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# Molecular mapping of five soybean genes involved in male-sterility, female-sterility

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**Abstract:** In soybean, asynaptic and desynaptic mutants lead to abnormal meiosis and fertility reduction. Several male-sterile, female-sterile mutants have been identified and studied in soybean, however, some of these mutants have not been mapped to locations on soybean chromosomes. The objectives of this study were to molecularly map five male-sterile, female-sterile genes (*st2*, *st4*, *st5*, *st6*, and *st7*) in soybean and compare the map locations of these genes with already mapped sterility genes. Microsatellite markers were used in bulked segregant analyses to locate all five male-sterile, female-sterile genes to soybean chromosomes, and markers from the corresponding chromosomes were used on $F_2$ populations to generate genetic linkage maps. The *st2*, *st4*, *st5*, *st6*, and *st7* genes were located on molecular linkage group (MLG) B1 (chromosome 11), MLG D1a (chromosome 01), MLG F (chromosome 13), MLG B2 (chromosome 14), and D1b (chromosome 02), respectively. The *st2*, *st4*, *st5*, *st6*, and *st7* genes were flanked to 10.3 (~399 kb), 6.3 (~164 kb), 3.9 (~11.8 Mb), 11.0 (~409 kb), and 5.3 cM (~224 kb), and the flanked regions contained 57, 17, 362, 52, and 17 predicted genes, respectively. Future characterization of candidate genes should facilitate identification of the male- and female-fertility genes, which may provide vital insights on structure and function of genes involved in the reproductive pathway in soybean.

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

**Résumé :** Chez le soya, des mutants asynaptiques et désynaptiques présentent des anomalies méiotiques et une réduction de la fertilité. Plusieurs mutants présentant une stérilité mâle et femelle ont été identifiés et étudiés, mais certains de ceux-ci n'ont pas été situés sur les chromosomes du soya. Les objectifs de ce travail étaient de réaliser la cartographie de cinq gènes de stérilité mâle et femelle (*st2*, *st4*, *st5*, *st6* et *st7*) chez le soya et de comparer la position de ces gènes avec celle d'autres gènes de stérilité déjà positionnés. Des marqueurs microsatellites ont été employés dans le cadre d'analyses de ségrégants en mélange afin d'assigner une position chromosomique aux cinq gènes de stérilité mâle et femelle. Les marqueurs situés sur ces chromosomes ont été employés sur des populations $F_2$ afin de générer des cartes génétiques. Les gènes *st2*, *st4*, *st5*, *st6* et *st7* ont été assignés respectivement aux groupes de liaison moléculaires (MLG) B1 (chromosome 11), MLG D1a (chromosome 01), MLG F (chromosome 13), MLG B2 (chromosome 14) et MLG D1b (chromosome 2). Les gènes *st2*, *st4*, *st5*, *st6* et *st7* ont été assignés à des intervalles de 10,3 (~399 kb), 6,3 (~164 kb), 3,9 (~11,8 Mb), 11,0 (~409 kb) et 5,3 cM (~224 kb); ces intervalles contenaient respectivement 57, 17, 362, 52 et 17 gènes prédits. Une future caractérisation de gènes candidats devrait faciliter l'identification des gènes de fertilité mâle et femelle, laquelle fournira un éclairage important sur la structure et la fonction des gènes impliqués dans la reproduction chez le soya. [Traduit par la Rédaction]

*Mots-clés : Glycine max*, stérilité, séquence simple répétée, carte génétique, stérilité mâle et femelle.

## Introduction

In soybean, asynaptic and desynaptic mutants lead to abnormal meiosis and fertility reduction. This is caused by abnormal pairing of chromosomes during the first meiotic prophase, also known as synapsis. Mutations in genes involved in synapsis can result in male-sterile, female-sterile plants, male-sterile, female-fertile plants, or male-fertile, female-sterile plants. Asynapsis refers to the lack of chromosome pairing in prophase I during meiosis, and desynapsis describes the situation in which chromosomes initially pair in early prophase I but fail to remain paired at later stages (Gottschalk and Kaul 1980*a*,

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**Table 1.** Male-sterile, female-sterile mutants in soybean with gene symbols and mapping locations.

