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ORIGINAL PAPER

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Molecular mapping of four ovule lethal mutants in soybean

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Abstract We report genetic mapping of four soybean ovule lethal mutants, PS-1, PS-2, PS-3, and PS-4, which had been identified as female partial-sterile mutants from a gene-tagging study. The four mutants had been classified into two mutation classes: (1) PS-1—sporophytic mutation affects sporophytically expressed genes; and (2) PS-2, PS-3, and PS-4 mutants—female gametophyte-specific mutations affect gametophytically expressed genes and are transmitted through the male, but not the female gametes. Molecular mapping demonstrated that these four mutant genes and previously reported female-partial sterile gene, *Fsp1*, are located independently on soybean molecular linkage groups (MLG-) using SSR markers. PS-1, designated as *Fsp2* and Genetic Type Collection number T364, is located between SSR markers Satt170 and Satt363 on MLG-C2 and linked by 13.9 cM and 12.1 cM, respectively. PS-2, designated as *Fsp3* and Genetic Type Collection number T365H, is located between SSR markers Satt538 and Satt429 on MLG-A2 and linked by 13.3 cM and 25.4 cM, respectively. PS-3, designated as *Fsp4* and Genetic Type Collection number T366H, is located on the terminus of MLG-F and linked to Sat 152 by 13.1 cM. PS-4, designated as *Fsp5* and Genetic Type Collection number T367H, is located between SSR markers Satt324 and Satt138 on MLG-G and linked by 19.6 cM and 7.5 cM, respectively. SSR markers adjacent to *Fsp3*, *Fsp4*, and *Fsp5* were distorted from a 1:2:1 ratio and fit a 1:1 ratio. The segregation distortions of SSR markers adjacent to *Fsp3*, *Fsp4*, and *Fsp5* are in support of male, but not female transmission of the *Fsp3*, *Fsp4*, and *Fsp5* gametes.

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Introduction

The plant life cycle alternates between the gametophyte (a multicellular haploid generation) and the sporophyte (a multicellular diploid generation). In plants, meiosis gives rise to haploid spores, which undergo cell proliferation and differentiation to develop into gametophytes. A major function of the gametophyte generation is to produce haploid gametes. Fusion of the egg cell with the sperm cell gives rise to the sporophyte, thereby completing the life cycle (Raven et al. 1992).

Gene products required for female gametophyte development and function could be encoded by genes expressed either within the female gametophyte or in the surrounding sporophytic cells of the ovule. Several sporophytic mutations disrupting ovule development and function have been identified (Robinson-Beers et al. 1992; Lang et al. 1994; Leon-Kloosterziel et al. 1994; Modrusan et al. 1994; Gaiser et al. 1995; Elliot et al. 1996; Klucher et al. 1996; Baker et al. 1997; Pereira et al. 1997a; Schneitz et al. 1997). In some of these mutants, female gametophyte development is altered. However, it is not clear whether these mutations affect female gametophyte development directly or as a secondary consequence of an effect on the ovule's surrounding sporophytic tissue.

Genetic studies also suggest that female gametophyte development depends on the activities of many female gametophyte-expressed genes. For example, studies in

maize have shown that most chromosomal deletions are deleterious to the female gametophyte, suggesting that genes essential for embryo sac viability are present throughout the maize genome (Patterson 1978, 1994; Coe et al. 1988; Buckner and Reeves 1994; Vizir et al. 1994; Vollbrecht and Hake 1995). Moreover, morphological analysis of embryo sacs harboring a variety of small chromosomal deletions has demonstrated that female gametophyte-expressed genes control fundamental aspects of female gametophyte development, including nuclear migration, the coordination of developmental events, and the establishment of polarity (Vollbrecht and Hake 1995). Many of these genes may perform regulatory functions.

Not long after the rediscovery of Mendel's laws of heredity in 1900, it was found that some mutations exhibit aberrant segregation patterns (e.g., Correns 1902). It was later determined that these mutations affect the gametophytic phase of the plant's life cycle (Brink and MacGillivray 1924; Jones 1924). Over the intervening years, it has become clear that plants contain two broad classes of mutations that exhibit fundamentally different segregation patterns: (1) sporophytic mutations affect sporophytically expressed genes and generally exhibit Mendelian 3:1 segregation patterns, and (2) gametophytic mutations affect gametophytically expressed genes and are not transmitted through the egg and/or sperm. As a consequence, gametophytic mutations exhibit apparent non-Mendelian segregation patterns and can only be passed from generation to generation as heterozygotes. Gametophytic mutations are classified into three subclasses: (1) affects the female gametophyte but not the male gametophyte, called a "female gametophyte-specific mutation"; (2) affects the male gametophyte but not the female gametophyte, called a "male gametophyte-specific mutation"; and (3) affects both gametophytes, called a "general gametophytic mutation." Kato and Palmer (2003) identified a new mutation (sub) class in soybean called "female partial sterility." The *Fsp1* mutation has maximum phenotypic expression of female partial sterility when heterozygous, rather than when homozygous. This trait transmits through both the male and female parents (Kato and Palmer 2003). The reasons for this female partial sterility were due to incomplete megagametophyte development: undeveloped polar nuclei, or they are developed but not in a position for fertilization; increased megagametophyte wall thickness; abnormal shape and/or premature degeneration of synergids and intact synergids throughout the life of the ovule; egg cell not well-developed or absent; and the megagametophyte remains uninucleate. Each of these abnormalities contributed to either lack of double fertilization or early megagametophyte abortion (Ilarslan et al. 2003).

