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Detection of Quantitative Trait Loci for Seed Size Traits in Soybean (*Glycine max* L.)

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ABSTRACT

Detection of validated and confirmed quantitative trait loci (QTLs) is essential to conduct more successful marker-assisted selection breeding programs. The objectives of this study were to determine the QTLs controlling seed size traits in soybean (*Glycine max* L.) and to compare those identified with the previously reported QTLs. One hundred thirty-five $F_{2:3}$ lines derived from the cross MJ0004-6 × R18500 were evaluated in the experimental fields of Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand in a randomized complete block design with two replications each in the 2011 and 2012 growing seasons. The population was genotyped by 149 polymorphic simple sequence repeat markers and the genetic map consisted of 129 simple sequence repeat loci which converged into 38 linkage groups covering 1156 cM of soybean genome. There were 16 quantitative trait loci (QTLs) significantly associated with seed size traits across two seasons with single QTLs explaining between 7.4% and 18.4% of the phenotypic variation. One novel QTL (*SL6*) on linkage group K was consistently mapped in both seasons. Most QTLs were environment-sensitive, revealing that the genotype × environment interaction played an important role in expression of seed size traits in soybean. The majority of QTLs detected in the research were consistent with earlier QTLs reported by previous researchers.

Keywords: seed size traits, linkage map, quantitative trait loci, simple sequence repeat, soybean

INTRODUCTION

Genetic improvement through plant breeding is an approach to increase soybean production. The most important objective in soybean breeding is to increase seed yield, which is the product of number of plants per unit area, number of seeds per plant and 100-seed weight. The 100-seed weight is affected by seed size as measured by seed length, width and thickness and seed size is also important in soy food products, including tofu, natto, miso and edamame (Hoeck *et al.,* 2003). Small-seeded soybeans are desirable for high quality soybean sprouts and natto production, whereas large-seeded ones are desirable for tofu, edamame and miso production (Wilson, 1995).

Cober *et al.* (1997) reported moderate to high heritabilities (59–79%) for seed size traits. Liang *et al*. (2005) showed that the inheritance of seed length, width and thickness are mainly controlled by cytoplasmic or maternal effects. Salas *et al.* (2006) mapped 19 significant

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quantitative trait locis (QTLs) on 10 linkage groups for seed size traits in three populations of recombinant inbred lines of soybean. Liang *et al*. (2008) mapped seven QTLs for seed length, three for seed width and three for seed thickness. Five hundred four $F_{2:3}$-$F_{2:7}$ families of soybean from the direct and reciprocal crosses of Lishuizhongzihuangdou × Nannong493-1 were evaluated in 2007–2011 to detect main-effect quantitative trait loci, QTL-by-environment, QTL-by-cytoplasm and QTL-by-QTL interactions for the soybean seed size traits (Yan *et al.,* 2013). The results indicated that the most important genetic component is main-effect quantitative trait loci. Recently, 257 soybean cultivars obtained from six geographical ecotypes in China were used to carry out association mapping for seed size traits using 135 microsatellite markers (Niu *et al.,* 2013). In total, 59 main-effect QTLs and 31 QTL-by-environment interactions were identified.

Seed size traits are complex and polygenic traits. Therefore the use of molecular markers for indirect selection of these traits may be helpful to breeders. The objectives of this study were to identify and characterize QTLs affecting seed size traits in soybean and to compare those QTLs with the previously reported QTLs.

MATERIALS AND METHODS

Plant materials

In total, 135 $F_{2:3}$ lines were developed from a cross between a large-seeded vegetable soybean breeding line from Thailand (MJ0004-6) with a 100-seed weight of 36.8 g and a small-seeded line from the Republic of the Union of Myanmar (R18500) with a 100-seed weight of 11.1 g. MJ0004-6 was developed from a cross between #75 (commercial vegetable soybean cultivar of Taiwan) and Chamame (Japanese vegetable soybean variety) by the Chiang Mai Field Crop Research Center, Thailand, while R18500 was collected from a local variety in Nyaung Kar village nearby the Chtone Bo Research farm, Myanmar. The $F_{2:3}$ population and their parents were grown in a randomized complete block design with two replications in the research fields of Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand (14°01′N, 99°59′E, 7.5m above mean sea level) in the 2011 and 2012 growing seasons (November 2011–March 2012 and November 2012–March 2013, respectively). Each plot was a single row 2 m long, constituting 7 plants in each row (a total of 14 plants for each line with the two replications), with a space of 20 cm between the adjacent plants.

Trait measurement

In both seasons, the seed size traits evaluated were seed length, seed width, seed thickness and seed volume. Seed length was defined as the longest distance across the seed parallel to the hilum. Seed width was determined as the longest distance across the seed from the hilum to the opposite site. Seed thickness was the longest distance from the top to bottom of the flat side of the seed. Twenty- four seeds were randomly sampled from each line and used to record seed size traits.

Statistical analyses

Trait means, ranges, standard deviations and a *t*-test for testing the significance of differences between the two parental lines for each trait were determined for both years as well as for the mean data. Pearson phenotypic correlation coefficients among traits were calculated using the R program (R Core Team, 2013). Frequency distributions of entry means over seasons were plotted for all traits.

DNA extraction and molecular marker development

Total genomic DNA samples of the parental plants and individual F_2 plants were extracted from fresh young leaf tissue using the modified cetyltrimethylammonium bromide (CTAB) method advocated by Lodhi *et al.* (1994).

