Theor Appl Genet (2011) 123:421–429
DOI 10.1007/s00122-011-1594-8

ORIGINAL PAPER

Genetic analysis and QTL detection of reproductive period and post-flowering photoperiod responses in soybean

Lirui Cheng · Ying Wang · Chunbin Zhang · Cunxiang Wu · Jianlong Xu · Huiying Zhu · Jiantian Leng · Yangnian Bai · Rongxia Guan · Wensheng Hou · Lijuan Zhang · Tianfu Han

Received: 26 December 2010 / Accepted: 4 April 2011 / Published online: 10 May 2011
© Springer-Verlag 2011

Abstract Reproductive period (RP) is an important trait of soybean [*Glycine max* (L.) Merr.] It is closely related to yield, quality and tolerances to environmental stresses. To investigate the inheritance and photoperiod response of RP in soybean, the F_1, F_2, and $F_{2:3}$ populations derived from nine crosses were developed. The inheritance of RP was analyzed through the joint segregation analysis. It was shown that the RP was controlled by one major gene plus polygenes. 181 recombinant inbred lines (RILs) generated from the cross of Xuyong Hongdou × Baohexuan 3 were further used for QTL mapping of RP under normal conditions across 3 environments, using 127 SSR markers. Four QTLs, designated *qRP-c-1*, *qRP-g-1*, *qRP-m-1* and *qRP-m-2*, were mapped on C1, G and M linkage groups, respectively. The QTL *qRP-c-1* on the linkage group C1 showed stable effect across environments and explained 25.6, 27.5 and 21.4% of the phenotypic variance in Nanjing 2002, Beijing 2003 and Beijing 2004, respectively. Under photoperiod-controlled conditions, *qRP-c-1*, and two different QTLs designated *qRP-l-1* and *qRP-o-1*, respectively, were mapped on the linkage groups L and O. *qRP-o-1* was detected under SD condition and can explained 10.70% of the phenotypic variance. *qRP-c-1* and *qRP-l-1* were detected under LD condition and for photoperiod sensitivity. The two major-effect QTLs can explain 19.03 and 19.00% of the phenotypic variance, respectively, under LD condition and 16.25 and 14.12%, respectively, for photoperiod sensitivity. Comparative mapping suggested that the two major-effect QTLs, *qRP-c-1* and *qRP-l-1*, might associate with *E8* or *GmCRY1a* and the maturity gene *E3* or *GmPhyA3,* respectively. These results could facilitate our understanding of the inheritance of RP and provide information on marker-assisted breeding for high yield and wide adaptation in soybean.

Communicated by I. Rajcan.

L. Cheng and Y. Wang contributed equally to this work

L. Cheng · Y. Wang · C. Zhang · C. Wu · J. Xu · H. Zhu · J. Leng · Y. Bai · R. Guan · W. Hou · T. Han (✉)
The National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), 12 Zhongguancun South Street, Beijing 100081, China
e-mail: tianfuhan@hotmail.com

L. Cheng · L. Zhang
College of Plant Science and Technology, Qingdao Agricultural University, Qingdao 266109, China

Introduction

The reproductive period (RP), a trait measured as days from flowering to maturity, is an important agronomic trait in soybean. RP is closely related to yield, quality and tolerances to environmental stresses. Increasing the length of RP has been suggested as an effective means for improving yield, since it has a positive correlation with many yield-determining factors (Johnson and Bernard 1962; Boote 1981; Hanson 1985; Smith and Nelson 1987; Curtis et al. 2000; Cober et al. 2003; Kantolic and Slafer 2001, 2005, 2007). Oil content and linoleic acid proportion increased and protein content and proportion of palmitic, oleic acids, and linolenic acids decreased with RP length under long day condition (Han et al. 1997). Mediating the length of RP can improve the tolerance of soybean to environmental stress. For instance, the spring-sowing varieties grown in Southern China have shorter RP, which reduces or avoids

the damage to seed quality caused by high temperature (Han et al. 1998).

Conventional genetic analysis indicated that soybean RP was a quantitative trait. The heritability of RP was different among populations and environments (Hanson 1992). However, genes with large effects are involved in the genetic determination of RP length. Using near isogenic lines (NILs), at least seven major genes for maturity duration (or period), *E1/e1* (Bernard 1971), *E2/e2* (Bernard 1971), *E3/e3* (Buzzell 1971), *E4/e4* (Buzzell and Voldeng 1980), *E5/e5* (McBlain and Bernard 1987), and *E7/e7* (Cober and Voldeng 2001) were identified to affect RP (Kumudini et al. 2007). Recently, Cober et al. (2010) reported a new locus (*E8/e8*) for early maturity. These genes might be different in genetic effects and interact with photoperiod for the control of RP (McBlain et al. 1987; Summerfield et al. 1998; Wang et al. 2008). In the soybean genome, the linkage group L was most frequently associated with RP (Keim et al. 1990; Mansur et al. 1993, 1996; Orf et al. 1999). In addition, the linkage groups J, C2, M, F, L, I, and C1 were also found to be associated with RP (Mansur et al. 1993, 1996; Orf et al. 1999; Xin et al. 2008).

