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Molecular Mapping of a Male-Sterile Gene in Soybean

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Molecular Mapping of a Male-Sterile Gene in Soybean

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ABSTRACT

A newly identified genic male-sterile mutant in soybean [*Glycine max* (L.) Merr.] has high seed set under natural field conditions and is potentially useful in breeding programs. Meiosis is normal in the mutant line. Sterility in this mutant is caused by failure of callose dissolution at the tetrad stage, which results in microspore abortion; however, little is known about the male-sterile gene at the molecular level. The objective of this study was to identify molecular markers linked with the male-sterile gene (ms) and to place the ms gene onto the soybean molecular genetic map. An F2 population of 107 individuals was constructed from a cross between the mutant msMOS (ms ms) and the cultivar Minsoy (Ms Ms). Two hundred seventy markers, including 219 RFLP and 51 SSRs, were evaluated. Of these, 102 RFLP probes and 31 SSR markers detected polymorphisms between the parents. The F2 population was screened for segregation of these polymorphic molecular markers. Analyses revealed that the male-sterile locus, designated 'ms', was located on linkage group D1b of the USDA/ISU soybean molecular genetic map. The availability of linked DNA markers will facilitate the genetic analysis of this male-sterility gene in relation to soybean breeding programs, and will be a starting point for the isolation of the ms gene by map-based cloning.

A MAJOR OBSTACLE to F1 hybrid soybean seed production is the intensive hand-labor requirement for large numbers of pollinations. Male sterility can be exploited to take advantage of insect pollination (Kaul, 1988). Cytoplasmic male sterility (cms) is the ideal system for hybrid seed production (Kaul, 1988). Cms has been reported in soybean (Davis, 1985; Sun et al., 1997) and is being studied for its potential in hybrid seed production. At least seven nuclear male-sterile mutants, including the mutant in this study, have been documented in soybean (Jin et al., 1997; Palmer et al., 1992).

There are several limitations in the use of nuclear male sterility for hybrid seed production. Because this type of sterility is predominantly determined by recessive alleles, only the homozygous recessive can be identified in segregating populations. As a result, when using the classical backcross method for interline transfer of male sterility, fertile homozygotes and fertile heterozygotes are not distinguishable in the segregating backcross generations. To solve this problem, a breeder must identify lines carrying the male-sterile allele by self-pollination. A second concern is that recessive nuclear male sterility must be propagated via the heterozygotes. Therefore, the yield of male-sterile progeny is limited to 50% in a backcross to ms ms, and 25% in an F2 generation.

Tight linkage between a male-sterile locus and a selectable marker locus will allow for the transfer of the male-sterile allele among breeding lines via uninterrupted backcrossing (for review see Horner and Palmer, 1995). If the marker could be identified in early growth stages, it would be possible to eliminate most if not all of the undesirable fertile plants before flowering.

For example, the close genetic linkage (2–4% recombination) between the phenotype markers purple hypocotyl and flower (*W1* locus), and male fertility (*Ms6* locus) has been used in hybrid soybean seed production (Lewers et al., 1996). The *W1_* seedlings have purple hypocotyls, and *w1 w1* seedlings have green hypocotyls. The *ms6 ms6* plants are male sterile and female fertile. This closely linked marker system has the benefit of allowing elimination, shortly after emergence of nearly all male-fertile individuals from evaluation plots, and allowing greater control of parental contributions during intermating. Therefore, half-sib recurrent-selection methods in soybean are compatible with this method (Lewers and Palmer, 1997).

The ms mutation (no gene symbol has been given to this mutant, because the mutant is not in the Soybean Genetic Type Collection and seed is only available from Midwest Oilseeds, Inc., Adel, IA 50003) described in this study is recessive, segregates as a single Mendelian locus, and is not linked to either of the two morphological markers; flower color (*W1* locus) and pubescence color (*T1* locus) (Jin et al., 1997). This mutant line has unusually high seed set in the field when compared with other male-sterile, female-fertile soybean lines. It is non-allelic to *ms1* through *ms6* (Jin et al., 1997). The simple and recessive inheritance of this male-sterile gene, as well as knowledge from previous extensive genetic and developmental studies (Jin et al., 1997), make this ms mutation an excellent system for further genetic and molecular studies. Molecular markers have been identified for a number of male-sterile genes in plants, such as rice (*Oryza sativa* L.) photoperiod-sensitive genic male-sterile genes (Subudhi et al., 1997; B Wang et al., 1995; J Wang et al., 1995; Zhang et al., 1994) and *ms14* from tomato (*Lycopersicon esculentum* Mill.) (Gorman et al., 1996). There are no reports of molecular markers linked to male-sterile genes in soybean.