The DNA was quantified against a lambda DNA on 1.0% agarose gel stained with ethidium bromide and diluted to 10 ng/µl. Simple sequence repeat (SSR) markers were synthesized following the sequences published on the SoyBase website (http://soybase.agron.iastate.edu/). In total, 506 SSR primers were used to survey for polymorphism among the parental lines. DNA amplification was performed using 2 µL of dH_2O, 1 µL of 10 × polymerase chain reaction (PCR) buffer, 2 µL of 1 mM of each deoxynucleotide, 2 µL of 5 pmol of each SSR primer, 0.8 µL of 25 mM $MgCl_2$, 0.2 µL of 1 U *Taq* DNA polymerase (Fermentas; Burlington, Ontario, Canada) and 2 µL of 10 ng/µL template DNA. PCR reactions were performed with a pre-denaturing at 94 °C for 2 min and denaturing at 94 °C for 30 s. The cycle was repeated 35 times, followed by annealing at 47 °C for 30 s, extension at 72 °C for 1 min and the final extension at 72 °C for 1 min. The PCR products were separated using electrophoresis on denaturing 5% polyacrylamide gels in 0.5 ×Tris-borate-ethylenediaminetetraacetic acid stained with silver stain.

Map construction and quantitative trait loci detection

A linkage map was constructed using the program JoinMap 3.0 (Van Ooijen, 2004). The genetic distance between markers was calculated using the Kosambi map function (Kosambi, 1944). Linkages among the adjacent markers were ensured at a minimum likelihood of odds (LOD) > 3.0 and a maximum distance < 50 centimorgan (cM). QTL analysis was performed following the composite interval mapping method (CIM) (Zeng, 1994) using the software application WinQTL Cartographer 2.5 (Wang *et al.,* 2007). A LOD score of 2.5 was set as a threshold for declaring the presence of a QTL. The phenotypic variation explained by QTLs and the additive and dominance effects of each QTL for all traits were calculated.

RESULTS

Phenotypic variation in parents and the population

The results of statistical analysis of seed size traits in the parents and population are shown in Table 1. For all traits, the differences between the two parents were highly significant. The combined data across two growing seasons of seed length (A), seed thickness (B), and seed width (C) were approximately normally distributed (Figure 1) suggesting that they were polygenically inherited. Transgressive segregation was observed only for seed length based on combined data.

Correlation among seed size traits

Correlation coefficients among seed size traits were calculated for each season (Table 2). All traits were highly correlated ($P < 0.01$) with each other in both seasons with the correlation coefficients ranging from 0.45 to 0.77.

Genetic map construction

Of the 506 SSR markers, 232 markers (46%) were polymorphic between the parental lines. Of these, 149 markers were used in the initial linkage map construction. Based on the relative positions of these markers on the reference genetic maps (http://soybase.agron.iastate.edu/), 129 markers with clear polymorphic bands were assigned onto 38 linkage groups. The coverage ratio of the linkage map constructed in this study to the whole soybean genome was 46% (1156 cM out of 2512 cM (Song *et al.,* 2004)). Twenty markers remained unlinked. Linkage groups were designated with names corresponding to the integrated public soybean genetic map (Song *et al.,* 2004). In this study, five linkage groups (B1, D1a, H, I and N) were consistent with those of Cregan *et al.* (1999), while the A1, A2, B2, C2, D1b, D2, G, J, K, L, M and O linkage groups were split into two subgroups and C1, E and F were divided into three subgroups. Most of the markers mapped in the population showed Mendelian segregation

Table 1 Statistical analysis of seed size traits in the parents and $F_{2:3}$ population

Traits	Years	Parents		Difference between parents **	$F_{2:3}$ population			
		MJ0004-6	R18500		Range	Mean ± SD	Skew	Kurt
Seed length (mm)	2011	9.6 ± 0.3	7.4 ± 0.5	2.2	6.9–9.9	8.4 ± 0.5	-0.06	0.48
	2012	9.1 ± 0.3	7.3 ± 0.5	1.8	7.4–9.6	8.3 ± 0.5	0.08	-0.50
	Combined	9.3 ± 0.3	7.4 ± 0.4	1.9	7.0–9.6	8.4 ± 0.4	0.06	0.60
Seed thickness (mm)	2011	7.0 ± 0.3	4.8 ± 0.3	2.2	4.7–6.5	5.7 ± 0.3	-0.02	-0.17
	2012	7.2 ± 0.4	4.6 ± 0.1	2.6	4.6–6.5	5.5 ± 0.3	0.21	0.37
	Combined	7.1 ± 0.3	4.7 ± 0.2	2.4	4.9–6.3	5.6 ± 0.2	0.17	0.15
Seed width (mm)	2011	8.6 ± 0.4	6.0 ± 0.3	2.6	6.0–8.2	7.1 ± 0.4	-0.17	0.53
	2012	8.4 ± 0.3	5.8 ± 0.1	2.6	6.2–8.2	7.1 ± 0.4	0.31	0.43
	Combined	8.5 ± 0.3	5.9 ± 0.2	2.6	6.2–8.0	7.1 ± 0.3	-0.14	0.68

** All traits measured among parents were significantly different at 0.01 probability level

(1:2:1), while 24 markers (16.0%) showed a significant deviation from the expected ratio.

