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ORIGINAL PAPER

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Quantitative trait loci analysis for the developmental behavior of Soybean (*Glycine max* L. Merr.)

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Abstract Quantitative trait loci (QTLs) identified so far in soybean were mainly derived in the final stage of plant development, which did not apply to the exploitation of genetic effects that were expressed during a specific developmental stage. Thus, the aim of this study was to identify conditional QTLs associated with yield traits at a specific developmental interval of soybean plant. The 143 recombinant inbred lines developed from the cross of soybean cultivars 'Charleston' and 'Dongnong 594' were used for the developmental QTLs analysis of pod number in the main stem and plant height by composite interval mapping method combined with mixed genetic model. The results indicated that the number and type of QTLs and their genetic effects for the two agronomic traits were different in a series of measuring stages. A total of 10 unconditional QTLs in 6 linkage groups and 5 conditional QTLs in 3 linkage groups were identified for the pod number of the main stem, while 13 unconditional QTLs in 7 linkage groups and 12 conditional QTLs in 6 linkage groups were identified for plant height. Many QTLs that were detected in the early stages were different from those detected at the later stages. Some QTLs existed only at one stage and others existed across two or three stages. Five marker intervals (satt509-satt251, sat_099-sat_113, sat_113-OPAW19_4, satt457-OPC10_85, sat_095-OPBA08_5) were proven to be associated both with the development of pod number in the main stem and the development of plant height. The present study suggested that the development of pods and plant height in soybean were governed by time-dependent gene expression.

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Introduction

Pod number per plant and plant height are important agronomic traits underlying soybean seed yield. Both traits are quantitatively inherited or in certain cases qualitatively inherited. Understanding the inheritance of these traits during plant development will lead to the elucidation of the mechanisms that controlled the formation of soybean yield. Moreover, the discovery of molecular QTL and QTL-assisted selection for these quantitative straits would immensely stimulate breeding procedure because traditional soybean breeding for quantitative traits was usually slow and difficult. Marker assisted selection (MAS) could potentially improve selection effect of yield traits that have low heritability by using markers with high heritability. In the past decades, many studies were focused on the inherence of pod number and plant height in soybean. Some reports described that the pod number of the main stem and plant height had lower inheritability than those of reproductive traits, however, they possessed a higher inheritability than those of quality traits (Wang et al. 1992). The broad-sense heritability of pod number of the main stem was estimated between 22 and 32.06% in F_2 and F_3 generations (Yang et al. 1975). Additive, dominant and epistatic effects were all found to be important for the formation of pods (Chen et al. 1984). In contrast to the pod number of the main stem, the broad heritability of plant height was higher, ranging between 45 and 75%. Additive gene effect dominated the inheritance

pattern of plant height (Wang et al. 1992; Yang et al. 1995). Additionally, the correlation coefficient between plant height and plant yield was relatively high with $r = 0.40$ ($P < 0.05$) than between pod number and plant yield (Wang 1963), and the correlation coefficient between pod number of the main stem and plant height was 0.60–0.75 ($P < 0.05$) (Wang et al. 1992).

Recently, PCR-based molecular markers, such as random amplified polymorphic DNA (RAPD) makers and simple sequence repeat (SSR) markers were apparently emphasized in marker-associated selection and genetic diversity analyses because they possess the potential of reducing the time, effort and expense required for molecular mapping. RAPD markers have been used for a variety of purposes including the construction of genetic linkage maps (Reiter et al. 1992), gene tagging, identification of cultivars (Nybom 1994), assessment of genetic variation in populations and species (Nesbitt et al. 1995) and to identify the desired genotypes during selection. However, there was some loss of information when RAPD markers were used because they are dominant rather than codominant (Sun et al. 2001). SSR marker is ubiquitous in eukaryotic genomes and its utility has been greatly facilitated by recent advances in polymerase chain reaction (PCR) technology. The high level of polymorphism, relative to restriction fragment length polymorphisms (RFLPs) and RAPDs, combined with a high-interspersion rate make them an abundant source of genetic markers (Gupta et al. 1999). The codominant, single-locus SSR markers are distributed throughout the soybean genome and the frequency of occurrence is one SSR for every 10 kb, though some clustering of markers is observed (Cregan et al. 1999). SSR analysis possesses potential advantages of reliability, reproducibility, discrimination, standardization and cost-effectiveness over RFLP analyses (Senior and Heun 1993; Smith et al. 1997). Recently, the development of an integrated linkage map of soybean (Cregan et al. 1999) based on SSR markers greatly facilitates QTL mapping.

