). Seed protein content in MPB ranged from 371 to 452 g kg⁻¹ with a mean of 413 g kg⁻¹ and in MPC ranged from 347 to 466 g kg⁻¹ with a mean of 412 g kg⁻¹ on dry weight basis. The RILs were normally distributed for seed protein in both mapping populations and there was no transgressive segregation for protein in

MPB population (Fig. 1a; Table 2). There was significant transgressive segregation for protein in MPC. There were significant differences ($P < 0.0001$) among RILs for seed oil content in both the MPB and MPC populations (Table 2). Seed oil content in MPB ranged from 163 to 203 g kg⁻¹ with a mean of 180 g kg⁻¹ and in MPC ranged from 156 to 206 g kg⁻¹ with a mean of 181 g kg⁻¹ on dry weight basis. The RILs were normally distributed for seed oil in both populations and transgressive segregation was not observed for oil (Fig. 1b; Table 2). Seed weight in MPB ranged from 8.7 to 17.5 g with a mean of 12.7 g per 100 seed and in MPC ranged from 6.2 to 16.4 g with mean a of 9.5 g per 100 seed. The parent PI 567516C had lower seed weight of 4.7 g per 100 seed. The RILs were normally distributed for seed weight in both the populations and transgressive segregation was not observed (Fig. 1c; Table 2).

In MPB, entry mean basis heritabilities for protein, oil, and seed weight were 0.63, 0.62, and 0.75, respectively. In MPC, heritabilities for protein, oil, and seed weight were 0.76, 0.66, and 0.86, respectively, indicating much of the variation observed among RIL was genetic (Table 2). In both populations, significant negative phenotypic ($r = -0.64$, $P < 0.0001$) and genotypic (in MPB, $r = -0.58$, $P < 0.0001$, and in MPC, $r = -0.75$, $P < 0.0001$) correlation between protein and oil was observed. Phenotypic and genotypic correlations for seed weight with protein and oil contents were nonsignificant and inconsistent (Table 3).

Quantitative Trait Loci Analyses for Seed Protein and Oil Contents and Seed Weight

Based on a genomewide permutation test, LOD threshold of 3.0 ($P = 0.05$), 3.1 ($P = 0.05$), and 3.1 ($P = 0.05$) were used to declare significant QTL for seed protein and oil contents and seed weight, respectively, in both MPB and MPC populations. A total of seven QTL for seed protein were mapped on Chrs. 3, 5, 6, 7, 8, and 15 with LOD scores ranging from 2.7 to 6.7 and total phenotypic variation explained by individual QTL ranged from 4.8 to 14.2% (Table 4). Among them, one significant protein QTL mapped on Chr. 6 (between markers BARC-059985-16274 and BARC-044639-08743) was consistent across environments and genetic backgrounds. The three other protein QTL mapped on Chrs. 5 (between

Protein g Kg⁻¹

Oil g Kg⁻¹

Seed weight g per 100 seed

Figure 1. Frequency distribution of (a) seed protein (g kg⁻¹), (b) oil content (g kg⁻¹), and (c) seed weight (g per 100 seed) of 216 F_{5:7} recombinant inbred lines (RILs) of Magellan (Parent 1 [P1]) × PI 438489B (Parent 2 [P2]) (MPB) (striped bar) and 156 F_{5:7} RILs of Magellan (P1) × PI 567516C (Parent 3 [P3]) (MPC) (solid bar) averaged over two environments in Missouri (Bradford Research and Extension Center and Delta Research Center) in summer 2008.

Table 3. Genetic (lower left diagonal) and phenotypic correlations (upper right diagonal) among protein, oil, and seed weight in F₅-derived 216 recombinant inbred lines (RILs) of Magellan × PI 438489B (MPB) and F₅-derived 156 RILs of Magellan × PI 567516C (MPC) averaged over two environments (Columbia, MO, and Portageville, MO) in 2008. The first number represents MPB (open) and the second number (in parenthesis) represents MPC populations.

