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REVIEW

Analysis of quantitative trait loci underlying the period of reproductive growth stages in soybean (*Glycine max* [L.] Merr.)

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Abstract Quantitative trait loci (QTLs) underlying reproductive growth stages are important for molecular breeding of soybeans [*Glycine max* (L.) Merr.]. Most of these QTLs identified so far derived from a single environment, and thus may be influenced by specific environmental conditions. In this study (from 2004 to 2005), analysis of QTLs underlying the period to reach a given reproductive growth stage was performed in three different environments (Harbin, Heilongjiang Province, China). QTL analysis was achieved with a recombination inbred line (RIL) population consisting of 153 lines. The RIL population derived from a cross between an American semi-dwarf cultivar (cv. Charleston) and a Chinese line with a short growth stage (cv. Dongnong 594). The growth stage data of soybean was recorded for each day. QTLs for all eight reproductive growth stages of soybean (R1 to R8) were analyzed by a composite interval mapping method combined with a mixed genetic model. Fifty-four QTLs displayed main effects and 56 QTL pairs showed epistatic effects. Two marker intervals (Satt173–Satt581, Satt402–Satt267), located on the linkage group O and D1a respectively, strongly influenced plant developmental processes during reproductive growth stages. The findings of this study open the possibility to modulate the structure of soybean growth stages by marker-assisted selection and pyramiding QTL analysis.

Keywords Soybean · Main effect · Epistatic effect · Reproductive growth stage

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Introduction

Soybean [*Glycine max* (L.) Merr.] is an important crop legume with a broad variety of cultivars. Inbred breeding has been a major strategy in soybean genetics to shorten the vegetation period and improve the grain yield in geographic regions with short summers. Soybean growth stages show high correlations with grain yield, plant height, pod number, and seed weight (Chen et al. 2004). The growth stages of

soybean consists of a vegetative stage and reproductive stage. The reproductive stage can be further divided into eight stages: R1 to R8 (Table 1). When soybeans reach a given growth stage at a given date of the vegetation period, the date of maturity, the yield and quality of soybean grains can be predicted. Hence, quantitative trait loci (QTLs) affecting reproductive growth stages and marker-assisted selection are important tools to develop new cultivars adapted to geographical regions with short vegetation periods (Tasma et al. 2001).

QTLs associated with reproductive growth stages are known to be influenced by day length, temperature, and plant genotype (Whigham and Minor 1978). Soybean cultivars adapted to specific geographical regions often differ in day length perception that affect the length of time required to reach a given growth stage (Cober et al. 1996). In the past decades, studies on soybean growth stages mainly focused on the inherence of traits associated with seed maturity. A classical study described that maturity traits showed a high variance of inheritability during early generations, whereas traits for maturity were stably inherited after the F_5 generation (Wang et al. 1963). In soybean, at least seven genes have been reported to affect flowering time and maturity (Cober et al. 1996). The genes are known as the *E*-series: *E*1 to *E*7 (Bernard et al. 1971; Buzell et al. 1971, 1980; McBlain et al. 1987; Bonato et al. 1999; Cober et al. 2001).

Several QTLs for reproductive growth stages, including QTLs for R1, R3, R7 and R8, have been identified with recombination inbred line (RIL) populations. Five markers on the linkage groups C1, C2, and D1 were found to be associated with maturity (including time of flowering) in a F_2 population derived from a cross between *Glycine max* and *Glycine soja*. The observed QTL explained 17–23% of total phenotypic variance (Shoemaker et al. 1995). With "Minsoy' × 'Noir 1", F_2 derived population, a major QTL for flowering time was identified on linkage group C2 and two minor QTLs for maturity on the linkage groups L and M (Mansur et al. 1996; Cregan et al. 1999). Additional QTLs for traits related to maturity (including time of flowering) were found on linkage group K (Lee et al. 1996). The observed QTL accounted for 26.2–31.2% of phenotypic variance. An F_2 population of "A81-356022 × PI468916" was used to identify a QTL associated with the trait "reproductive period" (Keim et al. 1990). QTL for "reproductive period" were also found on the linkage groups M, C1, C2, F, L and J using RIL populations of "Minsoy × Noir1", "Minsoy × Archer" and "Noir1 × Archer", respectively (Orf et al. 1999). Taken these findings together, they suggest that the putative QTLs for maturity traits (including those for flowering time) are population-specific. Different genomic regions (linkage groups) seem to control the same trait (s) in different mapping populations.