Quantitative trait loci analysis

All QTLs detected are listed in Table 3 and their locations are marked on the genetic map (Figure 2). In total, 13 different QTLs were detected across all traits in both seasons with single QTLs explaining between 7.4% and 18.4% of the phenotypic variations. The QTLs were distributed on nine linkage groups (A1, A2, B2, D1a, D1b, D2, F, J and K). One or more QTLs were identified on each linkage group, with linkage group D2 having the most (four QTLs) and linkage groups A1, B2, D1a, D1b, F and J having one QTL each.

Among the six QTLs found for seed length in this study, the largest QTL (*SL4*) was detected in the satt663-satt425 interval on linkage group F with a LOD score of 4.0 explaining 16.0% of the phenotypic variation in 2012, and was contributed by MJ0004-6. One stable QTL (*SL6*) was detected in the satt196-satt588 region for seed length on linkage group K, explaining 9.8 and 7.5% of the variation in 2011 and 2012, respectively, where the allele of MJ0004-6 increased the trait. Four seed thickness QTLs were identified on linkage groups A1, A2, D1b and D2. *ST2* was the largest QTL for seed width accounting for 16.2 and 15.7% of the total variation in 2011 and mean environmental data, respectively. Linkage groups A2 and D2 harbored three QTLs for seed width. The QTL (*SW1*) located on chromosome A2 in the satt632-satt493 region accounting for 14.7 and 16.2% of the variation in 2011 and mean environmental data, respectively, was found to be the largest QTL for seed thickness. In these QTLs, the alleles of MJ0004-6 increased seed thickness.

The QTL analyses through composite interval mapping in individual seasons demonstrated that four QTLs were detected in 2011 and nine QTLs were identified in 2012. Only one stable QTL was mapped across two seasons.

DISCUSSION

Parents of the mapping population used in this experiment were chosen based on their great differences in seed size traits. The results show highly significant correlations among seed size traits in both seasons. Earlier reports showed that seed size traits in soybean were highly correlated with each other (Salas *et al.*, 2006; Xu *et al.*, 2011).

The results of QTL analysis by CIM (Table 3), showed considerable differences between the significant levels across the two seasons, leading to the detection of many environmental-sensitive QTLs. *SL6* on linkage group K was the only stable QTL in the study. This QTL is rather distant to the *qSL-9* identified by Niu *et al.* (2013), thus it is likely to be independent and can be considered as a novel QTL. Because *SL6* was consistent across two seasons, molecular markers linked to this QTL are considered useful in increasing the frequency of desirable alleles in breeding for seed size. In agreement with the current result, Salas *et al.* (2006) detected only one stable QTL for seed size traits across populations and environments. Orf *et al.* (1999) also reported only one molecular marker significantly associated with seed size in three populations of soybean.

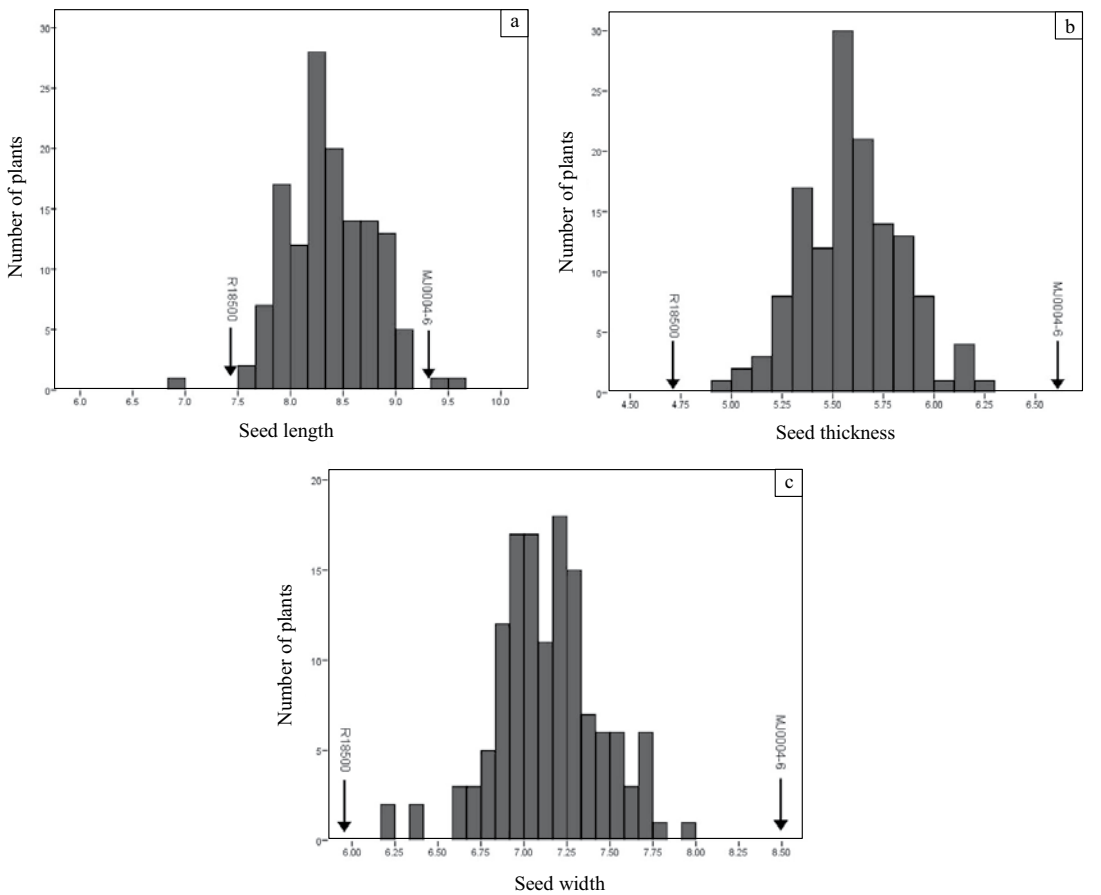

Figure 1 Frequency distributions of seed length (A), seed thickness (B), and seed width (C) (averaged across environments) in the $F_{2:3}$ population derived from the cross MJ004-6 x R18500. Parental means are marked with arrows.

