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Quantitative Trait Loci for Stem Length in Soybean Using a Microsatellite Markers

Hyeun Kyeung Kim*, Sung Taeg Kang[1], Hyeun Jong Kong[2] and In Soo Choi[2]

Yeongnam Agricultural Research Institute, NICS, RDA, Miryang 627-130, Korea
[1]Research Coordination Division RDA, Suwon 441-707, Korea
[2]Dept. of Plant Resources, Miryang National University, Miryang 627-130, Korea

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Identification of individual quantitative trait loci (QTL) is a prerequisite to application of marker-assisted selection for stem length. Two simple sequence repeat (SSR)-based linkage maps were constructed from recombination inbred line populations between cross of Keunolkong and Shinpaldalkong. Two parents used differed greatly in stem length, which were 30.57 cm and 49.75 cm in Keunolkong and Shinpaldalkong, respectively. Using the constructed maps, regression analysis and interval mapping were performed to identify QTLs conferring stem length. Four QTLs for stem length on linkage groups (LG) F, J, N and O were identified in the Keunolkong×Shinpaldalkong population and they totally explained 37.83% of variation for stem length. In the population, two major QTLs on LG J and O conditioning 14.25% and 10.68% of the phenotypic variation in stem length were determined and two QTLs with minor effect were detected on LG F and N. Identification of QTLs for stem length and mapping individual locus should facilitate to describe genetic mechanisms for stem length in different population. SSR markers tightly linked to QTLs for stem length allow to accelerate the elimination of deleterious genes and selection for desirable recombinants at early stage in crop breeding programs.

Key words – Stem length, Soybean, QTLs, Microsatellite marker,

The current molecular marker technology would provide the plant breeders and geneticist tools to facilitate overcome such problems that are present in conventional methodology. The most common DNA markers in plant are the RFLPs, RAPDs, AFLPs and SSR. In soybeans, the SSR markers would be an excellent complement to the RFLP makers for the research on the genetic analysis and the application to varietal improvement owing to the performance of high level of polymorphism, single locus nature and random distribution in the genome.

Although numerous investigations have been carried out on the inheritance of quantitative traits, plant breeders have little information on the number of genetic factors (loci) involved in the expression of the traits, the chromosomal location of those loci, and the relative magnitude of contribution of each locus. The molecular marker technology will also provide a powerful tool to facilitate the marker-assisted selection, and eventually enhance map-based cloning of agronomically important genes and/or traits.

The consensus map is being saturated with a total of over 1100 SSR makers available at Soybase website (http://soybase.agron.iastate. edu/) now in 2003 (personal communication with P. Cregan). After construction of the consensus map, many QTLs involving pest resistance, seed composition and agronomic traits have extensively reported in soybean. QTLs for soybean cyst nematode[20, 23], sudden death syndrome[17], root-knot nematode[13], and yield[2,3] have been included.

In many others crops including soybean, selection for major agronomic trait such as stem length has been extensively applied in breeding program for development of cultivar with superior performance and adaptation. A number of researchers[10,15,11,16] have identified QTLs for stem length in mature soybean plant.

The objective of the present research was to improve the breeding efficiency stem length in soybean. We identified additional QTLs associated with stem length in F_2 derived F_{10} population from a cross between Keunolkong and Shinpaldalkong.

Materials and methods

Plant materials

Keunolkong was selected from local cultivars, showing susceptibility to early maturity, and large seed size. Shinpaldalkong was selected from a cross of Will×(Elf×

***Corresponding author**
Tel : +82-55-350-1239, Fax : +82-55-353-3050
E-mail : hkkkim@freechal.com

SS74185), showing susceptibility to medium maturity, and it has a medium seed size.

For constructing genetic linkage map, the genetic materials consisted of the populations from F_2 derived RILs. They were developed by single seed descent (SSD) from reciprocal cross of Keunolkong × Shinpaldalkong. In SSD method, a single seed was chosen at random from each plant in each generation, starting with the F_2, through self pollination either in the greenhouse and/or in the field[15]. In F_9 generation, each plant was threshed individually and maintained as a separate RILs. The F_9 seeds were mutiplied and bulked. F_{10} seeds were used for field experiment in Yeongnam Agricultural Research Institute (YARI), NICS during the summer, 2001.

