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Identification of Quantitative Trait Loci Associated with Traits of Soybean for Sprout

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ABSTRACT

The identification of quantitative trait loci (QTL) has the potential to enhance the efficiency of improving food processing traits of soybean. In this study, 92 restriction fragment length polymorphism (RFLP) loci and two morphological markers (W_1 and T) were used to identify QTL associated with food processing traits of soybean for sprout in 83 F_2-derived lines from a cross of 'Pureun' x 'Jinpum 2'. The genetic map consisted of 76 loci which covered about 760 cM and converged into 20 linkage groups. Eighteen markers remained unlinked. Phenotypic data were collected for hypocotyl length, abnormal seedling rate, and sprout yield seven days after seed germination at 20℃. Based on the single-factor analysis of variance, eight independent markers were associated with hypocotyl length. Four of seven markers associated with abnormal seedling rate were identified as independent. Seven loci were associated with sprout yield. For three different traits, much of genetic variation was explained by the identified QTL in this population. Several RFLP markers in linkage group (LG) B1 were detected as being associated with three traits, providing a genetic explanation for the biological correlation of sprout yield with hypocotyl length ($r=0.407^{***}$) and with abnormal seedling rate ($r=-406^{***}$).

Keywords : quantitative trait loci, restriction fragment length polymorphism, sprout, hypocotyl length, abnormal seedling rate, sprout yield.

Soyfood has received much more attention, primarily due to its beneficial effects on human health. A large number of food preparations can be made from soybean for the human diets. Of the soybean products extensively used in the Orient such as soybean curd, soymilk, fermented food products, germinated proudcts, and soybean for cooking with rice, soybean sprout is a year-round vegetable (Wijeratne and Nelson, 1986). Also, soybean sprout is a popular food type in the Orient, especially in Korea.

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Soybean for sprout was characterized with small seed size of seeds, which was less than 12 gr in 100-seed weight (Kwon et al., 1972). This may be due to higher germination rate in small seed size of soybean (Kim et al., 1994). Also, it was general that small seed size of soybean gave the higher sprout yield. In addition to seed size, of importance are several major traits of soybean sprouts such as hypocotyl length, seed germinability, water absorption rate, and sprout yield(Kim et al., 1994; Kim, 1981). Kwon et al. (1981) reported that significant differences in sprout yield, hypocotyl length, and seed germinability among soybean genotypes. Also, improvement in seed germination rate was thought to be easy (Green and Pinnell, 1968). However, in spite of significant genotypic variation in traits associated with soybean sprout, soybean breeders have neglected soybean breeding programs for improving sprout traits of soybean seeds. These have been limited due to multiple gene control of these traits as well as the time consuming and expensive procedures for measuring cooking qualities. In addition, the quality determination of soybean for sprout requires a large amount of seed and destructive to the viability of the seed.

With the development of molecular markers, quantitative trait loci (QTL) can be identified in the plant genome (Tanksley et al., 1989). Desirable genes, associated with food processing traits, can be selected via their linkage to easily detectable markers. In our previous papers (Lee et al., 1997a; 1997b), genetic map was constructed using RFLP from a cross between special food-type soybean cultivars. One of soybean population was developed by crossing Pureun, a recommended soybean variety for sprout in Korea with Jinpum 2. Related to that, the genetic map based on this population was suggested to be useful to characterize the QTL associated with soybean sprout traits.

In this study, 92 RFLP loci and two morphological markers (W_1 and T) were used to identify QTL associated with hypocotyl length, abnormal seedling rate, and sprout yield in an F_2-derived soybean population generated from a cross between Pureun and Jinpum 2, which were major traits of soybean sprout.

MATERIALS AND METHODS

An F_2 soybean population, developed from a cross of Pureun x Jinpum 2, was used to construct genetic

linkage map. A total of 100 lines were developed from this population. Genetic segregation analysis for RFLP of each line of the population has been determined previously (Lee et al., 1997) using polymorphic probes from various sources, including cDNA and/or genomic clones of *Glycine max* (R. C. Shoemaker, USDA/Iowa State Univ.; K. G. Lark, Univ. of Utah; R. T. Nagao, Univ. of Georgia), *Vigna radiata* (N. D. Young, Univ. of Minnesota), *Phaseolus vulgaris* (J. M. Tohme, CIAT), *Arachis hypogaea* (G. D. Kochert, Univ. of Georgia), and *Medicago sativa* (G. D. Kochert). The DNA isolation, Southern blotting, and hybridization procedures have been described previously (Lee et al., 1997a). For the two pigmentation loci, flower color (W_1) and pubescence color (T), segregation data were also obtained in the F_3 population, and used to construct the genetic map. The linkage map, constructed using the Kosambi map function of Mapmaker in a previous paper (Lee et al., 1997a; 1997b), was used to determine the putative independent QTL, which was 30cM from another marker significantly associated.