A number of QTLs with agronomic traits have been identified in soybean, including morphological traits (Keim et al. 1990; Mansur et al. 1996; Lee et al. 1996; Zhang et al. 2004), reproductive traits (Keim et al. 1990; Mansur et al. 1996), seed quality traits (Mansur et al. 1993, 1996; Qiu et al. 1999), disease resistant traits (Concibido et al. 1994; Webb et al. 1995; Qiu et al. 1999; Yuan et al. 2002) and yield traits (Chung et al. 2003; Wang et al. 2004). Lee et al. (1996) detected a major QTL for Dt1 associated with plant height on linkage group L. This locus was also associated with lodging and explained 67.7% of the total variation for plant height and 56.4% of the total variation for lodging. Using recombinant inbred lines (RILs) from a cross of cultivars Noir and Minsoy, Mansur et al. (1996) determined a QTL for plant height, explaining 31.6% of the total variation. Zhang et al. (2004) detected eight QTLs associated with plant height. Three of these loci are located on C2 linkage group and explained more than 20% of the total variation. Two of them were also associated with pod number per node.

The QTLs that were identified so far in soybean were gained mainly based on phenotypic value at one specific developmental stage of plant, especially at the final stage of plant development. However, these QTLs could not account for the net genetic effects in a specific time interval of plant development, which are referred as the essential components for quantitative traits (Yan et al. 1998). According to the rational of developmental genetics, genes are expressed selectively at different growth stages. The conventional statistical results revealed that the development of morphological traits occurs through the actions and interactions of any genes that might behave differentially during growth periods, and that gene expression is modified by interaction with other genes and by the environment (Atchley and Zhu 1997). Zhu (1995) deduced a genetic model to evaluate net genetic effects of quantitative traits at a specific developmental stage. This genetic effect is referred to as conditional genetic effect and the QTL that is detected at a specific growth stage with conditional genetic effect is referred to as conditional QTL.

The association of developmental behavior of quantitative traits with molecular markers had been well studied in rice and cotton (Yan et al. 1998; Wu et al. 1999; Ye et al. 2003). However, such a study has not yet been carried out on soybean. The objectives of the presented study were to investigate the developmental behavior of pod numbers in the main stem and plant height using a soybean RILs population and to identify the conditional QTLs and unconditional QTLs for the two typical quantitative traits.

Materials and methods

Plant materials

A population of 143 $F_{2:10}$ RI lines derived from a cross between an USA cultivar Charleston (provided by Dr. Randall L. Nelson, Illinois University of USA) and a local cultivar Dongnong 594 (developed by Northeast Agriculture University of China) were used in the experiment. The basic characteristics of the two parents were listed in Table 1. The RIL population and their parents were grown in a randomized complete design at Harbin (45°N, fine-mesic chernozen soil) of China on May 3, 2004, with a row 3 m long and a space of 6 cm between two plants. There were two replicates in time, with five measurements per plant for pod number and six measurements per plant for plant height, for a total of 22×5 phenotypic observations per genotype (Five plants per genotype). Plant height (from the surface of the soil to the tip of the plant) was measured every 10 days for the central five plants of each line until nearly stopping growth. Pod numbers of the main stem were measured every 10 days for the same five plants from 15 days after flowering until the stage of bearing pods.

Table 1 Comparison of basic characters between parents

Parents	Pod setting habit	Flower color	Days of maturity	Plant height (cm)	100-seed weight (g)	Pod numbers
Charleston	Definite	Purple	135	59.56	13.43	41.20
Dongnong594	Semi-definite	White	120	74.56	17.80	52.75

RAPD analysis

The total DNA of each line was isolated from freeze-dried leaf tissue with cetyltrimethylammonium bromide (CTAB) method. RAPD analysis was done with random decamer primers obtained from Operon Technologies Inc., Alameda, CA, USA. PCR reaction contains 30 ng of genomic DNA, 300 nM of random primer, 1× reaction buffer, 3 mM $MgCl_2$, 0.4 mM dNTP, 0.6 U *Taq* DNA polymerase in 20 µl solution. DNA amplification was done using a thermal cycle profile of 4 min at 94°C followed by 41 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 1 min with a final extension of 10 min at 72°C. The PCR products were separated on a 1.5% (w/v) agarose gel and stained with ethidium bromide.

SSR analysis

Simple sequence repeat marker analysis was done with the primers developed by P.B. Cregan (USDA-ARS, Beltsville, MD, USA). The PCR amplification was performed with 15 µl of reaction mixture, containing 50 ng of genomic DNA, 1× reaction buffer, 3 mM $MgCl_2$, 0.4 mM dNTP, 300 nM each primer, 0.6 U Taq polymerase. The initial step of thermal cycle profile was at 94°C for 5 min followed by 36 cycles of 94°C for 40 s, 47°C for 40 s and 68°C for 40 s with a final extension for 5 min at 72°C. The PCR products were then separated on 6.0% (w/v) polyacrylamide gels and visualized by silver staining.

Data analysis

Mapmaker/EXP version 3.0 was used for genetic linkage analysis and the analysis of conditional and unconditional QTLs was performed using QTL Cartographer with composite interval mapping (CIM) module (Basten et al. 1996). Window size was 5 and 10 cM, respectively. The walk speed was 1 cM. The threshold of LOD score for evaluating the statistical significance of QTL effects were determined by 1,000 permutations using the Zmapqtl program in QTL Cartographer (Churchill and Doerge 1994). An LOD value corresponding to an experiment-wise threshold of $\alpha = 0.05$ was used to declare a QTL as significant. The estimate of the QTL position was the point of maximum LOD score in the region under consideration.