From a gene-tagging study in soybean, four female partial-sterile mutants, PS-1, PS-2, PS-3, and PS-4, were identified (Palmer et al. 1989). PS-1 mutant is associated with a single recessive gene and is classified as a sporophytic mutation, due to abnormal migration of the fused polar nucleus that prevents fertilization (Pereira et al. 1997a). PS-2 to PS-4 mutants are not fertilized, in spite of an intact egg apparatus and fused polar nuclei, and are expressed only when heterozygous at a single locus and transmit only through the male parent (Pereira et al. 1997b). Hence, PS-2 to PS-4 mutants are classified as female gametophyte-specific mutations. Allelism tests by cross-pollinations demonstrated that PS-1 is nonallelic to PS-2 to PS-4 (Pereira et al. 1997a). However, it was impossible to carry out the allelism tests among PS-2 to PS-4 mutants by cross-pollinations, because PS-2 to PS-4 mutations do not transmit the female partial-sterile gene when they are used as female parents (Pereira et al. 1997a). The allelic interaction among these mutants can be determined by the comparison of map location or DNA sequences of mutant genes.

The objectives of the present study were to determine the genetic map location of PS-1 to PS-4 mutant genes by molecular mapping using simple sequence repeat (SSR) markers.

Materials and methods

Plant materials

Cultivar Minsoy (PI 27890) (female parent) was crossed with PS-1, PS-2, PS-3, and PS-4 (Table 1) using standard soybean crossing techniques. All F_1 plants of the cross between Minsoy and PS-1 were fertile. While about 50% of the F_1 plants of the crosses between Minsoy and PS-2, PS-3, and PS-4 expressed female partial sterility, the remaining F_1 plants were fertile. F_2 plants of each female partial-sterile F_1 plant derived from these four crosses were used for the mapping study, and designated "POP-1," "POP-2," "POP-3," and "POP-4," respectively. POP-1, -2, and -3 F_2 seeds were planted at the Bruner Farm near Ames in May 2001.

Table 1 Soybean Genetic Type Collection T-number, PI number, and description of mutant strains in mapping study

Strain	Genotype	Phenotype	Parental origin	Where and when found	PI number
T364 (PS-1)	*fsp2 fsp2*	True breeding Female-partial sterile	Mutant in T322	By R.G. Palmer Ames, Iowa, USA, 1987	632946
T365H (PS-2)	*Fsp3 fsp3*	Female partial-sterile	Mutant in T322	By R.G. Palmer Ames, Iowa, USA, 1987	632947
T366H (PS-3)	*Fsp4 fsp4*	Female partial-sterile	Mutant in T322	By R.G. Palmer Ames, Iowa, USA, 1987	632948
T367H (PS-4)	*Fsp5 fsp5*	Female partial-sterile	Mutant in T322	By R.G. Palmer Ames, Iowa, USA, 1987	632949

Segregation for fertile/female partial-sterile plants was recorded at maturity. F_3 seeds from fertile and female partial-sterile F_2 plants were harvested individually. These F_3 seeds were planted at the University of Puerto Rico-Iowa State University Soybean Nursery, near Isabela in September 2001. Segregation of fertile/female partial-sterile plants in each F_3 line was recorded at maturity to confirm F_2-plant phenotypic classification. POP-4 F_2 seeds were planted in the USDA greenhouse at Iowa State University in January 2002. Segregation for fertile/female partial-sterile plants was recorded at maturity. F_3 seeds from fertile and female partial-sterile F_2 plants were harvested individually. These F_3 seeds were planted at the Bruner Farm near Ames in May 2002. Segregation of fertile/female partial-sterile plants in each F_3 line was recorded at maturity to confirm F_2-plant phenotypic classification.

SSR analysis

Soybean DNA was isolated from freeze-dried leaf tissue of parental and each POP-1, -2, -3, and -4 F_2 plants according to Keim et al. (1988). SSR markers (Akkaya et al. 1992) were evaluated. PCR reaction mixture contained 50 ng of soybean genomic DNA, 1.75 mM Mg^{2+}, 0.15 mM of sense and antisense primers, 150 mM of each nucleotide, 1×PCR buffer, and 0.5 U *Taq* DNA polymerase (Promega) in a total volume of 30 μl. Cycling consisted of 45 s at 94°C, 45 s at 47°C, and 45 s at 68°C for 32 cycles on a PTC-100 Programmable Thermal Controller (MJ Research). PCR products were run on 2.0% (w/v) Agarose 3:1 E776 (AMRESCO) gel in 1×TBE (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA) buffer with ethidium bromide incorporated in the gel. Alternatively, PCR products were run on a sequencing gel: 8% (w/v) acrylamide:bis-acrylamide (29:1), 5.6 M urea, and 30% (v/v) formamide in 1×TAE buffer.

