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QTL Mapping for Major Agronomic Traits across Two Years in Soybean (*Glycine max* L. Merr.)

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Abstract

The agronomic traits, such as days to flowering and maturity, plant height, 100-seed weight and seed filling period, are quantitatively inherited and important characters in soybean (*Glycine max* L. Merr.). A total of 126 F_5 recombinant inbred lines (RILs) developed from the cross of PI 171451 × Hwaeomputkong were used to identify quantitative trait loci (QTLs) for days to flowering (FD), days to maturity (MD), plant height (PH), 100-seed weight (SW), number of branches (NB) and seed filling period (FP). A total of 136 simple sequence repeat (SSR) markers segregated in a RIL population were distributed over 20 linkage groups (LGs), covering 1073.9 cM of the soybean genome with the average distance between adjacent markers of 7.9 cM. Five independent QTLs were identified for FD, three for MD, two for PH, three for SW, one for NB and one for FP. Of these, three QTLs were related to more than two traits of FD, MD, PH, NB and FP and mapped near the same positions on LGs H and O. Thus, these traits could be correlated with biologically controlled major QTLs in this soybean RIL population.

Key words: agronomic traits, linkage map, QTL, soybean, SSR marker

Introduction

Soybean (*Glycine max* L. Merr.) is the most economically important crop in the world due to its high content of high quality protein and oil for human and animal consumption as well as for industrial uses. Soybean oil accounts for 48% in the world market (Zhang et al. 2004). As a food and a component of drugs, soybean has been used in China for more than 5,000 years (Liu et al. 2008). Improvement of soybean yield and quality has become the major interest of plant breeders.

Days to flowering and days to maturity in soybean are important reproductive traits and valuable to development of soybean cultivars with superior performance and adaptation. Many agronomic traits are controlled by multiple genomic regions known as quantitative trait loci (QTLs). With the advent of molecular markers, like RFLP, AFLP, RAPD, and SSR, together with the convenience of the advanced analytical techniques, the molecular study of quantitative traits becomes facility in many plant species (Wang et al. 1999). Based on the construction of the soybean genetic linkage map, QTLs associated with numbers of agronomic traits have been mapped. Li et al. (2007) identified 21 unconditional and 16 conditional QTLs for seed protein content on 8 linkage groups (LGs) and 5 LGs, respectively. Also, they detected 21 unconditional and 16 conditional QTLs for seed oil content on 9 and 8 LGs, respectively. For plant height, 29 QTLs by 14 RFLP markers were detected using F_4 derived population from Young × PI 416937 (Lee et al. 1996). Based on recombinant inbred lines (RILs, F_8), Watanabe et al. (2004) constructed the genetic linkage map with molecular markers, RFLP, SSR and AFLP and 5 phenotypic markers. And, they detected 39 QTLs for all the reproductive development and seed quality traits of soybean, including three QTLs for flowering time (FT1-3) and four QTLs for days to maturity (HAV1-4). It was reported that at least seven genes influence days to flowering and maturity in soybean (Cober et al. 1996). Funatsuki et al. (2005) also reported three QTLs for flowering time, qFLT1, qFLT2 and qFLT3 positioned on LGs C2, L and I, respectively. Yamanaka

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Table 1. Statistical analysis of six agronomic traits for the parents and the RIL population derived from the cross PI 171451 × Hwaeomputkong.

Trait	Parent						RIL population					
	PI 171451			Hwaeomputkong			Mean ± S.D			Range		
	2006	2007	mean	2006	2007	mean	2006	2007	mean	2006	2007	mean
Days to flowering	78.0	86.0	82.0	54.0	45.0	49.5	65.8±7.4	64.0±7.5	65.2±7.2	54.0~83.0	45.0~90.0	49.5~86.5
Days to maturity	155.0	173.0	164.0	100.0	102.0	101.0	147.4±10.7	153.5±14.9	150.6±11.6	100.0~155.0	102.0~179.0	101.0~167.0
100-seed weight	13.8	12.3	13.1	26.7	29.8	28.3	21.6±3.2	21.9±3.5	21.7±3.1	12.6~29.3	14.7~33.4	13.8~30.7
Plant height	78.0	91.6	84.8	33.0	34.0	33.5	65.8±12.3	69.5±11.9	67.8±11.0	33.0~93.0	36.6~103.4	37.8~97.7
Numbers of branches	13.8	6.2	10.0	9.2	5.0	7.1	10.1±2.0	5.8±1.4	8.1±1.5	6.0~17.0	2.6~12.0	4.7~14.0
Seed filling period	77.0	87.0	82.0	46.0	57.0	51.5	81.6±8.5	89.2±11.6	85.9±8.2	46.0~99.0	56.0~106.0	51.5~99.0