	Protein	Oil	Seed weight
Protein		-0.64*** (-0.64***)	-0.02 ns† (0.2***)
Oil	-0.58*** (-0.75***)		-0.006 ns (0.04 ns)
Seed weight	-0.16* (0.38***)	0.07 ns (-0.27**)	

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†ns, not significant ($P > 0.05$).

BARC-041257-07953 and BARC-060051-16321), 7 (between BARC-014705-01623 and BARC-029825-06442), and 8 (between Satt632 and BARC-040339-07715) were detected in both environments and in one of the populations. One protein QTL mapped on Chr. 3 (between BARC-028645-05979 and BARC-055149-13089) was identified in both populations and in one of the environments. Two other QTL mapped on Chrs. 6 (between Satt433 and BARC-049601-09082) and 15 (between BARC-018923-03037 and BARC-025493-06513) were detected in only one population and one environment. In most of the cases, both PI 438489B and PI 567516C contributed positive alleles and in two cases Magellan contributed positive alleles (Chr. 3 and 6) that increase protein content.

A total of six QTL for seed oil content were identified and mapped on Chrs. 2, 3, 5, 6, and 15 (Table 4). Among them, one QTL on Chr. 5 (between markers BARC-041257-07953 and BARC-060051-16321) and another on Chr. 6 (between BARC-059985-16274 and Sat_336) were detected in both environments and genetic backgrounds. The oil QTL on Chr. 6 was consistent with the protein QTL identified in the same genomic region in both populations and environments. The oil QTL on Chr. 5 was consistent with the protein QTL identified in the same genomic region in one population and both environments. Two other QTL, one on Chr. 2 (between BARC-028373-05856 and Sat_198) and another on 15 (between markers BARC-039687-07541 and BARC-042349-08247), were detected in both environments and one of the populations. Two more QTL on Chrs. 3 and 15 were detected in one of the populations and environments. Cultivar Magellan contributed favorable alleles that increase oil content in all QTL except one on Chr. 3.

Four QTL associated with seed weight were mapped on Chrs. 1, 6, and 19 across environments and genetic backgrounds (Table 4). Among them, QTL on Chrs. 6 (between markers BARC-059997-16280 and Sat_336) and 19 (between BARC-014885-01914 and Satt527) were detected across environments and genetic backgrounds. In addition,

Table 4. Quantitative trait loci (QTL) for soybean seed protein and oil contents and seed weight were detected with multiple-QTL method in two recombinant inbred line mapping populations, Magellan × PI 438489B and Magellan × PI 567516C, evaluated in replicated trials in two environments, Bradford Research and Extension Center (BREC), Columbia, MO, and Delta Research Center (DRC), Portageville, MO, in summer 2008.

