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Analysis of Quantitative Trait Loci for Yield Component Traits in Soybean Using Recombinant Inbred Lines

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Seed and pod numbers are the main yield components in soybean. Selection for increased yield potential is main goal of plant breeding. The objective of this study was to identify quantitative trait loci (QTLs) that control pod number per plant, seed number per plant and pod in soybean. The 117 $F_{2:10}$ recombinant inbred lines (RILs) developed from a cross of 'Keunolkong' and 'Shinpaldalkong' were used. Two independent QTLs for pod number per plant were identified from linkage group (LG) F and L. Two QTLs for seed number per plant were located on LG F and L. Seed number per pod was related with three QTLs located on LG D1a, D1b and F. Pod and seed number per plant have two common QTLs on LG F and L.

Key words – Pod number, Seed number, Soybean, QTL, SSR marker

Introduction

Selection for increased yield potential is main goal of plant breeding. Much of the yield increase of soybean over the post 60 years has been due to genetic advances obtained by intercrossing existing varieties [20]. However, yield is a multigenic trait and yield potential of lines derived by intercrossing is difficult to be predicted without extensive field study [22].

Soybean grain yield is functional product of the mean number of plant per unit area, pods per plant, seeds per pod, and the average mass of the individual seed. Each of these components sequentially fixed during specific time frames of soybean onto genetic development [17]. But the genetic basis of yield improvement and pod number per plant, seed number per plant and seed number per pod are still obscure.

Recent developments in the use of the molecular markers can help plant breeding become more efficient [16]. Information concerning the inheritance and underlying genetic control of important seed components and yield traits in soybean will provide better understanding of the formation of those yield and seed constituents, and may help breeders accelerate the development of specialty varieties destined for yield increase.

Several researches were conducted using molecular markers to identify genomic regions and genetic effects of interesting genes; sucrose content [11,14], seed protein and oil [2,3,8], lodging [9,13,15], leaflet traits [10] and yield [3]. Breeder's use of such QTLs is more likely only after they are confirmed in diverse populations grown in diverse environments.

Although Zhang et al. [23] reported QTLs, those controlled the pods per node of soybean. QTL research targeted at pod number per plant, seed number per plant and seed number per pod is very rare. Therefore, the primary objective of the present research was to improve the breeding efficiency of yield increase in soybean. This study was conducted to identify simple sequence repeats (SSR) markers associated with QTLs and marker assisted selection (MAS) for pod number per plant, seed number per plant and pod in soybean.

Materials and Methods

Plant materials and field evaluation

Two well-characterized soybean cultivars, 'Keunolkong'

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and 'Shinpaldalkong' were used as parents to identify QTLs associated with yield component traits. 'Keunolkong', a cultivar developed from pure line selection of Korean local collection is characterized by early maturity, short stem length, and large seed size. 'Shinpaldalkong' is a cultivar developed from a cross of 'Will' × ('Elf' × SS74185) and has the characteristics of late maturity, long stem length, and small seed size.

A total of 117 $F_{2:10}$ recombinant inbred line (RIL) population was developed by single seed descent (SSD) each generation from individual F_2 plants in a cross of 'Keunolkong' × 'Shinpaldalkong' designated K/S. The F_{10} seeds of each line were planted in a randomized complete block design with two replications, at Yeongnam Agricultural Research Institute, NICS, RDA, Milyang, Korea, in 2001. Each plot was consisted of 1.5m long paired rows spaced 0.6m apart. In each rows, fifteen hills were planted with two seeds per hill.

Simple sequence length polymorphism analysis

Genomic DNA was isolated from fresh leaves following the procedure described by Keim et al. [7]. A total of 199 soybean SSR markers (http: //soybase.agron.iastate.edu/ssr.htm) were used with primer pairs to screen for polymorphism between parentals genotypes. PCR was performed in a total volume of 10 μl containing 25 ng of template DNA, 0.15 μM of each forward and reverse primers, 200 μM of each dNTP, 2 mM $MgCl_2$, 0.1% Triton X-100, 1× reaction buffer (10 mM Tris-HCl pH 8.5, 100 mM KCl) and 0.5 U of *Taq* DNA polymerase (BioBasic Taq Polymerase, Applied Bio Basic, Canada). Template DNA was initially denatured at 94℃ for 2 min, followed by 40 cycles of PCR amplification using the following conditions; denaturation at 94℃ for 25 sec, annealing at 47℃ for 25 sec and extension at 68℃ for 60 sec on a 96-Well GeneAmp PCR system 9700 (Applied Biosystems, USA). The segregation patterns of SSR markers were determined by electrophoresis on a 4% polyacrylamide gel. Afterward, the gel was stained with a silver sequencing kit (Promega, USA) and scored for map construction. Pigmentation colors for soybean plant pubescence and seed hilum were also scored as morphological markers.

