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QTL Analysis of Soybean Seed Weight Using RAPD and SSR Markers

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ABSTRACT

Soybean [*Glycine* max (L.) Merr.] seed weight is a important trait in cultivar development. Objective of this study was to identify and confirm quantitative trait loci (QTLs) for seed weight variation in the F2 and F2:3 generations. QTLs for seed weight were identified in F2 and F2:3 generations using interval mapping (MapMaker/QTL) and single-factor analysis of variance (ANOVA). In the F2 plant generation (i.e., F3 seed), three markers, OPL9a, OPM7a, and OPAC12 were significantly (P<0.01) associated with seed weight QTLs. In the F2:3 plant row generation (i.e., F4 seed), five markers, OPA9a, OPG19, OPL9b, OPP11, and Sat_085 were significantly (P<0.01) associated with seed weight QTLs. Two markers, OPL9a and OPL9b were significantly (P<0.05) associated with seed weight QTLs in both generations. Two QTLs on USDA soybean linkage group C1 and R were identified in both F2 and F2:3 generations using interval mapping. The linkage group C1 QTL explained 16% of the variation in seed weight in both generations, and the linkage group R QTL explained 39% and 41% of the variation for F2 and F2:3 generation, respectively. The linkage group C2 QTL identified in F2:3 generation explained 14.9% of variation. Linkage groups C1, C2 and R had previously been identified as harbouring seed size QTLs. The consistency of QTLs across generations and populations indicates that marker-assisted selection is possible in a soybean breeding program.

Key Words : RAPD, SSR, RFLP, QTL, Soybean seed size

INTRODUCTION

Traits of agronomic and economic importance are controlled by polygene or quantitative trait loci (QTLs). The phenotype of a quantitative character is a consequence of both genetic and environmental sources. Thus, in a polygenic system an individual gene substitution may well contribute a relatively small effect to the phenotype of the character (Mather and Jinks, 1971). An association of a simply inherited genetic markers with a quantitative trait in plants was first noted by Sax (1923) who observed that segregation for seed size associated with segregation for a seed coat color marker in beans (*Phaseolus vulgaris* L.). Thoday (1961) demonstrated that simply inherited gene markers can be used as tags to locate quantitative trait loci (QTLs). More systematic attempts to resolve quantitative traits into their individual genetic components were initially limited by the lack of polymorphic qualitative markers

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with a uniform distribution the genome. These limitations have been partly overcome by rapid developments in the area of molecular genetics. Molecular markers based upon DNA polymorphism have greatly simplified the genetic analysis of quantitative traits, providing a reliable and extensive framework of qualitative markers to which quantitative trait loci (QTLs) can be linked.

Because seed weight of soybean is a major yield component increasing of seed weight is often an important goal in breeding programs, particularly in cultivars developed for specialty seed markets. Seed weight QTLs have been identified and characterized in such legume crops as mungbean [*Vigna radiata* (L.) Wilczek] and cowpea [*Vigna unguiculata* (L.) Walp.] (Fatokun et al. 1992), lentil [*Lens culinaris*] (Tahir et al. 1994, Abbo et al. 1992), and pea [*Pisum sativum*] (Timmerman-Vaughan et al. 1996). Seed weight in soybean [*Glycine max* (L.) Merr.] is highly inherited quantitatively and governed almost entirely by additive gene action. High-yielding soybean cultivars with either a large or a small seed weight need to be developed for niche markets. Information on the association between genetic markers and seed weight should help breeders construct beneficial allelic combinations and accelerate the development of specialty cultivars. Quasi-isogenic lines differing in seed weight would be useful in exploring the often found negative relation between the seed weight and seed number components of seed yield.