| Gene | Phenotype | Map location |
|---|---|---|
| *st1* | Gene has been lost (Owen 1928) | Not known |
| *st2* | MSFS (asynaptic) (Hadley and Starnes 1964) | MLG B1 (This study) |
| *st3* | MSFS (asynaptic) (Hadley and Starnes 1964) | Not known |
| *st4* | MSFS (desynaptic) (Palmer 1974) | MLG D1a (This study) |
| *st5* | MSFS (desynaptic) (Palmer and Kaul 1983) | MLG F (This study) |
| *st6* | MSFS (desynaptic) (Graybosch et al. 1987) | MLG B2 (Speth et al. 2014; This study) |
| *st7* | MSFS (desynaptic) (Graybosch et al. 1987) | MLG D1b (This study) |
| *st8* | MSFS (desynaptic) (Palmer and Horner 2000) | MLG J (Kato and Palmer 2003; Raval et al. 2013) |
| *ASR-7-206* | MSFS (Palmer et al. 2008) | MLG G (Palmer et al. 2008) |
| *A03-2137* | MSFS (Slattery et al. 2011) | MLG B2 (Slattery et al. 2011) |
| *A05-133* | MSFS (Slattery et al. 2011) | MLG D1b (Slattery et al. 2011) |
| *A06-204* | MSFS (Slattery et al. 2011) | MLG G (Slattery et al. 2011) |
| *st_A06-2/6* | MSFS (Baumbach et al. 2012) | MLG D1a (Baumbach et al. 2012) |

**Note:** MSFS, male-sterile, female-sterile; MLG, molecular linkage group.



1980*b*; Koduru and Rao 1981). Several male-sterile, female-sterile mutants have been identified and studied in soybean (Table 1) (Owen 1928; Hadley and Starnes 1964; Palmer 1974; Palmer and Kaul 1983; Ilarsian et al. 1997; Palmer and Horner 2000; Palmer et al. 2008; Slattery et al. 2011; Baumbach et al. 2012). Some of these mutants have been mapped to locations on soybean chromosomes (Kato and Palmer 2003; Palmer et al. 2008; Slattery et al. 2011; Baumbach et al. 2012).

The *st1* mutant was the first male-sterile, female-sterile mutant identified in soybean (Owen 1928). Unfortunately, this mutant has been lost. *st2* (T241) was identified as a male-sterile, female-sterile mutation in strain S54-1714, having the varieties Lincoln, Richland, and CNS as parentage and displaying monogenic inheritance (Hadley and Starnes 1964). Sterile plants experienced early dropping of flowers followed by the development of small, fleshy but seedless pods (Palmer 1974). Pollen from sterile plants was analyzed for viability, and only 8.9% of pollen was determined to be viable. These sterile pollen grains varied greatly in size (Palmer 1974). This great range in pollen size in sterile plants is consistent with asynapsis.

*st4* was identified as a spontaneous mutation at Iowa State University and was assigned a genetic type collection T-number (T258) (Palmer 1974). *st4* produces sterile plants with fleshy but seedless pods. Cytology of the *st4* mutant at pachytene showed initial pairing of homologous chromosomes (Palmer 1974). At diakinesis, sterile plants were observed to have a complete lack of pairing. A major consequence of desynapsis is the production of gametes with various degrees of chromosome imbalance because of unequal chromosome distributions at anaphase I and II (Palmer 1974). The overall effect of this gametic imbalance is almost complete sterility in both meiocytes (Palmer 1974).

*st5* (T272) was also identified as a desynaptic mutant due to a complete absence of chromosome pairing at diakinesis (Palmer and Kaul 1983). The first noticeable characteristics of sterile plants were the abscission of early flowers and subsequent failure to produce normal pods (Palmer and Kaul 1983). During meiosis, desynaptic plants showed few chromosome pairs, however bivalents appeared as attenuated rods rather than associated homologues (Palmer and Kaul 1983). Univalent chromosomes were found scattered throughout the cell or adjacent to the metaphase plate. Pollen grains in sterile plants varied greatly in size and stained poorly with $I_2KI$ (Palmer and Kaul 1983).