Various QTLs underlying the period of a given reproductive growth stage have been mapped on different soybean linkage groups (Shoemaker et al. 1995; Tasma et al. 2001; Mansur et al. 1996; Cregan et al. 1999). QTLs affecting the eight reproductive

Table 1 Criteria for the reproductive growth stages R1 to R8 of soybean used in this study (according to Fehr and Caviness 1977; McWilliams et al. 1999)

No.	Name	Description
R1	Beginning bloom	At least one flower is located on the plant at any node on the main stem
R2	Full bloom	An open flower is seen at one of the two top nodes of the main stem
R3	Beginning pod	A pod on the upper four nodes is 3/16 inch long (=0.5 cm)
R4	Full pod	A pod at least 3/4 inch long (=2.0 cm) on at least one of the four upper nodes of the main stem
R5	Beginning seed	The seed at least 1/8 inch long (=0.33 cm) in one of the pods on one of the four upper nodes of the main stem
R6	Full seed	A pod containing a green seed that fills the pod cavity on at least one of the four top nodes on the main stem
R7	Beginning maturity	One normal pod on the main stem which obtains the mature color (brown or tan)
R8	Full mature	95% of the pods have reached their mature color

growth stages (Table 1) were not mapped simultaneously. Moreover, QTL–environment interactions remain to be elucidated. For example, the stability of QTLs associated with "maturity date" may alter in different environments.

In this lab, a RIL population, developed from a cross between an American semi-dwarf cultivar (cv. "Charleston") and a Chinese line with a short growth stage (cv. "Dongnong 594"), was used to locate QTL. Analysis of QTLs underlying all eight reproductive growth stages was performed with soybeans growing in three different environments for 2 years. Main-effect QTL, epistatic interactions and QTL–environment interactions were analyzed to reveal the genetic basis for reproductive growth and "maturity date" of soybean.

Materials and methods

The experimental RIL population used in this study consisted of 153 lines. The RIL population was developed using single-seed descents in F_2 and multi-seed descents from F_3 to F_{10} (Chen et al. 2005). The parents were the American semi-dwarf cultivar "Charleston" (kindly provided by Dr. Randall L. Nelson, Illinois University of USA), and "Dongnong 594" (developed by the Northeast Agriculture University of China), a cultivar with a short growth stage and protein-rich grains (Chen et al. 2005).

In 2004 and 2005, the RIL population and their parents were grown at a field site of Xiangfang Research Station (Northeast Agricultural University, Harbin, China). Soybeans were planted on May 3rd 2004 and May 4th 2005, respectively. Randomized blocks with a row of 3 m and a space of 6 cm between two plants were designed. In 2005, the RIL population and their parents were also grown in pots (without bottom) in a courtyard of the Northeast Agricultural University in Harbin (the diameter of the pots was 50 cm and the space between each plant was 6 cm). Trait data from plants grown at the field site and in pots were analyzed in a similar way: five plants from each line were randomly selected and their growth stage was daily monitored. Water of the field site was supplied form rain, and the pot in the courtyard was watered during the seed germination. No herbicide was used in this study. The day length criterion during growth period was 12–16 h. The date of each of the eight R stages (growth period) was determined for all lines using the average date from five plants per line. The criteria for the R stages are presented in Table 1 (Fehr and Caviness 1977; McWilliams et al. 1999).

One hundred and sixty-one simple sequences repeat (SSR) DNA markers were used to genotype the population. A linkage map spanning 1913.5 cM of the soybean genome and the average distance of 11.89 cM between adjacent markers, which was constructed by the Mapmaker/Exp V3.0 program (Lincoln et al. 1992). The SSR markers integrated into 20 linkage groups, named NEAUSRI-GMS, and the genetic map was constructed corresponding to the Cregan's genetic map with the same SSR primer pairs (Chen et al. 2005; Cregan et al. 1999).

QTL analysis was conducted by using the QTLMapper V2.0 program based on a mixed model approach with composite interval mapping module (Wang et al. 1999). The days from emergence to a given reproductive growth stage (Table 1) were used as input data, while different years were regarded as an environment factor. A threshold of $P \leq 0.005$ was considered as significant to define main effect QTL, epistatic QTL pairs, and QTL–environment (Q × E) interactions. Contribution rates (R^2) were estimated as percentage of variance explained by each locus (or epistatic pair) in relation to total phenotypic variance.

