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ORIGINAL PAPER

QTL identification of flowering time at three different latitudes reveals homeologous genomic regions that control flowering in soybean

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Abstract Since the genetic control of flowering time is very important in photoperiod-sensitive soybean (*Glycine max* (L.) Merr.), genes affecting flowering under different environment conditions have been identified and described. The objectives were to identify quantitative trait loci (QTLs) for flowering time in different latitudinal and climatic regions, and to understand how chromosomal rearrangement and genome organization contribute to flowering time in soybean. Recombinant inbred lines from a cross between late-flowering 'Jinpumkong 2' and early-flowering 'SS2-2' were used to evaluate the phenotypic data for days to flowering (DF) collected from Kamphaeng Saen, Thailand (14°01′N), Suwon, Korea (37°15′N), and Longjing, China (42°46′N). A weakly positive phenotypic correlation ($r = 0.36$) was found between DF in Korea and Thailand; however, a strong correlation ($r = 0.74$) was shown between Korea and China. After 178 simple sequence repeat (SSR) markers were placed on a genetic map spanning 2,551.7 cM, four independent DF QTLs were identified on different chromosomes (Chrs). Among them, three QTLs on Chrs 9, 13 and 16 were either Thailand- or Korea-specific. The DF QTL on Chr 6 was identified in both Korea and China, suggesting it is less environment-sensitive. Comparative analysis of four DF QTL regions revealed a syntenic relationship between two QTLs on Chrs 6 and 13. All five duplicated gene pairs clustered in the homeologous genomic regions were found to be involved in the flowering. Identification and comparative analysis of multiple DF QTLs from different environments will facilitate the significant improvement in soybean breeding programs with respect to control of flowering time.

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Introduction

Flowering time is a reproductive characteristic of major agronomic interest, since it is critical for plant adaptation to different latitudes. Classical soybean breeding programs have long recognized that the genetic control of flowering time plays an important role in efficient crop production under different latitudinal and climatic conditions (Curtis et al. 2000). In most plant species, flowering time is controlled by interactions between environmental and endogenous elements (Levy and Dean 1998). Under the conditions of short photoperiod and warm temperature, all lines flowered approximately at the same time. However, cooler temperatures under the 20 h photoperiod led to delayed flowering. The isolines with two or more dominant alleles flowered about 20 days earlier under cool conditions compared with warm conditions (Cober et al. 2001). Furthermore, at higher latitudes,

flowering is more closely associated with temperature and predictable rainfall patterns at certain times of the year. Photoperiods that are to short for the soybean variety under cultivation will cause reproductive growth to initiate before sufficient vegetative growth has occurred and thus, result in low yields and poor seed quality (Cregan and Hartwig 1984).

Molecular genetic marker analyses have identified quantitative trait loci (QTLs) for diverse traits (Dudley 1993; Cregan et al. 1999; Song et al. 2004). QTLs with the large phenotypic effects on flowering time have been positioned on the soybean genetic linkage map and some are located in more than one region (Soybase, http://soybase.org). The recombinant inbred line (RILs) populations of the soybean cultivars 'Minsoy' and 'Noir 1' were used to map QTLs for flowering time on Chrs 6 and 9 (previously as linkage groups (LGs) C2 and K, respectively; Mansur et al. 1996). Large effect QTLs for flowering time were also mapped on Chr 6, which is responsible for 40.7 and 47.4% of total phenotypic variation in two different single-cross populations of PI 317.336 × 'Corsoy' and PI 317.334B × 'Corsoy' (Tasma et al. 2001). With the parents 'Misuzudaizu' and 'Moshidou Gong 503', major flowering time QTLs were mapped to Chrs 6 (*FT1*), 10 (*FT2*), 20 (*FT3*) and 11 (*FT4*) (LGs C2, O, I and B1, respectively; Yamanaka et al. 2001). In soybean, classical methods were used to designate eight *E* loci (*E1–E8*) controlling flowering time and maturity; the locations of these loci include Chrs 6 (*E1* and *E7*), 10 (*E2*), 19 (*E3*), 20 (*E4*) and 4 (*E8*) (Bernard 1971; Buzzell 1971; Buzzell and Voldeng 1980; McBlain and Bernard 1987; Bonato and Vello 1999; Cober and Voldeng 2001; Cober et al. 2010). The *E1* locus is located at the same position as the previously identified *FT1* locus, and is tightly linked to *E7* and pubescence color locus *T* (Cober and Voldeng 2001; Yamanaka et al. 2001). The *E1*, *E3*, *E4, E5* and *E7* loci show particular involvement in the flowering response to long day (LD) length (Buzzell and Voldeng 1980; Molnar et al. 2003). Two single loci of *E6* and *J* have been reported to control long-juvenile traits that delayed flowering under short photoperiods (Bonato and Vello 1999; Ray et al. 1995). This trait may be important in increasing the adaptation range of soybean to low latitudes.