In a tissue culture study, two sterility mutants (*st6* and *st7*) were identified in 89 families generated from cotyledonary node tissue culture (Graybosch et al. 1987). Both of these mutants appeared in cultivar Calland that showed the male-sterile, female-sterile phenotype and was named Calland TC sterile (Ilarsian et al. 1997). Inheritance studies revealed that two redundant recessive genes (*st6* and *st7*) control sterility in the mutant (Ilarsian et al. 1997). Calland TC sterile was designated *St6st6st7st7* and assigned the genetic type collection number T331H. The mutant plant showed abnormalities in chromosome segregation, which resulted in nonviable pollen grains that were variable in size (Ilarsian et al. 1997). The sterility in the Calland TC sterile mutant was ascribed to desynapsis. During meiosis in Calland TC sterile, lagging chromosomes separated from the spindles during the early dyad stage or during postmetaphase resulting in the formation of some male cells with four nuclei and others with five to eight nuclei (Ilarsian et al. 1997). The *st6* gene was mapped to a 644-kb region on molecular linkage group (MLG) B2 (Speth et al. 2014).

The *st8* gene was identified among progeny of a mutable line, *w4-m* (Palmer and Horner 2000). The *W4* locus controls flower pigmentation in soybean. The unstable mutable *w4-m* allele is the result of a transposon insertion in the *W4* locus (Xu et al. 2010). When the transposon excises, the unstable allele reverts to *W4* and the flower regains pigmentation, however, the transposon lands in the *St8* locus, causing sterility (Palmer and Horner 2000). This sterility gene was inherited as a single recessive nuclear gene and was nonallelic to the known male-

sterile, female-sterile mutants *st2*, *st4*, *st5*, *st6*, and *st7* (Palmer and Horner 2000). The *st8* gene was mapped to a 62-kb region on MLG J; the mutation was caused by the insertion of an active soybean transposable element, *Tgm9* (Kato and Palmer 2003; Raval et al. 2013).

In addition to the above-mentioned male-sterile, female-sterile genes, five new gene loci (*ASR-7-206*, *A03-2137*, *A05-133*, *A06-204*, and *st_A06-2/6*) have been characterized and mapped in the last few years (Table 1). *ASR-7-206* and *A03-2137* were identified among the progenies of the mutable line *w4-m* and were mapped to MLG G and MLG B2, respectively (Palmer et al. 2008; Slattery et al. 2011). *A05-133* (W.R. Fehr's breeding project) and *A06-204* (S.R. Cianzio's breeding project) were identified as spontaneous mutations in two breeding populations at Iowa State University. *A05-133* and *A06-204* were mapped to MLG D1b and MLG G, respectively (Slattery et al. 2011). *st_A06-2/6* was identified in a transformation study in soybean and was mapped to MLG D1a (Baumbach et al. 2012).

The objectives of this study were to molecularly map five male-sterile, female-sterile genes (*st2*, *st4*, *st5*, *st6*, and *st7*) in soybean and compare the map locations of these genes with already mapped sterility genes.

## Materials and methods

### Plant materials

Three *st2* mapping populations (A10-1009, A10-1011, and A10-1065) consisting of 72, 72, and 40 $F_2$ plants, respectively, were generated by crossing Minsoy (*St2St2*) with the sterility mutant line T241H (*St2st2*). The $F_1$ plants were self-pollinated to generate $F_2$ populations, and segregating $F_2$ populations were used for molecular mapping. Two *st4* mapping populations (A10-1100 and A10-1130) consisting of 62 and 60 $F_2$ plants, respectively, were generated by crossing Minsoy (*St4St4*) with the sterility mutant line T258H (*St4st4*). For *st5*, a mapping population (A10-138) consisting of 95 $F_2$ plants was generated by crossing Minsoy (*St5St5*) with the sterility mutant line T272H (*St5st5*). Two *st6* mapping populations (A10-121 and A10-131) consisting of 63 and 93 $F_2$ plants, respectively, were generated by crossing Minsoy (*St6St6 st7st7*) or Manchu (*St6St6 st7st7*) with sterility mutant line T331H (*St6st6 st7st7*). Since *St6* and *St7* are redundant genes, mapping of *st6* and *st7* consisted of crossing T272 (*St6St6 St7St7*) with T331H (*St6st6 st7st7*) to generate two $F_2$ populations (segregating for *St6* and *St7*) consisting of 201 (A11-109) and 195 (A11-110) plants.