Table 2 Pearson phenotypic correlation coefficients among seed size traits in 2011 and 2012 (italic)

Trait	Seed length	Seed thickness
Seed thickness	0.45**	
	*0.54***	
Seed width	0.74**	0.69**
	*0.77***	*0.63***

** Significant at 0.01probability level

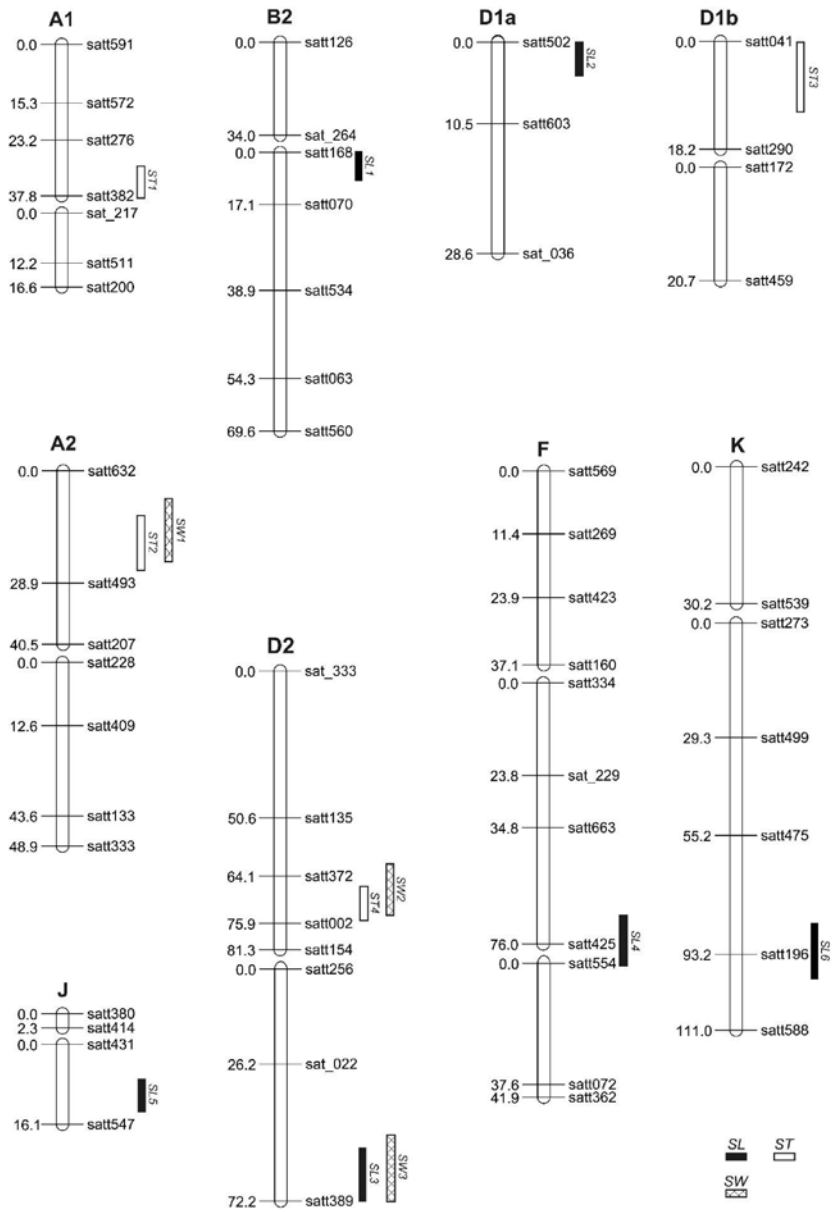

Figure 2 Locations of the QTLs associated with seed size traits in the $F_{2:3}$ population derived from the cross MJ0004-6 x R18500. Each marker name is shown on the right side of each linkage group and cumulative map distance (in centi-Morgan) is shown on the left side.

For practical breeding applications, QTLs should be validated in independent populations with different genetic backgrounds. Detection of QTLs controlling complex traits such as seed size traits without QTL confirmation experiments is inadequate for use in marker-assisted selection (MAS) breeding programs (Young, 1999). This study determined the QTLs using a mapping population with different genetic background and compared the QTLs with the previous reported QTLs found by other researchers.

