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Genetic mapping of QTLs conditioning soybean sprout yield and quality

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Abstract Soybean sprouts have been used as a food in the Orient since ancient times. In this study, 92 restriction fragment length polymorphism (RFLP) loci and two morphological markers (*W1* and *T*) were used to identify quantitative trait loci (QTLs) associated with soybean sprout-related traits in 100 F_2-derived lines from the cross of 'Pureunkong' × 'Jinpumkong 2'. The genetic map consisted of 76 loci which covered about 756 cM and converged into 20 linkage groups. Eighteen markers remained unlinked. Phenotypic data were collected in 1996 and 1997 for hypocotyl length, percentage of abnormal seedlings, and sprout yield 6 days after germination at 20°C. Hypocotyl length was determined as the average length from the point of initiation of the first secondary root to the point of attachment of the cotyledons. The number of decayed seeds and seedlings, plus the number of stunted seedlings (less than 2-cm growth), was recorded a s abnormal seedlings. Seed weight was determined based on the 50-seed sample. Sprout yield was recorded as the total fresh weight of soybean sprouts produced from the 50-seed sample divided by the dry weight of the 50-seed sample. Four QTLs were associated with sprout yield in the combined analysis across 2 years. For the QTL linked to L154 on the Linkage Group (LG) G the positive allele was derived from Pureunkong (R^2 = 0.19), whereas at the other three QTLs (A089 on LG B1, A668n on LG K and B046 on LG L) the positive alleles were from Jinpumkong 2. QTLs conditioning seed weight were linked to markers A802n (LG B1), A069 (LG E), Cr321 (LG F) and A235 (LG G). At these four markers, the Jinpumkong allele increased seed weight. Markers K011n on LG B1, *W1* on LG F and A757 on LG L were linked to QTLs conditioning hypocotyl length; and Bng119, K455n and K418n to QTLs conditioning the abnormal seedlings. The QTLs conditioning sprout yield were in the same genomic locations as the QTLs for seed weight identified in this population or from previously published research, indicating that QTLs for sprout yield are genetically linked to seed-weight QTLs or else that seed-weight QTLs pleiotropically condition sprout yield. These data demonstrate that effective marker-assisted selection may be feasible for enhancing sprout yield in a soybean. The transgressive segregation of sprout yield, as well as the existence of two QTLs conditioning greater than 10% of the phenotypic variation in sprout yields provides an opportunity to select for progeny lines with a greater sprout yield than currently preferred cultivars such as Pureunkong.

Keywords Soybean · Quantitative trait loci · Restriction fragment length polymorphism · Sprouting · Hypocotyl length · Abnormal seedling · Sprout yield · Single factor analysis of variance · Multiple regression analysis

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Introduction

Soybean seed is rich in protein and oil and is extensively used for human and animal consumption. Data showing the beneficial effects on human health of soybean in the diet has increased interest in and the consumption of soybean foods (Kitamura 1995). In the Orient soybean seeds are utilized in a large number of human foods, such as soybean curd, soymilk, soybean sprout, fermented food products, and soybean for cooking with rice. In many Asian countries germinated soybean sprouts are

served as a vegetable throughout the year, and they have been used in soups, salads and side dishes (Wjeratne and Nelson 1986; Liu 1997).

In Asia soybean sprouts are generally prepared by the following procedure (Liu 1997): (1) Soybean seeds were soaked in water at room temperature for 4 to 5 h, washed, and spread in several layers in a container with holes at the bottom for water drainage, (2) the container is then covered with a cloth and placed in a dark room, (3) seeds in the container are watered three to four times a day, (4) after 6 to 7 days at room temperature, the sprouts reach a seedling length of about 8 cm, and (5) they are then washed, de-hulled, and are ready for serving.

Soybean seeds for sprout production should possess a small seed weight. Kwon et al. (1972) reported that less than 120 mg seed^{-1} is desirable for producing sprouts. This may be partially due to the higher germination rate and the sprout yield of small-seeded cultivars (Kim et al. 1994). Other important traits of soybean seed utilized for sprouts include: hypocotyl length, seed germination, water absorption rate and sprout yield (Kim 1981; Kim et al. 1994).