Since multiple genes control flowering time in soybean, their combined effects may differ with environment. Many flowering time QTLs have been reported, and *E3* and *E4* loci have been identified as genes encoding phytochrome *A* (*GmphyA3* and *GmphyA2*; Liu et al. 2008; Watanabe et al. 2009). However, there have been limited numbers of studies for QTL mapping across several environmental conditions. Thus, there is a little understanding about how genomic segments or genes might interact under different environmental conditions, such as photoperiod and temperature. To improve soybean cultivation and yields, it is important to identify critical factors regulating flowering under various environmental conditions. Several researchers suggest that the multiple QTLs might have occurred via two rounds of soybean genome duplication. QTLs for seed protein and oil were located on Chrs 5, 6, 8, 15 and 18 and homeology was observed among these genomic regions encompassing related markers (Shoemaker et al. 1996). Homeologous genomic regions carrying lipoxygenase genes were found on four different chromosomes (Chrs 7, 8, 13 and 15), which harbored duplicate QTLs for several traits including corn earworm resistance and yield, as well as sucrose and oil contents (Shin et al. 2008). QTLs for resistance to six different bacterial leaf pustule (BLP) pathogens have been identified under both greenhouse and field conditions (Van et al. 2004), and four loci were conserved across homeologous genomic regions on Chrs 4, 5, 6, and 17 (Kim et al. 2009).

The objective of this work was to identify the genomic locations of environment-specific genes that regulate flowering time in soybean. We evaluated days to flowering (DF) using a RIL population derived from a cross between the late-flowering Jinpumkong 2 and early-flowering SS2-2 cultivars. Plants were grown in three different latitudinal locations, including Thailand, Korea and China. Genome synteny among DF QTL regions was investigated across different environments to explain environment-specific expression of the duplicated genes.

Materials and methods

Plant materials and DNA extraction

A total of 89 F_9 RILs derived from a cross between soybean cultivars Jinpumkong 2 (Kim et al. 1997) and SS2-2 (Kim et al. 2005) were used to construct a genetic linkage map and perform QTL analysis for DF. The cross was conducted at Kasetsart University (Kamphaeng Saen, Nakhon Pathom, Thailand) during the summer of 2004. Single-seed descent method was used to advance each generation for development of RILs.

Genomic DNA was extracted from the fresh leaves of young seedlings, according to the procedure described by Shure et al. (1983), with slight modifications. DNA concentration was determined using an ND-1000 NanoDrop (NanoDrop Technologies Inc., Wilmington, DE, USA). The final concentration of genomic DNA was adjusted to 10 ng/μl in Tris–EDTA buffer (pH 8.0), and then stored at −20°C until use.

Table 1 Planting date, temperature and day length at three different latitudinal locations during the soybean growth period

	Kamphaeng Saen, Thailand	Suwon, Korea	Longjing, China
Latitude (N)	14°01′	37°15′	42°46′
Planting date	16 November, 2009	27 May, 2009	10 May, 2009
Ranges of temperature (°C)	17.2–39.3	12.7–34.8	6.2–33.4
Ranges of day length (h)[a]	1205–1252	1139–1571	1205–1635

[a] Day length was calculated at sunrise–sunset and civil twilight (Downs and Thomas 1990)

Field experiments

During 2009, parental cultivars and 89 RILs were sown at the following latitudinal locations: Longjing, China, (42°46′N); Suwon, Korea (37°15′N); and Kamphaeng Saen, Thailand (14°01′N; Table 1). These sites had different planting dates, ranges of temperature and day lengths, i.e., 10th May (1205–1252 hours), 27th May (1139–1571 hours) and 16th November (1205–1635 hours), respectively (Table 1). Planting density and cultivation practices were performed according to the local standards. DF was recorded by measuring the number of days between planting and the day when half of the plants in each line flowered.

Simple sequence repeat (SSR) marker analysis

SSR markers were used to identify allelic variations at SSR loci from different soybean linkage groups. A total of 243 SSR markers were designed using Soybase (http://www.soybase.org/resources/ssr.php). PCRs were performed in a 5 µl reaction volume containing 10 ng genomic DNA, 1× reaction buffer (w/$MgCl_2$), 160 µM dNTP, 0.4 U *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA) and 0.5 µM of primer mixture. PCR were conducted using an MJ Research PCT-25 TM Thermal Controller (MJ Research, Watertown, MA, USA) at 94°C for 10 min, followed by 30 cycles of 94°C (25 s), 46°C (25 s) and 68°C (25 s), and then a final extension at 72°C for 10 min. PCR products were resolved using an ABI PRISM 3730xl automatic DNA sequencer (Applied Biosystems) and allelic differences in SSR markers were analyzed using GeneMapper version 3.7 software (Applied Biosystems) to determine the SSR genotypes of the parents and RILs.