In soybean, photoperiod played a key role in the length of post-flowering phase (Han and Wang 1995a; Han and Gai 1998; Han et al. 2006; Cober and Voldeng 2001; Summerfield et al. 1998; Kumudini et al. 2007; Liu and Abe 2010; Jiang et al. 2011). Long day increases RP. Post-flowering photoperiod sensitivity was quantitatively related to the length of exposure to long day condition (Kantolic and Slafer 2001, 2005). 16 QTLs for pre-flowering and maturity photoperiod insensitivity were mapped on linkage groups G and C2 using two RIL populations (Tasma et al. 2001). The major QTL on C2 explained 22.3 and 20.8% of the phenotypic variation in the two mapping populations, respectively. Among the *E* genes, *E1*, *E3,* and *E4* were considered to be related to photoperiod sensitivity (Saindon et al. 1989; Cober et al. 1999; Abe et al. 2003). The *E3* gene on linkage group L, which controls photoperiod sensitivity, has been cloned (Watanabe et al. 2009). The *E1* and *E4* on the linkage group C2 and I, respectively, were finely mapped (Yamanaka et al. 2005; Matsumura et al. 2008). The responsible gene for *E4* was identified by the candidate gene approach (Liu et al. 2008).

Although a number of QTLs associated with RP on soybean have been identified, our understanding of the genetics of RP, especially with the photoperiod effects on RP, is still limited. The aims of this study were to analyze the inheritance of RP using the F_1, F_2 and $F_{2:3}$ populations derived from nine crosses to evaluate the effects of genetic backgrounds on RP using parents with similar flowering dates but diverse RP, and to identify QTLs controlling RP with RILs.

Materials and methods

Plant materials

The F_1, F_2 and $F_{2:3}$ populations derived from nine crosses, viz. Xuyong Hongdou (Xu) × Baohexuan 3 (Bao), Xu × Wangjiang Huangdou (Wang), Xu × Dongguan Qingxi Huangdou (Dong), Xu × Nannong1138-2 (Nan), Xu × Batang Huangdou (Ba), Qianshan Kunlun Qingdou No.3 (Qian) × Dong, Qian × Zhengyuanmeng Dalupidou (Zheng), Qian × Ba, Qian × Ruili Shanghai Qingdou (Rui), were used to study the inheritance of RP and to evaluate the effects of genetic backgrounds on RP. The parents of these crosses had similar flowering dates but different maturity dates (Han et al. 1998). A total of 181 F_7 RILs derived from the cross of Xu × Bao by single seed descent method were used for QTL mapping of RP.

Field trials and phenotypic data scoring

The F_1, F_2 and $F_{2:3}$ populations of nine crosses were planted at Jiangpu Experimental Station, Nanjing Agricultural University, Nanjing (32.0°N, 118.8°E, 8.9 m in altitude), China on 14 June 1998 with a row length of 1.5 m, row space of 0.4 m and plant distance 5.0–7.0 cm.

The RILs were tested under three environments using randomized complete block designs with two replicates. One trial was conducted at Jiangpu Experimental Station, Nanjing Agricultural University, Nanjing, China, on 22 June 2002 (Nanjing 2002), using 0.5 × 0.5 m^2 hill-plots, 0.5 m inter-space per hill-plot and 6 plants per hill. Two trials were conducted at the Campus Experimental Farm, CAAS, Beijing (39.9°N, 116.4°E, 31.2 m in altitude), China. One was planted on 25 April 2003 (Beijing 2003) and the others was planted on 5 May 2004 (Beijing 2004). The plot size is 0.5 × 0.5 m^2 hill-plots, 0.5 m inter-space per hill-plots and 6 plants per hill.

RP was calculated as the period from the beginning bloom (R1) to the physiological maturity (R7). R1 was recorded when first flower emerged at any node on the main stem and R7 was recorded when the first normal pod on the main stem was mature (Fehr and Caviness 1977).

Post-flowering photoperiod sensitivity analysis

Each RIL was planted in two plastic pots at the campus of CAAS on 2 May, 2006, under short day (SD) condition (12 h). After R1, one pot for each line was still maintained at SD treatment, whereas the other pot was transferred to long day (LD) condition (16 h). The SD condition was created by transferring pots to dark room at 19:00, and was moved out at 7:00 in the next morning. The LD condition was created by the use of incandescent bulbs (Wu et al.

2006). The photon flux density of artificial illumination at the top of the canopy was 38.86 μmol m^{-2} s^{-1}as measured by LI-190SA (LI-COR Inc., USA). Seedlings were thinned to three plants per pot at the first trifoliolate stage.

Recommended weed and pest control measures were used for entire season, and irrigation was applied whenever needed. R1 and R7 were recorded every day. The days from R1 to R7 was calculated as an index to evaluate post-flowering photoperiod sensitivity for each line.

DNA extraction and SSR analysis

Genomic DNA was extracted from fresh soybean leaves of each line using SDS method (Keim et al. 1988). According to the sequences published on the SOYBASE website (http://soybase.agron.iastate.edu/), 856 pairs of SSR primers were synthesized for genotyping the RIL population. The PCR amplification volume is 20 μl aliquots, containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 150 μM of each of dNTPs, 3 pmol of each primer, 1.0 U *Taq* DNA polymerase and 40 ng of genomic DNA. The initial step was performed at 94°C for 5 min, followed by 94°C for 30 s, 47°C for 30 s and 72°C for 30 s for 35 cycles, and the last step was an extension at 72°C for 5 min. The amplified PCR products were separated on 6% w/v denaturing polyacrylamide gels, and the fragments were visualized by silver staining (Sanguinetti et al. 1994).

Genetic analysis

Joint segregation analysis (Zhang and Gai 2002) was used to analyze the inheritance using RP of the P_1, P_2, F_1, F_2 and $F_{2:3}$ generations of the nine crosses. Akaike's Information Criterion (AIC) and the likelihood ratio test were used to identify the major-gene and polygene models that best fit the segregation patterns of the different populations of a pair of parents. This method is developed based on the methods for fitting mixture distribution. It assumes that the trait distribution in a segregating population is a mixture distribution with component distributions determined by the segregation of the major genes. The related genetic parameters, including gene effects and genetic variances of major genes and polygenes, were obtained based on the estimates of component distributions.