To provide a complete comparison of QTLs found in this study to QTLs detected in the previous studies, locations of marker intervals associated with the QTLs conditioning soybean seed size traits in this study and in previous studies are shown in Tables 4, 5 and 6. The majority of QTLs from the current study were consistent with the earlier QTLs reported by previous researchers. For instance, *SL5* on linkage group J was consistent with the *qSL-16e-1* and *qSL-16e-2* identified by Niu *et al.* (2013) and the *qSL-16* reported by Xu *et al.* (2011). Linkage groups D2 and A2 harbor most QTLs (four and two QTLs, respectively) for seed size traits in this experiment. *ST2* and *SW1* on linkage group A2 found in the current study corresponded to the *qST-8* and *qSW-8-1* detected by Niu *et al.* (2013). Moreover, the QTLs in the current study for seed size traits on linkage group D2 (*SL3*, *SW3* and *ST4*) coincided well with the *qSL-17-1* reported by Xu *et al.* (2011), the *qSW-17e-1* identified by Niu *et al.* (2013) and the QTL located in the satt002-satt582 region found by Salas *et al.* (2006), respectively.

Co-located QTLs for seed size traits on linkage groups A2 and D2, and the correlations among these traits (Table 2) suggested that markers on these linkage groups can be simultaneously considered in MAS breeding programs. QTL clusters for seed size traits have also been

Table 3 QTLs conditioning seed size traits in the $F_{2:3}$ mapping population derived from MJ0004-6 x R18500

Trait	QTL	LG	Marker interval	Position (cM)	Years	LOD	Additive effect	Dominance effect	PVE (%)
Seed length	*SL1*	B2	satt168-satt070	0.6	2012	3.0	-0.18	-0.06	7.6
	SL2	D1a	satt502-satt603	1.4	2012	2.7	0.17	-0.06	7.4
	SL3	D2	sat_022-satt389	69.5	2012	2.8	0.20	-0.05	8.2
	SL4	F	satt663-satt425	49.2	2012	4.0	0.24	0.01	16.0
	SL5	J	satt431-satt547	10.9	2011	2.6	-0.20	0.18	10.0
	SL6	K	satt196-satt588	94.8	2011	3.0	0.95	0.28	9.8
					2012	2.6	0.41	0.21	7.5
					mean	5.2	0.76	0.27	15.0
Seed thickness	*ST1*	A1	satt276-satt382	34.0	mean	2.8	0.05	0.15	12.4
	ST2	A2	satt632-satt493	18.0	2011	2.8	0.16	0.14	16.2
					mean	3.0	0.11	0.10	15.7
	ST3	D1b	satt041-satt290	6.6	2012	2.8	0.14	-0.06	9.5
					mean	2.9	0.10	-0.06	11.1
	ST4	D2	satt372-satt002	71.1	2012	2.7	0.12	-0.10	9.4
Seed width	*SW1*	A2	satt632-satt493	14.6	2011	2.5	0.18	0.17	14.7
					mean	3.0	0.14	0.15	16.2
	SW2	D2	satt372-satt002	66.1	2012	2.8	0.10	-0.09	8.4
	SW3	D2	sat_022-satt389	69.5	2012	4.1	0.20	-0.03	13.5

Table 4 Locations of marker intervals associated with the QTLs conditioning soybean seed length in this study and in previous studies

