SSR Markers Linking to Seed Traits and Total Oil Content in Soybean

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Abstract

Soybean is an economic crop used as a main source of vegetable oil. Quality and quantity of oil in soybean seed is important as a raw material determining manufacturing cost. The markers associating with oil content are useful in selection for soybean lines with high oil. The aim of this research was to develop molecular markers linking to genes controlling seed traits and total oil content in soybean. An $F_{2:3}$ population comprising 186 families was developed from a cross between Pak Chong 2 and Laos 7122. The population was genotyped by 159 polymorphic SSR markers, and seeds were determined for oil content by hexane extraction method. QTL analysis was done by a simple regression method and composite interval mapping. Finally, 138 SSR markers were grouped into 30 linkage groups covering 1,921.1 cM of soybean genome. Twenty-one polymorphic markers remained unlinked. There were 10 QTLs located on linkage groups A1, C2, E, and G that found associating with nodes per plant, seed length, seed width, hundred seed weight and total oil content.

Keywords: molecular marker, *Glycine max*, QTL mapping, linkage map

Introduction

Soybean (*Glycine max* (L.) Merr.) is the most important vegetable oil and feeds crop in the world. The ranges of oil and protein are 19.0-23.5% and 34.9-39.6%, respectively (Liu, 1997; Hildebrand et al., 2008). Annually, Thailand imports over 3 million tons of soybean seed and cake from the US, Brazil, Argentina and China (Office of Agricultural Economics, 2010), because Thailand cannot produce enough to meet with her domestic demand. Approximately 85% of soybean produced in Thailand is used in vegetable oil industry. The main problem of Thai soybean itself is low yielding and medium oil content. Recently, breeders have used molecular marker technique as a tool for efficiently screening desirable traits in segregating plant populations. This technique could save time and money as compared to conventional phenotypic selection in the field. The objective of this research was to develop molecular markers linking to genes controlling oil content and seed traits in soybean.

Materials and Methods

Plant Materials and Phenotying

An $F_{2:3}$ population comprising 186 families was developed from crossing between Pak Chong 2 and Laos 7122 and grown in the field of Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. Data on agronomic traits were collected from 5 random plants of each F_3 family. Plant height (m) was measured from the ground to the tip of the central axis on main stem when 95% of plants in the plot attained maturity. Number of nodes on main stem per plant was counted and averaged data from the 5 plants were used in analysis. Seed length and seed width were measured in mm from ten seeds each of the 5 plants. One hundred seed weight (g) was measured from 100 seeds per plant.

Total oil content was determined from ground F_4 seed samples using hexane extraction method which had been optimized for 0.5 g soybean meal. A suitable condition for hexane extraction in 16×100 mm test tube was used to screen the population. Briefly, each sample was extracted by 3 ml hexane volume by shaking for 15 min and incubated for 3 h in each cycle. The extraction process was repeated 3 times and incubated overnight in the last step. Total oil content was presented in percentage of dry meal.

DNA Isolation and SSR Genotyping

Two to three grams of young trifoliolate leaves were collected in bulk from each parental line and individual F_2 plants. Genomic DNA was extracted by modified CTAB method recommended by Lodhi et al. (1994). DNA concentration was estimated on 0.8% agarose gel electrophoresis for 30 min at 100 V in 1x TBE buffer, stained with ethidium bromide. The DNA bands were compared with a known concentration of standard λ DNA and photographed under UV light using a gel documentation (SYNGENE, Genius). The stock DNA solution was adjusted to the working concentration of 10 ng μL^{-1} with TE buffer pH 8.0 and stored at -20ºC until use.

