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Abstract

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ABSTRACT

Markers linked to male-sterile, female-fertile loci on the soybean [*Glycine max* (L.) Merr.] molecular map would facilitate early identification of male-sterile plants. The objectives were to verify the chromosome location of the *ms2* (A00–63) mutation, to determine the allelism and the chromosome location of the suspected *ms2* [*ms?* (A00–39)] mutation, and to determine the chromosome location of the *ms9* (T359) mutation. Simple sequence repeat (SSR) markers were used to molecularly map the male-sterile, female-fertile loci reported in this study. Segregating F₂ populations were developed from crosses of *ms?ms?* (A00–39) × ‘Minsoy’ (PI 278901), *ms2ms2* (A00–63) × Minsoy, and Minsoy × *Ms9ms9* (T359H). The *ms?* (A00–39) locus was positioned on MLG O at 9.9 centiMorgans (cM) distance from the marker Sat_190. The *ms2* (A00–63) locus was positioned on molecular linkage group (MLG) O between markers Sat_190 and Scaa001, with a distance of 6.9 and 9.0 cM, respectively. The *ms9* locus was located on MLG N between markers Satt521 and Satt237, with a distance of 8.5 and 16.2 cM, respectively. Classical allelism tests confirmed that mutant *ms?* (A00–39) was allelic to *ms2* (A00–63). The A00–39 mutant line was assigned Genetic Type Collection number T375H and gene symbol *Ms2ms2* (Ames 2). Thus Genetic Type T360H, previously identified at Ames, Iowa, becomes *Ms2ms2* (Ames 1).

MUTATIONS that affect microsporogenesis and microgametogenesis causing male sterility have been described for many species (Gottschalk and Kaul, 1974), especially maize, (*Zea mays* L.) (Golubovskaya, 1979; 1989; Albertsen and Phillips, 1981), and *Arabidopsis thaliana* (Chaudhury et al., 1992; Dawson et al., 1993; He et al., 1996; Hulskamp et al., 1997; Taylor et al., 1998). Meiotic mutations in soybean (*Glycine max* L. Merrill) have been reported (Palmer et al., 2004). These include mutations affecting male and female meiosis (*st* mutants), meiotic and post-meiotic mutations that affect

only male development and cause male sterility by cytoplasmic genes (*cms*) or nuclear genes (*ms* mutants), and meiotic mutations that affect only the female and cause female partial sterility (*fsp* mutants).

Manual cross-pollination for the production of large quantities of hybrid seeds can be difficult and time consuming. Male-sterile mutants are considered a powerful tool in hybrid breeding programs. A *cms* system is the ideal system for hybrid seed production (Kaul, 1988). Two *cms* systems have been reported in soybean (Davis, 1985; Sun et al., 1997). One is being studied for its potential in hybrid seed production (Sun et al., 1997).

Alternatively, several methods have been proposed to use genic male-sterile plants to produce hybrid seed (Rao et al., 1990; Horner and Palmer, 1995). The use of CMS and genic nuclear male-sterile plants to produce hybrid soybean seed would require insect pollinators to transfer pollen from the male plants to the female plants. Genic male sterility in soybean is used to improve efficiency of hybridization by eliminating tedious hand emasculations, to enhance random mating for population development (Specht et al., 1985; St. Martin and Ehounou, 1989; Specht and Graef, 1992) and recurrent selection (Lewers and Palmer, 1997), and to facilitate the development of testers for inbred line evaluation and development (St. Martin et al., 1996; Ortiz-Perez, 2005). So far in soybean, nine nuclear male-sterile mutations have been reported (Palmer et al., 2004).

The male-sterile, female-fertile T259 mutant (*ms2ms2*) was found at Eldorado, Illinois (Bernard and Cremeens, 1975; Graybosch et al., 1984). The *ms2* mutation causes abortion of young microspores at the tetrad stage of development, probably due to abnormalities of the tapetal layer (Graybosch and Palmer, 1985). The T259H (*Ms2ms2*) mutant line was used to develop the *ms2* (‘Beeson’), *ms2* (‘Clark’), *ms2* (‘Wells’), and *ms2* (‘Williams’) near iso-genic lines (Bernard et al., 1991). The *ms2* (A00–63) mutant is a backcross-derived near-isogenic line of cultivar Beeson (Bernard et al., 1991). The T360 (*ms2ms2*) mutant was found at Ames, Iowa (Palmer, 2000). The *ms?* (A00–39) mutant was found in 1999 among progeny of a germinal revertant of T322 (*w_f-m*), identified as ARS-10–483. Based on anther phenotype, male-sterile plants in family A00–39 were suspected to carry an allele at the *ms2* locus.