For each mapping population, the fertile $F_2$ plants were threshed separately. Each fertile $F_2$ plant was progeny tested by planting 50 $F_{2:3}$ descendants. Segregation of fertile and sterile plants, or all fertile plants, in each $F_{2:3}$ line was recorded at maturity to determine each $F_2$-plant genotype (Table 2).

### Bulked segregant analyses (BSA)

To find the locations of the *st2*, *st4*, *st5*, *st6*, and *st7* genes, BSA were used (Michelmore et al. 1991). For

**Table 2.** Segregation patterns, chi-square values, and *P* values for the sterility populations.

| Populations | Parents | Gene | No. of $F_2$ plants | | | | | No. of $F_2$ families | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fertile | Sterile | $\chi^2$ (3:1) | $\chi^2$ (15:1) | *P* | All fertile | 15:1 | 3:1 | $\chi^2$ (1:2) | $\chi^2$ (7:4:4) | *P* |
| A10-1009, A10-1011, A10-1065 | Minsoy (*St2St2*) × T241H (*St2st2*) | *st2* | 133 | 51 | 0.73 | — | 0.39 | 45 | — | 88 | 0.02 | — | 0.90 |
| A10-1100, A10-1130 | Minsoy (*St4St4*) × T258H (*St4st4*) | *st4* | 100 | 22 | 3.16 | — | 0.08 | 25 | — | 75 | 3.12 | — | 0.08 |
| A10-138 | Minsoy (*St5St5*) × T272H (*St5st5*) | *st5* | 76 | 19 | 1.27 | — | 0.26 | 29 | — | 47 | 0.8 | — | 0.37 |
| A10-121, A10-131 | Minsoy/Manchu (*St6St6 st7st7*) × T331H (*St6st6 st7st7*) | *st6* | 84 | 72 | 37.2 | — | <0.01 | 27 | — | 55 | 0.01 | — | 0.94 |
| A11-109, A11-110 | T272 (*St6St6 St7St7*) × T331H (*St6st6 st7st7*) | *st6* and *st7* | 366 | 30 | — | 1.19 | 0.28 | 179 | 100 | 87 | — | 1.61 | 0.45 |

mapping populations *st2*, *st4*, *st5*, and *st6*, fertile and sterile bulks for BSA were prepared from randomly selected DNA samples of 10 homozygous fertile (fertile bulk) and 10 sterile (sterile bulk) $F_2$ plants. For the population that segregated for *st6* and *st7*, two sterile bulks consisting of 10 sterile $F_2$ plants each were prepared and tested against the fertile parent. 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. The bulks were tested with 900 simple sequence repeat (SSR) markers from the 20 soybean chromosomes.

### Molecular marker analysis

For SSR analysis, 30 ng DNA was used as the template 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.). The PCR conditions consisted of the following: 94 °C for 3 min; 11 cycles of 94 °C for 30 s, 58 °C for 30 s with an increment of –1 °C per cycle, and 72 °C for 1 min; 35 cycles of 94 °C for 30 s, 46 °C for 30 s, and 72 °C for 1 min; and a final 10 min at 72 °C. The PCR products were separated on a 4% agarose gel at 150 V for 1–3 h. The Mapmaker 2.0 program was used to determine genetic linkages and genetic distances (Kosambi 1943; Lander et al. 1987). Marker order was determined at a LOD threshold of 3.0. Sequence information for developing SSR markers was obtained from http://soybase.org/resources/ssr.php (Song et al. 2004, 2010). The software MapChart was used to create maps (Voorrips 2002).

### Scoring and data analysis

An "ABHCD" 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 fertile parent; "B" was assigned if it was homozygous for the alleles from the sterile parent; and "H" was assigned if the plant was heterozygous. In some cases "C" was assigned when it was not possible to resolve between the "B" or "H" banding pattern, and "D" was assigned when it was not possible to resolve between the "A" or "H" banding pattern.

For the mapping populations where both *st6* and *st7* were segregating (A11-109 and A11-110), families that were showing all sterile plants in $F_{2:3}$ progeny test were scored as "B". The families that segregated 3:1 in $F_{2:3}$ were scored as "C": either *St6st6* was heterozygous and *st7st7* was homozygous or *st6st6* was homozygous and *St7st7* was heterozygous. The $F_{2:3}$ families that segregated 15:1, indicating *St6st6* and *St7st7* both were heterozygous, were scored as "H". The $F_2$ plants that did not segregate for fertile and sterile plants in the $F_{2:3}$ generation (*St6St6 St7St7*, *St6St6 St7st7*, *St6St6 st7st7*, *St6st6 St7St7*, and *st6st6 St7St7*) were not used for mapping analysis.