The parental and F_4 seeds from each line were tested for traits associated with soybean sprout. For phenotypic trait measurements, 50 seeds per each line were germinated at 20℃ in the dark for 6 days with three replications. Then, data were collected for hypocotyl length, abnormal seedling rate, and sprout yield. Hypocotyl length was measured as the average length of plants from the top of root to the cotyledon. Abnormal seedling rate was recorded as the percentage of the seeds decayed plus hypocotyl-stunted to the total number of seeds. Sprout yield was expressed as the percentage of the total fresh weight of soybean sprout to the dry weight of seed material.

The association between marker and QTL was tested by single-factor ANOVA. For each of the 92 RFLP and two morphological marker loci, the marker class means were compared for the determination of significant difference ($P \leq 0.05$) using an F-test from the mean squares obtained from the GLM procedure of SAS (SAS Institute, Cary, NC). Two-way analysis of variance was also used to detect epistatic interactions between markers with significant associations.

RESULTS AND DISCUSSION

Genetic map

A soybean genetic linkage map was constructed with a total of 92 RFLP markers and two morphological loci for the Pureun × Jinpum 2 population using Mapmaker. The genetic map from this population, which had been described previously (Lee et al., 1997b), consisted of 76 markers which were genetically linked and were placed into 20 linkage groups covering 760 cM. Eighteen markers remained unlinked.

Hypocotyl length

Pureun and Jinpum 2 differed by 1.8 cm in hypocotyl length 6 days after germination. Wide variation (8.6 - 13.4 cm) occurred in hypocotyl length among progenies (Table 1). A total of 10 markers were associated with hypocotyl length (Table 2). Of these ten markers, eight represent putative independent QTL, and individually accounted for 5.6 to 11.0 % of the phenotypic variation. At all independent loci except W_1 in linkage group (LG) F, the Pureun allele did not increase hypocotyl length. The amount of variation explained by the seven independent markers sums to 69.8%. The heritability of hypocotyl length was 57%. Thus when combined, these seven markers explain most of the genetic variation for this trait.

Only three (Blt51-62n/A135-1, W1/A135-1, and Blt51-10n/A135-1) of the possible 28 two-way epistatic interactions were significant (Table 3). It is interesting to note that one of two markers was A135-1 in all significant epistatic combinations. The interaction between Blt51-62n/A135-1 loci resulted in greater hypocotyl length when in combination with the Jinpum 2 allele at the Blt51-62n and A135-1. The same trend was observed in the interaction between Blt51-10n/A135-1. Contrary to this, hypocotyl length was reduced in the interaction between W_1/A135-1 when the Jinpum 2 allele was in both two loci.

Abnormal seedling rate

Pureun and Jinpum 2 did not differ in abnormal

Table 1. Means and ranges of parental and F_2-derived progeny for the major traits, and their heritabilities.

Genotypes	Traits		
	Hypocotyl length	Abnormal seedling rate	Sprout yield
	cm	%	%
Pureunkong	12.9	1.67	515
Jinpumkong 2	11.1	1.67	452
Progeny range	8.6 ~ 13.4	0 ~ 16.7	400 ~ 621
Progeny mean	11.6	5.12	504
$LSD_{0.05}$	1.73	9.39	52.8
h^2(%)	57.0	44.4	80.9

Table 2. RFLP markers associated with variation in hypocotyl length.

RFLP locus	Linkage group[a]	*P*	R^2 (%)	Genotypic means[b](cm)		
				A/A	A/B	B/B
Blt51-62n[c]	B1	0.003	10.6	11.5		12.1
K011-2n[c]	B1	0.007	8.8	11.2	11.8	
Bng088-1	B1	0.042	7.7	11.3	11.6	12.0
A121-1[c]	C2	0.019	9.6	11.5	12.0	11.4
W_1[c]	F	0.017	9.9	12.1	11.8	11.3
Cr321-1	F	0.033	8.4	12.2	11.6	11.4
A199-2n[c]	F	0.038	5.8	11.3	11.8	
Blt51-10n[c]	L	0.033	5.6	11.6		12.1
Bng222-1[c]	M	0.035	8.2	11.7	11.9	11.3
A135-1[c]	Unlinked	0.011	11.0	11.6	11.9	11.1

[a] Based on the designation of Shoemaker and Specht (1995).
[b] A/A:homozygous Pureun; B/B:homozygous Jinpum 2.
[c] Putative independent QTL.

Table 3. Epistatic interactions between two markers associated with hypocotyl length.

Locus	Allele	Allele/Locus			*P*	R^2 (%)
		A/A[a]	A/B	B/B		
		------ cm -----				
		A135-1				
Blt51-62n/A135-1						
Blt51-62n	A/A or A/B	11.3	11.8	10.9	0.019	26.9
	B/B	12.0	12.0	12.9		
		A135-1				
W_1/A135-1						
W_1	A/A	12.1	12.0	11.8	0.019	31.5
	A/B	11.4	11.9	11.7		
	B/B	11.3	11.8	10.1		
		A135-1				
Blt51-10n/A135-1						
Blt51-10n	A/A or A/B	11.6	11.8	10.6	0.007	28.9
	B/B	11.5	12.1	12.3		

[a] A/A:homozygous Pureun; B/B:homozygous Jinpum 2.