Isozyme screening of near-isogenic male-sterile soybean lines, showed that the *ms2* locus was linked with the phosphoglucosyltransferase (*Pgm1*) locus with an estimated map distance of 18.73 ± 2.4 cM (Sneller et al., 1992). These two loci and the *E2* locus comprise classical linkage group (CLG 15) (Palmer et al., 2004). *Pgm1* is located on molecular linkage group (MLG) O.

Abbreviations: BSA, bulk segregant analysis; CLG, classical linkage group; cM, centiMorgans; MAS, marker-assisted selection; MLG, molecular linkage group; SSR, simple sequence repeats.

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The position of the *ms2* locus on MLG O has not been determined.

Another male-sterile, female-fertile mutant, T359 (*ms9ms9*), has pollen grains that stain very lightly without any evidence of colpi, although pollen grains are similar in size to mature pollen grains of fertile plants (Palmer, 2000). The T359 mutant has been used as a parent to produce large quantities of F₁ hybrid seed for heterosis tests (Ortiz-Perez, 2005). The *ms9* locus has not been located on the classical or the molecular soybean map.

The objectives were to verify the chromosome location of the *ms2* (A00–63) mutation, to determine the allelism and the chromosome location of the suspected *ms2* (A00–39) mutation, and to determine the chromosome location of the *ms9* (T359) mutation. These results should facilitate the transfer of the *ms2* and *ms9* alleles in soybean breeding programs by using marker-assisted selection (MAS).

MATERIALS AND METHODS

Allelism Test

Known heterozygotes of *Ms?ms?*, from family A00–39 used as male parents for the allelism test, were produced in 2002 by crossing *ms?ms?* × fertile sibling plants (*Ms?Ms?* or *Ms?ms?*) from families segregating fertile and sterile plants. Progeny testing of fertile F₁ plants confirmed the heterozygous genotype (*Ms?ms?*). For the allelism test, *ms2ms2* (A00–63) plants were used as female parents and *Ms?ms?* plants as male parents.

In the allelism test, if the F₁ plant segregation was in the ratio of 1 fertile:1 sterile (*Ms?ms?: ms?ms?*) plant and if the fertile (*Ms?ms?*) F₁ plants always segregated in the F₂ about 3:1, fertile:sterile plants, the unknown was considered allelic to the known tester. If all the F₁ plants were fertile and if the F₂ family classification was 1 (3:1, fertile:sterile plants): 1 (9:7, fertile:sterile plants), the unknown was considered nonallelic to the known tester. This suggests two separate and independent male-sterile, female-fertile mutant genes. The χ^2 test was performed to determine whether the observed data fit the expected ratio.

Progeny Testing

F₃ seeds from individual F₂ fertile plants were harvested, and planted in summer 2004 for Pop-1, and summer 2005 for Pop-2 and Pop-3 at the Bruner Farm, near Ames, Iowa (Table 1). Segregation for fertility/sterility in the progeny rows was used to determine the genotype of each fertile F₂ plant. The expected phenotypic ratio in the F₂ generation was 3:1 (male-fertile:male-sterile plants), and the expected genotypic ratio of the F_{2,3} population was 1:2:1, [(*Ms?Ms?:Ms?ms?:ms?ms?*), (*Ms2Ms2:Ms2ms2:ms2ms2*), and (*Ms9Ms9:Ms9ms9:ms9ms9*)].

Molecular Mapping Experiments

Mapping Populations

The segregating F₂ populations, Pop-1, Pop-2, and Pop-3 used for the molecular mapping experiments, were developed from the crosses of *ms?ms?* (A00–39) × Minsoy (PI 27890), *ms2ms2* (A00–63) × Minsoy, and from the cross of Minsoy × *Ms9ms9* (T359H), respectively (Table 1). Minsoy is a true breeding homozygous non-sterile (*MsMs*) genotype.

Segregating F₂ populations were classified at stage R1, at the beginning of flowering (Fehr and Caviness, 1979). Anthers from 5 to 10 plants per population were collected to identify presence/absence of pollen. On that basis only one segregating male-sterile F₂ population tracing to just one F₁ plant was selected as the mapping population. Individual F₂ plants were phenotypically scored as fertile or sterile at maturity. The genotype of each F₂ plant was determined in the F_{2,3} generation.