et al. (2001) identified four QTLs for flowering time (FT1-4) using the soybean linkage map of an F_2 population derived from a cross between Misuzudaizu and Moshidou Gong 503. With the same population, Yamanaka et al. (2000) suggested that FT1, the major QTL for flowering time, might be related to the E1, the flowering and maturity locus, because the locus of pubescence color, *T*, was mapped close to FT1 in this population and linked to the E1 locus on the soybean classical genetic map.

The objectives of this study were to identify QTLs for days to flowering, days to maturity, plant height, 100-seed weight, number of branches and seed filling period and to discuss correlations between these traits. We found several new genomic regions of QTLs for these agronomic traits and verified the positive correlation between days to flowering and maturity in this population.

Materials and Methods

Plant materials and agronomic traits

A total of 126 F_5 recombinant inbred lines (RILs) derived from the cross between PI 171451 and Hwaeomputkong, were used to construct a genetic linkage map and for QTL analysis of the major agronomic traits. PI 171451was resistant to insect with late maturity and Hwaeomputkong was a recommended variety for vegetable with early maturity (Hong et al. 1995). The RILs and their parents were planted in the field at the experimental farm of Seoul National University in Suwon, Korea during 2006 and 2007 growing seasons. Six major agronomic traits were measured, such as days to flowering (FD), days to maturity (MD), plant height (PH), 100-seed weight (SW), numbers of branches (NB) and seed filling period (FP) (Table 1). Seed filling period (FP) was calculated according to following formula:

Seed filling period (FP) = Days to maturity (MD) - Days to flowering (FD).

Simple sequence repeat (SSR) analysis

Fresh leaves of young seedlings of each parent and RIL grown in the greenhouse were harvested and used for extracting genomic DNA. After leaf samples were finely pulverized in a mortar with a pestle in the presence of liquid nitrogen, the frozen powder (about 750 µl) was transferred into 2-ml microcentrifuge tubes in liquid nitrogen and stored at -70 °C. Genomic DNA was isolated following the procedure as described by Shure et al. (1983). The final concentration of genomic DNA was adjusted to 50 ng/µl with Tris-EDTA buffer (pH 8.0) and stored at -20 °C until use.

A total of 153 normal SSR markers and 169 universal SSR markers were designed according to Soybase (http://www.soybase.com/resources/ssr.php). Normal SSR primers and fluorescent labeled M13 (-21) universal primers were manufactured by Illumina (San Diego, CA, USA). With normal SSR primers, PCR amplifications were carried out in a total volume of 5 µl containing 10 ng of genomic DNA, 10X buffer (w/$MgCl_2$), 2.5 mM dNTP, 2 unit AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA) and 5 µM primer mixture. The procedures of PCR amplification were as follow: 94 °C (10min), then 30 cycles at 94 °C (25 s) / 46 °C (25 s) / 68 °C (25 s), and a final extension at 72 °C for 10 min on MJ Research PCT-225™ Thermal Controller (MJ research, Watertown, MA, USA). For universal primers, the PCR reaction with M13 (-21) universal primer was performed as reported by Schuelke (2000) with a slight modification. Sequence-specific forward primers were fused with M13 (-21) universal sequence (18 bases, 5'- TGT AAA ACG ACG GCC AGT) at their 5'-ends and M13 (-21) universal primers were labeled at their 5'-end with blue (6-FAM), yellow (NED), red (PET) or green (VIC) fluorescent tags. Individual PCR amplifications were performed in a total volume of 5 µl containing 10 ng genomic DNA, 10X buffer (w/$MgCl_2$), 2.5 mM dNTP, 2 unit AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 µM of each reverse primer and fluorescent labeled with M13 (-21) primer, 1.25 µM of the forward primer with M13 tail. The conditions of PCR amplification were as follow: 94 °C (5 min), then 30 cycles at 94 °C (30 s) / 54 °C (45 s) / 72 °C (45 s), followed by 10 cycles at 94 °C (30 s) / 53 °C (45 s) / 72 °C (45 s) and the final extension at 72 °C for 10 min on an MJ Research PTC-225 Peltier Thermal Cycler (MJ Research, Watertown, MA, USA)