Result

Phenotypic variation and correlation

The heritability of eight reproductive stages was more than 99.9% (Wang et al. 1963). Phenotypic values (i.e. days required to reach the reproductive growth stages R1 to R8) in different environments are presented in Table 2. Phenotypic value of two parents appeared significantly different in three environments. As expected, the data from the RIL population varied at different reproductive growth stages and in different environments, but no significant trends were observed. All skew and kurtosis values (Table 2) were less than 1.0 in all environments, suggesting that the segregation of the eight traits was normally distributed. The correlation of R1 to R8 in different environment was

Table 2 Statistical analyses of eight traits (days required to reach the indicated growth stages) for the parents (Charleston and Dongnong 594) and the derived RIL population in three different environments

Trait	Environment	Charleston Dongnong 594		RIL Population			
		Mean		Mean	Variation range	Skew	Kurtosis
Days to R1	2004 field	53	45	50.37	40.00–66.00	−0.34	0.05
	2005 field	57.8	43.4	49.61	32.60–61.60	0.24	−0.26
	2005 pots	56.6	34.2	46.74	33.00–59.80	−0.21	−0.58
Days to R2	2004 field	59	52	56.19	48.00–74.00	−0.40	0.83
	2005 field	64.8	52.6	56.59	47.00–68.00	−0.45	−0.84
	2005 pots	58.6	41.2	50.57	35.20–66.00	0.32	0.21
Days to R3	2004 field	71	62	66.94	51.60–89.00	−0.18	−0.54
	2005 field	75	56.6	64.74	51.60–78.00	−0.36	−0.88
	2005 pots	75	47.4	61.82	41.80–76.80	0.24	−0.59
Days to R4	2004 field	77	70.6	73.75	56.00–96.00	0.19	−0.87
	2005 field	77	67.6	70.33	58.00–87.00	−0.45	−0.56
	2005 pots	78	50.4	66.88	43.40–80.00	0.26	−0.56
Days to R5	2004 field	84	76	79.60	63.00–102.00	−0.23	−0.94
	2005 field	85	71	75.20	59.40–88.00	−0.46	−0.51
	2005 pots	81	58.8	71.80	47.80–86.00	0.28	−0.49
Days to R6	2004 field	92	88	90.58	71.00–115.00	−0.30	−0.74
	2005 field	95	84	87.06	69.80–109.00	−0.47	−0.05
	2005 pots	95	78	82.35	58.00–97.00	0.47	0.54
Days to R7	2004 field	99	101	103.56	82.00–132.00	0.35	−0.26
	2005 field	127	113	112.03	99.00–132.00	0.78	0.54
	2005 pots	125	102	112.20	79.00–133.00	−0.62	0.61
Days to R8	2004 field	131	121	128.87	109.00–145.00	−0.54	−0.02
	2005 field	140	123	126.14	108.00–155.00	0.58	−0.67
	2005 pots	140	122	125.36	89.40–148.00	−0.54	0.65

Field site at Xiangfang Research Station in the years 2004 and 2005; pots in the courtyard of Northeast Agricultural University, Harbin in the year 2005

not similar (Table 3). Trend of correlation of R1 to R8 was higher and higher.

Main effect QTL

Eleven main effects QTLs were detected underlying trait R1, the number of days from emergence to reach growth stage R1. Three of these QTLs, located on the linkage groups A1 (Satt164–Satt042) and G (Satt288–Satt012, Satt394–Satt570), had no significant Q × E interaction. The total contribution rate of the three QTLs accounted for 7.51% of phenotypic variance. The remaining eight QTLs displayed significant Q × E interactions (Table 4 and Fig. 1). Six QTLs were found to positively influence the trait R2 (number of days from emergence to reach growth stage R2). One of them, located on the linkage group D1a (Satt402–Satt267), displayed no significant Q × E interaction. This QTL accounted for 5% of phenotypic variance. Ten QTLs were detected underlying trait R3. Two QTLs among them had no Q × E interactions and were located on the linkage groups A2 (Satt468–Satt327) and C2 (Sat_120–Sat_103). These two QTLs explained about 4.16% of phenotypic variance. Five QTLs were detected for trait R4 and only one of them, located on linkage group O (Satt173–Satt581), had no Q × E interaction. This QTL accounted for 11.92% of phenotypic variance. Seven QTLs, located on four different linkage