Unconditional QTL was assessed based on the phenotypic value at time $t[y_{(t)}]$ (Zeng 1993), in which the genetic effect was the accumulation of the individual gene effect from the initial time of plant growth to a time point t:

$$y_{j(t)} = \beta_{0(t)} + \beta^*_{(t)} X^*_j + \beta_{(t)} X_{ij} + \varepsilon_{j(t)},$$

where $y_{j(t)}$ is the phenotypic value of the jth individual measured at time t; $\beta_{0(t)}$ is the population mean at time t; $\beta^*_{(t)}$ is the accumulated QTL effect at time t; X^*_j is the coefficient for the QTL effect; $\beta_{i\ (t)}$ is the accumulated effect for the ith marker at time t; X_{ij} is the coefficient for the ith marker effect; $\varepsilon_{j(t)}$ is the residual error of the jth individual at time t.

Conditional phenotypic means $[y(t|t-1)]$ were obtained by mixed model approach in which the genetic effect contributed by the specific developmental stage between time $t-1$ to time t implied a net genetic effect in that stage rather than the genetic effect accumulation from the initial growth in the unconditional QTL (Zhu 1995):

$$y_{j(t|t-1)} = \beta_{0(t|t-1)} + \beta^*_{(t|t-1)} X^*_j + \sum_i \beta_{i(t|t-1)} X_{ij} + \varepsilon_{j(t|t-1)},$$

where $y_{j(t|t-1)}$ is the conditional phenotypic value of the jth individual; $\beta_{0(t|t-1)}$ is the conditional population mean; $\beta^*_{(t|t-1)}$ is the conditional QTL effect; X^*_j is the coefficient for conditional QTL effect; $\beta_{i(t|t-1)}$ is the conditional effect for the ith marker; X_{ij} is the coefficient for the ith marker effect; $\varepsilon_{j(t|t-1)}$ is the conditional residual error of the jth individual.

Conditional QTL analysis was conducted according to CIM along with the statistical method for the analysis of time−independent genetic effects (Zhu 1995).

Results

Phenotypic variation

Phenotypic values for pod number and plant height measured at different growth stages were shown in Table 2. The difference between the two parents for pod number of the main stem and plant height was significant at most measuring stages. The average number of pods in the main stem was quickly elevated from 10.97 at 15 days to 51.07 at 45 days, and then decreased to 41.54 at 55 days due to the abortion of some pods. Across all measuring stages, plant height for the parents and RIL population displayed a consistent increment. The average height of the RIL population increased from 10.11 cm at 30 days after seedling to 81.99 cm at

Table 2 Statistical analyses of two traits for parents and RIL population at different growth stages.

Traits	Stage (date)	Parent mean		RIL population			
		Charleston	Dongnong594	Mean	Var. range	Skew	Kurtosis
Pod number in the main stem	15D	4.65	34.67	10.97 ± 10.27	0.17–38.00	0.98	−0.05
	25D	35.50	49.67	32.17 ± 15.22	9.83–75.00	0.62	−0.37
	35D	62.75	67.50	45.61 ± 14.52	17.17–78.83	0.21	−0.81
	45D	51.17	61.50	51.07 ± 14.56	20.15–104.67	0.13	0.98
	55D	41.20	52.75	41.54 ± 13.32	9.67–72.25	−0.16	−0.37
Plant height	30D	6.73	10.72	10.11 ± 1.22	6.61–12.83	0.20	−0.19
	40D	14.11	18.78	18.88 ± 2.30	13.67–24.78	0.39	−0.01
	50D	22.00	29.11	29.43 ± 3.91	19.33–42.13	0.38	0.44
	60D	34.00	47.22	47.85 ± 6.64	30.11–63.56	−0.11	−0.03
	70D	51.33	64.78	68.82 ± 12.76	35.56–91.44	−0.67	−0.22
	80D	59.56	74.56	81.99 ± 21.10	36.67–113.67	−0.58	−0.72

80 days, including a rapid increment period from 50 to 70 days. The transgressive segregation for pod number of the main stem was more prominent than that for plant height at all measuring stages. In Table 2, both skew and kurtosis values of the two traits were less than 1.0 at all measuring stages, suggesting that the segregation of the two traits fit normal distribution for the two traits.

Construction of the genetic linkage map

To map QTLs for pod number and plant height, 199 molecular markers (including 164 SSR markers and 35 RAPD markers), which exhibited polymorphism between the two parents Charleston and Dongnong 594, were mapped onto 20 linkage groups (Fig. 1). The order of most markers is consistent with Cregan's map (Cregan et al. 1999). This genetic linkage map covered 3,067.28 cM and an average distance between markers was 15.65 cM with the longest distance 48.8 cM and the shortest distance 0.5 cM. The average number of markers on each linkage group was 9.7 with an average length 153.36 cM.