Twelve PCR products with different labels were mixed together and then 2 µl of mixed samples were combined with 7.7 µl of formamide and 0.3 µl of size standard. After denaturation at 95 °C for 5 min and placement on ice, PCR products were separated on an ABI 3730 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). GeneMapper 3.7 software (Applied Biosystems, Foster City, CA, USA) was used for accurate characterization of the alleles and automated data output.

Statistical analysis

The genetic linkage map was constructed using MAPMAKER/EXP version 3.0b (Lincoln et al. 1993) with Kosambi mapping function. Linkage was declared at LOD greater than or equal to 3.0 and a maximum distance of 50 cM. For calculating main-effect, QTLMapper version 1.6 was applied to identify loci affecting quantitative traits on the basis of composite interval analysis (Wang et al. 1999). The LOD score of 3.0 was selected as the threshold for the presence of a main-effect QTL on the basis of the total map distance and the average distance between markers. Simple correlation coefficients among six agronomic traits within the RIL population were calculated from mean values using Statistica 7.0 software (StatSoft, Inc. 2007).

Results

Statistical analysis of six agronomic traits

The results of statistical analysis of the major agronomic traits of parents and RILs were shown in Table 1. For all traits, except NB, the differences between the two parents were significant. Except SW, the female parent, PI 171451, had higher values in FD, MD, PH, NB and FP than those of Hwaeomputkong across two years. In addition, FD, NB and FP varied significantly between two years. In the RIL population, the transgressive segregation in all traits was observed. Nevertheless, the transgressive phenomena were prominent only for PH, NB and FP, agreeing with the previous report by Zhang et al. (2004).

Correlations among six major agronomic traits

Across two years' trials, correlations among FD, MD, SW, PH, NB and FP were significant ($P < 0.05$), except SW vs. MD, SW vs. PH and FP vs. NB. The correlation coefficients (r) were positive and greater than 0.63 for FD vs. MD, FD vs. PH and MD vs. PH, particularly FP vs. MD (Table 2 and Fig. 1). In contrast, SW was negative and significantly correlated with FD and NB ($P < 0.01$). No correlations for SW vs. MD and PH, and FP vs. NB were found in this study.

Marker segregation and map construction

Of 153 normal SSR markers tested, 94 (61.4%) were polymorphic between parents, PI 171451 and Hwaeomputkong and 107 (63.3%) out of 169 universal tested SSR markers showed polymorphisms between parents. In addition, among the markers showing polymorphisms between two parents, only 136 primers in total, including 66 normal and 70 universal SSR primers, were well segregated in the RIL population. Of the 136 SSR segregated markers in the RIL population, 63 markers did not follow the expected segregation ratio of 1:1 ($P < 0.05$). Of these, 37 were skewed toward the female parent 'PI 171451' allele and 26 were skewed toward the pollen parent 'Hwaeomputkong' allele.

The segregating markers were distributed over all 20 LGs (Fig. 2) and covered 1073.9 cM with an average distance between the adjacent marker loci of 7.9 cM. This map coverage did not include the unlinked anchored-markers. As a result, LG D1a contained only two markers. And, LGs C1, C2, E, I and L were divided into two parts for each due to no polymorphism of markers on some regions of each LG. LGs were designated with names corresponding to the integrated public soybean genetic map (Song et al. 2004).