Map construction and QTL detection

The program MAPMAKER/EXP VER. 3.0 (Lander and Bostein 1989) was used to construct the linkage map. A LOD score of 3.0 and maximum genetic distance of 50 cM were employed as a threshold to declare the linkage for grouping the markers. The linkage map was constructed using the Kosambi map function. The marker orders were assigned with 'Compare' and 'Try' commands. Linkage groups were named according to the designations of the consensus map of Song et al (2004). The 127 polymorphic SSR markers used were assigned to 29 linkage groups with a total coverage of 1,541.4 cM.

QTL analysis was conducted using the QTLmapper 1.6 software (Wang et al. 1999). RP data collected from different field environments (trials) were analyzed separately. The RP obtained from LD and SD conditions and its differences (LD-SD) were used to identify QTLs for post-flowering photoperiod sensitivity. LOD value of 2.5 was used for claiming a significant QTL based on permutation of 1,000 runs (Churchill and Doerge 1994).

Results

Genetic analysis of nine segregation populations

The flowering dates of the parents in each cross were similar, with differences ranging from 0.3 to 3.3 days among the parents. However, the parents showed great diversity in RP, with significant differences among all the pairs of parents (Table 1). The means of phenotypic values were intermediate between the parents in the F_1 populations, and close to the lower value parent in the F_2 and $F_{2:3}$ populations except in those of Xu × Nan cross, demonstrating that partial dominant genes tended to shorten RP in most of crosses (Table 2).

For all the nine crosses, joint segregation analysis using the F_1, F_2 and $F_{2:3}$ populations showed the best inheritance model was the mixed one additive major-gene and additive-dominance polygenes. However, the gene effects and heritabilities were different among the crosses. Additive effects of the major gene were negative in all crosses, indicating the alleles from the high-value parents prolonged RP. The heritabilities of major genes were in the ranges of 21.49–75.86% and 17.93–50.27% in the F_2 and and $F_{2:3}$ populations, respectively.

QTL mapping for RP under normal conditions

Phenotypic variation of the RILs

RILs showed significant transgressive segregation, especially in Beijing 2003 and Beijing 2004 (Fig. 1), which are under LD condition. Compared with the days to maturity in Nanjing 2002, the LD conditions in Beijing 2003 and Beijing 2004 caused significantly delayed maturity of 179 lines (ranging from 1 to 33.5 days) and 174 lines (ranging from 0.5 to 33.5 days), respectively. Surprisingly, significant earlier maturity were found for 2 (ranging from 0 to 0.5 days) and 7 lines (ranging from 0 to 5.5 days),

Table 1 Comparison of flowering time and reproductive period (RP) between parents used in genetic analysis

Cross	Flowering time			Reproductive period		
	Mean ± SD (♀)	Mean ± SD (♂)	Difference between parents	Mean ± SD (♀)	Mean ± SD (♂)	Difference between parents
Xu × Bao	46.3 ± 0.5	46.6 ± 2.2	0.3	46.5 ± 2.2	51.1 ± 2.1	4.6***
Xu × Nan		47.3 ± 0.8	1.0		52.2 ± 2.1	5.7***
Xu × Wang		43.6 ± 1.9	2.7		54.6 ± 2.8	8.1***
Xu × Dong		44.3 ± 1.7	2.0		56.3 ± 2.9	9.8***
Xu × Ba		48.4 ± 1.6	2.1		69.2 ± 2.0	22.7***
Qian × Dong	45.1 ± 1.2	44.3 ± 1.7	1.9	46.4 ± 1.9	56.3 ± 2.9	9.9***
Qian × Zheng		46.3 ± 1.3	0.9		62.9 ± 3.1	16.5***
Qian × Rui		47.6 ± 0.7	2.5		68.7 ± 2.3	22.3***
Qian × Ba		48.4 ± 1.6	3.3		69.2 ± 2.0	22.8***

*** Significant difference at the level of $P < 0.001$

Table 2 Genetic parameters estimated in RP for nine sets of populations using the joint segregation analysis (Phenotypic data were collected in Nanjing in 2002)

Cross	Generation	The number of lines	Mean ± SD (days)	Range (days)	a[a]	[a][b]	[d][c]	h^2_{mg}%[d]	h^2_{pg}%[e]
Xu × Bao	F_1	4	42.50 ± 1.73	40–44					
	F_2	202	44.08 ± 3.94	36–57	−0.93	−1.37	0.83	44.95	29.13
	$F_{2:3}$	46	46.18 ± 3.74	37–57				38.40	26.46
Xu × Wang	F_1	7	49.43 ± 2.23	47–53					
	F_2	48	47.91 ± 4.12	40–59	−1.97	−2.16	−2.02	30.79	31.36
	$F_{2:3}$	55	47.84 ± 5.97	34–70				17.93	53.67
Xu × Dong	F_1	4	51.25 ± 1.26	50–53					
	F_2	115	50.59 ± 4.22	41–68	−2.93	−0.72	2.34	25.17	48.83
	$F_{2:3}$	56	50.35 ± 4.56	35–66				27.56	33.13
Xu × Nan	F_1	3	51.00 ± 1.00	50–52					
	F_2	98	50.00 ± 4.00	42–69	−2.65	0.23	1.31	21.49	57.02
	$F_{2:3}$	49	49.84 ± 4.74	40–66				34.84	49.71
Xu × Ba	F_1	4	47.75 ± 3.30	44–52					
	F_2	53	54.83 ± 4.18	49–68	−4.59	−6.67	−4.12	38.81	43.91
	$F_{2:3}$	68	55.65 ± 6.05	42–74				34.99	37.15
Qian × Dong	F_1	9	51.60 ± 5.06	41–57					
	F_2	79	49.96 ± 3.48	42–59	−2.45	−1.58	−0.82	60.54	22.77
	$F_{2:3}$	129	50.10 ± 4.44	39–73				21.13	56.95
Qian × Zheng	F_1	7	54.43 ± 1.51	52–57					
	F_2	121	55.84 ± 3.86	48–66	−1.51	−6.25	3.34	62.07	9.37
	$F_{2:3}$	51	53.56 ± 3.58	42–71				25.99	60.14
Qian × Ba	F_1	5	59.40 ± 5.03	51–64					
	F_2	55	62.68 ± 7.14	49–75	−5.41	−6.88	7.20	75.86	3.80
	$F_{2:3}$	93	57.42 ± 8.28	38–81				47.29	29.53
Qian × Rui	F_1	3	55.67 ± 3.79	53–60					
	F_2	69	57.77 ± 5.98	47–73	−5.56	1.61	−1.20	75.06	7.34
	$F_{2:3}$	88	59.42 ± 6.87	43–77				50.27	20.31