After scoring the population, linkage analysis was conducted. Recombination frequencies were converted to map distances in centiMorgans (cM) using the Kosambi mapping function (Kosambi 1943). Recombination values were calculated to determine whether the SSR markers were linked to the gene of interest. Mapmaker 2.0 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.

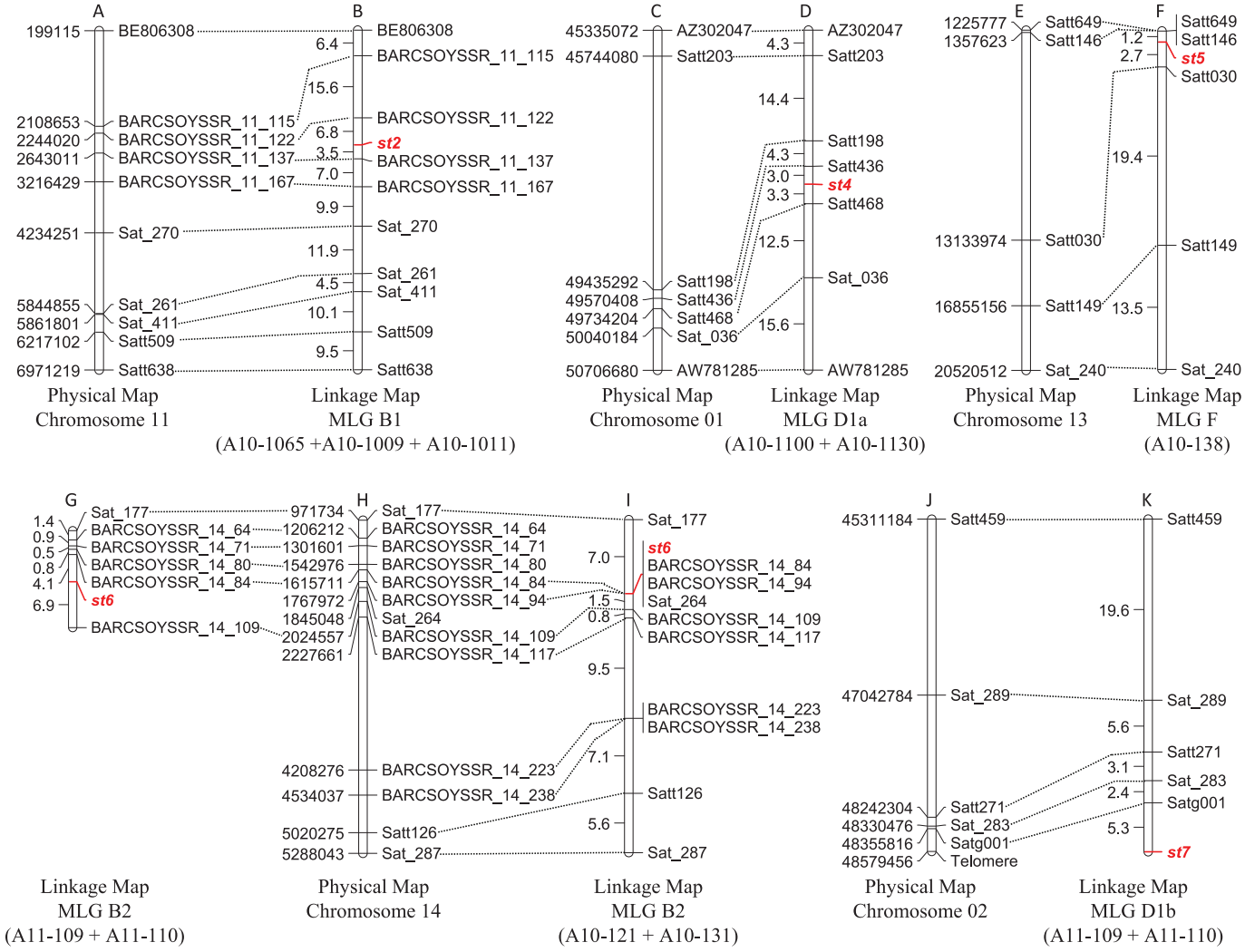
## Results

Male-sterility, female-sterility was evaluated in the $F_2$ and $F_{2:3}$ populations generated for the *st2*, *st4*, *st5*, *st6*, and *st7* genes. Three $F_2$ populations for *st2* (A10-1009, A10-1011, and A10-1065), two for *st4* (A10-1100 and A10-1130), and one for *st5* (A10-138) displayed 3:1 segregation for fertile and sterile plants with collective *P* values of 0.39, 0.08, and 0.26, respectively (Table 2). The progeny testing of the $F_2$ populations in $F_{2:3}$ for *st2*, *st4*, and *st5* showed 1:2 ratios of homozygous fertile to heterozygous fertile $F_2$ plants with *P* values of 0.90, 0.08, and 0.37, respectively. Two $F_2$ populations for *st6* (A10-121 and A10-131) displayed a combined *P* value less than 0.01 for the 3:1 segregation. The progeny testing in $F_{2:3}$ generation, however, displayed expected 1:2 ratio for homozygous fertile to heterozygous fertile plants with a *P* value of 0.94. The low *P* value in $F_2$ may have been due to sampling variation. The $F_2$ and $F_{2:3}$ segregations confirmed recessive monogenic inheritance of all four genes (Table 2).

Two $F_2$ populations (A11-109 and A11-110) that segregated both for *st6* and *st7* exhibited a good fit to the segregation ratio of 15:1 (fertile : sterile) with a collective *P* value of 0.28 (Table 2). Progeny testing in the $F_{2:3}$ generation displayed segregation ratio of 7:4:4 (all fertile (*St6_ St7_*, *St6St6 _ _ or _ _ St7St7*) : segregating 15 fertile to 1 sterile (*St6st6 St7st7*) : segregating 3 fertile to 1 sterile (*St6st6 st7st7 or st6st6 St7st7*)) with a *P* value of 0.45 (Table 2). These data confirmed duplicate dominance epistasis between *St6* and *St7*.