Map construction and statistical analysis

Means of traits, correlation, and analysis of variance were calculated by use of SAS software. On the basis of the segregation data subsets of SSR and morphological markers we constructed a linkage map with MapManager QT version 2.8 software [12]. Recombination fractions were converted to map distances by applying the Haldane map function [5]. Where possible, linkage groups were named according to their designations from a consensus USDA map [19].

The association between marker and QTL was tested according to the interval-mapping methods of Whittaker et al. [27], using MapManager QT and single-factor ANOVA (SF-ANOVA). An LOD threshold level of 3.0 was chosen as minimum to declaration of the presence of QTLs in a given genomic region. For each SSR and morphological marker, the class means for pod number per plant, seed number per plant and seed number per pod were compared for significance ($P < 0.05$) using an *F*-test from the Type Ⅲ mean squares, as obtained from the General Linear Model (GLM) procedure of SAS.

If SF-ANOVA identified two or more linked markers associated with the pod number per plant, seed number per plant and seed number per pod, multiple regression analysis was conducted by including all the significant markers on that linkage group in the model (i.e. SLG-Regr). Forward and step-wise selection procedures were applied in the regression analysis. Any significant ($P < 0.05$) marker that was retained in the SLG-Regr analysis was represented to identify unique QTLs on that linkage group. All significant markers from the SLG-Regr analysis were then combined into a multiple-linkage group regression (MLG-Regr) at $P < 0.05$ to determine the combination of independent markers that was explained the greatest amount of phenotypic variation in a given trait. Finally, the coefficient of determination (*R*2) obtained from MLG-Regr was used to provide an estimate of the percentage of phenotypic variation explained by the marker

Results and Discussion

Phenotypic evaluation and correlations of the pod number per plant, seed number per plant and seed number per pod

Grain or pod number per unit area are the main yield components in most annual crops [4,18]. Seed and pod numbers are the main yield components in soybean [6]. Variation of pod number per plant, seed number per plant and pod of RIL is presented in Table 1 and Fig. 1. Pod number per plant, seed number per plant and seed number per pod were normally distributed in the $F_{2:10}$ RIL. Pod

Table 1. Descriptive statistics for pod number per plant, seed number per plant and seed number per pod of 117 RILs of 'Keunolkong' × 'Shinpaldalkong'

Traits	Parents		K/S population	
	Keunolkong (mean ± SD)	Shinpaldalkong (mean ± SD)	Range	Mean ± SD
Pod number per plant	18.11±2.80	46.15±8.64	8.67~ 61.37	28.98±9.17
Seed number per plant	34.20±5.73	93.60±14.03	19.00~124.79	57.88±17.91
Seed number per pod	1.89±0.07	2.04±0.13	11.60~ 30.35	2.01±0.15