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 by the Kosambi (1944) function. On the basis of two-point analysis, MAPMAKER generated log-likelihood values for the most probable order.

Results and discussion

PS-1 mapping study

The $F_{2:3}$ segregation of homozygous for Minsoy:heterozygote:homozygous for PS-1 was 10:24:7 and followed a 1:2:1 ratio (χ^2=1.63, P=0.44). PS-1 was designated *Fsp-2* and assigned Genetic Type Collection number T364 (Table 1).

Initial screening of the F_2 population was conducted by selecting several SSR markers from each linkage group (Cregan et al. 1999). The markers were chosen to divide each linkage group into segments of less than 30 cM. A total of 206 SSR markers were tested. Among them, SSR marker Satt170 on MLG-C2 was identified as linked to the PS-1 mutant gene (LOD=4.97). An additional 25 markers, Satt227, Sat 062, Satt291, Satt322, Satt450, Satt363, Sat 076, Satt286, Satt277, Satt289, Satt100, Satt319, Satt460, Satt307, Sct 028, Satt316, Satt202, Satt371, Satt357, Satt205, Satt432, Satt281, Satt376, Satt489, and Satt079, from this linkage group (Cregan et al. 1999) were screened between parental lines. We detected polymorphisms using SSR markers Sat 062, Satt281, Satt291, Satt363, Satt277, Satt307, Satt489, Satt460, Satt079, Satt202, Satt371, and Satt357. On the basis of LOD scores generated from the MAPMAKER program, PS-1 mutant gene was linked to SSR markers Satt277, Satt307, Satt363, Satt489, Satt460, and Satt079, with LOD scores of 7.39, 7.39, 6.22, 4.98, 4.98, and 4.57, respectively. The most likely order of markers is shown in Fig. 1A. The PS-1 mutant gene, designated as *Fsp2*, was located between Satt170 and Satt363, and linked to by 13.9 cM and 12.1 cM, respectively. No polymorphisms were observed using SSR markers Satt227, Satt322, Satt450, Sat 076, Satt286, Satt289, Satt100, Satt319, Sct 028, Satt316, Satt205, Satt432, and Satt376. Segregation ratios of all SSR markers on MLG-C2 provided good fits to 1:2:1 (Table 2).

PS-2 mapping study

The $F_{2:3}$ segregation of homozygous for Minsoy to heterozygote was 57:54 and followed a 1:1 ratio (χ^2=0.08, P=0.78). PS-2 was designated *Fsp3* and assigned Genetic Type Collection number T365H (Table 1).

Initial screening of the F_2 population was conducted by selecting several SSR markers from each linkage group (Cregan et al. 1999). The markers were chosen to divide each linkage group into segments of less than 30 cM. A total of 221 SSR markers were tested. Among them, SSR marker Satt538 on MLG-A2 was identified as linked to the PS-2 mutant gene (LOD=38.30). An additional 36 markers, Satt390, Satt207, Satt480, Satt493, Satt589, Satt315, Satt187, GMEND2B, Satt341, Sat 129, Sat 115, Satt377, Satt525, Satt233, Satt508, Satt329, Satt327, Satt470, Satt133, Satt209, Satt455, Satt409, Satt228, Satt538, Satt378, Satt429, Sct 067, Satt177, Satt424, Satt089, Satt119, Satt437, Satt158, Satt421, Sat 040, and Satt333, from this linkage group (Cregan et al. 1999) were screened between parental lines. We detected polymorphisms using SSR markers Satt177, Satt424, Sat 115, Sat 129, Satt437, Satt409, Satt538, and Satt429. On the basis of LOD scores generated from the MAPMAKER program, PS-2 mutant gene was linked to SSR markers Satt409, and Satt429 with LOD scores of 12.86 and 7.08, respectively. The most likely order of markers is shown in Fig. 1B. The PS-2 mutant gene, designated as *Fsp3*, was located between Satt538 and Satt429, and linked to by 13.3 cM and 25.4 cM, respectively. Segregation ratios of five SSR markers, Satt177, Satt424, Satt115, Satt129, and Satt437, on MLG-A2 apart from PS-2 mutant gene by more than 65.9 cM provided good fits to 1:2:1 (Table 3). While segregation ratios of three SSR markers, Satt409, Satt538, and Satt429, linked to PS-2 mutant gene by less than 31.1 cM were distorted from 1:2:1 and provided good fits to 1:1.