Pop-1 and Pop-2 were grown during fall 2003 and fall 2004, respectively, in the USDA greenhouse at Iowa State University (Table 1). Seeds were placed on germination paper, and each seedling was transplanted to individual pots, having 116 F₂ plants for Pop-1 and 114 F₂ plants for Pop-2 (Table 1). Plants were subjected to artificial photoperiod after transplanting, i.e., 3 wk with 18-h photoperiod, 2 wk with a photoperiod of 16-h, 2 wk with 14-h photoperiod, and for the remainder of the growing period until harvest, plants were subjected to 13-h photoperiod. During summer 2004, 148 F₂ plants for Pop-3 were grown at the Bruner Farm near Ames, IA.

DNA Extraction

Two to three grams of young trifoliolates were collected from each F₂ plant of the three mapping populations, Pop-1, Pop-2, and Pop-3, stored in individual labeled plastic bags, and freeze-dried for 48 to 72 h. Dry leaves were transferred to 15 mL sterile propylene tubes, ground to powder using glass beads, and kept at –80°C until DNA extraction. The protocol for DNA extractions used in previous research was followed (Kato and Palmer, 2003a; 2003b; 2004).

Also during summer 2004, 50 seeds of each of the parent lines used to develop the populations were planted in the USDA greenhouse at Iowa State University. Young trifoliolates of each parental line were collected in bulk and prepared for DNA extraction using the same procedure used for F₂ plants.

SSR Analysis

The *ms2* gene was mapped to CLG 15 (Sneller et al., 1992), which corresponded to MLG O (Cregan et al., 1999). Therefore, for Pop-1 and Pop-2 only 20 markers from MLG O were selected to test for polymorphism, using as criterion for selection an approximate genetic distance of less than 25 cM between two adjacent markers. For Pop-3, the parents were screened with 229 markers from all 20 MLGs to test for polymorphisms. The markers were selected using the same criterion as for Pop-1 and Pop-2.

Table 1. The F₂ populations for mapping the soybean *ms?*, *ms2*, and *ms9* male-sterile genes.

Population	Cross		Source of the male-sterile mutant	Grown	Population size	Locus
	Female	Male				
Pop-1	<i>ms?ms?</i> (A00–39)	<i>MsMs</i> (Minsoy)	ASR-10–483–1†	Fall 2003	116	<i>ms?</i>
Pop-2	<i>ms2ms2</i> (A00–63)	<i>MsMs</i> (Minsoy)	L75–0587‡	Fall 2004	114	<i>ms2</i>
Pop-3	Minsoy (<i>MsMs</i>)	<i>Ms9ms9</i> (A00–73)	T359H§	Summer 2004	148	<i>ms9</i>

† Found among progeny of a germinal revertant of T322 (*w_A-m*).

‡ Isoline Collection; Beeson isolate, L75–0587; USDA-ARS, Urbana, Illinois.

§ T359H refers to Genetic Type Collection number; USDA-ARS, Urbana, Illinois.

At maturity, each F₂ plant was phenotypically scored as either sterile or fertile. The initial SSR screening was done as separate experiments for each of the populations using bulk segregant analysis (BSA) method (Michelmore et al., 1991). The contrasted bulks were DNA of 10 F₂ plants with sterile phenotype, and DNA of 10 F₂ plants with fertile phenotype. The initial molecular screening was made using the two parents and the two contrasted bulks for each SSR marker to find polymorphisms between parents of each cross, and to identify the SSR markers that could be linked to each of the *ms?*, *ms2*, and *ms9* loci.

Each PCR reaction mixture had a volume of 15 µL, including 1x PCR buffer, 1.8 µM of MgCl₂, 0.17 mM of each of dNTPs, 0.25 units *Taq* polymerase (Bioline, Inc., Randolph, MA), 0.3 µM of forward and reverse primers, and 50 ng of template DNA (10 ng/µL). PCR was done at a PTC-100TM Programmable Thermal Controller (MJ Research, Inc., Waltham, MA), PCR condition was 45 s at 94°C, 45 s at 47°C, and 45 s at 68°C for 32 cycles.