Segregating $F_2$ populations were selected for mapping. BSA was used on fertile and sterile bulks for each segregating population. Each of the five sterility genes (*st2*, *st4*, *st5*, *st6*, and *st7*) were located to soybean chromosomes using 900 SSR markers. For the *st2* populations, Sat_270 showed polymorphism between the bulks. Other markers located close to Sat_270 on MLG B1 were tested for polymorphism between the parents and bulks. Nine markers (BE806308, Sat_261, Sat_411, Satt509, BARCSOYSSR_11_115, BARCSOYSSR_11_122, BARCSOYSSR_11_137, BARCSOYSSR_11_167, and Satt638) showed polymorphism in the parents and were tested on the $F_2$ populations A10-1065, A10-1009, and A10-1011 for mapping. The *st2* gene was flanked by markers BARCSOYSSR_11_122 and BARCSOYSSR_11_137 with a genetic distance of 6.8 and 3.5 cM, respectively (Fig. 1B).

**Fig. 1.** Genetic linkage maps and sequenced-based physical maps of the soybean chromosomes showing locations of SSR markers close to the male-sterile, female-sterile loci *st2*, *st4*, *st5*, *st6*, and *st7*. (A) Physical map of chromosome 11. (B) Genetic linkage map of MLG B1 (chromosome 11) showing map location of *st2*. (C) Physical map of chromosome 01. (D) Genetic linkage map of MLG D1a (chromosome 01) showing map location of *st4*. (E) Physical map of chromosome 13. (F) Genetic linkage map of MLG F (chromosome 13) showing map location of *st5*. (G) Genetic linkage map of MLG B2 (chromosome 14) (populations A11-109 + A11-110) showing map location of *st6*. (H) Physical map of chromosome 14. (I) Genetic linkage map of MLG B2 (chromosome 14) (populations A10-121 + A10-131) showing map location of *st6*. (J) Physical map of chromosome 02. (K) Genetic linkage map of MLG D1b (chromosome 02) showing map location of *st7*. Names of the populations used for the genetic linkage mapping are shown in parenthesis below the linkage maps. Physical distances are shown in base pairs, and genetic distances are shown in centiMorgans.