Kwon et al. (1981) reported that there were significant differences in sprout yield, hypocotyl length and seed germination among soybean genotypes. Green and Pinnell (1968) found that selection was feasible for improved seed germination. However, in spite of significant genotypic variation in traits associated with soybean sprouts, soybean breeders have neglected selection of the germination-related traits for improving sprout production. Selection for these traits has been limited due to multiple gene control, as well as the time-consuming and expensive procedures for measuring these traits (Tan et al. 1999). In addition, the quality determination of soybean sprout requires a large amount of seed and is destructive to the viability of the seed. Thus, selection for these traits has been made only on the advanced lines on the basis of a seed germination test in a soybean breeding program for sprout.

With the advent of DNA marker technology, quantitative trait loci (QTLs) 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 paper (Lee et al. 1997), a genetic map was constructed using RFLP markers in a cross between two food-type soybean cultivars. A soybean population was developed using Pureunkong (Kim et al. 1996), a recommended soybean cultivar for sprout production in Korea, and Jinpumkong 2, which lacks seed lipoxygenase enzymes (Kim et al. 1997).

In this study, RFLP mapping was utilized to identify QTLs conditioning hypocotyl length, abnormal seedlings, seed weight and sprout yield in the F_2-derived lines created from a cross between Pureunkong and Jinpumkong 2. A secondary objective was to determine the relationship among sprout yield, seed weight, and sprout quality traits.

Materials and methods

One hundred F_2 plants from the cross of Pureunkong × Jinpumkong 2 were used to construct a genetic linkage map, and $F_{2:4}$ and $F_{2:5}$ seeds were employed for phenotypic evaluation of sprout yield and sprout quality traits. Pureunkong was selected as a parent for its small seed, green seed coat and green seed embryo, which are considered desirable seed traits for producing soybean sprouts (Kim et al. 1996). Jinpumkong 2 produces a high quality seed that lacks the beany taste of common cultivars (Kim et al. 1997). Both parents were chosen for their different soy food traits.

The procedures of RFLP mapping including DNA isolation, Southern blotting, and hybridization have been described previously (Lee et al. 1996a, b; Mian et al. 1996a). Briefly, RFLPs were surveyed from DNA isolated from lyophilized young leaves of parents grown in the greenhouse. The DNA was isolated from leaves according to the procedure of Keim et al. (1988), and digested overnight with each one of five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII or *Taq*I). Following electrophoresis of DNA fragments, a Southern blot was made by transfer to an uncharged nylon membrane. About 25 ng of the isolated DNA probe were labelled with ^{32}P using a random primer procedure, and hybridization was conducted overnight. RFLP markers that were polymorphic between the parents were surveyed on the 100 F_2 plants.

Data for two pigmentation markers, flower color and pubescence color, were also obtained. It was assumed that flower color (white or purple) was conditioned by the *W* locus and pubescence color (tawny or gray) by the *T* locus. The classification of flower and pubescence color on these $F_{2:3}$ lines allowed the identification of lines that were derived from either homozygous or heterozygous F_2 plants. A linkage map was constructed with RFLP and pigmentation marker data using the Kosambi map function of Mapmaker (Lander et al. 1987). For grouping markers into linkage groups, a minimum LOD of 3.0 and a maximum distance of 40 cM were used.

In 1996 and 1997 the parents and the F_2-derived lines were grown at the National Crop Experiment Station, Suwon, Republic of Korea. Seeds were planted in mid-May in 1996 and 1997. The experimental plot was arranged in a randomized complete block (RCBD) design with three replications. Seeds harvested in 1996 and 1997 from each line were evaluated for traits associated with sprout yield and quality. Soybean sprouts were produced in a growth chamber. The experimental unit consisted of a 50-seed sample of a line. The 50-seed samples were placed in a paper towel, were sprayed with water, wrapped with saran wrap to maintain near 100% relative humidity, and were germinated at 20°C in the dark for 6 days. After 6 days, the sprouts were transferred into the laboratory and data were collected for hypocotyl length, abnormal seedlings and sprout yield. Hypocotyl length was determined as the average length from the point of initiation of the first secondary root to the point of attachment of the cotyledons on ten seedlings. The number of decayed seeds and seedlings, plus the number of stunted seedlings (less than 2-cm growth) out of 50 seeds, was recorded as abnormal seedlings. Seed weight was determined only in 1996 based on the 50-seed sample. Sprout yield was recorded as the total fresh weight of soybean sprouts produced from the 50-seed sample divided by the dry weight of the 50-seed sample (g fresh weight per g dry weight). Methods employed for analyses of variance, regression, and heritability were reported previously (Bianchi-Hall et al. 1998). For the analysis of variance model blocks, years, and lines were considered random effects. The GLM procedure of SAS was used for statistical analyses (SAS 1990). Variance components for estimating heritability were obtained using the VARCOMP procedure of SAS (SAS 1990).