PCR products were separated on 2–5% agarose gels (Agarose 3:1, Amresco Inc., Solon, OH), containing 0.50 µg/mL ethidium bromide, in 1x TBE buffer (0.089M Tris base, 0.089M boric acid, and 0.002M EDTA). The percentage of agarose gels depended on the difficulty of band separation. The band patterns were checked and photographed under UV light.

Data Analysis

Each plant in the population was scored according to the alleles at the locus, i.e., a score of A was assigned if the plant was homozygous for alleles from Minsoy, B if it was homozygous for alleles from male-sterile mutants, and H if it was heterozygous. After scoring the population, recombination values were calculated to determine if a specific primer was linked to the gene of interest, using the Linkage-1 program (Suiter et al., 1983).

Mapmaker V3.0 (Whitehead Institute, Cambridge, MA) (Lander et al., 1987) program was used to make the initial map, using a minimum logarithm of the odd ratio (LOD score) of 3, for accepting linkage between two markers as suggested by Kato and Palmer (2004). Recombination frequencies were converted to map distances in centiMorgans (cM) using Kosambi map function (Kosambi, 1944). JoinMap 3.0 (Kyazma B.V., Wageningen, Netherlands) (Van Ooijen and Voorrips, 2001) was used to make an integrated map, and (or) to make a final map.

RESULTS AND DISCUSSION

Allelism Test

The allelism test (testcross or genetic complementation test) of *ms2ms2* (A00–63) × *Ms?ms?* (A00–39) gave

Table 2. Segregation of the *ms?* locus linked to SSR markers on soybean molecular linkage group (MLG) O in F₂ Pop-1 from the cross *ms?ms?* (A00–39) × Minsoy.

Marker	Distance [†] (cM)	Segregation [‡]			Number of plants	χ^2 (1:2:1)	P
		A	H	B			
Satt477	67.6	24	62	30	116	1.17	0.56
Satt592	48.3	25	63	28	116	1.02	0.60
Satt581	42.0	30	61	25	116	0.74	0.69
Satt153	22.2	29	59	28	116	0.05	0.97
Sat_231	12.7	29	63	24	116	1.29	0.52
Sat_109	12.1	28	63	25	116	1.02	0.60
Sat_190	9.9	25	69	22	116	4.33	0.11
<i>ms?</i>	0.0	30	64	22	116	2.34	0.31

[†] Distance to *ms?* locus.

[‡] Genotypes: A, homozygous for the allele from Minsoy; H, heterozygous; B, homozygous for the allele from the *ms?* locus.

Table 3. Segregation of the *ms2* locus linked to simple sequence repeats (SSR) markers on soybean molecular linkage group (MLG) O in F₂ Pop-2 from the cross *ms2ms2* (A00–63) × Minsoy.

Marker	Distance [†] (cM)	Segregation [‡]			Number of plants	χ^2 (1:2:1)	P
		A	H	B			
Sat_274	48.5	31	58	25	114	0.66	0.72
Satt153	30.2	28	64	22	114	2.35	0.31
Sat_231	19.6	35	53	26	114	1.98	0.37
Sat_109	10.3	33	57	24	114	1.42	0.49
Sat_190	6.9	33	55	26	114	1.00	0.61
<i>ms2</i>	0.0	33	55	26	114	1.00	0.61
Scaa001	9.0	36	55	23	114	3.10	0.21

[†] Distance to *ms2* locus.

[‡] Genotypes: A, homozygous for the allele from Minsoy; H, heterozygous; B, homozygous for the allele from the *ms2* locus.

32 fertile: 37 sterile F₁ plants, $\chi^2_{(1:1)} = 0.36$, $P = 0.55$. This 1:1 ratio is expected if the mutants are allelic. Progeny from 20 fertile F₁ plants segregated 3 fertile: 1 sterile in the F₂ generation. For the combined F₂ population, segregation was 1971 fertile: 683 sterile plants, $\chi^2_{(3:1)} = 0.76$, $P = 0.38$. Furthermore, a 9 fertile: 7 sterile plants class was not found in the F₂ generation. Thus two separate and independent male-sterile, female-sterile mutant genes were not evident. The conclusion is that the *ms?* allele in the *ms?ms?* (A00–39) mutant line is an allele at the *ms2* locus. The A00–39 mutant line was assigned Genetic Type Collection number T375H and the gene symbol *Ms2ms2* (Ames 2). Then Genetic Type T360H becomes *Ms2ms2* (Ames 1).