For *st4* mapping, Satt198 showed polymorphism between the bulks. Other markers in the vicinity of Satt198 on MLG D1a were tested on the parents and bulks for polymorphism. Of the markers tested, AZ302147, Satt203, Satt436, Satt468, Sat_036, and AW781285 detected polymorphism. The polymorphic markers were tested on the $F_2$ populations A10-1100 and A10-1130 for mapping. Markers Satt436 and Satt468 flanked the *st4* gene with a genetic distance of 3.0 and 3.3 cM, respectively (Fig. 1D).

Marker Satt030 detected polymorphism in the *st5* bulks. Other markers close to Satt030 on MLG F were then tested for polymorphism with the parents and bulks. Sat_240, Satt149, Satt649, and Satt146 all detected polymorphism and were used on the entire $F_2$ population A10-138 for mapping. Satt030 and Satt649/Satt146 flanked the *st5* gene with a genetic distance of 2.7 and 1.2 cM, respectively (Fig. 1F). The markers Satt649 and Satt146 showed cosegregation.

For the *st6* bulks, Sat_264 showed polymorphism. Other markers on MLG B2 were then tested for polymorphism between the parents and bulks. Sat_177, BARCSOYSSR_14_84, BARCSOYSSR_14_94, Sat_264, BARCSOYSSR_14_109, BARCSOYSSR_14_117, BARCSOYSSR_14_223, BARCSOYSSR_14_238, Satt126, and Sat_287 all detected polymorphism and were used on the $F_2$ pop-

ulations A10-121 and A1-131 for mapping. Markers, BARCSOYSSR_14_84, BARCSOYSSR_14_94, and Sat_264 showed complete linkage with *st6* (Fig. 1I).

The populations (A11-109 and A11-110) used for mapping *st7* also segregated for *st6*. We were able to map both genes in these populations. Instead of using a fertile and a sterile bulk for BSA, we used fertile parent T272 along with two sterile bulks. Each of the sterile bulks was constituted by mixing equal amount of DNA from 10 sterile plants. Two markers (BARCSOYSSR_14_84 (MLG B2) and Satg001 (MLG D1b)) showed polymorphism between T272 and the sterile bulks, indicating map location of the *st6* and *st7* genes. Twenty SSR markers on MLG B2 were tested on the parents and five (Sat_177, BARCSOYSSR_14_64, BARCSOYSSR_14_71, BARCSOYSSR_14_80, and BARCSOYSSR_14_109) detected polymorphism. Seventeen markers on MLG D1b were tested on the parents and four (Satt459, Sat_289, Satt271, and Sat_283) showed polymorphism. Polymorphic markers were used to assay DNA of 217 individual $F_2$ plants that segregated 15:1 or 3:1 or were all sterile (Figs. 1H and 1K). The map for MLG B2 showed that the *st6* locus was flanked by BARCSOYSSR_14_84 and BARCSOYSSR_14_109 (Fig. 1H), which is consistent with mapping populations A10-110 and A10-121 (Fig. 1I). Mapping results for MLG D1b revealed the location of *st7* between Satg001 and the telomere, with Satg001 at a distance of 5.3 cM (Fig. 1K).