QTL analysis for the pod number of the main stem

A total of 15 QTLs with LOD score greater than 3.4 for the pod number of the main stem were identified and were mapped on seven linkage groups (Table 3, Fig. 1). Ten of them were sorted into unconditional QTLs and five were sorted into conditional QTLs.

Unconditional QTL for the pod number of the main stem

A total of 10 unconditional QTLs were detected at 15, 25, 35 and 45 days, respectively, which responded to a rapid increment of pods at this time zone. Of these QTLs, pnD1a-1 (sat_099–sat_113) and pnD1a-2 (sat_113–OPAW19_400) were detected at two continuous stages. pnD1a-1 and pnD1a-2 on linkage group D1a accounted for more than 30% of the total variation at 45 days, which greatly facilitated the accumulation of pod numbers on plants. However, these QTLs were not detected at the subsequent stages (Table 3). No unconditional QTL was identified at the final measuring stage (55 days).

The unconditional QTLs that significantly affected pod numbers of the main stem varied at different developing stages. QTLs identified before 35 days did not show up after 35 days. None of the QTL was detected for over more than two stages probably due to the temporal expression of different genes that controlled the pod formation on different nodes (Table 3). The total additive effects of QTLs increased from 11.19 at 15 days to 30.00 at 45 days and subsequently decreased to 3.83 at the final stage (data not shown due to the lack of a significant QTL effect at the final stage (LOD score value = 3.0). This was in high coincidence with the phenomenon of the rapid increment of pod number at early stages, following a decrement of pod numbers caused by the abortion of young pods at the later stage.

Conditional QTL for pod number of the main stem

The analysis of conditional QTLs based on conditional phenotypic mean $[y(t|t-1)]$ is the result of the real temporal patterns of gene expression and provided inference for the net gene effects between time $t-1$ and t. Five conditional QTLs were identified at five measuring stages. Of them, pnD1a-1 expressed in two continuous developmental stages, 35D|25D and 45D|35D, indicating that the relevant genes were expressed continuously through two measuring stages rather than the cumulative result of genetic effects for the two specific growth stages in unconditional QTL. The net genetic effects were different at every specific stage (Table 3, Fig. 1). At 15D|initial, QTL pnF-1 (sat_114–sat_124) accounted for 27.01% of the total variation. At 35D|25D, conditional QTL pnD1a-1 explained for 14.1% of the total variation. The pnD1a-1 detected at 35D|25D and 45D|35D showed negative additive effects. Additionally, two conditional QTLs, pnB1-1 and pnB1-2, were identified

