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ARTICLE

Molecular mapping of three male-sterile, female-fertile mutants and generation of a comprehensive map of all known male sterility genes in soybean

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Abstract: In soybean, an environmentally stable male sterility system is vital for making hybrid seed production commercially viable. Eleven male-sterile, female-fertile mutants (*ms1*, *ms2*, *ms3*, *ms4*, *ms5*, *ms6*, *ms7*, *ms8*, *ms9*, *msMOS*, and *msp*) have been identified in soybean. Of these, eight (*ms2*, *ms3*, *ms5*, *ms7*, *ms8*, *ms9*, *msMOS*, and *msp*) have been mapped to soybean chromosomes. The objectives of this study were to (*i*) locate the *ms1*, *ms4*, and *ms6* genes to soybean chromosomes; (*ii*) generate genetic linkage maps of the regions containing these genes; and (*iii*) develop a comprehensive map of all known male-sterile, female-fertile genes in soybean. The bulked segregant analysis technique was used to locate genes to soybean chromosomes. Microsatellite markers from the corresponding chromosomes were used on F_2 populations to generate genetic linkage maps. The *ms1* and *ms6* genes were located on chromosome 13 (molecular linkage group F) and *ms4* was present on chromosome 2 (molecular linkage group D1b). Molecular analyses revealed markers Satt516, BARCSOYSSR_02_1539, and AW186493 were located closest to *ms1*, *ms4*, and *ms6*, respectively. The *ms1* and *ms6* genes, although present on the same chromosome, were independently assorting with a genetic distance of 73.7 cM. Using information from this study and compiled information from previously published male sterility genes in soybean, a comprehensive genetic linkage map was generated. Eleven male sterility genes were present on seven soybean chromosomes. Four genes were present in two regions on chromosome 2 (molecular linkage group D1b) and two genes were present on chromosome 13 (molecular linkage group F).

Key words: Glycine max, male sterility, simple sequence repeat, genetic linkage map, male-sterile female-fertile.

Résumé : Chez le soya, un système de stérilité mâle présentant une stabilité environnementale serait un atout clé pour rendre commercialement viable la production de semence hybride. Onze mutants présentant une stérilité mâle et une fertilité femelle (*ms1*, *ms2*, *ms3*, *ms4*, *ms5*, *ms6*, *ms7*, *ms8*, *ms9*, *msMOS* et *msp*) ont été identifiés chez le soya. Parmi ceux-ci, huit (*ms2*, *ms3*, *ms5*, *ms7*, *ms8*, *ms9*, *msMOS* et *msp*) ont été assignés à un chromosome. Les objectifs de ce travail étaient : (*i*) de situer les gènes ms1, *ms4* et *ms6* sur les chromosomes du soya ; (*ii*) de produire une carte génétique pour les régions contenant ces gènes ; et (*iii*) produire une carte complète de tous les gènes conférant une stérilité mâle et une fertilité femelle chez le soya. Une analyse des ségrégants en composite (BSA pour « bulked segregant analysis ») a été employée pour assigner chacun des gènes à un chromosome. Des marqueurs microsatellites logeant sur les chromosomes correspondants ont été examinés sur des populations F_2 pour produire des cartes génétiques. Les gènes *ms1* et *ms6* ont été assignés au chromosome 13 (groupe de liaison F) et *ms4* a été assigné au chromosome 2 (groupe de liaison D1b). Des analyses moléculaires ont révélé que les marqueurs Satt516, BARSOYSSR_02_1539 et AW186493 étaient respectivement les marqueurs les plus proches des gènes *ms1*, *ms4* et *ms6*. Les gènes *ms1* et *ms6*, bien que présents sur le même chromosome, présentaient une ségrégation indépendante et sont situés à 73,7 cM l'un de l'autre. En faisant appel à l'information produite au cours de ce travail et des études antérieures sur les gènes de stérilité mâle, une carte complète a été générée. Onze gènes de stérilité mâle sont présents sur sept chromosomes. Quatre gènes sont présents au sein de deux régions du chromosome 2 (groupe de liaison D1b) et deux gènes sont présents sur le chromosome 13 (groupe de liaison F). [Traduit par la Rédaction]

Mots-clés : Gycine max, stérilité mâle, séquences répétées simples, carte génétique, mâle stérile femelle fertile.