[a] Additive effect of major gene

[b] Additive effect of polygenes

[c] Dominance effect of polygenes

[d] Heritability of major gene

[e] Heritability of polygenes

respectively. There were significant differences between lines for each testing environments (Table 3). Across-experiment analysis revealed that there are highly significant differences among environments and lines, indicating environmental variation play an important role in phenotypic variation. Testing environment contributed more to the total phenotypic variation than line, suggesting that there was a common photoperiod response in RP (Table 4). Compared to SD condition, the RP was obviously increased under LD condition.

Xuyong Hongdou (P_1) was 6 days shorter and 5 days longer than Baohexuan 3 (P_2) under SD and LD conditions, respectively, indicating that Baohexuan 3 was more stable in RP than Xuyong Hongdou under different photoperiodic conditions (Fig. 1). Transgressive segregation for RP was observed in the RIL population in all testing environments. Under LD condition all lines had significantly increased RP, ranging from 2 to 107 days longer than under SD conditions.

Four QTLs were identified under normal conditions (natural sunshine) across three testing environments (Table 5). Among them, *qRP-c-1*, *qRP-g-1*, and *qRP-m-1* were mapped on linkage groups C1, G and M in Nanjing 2002, while *qRP-c-1* and *qRP-m-2* were mapped on linkage groups C1 and M in Beijing 2003 and Beijing 2004. The alleles from Bao at *qRP-c-1* and *qRP-m-1* loci prolonged RP, whereas those at *qRP-g-1* and *qRP-m-2* from Bao reduced RP. *qRP-c-1* in the interval of Sat_085-Satt294 on the linkage group C1 was identified across all three environments, and explained 25.6, 27.5, and 21.4% of the total phenotypic variance, respectively (Table 5), indicating a stable genetic effect in different environments.

QTL mapping under controlled conditions

Under LD condition, one QTL *qRP-l-1* for RP was mapped on linkage group L and explained 19.0% of the total phenotypic variance. Under SD condition, one QTL *qRP-o-1* for RP was identified in the interval of Satt259-Satt347 on linkage group O, explaining 10.7% of the total phenotypic variance. The alleles from Bao at these two loci shortened RP. One QTL *qRP-l-1* for post-flowering photoperiod response was identified on linkage group L, explaining 14.1% of total phenotypic variance. This QTL was located on the same interval with *qRP-l-1* for RP. The allele from Xu increased the photoperiod sensitivity in RP (Table 6; Fig. 2).

To reduce the influence of *qRP-l-1* on the detection of QTL *qRP-c-1*, we conducted mapping using two sub-populations homogenous for alternative alleles of *qRP-l-1* selected using the closest markers. The S1 population consisted of 88 individuals that have the P_2 alleles of Sat_150 and Satt561 markers, and the S2 population consisted of 42 individuals that have the P_1 alleles of Sat_150 and Satt561 markers. As Table 6 and Fig. 2 showed, under LD condition, one QTL *qRP-c-1* was identified in the

Table 3 Analysis of variance of reproductive period (RP) in soybean of three experiments

Experiment	Source	DF	MS	*P* value
Nanjing 2002	Lines	181	13.19	0.0001****
	Error	180	3.82	
Beijing 2003	Lines	181	65.03	0.0001****
	Error	180	26.14	
Beijing 2004	Lines	181	37.59	0.0005****
	Error	180	22.97	

**** Significantly difference at level of $P < 0.0001$

Table 4 Across-environment analysis of variance for reproductive period (RP) of 181 RIL lines tested in three environments

Condition	DF	MS	*P* value
Environment	2	13751.14	0.0001****
Line	180	44.99	0.0001****
Environment × Line	540	38.60	0.0001****
Error	902	28.81	