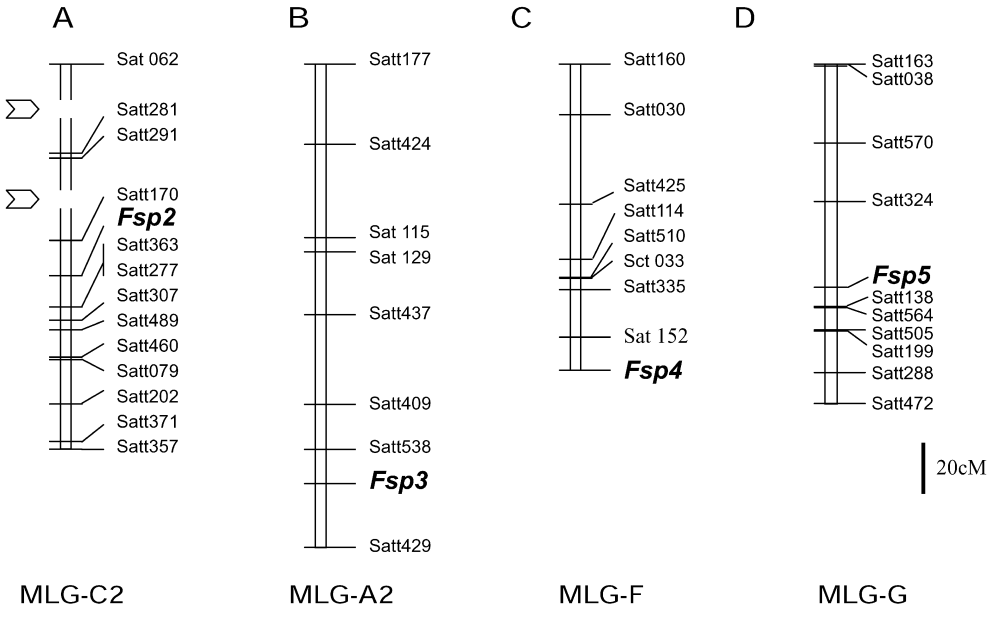

Fig. 1A–D Linkage maps of PS-1, PS-2, PS-3, and PS-4 soybean mutant genes. **A** Soybean molecular linkage group (MLG-) C2, showing the position of PS-1 mutant 1 gene designated as *Fsp2*. The *arrows* indicate the gaps where LOD scores are less than 3.0, calculated by MAPMAKER (Lander et al. 1987). The marker order of Sat 062, Satt281, and Satt291 was followed as in previous map (Cregan et al. 1999). **B** Soybean MLG-A2, showing the position of the PS-2 mutant gene, designated as *Fsp3*. **C** Soybean MLG-F, showing the position of PS-3 mutant gene, designated as *Fsp4*. **D** Soybean MLG-G, showing the position of the PS-4 mutant gene, designated as *Fsp5*

Table 2 Segregation of SSR markers linked to PS-1 mutant gene in an F_2 soybean population of a cross between cv. Minsoy and PS-1

Marker	Distance[a]	Observed number				χ^2	*P*
	(cM)	AA[b]	AB[b]	BB[b]	Total	(1:2:1)	
Sat 062	82.9	7	24	8	39	2.13	0.35
Satt281	48.1	11	19	8	38	0.47	0.79
Satt291	46.1	12	18	10	40	0.60	0.74
Satt170	13.9	12	21	7	40	1.35	0.51
Satt363	12.1	12	21	7	40	1.35	0.51
Satt277	17.2	10	23	7	40	1.35	0.51
Satt307	17.2	10	23	7	40	1.35	0.51
Satt489	21.2	11	21	6	38	1.74	0.42
Satt079	31.7	13	18	9	40	1.20	0.55
Satt460	32.7	12	19	9	40	0.55	0.76
Satt202	50.1	11	23	5	39	3.10	0.21
Satt371	64.8	13	18	9	40	1.20	0.55
Satt357	64.8	14	17	8	39	2.49	0.29

[a] Distance from PS-1 mutant gene, designated as *Fsp2*
[b] Genotypes: *AA* Minsoy, *AB* heterozygote, *BB* PS-1

Table 3 Segregation of SSR markers linked to PS-2 mutant gene in an F_2 soybean population of a cross between Minsoy and PS-2

Marker	Distance[a]	Observed number				χ^2	*P*
	(cM)	AA[b]	AB[b]	BB[b]	Total	(1:2:1)	
Satt177	150.0	36	50	25	111	3.27	0.19
Satt424	118.5	36	52	23	111	3.49	0.17
Sat 115	96.1	33	53	25	111	1.38	0.50
Sat 129	90.6	30	53	24	107	0.68	0.71
Satt437	65.9	37	52	21	110	4.98	0.08
Satt409	31.1	50	48	13	111	26.69	1.60
Satt538	13.3	57	53	1	111	56.73	<0.01
Satt429	25.4	56	52	3	111	51.05	<0.01

[a] Distance from PS-2 mutant gene, designated as *Fsp3*
[b] Genotypes: *AA* Minsoy, *AB* heterozygote, *BB* PS-2

Table 4 Segregation of SSR markers linked to PS-3 mutant gene in an F_2 soybean population of a cross between Minsoy and PS-3