Introduction

Male and female reproductive structures play an important role in the development of seeds in plants. Mutations in genes involved in development of stamens and (or) pistils can lead to male-sterile, female-sterile plants, male-sterile, female-fertile plants, or male-fertile, female-sterile plants. In soybean, 11 male-sterile, female-fertile (*ms1*, *ms2*, *ms3*, *ms4*, *ms5*, *ms6*, *ms7*, *ms8*, *ms9*, *msMOS*, and *msp*) mutants have been identified and studied (Palmer et al. 1978; Delannay and Palmer 1982; Graybosch and Palmer 1985, 1988; Horner and Palmer 1995; Jin et al. 1997, 1998; Palmer 2000; Cervantes-Martinez et al. 2007, 2009).

Soybean male-sterile, female-fertile mutants could be instrumental in making hybrid seeds (Ortiz-Perez et al. 2007). Hybrid crops have better yield potential than pure lines. At the recent International Conference on Utilization of Heterosis in Crops

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several reports were made on positive heterosis values for soybean yield utilizing male-sterile systems (Li et al. 2012; Sun 2012; Yang et al. 2012; Zong et al. 2012). Soybean is a self-pollinated crop; thus development of hybrid soybean will require removal of anthers from flowers before maturation. It is not commercially viable to produce a large quantity of hybrid seed using this technique. However, if plants are male-sterile, female-fertile, the manual removal of anthers is ultimately avoided. Of the 11 male-sterile, female-fertile mutants identified in soybean, eight (*ms2*, *ms3*, *ms5*, *ms7*, *ms8*, *ms9*, *msMOS*, and *msp*) have been genetically mapped to soybean chromosomes (Jin et al. 1998; Cervantes-Martinez et al. 2007, 2009; Frasch et al. 2011; Ott et al. 2013; J. Gosh, M.K. Bhattacharyya, R.G. Palmer, and D. Sandhu, personal communication).

The first male-sterile, female-fertile mutant gene (*ms1*) was reported in 1971 (Brim and Young 1971). The *ms1ms1* plants were distinguished as generally female-fertile and nearly entirely male-sterile. They produced a high frequency of twin seedlings (Kenworthy et al. 1973; Chen et al. 1985). Male sterility was due to the failure of cytokinesis after telophase II of meiosis, resulting in large, dark-staining coenocytic microspores (Albertsen and Palmer 1979). The *ms1* locus was genetically unstable and mutated at a higher rate in relation to other soybean genes (Palmer et al. 2004). The most evident feature of *ms1* abnormal gametophytes was the appearance of many cells at the egg-apparatus region of the mature megagametophyte (Kennell and Horner 1985).

The *ms2* mutant was found in Eldorado, Illinois (Bernard and Cremeens 1975). The *ms2* mutant gene caused abortion of young microspores during the tetrad stage of development, possibly due to abnormalities of tapetal layer cells (Graybosch and Palmer 1985). Sterile plants showed different levels of meiotic irregularities during chromosome segregation. The main cause of sterility was defective cytokinesis following second meiotic division (Bione et al. 2002). The *ms2* mutant was positioned on molecular linkage group O (Cervantes-Martinez et al. 2007).