Field evaluation of RILs

From Keunolkong × Shinpaldalkong, 117 F_2 derived F_{10} RILs and parent were arranged in a randomized complete block design with two replications at YARI. Each entry was planted in a 1.5 m long paired-row plot. Two seeds per hill were planted, with a spacing of 60 between row and 10cm between plants. The seeds were planted on June 12, 2001.

Molecular data

SSR primers were purchased from Perkin-Elmer, prepared at USDA Laboratory in Beltsville, MD, USA[1,4]. A total of 200 SSR primers were used to screen for parent polymorphism. A representative DNA from each RIL plants was obtained from 2~3 leaves from F_{10} plant. DNA was isolated using CTAB (hexadecyltrimethyl ammonium bromide) as described by Keim et al.[9]. With a slight modification, PCR reaction mixture contained 50 ng/μl soybean genomic DNA, 2 mM Mg^{2+}, 0.15 μM of 3' and 5' primers, 1 PCR buffer containing 100 mM KCl, 10 mM Tris-HC1 (pH8.5), 0.1% Triton X-100, and 0.5unit *Taq* DNA polymerase (AmpliTaq Gold polymerase, Applied Biosystems, Forster City, CA, USA) in a total volume of 10 μl. Cycling consisted of a 25-s denaturation at 94℃, 25-s annealing at 47℃, and 60-s extension at 68℃ for 40 cycles on a thermocycler (Pekin-Elemer Research model 9700). PCR products (5 μl/lane) were separated on a standard DNA sequencing gel containing 4% polyacrylamide, 8 M urea, and 0.5 × TBE, at 1,700V constant power for 1.5 to 2 hours.

After electrophoresis, the sequencing gel was subjected to silver staining according to the sequencing™ system's protocol (Promega Co., Madison, U.S.A.). When the size difference between DNA amplification products of the two alleles at an SSR locus was greater than 8 to 10bp in length, a 1.5% agarose + meta-phore agarose 1.5% gel with ethidium bromide was used instead of a sequencing gel.

Data analysis and QTL mapping

A linkage map was constructed with SSR and pigmentation marker data (pubescence and hilium color) using the Haldane map function[7] of Map Manager QT program (Ver.b2.3)[14], for grouping marker into linkage group, a minimum LOD of 3.0 and a maximum distance of 50 cM were used. The primary linkage group was determined on the basis of public USDA map information[6].

Map positions for QTLs were determined by analysis of variance[22] with Statistical Analysis System version 8.03 [19]. Analysis of marker-QTL associations was investigated by single factor analysis of variance (SF-ANOVA) in which marker-genotype groups were used as class variance. The means for each marker allelic group were compared using an F-test from the type-III mean squares obtained from the GLM procedure of SAS[19]. In addition, two-factor analysis of variance was used to detect significant ($P<0.05$) interaction (ie. epistasis) between all possible pairs of significant markers. When two more markers showing significant association with morphological characters linked to the same chromosome, multiple regression analysis (SLG-Regr) was conducted. Forward and stepwise selection procedures were applied in the regression analysis. All significant markers from SLG-Regr and unlinked single marker identified from SF-ANOVA were combined in a multiple linkage group regression model (MLG-Regr) at $P<0.05$ to determine the proportion of phenotypic variance explained by two or three QTLs. Phenotypic correlations were calculated with SAS[19].

Results and Discussion

Genetic map

The details on the genetic map of this population were reported in Kang[8]. The soybean genetic linkage map was constructed independently with 110 markers (108 SSR and 2 classical markers) for Keunolkong×Shinpaldalkong population. The genetic map from the Keunolkong×Shinpaldalkong population consisted of 94markers that were genetically linked and placed into 19 linkage groups covering about 1,890cM. The Morphological markers

(pubescence color and hilum color) used for constructing maps were mapped on the same location (*T* and *I* locus [5]) at linkage group (LG) C2 and A2, respectively, in Keunolkong×Shinpaldalkong population.