Molecular Mapping of the *ms2* (Pop-1) and *ms2* (Pop-2) Locus in Soybean

The molecular screening of the two parents and the two contrasted bulks for each SSR marker of MLG O of Pop-1, indicated linkage between the *ms2* locus and the SSR marker Sat_190 for MLG O. In Pop-2, linkage between the *ms2* locus and SSR markers Sat_190 and Scaa001 from MLG O was shown.

To construct a map for *ms2* (A00–39) and *ms2* (A00–63), additional polymorphic markers on MLG O were tested. The polymorphic SSR markers were Satt477, Satt592, Satt581, Satt153, Sat_231, Sat_109, and Sat_190 for Pop-1 (Table 2), and SSR markers Sat_274, Satt153,

Table 4. Segregation in F₂ Pop-1 and F₂ Pop-2 for the *ms?/ms2* locus linked to simple sequence repeats (SSR) markers from an integrated map of soybean molecular linkage group (MLG) O.

Marker	Distance [†] (cM)	Segregation [‡]			Number of plants	χ^2 (1:2:1)	P
		A	H	B			
Satt477	69.1	24	62	30	116	1.17	0.56
Satt592	49.8	25	63	28	116	1.02	0.60
Satt581	43.6	30	61	25	116	0.74	0.69
Sat_274	43.5	31	58	25	114	0.66	0.72
Satt153	24.5	29	61	25	115	0.70	0.70
Sat_231	13.9	32	58	25	115	0.86	0.65
Sat_109	11.2	30	60	25	115	0.65	0.72
Sat_190	8.4	29	62	24	115	1.14	0.57
<i>ms?/ms2</i>	0.0	31	60	24	115	1.07	0.59
Scaa001	8.6	36	55	23	114	2.34	0.31

[†] Distance to *ms?/ms2* locus.

[‡] Genotypes: A, homozygous for the allele from Minsoy; H, heterozygous; B, homozygous for the allele from the *ms?* or the *ms2* locus.

Sat_231, Sat_109, Sat_190 and Scaa001 for Pop-2 (Table 3).

The χ^2 calculations from the $F_{2:3}$ populations' progeny tests and the F_2 populations molecular screenings of Pop-1 and of Pop-2 with 116 and 114 individuals, respectively, showed a good fit to the expected genotypic ratio of 1 male-fertile, female-fertile homozygote: 2 heterozygotes: 1 male-sterile, female-fertile homozygote with $\chi^2_{(1:2:1)} = 2.34$ and $P = 0.31$ for Pop-1, and $\chi^2_{(1:2:1)} = 1.00$ and $P = 0.61$ for Pop-2 (Tables 2 and 3). Mapping data for Pop-1 and Pop-2 were combined and integrated using JoinMap 3.0 (Van Ooijen and Voorrips, 2001), resulting in a $\chi^2_{(1:2:1)}$ value of 1.07 with $P = 0.59$ (Table 4).

Population specific maps were made by JoinMap 3.0 (Van Ooijen and Voorrips, 2001), and showed that for Pop-1, the *ms?* locus was located at the position 9.9 cM from the marker Sat_190, (Fig. 1A). For Pop-2, the *ms2* locus was located between the markers Sat_190 and Scaa001 with a distance of 6.9 and 9.0 cM, respectively (Fig. 1B).

A map constructed to integrate both populations showed that the *ms2* locus was linked to markers Satt477, Satt592, Satt581, Sat_274, Satt153, Sat_231, Sat_109, Sat_190, and Scaa001, and by 8.4 cM from Sat_190 and 8.5 cM from Scaa001 (Fig. 1C and 1D). The results were positioned by JoinMap similar to the maps constructed by Mapmaker V3.0 (Lander et al., 1987).

Molecular Mapping of the *ms9* Locus (Pop-3)

The molecular screening of the two parents and the two contrasted bulks for Pop-3 using 229 SSR markers, indicated linkage between *ms9* and Satt 521 on MLG N. To construct a map for *ms9*, eight additional polymorphic markers on MLG N were tested. Linkage values were calculated between the *ms9* locus and polymorphic SSR markers Sat_033, Sat_266, Sat_236, Satt521, Satt237, Sat_091, Sat_239, Sat_241, and Sat_306.