Several reports have been published on soybean QTLs for seed weight. Mansur et al. (1993) found one unlinked RFLP marker linked to a QTL for seed weight using F5 families derived from the mating *G. max* cultivar Minsoy (24.7 g/100 seed) and *G. max* cultivar Noir1 (24.5 g/100 seed). This single marker explained 13% of the phenotypic variation for seed weight. Mian et al. (1996) also identified seed weight QTLs in two soybean populations derived from *G. max* by *G. max* matings. In both of these studies, the *G. max* parents had near-similar conventional seed weights and produced progenies whose seed weights were within the range that is commonly observed for conventional cultivars (i.e. 12 to 22 g/100seed). Maughan et al. (1996) used 77 molecular markers (covering 780 cM of the genome) to identify only two QTLs for seed weight which accounted for significant phenotypic variationin both F2 and F2:3 generations, respectively. In this study, a *G. max* breeding line with moderately large seeds (24 g/100 seed) was mated with *G. soja* plant introduction with very small seeds (1.5 g/100 seed). These authors observed that in both generations, the F2 and F2:3 seed weight variation was constrained towards smaller seed weight (i.e., 4 to 10 g/100 seed) with no recovery in the progeny of either parental seed weight. Orthologous seed weight QTLs were identified by Fatokun et al. (1992) in cowpea and mungbean based on RFLP markers and this orthologosity was extended lately to soybean by Maughan et al. (1996). In all of the foregoing studies, the *G. max* parental seed weights were not much different from the typical seed weights found in commodity-type soybean cultivars. It is not known whether a mating of *G. max* parents of quite different seed weights would lead to the same or different QTLs found in those studies. In this study, a population was derived from the mating of two *G. max* parents with substantial extremes in seed weight. Our objective was (1) to identify quantitative trait loci (QTLs) for seed weight variation in the F3 seed produced by F2 plants in a population that originated from the mating of the *G. max* cultivar Mercury (ca. 8 g/100 seed) and G. max plant introduction PI417.468 (ca. 36 g/100 seed), (2) to verify that those QTLs were still identifiable in the F4 seed produced by F3 plant progenies of the F2 plants, and (3) to determine if those QTLs were the same as those identified in previous studies.

MATERIALS AND METHODS

Plant Materials

The study was conducted with the population derived from a mating between the *G. max* cultivar Mercury (female parent) and *G. max* plant introduction PI 417.468 (male parent). Mercury, released in 1995 (Specht et al., 1995) has a very small seed weight (7.5-8.0 g/100 seed), whereas PI 417.468 has a large seed weight (34-38 g/100 seed). During the summer 1993, F1 hybrids were made in a field nursery located on the University of Nebraska-Lincoln (UNL) East Campus. The resultant F1 seeds were planted on 7 May 1994 in the UNL East Campus field nursery. F1 hybridity was confirmed based on pod color (i.e., recessive tan female mated to dominant brown male and female selfed is tan, F1 hybrid is brown). All F1 plants were individually harvested and those whose hybridity was confirmed were bulked. A random sample of 110 F2 seeds was planted on 17 May, 1995 in the UNL East Campus field nursery in a bordered block of six 25-seed plots that were 0.75 m x 0.90 m. At maturity, individual F2 plants were individually harvested. A random sample of 25 F3 seeds from each of the 110 F2 plants was planted in 0.90 m long plots spaced 0.75 m apart on 17 May, 1996 in the UNL East Campus field nursery. The experimental design was a randomized complete block with four replications of the 110 F2:3 lines. When the plants in each plot were matured, they were threshed in bulk and the harvested seed was air-dried for keeping a constant seed miosture.

DNA Isolation and Marker Assays

Total genomic DNA was isolated from young leaves collected from individual F2 plants that emerged in 1995 field nursery, and from the two parents grown in the 1994-95 greenhouse, by means of a modified CTAB procedures (Saghai Maroof et al., 1984). For the analysis of random amplified polymorphic DNA (RAPD) markers, one-thousand 10-mer oligonucleotide primers were obtained from Operon Techmologies, INC. (Alameda, Calif.). The 1,000 primers were grouped into 50 sets of 20 primers each (Operon kits A01-20 to Z01-20, and AA01-20 to AX01-20). The RAPD-PCR protocol followed that described Williams et al. (1990), except for minor modifications that were needed to optimize that protocol for soybean DNA. The PCR reaction was performed in a MJ Research PTC - 100 Thermocycler. The thermal profile was 2 cycles of 1 min at 92℃, 22 s at 42℃, and 70 s at 72℃, followed by 38 cycles of 16 s at 92℃, 22 s at 42℃, and 70 s at 72℃, then ending with 1 cycle of 5 min at 72℃ For the analysis of simple sequence repeat (SSR) markers, primer pairs were obtained from Perry B. Cregan (USDA-ARS, Soybean and Alfalfa Research Lab., Beltsville, MD). The SSR-PCR protocol followed that described by Akkaya et al. (1995), except that the reaction mixture was cooled to 4℃ and maintained at this temperature until gel electrophoresis. Amplification products were electrophoresed in 1.2% TBE agarose gels for RAPD markers, or in 2.5% TBE superfine resolution agarose gels for SSR markers. The gels were stained with ethidium bromide (EtBr) to reveal DNA segments (amplicons) of varying sizes (i.e., number of base pairs) that had been amplified by the PCR. Gels were photographed under transmitted UV light. All RAPD and SSR primers were first tested for polymorphism on the two parental DNAs. For RAPD marker nomenclature, the locus was given the primer name, that is, the OP prefix, followed by the kit letter, followed by the number within that kit. When a given RAPD primer detected more than one locus, a small case letter a and b (and c) were added as suffix designations. For SSR markers, the locus name indicates the bases in the repeat followed by a number.