Marker	Distance	Observed number				χ^2	*P*
	(cM)[a]	AA[b]	AB[b]	BB[b]	Total	(1:2:1)	
Satt160	119.9	31	48	37	116	4.07	0.13
Satt030	100.2	29	58	31	118	0.10	0.95
Satt425	65.2	30	65	23	118	2.05	0.36
Satt114	43.5	41	63	12	116	15.36	<0.01
Satt510	36.6	44	61	11	116	19.09	<0.01
Sct 033	36.2	45	58	11	114	20.32	<0.01
Satt335	31.7	45	63	10	118	21.31	<0.01
Sat 152	13.1	47	62	2	111	38.00	<0.01

[a] Distance from PS-3 mutant gene, designated as *Fsp4*
[b] Genotypes: *AA* Minsoy, *AB* heterozygote, *BB* PS-3

PS-3 mapping study

The $F_{2:3}$ segregation of homozygous for Minsoy to heterozygote was 49:71 and approximated a 1:1 ratio (χ^2=4.03, *P*=0.04). PS-3 was designated *Fsp4* and assigned Genetic Type Collection number T366H (Table 1).

Initial screening of the F_2 population was conducted by selecting several SSR markers from each linkage group (Cregan et al. 1999). The markers were chosen to divide each linkage group into segments of less than 30 cM. A total of 169 SSR markers were tested. Among them, SSR marker Satt510 on MLG-F was identified as linked to the PS-3 mutant gene (LOD=3.52). An additional 18 markers, Satt030, Satt160, Satt516, Satt425, Satt374, Satt595, Sat 133, Satt114, Satt334, Sct 033, Satt510, Satt335, Satt362, Satt072, Sct 188, Satt218, Sat 074, and Sat 152, from this linkage group (Cregan et al. , unpublished data) were screened between parental lines. We detected polymorphisms using SSR markers Satt030, Satt160, Satt425, Satt114, Sct 033, Satt335, and Sat 152. On the basis of LOD scores generated from the MAPMAKER program, PS-3 locus was linked to SSR markers Sct 033, Satt335, and Sat 152 with LOD scores of 4.00, 4.07, and 17.08, respectively. The most likely order of markers is shown in Fig. 1C. The PS-3 mutant gene, designated as *Fsp4*, was located on the terminus and linked to Sat 152 by 13.1 cM. No polymorphisms were observed using SSR markers Satt516, Satt374, Satt595, Sat 133, Satt334, Satt362, Satt072, Sct 188, Satt218, and Sat 074. Segregation ratios of three SSR markers, Satt160, Satt030 and Satt425, on MLG-F apart from PS-3 mutant gene by more than 65.2 cM provided good fits to 1:2:1 (Table 4). While segregation ratios of five SSR markers, Satt114, Satt510, Sct 033, Satt335, and Sat 152, linked to PS-3 mutant gene by less than 43.5 cM were distorted from 1:2:1 and provided fits to 1:1.

PS-4 mapping study

The $F_{2:3}$ segregation of homozygous for Minsoy to heterozygote was 53:59 and followed a 1:1 ratio (χ^2=0.32, *P*=0.57). PS-4 was designated *Fsp5* and assigned Genetic Type Collection number T367H (Table 1).

Initial screening of the F_2 population was conducted by selecting several SSR markers from each linkage group (Cregan et al. 1999). The markers were chosen to divide each linkage group into segments of less than 30 cM. A total of 234 SSR markers were tested. Among them, SSR marker Satt324 on MLG-G was identified as linked to the PS-4 mutant gene (LOD=9.01). An additional 31 markers, Satt038, Satt309, Satt570, Satt356, Satt217, Satt235, Sat 131, Satt394, Satt594, Satt427, Satt564, Satt533, Satt504, Satt303, Satt566, Satt131, Satt340, Satt501, Satt505, Satt400, Satt199, Satt012, Satt503, Satt517, Satt288, Satt472, Satt163 Satt130, Satt115, Satt138, and Satt191, from this linkage group (Cregan et al. 1999) were screened between parental lines. We detected polymorphisms using SSR markers Satt038, Satt570, Satt564, Satt505, Satt199, Satt288, Satt472, Satt163, and Satt138. On the basis of LOD scores generated from the MAPMAKER program, PS-4 mutant gene was linked to SSR markers, Satt138, Satt564, Satt505, Satt199, and Satt288 with LOD scores of 24.61, 24.61, 16.50, 15.70 and 4.77, respectively. The most likely order of markers is shown in Fig. 1D. The PS-4 mutant gene, designated as *Fsp5*, was located between Satt324 and Satt138 and linked by 19.6 cM and 7.5 cM, respectively. No polymorphisms were observed using SSR markers Satt309, Satt356, Satt217, Satt235, Satt394, Satt594, Satt427, Satt533, Satt504, Satt303, Satt566, Satt131, Satt340, Satt501, Satt400, Satt012, Satt503, Satt517, Satt130, Satt115, and Satt191. Segregation ratios of three SSR markers Satt038, Satt163, and Satt570, on MLG-G apart from PS-4 mutant gene by more than 42.2 cM provided good fits to 1:2:1 (Table 5). While segregation ratios of seven SSR markers, Satt324, Satt138, Satt564, Satt505, Satt199, Satt288, and Satt472, linked to PS-4 mutant gene by less than 45.8 cM were distorted from 1:2:1 and provided fits to 1:1.