Table 3 The correlation of R1 to R8 in different environments

		2004f	2005F	2005P			2004F	2005F	2005P
R1	2004f	1			R5	2004F	1		
	2005F	−0.325**	1			2005F	−0.012	1	
	2005P	−0.067	0.122**	1		2005P	0.888*	0.028	1
R2	2004F	1			R6	2004F	1		
	2005F	−0.25**	1			2005F	0.14**	1	
	2005P	0.224**	0.025	1		2005P	0.945**	0.155**	1
R3	2004F	1			R7	2004F	1		
	2005F	−0.144**	1			2005F	0.419**	1	
	2005P	0.754**	−0.048	1		2005P	0.979**	0.421**	1
R4	2004F	1			R8	2004F	1		
	2005F	−0.74	1			2005F	0.544**	1	
	2005P	0.873**	−0.016	1		2005P	0.986**	0.544**	1

* Significance level reaches 0.05; ** significance level reaches 0.01

groups, were identified to be associated with trait R5. Three of them were located on the linkage group D1a, and only one of them on linkage group F (Satt252–Satt269) had no Q × E interaction. For trait R6, six QTLs were identified. One of them (Satt094–Satt556) on linkage group B2 had no Q × E interaction. Its contribution rate was 2.48% of phenotypic variance. Four QTLs were found for trait R7. Three of them located on linkage group A2 (Satt468–Satt327), D1a (Satt402–Satt267) and O (Satt173–Satt581) displayed no Q × E interactions and could explain 20.79% of phenotypic variance. Five QTL underlying trait R8 were identified, but all displayed Q × E interactions. One of them, located on linkage group A1, exhibited a relatively weak Q × E interaction. Taken together, 54 main QTL associated with reproductive growth stages were found in this study. The QTL on the marker interval Satt173–Satt581 on the linkage group O was associated with all eight traits. The QTL on the interval Satt402–Satt267 on the linkage group D1a was found for the traits R1 to R5 and R7 (Table 4).

QTL pairs with epistatic effects

Eight QTL pairs affecting trait R1 displayed significant epistatic effects (values between −1.65% and 0.58%). Five main effect QTLs were involved in these eight QTL pairs, whereas two QTL pairs did not display significant epistasis (Table 5). Seven epistatic QTL pairs were associated with trait R2. Three showed negative epistasis and four pairs showed positive epistasis. Their contribution rates varied from 0.07% to 14.52%. Epistatic interactions were found for 11 QTL pairs associated with trait R3 and nine pairs displayed positive epistasis. For trait R4, six QTL pairs showed negative epistasis. Interestingly, QTLs mapped on linkage group D1a were involved in five epistatic interactions. Seven QTL pairs were found for trait R5. Five pairs displayed negative epistasis and QTLs located on linkage group D1a were present in four of them. Three QTL pairs were associated with trait R6, but only one showed positive epistasis. Epistasis analysis for trait R7 revealed nine QTL pairs and three of them displayed significant positive epistasis. QTLs located on the linkage group D1a contributed to four epistatic interactions. Five epistatic QTL pairs were associated with trait R8. Four QTL pairs displayed positive epistasis. For three of the four pairs, the effect of the epistatic interaction was high (about 3%). QTLs from the linkage group D1a were involved in these three epistatic interactions. Taken together, 56 pairs of QTL exhibited epistatic effects in our study.

Discussion

QTLs associated with all eight reproductive growth stages of soybean were mapped in this study. QTL