The associations between markers and sprout traits were evaluated using single-factor analysis of variance (SF-ANOVA) for data within each year and combined across years. For each of the RFLP and morphological markers, the marker class means for sprout traits (A_1A_1; A_1A_2; A_2A_2) were compared for significance ($P < 0.05$) using an *F*-test from the Type-III mean squares obtained from the GLM Procedure of SAS (SAS 1990). In addition, two-factor analysis of variance was used to detect significant

($P < 0.05$) interactions (i.e. epitasis) between all possible pairs of significant markers.

If SF-ANOVA identified two or more linked markers associated with the same sprout trait, a multiple regression analysis was conducted by including all the significant markers on that linkage group in the model (SLG-Regr). Forward and stepwise selection procedures were applied in the regression analysis. The significant ($P < 0.05$) markers that were retained in the SLG-Regr analysis were assumed to identify unique QTLs on that linkage group. All significant markers from the SLG-Regr analyses and unlinked single markers identified from SF-ANOVA were combined in a multiple linkage-group regression model (MLG-Regr) at $P < 0.05$ to determine the combination of independent markers that explained the greatest amount of phenotypic variation in a given trait. This probability level was selected to enhance our ability to detect QTLs associated with sprout-related traits. The coefficient of determination (R^2) from MLG-Regr was used to provide an estimate of the percent of phenotypic variation explained by the markers.

Results and discussion

Genetic map

The DNA of Pureunkong and Jinpumkong 2 was digested with five restriction enzymes and analyzed for polymorphisms. A RFLP was detected with 125 of the 246 probes. Thus, 51% of the probes detected a polymorphism with at least one of the five restriction enzymes, which is higher than in previou s studies (Apuya et al. 1988; Concibido et al. 1994; Lee et al. 1996a). This higher polymorphism frequency is probably due to the larger amount of genetic diversity between Pureunkong and Jinpumkong 2. Of the 92 markers, 63 were expressed in a co-dominant manner.

The genetic linkage map from the Purenukong × Jinpumkong 2 population has been described previously (Lee et al. 1997). Briefly, a total of 92 RFLP markers and two pigmentation loci (*W1* and *T*) were used to create the genetic map. The genetic map consisted of 76 linked markers which formed 20 linkage groups and accounted for a total of 756 cM. Eighteen markers remained unlinked. The flower-color marker mapped on Linkage Group (LG) F at the same location as the *W1* locus, and pubescence color mapped on LG C2 at the previously reported location of the *T* locus (Cregan et al. 1999).

Sprout yield

There was significant variation in sprout yield among the lines (Table 1), which had a range of 2.6 g with some lines producing up to a 1.4-g greater sprout yield than Pureunkong. Pureunkong yielded 4.9 g of sprouts compared with 4.1 g for Jinpumkong 2 (Table 2). None of the lines produced a significantly less sprout yield than Jinpumkong 2.

The SF-ANOVA identified ten markers as potentially associated with QTLs for sprout yield at $P < 0.05$ (Table 3). Individually, these markers accounted for 5 to 19% of the phenotypic variation. The Pureunkong alleles increased sprout yield at QTLs identified on LG G and M, whereas Jinpumkong 2 provided the positive alleles at QTLs on LG B1, K and L. There were no epistatic interactions identified among the 45 two-way combinations of the ten significant markers.