Homoeologous (orthologous) and/or paralogous relationships

The present study demonstrated that the four ovule lethal mutants, PS-1, PS-2, PS-3, and PS-4, were associated

Table 5 Segregation of SSR markers linked to PS-4 mutant gene in an F_2 soybean population of a cross between Minsoy and PS-4

Marker	Distance[a]	Observed number				χ^2	*P*
	(cM)	AA[b]	AB[b]	BB[b]	Total	(1:2:1)	
Satt163	59.1	30	50	32	112	1.36	0.51
Satt038	58.3	29	50	33	112	1.57	0.46
Satt570	42.2	30	63	19	112	3.91	0.14
Satt324	19.6	41	63	8	112	21.20	<0.01
Satt138	7.5	51	61	0	112	47.34	<0.01
Satt564	7.9	51	61	0	112	47.34	<0.01
Satt505	16.7	55	55	2	112	50.20	<0.01
Satt199	17.1	54	56	2	112	48.29	<0.01
Satt288	33.8	53	44	15	112	30.93	<0.01
Satt472	45.8	45	51	16	112	15.91	<0.01

[a] Distance from PS-3 mutant gene, designated as *Fsp5*
[b] Genotypes: *AA* Minsoy, *AB* heterozygote, *BB* PS-4

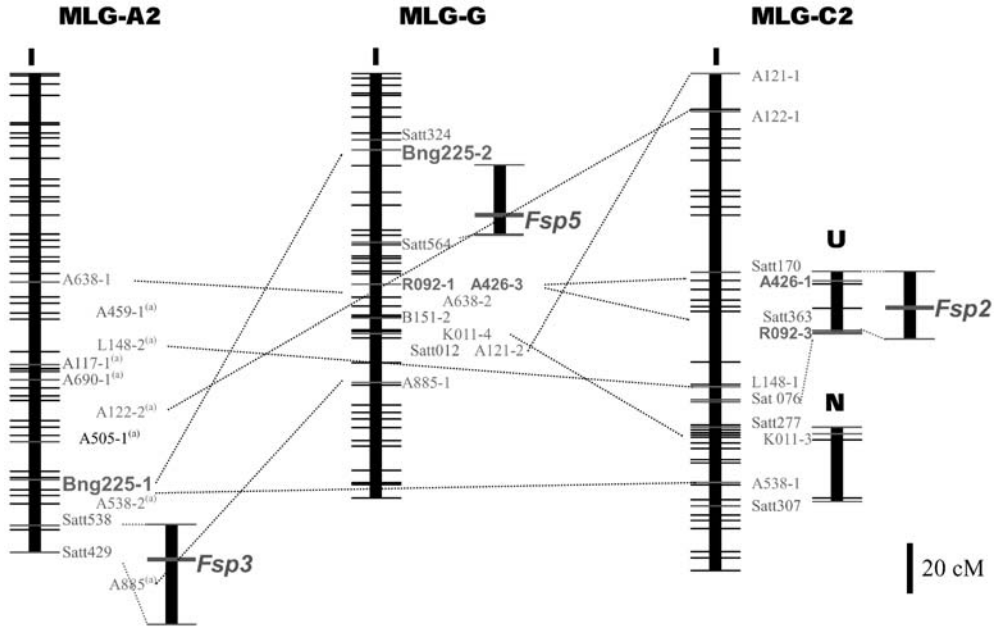

Fig. 2 Comparative maps among MLG-A2, -G, and -C2, based on the previous study (Shoemaker et al. 1996; http://129.186.26.94/HTML%20scripts/dupregions.html). The *bolded I* represents the USDA Iowa State University map (Cregan et al. 1999). The *bolded U* represents the University of Utah map (Cregan et al. 1999). The *bolded N* represents the University of Nebraska map (Cregan et al. 1999). Positions of *Fsp3*, *Fsp5*, and *Fsp2* are presented in *blue*. Homoeologous RFLP markers are indicated in *red*. *(a)* represents the approximate positions of each marker demonstrated in the SoyBase homepage (http://129.186.26.94/HTML%20scripts/dupregions.html)

with four independent loci, designated as *Fsp2*, *Fsp3*, *Fsp4*, and *Fsp5*, on soybean molecular linkage groups C2, A2, F, and G, respectively. In addition, these four loci were not associated with the *Fsp1* locus on molecular linkage group D1+W (Kato and Palmer 2003). The map positions of the five *Fsp* genes demonstrated that these were nonallelic relationships.

Cytological studies on PS-2, PS-3, and PS-4 mutants indicated that all three mutants had an abnormal megagametogenesis at 4–5 days post-anthesis stage (Pereira et al. 1997b). No differences in cytological studies were identified among PS-2, PS-3, and PS-4 mutants, suggesting the possibility that these three genes are expressed with genetic interaction(s) at the same stage.