SSR markers were synthesized following the sequences published on the Soybase website (http://soybase.org). Four hundred and twenty-eight SSR primers were used to survey for polymorphism among the parental lines. The polymorphic markers were later used to genotype the $F_{2:3}$ population. Polymerase chain reaction (PCR) was performed in a 10 μL volume containing 1x *Taq* buffer with $(NH_4)_2SO_4$, 40 ng DNA, 2.5 μM forward and reverse primers, 20 mM $MgCl_2$, 200 μM each dNTP, 1 unit *Taq* DNA polymerase (Fermentas, Lithuania). PCR reaction was conducted in a thermocycler of MJ Research model PTC-100^{TM} (MJ Research, Inc., Watertown, USA.). The reactions were pre-denatured at 94ºC for 2 min and denatured at 94ºC for 30 s. The cycle was repeated 35 times, then annealed for 30 s at 47-55ºC, depending on SSR primers, elongation at 72ºC for 1 min and the final elongation was held at 72ºC for 10 min. Amplified PCR product was separated by electrophoresis on denaturing 5% polyacrylamide gel in 0.5x TBE buffer. The polyacrylamide gel was cast using the Bio-Rad 38 × 50 cm gel apparatus and electrophoresed at a constant power of 70 W for 3-4 h depending on the PCR product size. The gel was stained with silver stain solution and visually scored. The polymorphic SSR data were used to construct a linkage map. The marker data were scored in each SSR loci, giving A for homozygous alleles inherited from the female parent (Pak Chong 2), giving B for homozygous alleles inherited from the male parent (Laos 7122), giving H for heterozygous alleles from both parents. Only the primers with clear and repeatable in the parents were used to screen the $F_{2:3}$ population.

Data Analysis

Segregation of each SSR primer in the $F_{2:3}$ population was tested for goodness of fit against a 1:2:1 ratio by a Chi-square. The marker loci that fitted with the ratio were used to construct the linkage map using JoinMap 3.0 software (Van Ooijen and Voorrips, 2001). The specified parameters included the map distance in Kosambi function with the linkage criteria, a LOD score of greater than 3.0 and the maximum genetic distance of 50 cM. The error detection probability level was set at 5%. All SSR markers were initially tested for their significance by simple regression analysis (R software version 2.8.1, 2006). Then, a QTL mapping was performed by composite interval mapping (CIM) using Window QTL Cartographer 2.5 software (Wang et al., 2007). A total of 1,000 permutations was performed on each trait for the

empirical logarithm of the odd (LOD) threshold at the significant level 0.05. The QTL position was considered from LOD peaks at individual loci detected by maximum likelihood test at an increment of 1.0 cM walking between the adjacent linked markers.

Results and Discussion

Phenotypes of Seed Traits and Oil Content

All traits in the F_2 population showed continuous distribution (Figure 1). Plant height, seed length, seed width, one hundred seed weight were normally distributed, whereas the other traits skewed toward one end. The traits with normal distribution varied continuously from 0.43-1.57 m, 7.17-8.41 mm, 5.91-6.78 mm and 12.52-19.62 g, respectively (Table 1). The normality test of the $F_{2:3}$ population indicated that number of nodes on main stem and total oil content were not normally distributed with the range of 12-25 nodes and 15.03-25.31%. Transgressive segregation was observed in all traits, revealing that the parents carry on alleles which contribute the effect in different direction when recombined in the hybrids. It is an important mechanism contributing to adaptive evolution (Rieseberg et al., 1999; Rieseberg et al., 2003; Bell and Travis, 2005).