Genetic mapping and QTL identification

The soybean genetic map was constructed with MAPMAKER/EXP version 3.0b (Lander et al. 1993) and genetic distance was computed using the Kosambi mapping function. A logarithm of the odds (LOD) score of 3.0 and maximum distance of 50 cM was used as the threshold to group markers into chromosomes. Segregation of SSR markers in the 89 RILs was evaluated with a Chi-square (χ^2) test. The genetic association between markers and DF was analyzed by single factor analysis of variance (SF-ANOVA; PROC GLM; SAS version 9.1, SAS Institute Inc 2002). QTL Mapper version 1.6 was used for interval mapping to determine and estimate QTL positions from DF and marker data (Wang et al. 2004b). Following the composite interval analysis, an LOD score of 2.4 was used to identify the presence of a major QTL.

Sequence analysis of QTL regions

Genomic regions were examined (1 Mb up- and downstream) around the four DF QTLs identified in analyses. The QTLs were located at the following intervals: Satt202–Satt371 (Gm06: 47820410..49159840) on Chr 6; Satt055–Satt417 (Gm 9:10894174..17540158) on Chr 9; Satt206–Satt595 (Gm13: 10894174..17540158) on Chr 13; and Satt244–Satt431 (Gm16: 33327177..35718642) on Chr 16. Sequences in the four QTL regions were downloaded from the soybean reference genome sequence database (http://www.phytozome.net/soybean.php). Sequence alignment was performed with Mercator and visualized with Gbrowse_syn, developed within the GMOD framework (Stein et al. 2002; Dewey 2007). Synteny between genomic regions in different chromosomes was determined by retrieving the ClusterID of all genes belonging to the QTL regions and those genes showing the same ClusterID were regarded as homologs. The ClusterIDs were downloaded from the website Phytozome Biomart (http://www.phytozome.net/biomart/martview/).

Results

Phenotype data analysis

A wide range of phenotypic variation was observed for DFs from parental cultivars and RILs at each of the three planting locations (Fig. 1). In Korea, the parental cultivars were phenotypically different with respect to DF, plant height and number of nodes on main stem. SS2-2 flowered 13 days earlier than Jinpumkong 2 and it formed short plants with few nodes, whereas Jinpumkong 2 grew tall and produced more nodes. In Thailand, Jinpumkong 2 flowered one day earlier than SS2-2, whereas it flowered 25 days later in China. With respect to DF, the RIL population showed a narrow phenotypic range in Thailand, i.e., from 25 to 38 days, whereas DF ranged from 39 to 74 days in

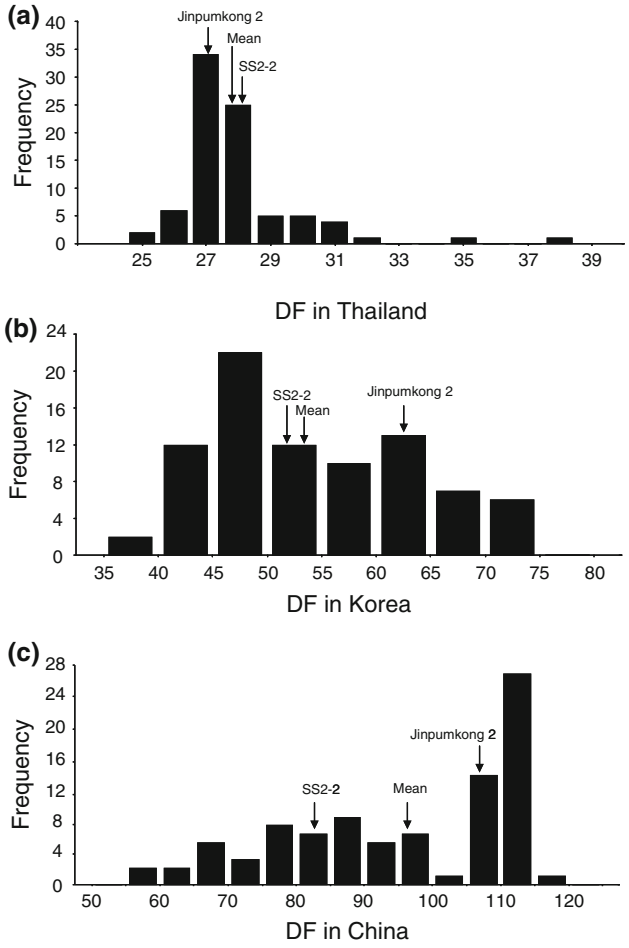

Fig. 1 Frequency distribution of days to flowering for 89 RILs derived from Jinpumkong 2 × SS2-2 grown at three different latitudes: **a** Thailand, **b** Korea and **c** China