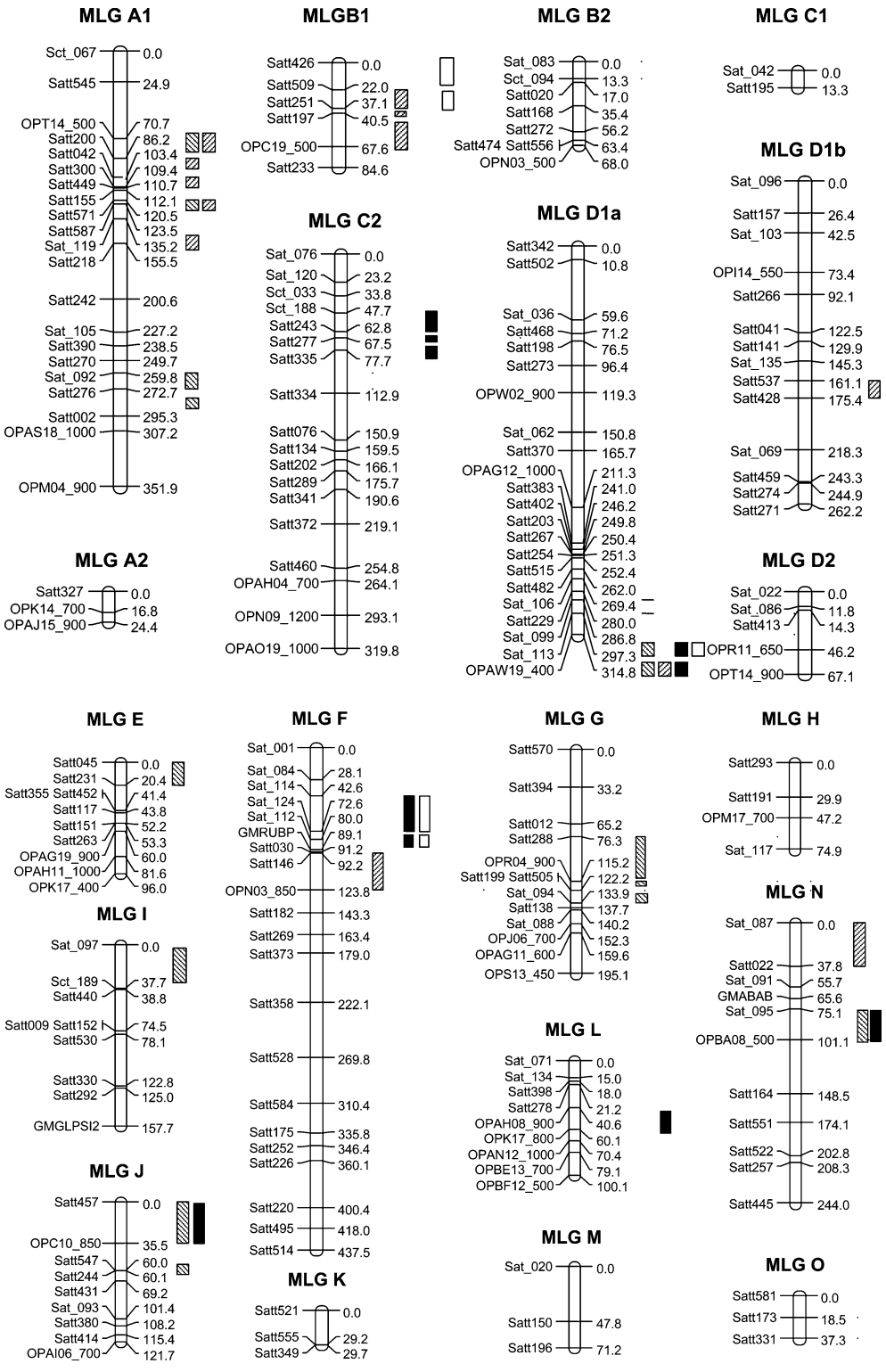

Fig. 1 Genetic linkage map of soybean developed with SSR and RAPD markers from cross Carleston × Dongnong 594 derived lines. ▮ Unconditional QTL for pod number of the main stem; ▯ conditional QTL for pod number of the main stem; ▧ Unconditional QTL for plant height; ▨ conditional QTL for plant height

at the final measuring stage (55D|45D) and mapped onto linkage groups B1. These QTLs, respectively, accounted for 20% of the total variation. The data showed that the conditional QTLs detected prior to 35 days mostly possessed positive net additive effects, whereas the conditional QTLs detected later than 35 days mostly possessed negative net additive effects (Table 3).

QTL analysis for plant height

Unconditional QTL for plant height

Thirteen unconditional QTLs for plant height were identified and anchored on 7 linkage groups (Table 4, Fig 1). Of them, one QTL (htG-3) was detected at three

Table 3 Unconditional and conditional QTLs for pod number of the main stem at different growth stages

QTL	Marker interval	Type of QTL	15D			25D			35D			45D			55D		
			LOD	A[a]	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)
pn B1-1	Satt426–satt509	t[b]															
		$t\|t-1$[c]													3.76	−5.18	20.90
pn B1-2	Satt509–satt251	t															
		$t\|t-1$													4.50	−5.29	19.89
pn C2-1	sct_188–satt243	t				6.54	−8.18	25.63									
		$t\|t-1$															
pn C2-2	Satt243–satt277	t				6.63	−6.99	18.08									
		$t\|t-1$															
pn C2-3	Satt277−satt335	t				6.83	−7.74	22.25									
		$t\|t-1$															
pn D1a-1	sat_099–sat_113	t							4.43	−5.97	13.05	11.11	−9.25	34.21			
		$t\|t-1$							4.53	−3.54	14.10	3.50	−2.97	11.41			
pn D1a-2	Sat_113–OPAW19_4	t							4.49	−6.28	14.91	10.61	−9.01	33.07			
		$t\|t-1$															
pn F-1	sat_114–satt_124	t	4.12	6.47	27.01												
		$t\|t-1$	4.12	6.47	27.01												
pn F-2	sat_124–sat_112	t	4.30	4.72	12.78												
		$t\|t-1$	4.30	4.72	12.78												
pn J	Satt457–OPC10_85	t							4.12	5.25	11.35						
		$t\|t-1$															
pn L	OPAH08_9–OPK17_80	t				3.89	−6.69	12.24									
		$t\|t-1$															
pn N	sat_095–OPBA08_5	t										3.95	6.15	17.00			
		$t\|t-1$															

[a]The additive effect
[b]Unconditional QTL
[c]Conditional QTL

different growth stages and six QTLs (htA1-1, htA1-3, htD1a-1, htD1a-2, htG-2 and htN-2) were detected at two different growth stages. The number of unconditional QTLs and their accumulation of genetic effects increased consistently from measuring stage 30–70 days with QTL numbers from 0 to 9 and total genetic effects from 0 to 44.44, which were paralleled with the continuous increment of plant height at those growth stages. Two unconditional QTLs (htJ-1 and htG-1) in this experiment, accounted for more than 20% of the phenotypic variation. At the final measuring stage (80 days), six unconditional QTLs were detected and grouped onto chromosome A1 and D1a. Of them, htD1a-1 (sat_099–sat_113) and htD1a-2 (sat_113–OPAW19_4) together explained for 20% of the phenotypic variation.