The *ms3* mutant was inherited as a single recessive gene with normal meiosis I and II (Chaudhari and Davis 1977; Palmer et al. 1980). Male sterility was due to the abortion of microspores initiated by failure of callose dissolution at the tetrad stage (Jin et al. 1997). Microspores were generated and pollen walls were initiated, but the microspores rapidly lost all cytoplasm. At anther maturity, walls surrounding empty space represented pollen grains (Jin et al. 1997). The omission of the columellar layer was how the pollen wall differed in male-fertile versus male-sterile plants (Graybosch and Palmer 1988). Initially, at the tetrad stages, tapetal cells appeared normal; however, as microspore degeneration occurred, tapetal cells either collapsed or accumulated an electron-dense material (Nakashima et al. 1984). The *ms3* system may have led to the blockage of intercellular transport of sporopollenin precursors from the tapetum. Overload in the tapetum due to the failure of the microspores to adapt and metabolize sporopollenin precursors correctly could have caused intracellular polymerization (Graybosch and Palmer 1988). The *ms3* gene was mapped to Gm02, molecular linkage group D1b (Cervantes-Martinez et al. 2009).

Anthers from *ms4ms4* plants were slightly smaller and whiter than anthers from fertile plants, which were yellow (Delannay and Palmer 1982). The anthers appeared to consist of degenerated empty microspores. The *ms4* microspore development experienced a lack of meiocyte division into tetrads after telophase II, associated with an early development of a pollen-like wall (Delannay and Palmer 1982). Early degeneration of coenocytic microspores also occurred. In *ms4*, nuclear fusion and mitotic divisions within pollen mother cells could have led to the formation of giant bicellular pollen. If these pollen grains were viable, polyploidy would result (Graybosch and Palmer 1985).

The *ms5* mutant was identified in a mutagenesis study where seeds were treated with fast neutron irradiation (Buss 1983). Cytological analysis revealed normal microsporogenesis and microgametogenesis, but abnormal development at late-microspore stage (Ott et al. 2013). The *ms5* mutant was mapped to molecular linkage group B1 (Ott et al. 2013). The *ms5* mutant showed association with the cotyledon color gene *D2*, which made it possible to use green cotyledon to identify the *ms5* plants (Ott et al. 2013).

The *ms6* mutant showed abnormalities in tapetum development in anthers; however, female reproduction was completely normal (Skorupska and Palmer 1989). The *ms6* gene showed linkage with the flower color locus *w1* (Palmer et al. 1998). The *ms6* allele was unique from other male-sterile, female-fertile genes due to its pleiotropic effect on smaller flower size (Skorupska and Palmer 1989).

The *ms7* gene was identified from the progeny of a mutable line (*w4-m*) harboring an endogenous transposable element, *Tgm9* (Chen and Palmer 1996; Xu et al. 2010). Anthers from the *ms7* mutant plants were devoid of pollen grains, and dehisced anthers failed to release any aborted pollen grains or remnants of pollen grains (Palmer 2000). The *ms7* gene has been characterized recently using transposon tagging and has been putatively localized to molecular linkage group K (J. Gosh, M.K. Bhattacharyya, R.G. Palmer, and D. Sandhu, personal communication).

The *ms8* is a unique mutant that is environmentally sensitive (Palmer 2000). Both day and night temperatures affected the sterility expression of the *ms8* allele (Perez-Sackett and Palmer 2012). The degree of sterility varied from 0–92% at different temperatures. Sterility was greater in *ms8ms8* plants that were grown at the night temperature of 25 °C and the day temperature in the range of 30–35 °C (Perez-Sackett and Palmer 2012). The highest selfed-seed set was seen when the change in day and night temperature was approximately 6 °C. The *ms8* gene was located on chromosome 7 (molecular linkage group M) flanked by Sat_389 and the telomere (Frasch et al. 2011). The corresponding region was physically 160 Kb and contained 13 genes, of which, three were known to play a role in cell division.

The *ms9* mutant plants showed light-colored pollen grains as compared with the fertile plants in I_2KI staining, although there was no difference in the size of pollen grains (Palmer 2000). Colpi were not visible in the sterile pollen grains. The *ms9* mutant was positioned on molecular linkage group N (Cervantes-Martinez et al. 2007).

Another environment sensitive mutant, *msp*, displayed variation in anther appearance. High temperature promoted fertility in *mspmsp* plants (Stelly and Palmer 1980). For *mspmsp* plants, an increase in male fertility was found to be concomitant with a decrease in night temperatures (Carlson and Williams 1985). The plants that were completely sterile had small, brown anthers. Degenerating microspores and pollen grains remain clumped. Although abnormalities occurred throughout the development of sporogenous tissue, abnormalities peaked near pachytene stage of meiosis (Stelly and Palmer 1982). The *msp* gene was mapped to molecular linkage group D1b (Frasch et al. 2011).