Identification of QTLs for Stem Length RILs

Variation in stem length, in RIL population of Keunolkong×Shinpaldalkong is presented in Fig. 1. There was significant variation in stem length among the RILs, which had a range of 22.57~68.53 cm. The two parents used differed greatly in stem length, which were 30.57 cm and 49.75 cm in Keunolkong and Shinpaldalkong, respectively. For the above traits, values among RIL progenies were greater and smaller than those of the two parents, indicating transgressive segregation.

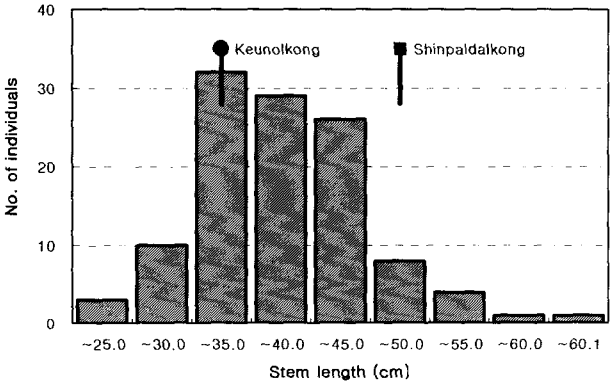

Fig. 1. Frequency distribution of stem length in F_2 derived F_{10} RILs of Keunolkong×Shinpaldalkong.

Based on SF-ANOVA, thirteen markers were significantly ($P<0.05$) associated with stem length in this population (Table 1, Fig. 2). Four markers were mapped on LG J, two markers on LG H, N, and O, and others on LG D2, F, and G. Individually, these markers accounted for 3.68 to 13.71% of the variation in stem length. The Keunolkong

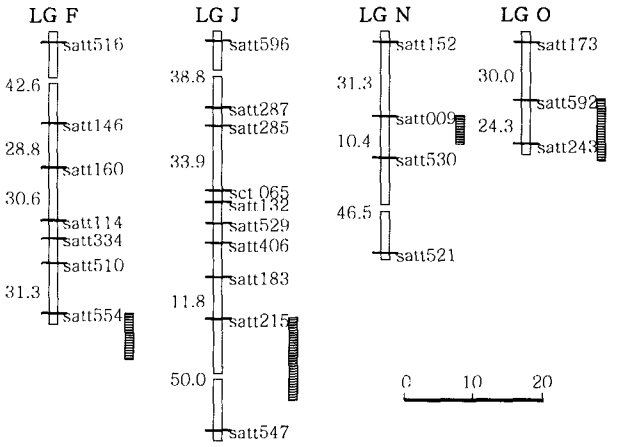

Fig. 2. QTL positions for stem length from Keunolkong×Shinpaldalkong. The map, showing marker position on the right hand side and estimated map distance (cM) on the left-hand side, was adopted from the public soybean map (Cregan et al., 1999). The length of vertical bars indicates R^2 values for the loci.

Table 1. Markers linked to QTLs associated with the stem length in F_2 derived F_{10} RILs of Keunolkong×Shinpaldalkong

Markers	LG	SF-ANOVA[a]		Alleic means		SLG-Regra		MLG-Regra	
		P	R^2 (%)	K/K[c]	S/S[c]	*P*	R^2 (%)	*P*	R^2 (%)
satt372	D2	0.0436	3.68	40.77	37.32	NA[b]	-	-	-
satt554	F	0.0402	3.91	36.71	39.57	NA	-	0.0027	7.94
satt472	G	0.0454	3.87	36.88	39.80	NA	-	-	-
satt442	H	0.0114	5.73	36.36	39.87	0.0076	6.66	-	-
satt541	H	0.0167	5.34	36.30	36.69	-	-	-	-
satt287	J	0.0363	4.23	39.51	36.56	0.0372	4.01	-	-
satt132	J	0.0258	4.48	39.42	36.36	-	-	-	-
satt406	J	0.0281	4.60	39.26	36.12	-	-	-	-
satt215	J	0.0001	12.88	40.29	35.03	0.0016	9.79	0.0004	14.25
satt009	N	0.0001	12.89	40.76	35.59	0.0002	12.46	0.0135	4.96
satt530	N	0.0321	4.26	39.90	36.88	-	-	-	-
satt592	O	<0.0001	13.71	35.66	51.17	0.0002	13.65	0.0010	10.68
satt243	O	0.0003	11.91	35.22	40.29	0.0255	4.35	-	-
Total									37.83