SLG-Regr for the five markers on LG G and the two markers on LG L retained L154 on LG G and B046 on LG L, indicating the existence of a single QTL for sprout yield on each linkage group. MLG-Regr analysis with

Table 1 Mean squares from the analysis of variance for sprout-related traits

Source	Sprout yield		Seed weight		Hypocotyl length		Abnormal seedling	
	df	Mean squares[a]	*df*	Mean squares[a]	*df*	Mean squares[a]	*df*	Mean squares[a]
Block			2	209*				
Year	1	136*			1	1,176*	1	48,503*
Block (Year)	4	9*			4	151*	4	2,639*
Lines	84	1*	84	3716**	84	3*	84	233**
Line × Year	84	0.3*			84	2	84	202
Error	349	0.2	180	50	346	1	349	141

[a] *, and ** significant at $P < 0.05$, and 0.01, respectively

Table 2 Means and ranges of parental and F_2-derived progeny for soybean sprout-related traits combined over 2 years

Item	Sprout yield (g fresh wt./ g dry wt.)	Seed weight (mg seed^{-1})	Hypocotyl length (cm)	Abnormal seedling (%)
Pureunkong	4.9	159	10.8	11
Jinpumkong 2	4.1	204	9.8	15
Progeny range	3.7–6.3	113–237	8.7–12.4	3–34
Progeny mean	4.5	184	10.2	14.4
$LSD_{0.05}$	0.7	8	1.5	16.4
H^2(%)	72	95	34	13

Table 3 Markers linked to QTLs associated with sprout yield in the combined data over 2 years from a F_2-derived population of Pureunkong × Jinpumkong 2

Markers	LG	SF-ANOVA[a]		Allelic means[b] (g fresh wt. / g dry wt.)			SLG-Regr[a]		MLG-Regr[a]	
		P	R^2 (%)	P/P	P/J	J/J	*P*	R^2 (%)	*P*	R^2 (%)
A089	B1	< 0.001	19	4.3	4.5	4.8	NA[c]	–	< 0.001	19
A235	G	0.009	11	4.7	4.5	4.4	–	–	–	–
Bng205	G	0.016	10	4.8	4.4	4.5	–	–	–	–
K493	G	0.024	9	4.8	4.5	4.4	–	–	–	–
A885	G	0.038	8	4.8	4.5	4.5	–	–	–	–
L154	G	0.039	8	4.7	4.5	4.4	0.002	12	0.001	11
A668n	K	0.035	5	4.5		4.7	NA	–	0.035	4
A757	L	0.019	10	4.3	4.6	4.5	–	–	–	–
B046	L	0.045	8	4.4	4.5	4.8	0.048	5	0.025	4
Bng222	M	0.039	8	4.5	4.6	4.4	NA	–	–	
Total										38

[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] P/P: homozygous Pureunkong, J/J: homozygous Jinpumkong 2, P/J heterozygous
[c] Not applicable. Not linked to other markers

Table 4 Markers linked to QTLs associated with seed weight from a F_2-derived population of a Pureunkong × Jinpumkong 2

Markers	LG	SF-ANOVA[a]		Allelic means[b] (mg seed^{-1})			SLG-Regr[a]		MLG-Regr[a]	
		P	R^2 (%)	P/P	P/J	J/J	*P*	R^2 (%)	*P*	R^2 (%)
A089	B1	0.013	11	191	185	173	0.004	10	–	–
A802n	B1	0.009	8	181		195	0.002	6	0.008	8
Bng068	B1	0.034	8	191	178	188	–	–	–	–
A095	D2	0.016	10	182	191	176	NA[c]	–	–	–
A069	E	0.015	10	180	181	196	NA	–	0.031	4
Cr321	F	0.005	13	171	184	198	NA	–	0.001	13
A235	G	0.035	8	176	185	192	NA	–	0.005	8
Total										33

[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] P/P: homozygous Pureunkong, J/J: homozygous Jinpumkong 2, P/J heterozygous
[c] Not applicable. Not linked to other markers

the five independent markers (A089, L154, A668n, B046 and Bng222) retained four of these five RFLP markers (Table 3). For the QTL linked to L154 the positive allele was derived from Pureunkong, whereas at the other three QTLs (A089, A668n and B046) the positive alleles were from Jinpumkong 2. The variance-component heritability of sprout yield (selection unit = 2 years, three replications/year) was 72% (Table 2), whereas the MLG-Regr accounted for 38% of the phenotypic variation. This would suggest that the other QTLs remain undetected, the markers were not linked to the four detected QTLs, the heritability estimate was poor, or that the epistatic effects remain undetected.

The mapping results from this study provide evidence that sprout yield can be improved though selection. For example, the sprout yield of Pureunkong, a cultivar currently used for sprout production, could be improved by the incorporation of the Jinpumkong 2 alleles at sprout yield QTLs linked to A089 on LG B1, A668n on LG K, and B046 on LG L.