Table 4 Main effect QTLs identified in this study

Trait	Chrom	Interval	LOD	A	Environments			R^2 (%)
					2004 field	2005 field	2005 pots	
Days to R1	A1	Satt164–Satt042	4.39	−1.00	–	–	–	2.12
	A2	Satt468–Satt327	6.67	1.22	–	–	0.78*	3.21
	B1	Satt251–Satt229	2.80	1.42	–	−0.85**	1.41**	4.32
	C2	Satt243–Satt341	2.50	1.81	–	1.36**	−0.82**	6.24
	C2	Satt335–Sat_120	2.51	1.36	–	0.94**	−0.59	3.12
	D1a	Satt182–Satt495	5.71	1.25	–	−0.36	0.13	3.11
	D1a	Satt402–Satt267	4.76	2.03	–	0.28	−0.17	7.00
	D1b	Satt266–Satt157	4.12	1.74	–	−0.84**	1.04**	5.00
	G	Satt288–Satt012	4.71	0.98	–	–	–	2.23
	G	Satt394–Satt570	5.43	1.24	–	–	–	3.16
	O	Satt173–Satt581	8.78	1.68	0.15	0.29	−0.44	5.15
Days to R2	D1a	Satt182–Satt495	8.28	1.32	0.31	−0.82**	0.51	3.25
	D1a	Satt495–Satt584	6.41	1.18	0.15	−0.38	0.23	3.33
	D1a	Satt402–Satt267	3.00	1.56	–	–	–	5.00
	D1a	Satt203–Satt198	3.21	1.79	−0.11	−0.59*	0.70**	6.00
	D1b	Satt266–Satt157	2.93	1.59	0.06	−0.64*	0.58*	5.34
	O	Satt173–Satt581	5.78	2.16	−0.15	0.67*	−0.52	9.42
Days to R3	A1	Satt164–Satt042	6.38	−1.67	−0.39	0.01	0.38	2.14
	A2	Satt468–Satt327	6.01	1.67	–	–	–	2.02
	B1	Satt251–Satt229	2.91	2.27	0.11	−0.98*	0.87*	4.04
	C2	Satt335–Sat_120	4.74	2.37	−0.42	0.89**	−0.47	5.03
	C2	Sat_120–Sat_103	5.56	1.61	–	–	–	2.02
	D1a	Satt182–Satt495	8.28	2.04	0.90*		−0.90*	4.00
	D1a	Satt495–Satt584	5.61	1.62	1.20**	−0.75	−0.46	2.00
	D1a	Satt402–Satt267	4.62	2.71	1.12**	−0.61	−0.51	6.16
	D1b	Satt428–Satt266	9.71	2.17	1.49**	−1.15**	−0.34	4.00
	O	Satt173–Satt581	6.60	3.33	0.13	−0.99*	0.86*	10.0
Days to R4	C1	Satt195–Sat_042	4.64	−1.15	−0.47	0.514	−0.05	1.33
	D1a	Satt402–Satt267	3.04	2.3	0.71	−0.375	−0.34	5.33
	D1a	Satt203–Satt198	12.4	2.63	0.26	−0.61	0.35	6.97
	D1b	Satt428–Satt266	3.28	2.65	1.43**	−1.86	0.43	7.07
	O	Satt173–Satt581	5.57	3.44	–	–	–	11.92
Days to R5	B1	Satt251–Satt229	5.53	1.51	−0.44	−0.82*	1.26**	2.01
	D1a	Satt182–Satt495	6.8	1.85	1.02*	−1.02*	–	3.03
	D1a	Satt495–Satt584	7.35	2	0.79	−0.67	−0.11	3.53
	D1a	Satt402–Satt267	14.4	2.53	0.03	−0.27	0.24	5.64
	D1b	Satt428–Satt266	2.70	2.56	1.13**	−1.25**	0.12	5.81
	F	Satt252–Satt269	4.46	1.44	–	–	–	1.84
	O	Satt173–Satt581	5.25	3.33	−0.16	0.19	−0.03	9.83
Days to R6	B2	Satt094–Satt556	5.5	−1.65	–	–	–	2.48
	C1	Satt195–Sat_042	5.91	−1.35	−0.63	0.45	0.17	1.67
	D1a	Satt495–Satt584	4.69	1.67	0.51	−0.35	−0.16	2.54
	D1a	Satt203–Satt198	8.23	2.47	−0.03	−0.07	0.09	5.58

Table 4 continued

Trait	Chrom	Interval	LOD	A	Environments			R^2 (%)
					2004 field	2005 field	2005 pots	
	D1b	Satt428–Satt266	8.6	2.2	1.69**	−1.29**	−0.39	4.41
	O	Satt173–Satt581	7.48	3.99	0.02	0.55	−0.56	14.56
Days to R7	A2	Satt468–Satt327	5.63	2.1	–	–	–	4.16
	B1	Satt251–Satt229	4.5	1.67	−0.15	−1.18*	1.33**	2.62
	D1a	Satt402–Satt267	8.27	2.17	–	–	–	4.45
	O	Satt173–Satt581	5.22	3.6	–	–	–	12.18
Days to R8	A1	Satt390–Satt218	2.53	1.9	0.02	0.025	−0.04	2.67
	B1	Satt251–Satt229	5.34	2.08	1.05	−1.96**	0.91	3.18
	D1a	Satt495–Satt584	4.94	1.92	0.29	−0.42	0.13	2.71
	D1a	Satt203–Satt198	2.84	3.09	0.27	0.41	−0.68	7.04
	O	Satt173–Satt581	8.27	5.07	−0.50	0.34	0.16	18.93

* Significance level reaches 0.05; ** significance level reaches 0.01

analysis for these traits was concurrently performed in three environments. The interval Satt173–Satt581 on linkage group O contained a QTL site that controlled all eight traits examined in our study. This consistency strongly suggests that eight reproductive growth stages may be controlled by same gene (or gene cluster) in the interval Satt173–Satt581. Hence, this region may contain important gene(s) that control plant development and seed maturity during all reproductive stages.