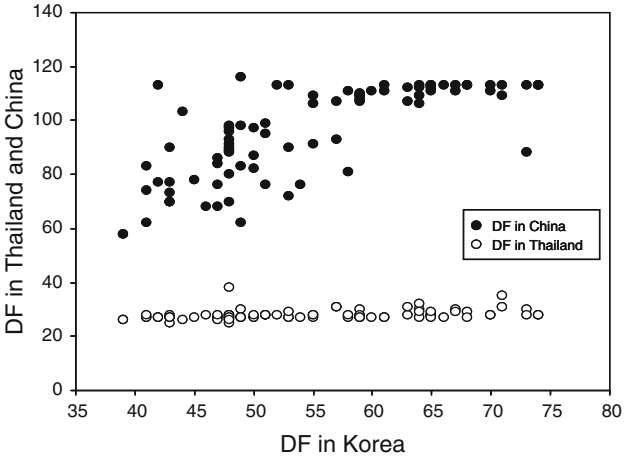

Fig. 2 Correlation between DF and locations at three different latitudes. A strong correlation was found between Korea and China, but only a weak correlation was evident between Korea and Thailand

Korea and from 58 to 116 days in China, with mean values of 28, 54 and 96 days, respectively (Fig. 1). Therefore, DF showed greater variability with the increasing latitude. Interestingly, the frequencies of DF did not always show a normal distribution. Thailand exhibited a nearly continuous quantitative DF trait and promoted flowering. DFs between China and Korea were somewhat correlated ($r = 0.74$), but only a weak correlation ($r = 0.36$) between Korea and Thailand was shown (Fig. 2). Photoperiod and temperature clearly interact with the whole life cycle. Short days (SD) combined with high temperatures resulted in the shortest time to flowering, whereas low temperatures and long day (LD) conditions delayed flowering.

Genetic map construction

Among the 243 SSR markers investigated, 189 (77.8%) showed polymorphisms between the mapping parents. These markers were used to construct a genetic linkage map for the soybean RIL population from Jinpumkong 2 and SS2-2. DF QTL analysis was performed using 178 (73.3%) polymorphic markers linked across all 20 soybean chromosomes. The other 11 SSR markers remained unlinked to the genetic map and were not used for the analysis. The map covered 2,551.7 cM and on average, adjacent SSR markers were 14.3 cM apart (data not shown). The order of markers was consistent with the USDA soybean linkage map developed by Choi et al. (2007).

QTL mapping for DF

SF-ANOVA and QTL Mapper version 1.6 were used to identify QTLs for DF. For the three different latitudinal locations, SF-ANOVA identified 11 significant ($P < 0.01$) DF QTLs across 8 chromosomes (Table 2). DF QTLs associated with the markers, Satt041 (Chr 2) and Satt202 (Chr 6), were detected in both Korea and China, with the latter marker accounting for a higher phenotypic variation than Satt041 at these locations. DF QTLs linked to Satt055 and Satt417 on Chr 9 were identified for Thailand and Korea, with the latter marker explaining the highest phenotypic variation (23.6%) in Thailand. In addition, DF QTLs associated with Satt244 (Chr 16) and Sat_337 (Chr 4) were found in Thailand and accounted for 8.9 and 18.3% of variation, respectively (Table 2). In Korea, QTLs for DF explained 11.9, 13.2, and 15.3% of phenotypic variation and were linked to Satt633 (Chr 10), Satt146 (Chr 13) and Satt206 (Chr 13), respectively. In China, two additional DF QTLs were identified on Chrs 5 and 13, and these were linked to Satt591 and Satt114.

Interval mapping with QTL Mapper version1.6 software was used to determine the genomic regions linked to DF.

Table 2 SF-ANOVA results of DF-associated QTLs in a soybean RIL population derived from Jinpumkong 2 × SS2-2 ($P < 0.01$)

Marker	Chr	Map position (cM)[a]	Thailand		Korea		China		Reported QTLs		
			P value	R^2 (%)	*P* value	R^2 (%)	*P* value	R^2 (%)	Marker	Map position (cM)[a]	References
Satt041	2	84.0	–	–	0.0066	8.6	0.0085	8.3	–	–	–
Sat_337	4	32.1	0.0004	18.3	–	–	–	–	K472_1	53.9	Keim et al. (1990)
Satt591	6	31.1	–	–	–	–	0.0099	10.9	Satt382	26.4	Kabelka et al. (2004)
Satt202	6	126.2	–	–	0.0017	14.7	<0.0001	22.3	K474_1	113.0	Keim et al. (1990)
Satt055	9	33.0	0.003	10.6	0.0052	9.0	–	–	C009_2	47.2	Mansur et al. (1996)
Satt417	9	46.2	<0.0001	23.6	0.0047	12.1	–	–	C009_2	47.2	Mansur et al. (1996)
Satt633	10	56.9	–	–	0.0012	11.9	–	–	138GA26	105.4	Yamanaka et al. (2001)
Satt146	13	1.9	–	–	0.005	13.2	–	–	BLT030_1	7.2	Specht et al. (2001)
Satt206	13	27.0	–	–	0.0002	15.3	–	–	BLT030_1	7.2	Specht et al. (2001)
Satt114	13	63.7	–	–	–	–	0.0086	8.3	–	–	–
Satt244	16	65.0	0.01	8.9	–	–	–	–	Satt380	43.0	Tasma et al. (2001)