Seed weight

The seed of Pureunkong averaged 45 mg seed^{-1} smaller than Jinpumkong 2 (Table 2). There were F_2-derived lines with up to 46-mg smaller seed than Pureunkong and 34-mg larger seed than Jinpumkong 2. The heritability for seed weight was 95% (selection unit = 1 year and three replications).

Based on the SF-ANOVA, seven markers were detected as potentially linked to QTLs for seed weight

Table 5 Markers linked to QTLs associated with hypocotyl length in the combined data over 2 years from a F_2-derived population of Pureunkong × Jinpumkong 2

Markers	LG	SF-ANOVA[a]		Allelic means[b] (cm)			MLG-Regr[a]	
		P	R^2 (%)	P/P	P/J	J/J	*P*	R^2 (%)
K011n	B1	0.013	11	9.9	10.4		0.006	10
K300	C1	0.009	8	10.2	10.5	10.0	–	–
W1	F	0.034	8	10.6	10.3	10.0	0.008	8
A757	L	0.016	10	10.0	10.2	10.7	0.026	6
Blt051	Unknown	0.015	10	10.3	10.4	9.9	–	–
Total								24

[a] SF-ANOVA: single factor analysis of variance, MLG-Regr: multiple regression with all significant markers from the SF-ANOVA

[b] P/P: homozygous Pureunkong, J/J: homozygous Jinpumkong 2, P/J heterozygous

(Table 4). Individually, these markers accounted for 8 to 13% of the variation among the F_2-derived lines. At two of the markers on LG B1 (A089 and Bng068) and the marker on LG D2, alleles from Jinpumkong 2 reduced the seed weight. At the other four markers the Pureunkong allele resulted in reduced seed weight. There were no epistatic interactions identified among the 21 possible two-way combinations between these seven markers.

SLG-Regr for the three markers on LG B1 retained markers A089 and A802n in the model (Table 4). This analysis indicated that A089 and A802n have detected unique QTLs. Additional support for two unique QTLs on LG B1 is provided by examination of the allelic means at these markers. At the QTL detected by A089 the allele for small seed is contributed by Jinpumkong 2, while the allele for small seed at A802n is contributed by Pureunkong. MLG-Regr analysis with A089, A802n and the other four markers on linkage groups D2, E, F and G confirmed that QTLs conditioning seed weight were linked to markers A802n (LG B1), A069 (LG E), Cr321 (LG F) and A235 (LG G). At these four marker loci, the Jinpumkong 2 allele increased seed weight. The multiple regression analysis accounted for 33% of the variation in seed weight among the progeny. These four markers accounted for approximately 35% (33/95 = 35%) of the genetic variation for seed weight. Thus, there are either undetected QTLs in this population or the four markers that detected seed-weight QTLs in this population are not closely linked to the detected QTLs.

Three of four seed weight QTLs detected in this study may have been identified in previous studies (Maughan et al. 1996; Mian et al. 1996b; Orf et al. 1999). The A802n marker for seed weight on LG B1 is approximately 20 cM from a seed-weight QTL identified by marker T028 in the Noir × Archer population (Orf et al. 1999) (Fig. 1). Cr321 on LG F is 6 cM from a seed-weight QTL detected with Blt025 in the Young × PI416937 population, and A235 on LG G was also detected as being associated with seed weight in the PI97100 × Coker 237 population (Mian et al. 1996b). Our study did identify a seed-weight QTL on LG E that has not been previously mapped.

Hypocotyl length

The hypocotyl length of Pureunkong was 10.8 cm, and that of Jinpumkong 2 was 9.8 cm (Table 2), There was significant variation among the F_2-derived lines for this trait (Table 1).

The initial SF-ANOVA analysis of hypocotyl length detected four RFLP markers and the *W1* marker as potentially linked to the QTLs conditioning hypocotyl length (Table 5). Each of these markers is located on a different linkage group. Individually, these markers accounted for 8 to 11% of the variation in hypocotyl length. At all QTLs, except for the QTL detected by Blt051, the Jinpumkong 2 allele increased hypocotyl length. All possible combinations of the five markers associated with hypocotyl length were tested for two-factor interactions to detect epistasis. Epistasis was not present for any of these markers.