The *msMOS* mutant is entirely male sterile (Jin et al. 1997). Sterility in *msMOS* may be due to low levels of callose, molecularly flawed callose, or inactive callose in locular fluid (Jin et al. 1997). After meiotic prophase I, irregularities in tapetum of the male-sterile anthers were noted at the tetrad stage and included abnormal formation of vacuoles. The most evident irregularities of tapetal cells were cell enlargement, the accumulation of sporopollenin, and early degeneration. Later in development, there was only a small amount of cytoplasm remaining in the male-sterile anthers (Jin et al. 1997). The *msMOS* mutant was located on molecular linkage group D1b (Cervantes-Martinez et al. 2009).

All these male-sterile, female-fertile genes are nonallelic and were inherited as single-recessive genes. However, differences exist among these mutants allowing identification of different male-sterile, female-fertile mutants.

Environmentally sensitive genic male-sterile lines have been identified and successfully used in agricultural industry for

several crop plants, such as rice and corn. Some of these genes have been characterized and genetically mapped to chromosomes (Wang et al. 2003; Lee et al. 2005).

Characterization of a stable male-sterile line may become instrumental in making hybrid seed economically viable in soybean. Insects have been shown to transfer pollen from male-fertile plants to male-sterile plants (Palmer et al. 2001). Therefore, the first step for characterizing male sterility genes is to set up a sterility system for possible commercial application. Then, simple sequence repeat (SSR) markers linked to genetic male sterility genes can play important roles in early identification and removal of male-fertile plants before flowering (Horner and Palmer 1995). In addition, these kinds of molecular markers also can be used for male-sterile line selection in backcrossing and recurrent selection breeding programs (Lewers and Palmer 1997). It is possible to facilitate identification of morphological markers linked to the genes for development of genetic linkage maps for male sterility genes, which can be utilized for quick and easier selection of male-sterile lines (Ott et al. 2013).

The objectives of this study were to (*i*) locate the *ms1*, *ms4*, and *ms6* genes to soybean chromosomes; (*ii*) generate genetic linkage maps of the regions containing these genes; and (*iii*) develop a comprehensive map of all known male-sterile, female-fertile genes in soybean.

Materials and methods

Development of genetic materials

For *ms1* and *ms6* mapping populations, Manchu (PI 30593) was used as a female parent in crosses with T266H (Genetic Type Collection number) and T295H, respectively. For the *ms4* population, Minsoy (PI 27890) was used as the female parent in a cross with T274H. Seeds for the parents were obtained from the USDA Soybean Germplasm Collection (www.ars-grin.gov/npgs/). The F_1 plants were self-pollinated to generate F_2 populations and segregating F_2 populations used for molecular mapping. Anthers from 5–10 plants per F_2 population were collected and stained with I_2KI for starch (Jensen 1962) to identify the segregating F_2 populations. At maturity, each F_2 plant was phenotypically scored as either male-fertile or male-sterile based on seed set. The fertile F_2 plants were single-plant threshed. The genotype of each fertile F_2 plant was determined in the $F_{2:3}$ generation.

Molecular mapping and analysis

CTAB method was used for DNA extractions from parents and the mapping populations (Sandhu et al. 2004). Bulked segregant analysis was used to find chromosomal location of each gene (Michelmore et al. 1991). DNA from 10 homozygous fertile or 10 homozygous sterile F_2 plants was mixed to make sterile and fertile bulks. The DNA bulks were prepared by pooling 1 μg DNA from each F_2 plant. Each DNA bulk was diluted to a final concentration of 50 ng DNA/μL. Bulks were tested with SSR markers covering the entire soybean genome. A total of 700 SSR markers from the 20 soybean chromosomes were used to check for polymorphism between the fertile and sterile bulks.