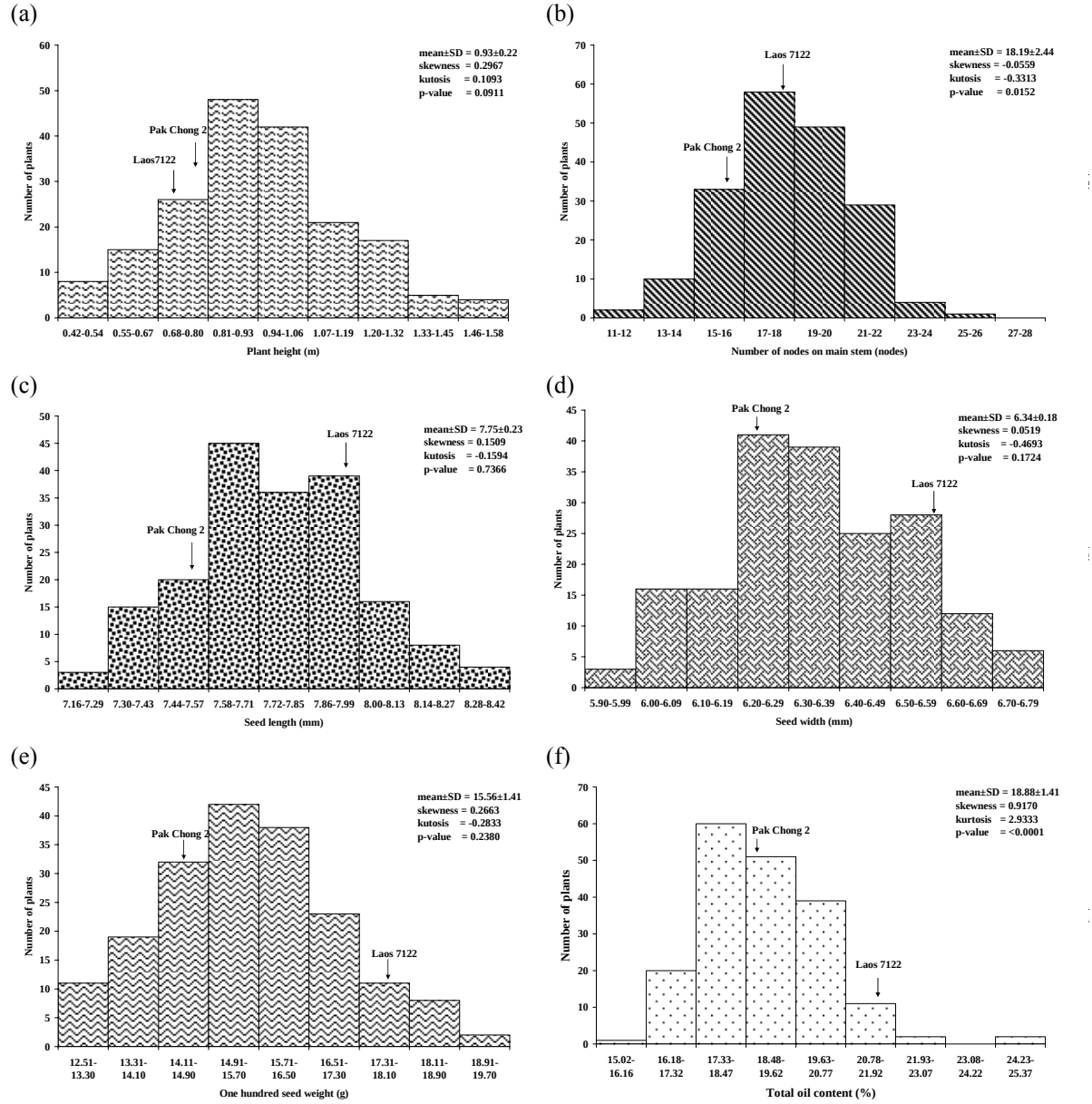

Figure 1 Frequency distribution of plant height, number of nodes on main stem, seed length, seed width, 100 seed weight and total oil content in the $F_{2:3}$ population from the cross Pak Chong 2 × Laos 7122.

Table 1 Mean and range of plant height, number of nodes on main stem, seed length, seed width, 100-seed weight and total oil content in $F_{2:3}$ population from the soybean cross Pak Chong 2 × Laos 7122.