Since each of these markers was unlinked, the five significant markers from the SF-ANOVA were included in the MLG-Regr analysis. This analysis confirmed the linkage of marker K011n on LG B1, W1 on LG F, and A757 on LG L to QTLs conditioning hypocotyl length. The heritability of hypocotyl length (selection unit = 2 years, three replications/year) was 34%, whereas the multiple regression analysis accounted for 24% of the phenotypic variation.

Abnormal seedlings

There was a large line × year interaction for abnormal seedlings (Table 1). Pureunkong and Jinpumkong 2 did not significantly differ in abnormal seedlings, but there was a range of 3 to 34% amon g the F_2-derived lines (Table 2). Some of the F_2 -derived lines did produce significantly more abnormal seedlings than either parent. The heritability of abnormal seedlings was only 13% (selection unit = 2 years and three replications/year).

Four markers were identified by SF-ANOVA as being potentially associated with QTLs conditioning abnormal seedlings (Table 6). There was no epistatic interaction

Table 6 Markers linked to QTLs associated with abnormal seedlings in the combined data over 2 years from a F_2-derived population of Pureunkong × Jinpumkong 2

Markers	LG	SF-ANOVA[a]		Allelic means[b] (%)			MLG-Regr[a]	
		P	R^2 (%)	P/P	P/J	J/J	*P*	R^2 (%)
Bng119	B1	0.045	8	12.1	14.6	16.9	0.013	8
K455n	C2	0.028	6	13.5		17.2	0.030	5
Bng222	M	0.005	12	15.0	12.0	17.2	-	–
K418n	N	0.032	6	16.8	13.4		0.008	8
Total								21

[a] SF-ANOVA: single factor analysis of variance, MLG-Regr: multiple regression with all significant markers from the SF-ANOVA

[b] P/P: homozygous Pureunkong, J/J: homozygous Jinpumkong 2, P/J heterozygous

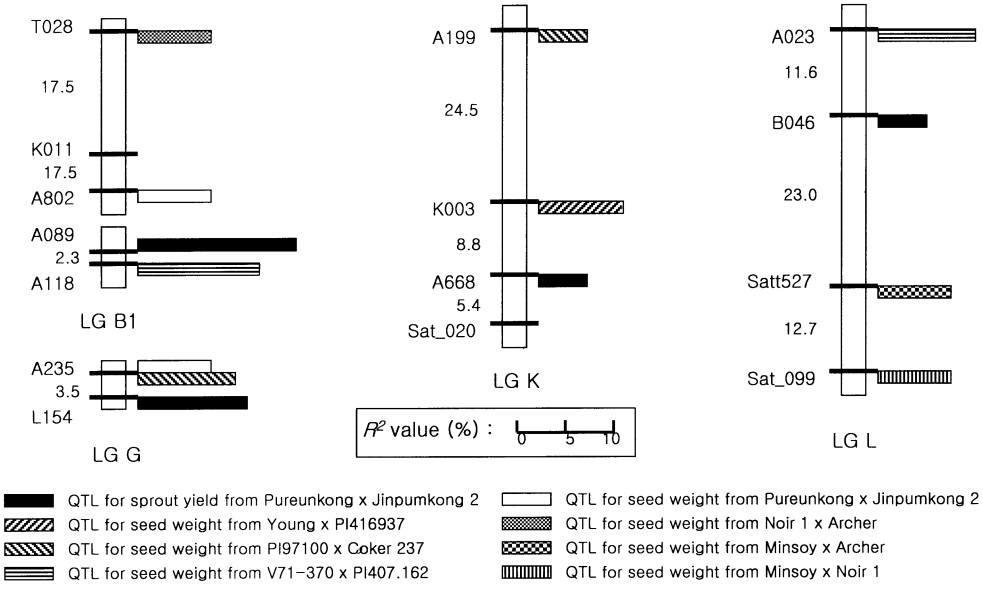

Fig. 1 Comparison of QTLs for sprout yield and seed weight from the Pureunkong and Jinpumkong 2 population with those for seed weight derived from Young × PI416937 and PI97100 × Coker 237 (Mian et al. 1996), V71–370 × PI407.162 (Maughan et al. 1996), and Noir 1 × Archer, Minsoy × Archer and Minsoy × Noir 1 (Orf et al. 1999). The composite map, showing marker positions and the estimated map distance (cM) on the left-hand side, was adapted from the public soybean map (Cregan et al. 1999) and is based on the average of recombination across populations. The length of horizontal bars indicates R^2 values for the loci

identified among the six two-way combinations of the four markers. At all marker loci, except K418n on LG N, the Pureunkong allele reduced the percent of abnormal seedlings. The four significant markers were included as independent variables in MLG-Regr analysis. This analysis confirmed the linkage of Bng119, K455n and K418n to QTLs conditioning the percent of abnormal seedlings. The multiple regression model accounted for 21% of the phenotypic variation (Table 6).