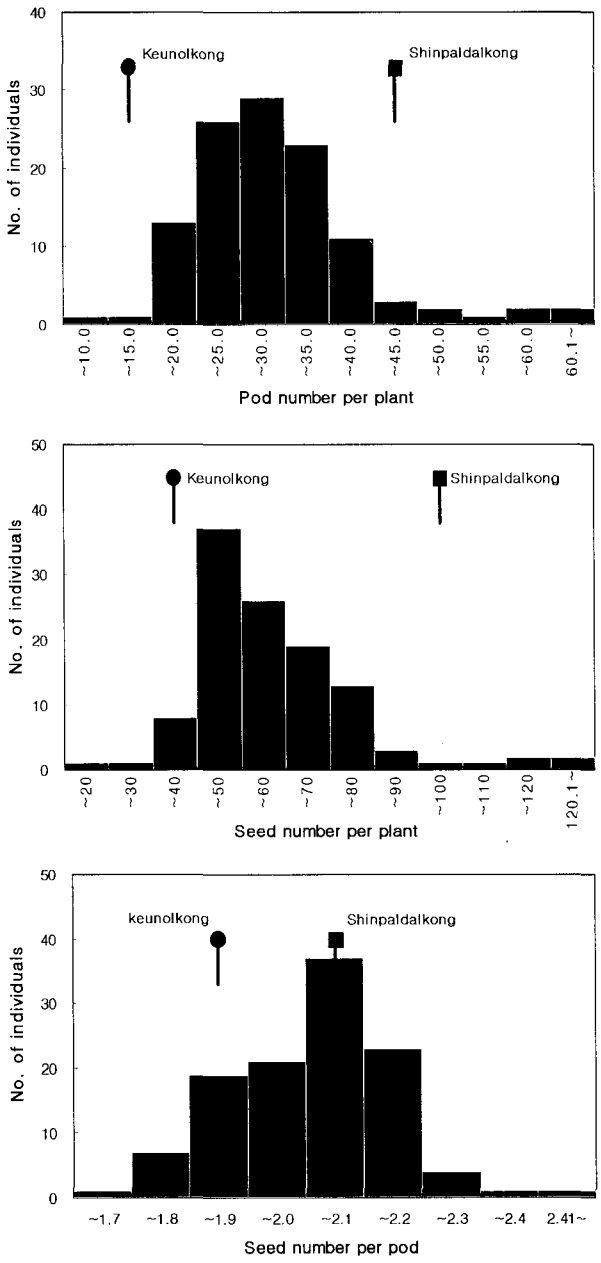

Fig. 1. Frequency distribution of pod number per plant, seed number per plant and seed number per pod of 117 recombinant inbred lines (RILs) of 'Keunolkong' × 'Shinpaldalkong'.

number per plant was ranged from 8.67 to 61.37, seed number per plant was 19.0 to 124.79 and seed number per pod was distributed from 1.37 to 2.58. Pod number per plant, seed number per plant and seed number per pod of 'Shinpaldalkong' (46.15, 93.6 and 2.04, respectively) were higher than that of 'Keunolkong' (18.11, 34.20 and 1.89, respectively). Pod number per plant, seed number per plant and seed number per pod showed normal distribution that is a typical of polygenic inheritance. There was transgressive segregationfor all traits suggesting that it will be possible to advance by breeding efforts.

The pod number per plant content was positively correlated with seed number per plant (r=973, p<0.001), but seed number of pod was negatively correlated (r=-0.267, p<0.01) (Table 2). Chang et al. [1] reported seven quantitative traits except 100 seed weight were effect for factor that is concerned in yields. Degree of correlation of pod number per plant and seed number per plant etc. showed high among them. Therefore, may increase effects of breeding selection if use QTLs associated with these traits.

Distribution of QTLs associated with pod number per plant, seed number per plant and pod

The linkage map of K/S population consisted with 19

Table 2. Correlation coefficients among pod number per plant, seed number per plant, seed number per pod and seed weight in 117 RILs of 'Keunolkong' × 'Shinpaldalkong

	Pod number per plant	Seed number per plant	Seed number per pod
Seed numbe per plant	0.973***		
Seed number per pod	-0.267**	-0.047ns	
Seed weight (g/100 seeds)	-0.395***	-0.412***	-0.075ns

: significant at the 0.01% level, * Significant at the 0.001% level, ns: Not significant ($P > 0.05$)

linkage groups spanning 1,890 cM of map distance that contained 110 markers, including 108 SSR markers and two morphological markers. The average map distance between markers was 17.2cM. Here, we present LGs those include tentative QTLs (Fig. 2).

The pod number per plant was distributed between 8.67 and 61.37 in the K/S population (Table 1). According to the SF-ANOVA analysis, seven markers were found significantly associated with pod number per plant. MLG-Regr analysis showed that two QTLs markers, satt510 on LG F and sct 010 on LG L were significantly associated with pod number per plant (Table 3). One major