Data analysis and QTL mapping

Seed weights for two generations (F2 and F2:3) were measured by randomly sampling 100 seeds produced by

each F2 plant and each F2:3 family. In the F2:3 families, four estimates were averaged to obtain a mean seed weight for use in QTL mapping. A linkage map of RAPD and SSR markers was constructed by applying the computer program MAPMAKER v. 3.0 (Lander et al., 1987) to the F2 plant marker data. F2 genotypes were coded as A (homozygous for an allele from the PI417.468 parent), B (homozygous for an allele from the Mercury parent), or H (heterozygous). For grouping markers into linkage groups, a minimum LOD of 3.0 and a maximum distance of 50 cM were employed.

Single-factor analysis of variance (ANOVA) were performed for each marker locus to discern the effect of its alleles on seed weight (PROC GLM, SAS). For the codominant markers (SSRs and several RAPDs), three phenotypic classes were distinguishable (i.e., homozygous A or B and heterozygous H), whereas only two phenotypic classes for the dominant markers (most RAPDs) were distinguishable (i.e., D vs B when allele B was a recessive null, or C vs A when allele A was the recessive null). A simple F-test was used to determine if the least square, seed weight means for the marker types distinguished at a given locus were statistically different. An F-test significance level of $P<0.01$ was chosen for declaring that a marker was linked to a seed weight QTL. The possibility of false positives (Type I Error) must be considered, since the number of marker loci exceeded the number of F2 plants (i.e., overparameterization of the model). Of course, a lesser probability value (say, $P<0.001$) would, of course, be more conservative, but since we were also interested in identifying QTLs that had been identified by others, we chose a $P<0.01$ level. An interval mapping technique (Lander and Botstein, 1989) was also performed using the computer program MAPMAKER-QTL v. 1.1 (Lincoln et al. 1992). This QTL analysis was performed on both the F2 generation and on the mean values of the F2:3 lines. A LOD score of 2.0 was chosen as the threshold to be used to declare the presence of a QTL in any given the interval between two adjacent markers.