Trait	Mean±SD			Population range
	Pak Chong 2	Laos 7122	$F_{2:3}$ population	
Plant height (mm)	0.77±0.03	0.70±0.04	0.93±0.22	0.43-1.57
Number of nodes	16±1.14	18±1.25	18.19±2.44	12-25
Seed length (mm)	7.51±0.05	7.90±0.35	7.75±0.23	7.17-8.41
Seed width (mm)	6.22±0.05	6.59±0.08	6.34±0.18	5.91-6.78
100 seed weight (g)	14.49±1.02	17.96±0.93	15.56±1.41	12.52-19.62
Total oil content (%)	18.69±0.21	21.75±0.53	18.88±1.41	15.03-25.31

SSR Genotyping

Among 428 SSR primers surveyed in the parental lines, 240 (56.07%) were polymorphic. Of these, 159 markers (66.25% of polymorphic markers) showed polymorphism among 186 $F_{2:3}$ families. Based on the χ^2 test, out of 159 SSR markers, 112 (70.44% of polymorphic markers) showed Mendelian segregation of 1:2:1, while the rest 47 markers exhibited distortion ratios.

Linkage Analysis

One hundred and thirty-eight SSR markers were mapped onto 30 linkage groups (LGs) and covered 1,921.1 cM of soybean genome (Figure 2). Twenty-one polymorphic markers remained unlinked. The genetic map represented approximately two third of the consensus soybean map (2,523.6 cM; Song et al., 2004; Soybase, 2005). The linkage groups consist of 2 to 11 SSR markers with an average distance of 14 cM between the adjacent loci. The length of the LGs varied from 13.9 to 218.0 cM. The shortest LG was F-1 (sub-group) while the longest one was D1b. The markers were dispersed but did not cover throughout the soybean genome. There were many gaps up to 50 cM in some LGs such as C1, because the map was constructed from the markers that may not be well-spreading in the soybean genome.

Figure 2 showed 11 LGs that were consistent with the consensus map of Cregan et al. (1999), consisting of A1, B1, B2, C1, D1b, G, H, I, K, L and N. Eight LGs, viz A2, C2, D2, E, F, J, M and O were split into 2 sub-groups, while D1a was divided into 3 sub-groups. Most markers constructed in LGs and the map position in this research corresponded well with the soybean composite map (Soybase, 2005), with some variation in linkage distance between the markers. Some markers were mapped on the same LG as the reference map but the order of the markers was somewhat different, especially among the closely linked markers. Gene duplication was one reason causing marker allocation in different positions (Shoemaker et al., 1996; Soybase, 2005). Some markers with segregation distortion may also affect the map position.

QTL Analysis

Both simple regression analysis (Table 2) and composite interval mapping (Table 3) were used to identify SSR markers associated with the traits under study. The results of simple regression analysis showed that there were two SSR markers on LG-A1 including Satt236 and Satt258 accounting for 7.90% and 6.12% of the total variation in plant height, respectively. Three markers consisting of Satt313, Satt418 and Satt523 on LG-L also linked to this trait (Table 2). However, analysis by CIM revealed no QTL for plant height but at least one QTL was detected for the other traits. The main reason that we were unable to locate the QTL for plant height because the difference between the parents was too low (0.77 m for Pak Chong 2 and0.70 m for Laos 7122). The QTLs for plant height were reportedly located on linkage group B1 (Zhang et al., 2004), N (Reinprecht et al., 2006), F (Reinprecht et al., 2006), C2 (Zhang et al., 2004; Reinprecht et al., 2006), D1b (Kabelka et al., 2004; Reinprecht et al., 2006), M (Zhang et al., 2004) and O (Reinprecht et al., 2006).