Table 4. RFLP markers associated with variation in abnormal seedling rate.

RFLP locus	Linkage group[a]	*P*	R^2 (%)	Genotypic means[b](%)		
				A/A	A/B	B/B
A109-1[c]	B1	0.001	16.9	5.1	3.9	9.2
Bng119-1	B1	0.009	11.3	5.2	3.9	7.8
A381-1	B1	0.033	8.3	4.9	4.1	7.6
B031-1	B1	0.032	8.3	4.9	4.1	7.5
A757-2[c]	L	0.038	8.3	6.2	3.9	6.8
K418-2n[c]	N	0.028	5.8	7.0	4.5	
Bng068-1[c]	Unlinked	0.028	8.8	4.0	4.6	7.4

[a] Based on the designation of Shoemaker and Specht (1995).
[b] A/A: homozygous Pureun; B/B: homozygous Jinpum 2.
[c] Putative independent QTL.

Table 5. RFLP markers associated with variation in sprout yield.

RFLP locus	Linkage group[a]	P	R^2 (%)	Genotypic means[b](%)		
				A/A	A/B	B/B
A089-1[c]	B1	0.005	12.9	485	507	525
Bng205-1[c]	B1	0.025	8.9	488	502	525
A708-2[c]	F	0.041	7.7	526	504	492
A235-1[c]	G	0.005	12.6	528	500	486
Bng205-2[c]	G	0.012	10.6	528	492	506
L154-1	G	0.012	10.4	526	502	487
A757-2[c]	L	0.001	15.9	481	519	489

[a] Based on the designation of Shoemaker and Specht (1995).
[b] A/A:homozygous Pureun; B/B:homozygous Jinpum 2.
[c] Putative independent QTL.

Table 6. Epistatic interactions between two markers associated with sprout yield.

Locus	Allele	Allele/Locus			P	R^2 (%)
		A/A[a]	A/B	B/B		
		---------- % ----------				
		A235-1				
A708-2/A235-1						
A708-2	A/A	540	511	519	0.036	29.8
	A/B	547	497	477		
	B/B	475	501	470		

[a] A/A:homozygous Pureun; B/B:homozygous Jinpum 2.

seedling rate, which was 1.67% in both varieties. However, transgressive variation of 16.7% occurred among progenies(Table 1). Of the seven RFLP loci associated with abnormal seedling rate, four markers were genetically independent (Table 4). At all loci except K418-2n, the Jinpum 2 allele increased the abnormal seedling rate. The total amount of variation explained by the four independent markers was 39.8%. The heritability of abnormal seedling rate was 44.4%, indicating that much of genetic variation was explained by these four independent markers. There were no epistatic interactions identified among the six two-way combinations between two independent markers.

Sprout yield

Pureun and Jinpum 2 differed by 63% in sprout yield. However, transgressive variation of 221% occurred among progenies (Table 1). For sprout yield, seven markers were detected. Six of these markers were identified as independent (Table 5). The Pureun allele increased sprout yield at three independent marker loci (A708-2, A235-1, and Bng205-2) in LG F and G, whereas the Jinpum 2 allele did at two independent marker loci (A089-1 and Bng205-2) in LG B1. Summed together, these six independent markers explained 69% of the total variation for sprout yield. Of the three traits associated with soybean sprout, higher heritability was observed in sprout yield. The heritability of sprout yield was 80.9%, suggesting that, though not complete, most of genetic variation for sprout yield was explained by these six independent QTL.

Of the possible 21 two-way epistatic interactions, only one (A708-2/A235-1) was significant (Table 6). When a line contained the Pureun alleles at both the A708-2 and A235-2 locus, its sprout yield was greater than would be expected due to additive gene action. Lines containing the Pureun alleles at the A235-1 locus and heterozygous alleles at the A708-2 also showed greater sprout yield than would be predicted.

Relationship among traits

Hypocotyl length, abnormal seedling rate, and sprout yield were interrelated in that higher sprout yield showed a positive association with greater hypocotyl length ($r=0.407^{***}$) and lower incidence of abnormal seedling rate ($r=0.406^{***}$). Several RFLP markers in LG B1 were detected as being associated with three traits. These loci could provide genetic basis for the correlations among three traits.

Soybean was known to have a fairly large genome size (about 3,000 cM). However, the results from this study support that QTL detected from about 100 markers mapped in a soybean population were enough

to explain the genetic variation for polygenic traits associated with food processing traits of soybean for sprout. The data also provide evidence for a marker-assisted selection for polygenic traits in a segregating population of soybean breeding program.

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