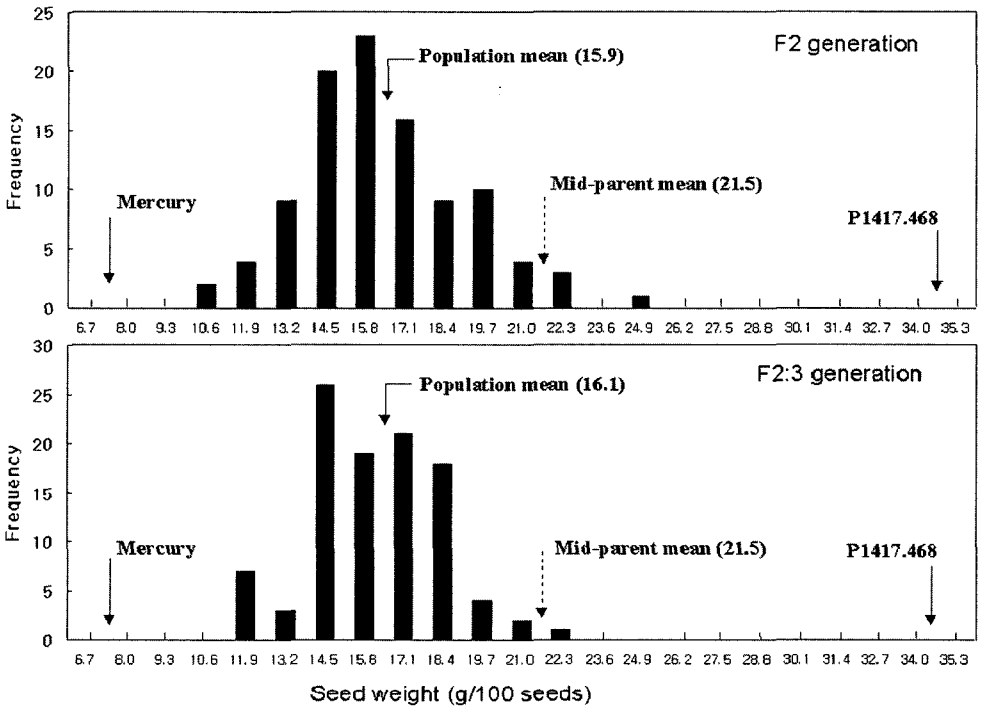

Fig.1. Frequency distribution of 107 F2 and F2:3 generations for mean seed weight.