Relationship of sprout yield with other traits

It was of specific interest to determine the relationship of QTLs conditioning sprout yield to the QTLs for seed weight identified in this population or from previously published maps (Mansur et al. 1993, 1996; Maughan et al. 1996; Mian et al. 1996b; Orf et al. 1999). To determine if the QTLs for sprout yield and seed weight in this study were similar to those for seed weight from the previous studies, the relative QTL positions were drawn in Fig. 1 on the basis of the integrated genetic map (Cregan et al. 1999).

The A089 marker on LG B1 for sprout yield in the population used in this study was approximately 2 cM from the A118 marker for seed weight in the V71–370 × PI407.162 population (Maughan et al. 1996). L154 on LG-G, which was associated with sprout yield, was near the A235 locus for seed weight in the Pureunkong × Jinpoumkong 2 and PI97100 × Coker 237 populatio ns. The sprout yield QTL linked to A668 on LG K is near the K003 marker for seed weight in the Young × PI 416937 population. On LG L, B046 for sprout yield is near A023 and Satt527, which were associated with seed weight in the V71–370 × PI407.162 (Maughan et al. 1996) and the Minsoy × Archer (Orf et al. 1999) populations, respectively. The QTL for sprout yield detected by B046 was also in the same position as the QTL for seed weight in the genomic region between EV2 and *Dt1* detected in the PI 97100 × Coker 237 population using interval mapping (Mian et al. 1996b). The four QTLs for sprout yield identified in this study mapped near the QTLs for seed weight in this or pre-

Table 7 Effect of divergent selection for seed weight on sprout yield in the F_2-derived population from Pureunkong × Jinpumkong 2

Seed weight group	Number of lines	Seed weight (mg seed^{-1})		Sprout yield (g fresh wt. / g dry wt.)	
		Mean	Range	Mean	Range
Smallest	10	148	113–164	5.2	4.8–6.3
Largest	10	217	205–237	4.0	3.1–4.5
$LSD_{0.05}$		8		0.1	

vious studies, indicating that QTLs for sprout yield are genetically linked to the seed-weight QTLs or that differences in seed weight result in differences in sprout yield.

In this study seed weight was negatively associated with sprout yield (r = –0.763***). The strength of this association can be seen by results from divergent selection for weight among the 100 lines examined. The ten lines with the smallest seed weight averaged a 5.2-g sprout yield compared to an average of 4.0 g for the ten lines with the largest seed weight (Table 7).

Producers of soybean sprouts prefer cultivars with a small seed weight. It is generally accepted that sprout yield is greater when smaller seeds are germinated (Kim et al. 1994; Kwon et al. 1981). Prior to the initiation of formal breeding programs for improved soybean cultivars in South Korea, soybean growers had chosen small-seeded soybean cultivars for sprout production. Empirically, they recognized that small seeds produced a higher sprout yield. The identification of common markers linked to QTLs for both sprout yield and seed weight suggest either pleiotropy or linkage a as genetic explanation for the selection of soybean cultivars with a seed weight less than 12 mg seed^{-1} to maximize soybean sprout yield (Kwon et al. 1972).

Sprout yield and abnormal seedlings were interrelated in that greater sprout yield showed a negative association with a higher incidence of abnormal seedlings (r = –0.337**). Although not detected in the MLG-Regr analysis, marker Bng222 on LG M was detected by SF-ANOVA as being associated with both traits. The Jinpumkong 2 allele was associated with less seedling damage and a greater sprout yield. This QTL could provide the genetic basis for the correlations among the three traits.

These data indicate that effective marker-assisted selection may be feasible for enhancing sprout yield in a soybean. The transgressive segregation of sprout yield, as well as the existence of two QTLs conditioning greater than 10% of the phenotypic variation in sprout yield, provides an opportunity to select lines with a greater sprout yield than the currently preferred cultivars such as Pureunkong. However, markers detecting variation near the $P < 0.05$ level will require further confirmation.

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