For SSR analysis, 30 ng DNA was used in a 10 μL reaction containing 1× reaction buffer (10 mmol/L Tris-HCl, 50 mmol/L KCl, pH 8.3), 2.0 mmol/L $MgCl_2$, 0.25 μmol/L of each primer, 200 μmol/L of each dNTP, and 0.25 U of *Biolase* DNA polymerase (Bioline USA Inc., Taunton, Mass., USA). The PCR conditions were as follows: 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 47 °C, and 1 min at 72 °C; followed by 8 min at 72 °C. The PCR products were separated on a 4% agarose gel and photographed using a gel documentation system.

Scoring and data analysis

An "ABH" scoring system was used for each F_2 plant, based on SSR alleles at the locus depending on the F_2 population. In detail, a score of "A" was assigned if the plant was homozygous for the alleles from the male-fertile parent, "B" was assigned if it was homozygous for the alleles from the male-sterile parent, and "H" was assigned if the plant was heterozygous. After scoring the population, linkage analysis was conducted. Recombination frequencies were converted to map distances in centiMorgans (cM) using the Kosambi map function (Kosambi 1943). Recombination values were calculated to determine whether the SSR markers were linked to the gene of interest. Mapmaker V3.0 was used to make final maps (Lander et al. 1987). A minimum logarithm of the odd score of 3 was used for accepting linkage between two markers.

Results

Male sterility was evaluated on F_2 populations generated for the *ms1*, *ms4*, and *ms6* genes. The *ms1* and *ms6* F_2 populations consisted of 120 plants each and the *ms4* population consisted of 94 plants. Segregation for each F_2 population displayed a 3:1 ratio for fertile and sterile plants with *p* values of 0.14, 0.72, and 0.53 for the *ms1*, *ms4*, and *ms6* genes, respectively (Table 1). Progeny testing of male-fertile, female-fertile F_2 plants showed a 1:2 ratio of homozygous fertile: heterozygous fertile F_2 plants with *p* values of 0.35, 0.30, and 0.51 for the *ms1*, *ms4*, and *ms6* genes, respectively (Table 1). The F_2 and $F_{2:3}$ segregations confirmed recessive monogenic inheritance of all three genes (Table 1).

Bulked segregant analysis was used with the fertile and sterile bulks for all populations to locate the *ms1*, *ms4*, and *ms6* genes to soybean chromosomes using 700 SSR markers. SSR markers Satt595 and AW186493 showed clear polymorphisms between the bulks for the *ms1* and *ms6* genes, respectively (Fig. 1). In bulked segregant analysis, polymorphic markers between the bulks indicate proximity of the markers to the gene of interest. Both of these markers were located on chromosome 16 (molecular linkage group F) (Song et al. 2004), suggesting that both *ms1* and *ms6* were present on chromosome 16. Similarly, marker BARCSOYSSR_02_1477 detected polymorphism in bulked segregant analysis between fertile and sterile bulks for the *ms4* population (Fig. 1). BARCSOYSSR_02_1477 was present on chromosome 2 (molecular linkage group D1b) (Song et al. 2004), suggesting that *ms4* was present on chromosome 2.

For all these three genes, markers present around the region of polymorphic markers were tested on parents of each F_2 population for polymorphisms. For the *ms1* gene, six markers on molecular linkage group F (Satt423, Satt146, Satt030, Satt516, Satt595, and Sat_133) showed polymorphism between parents. All the polymorphic markers in the region were tested on the whole F_2 population (supplementary data, Table S1)[1]. Linkage analysis showed that the *ms1* gene was flanked by Satt516 and Satt595 (Fig. 2*a*). The closest marker was Satt516, which was 7.5 cM from the gene of interest. For *ms4*, nine markers (Satt546, BARCSOYSSR_02_1450, BARCSOYSSR_02_1469, BARCSOYSSR_02_1477, Satt703, BARCSOYSSR_02_1539, BARCSOYSSR_02_1547, Sat_183, and GMES6822) in the region showed polymorphisms between parents (Table S2). BARCSOYSSR_02_1439 showed closest association with the gene of interest and was at a distance of 5.5 cM (Fig. 2*b*). For the *ms6* gene, five markers (BE806387, AW186493, Satt149, Satt030, and Satt516) detected polymorphisms and Satt149 mapped at a closest distance of 5.0 cM from the gene of interest (Fig. 2*a*; Table S3).