LG	Marker interval	Position (cM[a])	LOD	PVE (%)	References
A1	satt382	27.8	-	> 10.0	Niu *et al.* (2013)
A2	satt207-satt315	26.5-45.3	4.0	9.6	Salas *et al.* (2006)
A2	BE820148-sat_162	35.9-51.9	2.7	7.7	Hu *et al.* (2013)
A2	AW132402	67.9	-	< 10.0	Niu *et al.* (2013)
A2	satt508-satt421	108.8-116	3.7	8.6	Salas *et al.* (2006)
B1	satt453	124.0	-	< 10.0	Niu *et al.* (2013)
B2	satt168-satt070	55.2-72.8	3.0	7.6	This study
C1	SOYGPATR-satt578	10.3-65.1	4.2	10.8	Salas *et al.* (2006)
C1	A463_1-K001_1	21.0-31.3	3.6	8.3	Salas *et al.* (2006)
C1	L192_1-satt136	73.2-75.1	3.9	9.4	Salas *et al.* (2006)
C1	AW277661	74.8	-	< 10.0	Niu *et al.* (2013)
C1	sat_077-satt338	76.0-123.8	3.3	7.6	Salas *et al.* (2006)
C2	satt640-satt422	30.5-44.7	2.6	7.1	Xu *et al.* (2011)
C2	satt277-satt489	107.6-113.4	3.8	8.9	Salas *et al.* (2006)
C2	satt289	112.3	-	< 10.0	Niu *et al.* (2013)
D1a	satt502-satt603	49.8-54.5	2.7	7.4	This study
D1a	satt254	56.4	-	< 10.0	Niu *et al.* (2013)
D1a	sat_160	104.3	-	< 10.0	Niu *et al.* (2013)
D1b	satt216	19.4	-	< 10.0	Niu *et al.* (2013)
D1b	satt611-sat_135	70.6-74.0	2.6	5.1	Hu *et al.* (2013)
D2	sat_022-satt389	79.2-120.3	2.8	8.2	This study
D2	satt226-sat_354	85.2-93.7	3.1	9.2	Xu *et al.* (2011)
E	sat_112-G214_10	8.7-17.45	3.2	7.4	Salas *et al.* (2006)
E	sat_381	64.2	-	< 10.0	Niu *et al.* (2013)
F	satt423-W1	20.6-28.9	4.1	9.5	Salas *et al.* (2006)
F	satt663-satt425	43.4-56.2	4.0	16.0	This study
F	satt335-satt072	77.7-87.0	3.9	9.2	Salas *et al.* (2006)
G	satt309-satt688	4.5-12.5	2.8	5.0	Xu *et al.* (2011)
H	satt181-satt434	87.6-105.7	3.6	8.8	Salas *et al.* (2006)
I	sat_419	98.1	-	< 10.0	Niu *et al.* (2013)
J	satt431-satt547	67.8-78.6	2.6	10.0	This study
J	sat_224	75.1	-	< 10.0	Niu *et al.* (2013)
J	satt431	78.6	-	< 10.0	Niu *et al.* (2013)
J	sat_224-sat_394	75.1-89.4	2.6	5.8	Xu *et al.* (2011)
K	satt247	44.0	-	< 10.0	Niu *et al.* (2013)
K	satt196-satt588	104.8-117.0	5.2	15.0	This study
L	satt166	66.5	-	< 10.0	Niu *et al.* (2013)
L	satt527-satt166	66.5-70.4	4.8	11.4	Salas *et al.* (2006)
L	satt527-satt166	66.5-70.4	2.9	6.6	Xu *et al.* (2011)
L	sat_099-G173_1	78.2-86.6	4.0	9.2	Salas *et al.* (2006)
M	sat_391	1.0	-	< 10.0	Niu *et al.* (2013)
M	satt590-satt245	7.8-53.5	4.0	8.0	Hu *et al.* (2013)
M	satt150-satt567	18.6-33.5	4.0	9.6	Salas *et al.* (2006)
M	satt567-R079_1	33.5-39.0	4.9	11.3	Salas *et al.* (2006)
M	satt245	53.5	-	< 10.0	Niu *et al.* (2013)
M	sat_003-satt494	62.3-71.7	3.8	8.8	Salas *et al.* (2006)
N	satt255	76.5	-	< 10.0	Niu *et al.* (2013)
N	satt234-satt022	84.6-102.1	4.9	11.2	Salas *et al.* (2006)
O	satt173-satt094	56.6-58.4	2.9	5.4	Xu *et al.* (2011)
O	satt331-satt592	93.4-100.4	2.7	4.5	Xu *et al.* (2011)
O	satt592	100.4	-	< 10.0	Niu *et al.* (2013)
O	satt592-sat_274	100.4-107.6	3.8	6.6	Xu *et al.* (2011)

[a] Positions of marker intervals are derived from Song *et al.* (2004)

Table 5 Locations of marker intervals associated with the QTLs conditioning soybean seed thickness in this study and in previous studies

LG	Marker interval	Position (cM[a])	LOD	PVE (%)	References
A1	satt276-satt382	17.2-26.4	2.8	12.4	This study
A1	sat_344	19.4	-	< 10.0	Niu *et al.* (2013)
A1	satt382	26.4	-	> 10.0	Niu *et al.* (2013)
A1	satt449-sat_356	27.8-42.8	2.9	9.8	Xu *et al.* (2011)
A1	A053_2-R183_1	34.6-42.4	3.0	7.1	Salas *et al.* (2006)
A1	A975_1-K636_2	75.4-81.5	4.5	10.4	Salas *et al.* (2006)
A1	sat_267	78.4	-	< 10.0	Niu *et al.* (2013)
A1	satt200	92.9	-	< 10.0	Niu *et al.* (2013)
A2	satt632-satt493	35.0-51.5	3.0	15.7	This study
A2	satt632	51.5	-	< 10.0	Niu *et al.* (2013)
A2	AW132402	67.9	-	< 10.0	Niu *et al.* (2013)
A2	satt508-satt421	108.8-116.0	3.1	7.2	Salas *et al.* (2006)
B1	T028_1-satt509	5.1-32.5	3.1	7.2	Salas *et al.* (2006)
B2	satt687	113.6	-	< 10.0	Niu *et al.* (2013)
C1	satt565	0.0	-	< 10.0	Niu *et al.* (2013)
C1	SOYGPATR-satt578	10.3-65.1	4.4	11.2	Salas *et al.* (2006)
C1	L192_1-satt136	73.2-75.1	4.8	11.5	Salas *et al.* (2006)
C1	AW277661	74.8	-	< 10.0	Niu *et al.* (2013)
C1	sat_077-satt338	76.0-123.8	5.7	12.9	Salas *et al.* (2006)
C1	A063_1	90.7	3.2	7.5	Salas *et al.* (2006)
C2	L199_2-sat_062	23.3-30.8	5.5	12.5	Salas *et al.* (2006)
C2	satt640-satt422	30.5-44.7	3.7	7.8	Xu *et al.* (2011)
C2	satt291-A426_1	45.8-79.1	5.3	12.1	Salas *et al.* (2006)
C2	satt307	121.3	-	< 10.0	Niu *et al.* (2013)
D1b	satt216	19.4	-	< 10.0	Niu *et al.* (2013)
D1b	sat_351	20.6	4.0	8.9	Hu *et al.* (2013)
D1b	satt041-satt290	73.3-84.0	2.9	11.1	This study
D2	satt372-satt002	39.3-47.7	2.7	9.4	This study
D2	satt002-satt582	47.7-53.8	3.5	8.2	Salas *et al.* (2006)
D2	satt256	124.3	-	< 10.0	Niu *et al.* (2013)
E	sat_381	64.2	-	< 10.0	Niu *et al.* (2013)
F	AW186493	21.0	-	< 10.0	Niu *et al.* (2013)
F	L050_14-sct_033	71.4-74.1	3.3	7.7	Salas *et al.* (2006)
G	satt309-satt688	4.5-12.5	3.0	5.4	Xu *et al.* (2011)
G	satt352	50.5	-	< 10.0	Niu *et al.* (2013)
G	sat_372	107.8	-	< 10.0	Niu *et al.* (2013)
I	sat_418-sat_419	74.3-98.1	2.9	6.0	Xu *et al.* (2011)
I	sat_419	98.1	-	< 10.0	Niu *et al.* (2013)
J	sat_224	75.1	-	< 10.0	Niu *et al.* (2013)
K	satt247	44.0	-	< 10.0	Niu *et al.* (2013)
L	satt527-satt166	66.5-70.4	4.5	10.7	Salas *et al.* (2006)
L	satt166-satt006	66.5-92.0	4.0	9.3	Salas *et al.* (2006)
M	satt150-satt567	18.6-33.5	3.4	8.2	Salas *et al.* (2006)
M	satt150-sat_258	18.6-60.5	2.9	9.0	Xu *et al.* (2011)
M	sat_258-satt463	50.1-60.5	4.3	11.3	Xu *et al.* (2011)
M	sat_256	74.5	-	< 10.0	Niu *et al.* (2013)
N	sat_280	43.5	-	< 10.0	Niu *et al.* (2013)
N	satt255-satt237	75.0-76.5	3.3	6.4	Xu *et al.* (1120)
N	satt339-satt022	75.9-102.1	2.7	5.7	Xu *et al.* (2011)