[a]SF-ANOVA : single factor analysis of variance.
SLG-Regr : multiple regression with markers on each linkage group.
MLG-Regr : multiple regression with all significant markers from the SLG-Regr model.
[b]NA : Not applicable. Not linked to other markers.
[c]K/K : Keunolkong, S/S : Shinpaldalkon.

allele increased stem length at QTLs identified on LG D2, J, and O where as Shinpaldalkong provided the positive alleles at QTLs identified on LG F, G, H, and O. SLG-Regr for the four markers on LG J and two markers on LG H, N, and O retained satt287 and satt215 on LG J, and satt442 on LG H, and satt009 on LG N, and satt592 and satt243 on LG O, showing the existence of single QTL for days to stem length on each of the linkage group. MLG-Regr analysis with nine independent markers retained satt009, satt215, satt554, and satt592. The total amount of variation explained by above four markers was 37.83%.

In an F_2 population derived from the cross between *G. max* and *G. soja*, Keim et al.[9] identified RFLP markers associated with stem length. They found that only one marker, K18 (unknown LG), was associated with variation in stem length. Lee et al.[12] reported QTL for plant height (same as stem length) was associated with LG A2, B1, C1, D1, F, and L from the cross between 'Young' and 'PI416397'. Recently, several QTLs for stem length on LG C2 and F[14], LG C2, D1b+W, L, and M[18], LG I[21], and LG K[23] have been reported.

All the two-way combinations using these markers were tested for significant epistatic interaction. Eighth pairs showed a significant interaction ($P<0.05$) (Table 2). The interaction between satt554/satt530 and between satt472/satt215 loci resulted in longer stem length for lines with the Shinpaldalkong/Keunolkong allelic configuration at satt554/satt530, satt472/satt215 loci. For combination between satt530 and satt592 loci, the allele combination of Keunolkong/Shinpaldalkong result in longer stem length.

The interval mapping analysis for detection of QTL

Table 2. Epistatic interactions between two markers associated with the stem length in Keunolkong×Shinpaldalkong

SSR locus	Allele	Allele/locus		P	R2 (%)
		Keunolkong	Shinpaldalkong		
satt554 / satt442		satt554		0.0453	13.87
satt442	Keunolkong	36.32	36.41		
	Shinpaldalkong	37.32	42.87		
satt554 / satt530		satt554		0.0130	18.01
satt530	Keunolkong	37.11	44.98		
	Shinpaldalkong	36.30	37.48		
satt554 / satt592		satt554		0.0321	22.65
satt592	Keunolkong	35.26	36.21		
	Shinpaldalkong	38.17	44.75		
satt554 / satt243		satt554		0.0218	17.64
satt243	Keunolkong	36.11	34.80		
	Shinpaldalkong	37.55	42.56		
satt472 / satt541		satt472		0.0465	13.95
satt541	Keunolkong	36.41	36.51		
	Shinpaldalkong	37.48	43.34		
satt472 / satt215		satt472		0.0014	25.14
satt215	Keunolkong	37.64	43.63		
	Shinpaldalkong	36.00	33.41		
satt472 / satt592		satt472		0.0366	19.02
satt592	Keunolkong	35.95	35.48		
	Shinpaldalkong	38.32	43.78		
satt530 / satt592		satt530		0.0005	26.67
satt592	Keunolkong	34.97	36.38		
	Shinpaldalkong	45.67	37.85		