Conditional QTLs for plant height

Twelve conditional QTLs were identified for plant height and mapped on six chromosomes (Table 4, Fig. 1). Maximally three QTLs were found in linkage group A1, covering four measuring stages. QTL htA1-1 and htA1-3 were detected at two different measuring stages (Table 4, Fig. 1). Two conditional QTLs detected at stage 80D|70D were mapped in linkage group A1. Of them, QTL htA1-5 accounted for 11.01% of the total variation. The number of conditional QTLs and their net genetic effects altered during plant development. The maximal numbers of QTLs and relative net genetic effects occurred at stage 40D|30D and 70D|60D.

Discussion

According to the theory of developmental genetics, different genes will be dynamically expressed at different growth stages. Previous researches on soybean QTLs were focused on single developmental stage, usually the mature stage, in which most of the genetic information may be undetected. Instead, the analysis of conditional QTLs in different developmental stages will allow us to reveal the gene expressions in a dynamic pattern. In our study, the numbers of QTLs related to soybean pod number of the main stem and plant height, and their genetic effects were varied at different measuring stages, especially at earlier stages. However, most genes controlling production of pod did not express or their genetic effects were not significant at 55 days (near mature stage). Only one unconditional QTL, pnG (located in chromosome G), for pod number of the main stem was detected at near mature stage when threshold LOD score was set to 3.0 (data not shown) and some major QTL, like htJ-1, that significantly affected plant height was only detected at the earlier measuring stage but undetected at the near mature stage. This finding indicated that the parallel between gene expression and dynamic

Table 4 Unconditional and conditional QTLs for plant height at different growth stages

QTL	Marker Interval	Type of QTL	30D			40D			50D			60D			70D			80D		
			LOD	A[a]	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)	LOD	A	R^2 (%)
htA1-1	Satt200–Satt042	t[b]													7.26	5.04	13.47	7.46	8.73	13.31
		$t\|t-1$[c]				5.15	0.65	17.20				5.41	1.34	18.97						
htA1-2	Satt042–Satt300	t																		
		$t\|t-1$				6.51	0.65	17.73												
htA1-3	Satt155–Satt571	t													5.16	3.65	7.71	3.42	5.73	4.76
		$t\|t-1$				7.37	0.65	17.16										3.54	2.64	10.04
htA1-4	Satt587–Sat_119	t																		
		$t\|t-1$																3.80	2.85	12.14
htA1-5	Sat_092–Satt276	t																5.29	8.44	11.01
		$t\|t-1$																		
htA1-6	Satt276–Satt002	t																4.62	7.44	8.83
		$t\|t-1$																		
htB1-1	Satt509–Satt251	t																		
		$t\|t-1$													7.53	−4.36	19.69			
htB1-2	Satt251–Satt197	t																		
		$t\|t-1$													7.47	−4.28	19.43			
htB1-3	Satt197–OPC19_50	t																		
		$t\|t-1$				5.05	−0.71	12.81							4.63	−3.78	16.09			
htD1a-1	Sat_099–Sat_113	t													8.61	−5.42	14.61	13.14	−10.81	21.38
		$t\|t-1$																		
htD1a-2	Sat_113–OPAW19_4	t													9.02	−5.85	18.14	14.17	−12.30	27.72
		$t\|t-1$										3.58	−1.07	11.45						
htD1b	Satt537–Satt428	t																		
		$t\|t-1$										3.59	−0.99	10.48						
the	Satt045–Satt231	t													6.09	−4.10	9.82			
		$t\|t-1$																		
htF-1	Satt146–OPN03_85	t																		
		$t\|t-1$				3.47	−0.57	13.81												
htG-1	Satt288–OPR04_90	t							5.88	2.10	22.85				8.24	5.98	9.30			
		$t\|t-1$																		
htG-2	OPR04_90–Satt199	t										5.82	2.70	14.19	8.51	5.27	14.29			
		$t\|t-1$																		
htG-3	Satt505–Sat_094	t							3.82	1.60	13.62	4.11	2.37	11.80	7.08	5.09	14.64			
		$t\|t-1$																		
htI-1	Sat_097–Sct_189	t				3.83	0.85	12.31												
		$t\|t-1$																		
htJ-1	Satt457–OPC10_85	t				7.63	1.29	22.06												
		$t\|t-1$																		
htJ-2	Satt547–Satt244	t				5.51	−1.15	14.57												
		$t\|t-1$																		
htN-1	Sat_087–Satt022	t																		
		$t\|t-1$													7.19	4.17	33.28			
htN-2	Sat_095–OPBA08_5	t										5.31	2.87	16.74	3.47	4.04	8.52			
		$t\|t-1$																		

[a] Additive effect
[b] Unconditional QTL
[c] Conditional QTL

QTLs existed and some major QTLs of soybean might be neglected by the previous studies that only surveyed the mature stage. A similar conclusion was also reported by Yan et al. (1998) for rice tiller trait.