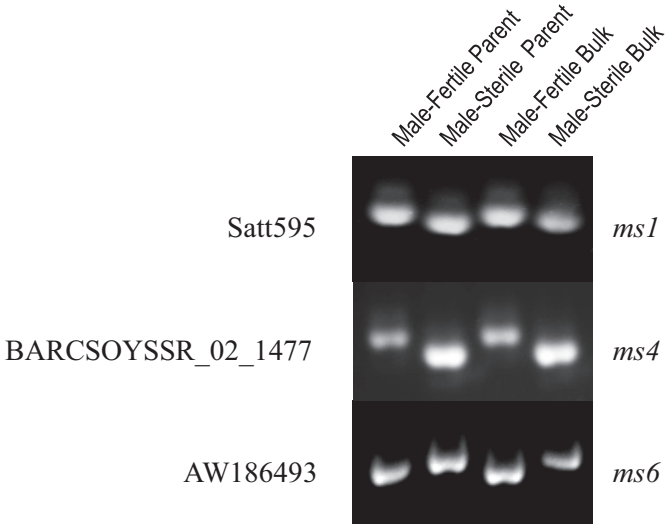
[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2014-0018.

Table 1. Segregation patterns, chi-square, and *p* values for populations of male-sterile, female-fertile *ms1*, *ms4*, and *ms6* mutant lines of soybean.

			No. of F_2 plants				No. of F_2 families			
Population	Parents	Gene	Fertile	Sterile	χ^2 (3:1)	*p*	Homozygous fertile	Heterozygous fertile	χ^2 (1:2)	*p*
A12-129	Manchu × T266H	*ms1*	97	23	2.18	0.14	36	59	0.88	0.35
A12-100	Minsoy × T274H	*ms4*	69	25	0.13	0.72	26	41	1.08	0.30
A12-124	Manchu × T295H	*ms6*	93	27	0.40	0.53	34	59	0.44	0.51

Note: Homozygous F_2 families consisted of all male-fertile, female-fertile plants. Segregating heterozygous F_2 families consisted of approximately 3 fertile: 1 sterile plants.

Fig. 1. Bulked segregant analysis results showing identification of SSR markers linked to the *ms1*, *ms4*, and *ms6* genes. SSR markers show polymorphisms between the male-fertile and male-sterile bulks, suggesting close association between the male sterility genes and the markers. For *ms1*, Manchu (*Ms1Ms1*) was the male-fertile parent and T266H (*Ms1ms1*) was the male-sterile parent. For *ms4*, Minsoy (*Ms4Ms4*) was the male-fertile parent and T274H (*Ms4ms4*) was the male-sterile parent. For *ms6*, Manchu (*Ms6Ms6*) was the male-fertile parent and T295H (*Ms6ms6*) was the male-sterile parent. Fertile bulk, bulk of 10 homozygous male-fertile F_2 plants; Sterile bulk, bulk of 10 male-sterile F_2 plants.