Table 2. Simple correlations among days to flowering, days to maturity, 100-seed weight, plant height, numbers of branches and seed filling period based on mean values of field data of 2006 and 2007 growing seasons.

	Days to flowering	Days to maturity	100-seed weight	Plant height	Numbers of branches
Days to maturity	0.74**				
100-seed weight	-0.22*	0.02			
Plant height	0.60**	0.63**	-0.16		
Numbers of branches	0.48**	0.38**	-0.29**	0.48**	
Seed filling period	0.25**	0.84**	0.20*	0.42**	0.15

* and ** indicates significance level of correlations at $P < 0.05$ and 0.01.

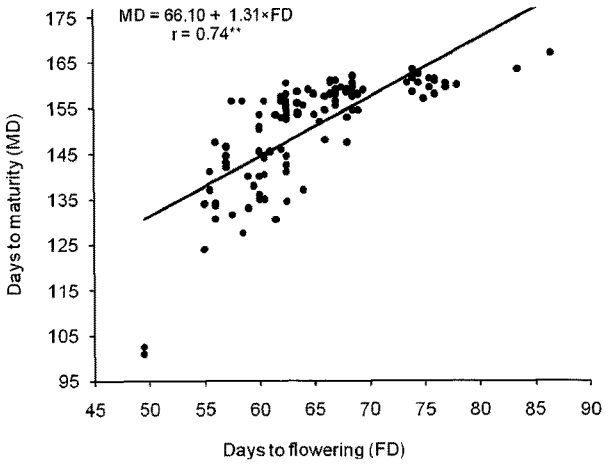

Fig. 1. Regression of days to maturity (MD, days) on the days to flowering (FD, days) for recombinant inbred lines (RILs) derived from the cross PI 171451 × Hwaeomputkong.

** indicates significance level of correlations at $P < 0.01$.

QTLs for six agronomic traits

In this study, a total of 15 QTLs with LOD scores greater than 3 for all six major agronomic traits were detected (Table 3, Fig. 2). These QTLs were distributed over nine LGs, B1, C2, D1b, E, F, H, L, N and O. Among these, nine QTLs for FD and MD and PH and SW were identified in 2006 and eight QTLs for FD and MD and PH and FP were detected in 2007. No QTL for NB was identified, if data analyzed separately in either 2006 or 2007, but one QTL was detected with mean values of data combined with these two years. Also, two QTLs for SW were detected based on mean values of these two years, which not detected in either 2006 or 2007.