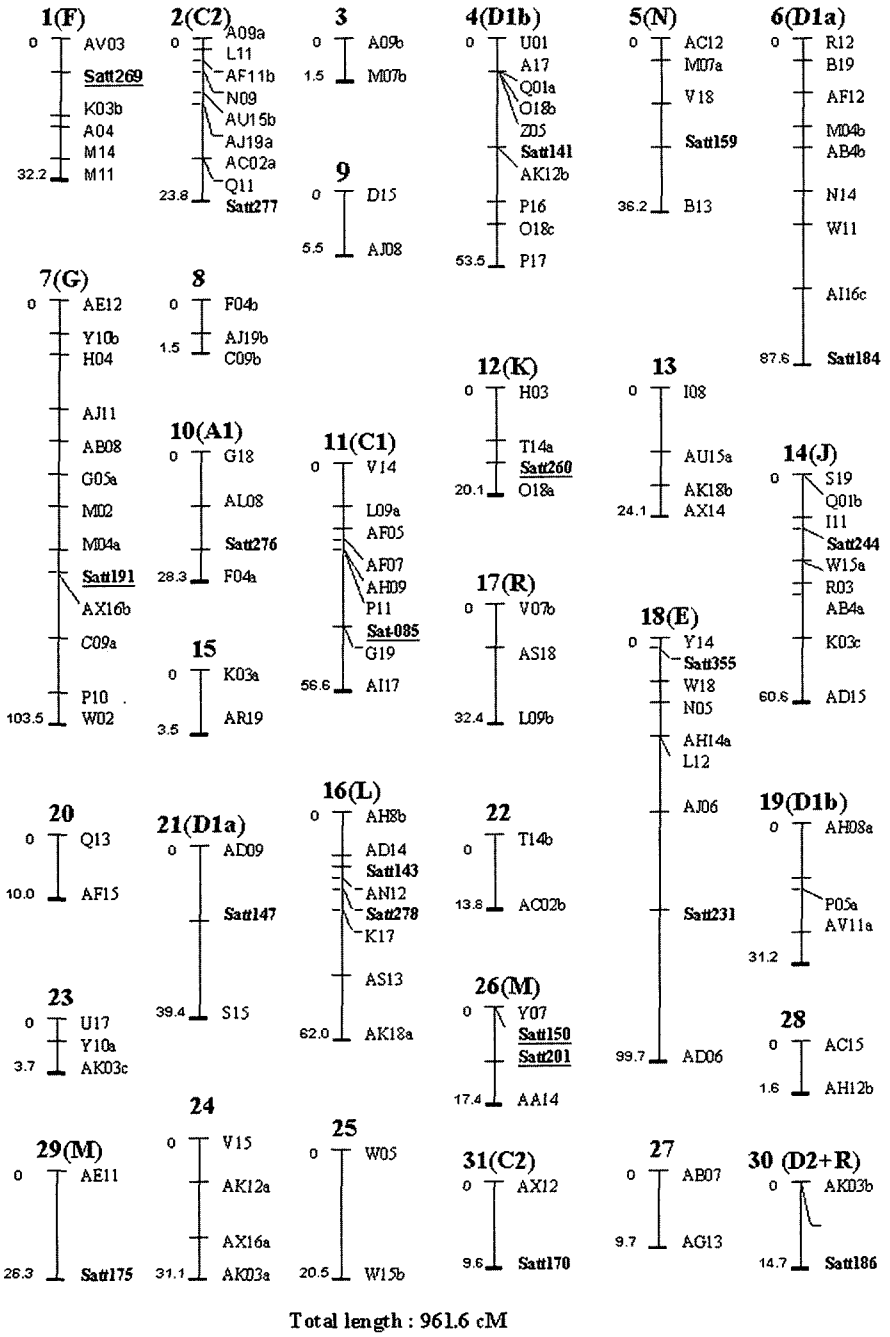

Fig. 2. Soybean linkage map with 124 RAPD and 22 SSR markers constructed from a segregating F2 population derived from mating of small-seed parent Mercury and large-seed parent PI417.468. The linkage group names (LG1 to LG31) are listed at the top of each group, with map distances shown on the left and marker positions on the right. The SSR markers are underscored. The correspondent linkage group in public soybean map (Shoemaker et al., 1995) is shown parenthesis.

RESULTS

Phenotypic analysis of seed weight

The seed weight frequency distributions in the 107 F2 plants and F2:3 lines are shown in Fig.1. Seed weight in both generations varied in a fashion that suggested a continuous, polygenic inheritance. Seed weight ranged from 10.5 to 22.5 g/100 seed in the F2 generation and from 11.9 to 21.7 g/100 seed in the F2:3 generations. The means of the F2 and F2:3 generations were 15.9 and 16.1 g/100 seed, respectively. A seed weight equal to that of either parent was not observed in the progeny of either generation. The expected mid-parental values for the F2 and F2:3 generations were 20.7 and 23.0 g/100 seed, respectively. In both generations, the seed weight means for each population were skewed towards small-seed parent.

Linkage map and QTL analysis

Of the 1000 RAPD primers tested on the two parents, only 210 primers revealed a parental polymorphism, and of those, only 165 primers produced amplicons that were scorable as either codominant 1:2:1 segregations or dominant 3:1 segregations. For 30 RAPD primers, the F2 amplicon segregation pattern was interpretable as two or more marker loci. As a result, the RAPD-PCR assay resulted in the identification of 195 RAPD marker loci that segregated in this population. Of the 180 SSR primers tested, only 24 primers revealed a parental polymorphism that in the progeny produced a scorable

Table 1. RAPD and SSR loci putatively associated with seed weight QTLs in the two generations detected by a single-factor analysis of variance

Markers	Linkage group[1]	Generation[2]	F-test probability	Allelic means and standard deviations	
				Mercury	PI 417.468
OPA9a	2(C2)	F2	0.123	15.07±0.64	16.23±0.37
		F2 : 3	0.004	14.79±0.53	16.57±0.30
OPM7a	5(N)	F2	0.005	13.89±0.77	16.31±0.33
		F2 : 3	0.282	15.42±0.70	16.25±0.30
OPAC12	5(N)	F2	0.005	13.89±0.77	16.32±0.33
		F2 : 3	0.282	15.42±0.70	16.25±0.30
OPG19	11(C1)	F2	0.069	14.96±0.62	16.29±0.37
		F2 : 3	0.002	14.73±0.50	16.63±0.30
OPL9a	11(C1)	F2	0.006	15.48±0.35	17.59±0.65
		F2 : 3	0.019	15.78±0.30	17.35±0.57
OPP11	11(C1)	F2	0.066	14.95±0.61	16.30±0.37
		F2 : 3	0.004	14.84±0.51	16.59±0.30
Sat-085	11(C1)	F2	0.055	14.95±0.61	17.36±0.76
		F2 : 3	0.007	14.73±0.51	16.99±0.63
OPL9b	17(R)	F2	0.012	14.74±0.55	16.48±0.37
		F2 : 3	0.008	15.05±0.47	16.62±0.32

[1] Filial plant generation. Seed weight measured on seed produced by F2 plants or F2 : 3 lines.