The objectives of our research were (i) to identify

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Abbreviations: cM, centimorgan; cms, cytoplasmic male sterility; LOD, logarithm of odds ratio; ms, male sterility; MAS, marker-assisted selection; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

molecular markers linked to the male-sterile gene, ms; and (ii) to map the locus within the soybean genome. The results will facilitate the transfer of the ms allele in soybean breeding programs by using markers closely linked to ms to distinguish Ms ms from Ms Ms plants, and will help to characterize the behavior of the ms gene in various genetic backgrounds. We anticipate that as the soybean molecular map becomes more saturated, and additional markers are identified that are even more closely linked to ms, these markers will be useful in isolating the ms gene by map-based cloning.

MATERIAL AND METHODS

Plant Material

Four cultivars [Williams 82, Harosoy, Noir I (PI 290136), and Minsoy (PI 27890)] and the male-sterile mutant (msMOS) were screened with 219 mapped RFLP probes and 51 SSRs to survey for DNA polymorphisms between msMOS and other cultivars. Seeds of the male-sterile, female-fertile soybean were obtained from Midwest Oilseeds, Inc. This process demonstrated that the mutant msMOS and Minsoy possessed a relatively high level of polymorphism (49%). A cross between msMOS and Minsoy was subsequently made during the summer of 1995. The F1 seeds were planted at the University of Puerto Rico Soybean Breeding Nursery, at the Isabela Substation, Isabela, PR. F2 plants were grown in the growth chamber at photoperiods of 16 h light for the first 4 wk, 14 h for 2 wk, and 13 h until mature, and at 30°C daytime, and 24°C nighttime temperatures. Fertile F2 plants were single-plant threshed and F3 seeds were planted in Puerto Rico for classifying male sterility / fertility at maturity on the basis of the successful seed set.

Male-fertile and male-sterile plants were identified at flowering by squashing late-stage anthers in an aqueous solution of I_2KI (Jensen, 1962). Anthers from male-fertile plants displayed densely staining rounded pollen grains, whereas anthers from male-sterile plants were void of densely staining rounded pollen grains (Jin et al., 1997). Two to three flowers per plant were evaluated on different days. Chi-square tests were performed to determine the goodness of fit of the phenotype of the F2 generation to a 3:1 ratio and of the F3 generation to a 1:2:1 ratio to identify the F2 genotype.

RFLP and SSR Analyses

Soybean DNA was isolated from freeze-dried leaf tissue of parental, F1, and F2 plants according to Keim et al. (1988), and digested with five restriction endonucleases (*Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, and *Taq*I). Digested DNA was separated by agarose gel electrophoresis (10 mg/lane, 0.8% [w/v] agarose), and transferred onto a Zeta Probe Nylon membrane (BioRad, Hercules, CA) according to Sambrook et al. (1989). Blots were hybridized with randomly primed ^{32}P-dCTP-labeled probes. Hybridizations and washes were performed at 65 and 60°C, respectively, according to Zeta Probe recommendations (Bio-Rad). Preliminary screening of parental DNA identified polymorphic clones used to collect RFLP data from the F2 progeny. The segregation of alleles at each locus was tested by chi-square analysis to determine the fit to expected ratios. Segregation data were collected for 102 clones, including 90 from soybean (Shoemaker and Olson, 1993; Shoemaker et al., 1997) and 12 from common bean (*Phaseolis vulgaris* L.) (Vallejos et al., 1992) or mung bean [*Vigna radiata* (L.) Wilczek] (Menancio-Hautea et al., 1993).

Simple-sequence repeat (SSR) markers (Akkaya et al., 1995) also were evaluated, thus bringing the number of markers evaluated to 133. For SSR analysis, PCR reaction mixtures contained 60 ng of soybean genomic DNA, 1.5 m*M* Mg^{2+}, 0.3 m*M* of sense and antisense primers, 200 m*M* of each nucleotide, 1× PCR buffer, and 0.5 U *Taq* DNA polymerase in a total volume of 20 mL. Cycling consisted of 30 sec at 94°C, 30 sec at 47°C, and 30 sec at 68°C for 45 cycles on a Perkin-Elmer 960 Thermal Cycler (Perkin-Elmer, Norwalk, CT). PCR products were run on 2.5 to 3.5% (w/v) (depending on the sizes of the polymorphic fragments of the two bands) Metaphor (FMC BioProducts, Rockland, ME) agarose gel in TBE (0.089 *M* Tris-borate, 0.089 *M* boric acid, 0.002 *M* EDTA) buffer with ethidium bromide incorporated in the gel.

Linkage Analysis

The Mapmaker program (Lander et al., 1987) was used to construct a linkage map. A LOD score of 3 was used as the lower limit for accepting linkage between two markers. Recombination frequencies were converted to map distances in centimorgans (cM) by the Kosambi (Kosambi, 1944) function. On the basis of two-point analyses, Mapmaker generated log-likelihood values for the most probable order.