The $F_{2:3}$ progeny test and the F_2 population molecular data from 148 plants gave χ^2 calculations that showed a good fit to the genotypic ratio of 1:2:1. This indicated that the $F_{2:3}$ segregation for the *ms9* gene fit a 1 male-fertile, female-fertile homozygote: 2 heterozygotes: 1 male-sterile, female-fertile homozygote ratio with $\chi^2_{(1:2:1)} = 4.75$ and $P = 0.09$ (Table 5). A map was constructed using Mapmaker V3.0 (Lander et al., 1987). The *ms9* locus was located between markers Satt521 and Satt237 and linked to each by 8.5 cM and 16.2 cM, respectively (Fig. 2).

Molecular Markers Tightly Linked to *ms2* and *ms9*

The *ms2ms2* (A00-63) and the *ms2ms2* (A00-39) plants display very different out-crossed seed-set behaviors with insect pollinators even though they are alleles

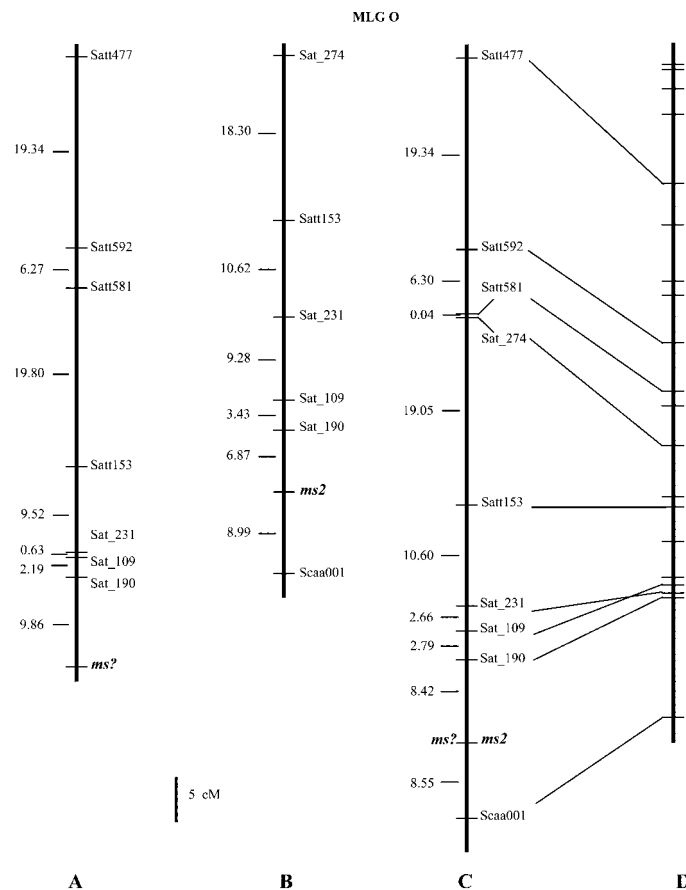


Fig. 1. Soybean molecular linkage group O constructed by JoinMap. A) *ms?* locus from Pop-1, cross *ms?ms?* (A00-39) × Minsoy; B) *ms2* locus from Pop-2, cross *ms2ms2* (Beeson) × Minsoy; C) Integrated map of the two populations for the *ms?* and *ms2* loci; D) molecular linkage group (MLG) O from the USDA-ARS-ISU map (Cregan et al., 1999; Song et al., 2004). Distances shown in centiMorgans (cM).

Table 5. Segregation of the *ms9* locus linked to simple sequence repeats (SSR) markers on soybean molecular linkage group (MLG) N in F₂ Pop-3 from the cross Minsoy × *Ms9ms9* (T359H).

Marker	Distance† (cM)	Segregation‡			Number of plants	χ^2 (1:2:1)	P
		A	H	B			
Sat_033	61.4	27	79	42	148	3.71	0.16
Sat_266	54.7	33	72	43	148	1.46	0.48
Sat_236	31.2	41	77	30	148	1.87	0.39
Satt521	8.5	31	76	41	148	1.45	0.48
<i>ms9</i>	0.0	39	62	47	148	4.75	0.09
Satt237	16.2	37	73	38	148	0.04	0.98
Sat_091	21.1	34	73	41	148	0.68	0.71
Sat_239	37.7	40	63	45	148	3.60	0.16
Sat_241	45.9	36	72	40	148	0.32	0.85
Sat_306	53.7	35	74	39	148	0.22	0.90

† Distance to *ms9* locus.