To identify the genetic relationships among these *Fsp* genes, comparison of the map positions via homoeologous DNA markers and/or DNA sequences of the *Fsp* genes themselves could be done. At present, we can discuss only the comparison of map positions of the *Fsp* genes. Shoemaker et al. (1996) demonstrated multiple homoeologous regions throughout the soybean genome. Each *Fsp* gene was not located in the homoeologous regions directly (Shoemaker et al. 1996). Using SoyBase (http://soybase.agron.iastate.edu/HTML%20scripts/dupregions.html), we searched for the common RFLP markers on soybean MLG-C2, -A2, -F, -G, and -D1b+W. MLG-C2 and -A2 carry three common RFLP clones, A122, L148, and A538, respectively. MLG-A2 and -G carry three common RFLP markers, Bng225,

A638, and A885, respectively. MLG-C2 and -G carry four common RFLP markers, A121, R092, A426, and K011, respectively. There were no common RFLP markers on MKG-F and the others, and MLG-D1b+W and the others, respectively. In combination with mapping data from Cregan et al. (1999), we summarized the comparative maps between MLG-A2, -G, and -C2 in Fig. 2. Two common RFLP markers, Bng225 and A885, are close to the *Fsp3* locus. These two RFLP markers also are located on MLG-G. The position of A885 is far from *Fsp5*, while Bng225 is close to *Fsp5*. Close linkage relationships between a common RFLP marker, Bng225, and two *Fsp* loci on each chromosome suggest the possibility that *Fsp3* and *Fsp5* loci are located on the duplicated region on each chromosome. This means that these two *Fsp* genes are paralogues or homologues that are not located on the homoeologous regions directly. It is necessary to identify the DNA sequences of the *Fsp* genes before making conclusions regarding the relationships between these two *Fsp* genes.

On the other hand, two common RFLP markers, A426 and R092, are located in the vicinity of the genomic region of the *Fsp2* locus. These two RFLP markers are located at a single locus on MLG-G in the USDA/Iowa State University map (Cregan et al. 1999) and estimated to be apart by more than 30 cM from *Fsp5*. These data suggest the possibility that the *Fsp2* and *Fsp5* genes had been located on the same ancestral chromosome before the whole genome duplication event (Shoemaker et al. 1996).

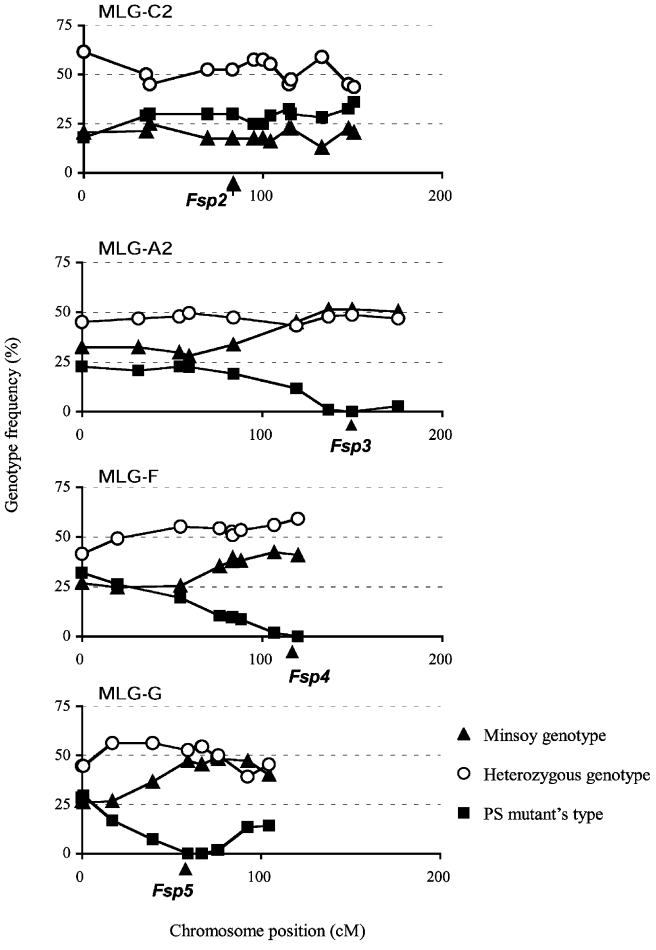

Fig. 3 Genotype frequencies as a function of the MLG-C2, -A2, -F, and -G. Frequencies of genotype in F_2 plants derived from crosses of Minsoy and PS-1 (MLG-C2), PS-2 (MLG-A2), PS-3 (MLG-F), and PS-4 (MLG-G) are plotted along the genetic linkage map with a Mendelian skewness curve applied. For each linkage group, the left end of the *x*-axis corresponds to the top end of each linkage group in Fig. 1, and the *y*-axis indicates the genotype percentages observed for each marker. The *legend* indicates the genotype that each line represents. In normal segregation, the heterozygous and homozygous genotypes account for 50% and 25%+25%, respectively