Population [†]	Environment	Chr. [‡] no.	Marker interval of QTL position	Map position [§] (cM)	Confidence interval [¶] (cM)	LOD [#]	R ² (%)	Additive effect ^{††}	Total PVE ^{‡‡} (%)	
Protein content										
MPB	BREC	3	BARC-016199-02307_BARC-053313-11792	22.3	21.0–23.6	3.3	5.6	0.47	21.5	
		6	BARC-059985-16274_BARC-044639-08743	34.4	33.3–35.5	3.1	5.3	−0.46		
		7	BARC-014705-01623_BARC-029825-06442	44.3	42.6–46.0	3.7	6.8	−0.54		
	DRC	6	Satt281_BARC-059997-16280	38.9	38.2–39.6	2.7	4.8	−0.35	15.1	
		15	BARC-018923-03037_BARC-025493-06513	11.9	9.9–13.9	4.0	7.5	−0.44		
MPC	BREC	7	BARC-014705-01623_BARC-029825-06442	44.3	43.3–45.3	3.0	7.0	−0.45	34.4	
		3	BARC-028645-05979_BARC-055149-13089	21.1	19.7–22.5	3.4	6.7	0.70		
		5	Satt236_BARC-041257-07953	80.8	79.6–82.0	3.2	6.4	−0.70		
		6	Satt433_BARC-049601-09082	114.9	113.6–116.2	3.3	7.6	0.85		
	DRC	8	Satt632_BARC-040339-07715	46.3	41.6–51.0	6.7	14.2	−1.04		35.0
		5	BARC-041257-07953_BARC-060051-16321	78.8	76.8–80.8	4.0	7.7	−0.63		
		6	BARC-059985-16274_Satt291	34.4	31.7–37.1	4.7	9.0	−0.63		
		8	Satt632_BARC-010097-00518	46.3	44.7–47.9	3.6	6.8	−0.55		
Oil content										
MPB	BREC	3	BARC-055637-13558_BARC-057823-14942	27.6	26.5–28.7	3.1	5.6	−0.24	20.1	
		6	BARC-059985-16274_BARC-044639-08743	34.4	32.2–36.6	4.2	7.4	0.27		
		15	BARC-039687-07541_BARC-042349-08247	18.7	17.7–19.7	3.0	5.3	0.23		
	DRC	5	BARC-041257-07953_BARC-060051-16321	78.8	76.6–81.0	4.2	7.1	0.25	24.5	
		6	BARC-059985-16274_BARC-044639-08743	34.4	30.8–38.0	5.6	9.3	0.27		
MPC	BREC	15	BARC-039687-07541_BARC-042349-08247	18.7	15.1–22.3	5.6	9.7	0.28	25.1	
		2	BARC-044747-08795_BARC-054149-12354	126.2	124.0–128.4	4.2	10.5	0.42		
		5	BARC-041257-07953_BARC-060051-16321	78.8	77.4–80.2	3.4	8.4	0.38		
	DRC	15	BARC-018901-03270_BARC-038977-07417	47.2	46.0–48.4	3.2	9.2	0.41		
		2	BARC-028373-05856_Sat_198	116.5	116.0–117.0	2.5	6.1	0.29		24.7
		5	BARC-041257-07953_BARC-060051-16321	78.8	78.3–79.3	2.5	5.7	0.29		
Seed weight										
MPB	BREC	6	BARC-056271-14211_BARC-057469-14770	41.5	39.7–43.3	3.8	6.4	0.49	24.5	
		19	Satt527_BARC-047496-12943	61.5	59.6–63.4	3.9	7.0	0.54		
		1	BARC-045297-08928_Satt184	16.7	14.7–18.7	4.0	6.2	−0.47		
	DRC	6	BARC-016957-02165_Satt281	26.1	21.4–30.8	6.7	14.2	0.69		25.5
		19	Satt527_BARC-047496-12943	61.5	56.1–66.9	7.4	13.2	0.62		
MPC	BREC	6	BARC-059997-16280_Sat_336	35.4	34.9–35.9	2.5	5.9	0.47	23.2	
		19	BARC-014885-01914_Satt527	61.5	58.8–64.2	4.7	9.8	0.53		
		1	BARC-038883-07384_Satt184	15.9	14.1–17.7	3.8	7.9	0.48		
	DRC	6	BARC-059997-16280_Sat_336	35.4	34.9–35.9	2.5	5.9	0.40		23.2
		19	BARC-014885-01914_Satt527	61.5	60.3–62.7	3.2	7.3	0.41		

[†]MPB, Magellan × PI 438489B; MPC, Magellan × PI 567516C.

[‡]Chr., chromosome.

[§]The QTL position was determined based on genetic linkage map constructed in the present study.

[¶]Confidence interval for QTL location was estimated using a 1 – LOD support interval value as described by van Ooijen (1992).

[#]LOD, logarithm of the odds.

^{††}Positive sign indicates alleles from Magellan and negative sign indicates alleles from PI 438489B or PI 567516C.

^{‡‡}PVE, phenotypic variation explained.

one more QTL on Chr. 6 (between BARC-016957-02165 and Satt281) was identified in a single population and single environment. Another seed weight QTL mapped on Chr. 1 (between BARC-045297-08928 and Satt184) was identified in both populations but only in one environment, BREC. Remarkably, one QTL for protein and oil contents was also mapped in the same genomic region as QTL associated with seed weight on Chr. 6, an important location embracing QTL for three seed traits. Cultivar Magellan contributed

favorable alleles (except on Chr. 1) and had large additive effects that increased seed weight.