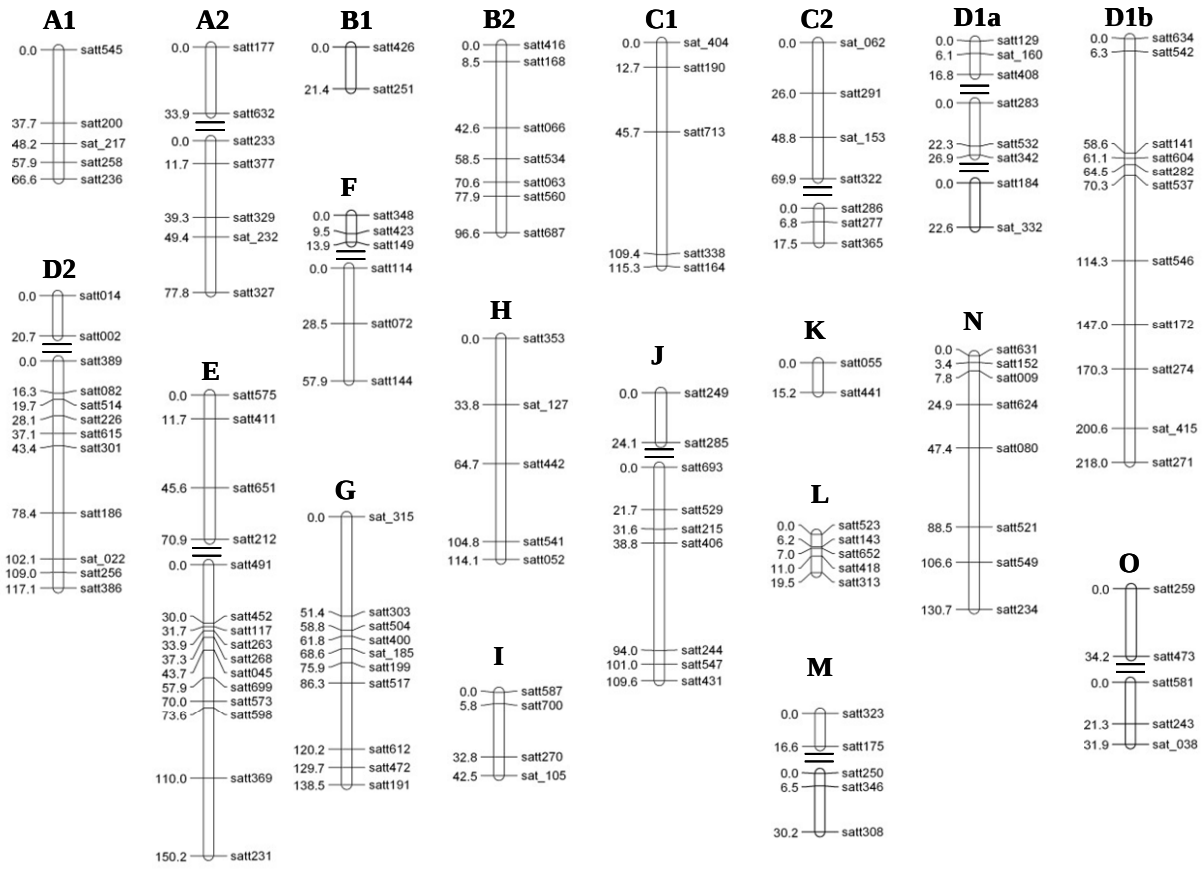

Figure 2 A soybean genetic linkage map developed from $F_{2:3}$ population from the cross Pak Chong 2 × Laos 7122 comprising 138 SSR markers grouping into 30 linkage groups and spanning 1,921.1 cM. The map shows marker position and estimated distance (cM) on the left-hand side and marker name on the right-hand side.

There were three markers from simple regression analysis linked to number of nodes on main stem on LG-C2 (Sat_153), D1b (Satt537) and L (Satt313), but only one QTL from CIM were found flanking by Sat_153 and Satt322 on LG-C2 and explained 9.68% of total variation in this trait. Zhang et al. (2004) found QTLs of this trait on LG-A2, B1, C2, F and I.

SSR markers associated with seed length were initially identified on LG-B2, E, F, G and H via simple regression analysis (Table 2). CIM analysis located two QTLs on LG-C2 (Satt291 and Sat_153) and G (Sat_315 and Satt303), explaining 8.13 and 11.10%, respectively via CIM. Only Satt303 on LG-G was found significant from both analytical methods.