**** Significantly difference at level of $P < 0.0001$

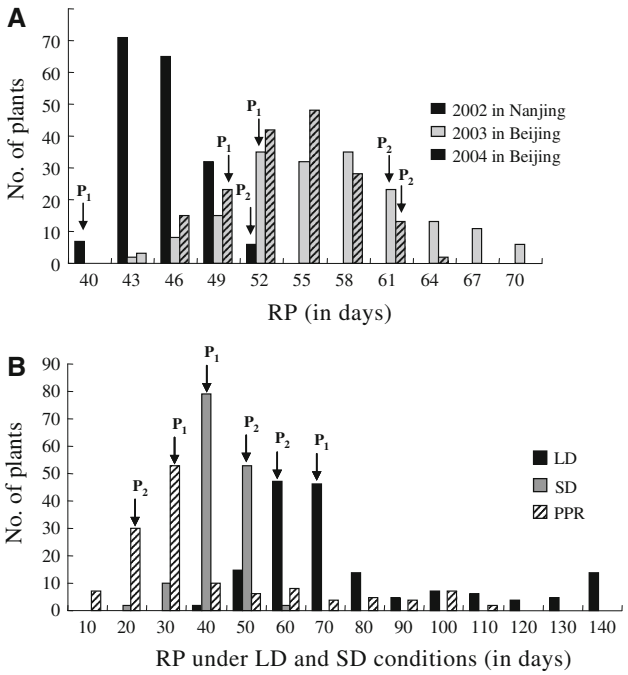

Fig. 1 Frequency distribution of the reproductive period (RP) in different locations across years and different photoperiod conditions in the RILs derived from Xuyong Hongdou (P_1)/Baohexuan 3 (P_2). LD and SD mean long duration (16 h) and short duration (12 h) after beginning bloom (R1), respectively. *PPR* post-flowering photoperiod response, calculated as days from R1 to R7 under LD condition minus days from R1 to R7 under SD condition

Table 5 QTLs affecting reproductive period (RP, in days) identified in the RILs population of Xuyong Hongdou (Xu) × Baohexuan 3 (Bao) in normal conditions in 2002, 2003 and 2004

QTL[a]	Location	Year	QTL interval	LG	LOD	Add[c]	R^2 (%)[b]	Reported QTL[d]
qRP-c-1	Nanjing	2002	Sat_085-Satt294 (76.9 ~ 78.7)	C1	7.19	−1.23	25.6	E8, Reprod3-4, Reprod2-2
qRP-c-1	Beijing	2003	Sat_085-Satt294 (76.9 ~ 78.7)	C1	6.43	−2.87	27.5	E8, Reprod3-4, Reprod2-2
qRP-c-1	Beijing	2004	Sat_085-Satt294 (76.9 ~ 78.7)	C1	4.75	−2.09	21.4	E8, Reprod3-4, Reprod2-2
qRP-g-1	Nanjing	2002	Satt503-Satt191 (68.8 ~ 96.6)	G	4.45	0.7	10.2	
qRP-m-1	Nanjing	2002	Satt536-Satt175 (61.5 ~ 72.3)	M	2.54	−0.56	5.41	Pod mat10-3
qRP-m-2	Beijing	2003	Satt540-Satt435 (10.1 ~ 12.9)	M	2.58	1.3	5.79	

[a] QTLs were detected using QTLmapper 1.6 CIM using a LOD threshold of 2.5 (1,000 permutations and a type I error of 5 %)

[b] Percentage of the phenotypic variation explained by the QTL

[c] Additive effect of the QTL

[d] QTLs from http://www.soybase.org

Table 6 QTLs affecting reproductive period (RP, in days) identified in the RILs population of Xuyong Hongdou (Xu) × Baohexuan 3 (Bao) under SD and LD conditions in 2006

QTL	Population	Condition	QTL interval	LG	LOD	Add	R^2 (%)	Reported QTL
qRP-o-1	Whole	SD	Satt259-Satt347 (39.8.4 ~ 42.3)	O	3.48	1.75	10.70	
qRP-c-1	S1[a]	LD	Sat_085-Satt294 (76.9 ~ 78.7)	C1	3.05	−10.81	19.03	E8, Reprod3-4, Reprod2-2
qRP-l-1	Whole	LD	Sat_150-Satt561 (53.7 ~ 71.4)	L	6.02	10.90	19.00	E3, Pod mat14-1, Pod mat15-1, R5 1-2
qRP-c-1	S1	PPR[b]	Sat_085-Satt294 (76.9 ~ 78.7)	C1	2.54	−9.59	16.25	E8, Reprod3-4, Reprod2-2
qRP-l-1	Whole	PPR	Sat_150-Satt561 (53.7 ~ 71.4)	L	2.62	4.16	14.12	E3, Pod mat14-1, Pod mat15-1, R5 1-2

[a] S1 consisted of 88 individuals that have the P_2 alleles of Sat_150 and Satt561 markers

[b] Post-flowering photoperiod response, calculated as days from R1 to R7 under LD condition minus days from R1 to R7 under SD condition

interval of Sat_085-Satt294 on linkage group C1, explaining 19.03% of the total phenotypic variance only in the S1 population. The allele from Bao prolonged RP. Meanwhile, the QTL was located on the same interval with the QTL for post-flowering photoperiod response, explaining 16.25% of total phenotypic variance in the same population. The allele from Bao increased the photoperiod sensitivity in RP.

Discussion

The development of high yielding varieties is a key objective in soybean breeding. The length of RP was considered to be highly related to the number of seeds and photosynthetic availability (Hanson 1985). In addition, the chemical compositions of soybean seeds are also closely related to the duration of RP (Han et al. 1997). Therefore, the manipulation of RP is important.

In this study, we used crosses between parents with similar flowering dates but different maturity dates and thus different RP to reduce the influence of flowering time on RP. It was found that the inheritance of RP follows the model of one major gene plus polygenes in all the nine crosses. However, the major-gene heritability in the F_2 and $F_{2:3}$ generations varied greatly among crosses, indicating that the expression of the major gene for RP was affected by the genetic background. The heritability of RP depended