Segregation distortions

Previous genetic studies (Pereira et al. 1997a, 1997b) demonstrated that PS-1 is transmitted through the male and female parents, while PS-2, PS-3, and PS-4 were transmitted only through the male parent but not the female parent. Genotype frequencies of all segregated markers on MLG-C2 were good fits to the Mendelian ratio of 1:2:1 (Table 1, Fig. 3). SSR markers linked to *Fsp3* and *Fsp5* segregated in support of female gametophyte-specific mutations. However, as mentioned in the PS-3 mapping study of the *Fsp4* locus, the heterozygous type was more frequent than the Minsoy type and did not fit a 1:1 ratio at the 5% level (Table 4, Fig. 3). The SSR marker Sat 152 was linked to *Fsp4* by 13.1 cM, in spite of the fact that the PS-3 genotype was only 2 among 111 F_2 plants in support of *Fsp4* being transmitted only through the male parent. In the previous genetic analyses of the crosses between PS-3 and Harosoy-*w4* or CD-5 mutant, the *Fsp4* locus segregated as a 1:1 ratio (Pereira et al. 1997a). These data show that the present segregation distortion was not due to the pleiotropic effect of *Fsp4*. Since *Fsp4* is transmitted only through the male parent but not the female parent, this skewed segregation around the *Fsp4*/Sat 152 region towards the PS-3 allele is due to another genetic factor (locus) affecting segregation distortion through the male parent by allelic interaction between Minsoy and PS-3. In this hypothesis, allelic interaction at this locus caused the reduced transmission of the Minsoy allele around this genomic region, perhaps by male gametophytic selection, such as selective abortion of male gamete or selective fertilization.

Recombination frequency around *Fsp* genes

We compared the map distances between the marker intervals among each *Fsp* gene with previous mapping studies, including three independent maps of USDA/Iowa State University, University of Utah, and University of Nebraska (Cregan et al. 1999) (Table 6). Because, in the present study, *Fsp4* was located on the terminus of the MLG-F, we could not compare the map distance around

Table 6 Comparison of the map distances of the marker intervals locating the *Fsp2*, *Fsp3*, and *Fsp5* loci

Locus	MLG[a]	Marker interval[b]	Map distance (cM)[c]			
			PS	ISU	Utah	Nebraska
Fsp2	C2	Satt170/Satt363	25.7	49.7	22.6	no data
Fsp3	A2	Satt538/Satt429	28.3	10.5	0.0	no data
Fsp5	G	Satt324/Satt138	23.5	no data	21.6	30.7

[a] Molecular linkage group (Cregan et al. 1999)
[b] Each *Fsp* locus was located between the marker interval
[c] PS was calculated in the present study. ISU was based on the USDA/Iowa State University map (Cregan et al. 1999). Utah was based on the University of Utah map (Cregan et al. 1999). Nebraska was based on the University of Nebraska map (Cregan et al. 1999)

Fsp4. In Table 6, we calculated map distance between each marker interval directly. Since *Fsp3* and *Fsp5* genomic regions were transmitted through only the female from F_1 to F_2 plants, only the map distances that originated from the recombination frequency of male parent in F_1 generation of POP-2 and POP-4 were demonstrated. The map distances in the USDA/Iowa State University, University of Utah, and University of Nebraska maps had been calculated from the recombination frequencies in both male and female parents in F_1 generation and/or a series of generations, respectively.

Among these comparisons, 28.3 cM of the marker interval around *Fsp3* was consistently greater than two previous reported maps: 10.5 cM of USDA/Iowa State University map and 0.0 cM of University of Utah map (Cregan et al. 1999). No differences between the map distances of other marker intervals on MLG-A2, Satt177 and Satt424, Satt424 and Sat 115, Sat 115 and Sat 129, Sat 129, and Satt437, Satt437 and Satt409, and Satt409 and Satt538, in POP-2 (data not shown) were detected. From this fact, we could simultaneously eliminate environmental effects, such as temperature stress, on differences of recombination frequency (Zetka and Rose 1990). An increase in recombination around *Fsp3* in male meiosis was identified. Alternatively, there was no difference of recombination around *Fsp5* gene. *Fsp5* is located on the central region of the linkage map, while *Fsp3* is located on the terminal region of the linkage map. In *Brassica nigra*, Lagercrantz and Lydiate (1995) suggested that male recombination is enhanced in the terminal regions of the chromosomes, and female recombination is generally more frequent adjacent to areas of high marker density, which are probably centromeric. A similar phenomenon has been observed in humans, where recombination frequencies are generally higher in female meiosis, but where male recombination frequencies are higher in terminal regions of many chromosomes (Bowden et al. 1989; Julier et al. 1990; Keith et al. 1990; Wright et al. 1990; Weiffenbach et al. 1991). In future studies, we need to clarify whether the increased recombination in male meiosis around the *Fsp3* gene is due to a genic effect of the *Fsp3* gene or another gene, or chromosomal position effect as demonstrated in other organisms.

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