DISCUSSION

In the MPB and MPC populations of this study, mean protein contents were 413 and 412 g kg^{–1} and mean oil contents were 180 and 181 g kg^{–1}, respectively, and our results were in good agreement with previous studies (Fasoula et al., 2004; Hyten et al., 2004; Panthee et al.,

2005) and within the range of values of the Soybean Germplasm Collection (Wilson, 2004). Heritability for protein and oil contents and seed weight in our study was moderately high and similar to those reported by Hyten et al. (2004) and Panthee et al. (2005). The heritability observed in our populations indicated that much of the variation was genetic; therefore, selection response would be reasonable for achieving genetic gain.

We observed significant negative phenotypic and genotypic correlation between seed protein and oil contents in both mapping populations as reported in other studies (Chung et al., 2003; Hyten et al., 2004; Mansur et al., 1996; Panthee et al., 2005). These genotypic correlations indicate that it is difficult to simultaneously increase protein and oil contents and seed yield. Large variations in correlation (positive and negative as well as significant and nonsignificant) for seed weight with protein and oil have been reported (Reinprecht et al., 2006; Specht et al., 2001).

Due to a strong negative correlation between soybean seed protein and oil contents observed in this study and consistent with previous reports, we predicted that QTL and/or gene or genes associated with protein and oil contents may have pleiotropic effects. In our study, both traits were associated with the same genetic markers at some of the detected QTL across environments and genetic backgrounds. Quantitative trait loci with pleiotropic effects for protein and oil have been reported in several earlier studies (Brummer et al., 1997; Chung et al., 2003; Diers et al., 1992; Hyten et al., 2004; Mansur et al., 1996; Orf et al., 1999; Sebolt et al., 2000). However, the reverse was also true; a number of protein and oil QTL were identified independently in diverse genetic backgrounds and mapped in different genomic regions (Brummer et al., 1997; Mansur et al., 1993; Specht et al., 2001). So far no validated QTL was discovered with pleiotropic effects that simultaneously increased both seed protein and oil contents. We have compared QTL detected and mapped in our study with QTL previously reported within a 10-cM vicinity. The soybean consensus genetic map (Hyten et al., 2010) was used for comparison and discussion because the consensus map contains all SNP markers of the USLP 1.0 array (Hyten et al., 2010) used in the present study. Also, we used the soybean composite genetic map (Song et al., 2004) for cross-comparison because it contains more restriction fragment length polymorphism (RFLP) and SSR markers and a few SNP markers. Three SNP markers (BARC-059985-16274, BARC-044639-08743, and BARC-059997-16280) located on Chr. 6 were consistently associated with protein and oil QTL in both populations and environments (Table 4). The SNP marker BARC-059997-16280 was also associated with seed weight QTL detected in both environments. One SSR marker, Sat_336, was associated with both oil and seed weight QTL and another SSR marker, Satt281, was also associated with protein and seed weight QTL. In this case,

protein, oil, and seed weight QTL mapped to a common genomic region on Chr. 6. In same genomic region, Shi et al. (2010) earlier mapped protein and oil QTL and Orf et al. (1999) mapped oil QTL (Oil 9-2). So far, no seed weight QTL has been reported in this region. With the consistency and confirmation obtained in our study, it was concluded that this was a novel QTL impacting the seed weight trait. One protein QTL (between markers BARC-041257-07953 and BARC-060051-16321) located on Chr. 5 was associated with seed oil QTL in both populations and environments. Previously, protein (Pro 2-1, Pro 9-1, and Pro 12-1), oil (Oil 3-2, Oil 10-1, and Oil 13-1), and seed weight (Sd wt 1-3 and Sd wt 10-1) QTL were mapped in the same genomic regions (Mansur et al., 1996; Orf et al., 1999; Specht et al., 2001). We were able to detect and consistently map protein and oil QTL on Chrs. 5 and 6 while using different genetic populations grown in different environments. Our results confirm similar findings of earlier genetic mapping studies.