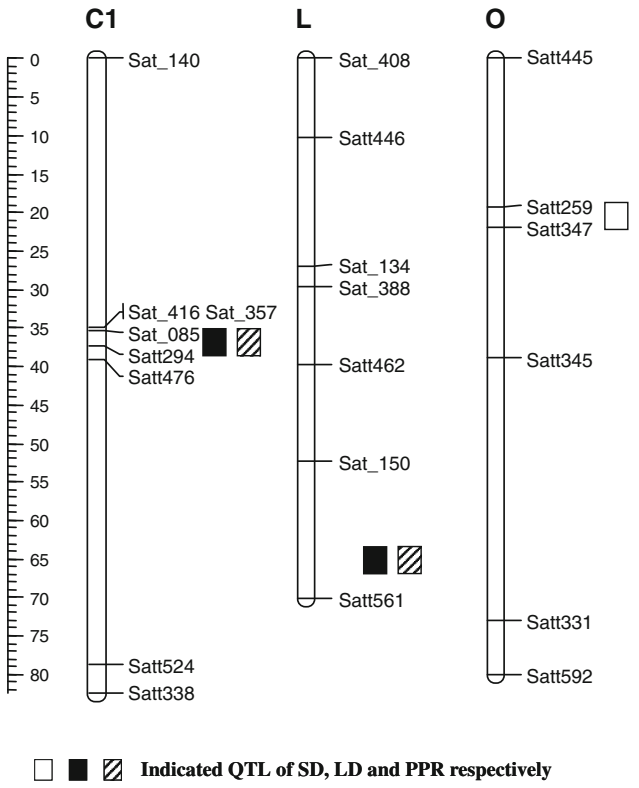

Fig. 2 Linkage group locations of main effect QTLs influencing RP under SD and LD conditions

on the genetic background and environmental factors (Metz et al. 1985). Joint segregation analysis does not need the genotype data and as a result is more economic than QTL mapping and allows the study of the effect of genetic background on major gene expression using many different parental lines. The joint segregation analysis can be used jointly with the bulk segregant analysis (BSA) to reduce the cost of gene tagging. If major genes are proven to be involved in the determination of a trait by the joint segregation analysis, BSA can be used to quickly and economically tag the major genes. In addition, the results from QTL mapping and the joint segregation analysis can be cross-validate the existence of the major gene. In this study, QTL mapping using a RIL population derived from Xu × Bao also identified major QTL for RP and demonstrated the presence of other QTLs under all three environments.

Six QTLs in total were identified to associate with RP and located on the linkage groups of C1, G, M, L and O.

A major-effect QTL (*qRP-c-1*) was detected in a small region of 1.8 cM on linkage group C1 in all three environments. The closest marker is Sat_085, which is close to the locus reported by Watanabe et al. (2004) and the locus *E8/e8* reported by Cober et al. (2010). Matsumura et al. (2009) reported that the Sat_085 marker is closely linked to the gene *GmCRY1a,* which plays a predominant role in photoperiodic flowering and latitudinal distribution of soybean varieties (Zhang et al. 2008). *GmCRY1a* controls the production of cryptochromes that were considered to mediate light-regulated plant development and growth (Guo et al. 1998). Therefore, *qRP-c-1* is most likely related to *GmCRY1a* and plays important role in soybean development during reproductive period.

qRP-c-1 was detected in all three environments under normal (natural sunshine) conditions. However, it was not identified under controlled LD (16 h) and SD (12 h) conditions using the RIL population. So we remapped the QTLs using two selected populations. The major QTL, *qRP-c-1,* were identified for PR under LD condition and for PPR in the S1 population, but not in S2 population. As the results showed, the allele at *qRP-l-1* from P_1 increased RP under LD condition and photoperiod sensitivity. In the S2 population, the alleles of Sat_150 and Satt561 markers all come from the P_1. Therefore, in the S2 population, the allele from P_1 at the *qRP-l*-1 locus has a negative effect on detection of *qRP-c-1* under LD condition. Conversely, in the S1 population, the major QTL, *qRP-c-1,* was identified due to the elimination of influence at *qRP-l-1* locus. Therefore, *qRP-c-1* is also an important locus related to photoperiod sensitivity in soybean although it is only detected in S1 population under LD condition. Meanwhile, in our study, the segregation distortion was detected for all markers on the C1 linkage group and the Sat_150 on L linkage group in random RIL population. The RILs derived from the cross of Xu × Bao were produced by single seed descent method without intentional selection. However, some of the lines could not produce mature seeds before frost and consequently were abandoned during selfing. So the RIL population used in mapping was unintentionally selected by environmental factors, primarily photoperiod and temperature. The two major QTLs on the C1 and L linkage groups are related to photoperiod and caused significant distortion in markers surrounding these QTLs. To a certain extent, the significant segregation distortions observed for markers on the C1 and L linkage groups can be explained as the existence of QTL effecting on RP and maturity.

Watanabe et al. (2009) cloned the gene *E3-GmphyA3* associated with soybean maturity. The major-effect QTL, *qRP-l-1*, was identified for photoperiod-sensitivity of RP based on phenotypic differences between LD and SD and was highly likely to be related to *E3*. Meanwhile, *qRP-c-1* is most likely related to *E8* and plays important role in soybean development during reproductive period. Kumudini et al. (2007) believed that RP was positively related to a number of dominant E-gene alleles. In the current study, these lines containing the *E3E3E8E8* alleles are more photoperiodic sensitivity in RP, indicating that all these

additive effects from different E-genes are responsible for the duration of reproductive period stage under photoperiod conditions.

Two QTLs for RP, *qRP-m-1* and *qRP-m-2* were located to linkage group M. Mansur et al. (1996) also mapped one RP QTL on the linkage group M. However, *qRP-m-1* and *qRP-m-2* are distant to the locus identified by Mansur et al. (1996), suggesting that the three loci were different.