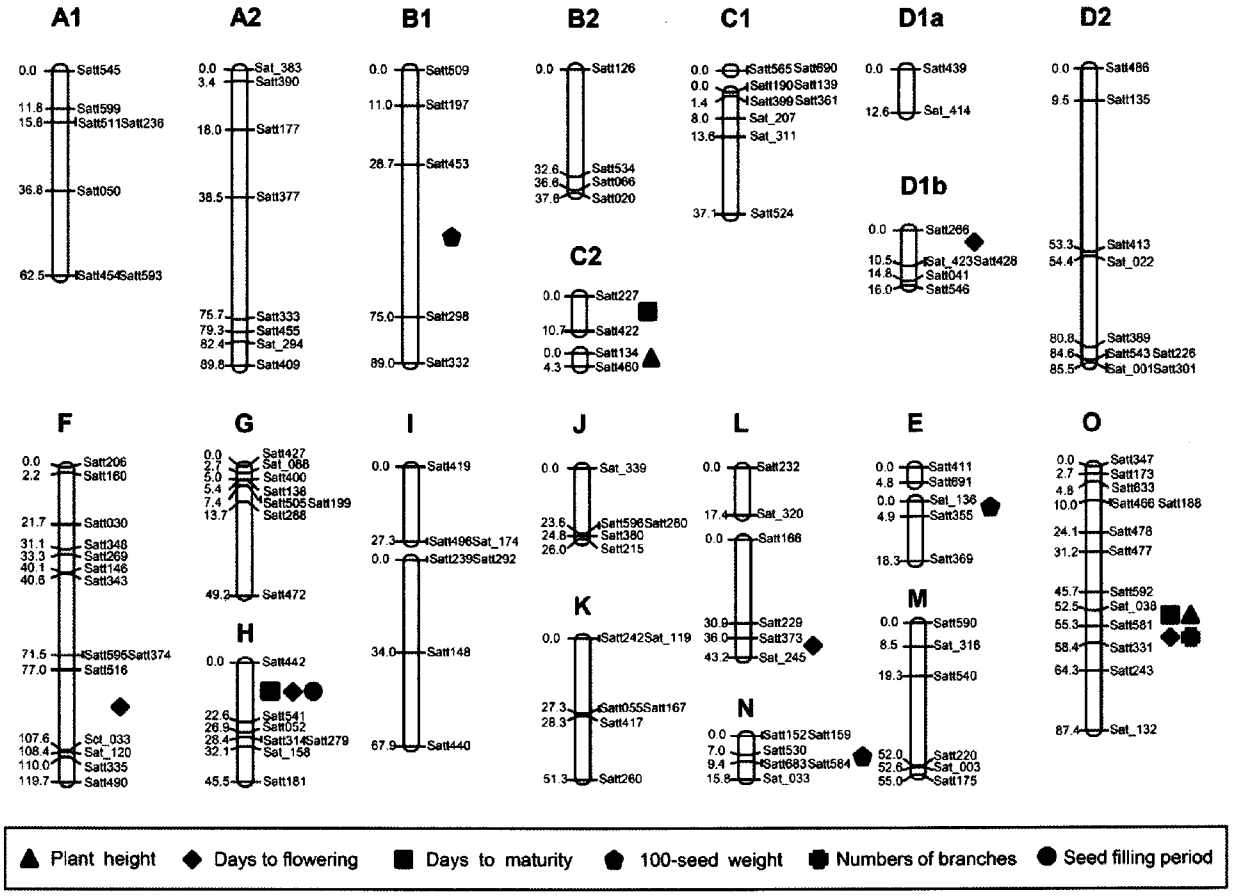

Fig. 2. Linkage map based on a RIL population derived from the cross PI 171451 × Hwaeomputkong

For FD, five QTLs were mapped on LGs D1b, F, H, L and O (Fig. 2). Three QTLs for MD were mapped on three LGs C2, H and O. For PH, one QTL was located on LG C2 and the other was located on LG O. In addition, three QTLs for SW were distributed over LGs B1, E and N. One QTL each for NB and FP were located on the LGs O and H, respectively. Among the QTLs detected in this study, the QTL for PH between Satt134-Satt460 accounted for a maximum value of 30.0% of total phenotypic variance.

In addition, two QTLs for FD on LGs L and O, two QTLs for MD on LGs H and O and one QTL for PH on LG C2 were all detected in both 2006 and 2007. Also, these QTLs were identified, if combined data with mean values of these two years were used, indicating that these five QTLs expressed stably across environments.

Discussion

Days to flowering in soybean is a character that may affect plant height, days to maturity, and mainly grain yield as well as other important agronomic characters (Oliveira et al. 1999). In this study, five QTLs for FD were detected. One of these with the LOD score of 8.18 was mapped between Satt373 and Sat_245 on LG L and this region was consistent with the QTL qFLT2 reported by Funatsuki et al. (2005). Another QTL with the LOD score of 8.94 was mapped between Satt581 and Satt331 on LG O. Both QTLs accounted for more than 20% of total phenotypic variance and interpreted as stable QTLs. Compared with these stable QTLs above, three other QTLs for FD were detected on LGs Db1, F and H only in 2006 and accounted for only 6.5 to 8.1% of total phenotypic variance. However, no QTLs for FD on LGs C2 and B1 were detected, which was reported by Zhang et al. (2004).

For MD, one QTL at the marker interval Satt442-Satt541 on LG H was fisrtly indentified in this study. But, the other two QTLs on LGs C2 and O were mapped near the previous QTLs reported by Specht et al. (2001) and Wang et al. (2004) (< 10 cM). In this study, one of three QTLs for PH located between Satt134 and Satt460 on LG C2 accounted for 29.87% of total phenotypic variance. This QTL was consistent with the previous reports (Kabelka et al. 2004; Orf et al. 1999; Specht et al. 2001; Wang et al. 2004). This suggested that some gene(s) controlling PH were likely located in this region on LG C2.