One SSR marker (Satt632) located on Chr. 8 was consistently associated with protein QTL in MPC at both locations. Although a large number of protein and oil QTL have been mapped in the soybean genome, only a few were mapped on Chr. 8. Earlier, one oil (Oil 1-1) QTL (Mansur et al., 1993), protein (Pro 26-1) QTL (Reinprecht et al., 2006), and seed weight QTL (Sd wt 4-5, Sd wt 6-2, and Sd wt 13-1) (Hoeck et al., 2003; Maughan et al., 1996; Orf et al., 1999) were mapped close to Satt632 detected in our study. Two SNP markers (BARC-014705-01623 and BARC-029825-06442) located on Chr. 7 were associated with seed protein in the MPB population in both environments. Three protein (Pro 12-4, Pro 13-4, and Pro 24-4) QTL (Csanadi et al., 2001; Hyten et al., 2004; Specht et al., 2001) and one oil (Oil 23-6) QTL (Hyten et al., 2004) were mapped about 12 cM downstream of SNP marker BARC-014705-01623. On Chr. 3, one protein QTL (between BARC-016199-02307 and BARC-053313-11792 in MPB and between BARC-028645-05979 and BARC-055149-13089 in MPC) detected at BREC and oil QTL (between BARC-055637-13558 and BARC-057823-14942) were detected in one location and in one population. Lee et al. (1996) reported two protein QTL (Pro 4-8 and Pro 4-9) and Qi et al. (2011) detected two oil QTL (Oil 24-5 and Oil 24-13) in this region. On Chr. 2, an oil QTL (between markers BARC-044747-08795 and BARC-054149-12354) was identified in the MPC population in both environments. Two oil QTL earlier reported (Oil 19-1 and Oil 24-3) were mapped close to above SNP markers (Panthee et al., 2005; Qi et al., 2011). On Chr. 15, an oil QTL (between BARC-039687-07541 and BARC-042349-08247) was detected in the MPB population at both locations and another protein QTL (between BARC-018923-03037 and BARC-025493-06513) was identified at DRC in the MPB population and mapped close to the

oil QTL. Earlier, protein and oil contents and seed weight QTL were mapped in the same genomic region (Diers et al., 1992; Lee et al., 1996; Qi et al., 2011).

An important QTL for seed weight was mapped on Chr. 19 (between marker Satt527 and BARC-047496-12943 and marker BARC-014885-01914 and Satt527), which was detected in both genetic backgrounds and environments and explained total phenotypic variation of about 10% (Table 4). This result was in agreement with previously reported seed weight QTL (Sd wt 5-1 and Sd wt 15-7) (Hyten et al., 2004; Orf et al., 1999). In our study, another seed weight QTL (BARC-045297-08928_Satt184) was consistently mapped on Chr. 1 in the MPB population across environments. The result was in good agreement with the findings as reported by Panthee et al. (2005).

We were able to successfully detect several major QTL for seed protein and oil contents and seed weight in one mapping population and were able to confirm the consistency of genetic position with confidence interval estimation (van Ooijen, 1992) in another genetic background in two different environments. Although we did not find new QTL for protein or oil contents, however, in the present study we were able to reconfirm previous findings. Reconfirmed protein and oil QTL on Chrs. 5 and 6 flanked by SSR and SNP markers may be important targets, leading to the identification of candidate genes involved in modification of protein and oil using genetic and genomic approaches. We did find a new QTL for seed weight on Chr. 6 at the same genomic region of protein and oil QTL. Reconfirmed seed weight QTL on Chr. 19 along with the new QTL warrant further investigation. Simple sequence repeat and SNP markers tightly associated with QTL regions without pleiotrophic effects may be used in marker-assisted breeding programs to develop new soybean germplasm and cultivars with high protein and oil contents. Moreover, these genetic markers would facilitate development of near-isogenic lines using marker-assisted backcrossing, which could lead to fine mapping and map-based gene cloning for high protein and oil contents and higher seed weight in soybean.

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