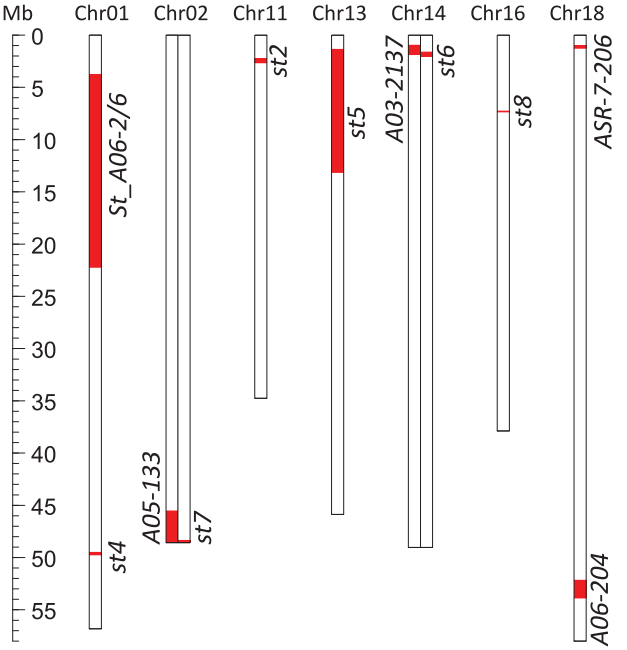
## Discussion

In this study, five male-sterile, female-sterile genes were molecularly mapped in the soybean genome. For the physical localization of the genes, physical maps of related chromosomes were generated based on soybean genome sequencing information (Schmutz et al. 2010) (http://www.phytozome.net/). The *st2* gene is flanked by BARCSOYSSR_11_122 and BARCSOYSSR_11_137 on chromosome 11, which corresponds to a physical distance of ~398 kb and is home to 57 predicted genes (Fig. 1A). *st5* was flanked by Satt146 and Satt030 on chromosome 13 with a physical distance of ~11.8 Mb and is home to 362 predicted genes. The *st6* gene is flanked by markers BARCSOYSSR_14_84 and BARCSOYSSR_14_109 on chromosome 14, which corresponds to a region ~409 kb in physical distance and is home to 52 predicted genes. In a previous study, *st6* was located between Sat_177 and BARCSOYSSR_14_84 (Speth et al. 2014). Although the gene mapped to the same region on the chromosome, but due of the use of two different populations and additional markers in the current study, we were able to locate the *st6* gene to a smaller physical region.

The *st4* and *st7* genes were located to small physical regions on chromosome 1 and chromosome 2, respectively, which made it possible to identify putative candidates for these genes (Fig. 1). The *st4* gene is present in a region flanked by markers Satt436 and Satt468, which corresponds to a physical distance of ~164 kb and contains 17 predicted genes (Table S1[1]). One of these 17 predicted genes (*Glyma.01G159200*) is of interest because it codes for a member of RWP-RK DOMAIN (RKD) transcription factor family. RKDs are known to play important roles in male gametogenesis, control of egg cell functions, and cell division (Waki et al. 2013; Chardin et al. 2014). The *st7* gene is flanked by marker Satg001 and the telomere on chromosome 2, which corresponds to a physical distance of ~224 kb and contains 17 predicted genes (Table S2[1]). Interestingly, one of the genes present in this region (*Glyma.02G311000*) also codes for a member of the RKD transcription factor family. It is possible that these two genes are epistatic to each other and may be involved in the same reproductive pathway in soybean.

Previously mapped sterility genes include *st8*, *ASR-7-206*, *A03-2137*, *A05-133*, *A06-204*, and *st_A06-2/6*, respectively located on MLG J (chromosome 16) (Kato and Palmer 2003), MLG G (chromosome 18) (Palmer et al. 2008), MLG B2 (chromosome 14) (Slattery et al. 2011), MLG

**Fig. 2.** Physical maps of the soybean chromosomes showing locations of all known male-sterility, female-sterility genes. The scale on the left represents length of the chromosomes in million basepairs (Mb). The highlighted sections in the bars represent physical regions where the genes are located (Kato and Palmer 2003; Palmer et al. 2008; Slattery et al. 2011; Baumbach et al. 2012; Speth et al. 2014). For chromosomes 02 and 14, two bars are drawn to show overlaps between the regions containing two closely located genes.




[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2015-0044.

D1b (chromosome 02) (Slattery et al. 2011), MLG G (chromosome 18) (Slattery et al. 2011), and MLG D1a (chromosome 01) (Baumbach et al. 2012). While three of the genes mapped in the present study are on the same chromosomes as previously mapped sterility genes, the differences in locations can be used to examine if they are unique genes. The *st4* and *st_A06-2/6* genes are present on chromosome 1, but they are separated by a distance of ~28.8 Mb, suggesting that they are unique genes (Fig. 2). However, *st7* and *A05-133* are in the similar region on chromosome 2, indicating the possibility of *A05-133* and *st7* being allelic (Fig. 2). *st6* and *A03-2137* are also present in the similar region on chromosome 14, suggesting that they may also be allelic (Fig. 2). Complementation tests between these mutants should be able to confirm these possibilities.

This study lays a foundation for fine mapping and map-based cloning of male-sterile, female-sterile genes in soybean. Future studies focusing on characterization of the candidate genes may result in cloning of these genes, which could shed light on the reproductive pathway in soybean and other plants. In the long run, it may help in the development of stable male-sterile lines that may become instrumental in exploiting heterosis in soybean.

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