[a] Estimated position (cM) was inferred from the publically available USDA map (Choi et al. 2007) and reported flowering QTLs were represented within 50 cM

On Chrs 9, 13 and 16, location-specific DF QTLs were linked precisely with the adjacent SSR markers and the LOD scores, R^2 values and additive effects are presented in Table 3. The chromosomal locations of previously identified QTLs nearby are shown in Fig. 3. In Thailand, two QTLs flanked by Satt055–Satt417 (Chr 9) and Satt244–Satt431 (Chr 16) explained 12.6 and 18.1% of DF variation, respectively. In Thailand, the SS2-2 alleles at these two QTLs showed negative or weak positive effects on DF. A Korea-specific DF QTL was found between Satt206 and Satt595 (Chr 13) and it explained 15.4% of phenotypic variation and early-flowering SS2-2 contributed an allele with a negative effect on DF at this QTL. No China-specific DF QTLs were identified. QTL Mapper version 1.6 detected a QTL for DF in the Satt202–Satt371 interval (Chr 6) from both Korea and China. This environmentally less sensitive QTL explained 13.7 and 28.4% of phenotypic variation in DF for Korea and China, respectively, and the maximum additive effect and highest phenotypic variation were observed in China (Table 3). The identification of an environment-less sensitive QTL for Korea and China appears to be in agreement with the positive correlation for DF observed between these locations.

Comparative analysis of four QTL regions for DF

Comparative analysis was performed on soybean genomic sequences surrounding the four DF QTL regions indentified by interval mapping. Alignment of these sequences revealed a syntenic relationship between two QTL regions on Chrs 6 and 13 (Fig. 4). On Chr 6, a 2.04 Mb region from Satt202 to Satt371 (Gm06:47820410..49860500) was examined and about 60 kb (Gm06:49800750.. 49860500) was found to be co-linear with a 317 kb region (Gm13:21919772..22237188) flanked by Satt206 and Satt595 on Chr 13. The co-linearity consisted of three distinct segment pairs, A–A′, B–B′ and C–C′, which showed different levels of synteny between the homeologous DF QTL regions. On Chr 13, a partial inversion of segment order was also observed, i.e., A–B–C on Chr 6 versus A′–C′–B′. Due to indels and inversions, only a relatively weak synteny was observed in the segment pair A–A′. Although three large insertion/deletion events were observed between the B and B′ segments, a strong conservation was observed between regions harboring the genes Glyma06g47320, Gly ma06g47330, Glyma06g47340 and Glyma06g47350 on Chr 6 and the B′ segment on Chr 13. The greatest synteny was found between the segment pair C–C′, although segment C on Chr 6 was five times longer because small heat shock protein, leucine-rich repeat and zinc finger motif were inserted after duplications.

Six duplicated gene pairs were clustered in homeologous DF QTL regions on Chrs 6 and 13. Thus, the Korea-specific QTL for DF on Chr 13 may be associated with genome duplication. On Chrs 6 and 13, the flowering-related duplicated genes showed 80–99% homology at the amino acid level. For the paralogous pair, Glyma06g47300–Glyma13g18580, their duplicated genes, Glyma09g41040 on Chr 9 and Glyma16g34610 on Chr 16 were detected in the Thailand-specific DF QTL regions. But, any significant syntenic block was not found around these DF QTL regions on Chrs 9 and 16.