RESULTS

Parental Survey

The four cultivars were screened with 219 mapped RFLP probes. Restriction patterns were compared with those obtained from the same digestion of msMOS with five restriction endonucleases (*Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, and *Taq*I). The msMOS and Minsoy combination demonstrated the highest level of polymorphism (49%, data not shown). Therefore, Minsoy was chosen as the male parent to cross with msMOS for constructing an F2 population.

Segregation of ms in the F2 Generation

The 19 F1 plants were fertile. The segregation of fertile to sterile plants in the F2 followed a 3:1 ratio (data not shown). This confirmed the observation of Jin et al. (1997) that ms is a recessive gene. Only F2 seeds from a single F1 plant were used in mapping. Following F2 progeny testing the data demonstrated that the population of 107 F2 individuals showed a 1:2:1 genotypic segregation ($\chi^2 = 0.33$, Table 1).

Identification of RFLP and SSR Markers Linked to ms

Initial screening of the F2 population was conducted by selecting several RFLP and SSR markers from each linkage group (Shoemaker et al., 1997). The markers were chosen to divide each linkage group into segments of less than 20 cM. Two-point analyses indicated that the ms gene was linked to SSR marker Satt005 (LOD 38.9) on linkage group D1b (Cregan et al., 1998, unpublished). Additional markers from the linkage group were screened against the F2 population (Satt157, Satt296, Satt412, Satt266, A605, A747, Bng47, Mng137, Satt141, Satt189, Satt290, B194, L161, and K411). On the basis of LOD scores generated from the Mapmaker program, we found that the ms locus was linked to RFLP marker, Bng047, and SSR markers Satt157, Satt412, Satt005 and Satt290 with the LOD scores of 31.5, 7.9, 10.7, 38.9, and 48.7, respectively. The most likely order of these

Table 1. Segregation of the ms (male sterility) locus, and linked restriction fragment length polymorphisms and simple sequence repeat markers in an F2 soybean population from msMOS × Minsoy.

Traits or markers	No. of F2 plants†	Observed no.					χ^2	
		AA‡	HH‡	BB‡	AA‡	CC‡	1:2:1	0.38
ms	107	25	55	27			0.33	
Satt157	101	24	56	21			1.81	
Satt412	106	26	57	23			0.29	
Bng047	107				24	83		0.38
Satt005	107	23	60	24			1.60	
Satt290	107	25	56	26			0.25	

† For Satt157, six plants were not scorable, and for Satt412, one plant was not scorable.
‡ Genotypes: AA = msMOS; HH = heterozygous; BB = Minsoy; CC = not AA.

markers is shown in Fig. 1. No polymorphisms were detected at loci: A605, A747, Mng137, and B194 using the restriction endonucleases *Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, *Taq*I, *Acc*I, *Alu*I, *Hha*I, *Hae*III, *Ssp*I, and *Bam*HI, nor were polymorphisms observed using the SSR markers: Satt296, Satt266, Satt141, and Satt189. Although markers K411 and L161 were polymorphic in this cross, they segregated independently of ms. Segregation ratios of all RFLP and SSR markers provided good fits to the 1:2:1 ratio or the 3:1 ratio. Only those markers linked with the ms gene are shown in Table 1.

DISCUSSION

We mapped the nuclear male-sterile gene, ms, to linkage group D1b. The gene was flanked by SSR markers Satt157 and Satt412. Male-sterile genes have been mapped in several different species (Subudhi et al., 1997;