[2] Linkage group number shown in Fig. 2. The correspondent linkage group in thepublic soybean map (Shoemaker et al., 1995) is shown in parenthesis.

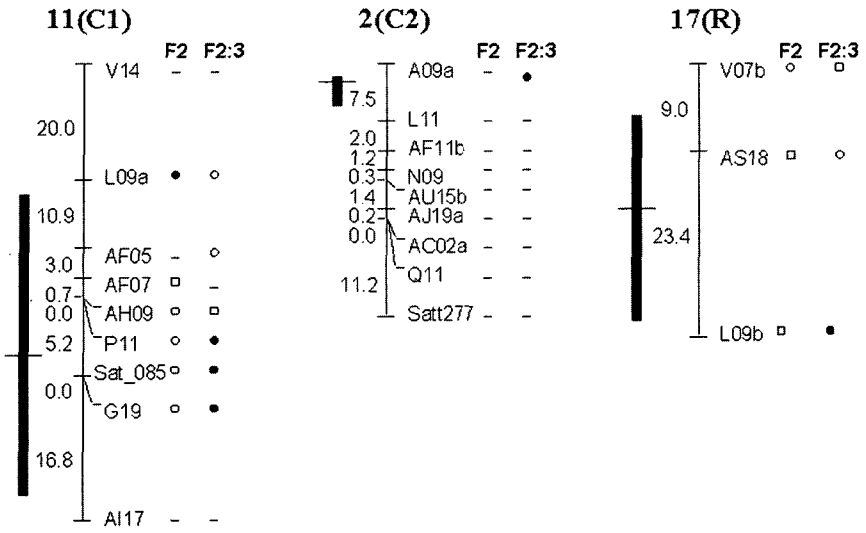

Fig 3. Three linkage groups identified as having putative seed weight QTLs in a population derived from mating of Mercury and PI417.468 mating. Map distance (cM) is shown on the left and markers are shown on the right of each linkage group. Solid bar on the left denotes a QTL region exceeding LOD 2.0. Significance of the F-test is indicated (solid circle for $P \leq 0.01$, open circle for $0.01<P \leq 0.05$ and open square for $0.05<P<0.10$).

codominant 1:2:1 segregation pattern. Only 124 of the segregating RAPD markers and only 22 SSR markers were found to be genetically linked. These markers coalesced into 31 linkage groups (Fig.2). The linkage map spanned 962 cM, with markers separated by an average distance of about 6.5 cM in Haldane map units (Haldane, 1919). The results of single-factor analysis are presented in Table 1. In the F2 generation, the F-tests were significant at $P<0.01$ for three markers: OPL9a and OPM7a on linkage group 11 and OPAC12 on linkage group 5. In the F2:3 generation, the F-tests were significant for five markers: OPA9a on linkage group 2, OPL9b on linkage group 17, OPG19, OPP11, and Sat_085 on linkage group 11. Thirteen markers in F2 generation and eighteen markers in F2:3 generation had F-tests that were significant at $P<0.05$ (data are not shown), but only three of these markers, OPL9a, OPL9b, and Sat_085, had F-tests that were significant at $P<0.05$ in both generations.

Interval mapping using the F2 and F2:3 data revealed only two and three putative QTLs for seed weight, respectively (Fig.2). The peak QTL region on linkage group 17, which was near OPL9b, explained 39.6% and 41.6% of the variation for seed weight in F2 and F2:3 generation, respectively. The peak QTL region on linkage group 11, which was located near Sat_085, accounted for 16.2% and 16.8% of the variation for seed weight in F2 and F2:3 generation, respectively. The interval on linkage group 2, which was near RAPD marker OPA9a, explained 14.9% of the variation for seed weight in F2:3 generation.

The three above mentioned linkage groups are depicted in Fig.3, along with the putative QTL positions identified by interval analysis and the markers with significant F-tests in the single factor analyses.