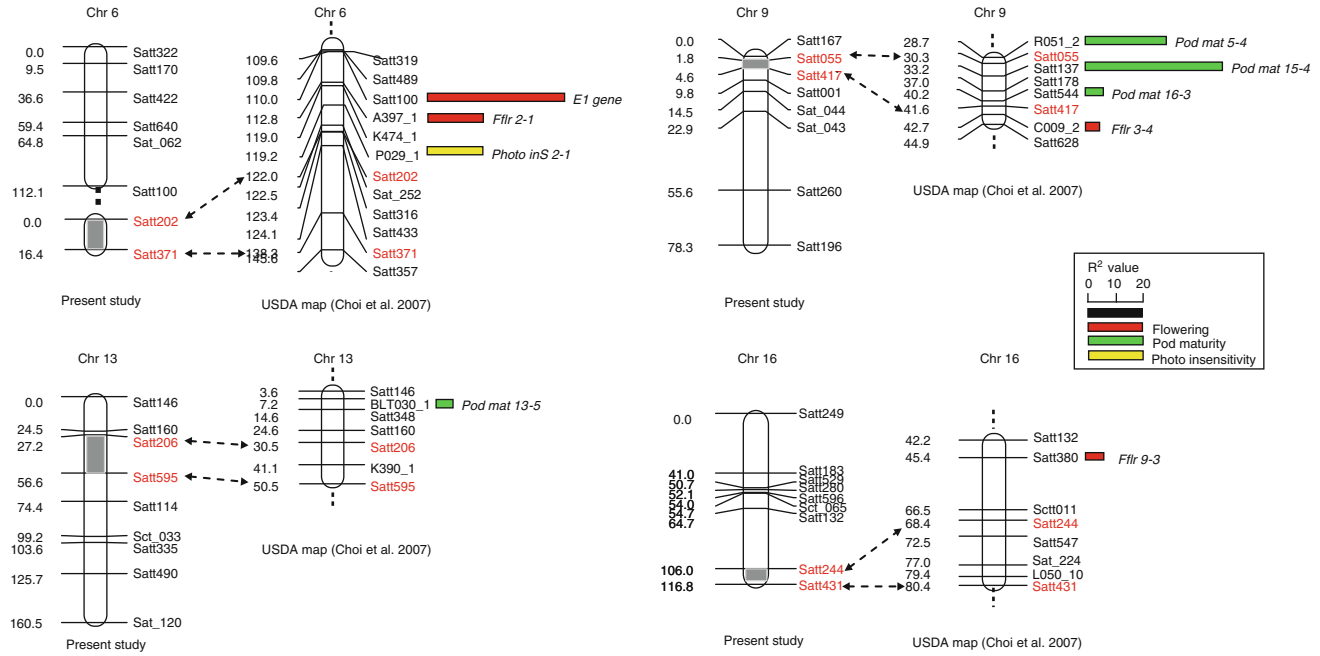

Fig. 3 Map showing comparable regions between this study and the USDA soybean genetic map (Choi et al. 2007). Positions of QTLs and markers linked to QTLs on Chrs 6, 9, 13 and 16. Four QTLs located on independent chromosomes are indicated and represent Thailand (Chrs 9 and 16), Korea (Chr 13) and China (Chr 6). QTLs identified in the present study are labeled in *gray*. *Red*, *green* and *yellow* indicate previously reported QTLs for flowering, pod maturity and photoperiod insensitivity, respectively

Table 3 QTLs with major effects on DF in a soybean RIL population derived from Jinpumkong 2 × SS2-2

Interval[a]	Chr	Length (cM)	Position (cM)[b]	Thailand			Korea			China		
				LOD[c]	Add[d]	R^2 (%)[e]	LOD[c]	Add[d]	R^2 (%)[e]	LOD[c]	Add[d]	R^2 (%)[e]
Satt202–Satt371	6	16.4	0	–	–	–	2.65	3.61	13.7	5.30	9.10	28.4
Satt055–Satt417	9	1.8	2	2.47	−0.71	12.6	–	–	–	–	–	–
Satt206–Satt595	13	29.4	2	–	–	–	2.90	−3.87	15.4	–	–	–
Satt244–Satt431	16	10.8	4	2.88	0.86	18.1	–	–	–	–	–	–

[a] Interval between markers (cM) flanking the QTL

[b] QTL distance from the first marker

[c] Maximum-likelihood LOD score for the individual QTLs

[d] The allelic genetic effect

[e] Phenotypic variance explained by the QTL

Discussion

Most soybean (*G. max*) cultivars require SD conditions for floral induction and flowering is normally suppressed by LD conditions. The mapping parents, soybean cultivars Jinpumkong 2 and SS2-2, belong to the Korean maturity group V (Ha et al. 2009). When these parental cultivars and the 89 RILs were grown under SD conditions, such as those in Kamphaeng Saen, Thailand (14°01′N), flowering occurred at approximately the same time (Fig. 1). However, flowering was much later when these plants were grown under LD conditions, such as in Longjing, China (42°46′N). This sensitivity to day length restricts the range in which soybean can be cultivated. Thus, it is important to understand the effects of day length on flowering if cultivars are to be developed for more extreme day length conditions. Two loci, *J* and *E6*, have been reported to control this response (Bonato and Vello 1999; Ray et al. 1995) and the long-juvenile trait is critically important at low latitudes where day length is always near 12 h. In a lower latitude, tropical location like Thailand, planting starts in November because flowering time is affected by warmer temperature rather than photoperiod (Carpentieri-Pipolo et al. 2002). The Thailand site was planted into a shortening day length