Another QTL associated with the traits R1 and R3 was located on linkage group C2. This QTL is near the region of a previously reported QTL affecting the traits R1 to R7 (Keim et al. 1990; Mansur et al. 1996; Tasma et al. 2001; Yamanaka et al. 2005).

The QTL associated with trait R2 were almost all located on the linkage group D1 (D1a + D1b) and only one was located on the linkage group O. Moreover, at least three QTLs on the linkage group D1 were identified for the traits R4, R6 and R8. Hence, linkage group D1 seems to contain a set of important genes involved in control of flowering time, formation of pods and seed maturity.

This paper underlines the importance of QTL analysis in different environments. For trait R3, mapped two QTLs were located on linkage group C2, i.e. near the region that has been reported by Tasma et al. (2001). One of them, QTL (Sat_120–Sat_103) had no Q × E interaction. This QTL seems to be more reliable than other QTL that have been only detected in a single environment. In this study also identified a QTL with no Q × E interaction associated with trait R4 on linkage O (Satt173–Satt581). For trait R5, a QTL on linkage group F (Satt252–Satt269), also displayed no Q × E interaction. Finally, in this study found three QTL without Q × E interaction for trait R7 and these loci were not identical to those reported previously by Tasma et al. (2001). The result suggested that effects from a single environment might negatively act on QTL identification. In contrast to these few QTL lacking Q × E interactions, many QTLs with Q × E interactions were found in this study. Future work with "high density linkage map" and "chromosome segment substitution lines" will be required to test whether these putative QTL have significant effects on reproductive growth stages.

To the E and J gene, the interval (Satt173–Satt581) on O linkage may was the E2 gene (Summerfield et al. 1998). The interval (Satt335–Sat_120) and (Sat_120–Sat_103) on C2 linkage were near the E1 gene (Yamanaka et al. 2001). No region was found has relation with J gene (http://bioinformatics.siu.edu). The interval (Satt468–Satt327) on A2 was found has relation with oil QTL 13-2(Specht et al. 2001). The interval (Satt182–Satt495) on D1a has relation with QTL R3 2-3 (Tasma et al. 2001, http://bioinformatics.siu.edu).

The findings of this work also demonstrate that certain QTL pairs display strong epistatic effects. To our knowledge, epistatic QTL underlying reproductive growth stages of soybean have not been reported so far. Interestingly, certain epistatic QTL pairs were