It is well known that photoperiod plays an important role in plant development during post-flowering phase. However, previous studies of photoperiod response in soybean mostly have focused on the pre-flowering stage and the knowledge of the post-flowering photoperiod response is fragmentary and far less comprehensive. Han and Wang (1995b) suggested that soybean has the similar signal receiving mechanism before and after flowering and that the effect of photoperiod persist at all reproductive development stages, from the onset of flowering to seed filling and maturation, that is, the genes controlling the post-flowering period and time to flowering are similar in soybean (pleiotrophic effect). In this study, the two QTLs, *qRP-c-1* and *qRP-l-1* identified for the duration of the post-flowering period also control the flowering time (flowering data not shown). Thus, it is likely that the post-flowering photoperiod signals are also mediated by the phytochromes and crytochromes similar to those before flowering.

Photoperiod sensitivity must be taken into consideration when varieties are grown outside of their adaptation areas due to the effect of photoperiod on RP. Soybean is a facultative photoperiod-sensitive crop, although there are some varieties with stable RP under different photoperiodic conditions. Photoperiod-insensitive breeding materials are good resources for the improvement of geographical adaptation. In this study, *qRP-c-1* and *qRP-l-1* were identified as two major QTLs associated with photoperiod insensitivity of RP and these two QTLs are in the regions where QTLs for RP have been located by others using different populations tested under various conditions. Therefore, marker-assisted selection against the region harboring the photoperiod-insensitive alleles at *qRP-c-1* and *qRP-l-1* from P_1 and P_2 could be explored to select for photoperiod-insensitive individuals/lines and develop soybean varieties with wide geographical adaptation.

Acknowledgments The authors are grateful to Drs. Xianchuan Xia and Guoyou Ye for valuable suggestions, Miss Guangming Yang for language improvement, Mr. Fengyuan Chen and Zhenjing Ren for field management, Professor Junyi Gai for providing the parent seeds. This work was supported by the National Basic Research Program of China (2009CB118400), Nature Science Foundation of China (30471054), and the Earmarked Fund for China Agriculture Research System (CARS-04).

References

Abe JD, Xu D, Miyano A, Komatsu K, Kanazawa A, Shimamoto Y (2003) Photoperiod-insensitive Japanese soybean landraces differ at two maturity loci. Crop Sci 43:1300–1304

Bernard RL (1971) Two genes for time of flowering and maturity in soybean. Crop Sci 11:242–244

Boote KJ (1981) Response of soybean in different maturity groups to march planting in southern USA. Agron J 73:854–859

Buzzell RL (1971) Inheritance of soybean flowering response to fluorescent-daylength conditions. Can J Genet Cytol 13:703–707

Buzzell RL, Voldeng HD (1980) Inheritance of insensitivity to long daylength. Soybean Genet Newsl 7:26–29

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cober ER, Molnar SJ, Charette M, Voldeng HD (2010) A new locus for early maturity in soybean. Crop Sci 50:524–527

Cober ER, Tanner JW, Voldeng HD (1999) Soybean photoperiod-sensitivity loci respond differentially to light quality. Crop Sci 36:606–610

Cober ER, Voldeng HD (2001) A new soybean maturity and photoperiod sensitivity locus linked to *E1* and *T*. Crop Sci 41:698–701

Cooper RL (2003) A delayed flowering barrier to higher soybean yields. Field Crops Res 82:27–35

Curtis DF, Tanner JW, Luzzi BM, Hume DJ (2000) Agronomic and phenological differences of soybean isolines differing in maturity and growth habit. Crop Sci 40:1624–1629

Fehr WR, Caviness CE (1977) Stages of soybean development. Agric and Home Economics Exp. Stn. Spec. Rep. 80. Iowa State University, Ames

Guo H, Yang H, Mockler TC, Lin C (1998) Regulation of flowering time by *Arabidopsis* photoreceptors. Science 279:1360–1363

Han T, Wang J (1995a) Pre- and post-flowering photoperiod response in early soybean varieties. Soybean Genet Newsl 22:89–92

Han T, Wang J (1995b) Studies on the post-flowering photoperiodic responses in soybean. Acta Bot Sin 37:863–869

Han T, Wang J, Yang Q, Gai J (1997) Effects of post-flowering photoperiod on chemical composition of soybeans. Sci Agron Sin 30:47–53

Han T, Gai J (1998) Post-flowering photoperiod response of soybean varieties with similar flowering dates and different maturity dates. Soybean Genet Newsl 25:48–49

Han T, Gai J, Cheng F, Qiu J (1998) Photoperiod response and agronomic characters of soybean varieties with different growth period structures. Acta Agron Sin 24:550–557

Han T, Wu C, Tong Z, Mentreddy RS, Tan K, Gai J (2006) Post flowering photoperiod regulates vegetative growth and reproductive development of soybean. Environ Exp Bot 55:120–129

Hanson WD (1985) Association of seed yield with partitioned lengths of the reproductive period in soybean genotypes. Crop Sci 25:525–529

Hanson WD (1992) Phenotypic recurrent selection for modified reproductive period in soybean. Crop Sci 32:968–972

Johnson HW, Bernard RL (1962) Soybean genetic and breeding. Adv Agron 14:149–221

Kantolic AG, Slafer GA (2001) Photoperiod sensitivity after flowering and seed number determination in indeterminate soybean cultivars. Field Crops Res 72:109–118

Kantolic AG, Slafer GA (2005) Reproductive development and yield components in indeterminate soybean as affected by post-flowering photoperiod. Field Crops Res 93:212–222