Discussion

This study was undertaken to develop a comprehensive map for all known male-sterile, female-fertile genes in soybean. There are 11 male-sterile, female-fertile genes identified in soybean and eight have been mapped to soybean chromosomes (Delannay and Palmer 1982; Graybosch and Palmer 1985, 1988; Horner and Palmer 1995; Jin et al. 1998; Palmer 2000; Kato and Palmer 2003; Palmer et al. 2004; Cervantes-Martinez et al. 2007, 2009; J. Gosh, M.K. Bhattacharyya, Reid Palmer, and D. Sandhu, personal communication). For three genes (*ms1*, *ms4*, and *ms6*) map locations were unknown. We used the bulked segregant analysis technique to locate these male-sterile, female-fertile genes to soybean chromosomes. The *ms1* and *ms6* genes mapped to molecular linkage group F (chromosome 13) and *ms4* mapped to molecular linkage group D1b (chromosome 2) (Fig. 2). Although *ms1* and *ms6* were present on the same chromosome, they were located far apart and segregated independently of each other. The SSR marker Satt030 was present on both maps. The *ms1* gene was located 15.3 cM down from this marker and *ms6* was located 58.4 cM up, with a genetic distance of 73.7 cM between the genes (Fig. 2*a*). Our observations confirmed earlier studies that showed that *ms1* and *ms6* are on the same linkage group, but assort independently from each other (Skorupska and Palmer 1989). In soybean, flower color locus *w1* was linked with *ms6* (Palmer et al. 1998). We also mapped the *w1* locus on the *ms6* mapping population. Results confirmed that *w1* and *ms6* were present on the same chromosome and were 15.7 cM apart (Fig. 2a). In this study, the distance between *w1* and *ms6* was significantly higher than the previously reported distance of 2–5 cM in different mapping populations (Palmer et al. 1998). The higher

Fig. 2. Genetic linkage maps and sequenced based physical maps of the soybean chromosomes showing locations of SSR markers close to the male-sterile, female-fertile loci *ms1*, *ms4*, and *ms6*. (*a*) Maps of molecular linkage group F (chromosome Gm16) showing map locations of *ms1* and *ms6*. (*b*) Map of molecular linkage group D1b (chromosome Gm02) showing map location of *ms4*. Physical distances are shown in base pairs, and genetic distances are shown in centiMorgans.

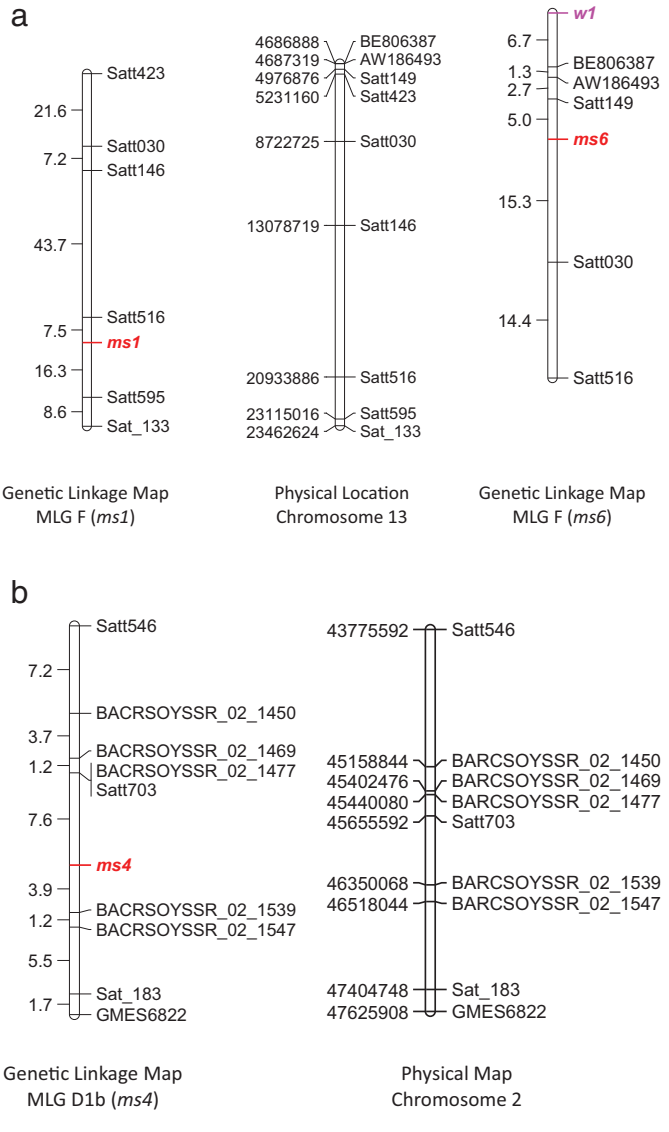
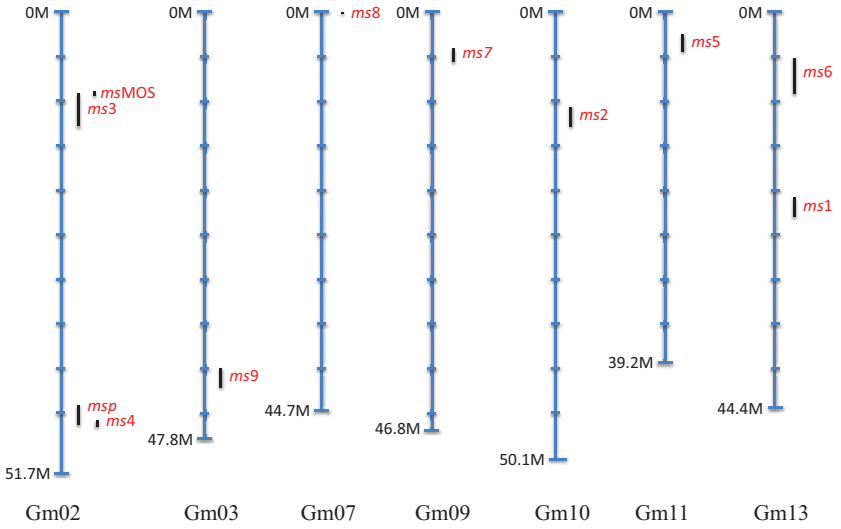