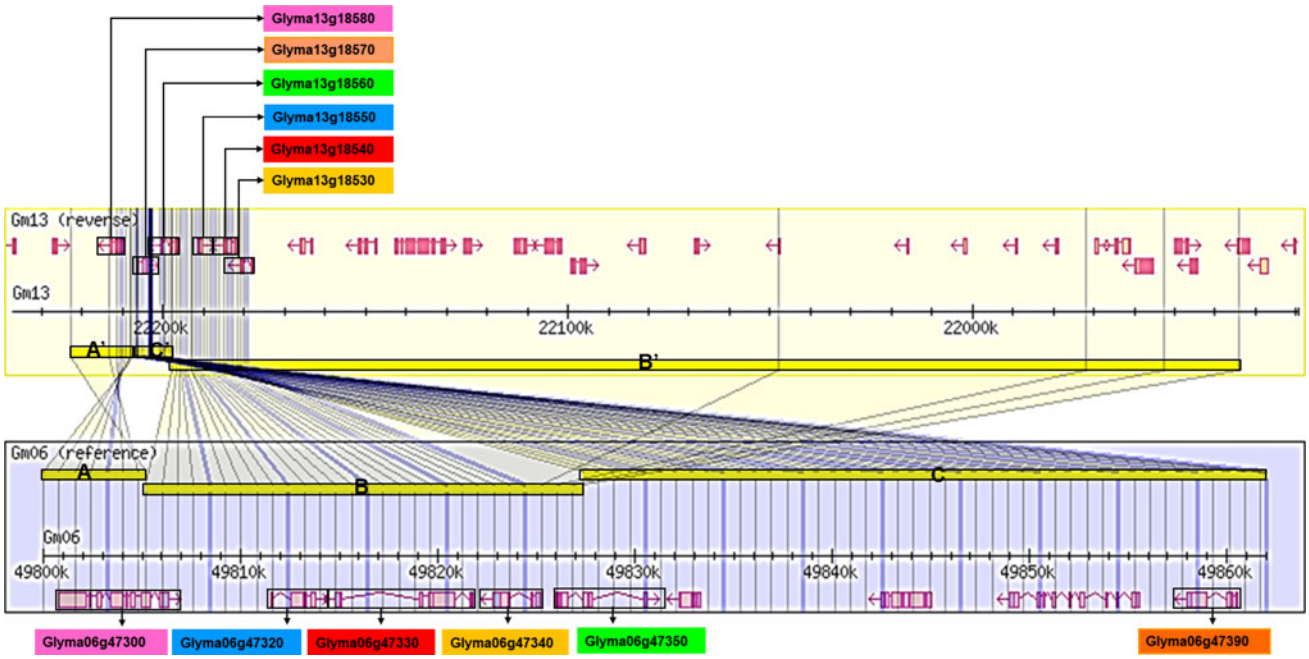

Fig. 4 Alignment of homeologous regions in Chr 6 (Gm06:49800750..49860499) and 13 (Gm:21919772..22237188) that contain six duplicated genes. The regions of Chr 13 are the interval of QTL for DF in Korea, and regions of Chr 6 distant from QTL regions about 650 kb. The regions in Chrs 6 and 13 represent forward and reverse directions of homeologous sequences, respectively. *Dark blue lines* indicate the syntenic region between chromosomes and *same color boxes* show putative duplicated genes. A versus A′, B versus B′ and C versus C′

while the Korea and China sites were planted into a lengthening day length. Therefore, the high temperatures during the night in Thailand have more significant effect on the induction of flowering. In this context, the delayed flowering in China compared to Korea might be caused by cooler temperature in the early vegetative stage (Fig. 1) as well as the longer photoperiod.

In the present study, a RIL population of Jinpumkong 2 × SS2-2 was used for QTL mapping of flowering time at three different latitudinal locations. Four DF-associated QTLs were identified on independent chromosomes (Table 3). Three of the QTLs were location-specific, with two identified in Thailand and one in Korea. In Thailand, a DF QTL was detected in the Satt055–Satt417 interval on Chr 9. It corresponds to the previously reported first flowering QTL, *Fflr3-4*, which was 1.5 cM from Satt417 (Mansur et al. 1996). Two QTLs for pod maturity, *Pod mat15-4* and *16-3*, were linked to Satt137 and Satt544, respectively. These QTLs were also located between Satt055 and Satt417; *Pod mat 5-4,* the pod maturity QTL, was positioned at marker R051_2, which is 1.6 cM from Satt055 (Lee et al. 1996; Kabelka et al. 2004; Wang et al. 2004a). The other Thailand-specific DF QTL in the Satt244–Satt431 interval (Chr 16) was equivalent to the first flowering QTL, *Fflr9_3*, which was linked to Satt380, 23.0 cM from Satt244 (Tasma et al. 2001). The Korea-specific QTL flanked by Satt206 and Satt595 appeared to align with *Pod mat 13-5* for pod maturity, which was linked to the marker BLT030_1 (Specht et al. 2001).

A DF QTL flanked by Satt202 and Satt371 (Chr 6) was also identified from both Korea and China (Table 3). Even though the temperature during the growing season in China ranged between 6.2 and 33.4°C with the day length ranging between 1205 and 1635 hours, and in Korea ranging was shown to be 12.7–34.8°C with the day length 1139–1571 hours (Table 1). The mapping parents and RILs showed distinct phenotypic variations. Specifically, the latest flowering date in China was up to 45 days later than that of Korea (Fig. 1). This indicated that this QTL was less environment-sensitive, despite the clear differences in latitude and climate between locations. Remarkably, this DF-associated QTL located on a genomic region containing the *Photo ins 2-1* locus. *Photo ins 2-1* controls photoperiod insensitivity and is located 2.8 cM from Satt202, which is linked to the P029_1 marker (Tasma et al. 2001) (Fig. 3). Furthermore, the flowering time locus *E1* and the first flowering locus *Fflr2-1* locate to the markers Satt100 and A397_1, which are 12.0 and 9.2 cM from Satt202, respectively (Fig. 3) (Mansur et al. 1993).