DISCUSSION

Seed weight is a primary consideration in the development of many types of specialty cultivars in soybean. Seed weight is a highly heritable character in this species. Seed weight in the F2 and F2:3 generations

showed a continous distribution (Fig.1). The mean values of F2 and F2:3 generations were less than the value of the mid-parent, and there was no recovery of parental types in the F2 and F2:3 generations. This observation is consistent with an additive gene model of many loci whose alleles have small effects. The frequency distributions of F2 and F2:3 generations show a partial dominance of the genetic factors for small seed weight. These results are consistent with previous reports (Ting. 1946; Williams. 1948; Weber. 1950; Bravo et al. 1981). However, Mian et al. (1996) observed transgressive segregation for seed weight in two soybean populations developed from the parents with similar seed weight.

A Soybean linkage map consisting of 124 RAPD and 22 SSR markers has been presented in this paper. Most of RAPD markers segregated in a dominant fashion, whereas all SSR markers and a few RAPD markers segregated in a codominant fashion. Of the 146 markers mapped in this population, 13 markers in the F2, and 18 markers in the F2:3, had F-tests that were significant at the $P \leq 0.05$ level (data not shown). Under the null hypothesis, chance alone would lead to the detection of about 7 or 8 markers as being associated with QTLs (i.e., 148 x 0.05). Distinguishing these 7 or 8 false positives from the true positives is contigent upon "QTL reproducibility", of which there are several forms. Replicate F2 (or Fn) populations from the same mating tested together in more than one environment is the best of QTL reproducibility. QTL reproducibility across filial generations is strong evidence of a true QTL. Reproducibility of a QTL over several adjacent markers in a given linkage group is also good evidence. Finally, the reproducibility of a QTL in the literature by researchers working with different populations can provide greater certainty as to whether that QTL is real. We have taken those approaches here. Using both MapMaker-QTL ($LOD \geq 2.0$) and single-factor analysis ($P \leq 0.01$), we identified three markers in linkage groups 5(N) and 11(C1) that were associated with QTLs for seed weight in the F2 generation, and subsequently identified five markers in linkage groups 2(C2), 11(C1), and 17(R) that were associated with QTLs for seed weight in the F2:3 generation. Only three of these markers, OPL09a and Sat_085 of linkage group 11(C1), and OPL09b of linkage group 17(R) had significant F-tests in the single factor analysis of both the F2 and F2:3 generations (Table 1). Relative to interval mapping, two regions (one on the linkage group 11 and another on linkage group 17) were identified as having putative QTLs for seed weight in both generations. Given the imprecision associated with QTL localization, the F2 and F2:3 QTLs identified on linkage group 11 are probably manifestations of a single QTL. This is also likely for the QTLs on linkage group 17. The results indicate that seed weight QTLs on linkage groups 11 and 17 persisted across generations. Linkage groups 11 and 17 are equivalent to linkage groups C1 and R of the public soybean map. Mian et al. (1996) reported that C1 and R had markers associated significantly with seed weight in population 1 and population 2, respectively.

In our population, one seed weight QTL on linkage group 2 was detected by both single-factor and interval analysis in the F2:3 generation but not the F2 generation. Linkage group 2 is equivalent to linkage group C2, which was reported by Mian et al. (1996) to have a seed weight QTL. Experimental error associated with measurement of seed size in our F2 generation may have been a factor since some F2 plants produced less than 100 seed (i.e., 100-seed weight for these was extrapolated from the weight of the actual number of seed). Inconsistency of QTLs associated with agronomic traits across population and environment in soybean has been reported by Lee et al. (1996) and Mian et al. (1996). Two markers (OPM7a and OPAC12 on linkage group 5) had significant F-tests in the single-factor analysis of the F2 generation. However, interval

analyses detected no QTLs of LOD 2.0 of better in linkage group 5. In addition, no significant QTLs in linkage group 5 were detected by either analysis method in the F2:3 generation. Linkage group 5 is equivalent to linkage group N. Neither Mian et al. (1996) nor Maughan et al. (1996) found seed weight QTLs on N. Given the fact that the F2 QTL on linkage group 5 was absent in the F3, it is likely a false positive. Of the remaining markers that were significant at $P \leq 0.05$ in the F2 and F2:3 generation, none were significant in both the F2 and F2:3. This non-reproducibility across generations led us to infer that these are false positives. In addition, none of the $P \leq 0.05$ markers were located in the QTL regions reported by Mian et al. (1996) and Maughan et al. (1996). The consistency of QTLs across generations and populations indicates that marker-assisted selection is possible in a soybean breeding program.

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