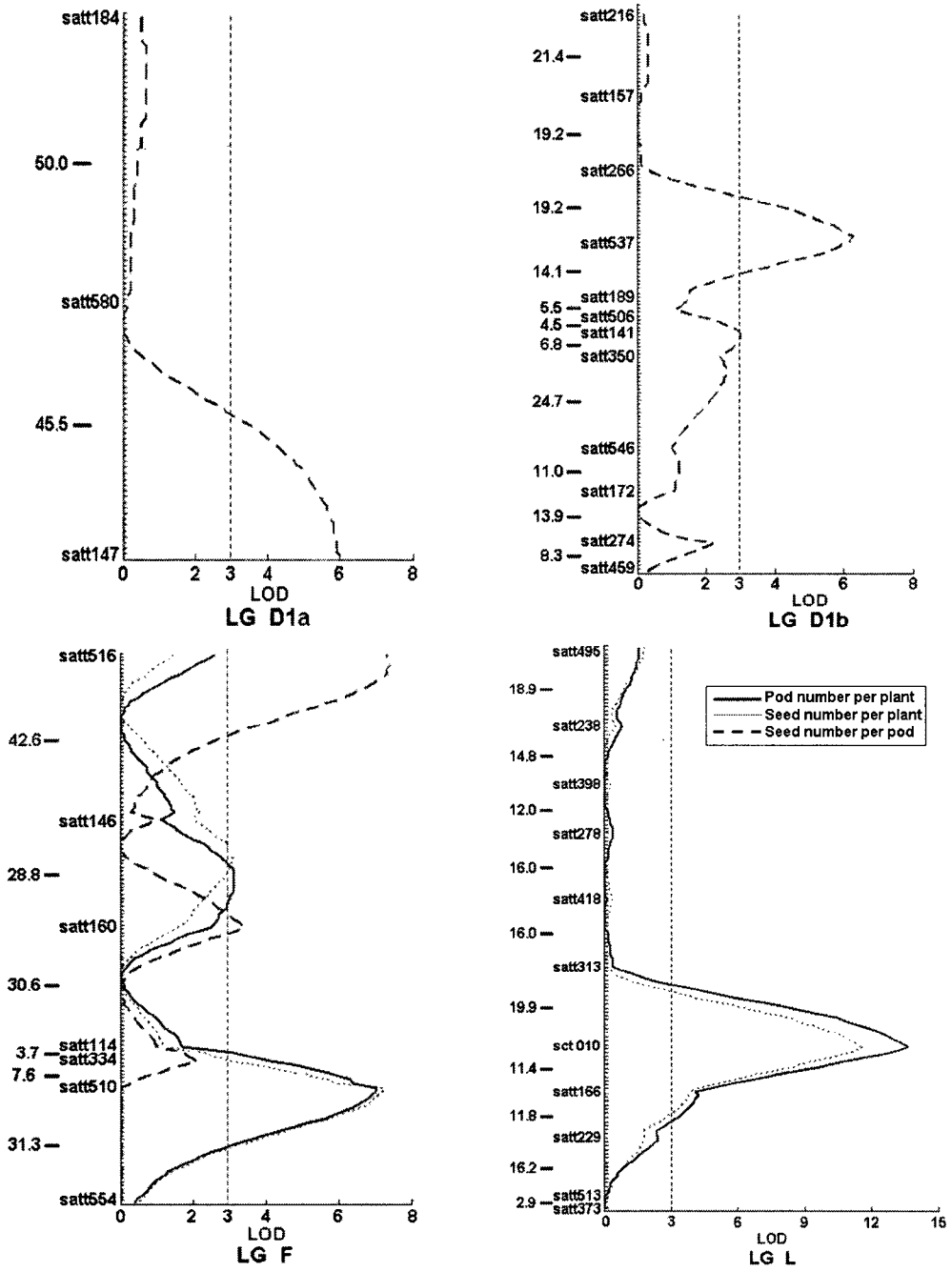

Fig. 2. Map location of QTLs determining pod number per plant, seed number per plant and seed number per pod of 117 RILs of 'Keunolkong' × 'Shinpaldalkong'. LOD score profile from composition interval mapping of pod number per plant, seed number per plant and seed number per pod on LG D1a, D1b, F and L. The y-axis indicated to genetic map with distance expressed in centiMorgan(cM). The vertical dotted line indicated to the LOD value threshold of 3.0.

Table 3. Marker distributions and QTLs associated with the pod number per plant, seed number per plant and seed number per pod of 117 RILs of 'Keunolkong' × 'Shinpaldalkong