[a] Positions of marker intervals are derived from Song *et al.* (2004)

Table 6 Locations of marker intervals associated with the QTLs conditioning soybean seed width in this study and in previous studies

LG	Marker interval	Position (cM[a])	LOD	PVE (%)	References
A1	sat_344	19.4	-	< 10.0	Niu *et al.* (2013)
A1	satt449-sat_356	27.8-42.8	3.4	9.8	Xu *et al.* (2011)
A1	satt454	28.1	-	< 10.0	Niu *et al.* (2013)
A2	satt632-satt493	35.0-51.5	3.0	15.7	This study
A2	satt632	51.5	-	< 10.0	Niu *et al.* (2013)
A2	AW132402	67.9	-	< 10.0	Niu *et al.* (2013)
B2	sat_355-satt070	64.6-72.8	2.6	28.2	Xu *et al.* (2011)
C1	sat_077-satt338	76.0-123.8	8.4	18.5	Salas *et al.* (2006)
C1	G214_24-satt399	76.2-77.3	6.3	14.7	Salas *et al.* (2006)
C1	A063_1	90.7	5.5	12.5	Salas *et al.* (2006)
C2	L199_2-sat_062	23.3-30.8	6.8	15.4	Salas *et al.* (2006)
C2	satt640-satt422	30.5-44.7	2.6	5.7	Xu *et al.* (2011)
C2	satt291-A426_1	45.8-79.1	3.6	8.3	Salas *et al.* (2006)
C2	satt307	121.3	-	< 10.0	Niu *et al.* (2013)
D1a	satt254	56.4	-	< 10.0	Niu *et al.* (2013)
D1a	satt077	77.5	-	< 10.0	Niu *et al.* (2013)
D1b	sat_096-satt095	0.0-25.6	3.1	7.9	Salas *et al.* (2006)
D1b	satt157-sat_254	37.1-46.9	3.7	17.9	Xu *et al.* (2011)
D1b	sat_254	46.9	-	< 10.0	Niu *et al.* (2013)
D2	satt372-satt002	39.3-47.7	2.8	8.4	This study
D2	L072_1-A401_2	7.8-17.2	3.5	8.5	Salas *et al.* (2006)
D2	sat_022-satt389	79.2-120.3	4.1	13.5	This study
D2	sat_365	87.4	-	< 10.0	Niu *et al.* (2013)
D2	satt413	113.6	-	< 10.0	Niu *et al.* (2013)
E	B2-satt573	35.8-52.0	4.3	9.9	Salas *et al.* (2006)
E	satt369-satt553	56.3-67.9	4.8	11.0	Salas *et al.* (2006)
F	AW186493	21.0	-	< 10.0	Niu *et al.* (2013)
G	satt309-satt688	4.5-12.5	3.1	5.6	Xu *et al.* (2011)
H	satt052-satt253	64.1-67.2	3.4	8.0	Salas *et al.* (2006)
H	satt142	86.5	-	< 10.0	Niu *et al.* (2013)
H	satt142-satt434	86.5-105.7	4.1	9.4	Salas *et al.* (2006)
I	satt419	21.9	-	< 10.0	Niu *et al.* (2013)
I	sat_418- sat_419	74.3-98.1	3.2	9.3	Xu *et al.* (2011)
I	sat_419	98.1	-	< 10.0	Niu *et al.* (2013)
L	satt284	38.2	-	< 10.0	Niu *et al.* (2013)
L	satt166-satt006	66.5-92.0	6.2	13.9	Salas *et al.* (2006)
L	G173_1-Dt1	86.6-89.1	3.8	8.9	Salas *et al.* (2006)
M	satt150-satt567	18.6-33.5	3.8	9.1	Salas *et al.* (2006)
M	satt323-satt220	56.3-60.0	3.6	6.5	Xu *et al.* (2011)
M	sat_256	74.5	-	< 10.0	Niu *et al.* (2013)
M	satt250	107.7	-	> 10.0	Niu *et al.* (2013)
N	sat_280	43.5	-	< 10.0	Niu *et al.* (2013)
N	satt255-satt237	75.0-76.5	3.4	5.7	Xu *et al.* (2011)
O	satt592	100.4	-	< 10.0	Niu *et al.* (2013)
O	satt592-sat_274	100.4-107.6	3.1	7.7	Xu *et al.* (2011)