The significance of this research was to introduce conditional QTL into soybean QTL analysis. Unconditional QTLs based on time t phenotypic mean [$y(t)$] only provided inference for the accumulative gene effects (multi-genes expression and interactions) from initial time to time t, which could not appropriately explain expression of genes at the specific developmental stage between time $t-1$ and t; whereas, conditional QTLs reflected the net genetic effects contributed in a specific time zoon $t-1$ to t. In the present study, conditional QTLs for pod number of the main stem at 15D|initial was theoretically equal to unconditional QTLs at 15D. Conditional QTLs associated to pod number of the main stem and plant height were mostly different from their unconditional QTLs. Only six QTLs detected were identical in both conditional and unconditional cases. For example, htA1-1 loci for plant height identified in two consistent stages, 70 and 80 days, by unconditional mapping method was also detected by conditional QTL mapping in 40D|30D and 60D|50D stages.

Our study revealed that gene expression exhibited multiple patterns. Some gene effects were maintained for a longer time and others disappeared quickly. For instance, QTL htA1-1 was detected at two consistent stages of plant development, which could reflect a continuous expression of genes, and QTL htN-1, on the other hand, was detected only at one stage, which indicated that gene expression at a specific measuring stage. In contrast with our results, some QTLs developed for the number of rice tillers existed consistently for more than five measuring stages (Yan et al. 1998). The difference might be due to the different background of genetics in monocot and dicot plants.

Since the unconditional QTLs explained the accumulative gene actions from initial time to the time t, the variation of accumulative gene effects might be diminished if genes with the opposite genetic effects were expressed at the same or close locations. It might explain why many conditional QTLs were not detected in unconditional mapping method, especially at the mature stage in this study (Yan et al. 1998). To breed high-yield cultivars, a new approach was to identify the major QTLs that facilitated high yield at different developmental stages before maturity, rather than using the QTLs detected at mature stage, which will reduce the discrete caused by opposite genetic effects. In addition, some conditional QTLs were found to appear at interval times, for example, htA1-1 appeared both at 40D and 60D. This phenomenon indicated that some genes might repeat their expression at different time zones.

In this study, conditional QTLs for pod numbers detected prior to 35 days mostly possessed positive net additive effects, whereas conditional QTLs detected later than 35 days mostly possessed negative net additive effects. Meanwhile, the total numbers of QTLs reduced from maximum four at 45 days to two at 55 days (Table 3). This result was paralleled with the phenomenon that pod number appeared rapidly prior to 35 days and the abortion of young pods occurred after the growth stage of 35 days.

Mansur et al. (1996) found that some of the QTLs on the same linkage group were close to each other to form a cluster and the total genetic effects of these loci could be large for agronomic traits of soybean. This was supported in our study for pod number in the main stem and plant height, such as htA1 1-6 and pnC2 1-3. We mapped two major QTLs (pnB1-1 and pnB1-2) on linkage group B1 at the final measuring stage, which could explain about 20% of the total variation for pod number of the main stem. Zhang et al. (2004) found a QTL on linkage group J associated with formation of pod per node. We also mapped a QTL (pnJ) for pod number of the main stem on the same linkage group. Moreover, six unconditional QTLs controlling plant height of soybean were identified at final measuring stage in this report. Of these, htD1a-1 and htD1a-2 on linkage group D1a explained 21.38 and 27.72% of the total variation, respectively. These two QTLs could be a key chromosome region (sat_099–OPAW19_4) to control plant height in soybean. The same linkage group was found to be associated with plant height by Lee et al (1996). Similar to the report of Zhang et al. (2004), we also mapped three QTLs (htB1-1: satt509–satt251, htB1-2: satt251–satt197, htB1-3: satt197–OPC19_50) on linkage group B1 for plant height. They explained 50% of the total variation for plant height. In this study, no QTL for plant height was found to be closely linked with published maturity QTLs according to a search in SoyBase (http://www.soybase.org), except for QTL htF-2 which was detected to be associated with maturity QTL, POD MA 9-3 cited from Orf et al. (1999) at LOD score 3.24 and located in linkage group F, with a distance of 36 cM between the two QTLs.

Although QTLs that significantly affect pod number of the main stem varied from the QTLs controlling plant height at different plant growth stages, five QTLs were identified that significantly affected both pod number of the main stem and plant height (Fig. 1). Of them, pnD1a-1 (or htD1a-1) (satt_099–sat_113) and pnD1a-2 (or htD1a-2) (sat113–OPAW19_400) were detected at two or three different measuring stages for the two traits and explained more than 20% of the total variation. Combining the unconditional and conditional QTL mapping results, we found that the two QTLs firstly affected plant height, and then affected the formation of pods.