‡ Genotypes: A, homozygous for the allele from Minsoy; H, heterozygous; B, homozygous for the allele from the *ms9* locus.

at the same locus. The *ms2ms2* (A00–63) plants have low cross-pollinated seed set (about 40 seed per male-sterile plant). The *ms2* (A00–39) plants have high cross-pollinated seed set (about 120 seed per male-sterile

plant) (Ortiz-Perez, 2005). This differential behavior is being studied.

The present study identified molecular markers closely linked to the *ms2* and *ms9* loci, respectively. Among nine soybean male-sterile mutant genes, only two mutant genes (MWO *ms*, MLG D1b and *ms3*, MLG D1b) had been mapped using molecular markers (Jin et al., 1998; Cervantes-Martinez, 2005). Molecular markers are now available to introgress these alleles into breeding lines by the backcross procedure.

The allelism tests confirmed that the *ms?* mutation of A00–39 was allelic to the *ms2* allele of the A00–63 *ms2* mutant line. Both male-sterile, female-fertile mutants were mapped, as expected, to MLG O. A total length of 16.9 cM between Sat_190 and Scaa001 in the present study was similar to the 16.6 cM in the saturated molecular linkage map (Song et al., 2004). In this chromosomal region, two RFLP markers, A644_1 and A955_2, had been reported (Song et al., 2004). Thus, these four markers are potential molecular markers for MAS to determine if *Ms2Ms2* or *Ms2ms2* is used in the backcross-breeding programs and to narrow the genetic map position for the map based cloning of *ms2*.

The *ms9* locus was located between Satt521 and Satt237 on molecular linkage group N, and linked to each by 8.5 cM and 16.2 cM, respectively (Fig. 2). In a total length of 24.7 cM between Satt521 and Satt237 in the present study, an RFLP marker of BLT015_1 and two SSR markers, Satt660 and GMABAB, had been reported (Song et al., 2004). These five markers are also potential molecular markers for MAS to determine if *Ms9Ms9* or *Ms9ms9* is used in the backcross breeding program and to narrow the genetic map position for the map based cloning of *ms9*.

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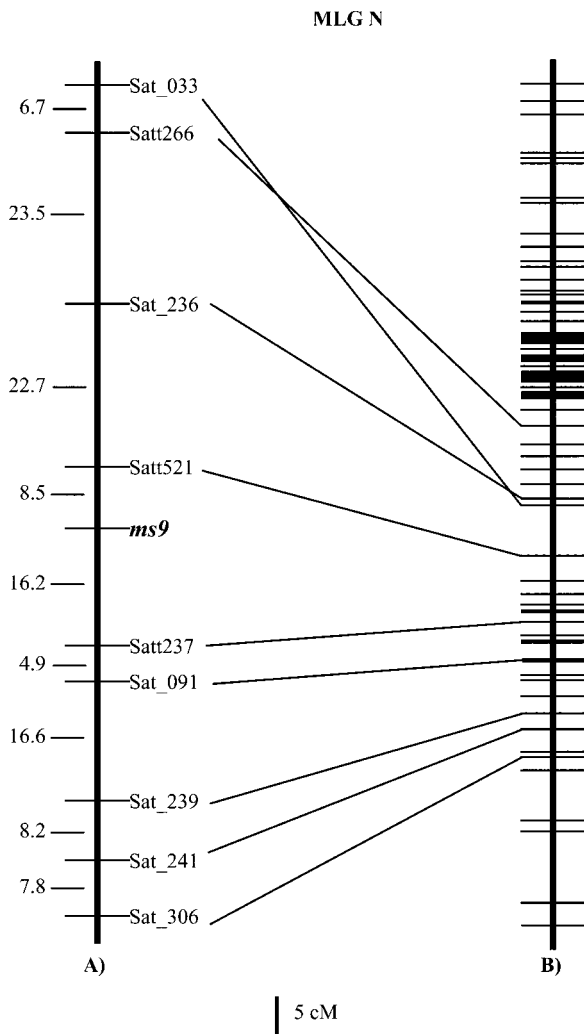


Fig. 2. Soybean molecular linkage group N constructed by Mapmaker. A) *ms9* locus, from the cross Minsoy × T359H *Ms9ms9*; B) MLG N from the USDA-ARS-ISU map (Cregan et al., 1999; Song et al., 2004). Distances showed in centiMorgans (cM).

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