[a] Positions of marker intervals are derived from Song *et al.* (2004)

previously reported in soybean; for example, eight clusters on linkage groups A1, C2, D2, F, G, N, I and O identified by Xu *et al.* (2011), and 12 clusters detected by Niu *et al.* (2013).

CONCLUSION

This study was conducted to identify genetic loci associated with seed size traits in soybean and also to clarify the consistency of the detected QTLs with those found by previous researchers. Most QTLs were environment-sensitive. Linkage groups A2 and D2 harbor co-located QTLs for seed size traits. Thus markers on these linkage groups can be simultaneously considered in MAS breeding programs. Most of the detected QTLs in the current research were consistent with earlier QTLs reported by previous researchers. Yet, one additional and stable QTL (*SL6*) has been identified on linkage group K for seed length in this experiment.

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LITERATURE CITED

Cober, E.R., H.D. Voldeng and J.A. Fregeau-Reid. 1997. Heritability of seed shape and seed size in soybean. **Crop Sci.** 37: 1767–1769.

Cregan P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler N. Kaya, T.T. Vantoai, D.G. Lohnes and J. Chung. 1999. An integrated genetic linkage map of the soybean genome. **Crop Sci.** 39: 1464–1490.

Hoeck, J.A., W.R. Fehr, R.C. Shoemaker, G.A. Welke, S.L. Johnson and S.R. Cianzio. 2003. Molecular marker analysis of seed size in soybean. **Crop Sci.** 43: 68–74.

Kosambi, D. 1944. The estimation of map distances from recombination values. **Ann. Eugen.** 12: 172–175.

Liang, H.Z., W.D. Li, H. Wang and X.J. Fang. 2005. Genetic effects on seed traits in soybean. **Acta Genetica Sin.** 32: 1199–1204.

Liang, H.Z., S.F. Wang, Y.L. Yu, T.F. Wang, P.T. Gong, X.J. Fang, X.Y. Liu, S.J. Zhao, M.C. Zhang and W.D. Li. 2008. Mapping quantitative trait loci for six seed shape traits in soybean. **Henan. Agric. Sci.** 45: 54–60.

Lodhi, M.A., G.N. Ye, N.F. Weeden and B.I. Reisch. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. **Plant Mol. Biol. Rep.** 12: 6–13.

Niu, Y., Y. Xu, X.F. Liu, S.X. Yang, S.P. Wei, F.T. Xie and Y.M. Zhang. 2013. Association mapping for seed size and shape traits in soybean cultivars. Mol. Breed. 31: 785–794.

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

R Core Team. 2013. R: A language and environment for statistical computing. **R Foundation for Statistical Computing, Vienna, Austria.** [Available from: http://www.R-project.org/].

Salas, P., J.C. Oyarzo-Llaipen, D. Wang, K. Chase and L. Mansur. 2006. Genetic mapping of seed shape in three populations of recombinant inbred lines of soybean (*Glycine max* L. Merr.). **Theor. Appl. Genet.** 113: 1459–1466.

Song, Q.J., L.F. Marek, R.C. Shoemaker, K.G. Lark, V.C. Concibido, X. Delannay, J.E. Specht and P.B. Cregan. 2004. A new integrated genetic linkage map of the soybean. **Theor. Appl. Genet.** 109: 122–128.

Van Ooijen, J.W. 2004. **MapQTL 5, Software for the Mapping of Quantitative Trait Loci in Experimental Populations.** Kyazma B.V. Wageningen, the Netherlands.

Wang, S., C.J. Basten and Z.B. Zeng. 2007. **Windows QTL cartographer 2.5.** Department of Statistics, North Carolina State University, Raleigh, NC, USA. [Available from http://statgen.ncsu.edu/qtlcart/WQTLCart.htm].

Wilson, D.O.J. 1995. Storage of orthodox seeds. *In* Basra A.S. (eds.) **Seed quality: basic mechanisms, agricultural implications.** Food Products Press. New York, NY, USA.

Xu, Y., H.N. Li, G.J. Li, X. Wang, L.G. Cheng and Y.M. Zhang. 2011. Mapping quantitative trait loci for seed size traits in soybean (*Glycine max* L. Merr.). **Theor. Appl. Genet.** 122: 581–594.

Yan, N., S.Q. Xie, Q.C. Geng, Y. Xu, G.J. Li, B. Liu, X. Wang, Q.G. Li and Y.M. Zhang. 2013. Genetic basics of seed traits in soybean with Bayes hierarchical generalized linear model method. **Acta Genetica Sin.** 39: 258–268.

Young, N.D. 1999. A cautiously optimistic vision for marker-assisted breeding. **Mol. Breed.** 5: 505–515.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. **Genetics** 136: 1457–1466.