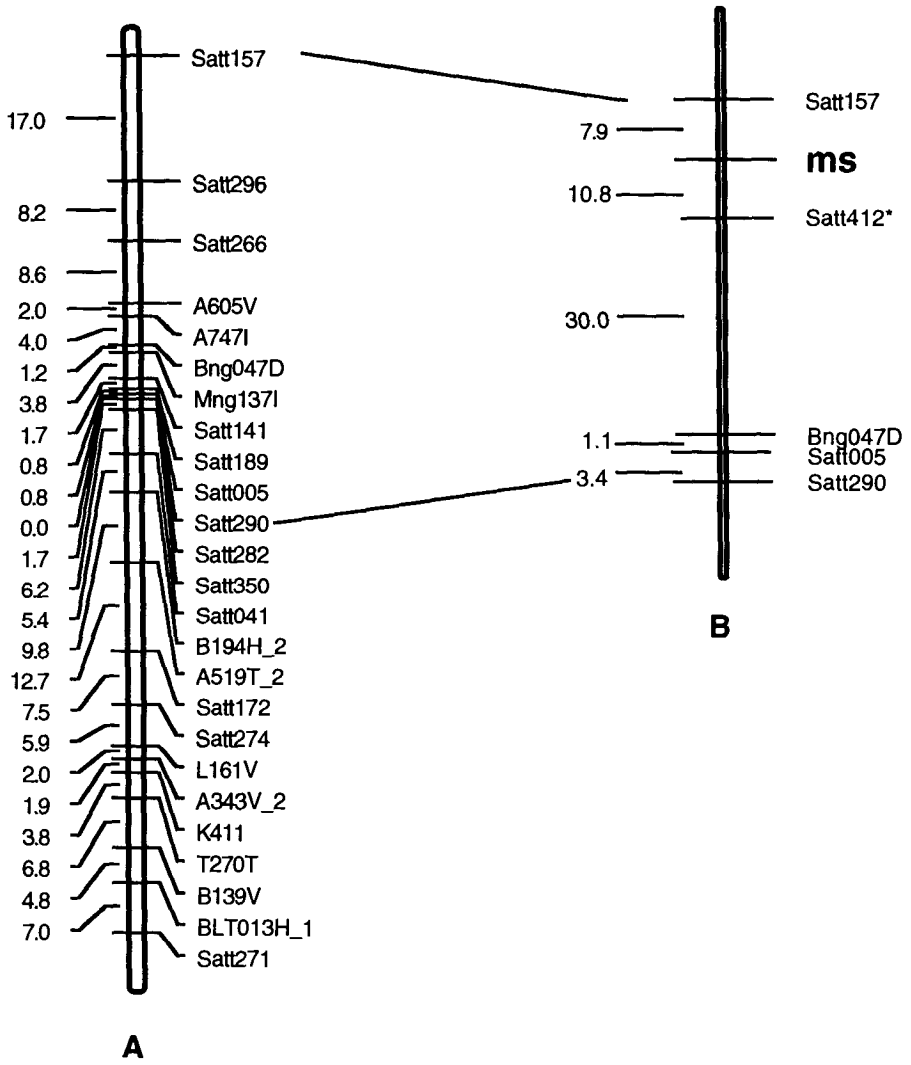

Fig. 1. Linkage map of linkage group D1b of the USDA/ARS/ISU molecular genetic map. Distances are shown in centimorgans. (A) A linkage map of linkage group D1b constructed from the *G. max* × *G. soja* population, where ms was placed between SSR markers Satt157 and Satt412. (B) Location of the ms locus, from the cross of msMOS × Minsoy, on linkage group D1b. *Satt412 has not been mapped in the *G. max* × *G. soja* population.

Gorman et al., 1996; Wang et al., 1995; Zhang et al., 1994); however , this is the first report on the molecular mapping of a soybean monogenic male-sterile gene.

In an independent population, the distance between Satt157 to Bng047 has been shown to be 33.4 cM (Cregan et al., 1999) whereas in our population we calculated the distance to be 48.7 cM. These differences may arise from the relatively low sample number or may be complicated by different recombination frequencies in this region in the specific genetic material used for our crosses (Mock, 1972; Palmer et al., 1998). Additionally, recombination distortions may have occurred because of different environment-genotype interactions at the time of F1 meiosis (Stephens, 1950; Williams et al., 1995).

Soybean nuclear male sterility is controlled predominantly by single recessive genes and the presence of male-sterile genes (heterozygotes) in breeding lines can only be detected by progeny testing. There are no reports of dominant male-sterile genes in soybean. Presently, breeders use self pollination alternating with backcrossing to identify lines carrying the male-sterile allele (Palmer et al., 1998). DNA markers linked to the ms locus provide a useful approach for early and accurate identification of lines carrying a male-sterile allele and they eliminate the necessity of several seasons of self-pollination. These markers should provide plant breeders with an efficient method for rapid transfer of the ms gene to elite soybean germplasm.

SSRs are excellent genetic markers in that they are highly abundant and highly polymorphic (Akkaya et al., 1992; Tautz, 1989). They are evenly distributed throughout the genome (Weber, 1990), generally detect only a single genetic locus, and are disseminated easily among laboratories by publishing primer sequences. In this study, SSR markers were scored with agarose electrophoresis without the use of radioisotopes. Such ease in screening for SSRs should further facilitate their use in practical plant breeding.

Pollen development in higher plants is a complex process (reviewed in McCormick, 1993). Four male-sterile genes have been cloned (Aarts et al., 1993; Albertsen et al., 1996; Gorman et al., 1996; Moffatt and Somerville, 1988), but because of the complexities of male gametophyte development, the mechanism(s) of nuclear-encoded male sterility is (are) not clear. It will be essential to clone and characterize a large number of male-sterile gene products from a number of species to understand the myriad functions of male-sterile genes. Map-based cloning is a reasonable strategy for isolation of male-sterile genes in soybean because the technique requires only that the target gene has a clear phenotype and that its position on a genetic map be known. The placement of the ms phenotype onto the soybean genetic map is a first step in this process.

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