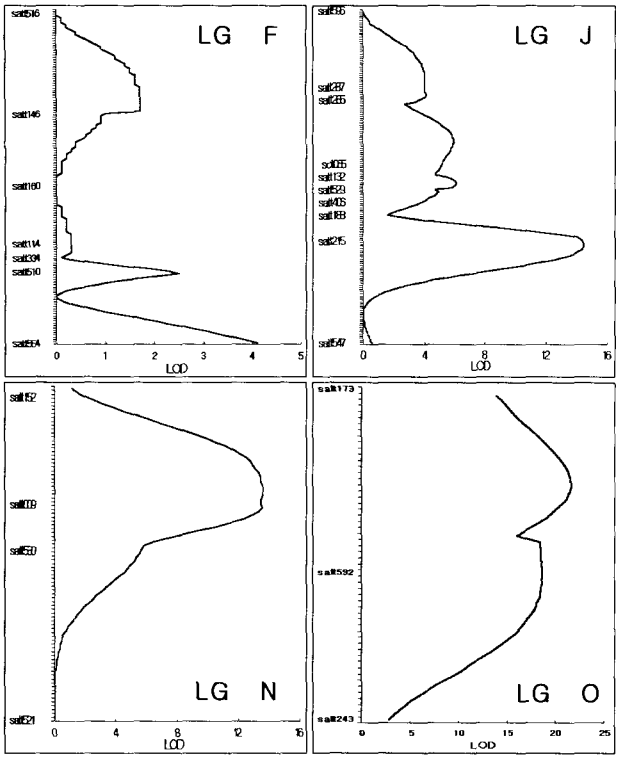

Fig. 3. Interval mapping for QTLs conferring the stem length in F_2 derived F_{10} RILs of Keunolkong×Shinpaldalkong.

positions on linkage map indicated that the most possible location of this QTL were at or near the satt554, satt215, satt009, and satt592 on LG F, J, N, and O, respectively (Fig. 3). The LOD scores for these QTLs at this location were 4.1 and 7.94% at LG F, 14.3 and 14.25% at LG J, 13.5 and 4.96% at LG N, and 18.3 and 10.68% at LG O of the phenotypic variation in stem length were explained.

In general, interval mapping detected the loci with large effects but missed the loci with small effects. The small effects loci, however, could be detected by the interval mapping method when LOD score was <2.4. Two major and one or two minor QTLs for stem length were identified. The underestimation of QTL by interval mapping might be due to low saturation of the genetic map in present study.

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초록 : 콩에서 Microsatellite 마커를 이용한 양적형질 유전자의 분석

김현경* · 강성택[1] · 공현정[2] · 최인수[2]
(농촌진흥청 작물과학원 영남농업연구소, [1]농촌진흥청 연구개발국 연구관리과, [2]밀양대학교 식물자원학과)

콩에서 경장과 연관된 DNA 표지인자를 개발하여 품종육성에 활용함으로서 육종효율 증진에 기여하고자 수행하였다. 본 시험은 육성된 큰올콩과 신팔달콩의 RIL 계통 및 SSR marker를 이용하여 유전자지도를 작성하고, 이를 바탕으로 경장과 관련된 양적형질 유전자좌(QTL)를 탐색하였다. 시험재료로 이용된 큰올콩과 신팔달콩은 경장이 각각 30.57 cm와 49.75 cm로 매우 큰 차이를 보였다. 경장과 연관된 QTL은 개별마커들과의 분산분석 결과, 연관군 F, J, N 및 O에서 전체변이의 37.83%를 설명할 수 있는 4개의 QTL을 탐색하였다. 특히, 연관군 J와 O에서 각각 14.25%와 10.68%를 설명할 수 있는 주요 QTL을 확인하였다. 따라서 경장 관련 QTL중 연관군 J와 O에서 확인된 주요 QTL은 품종 육성과정에서 경장 관련 선발 마커로서 활용가치가 높은 것으로 판단된다.