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References

Apuya NR, Frazier T, Keim P, Roth EJ, Lark KG (1988) Restriction fragment length polymorphisms as genetic marker in soybean (*Glycine* max L. Merr). Theor Appl Genet 75:889–901

Atchley WR, Zhu J (1997) Developmental quantitative genetics, conditional epigenetic variability and growth in mice. Genetics 147:765–776

Basten CJ, Weir BS, Zeng ZB (1996) QTL cartographer. North Carolina State University, NC

Chen HH (1984) Analysis of gene effects for important agronomic traits in soybean. Soybean Sci 3:91–99

Chung J, Babka HL, Graef GL, Staswick PE, Lee DJ, Cregan PB, Shoemaker RC, Specht JE (2003) The seed protein, oil, and yield QTL on Soybean linkage group I. Crop Sci 43:1053–1067

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

Concibido VC, Denny RL, Boutin SR, Houtea R, Orf JH, Young ND (1994) DNA marker analysis of loci underlying resistance to soybean cyst nematode (Heterodera glycines Ichinohe). Crop Sci 34:240–246

Cregan PB, Jarvik T, Bush AL, et al (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: association between marker loci and variation in quantitative trait. Genetics 126:735–742

Lee SH, Bailey MA, Mian MAR, Shipe ER, Ashley DA, Parrot WA, Hussey RS, Boerma HR (1996) Identification of quantitative trait loci for plant height, lodging, and maturity in a soybean population segregating for growth habit. Theor Appl Genet 92:516–523

Mansur LM, Lark KG, Kross HK, Oliveira L (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

Nesbitt KA, Potts BM, Vaillancourt RE, West AK, Reid JB (1995) Partioning and distribution of RAPD variation in a forest tree species *Eucalyptus globules* (Myrtaceae). Heredity 74:628–637

Nybom H (1994) DNA fingerprinting—a useful tool in fruit breeding. Euphytica 77:59–64

Orf JH, Chase K, Jarvik T, Mansur LM, Cregan PB, Adler FR, Lark KG (1999) Genetics of soybean agronomic traits: I comparison of three related recombinant inbred populations. Crop Sci 39(6):1642–1651

Qiu BX, Arelli PR, Slepor DA (1999) RFLP markers associated with soybean cyst nematode resistance and seed composition in a "Peking" × "Essex" population. Theor Appl Genet 98:356–364

Reiter RS, Williams GK, Feldmann KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884–889

Shoemaker RC, Olson TC (1993) Molecular linkage map of soybean (*Glycine max* L. merr). In: O' Brien SJ (eds) Genetic maps: locus maps of complex genomes. Cold Spring Harbor Press, Cold Spring Harbor, pp 131–138

Shoemaker RC, Specht JE (1995) Integration of the soybean molecular and classical genetic linkage groups. Crop Sci 35:436–446

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovitch S, Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet 95:163–173

Sun GL, William M, Liu J, Kasha KJ, Pauls KP (2001) Microsatellite and RAPD polymorphisms in Ontario corn hybrids are related to the commercial sources and maturity ratings. Mol Breed 7:13–24

Wang LZ, Wang JL (1992) Genetics and breeding in Soybean, 1st edn. Science press, Peking, pp 60–65

Wang S (1963) Study on correlation between yield and type of plant in soybean. Agric Sci Sanxi 1:8–10

Wang D, Graef GL, Procopiuk AM, Diers BW (2004) Identification of putative QTL that underlie yield in interspecific soybean backcross populations. Theor Appl Genet 108:458–467

Webb DM, Baltalzar BM, Rao-Arelli AP, Schupp J, Clayton K, Keim P, Beavis WD (1995) Genetic mapping of soybean cyst nematode race-3 resistance loci in soybean PI437654. Theor Appl Genet 91:574–581

Wu W, Li W, Tang D, Lu H, Worland AJ (1999) Time-related mapping of quantitative trait loci underlying tiller number in rice. Genetics 151:297–303

Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1998) Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza stativa* L). Theor Appl Genet 97:267–274

Yang QK (1975) Analysis on genetic variation in early generation of the cross materials for important agronomic traits. Acta Genet Sin 2:225–230

Yang Q, Wang JL (1995) The analysis of genetic parameter for different types of soybean by diallel crosses. Sci Agric Sin 28:76–80

Ye Z, Lu Z, Zhu J (2003) Genetic analysis for developmental behavior of some seed quality traits in upland cotton. Euphytica 129: 183–191

Yuan J, Njiti VN, Meksem K, Iqbal MJ, Triwitayakorn K, Kassem MA, Davis GT, Schmidt ME, Lighhfoot DA (2002) Quantitative trait loci in two soybean recombinant inbred line populations segregating for yield and disease resistance. Crop Sci 42:271–277

Zeng ZB 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zhang WK, Wang YJ, Luo GZ, Zhang JS, He CY, Wu XL, Gai JY, Chen SY (2004) QTL mapping of ten agronomic traits on the soybean (*Glycine max* L. merr) genetic map and their association with EST markers. Theor Appl Genet 108:1131–1139

Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. Genetics 141:1633–1639