It was possible one QTL was related to more than one trait (Zhang et al. 2004). Several QTLs of various traits were mapped on the same loci. In this study, two QTLs, one for MD and another for PH, were located at the same marker interval, Sat_038-Satt581 on LG O. And, two QTLs for FD and NB were mapped at the same marker interval, Satt581-Satt331 on LG O. These QTLs were inferred as being related to two or more agro-

Table 3. QTLs of six agronomic traits in the RIL population derived from the cross PI 171451 × Hwaeomputkong.

Trait	Interval	LG	2006			2007			Mean		
			LOD	Additive effect	R^2 (%)	LOD	Additive effect	R^2 (%)	LOD	Additive effect	R^2 (%)
Days to flowering	Satt266-Sat_423	D1b	3.8	-2.47	6.2	-	-	-	3.45	-2.12	8.0
	Satt516-Sct_033	F	4.09	2.47	7.6	-	-	-	-	-	-
	Satt442-Satt541	H	4.03	2.70	6.5	-	-	-	-	-	-
	Satt373-Sat_245	L	9.32	3.71	15.3	5.49	3.60	18. 5	8.18	3.47	20.5
	Satt581-Satt331	O	7.49	3.59	14.8	5.12	3.82	16.6	8.94	4.06	22.6
Days to maturity	Satt227-Satt422	C2	-	-	-	5.24	-6.69	10.5	5.39	-5.13	12.0
	Satt442-Satt541	H	3.96	4.86	8.3	7.42	7.68	12.2	6.62	5.88	10.8
	Sat_038-Satt581	O	3.12	4.02	10.5	3.78	5.61	11.0	4.13	4.64	11.3
Plant height	Satt134-Satt460	C2	7.19	7.10	25.3	5.7	5.85	20.5	8.76	6.63	29.9
	Sat_038-Satt581	O	4.65	5.87	13.6	-	-	-	-	-	-
100-seed weight	Satt453-Satt298	B1	3.6	-1.41	13.0	-	-	-	-	-	-
	Sat_136-Satt355	E	-	-	-	-	-	-	3.33	1.19	6.6
	Satt530-Satt683	N	-	-	-	-	-	-	3.28	-1.07	20.5
Numbers of branches	Satt581-Satt331	O	-	-	-	-	-	-	4	0.72	7.5
Seed filling period	Satt442-Satt541	H	-	-	-	5.35	6.26	16.1	3.81	4.19	9.4

nomic traits. Previous reports exhibited that FD and MD in soybean are highly correlated (Mansur et al. 1996; Orf et al. 1999). Similar results were obtained in this study, e.g., r = 0.74** for FD vs. MD, in agreement with the QTL data. And, the cluster QTLs on the same linkage groups could interpret significant correlations among corresponding traits based on field data.

As a vegetable soybean, 100-seed-weight of Hwaeomputkong is only one trait, which showed higher value than that of PI 171451. In this study, three QTLs for SW were mapped on each of LGs B1, E and N, respectively. However, the QTL at the marker interval Satt453-Satt298 on LG B1 was detected only in 2006 and the other two on LGs E and N were detected based on mean values of 2006 and 2007. The LOD scores for these QTLs were smaller than 3.6. Nevertheless, the QTLs for SW detected on LGs B1 and E were consistent with the previous reports (Maughan et al. 1996; Mian et al. 1996; Orf et al. 1999) and could be inferred as the real QTLs.

In conclusion, LGs B1, C2, D1b, E, F, H, L, N and O had the QTLs for days to flowering, days to maturity, plant height, 100-seed weight, numbers of branches and seed filling period. However, most of QTLs, particularly those for days to flowering, days to maturity and plant height, clustered on two LGs H and O. The QTLs mapped at same marker loci on LGs H and O were likely related to two or more agronomic traits in this soybean population.

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