On the basis of simple regression analysis, there were many SSR markers associated with seed width located on LG-A2, B2, C2, E, G and L (Table 2). By CIM, there were three QTLs for this trait locating on LG-C2 (Satt291 and Sat_153), E (Satt045 and Satt699) and G (Satt303 and Satt504). They respectively explained 10.88, 7.56 and 10.11% of the variation in seed width. Most markers which reported from CIM were the same as reported by simple regression analysis, except Sat_153.

Nine SSR markers linking to 100-seed weight were located on LG-E and G by simple regression analysis (Table 2). Linkage groups C2 and E harbor QTLs for this trait between Satt291 and Sat_153 and Satt699 and Satt573 in the order by CIM. The QTLs accounted for 10.47 and 10.69% of the variation in seed weight, respectively (Figure 3 and Table 3). Only QTL on LG-E via CIM was located on the same region as reported via simple regression analysis. QTLs for seed mass were earlier reported on LG-A2 (Zhang et al., 2004), B1 (Zhang et al., 2004), B2 (Watanabe et al., 2004), C2 (Hyten et al., 2004; Watanabe et al., 2004;

Table 2 Simple regression for effect of individual markers on QTLs locating on 4 soybean linkage groups.

SSR	LG	$\%R^2$ of trait					
		Plant height	Number of nodes	Seed length	Seed width	100-Seed weight	Total oil content
Sat_217	A1	-	-	-	-	-	21.99
Satt200	A1	-	-	-	-	-	17.95
Satt236	A1	7.90	-	-	-	-	17.30
Satt258	A1	6.12	-	-		-	23.92
Sat_332	A2	-	-	-	5.97	-	-
Satt083	B2	-	-	5.64	6.22	-	-
Sat_062	C2	-	-	-	7.36	-	-
Sat_153	C2	-	5.40	-	-	-	-
Satt291	C2	-	-	-	6.53	-	-
Satt537	D1b	-	6.19	-	-	-	-
Satt045	E	-	-	-	6.82	7.30	-
Satt117	E	-	-	-	6.45	-	-
Satt263	E	-	-	-	7.20	6.43	-
Satt268	E	-	-	-	5.32	-	-
Satt369	E	-	-	-	8.22	5.29	-
Satt452	E	-	-	-	7.82	5.71	-
Satt573	E	-	-	-	5.97	8.89	-
Satt598	E	-	-	-	5.65	6.72	-
Satt699	E	-	-	5.60	9.64	10.00	-
Satt348	F	-	-	7.11	-	-	-
Sat_185	G	-	-	5.01	5.06	-	-
Satt303	G	-	-	6.97	8.22	5.76	-
Satt400	G	-	-	-	5.21	-	-
Satt504	G	-	-	5.87	7.22	5.66	-
Sat_218	H	-	-	5.40	-	-	-
Satt143	L	-	-	-	5.04	-	-
Satt313	L	6.83	5.10	-	-	-	-
Satt418	L	6.45	-	-	-	-	-
Satt523	L	5.46	-	-	-	-	-

Reinprecht et al., 2006), D1a (Hyten et al., 2004; Panthee et al., 2005), D2 (Zhang et al., 2004; Panthee et al., 2005; Liu et al., 2007), F (Hyten et al., 2004), G (Hyten et al., 2004), H (Watanabe et al., 2004; Liu et al., 2007), I (Hyten et al., 2004; Reinprecht et al., 2006), J (Reinpreht et al., 2006), K (Hyten et al., 2004; Watanabe et al., 2004), L (Hyten et al., 2004), M (Liu et al., 2007) and O (Liu et al., 2007).

Three QTLs consisting of *SN6.1, SeedL6.1, SW6.1* were co-located on LG-C2 with the additive effect of -1.0013 nodes, 0.0739 mm and 0.5389 g, respectively. The alleles increasing number of nodes on main stem were from Laos 7122, while those increasing seed length and seed weigh were from Pak Chong 2. *SeedW6.1* was overlapped to these QTLs with the additive effect of 0.0769 mm (Table 3). *SeedW15.1* and *SW15.1* co-located on

Table 3 Description of the QTLs controlling traits in soybean under study on 4 LGs, as analyzed by composite interval mapping.