Soybean shows the vestiges of ancient polyploidy (paleopolyploidy) events in its genome (Schmutz et al. 2010). Soybean underwent two polyploidy events, an ancient duplication about 59 million years ago (MYA) and a more recent whole genome duplication at 14 MYA. Sequence

conservation in homeologous chromosomal segments suggests that duplicated genes retain function in soybean, resulting in the identification of multiple QTLs associated with agronomically important traits (Kim et al. 2009; Liu et al. 2008; Shin et al. 2008; Shoemaker et al. 1996; Watanabe et al. 2009).

In the present study, comparative analysis of four DF QTL regions revealed sequence synteny between two loci positioned on Chrs 6 and 13. One of these loci was identified in both Korea and China, while the other was found only in Korea. Recently, we identified a homeologous tetrad in the genomic region surrounding the BLP resistance gene (*rxp*) (Kim et al. 2009). The individual regions were located on Chrs 4, 5, 6 and 17, as a result of two rounds of genome duplication. The *rxp* homeologous region in the Sat_213–Satt277 interval, which ranges from 89.0 to 104.1 cM on Chr 6, had a counterpart located on Chr 4. Although the DF QTL region identified on Chr 6 was positioned from 122.0 to 138.3 cM (Fig. 3), it showed some colinearity with Chr 4. Only two genes, Glyma04g16040 and Glyma04g16100 on Chr 4, exhibited sequence homology of >80% with Glyma06g47300 (E1–E2 ATPase) and Glyma06g47330 (WD domain) in the DF QTL region of Chr 6, respectively (data not shown). This limited level of conservation may be attributed to complicated chromosomal rearrangements, including segmental deletion/insertion, inversion or translocation events, which have occurred in the soybean genome (Kim et al. 2009).

At present, in soybean, a high level of homology between duplicated *GmPhyA* is well described. One *GmPhyA* was mapped on Chr 10 using a SNP marker as *GmPhyA1* by Choi et al. (2007). *GmPhyA2*, the gene for the *E4* locus, and *GmPhyA3* (*E3*) were mapped on Chrs 20 and 19, respectively (Liu et al. 2008). The *GmPhyA1* gene (Chr 10) is homeologous to the region harboring *GmPhyA2* (Chr 20), and the region homeologous to *GmPhyA3* (Chr 19) was present on Chr 3 (Watanabe et al. 2009).

Our findings indicate that DF loci in two homeologous regions on Chr 6 and 13 have maintained ancestral gene function controlling flowering time, and that they have diverged in an environment-specific manner. Conservation of multiple QTLs across homeologous regions has been observed for other traits in soybean, including soybean cyst nematode resistance, corn earworm resistance, seed protein content, seed size, yield and oil content (Shin et al. 2008; Kim et al. 2009). Furthermore, several studies have used QTL mapping to show relationships between genes or genome duplication events and the functional conservation/diversification of flowering time in *Brassica* species. It is thought that these multiple QTLs for flowering time may represent copies of a single ancestral gene, which was possibly a homolog of *Arabidopsis thaliana CONSTANS* (*CO*) (Axelsson et al. 2001). The polyploid *Brassica* genome contains four copies of *FLOWERING LOCUS C* (*FLC*) that correspond to several flowering time loci on different linkage groups (Kole et al. 2001; Schranz et al. 2002). These *FLC* paralogs may perform similar functions in the regulation of flowering time, and multiple genes could have an additive effect (Schranz et al. 2002). In addition, three *FLOWERING LOCUS T* paralogs are associated with two major QTL clusters for flowering time in *Brassica napa*, and these clusters modulate functional differences in flowering time between winter and spring cultivars of oilseed *Brassica* (Wang et al. 2009).

In conclusion, using the soybean cultivars, Jinpumkong 2 and SS2-2, as well as 89 RILs, grown at three different latitudes, QTL mapping with the evaluation of variations in DF resulted in the identification of environment-sensitive and less-sensitive QTLs that modulate flowering time in soybean. The soybean genome generally contains multiple copies of genes that control agronomically important traits such as flowering, disease resistance and yield. Comparative analysis of the DF loci enabled elucidation of QTLs resulting from genome duplication, and potential candidate genes were predicted by finding duplicate genes in homeologous DF QTL regions. Thus, QTL mapping combined with comparative analysis provides a mean for understanding the functional conservation and diversification of replicated genes associated with polygenic traits and these insights will accelerate the pace of soybean improvement.

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