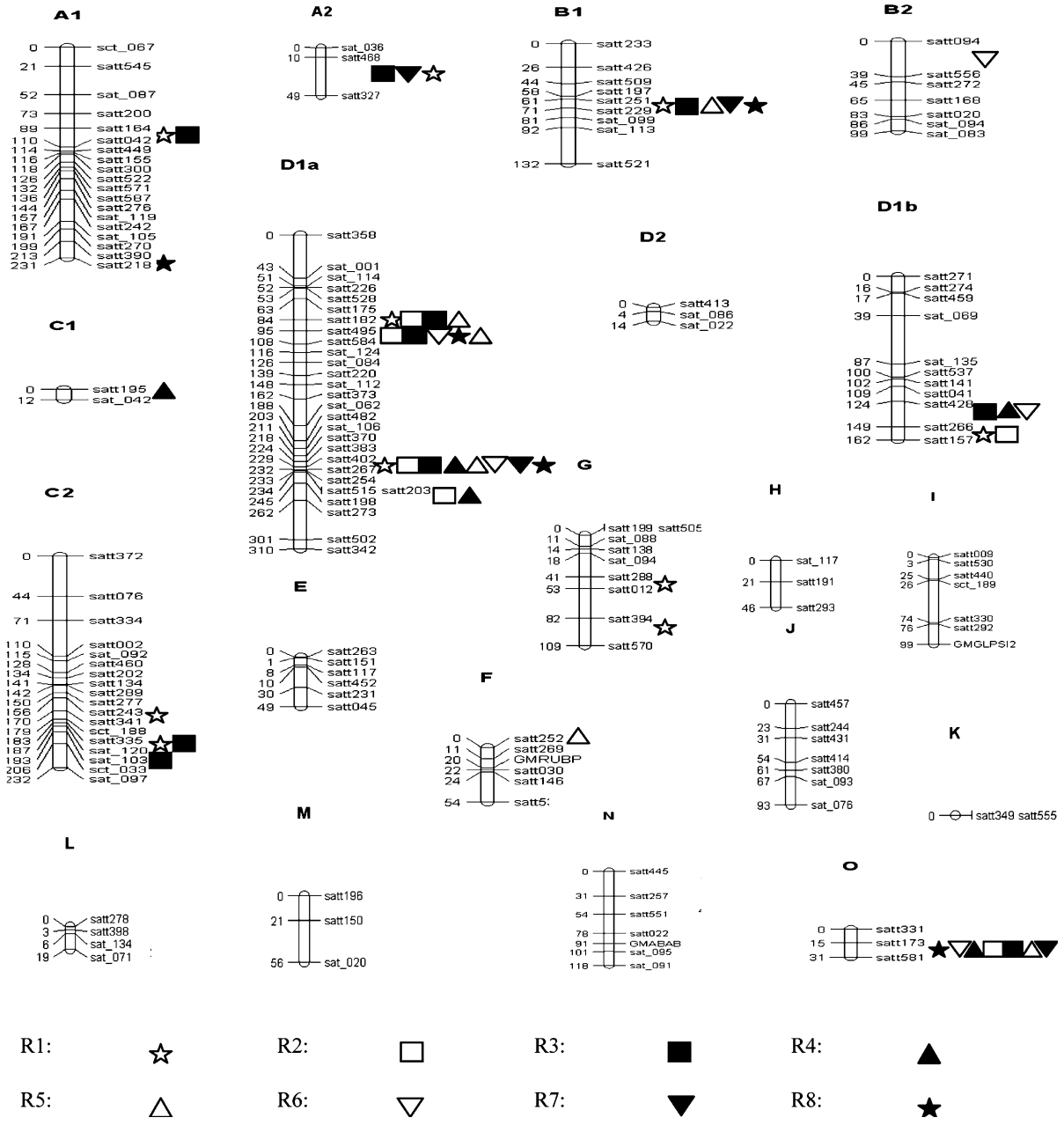

Fig. 1 Chromosomal locations of regions associated with reproductive stages of soybean

detected for all investigated traits. Many QTLs involved in epistatic interactions were mapped on linkage group D1b. It is suggested that the QTLs located on D1b are key QTLs with strong effects on plant developmental processes during reproductive growth stages.

Taken together, this study resulted in identification of 54 main QTLs and 56 epistatic QTL pairs affecting the period required to reach a specific growth stage during flowering, pod formation and seed ripening. The obtained form deferred the basis for future attempts to modulate the structure of soybean growth