Trait	QTL	LG	Position (cM)	Flanking markers	LOD	PVE (%)	Additive effect	Dominant effect
Number of nodes	*SN6.1*	C2	53.8	Sat_153-Satt322	3.7	9.68	-1.0013	0.3697
Seed length (mm)	*SeedL6.1*	C2	48.8	Satt291-Sat_153	3.5	8.13	0.0739	-0.0811
	SeedL18.1	G	51.4	Sat_315-Satt303	3.5	11.10	-0.0838	0.0962
Seed width (mm)	*SeedW6.1*	C2	34.0	Satt291-Sat_153	3.6	10.88	0.0769	-0.0372
	SeedW15.1	E	56.8	Satt045-Satt699	3.6	7.56	-0.0729	-0.0001
	SeedW18.1	G	52.4	Satt303-Satt504	3.6	10.11	-0.0811	0.0187
100-seed weight (g)	*SW6.1*	C2	43.0	Satt291-Sat_153	3.5	10.47	0.5389	-0.4446
	SW15.1	E	60.9	Satt699-Satt573	3.5	10.69	-0.6590	-0.0088
Total oil content (%)	*OL5.1*	A1	45.7	Satt200-Sat_217	3.5	27.16	0.9482	-0.5908
	OL5.2	A1	54.2	Sat_217-Satt258	3.5	45.18	1.0323	-0.4690

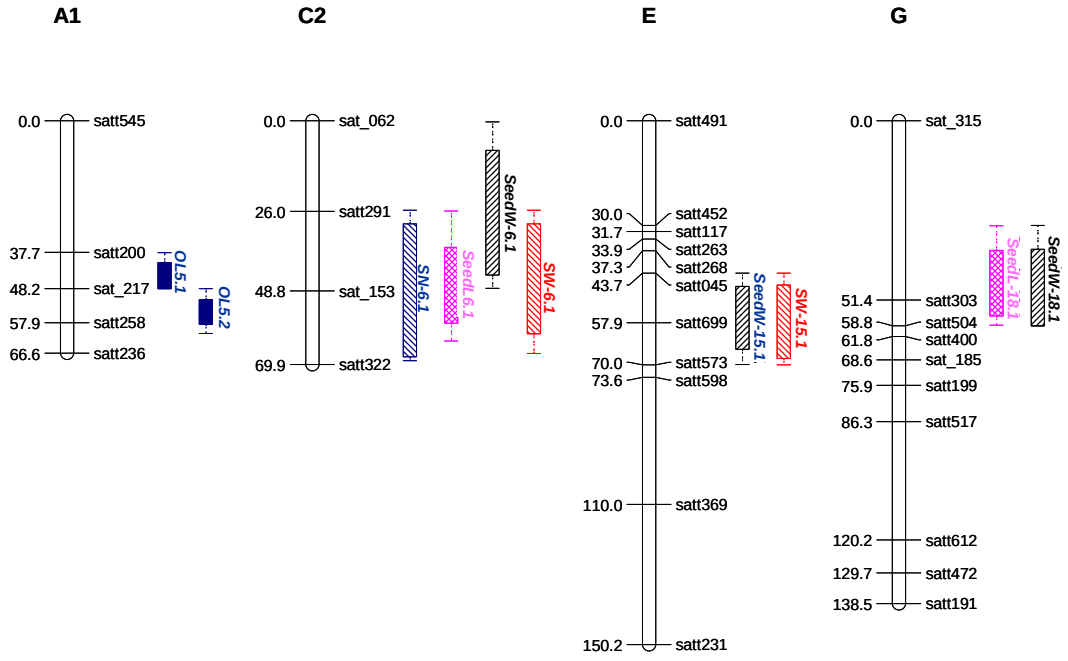

Figure 3 QTLs of 6 traits detected from $F_{2:3}$ population derived from Pak Chong 2 × Laos 7122 and found locating on four linkage groups. These QTLs were found conditioning number of nodes on main stem (*SN*), seed length (*SeedL*), seed width (*SeedW*), one hundred seed weight (*SW*), total oil percentage (*OL*).