Traits	LG	Markers	SF-ANOVA[a]		Alleic means		SLG-Regra		MLG-Regra	
			P	R^2 (%)	K/K[c]	S/S[c]	P	R^2 (%)	P	R^2 (%)
Pod number per plant	F	satt334	0.039	3.84	20.87	27.28	-	-	-	-
	F	satt510	0.016	5.28	31.35	27.11	0.013	5.63	0.007	6.55
	J	satt287	0.019	5.25	30.84	26.67	0.021	5.24	-	-
	J	satt285	0.038	3.93	30.87	27.22	-	-	-	-
	J	satt132	0.048	3.54	30.49	26.99	-	-	-	-
	J	satt215	0.025	4.47	30.69	26.79	-	-	-	-
	L	sct 010	<0.001	12.11	25.21	31.66	NA[b]	-	0.001	10.07
Seed number er plant	F	satt510	0.017	5.21	62.43	54.20	NA	_	0.010	6.16
	I	satt440	0.024	4.60	61.47	53.79	NA	-	-	-
	J	satt287	0.017	5.50	61.59	53.32	0.037	4.45	-	-
	J	satt285	0.024	4.63	61.87	54.14	-	-	-	-
	J	satt406	0.049	3.73	60.24	53.32	-	-	-	-
	J	satt215	0.018	5.02	61.40	53.35	-	-	-	-
	L	sct 010	<0.001	10.52	50.99	62.74	NA	-	0.004	8.19
Seed number per pod	A1	satt276	0.219	4.86	1.96	2.03	NA	-	-	-
	B2	satt556	0.023	4.85	2.04	1.96	NA	-	-	-
	D1a	satt147	0.016	5.29	2.04	1.96	NA	-	0.030	4.96
	D1b	satt537	0.014	5.73	1.96	2.03	NA	-	0.027	4.95
	F	satt516	0.008	6.56	1.98	2.05	NA	-	0.018	6.22

[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, NA: Not applicable. Not linked to other markers.
[b]K/K: Keunolkong, S/S: Shinpaldalkong.

QTL, sct 010 in LG L, explained 10.07% of phenotypic variation while other showed only minor effects. The QTLs LG A1, C1, C2 and I were consistent with those reported by Zhang et al. [23]. However we did not find any QTLs for pod number per plant on LG A1, C1, C2 and I in this present investigation.

There were no available data from previous studies as to the QTLs for seed number per plant and seed number per pod. In this study, seed number per plant was distributed between 19.0 and 124.79 in the K/S population (Table 1). According to the SF-ANOVA analysis, seven markers were found significantly associated with seed number per plant. MLG-Regr analysis showed that two QTLs markers, satt510 on LG F and sct 010 on LG L were significantly associated with seed number per plant (Table 3). One major QTL, sct 010 in LG L, explained 8.19% of phenotypic variation while other showed only minor effects.

Seed number per pod of RIL were distributed between 1.37 and 2.58 (Table 1). Fiver SSR markers were significantly related with seed number per pod in SF-ANOVA analysis with R^2 = 4.85 to 6.56%. Three minor QTLs for seed number per pod were selected after MGL-Regr analysis (Table 3).

The results of this study reveal a highly positive correction (r=0.973) between pod number per plant and seed number per plant. Two common QTLs on LG F and L are associated with both pod number per plant and seed number per plant. The QTLs located between sct010 and satt166 on LG L ,and satt510 and satt554 on LG F were associated with both traits (Fig. 2). Pod number per plant and seed number per plant we observed that same allele effects depended on the chromosomal background. For example, 'Keunolkong' -derived marker allele on LG F increased the pod and seed number per plant. The same effect was seen with the 'Shinpaldlakong' -derived marker allele on LG L. In contrast, the allele effects derived from 'Shinpaldlakong' in LG F were weaker than that of 'Keunolkong' for increasing either pod and seed number per plant. These QTLs add to yield component traits information on the genetic control of these traits.

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초록 : 콩에서 수량구성요인과 관련된 양적형질유전자좌의 분석

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콩에서 종자의 수와 협의 수가 주된 수량구성요소이다. 그리고 육종의 주된 목표는 수량을 증가시키기 위한 요인들을 선발하는 것이다. 따라서 본 연구의 목적은 큰올콩과 신팔달콩의 F_{10}세대의 RIL계통을 이용하여 주당협수와 주당립수 및 협당립수를 조절하는 양적형질유전자좌(QTL)를 확인하는 것이다. 주당협수와 연관된 QTL은 개별 마커들과의 분산분석 결과, 연관군 F와 L에서 2개의 QTL을 탐색하였으며, 주당립수도 연관군 F와 L에서 2개의 QTL을 탐색하였다. 협당립수는 연관군 D1a와 D1b 및 F에서 3개의 QTL을 탐색하였다. 따라서 주당협수와 주당립수와 관련된 QTL 연관군 F와 L에서 공통으로 탐색되었는데, 이는 품종 육성과정에서 이들 형질과 관련한 선발 마커로서 활용 가치가 높은 것으로 판단된다.