Fig. 3. Physical maps of the soybean chromosomes showing locations of all known male-sterile, female-fertile genes. The length of the bar next to the gene name represents the physical region where the gene is located (Jin et al. 1998; Cervantes-Martinez et al. 2007, 2009; Frasch et al. 2011; Ott et al. 2013). The putative location of the *ms7* gene is determined based on a transposon tagging study (J. Gosh, M.K. Bhattacharyya, R.G. Palmer, and D. Sandhu, personal communication).



genetic distance in this study may have resulted due to the presence of multiple markers between *ms6* and *w1* (Fig. 2*a*).

A physical map for each chromosome was generated using soybean genome sequence information (Schmutz et al. 2010; http://www.phytozome.net/). The *ms1* gene was flanked by Satt516 and Satt595, which were 23.8 cM apart. Physically, the region was ~2.2 Mb and contained 150 predicted genes (Fig. 2*a*). The *ms6* gene was flanked by Satt149 and Satt030 with a genetic distance of 20.3 cM, which corresponded to ~3.7 Mb and 268 predicted genes. On a sequence based physical map, *ms1* and *ms6* were at least 12 million base pairs away from each other (Fig. 2*a*). For *ms4*, the gene was flanked by Satt703 and BARCSOYSSR_02_1539, which encompassed an 11.5 cM region on chromosome 2. This region on the physical map was about 694 kb, which contained 88 predicted genes.

Comparison of map locations of male-sterile, female-fertile genes revealed that 11 genes were located on seven soybean chromosomes (Fig. 3). Four male-sterile, female-fertile genes (*ms3*, *msMOS*, *ms5*, and *msp*) were present in two regions on chromosome 2. Although, these two gene regions were present on the same chromosome, they were at least 32 Mb apart (Fig. 3). The *ms1* and *ms6* genes were present on chromosome 13, but they were more than 12 Mb apart from each other (Fig. 3). Chromosomes 3, 7, 9, 10, and 11 contained *ms9*, *ms8*, *ms7*, *ms2*, and *ms5*, respectively (Fig. 3).

In conclusion, mapping of these male-sterile, female-fertile genes may provide a way for fine mapping and map-based cloning of these genes. Cloning and characterization of male-sterile, female-fertile genes will advance our knowledge about reproductive biology of soybean and other crop plants. In the long run, it may help in the establishment of a stable male sterility system in soybean that can be exploited to develop hybrid soybean.

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