LG-E with the additive effect of -0.0729 mm and -0.6590 g respectively. The alleles that increase seed width and seed weight were both from Laos 7122. *SeedL18.1* and *SeedW18.1* co-located on LG-G with the additive effect of -0.0838 mm and -0.0811 mm, respectively. The alleles increased seed length and seed width were from Laos 7122. Alleles from Pak Chong 2 increased seed length, seed width and seed weight but decreased number of nodes on main stem. These relationships showed that it was rather difficult to improve all these traits at the same time.

Our research found one QTL for number of nodes on main stem, two QTLs for seed length, three QTLs for seed width and two QTLs for 100-seed weight. Although many markers were found, their contribution was small and no major QTL was observed.

Similar results from simple regression analysis and CIM were detected in two major QTLs linked to total oil content on LG-A1. One QTL locates between Satt200 and Sat_217 which explains 27.16% of the total variation, while the other locates between Sat_217 and Satt258 and accounts for 45.18%. Although the difference in oil content between parents is not large, it is possible that they are different in loci controlling this trait and thus causing transgressive segregation among the progenies. Diers et al. (1992) reported markers linked to oil content on LG-A. The other QTLs for oil content were found on LG-A2 (Tajuddin et al., 2003), B2 (Tajuddin et al., 2003), C1 (Lee et al., 1996), C2 (Hyten et al., 2004), D1a (Hyten et al., 2004), D1b (Kabelka et al., 2004; Panthee et al., 2005), D2 (Hyten et al., 2004), E (Lee et al., 1996; Reinprecht et al., 2006; Shibata et al., 2008), H (Lee et al., 1996; Panthee et al., 2005), I (Lee et al., 1996; Tajuddin et al., 2003; Shibata et al., 2008), J (Tajuddin et al., 2003), G (Lee et al., 1996; Reinprecht et al., 2006), L (Hyten et al., 2004; Reinprecht et al., 2006), M (Tajuddin et al., 2003; Hyten et al., 2004) and O (Tajuddin et al., 2003, Panthee et al., 2005).

None of markers located on LG-A1 has been reported linking to total oil content in the past so Satt200, Sat_217 and Satt258 are novel markers. Two QTLs of total oil content linking to *OL5.1* and *OL5.2* were overlapped on LG-A1. Their additive effects were 0.9482% and 1.0323%, respectively. Both alleles from Pak Chong 2 increased total oil content. Figure 3 presents four linkage groups viz. A1, C2, E and G harboring 10 QTLs of six traits upon CIM. The traits revealed no QTL on the other 16 linkage groups, viz. A2, B1, B2, C1, D1a, D1b, F, G, H, I, J, K, L, M, N and O.

Conclusions

Two major QTLs for total oil content on LG-A1 were identified and thus can be used in breeding to improve oil content in soybean.

Acknowledgments

This research was supported by (1) The Royal Golden Jubilee (RGJ) Scholarship and The Project on Genetics and Breeding of Field Legumes for Thailand of the Thailand Research Fund (TRF), (2) the Center for Agricultural Biotechnology of Kasetsart University, (3) Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Thailand. The authors acknowledge Dr. Joseph W Burton, Dr. Jim B Holland and Dr. Andrea J Cardinal from the USDA-ARS and the North Carolina Agricultural Research Service (Raleigh, NC) for technical assistance and helpful suggestions, and Dr. Amornrat Promboon and Dr. Chongrak Kaewprasit of Kasetsart University for their kind advices.

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Manuscript received 16 August 2011, accepted 2 December 2011