Kantolic AG, Slafer GA (2007) Development and seed number in indeterminate soybean as affected by timing and duration of

exposure to long photoperiods after flowering. Ann Bot 99:925–933
Keim P, Diem BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean association between marker loci and variation in quantitative traits. Genetics 126:735–742
Keim P, Olson TC, Shoemaker RC (1988) A rapid protocol for isolating soybean DNA. Soybean Genet Newsl 15:150–152
Kumudini SV, Pallikonda PK, Steele C (2007) Photoperiod and E-genes influence the duration of the reproductive phase in soybean. Crop Sci 47:1510–1517
Lander ES, Bostein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP maps. Genetics 121:185–199
Liu BH, Abe J (2010) QTL mapping for photoperiod insensitivity of a Japanese soybean landrace Sakamotowase. J Hered 101:251–256
Liu BH, Kanazawa A, Matsumura H, Takahashi R, Harada K (2008) Genetic redundancy in soybean photoresponses associated with duplication of phytochrome a gene. Genetics 180:995–1007
Mansur LM, Lark KG, Kross H, Oliveira A (1993) Interval mapping of quantitative trait loci for reproductive morphological, and seed traits of soybean (*Glycine max* (L.) Merr.). Theor Appl Genet 86:907–913
Mansur LM, Orf JH, Chase K, Jarvik T, Cregan PB, Lark KG (1996) Genetic mapping of agronomic traits using recombinant inbred lines of soybean. Crop Sci 36:1327–1336
Matsumura H, Liu BH, Abe J, Takahashi R (2008) AFLP mapping of soybean maturity gene *E4*. J Hered 99:193–197
Matsumura H, Kitajima H, Akada S, Abe J, Minaka N, Takahashi R (2009) Molecular cloning and linkage mapping of cryptochrome multigene family in soybean. Plant Genome 2:271–281
McBlain BA, Bernard RL (1987) A new gene affecting the time of flowering and maturity in soybean. Heredity 78:160–162
McBlain BA, Hesketh JD, Bernard RL (1987) Genetic effects on reproductive phenology in soybean isolines differing in maturity genes. Can J Plant Sci 67:105–116
Metz GL, Green DE, Shibles RM (1985) Reproductive duration and date of maturity in populations of three wide soybean crosses. Crop Sci 25:171–176
Orf JH, Chase K, Jarvik T, Mansur LM, Cregan PB, Adler FR, Lark KG (1999) Genetics of soybean agronomic traits: comparison of three related recombinant inbred populations. Crop Sci 9:1642–1651
Saindon G, Voldeng HD, Beversdorf WD, Buzzel RI (1989) Genetic control of long day length response in soybean. Crop Sci 29:1436–1439
Sanguinetti CJ, Dias Neto E, Simpson AJG (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17:915–919
Smith JR, Nelson RL (1987) Predicting yield from early generation estimates of reproductive growth periods in soybean. Crop Sci 27:471–474
Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128
Summerfield RJ, Asumadu H, Ellis RH, Qi A (1998) Characterization of the photoperiodic response of post-flowering development in maturity isolines of soyabean [*Glycine max* (L.) Merr.] 'Clark'. Ann Bot 82:765–771
Tasma IM, Lorenzen LL, Green DE, Shoemaker RC (2001) Mapping genetic loci for flowering time, maturity and photoperiod insensitivity in soybean. Mol Breed 8:25–35
Tasma IM, Shoemaker RC (2003) Mapping flowering time gene homologues in soybean and their association with maturity (*E*) loci. Crop Sci 43:319–328
Wang DL, Zhu J, Li ZK, Paterson AH (1999) Mapping QTL with epistatic effects and QTL × environment interaction by mixed linear model approaches. Theor Appl Genet 99:1255–1264
Wang Y, Wu C, Zhang X, Wang Y, Han T (2008) Effects of soybean major maturity genes under different photoperiods. Acta Agron Sin 34:1160–1168
Watanabe S, Tajuddin T, Yamanaka N, Hayshi M, Harada K (2004) Analysis of QTLs for reproductive development and seed quality traits in soybean using recombinant inbred lines. Breed Sci 54:399–407
Watanabe S, Hideshima R, Xia Z, Tsubokura Y, Sato S, Nakamoto Y, Yamanaka N, Takahashi R, Ishimoto M, Anai T, Tabata S, Harada K (2009) Map-based cloning of the gene associated with soybean maturity locus E3. Genetics 182:1251–1262
Wu C, Ma Q, Yam K-M, Xu Y, Han T, Lam H-M, Chong K (2006) In situ expression of the *GmNMH7* gene is photoperiod-dependent in a unique soybean [*Glycine max* (L.) Merr.] flowering reversion system. Planta 223:725–735
Xin DW, Qiu HM, Shan DP, Shan CY, Liu CY, Hu GH, Staehelin C, Chen QS (2008) Analysis of quantitative trait loci underlying the period of reproductive growth stages in soybean [*Glycine max* (L.) Merr.]. Euphytica 162:155–165
Yamanaka N, Watanade S, Toda K, Hayashi M, Fuchigami H, Takahashi R, Harada K (2005) Fine mapping of the *FT1* locus for soybean flowering time using a residual heterozygous line derived from a recombinant inbred line. Theor Appl Genet 110:634–639
Yan J, Wu C, Zhang L, Hu P, Hou W, Zu W, Han T (2011) Long-day effects on the terminal inflorescence development of a photoperiod-sensitive soybean [*Glycine max* (L.) Merr.] variety. Plant Sci 180:504–510
Zhang Q, Li H, Li R, Hu R, Fan C, Chen F, Wang Z, Liu X, Fu Y, Lin C (2008) Association of the circadian rhythmic expression of *GmCRY1a* with a latitudinal cline in photoperiodic flowering of soybean. Proc Natl Acad Sci USA 105:21028–21033
Zhang Y, Gai J (2002) An expansion of the joint segregation analysis of P_1, P_2, F_1, F_2 and $F_{2:3}$ generations in the mixed major gene plus polygenes inheritance analysis of quantitative traits. J Biomath 17:363–368