Table 5 QTL pairs with epistatic effects identified in this study

Trait	Chrom	Interval	Chrom	Interval	LOD	AA	R^2 (%)
Days to R1	A1	Satt164–Satt042	B1	Satt251–Satt229	2.82	0.48	0.35
	A1	Satt300–Satt522	A2	Satt173–Satt581	9.02	0.58	0.51
	A2	Satt468–Satt327	D1a	Satt175–Satt182	7.85	0.10	0.02
	B1	Satt229–Sat_099	A2	Satt244–Satt431	5.70	−1.65**	4.15
	C2	Sct_188–Satt335	G	Satt_094–Satt288	3.43	−1.00**	1.53
	D1a	Satt358–Sat_001	D1b	Satt266–Satt157	5.00	−0.78	0.92
	D1a	Satt182–Satt495	D1a	Satt203–Satt198	8.80	−0.55	0.46
	D1a	Satt402–Satt267	A1	Satt196–Satt150	4.56	−0.36	0.2
Days to R2	A1	Sat_119–Satt242	D1a	Satt182–Satt495	8.82	−0.51*	0.34
	C1	Satt195–Sat_042	D1a	Satt182–Satt495	2.54	−1.01**	1.38
	C2	Sat_103–Sct_033	A1	Satt457–Satt244	9.29	1.55**	3.21
	D1a	Sat_001–Sat_114	D1b	Satt266–Satt157	3.51	−0.65	0.57
	D1a	Satt383–Satt402	G	Satt288–Satt012	2.84	0.64**	0.55
	D1a	Satt203–Satt198	A2	Satt150–Sat_020	9.39	3.29*	14.52
	E	Satt151–Satt117	A2	Satt173–Satt581	5.32	0.23	0.07
Days to R3	A1	Satt164–Satt042	A2	Satt191–Satt293	8.39	0.01	0.01
	A1	Satt300–Satt522	A2	Satt173–Satt581	5.97	0.72	0.35
	A1	Sat_119–Satt242	C1	Satt195–Sat_042	7.18	0.72**	0.35
	A1	Satt390–Satt218	A2	Satt468–Satt327	3.46	−0.98**	0.65
	B1	Satt229–Sat_099	D1a	Satt182–Satt495	3.82	0.87**	0.51
	C2	Satt335–Sat_120	G	Satt288–Satt012	3.74	0.35	0.08
	D1a	Satt358–Sat_001	D1a	Satt383–Satt402	4.69	−1.79**	2.14
	D1a	Satt182–Satt495	D1a	Satt203–Satt198	2.59	−0.25	0.04
	D1a	Satt182–Satt495	A1	Satt196–Satt150	2.75	0.34	0.08
	D1a	Satt584–Sat_124	D1b	Satt266–Satt157	2.84	0.67	0.3
	D1b	Satt428–Satt266	E	Satt151–Satt117	7.8	0.06	0.2
Days to R4	C1	Satt195–Sat_042	D1a	Satt182–Satt495	2.83	−1.9**	0.31
	D1a	Sat_001–Sat_114	D1b	Satt266–Satt157	2.66	−0.99**	0.84
	D1a	Satt373–Sat_062	D1a	Satt370–Satt383	6.65	−1.41	1.63
	D1a	Satt402–Satt267	G	Satt_094–Satt288	2.91	−0.49	0.21
	D1a	Satt203–Satt198	A2	Satt150–Sat_020	8.56	−0.17	0.03
	E	Satt151–Satt117	A2	Satt173–Satt581	5.32	−0.20	0.04
Days to R5	A1	Sat_119–Satt242	D1a	Satt495–Satt584	9.07	0.00	0.1
	B1	Satt229–Sat_099	A2	Satt244–Satt431	16.8	−2.7**	5.39
	C1	Satt195–Sat_042	D1a	Satt182–Satt495	2.96	−1.72**	2.18
	D1a	Sat_001–Sat_114	D1b	Satt266–Satt157	2.56	−0.83	0.51
	D1a	Sat_112–Satt373	F	Satt252–Satt269	4.37	3.25**	7.82
	D1a	Satt402–Satt267	G	Satt_094–Satt288	2.42	−0.38	0.11
	E	Satt151–Satt117	A2	Satt173–Satt581	4.86	−0.13	0.01
Days to R6	A1	Satt571–Satt587	D1a	Satt203–Satt198	6.7	−1.14**	1.78
	A1	Satt276–Sat_119	D2	Sat_086–Sat_022	6.87	1.61**	3.56
	D1a	Satt495–Satt584	G	Satt_094–Satt288	8.24	−1.46**	2.94

Table 5 continued

Trait	Chrom	Interval	Chrom	Interval	LOD	AA	R^2 (%)
Days to R7	A1	Satt449–Satt155	A1	Satt390–Satt218	2.58	−1**	0.56
	A1	Satt390–Satt218	A2	Satt468–Satt327	2.68	−0.53	0.16
	B1	Satt251–Satt229	D1a	Satt175–Satt182	2.74	3.31**	6.24
	D1a	Satt584–Sat_124	D1b	Satt266–Satt157	2.72	−0.92	0.49
	D1a	Satt402–Satt267	G	Satt_094–Satt288	8.76	−0.23	0.03
	D1a	Satt203–Satt198	G	Satt012–Satt394	8.78	−1.36**	1.06
	D1b	Sat_135–Satt537	A1	Satt278–Satt398	9.21	2.21**	2.8
	D1b	Satt266–Satt157	A2	Satt173–Satt581	5.80	−1.62	1.5
	G	Satt394–Satt570	A1	Satt196–Satt150	8.47	1.27**	0.92
Days to R8	A1	Sat_119–Satt242	D1a	Satt203–Satt198	2.80	0.10	0.01
	A1	Satt390-Satt218	D1a	Satt495–Satt584	6.19	3.17**	6.06
	B1	Satt251–Satt229	D1a	Satt175–Satt182	9.1	3.26**	6.42
	D1a	Sat_112–Satt373	F	Satt252–Satt269	2.78	3.44**	7.13
	G	Satt288–Satt012	A2	Satt173–Satt581	7.32	−1.07*	0.69

* Significance level reaches 0.05; ** significance level reaches 0.01

stages by marker-assisted selection and pyramiding QTL analysis.

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