A Pleiotropic Mutant for Male Sterility and Spindly Branching at the *sb* Locus in Common Bean

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Abstract. The inheritance of an induced mutant for spindly branch and male sterility (SBMS) was investigated in common bean (*Phaseolus vulgaris* L.) in F₂ and backcross populations. The results support the hypothesis that the mutant is controlled by a single recessive gene. Extensive breeding work with SBMS, involving several thousand F₂ progeny, produced no recombinant of the types expected if two closely linked genes controlled the character. Therefore, a single pleiotropic gene apparently controls SBMS. Allelism tests demonstrated that SBMS is allelic with *sb* but not with *sb-2* and *sb-3*. The gene symbol *sb^m* is proposed for SBMS because it is a new allele at *sb*, with the order of dominance being *Sb* > *sb* > *sb^m*. Various ways to exploit the new mutant for marked male sterility are discussed.

The linkage map of common bean includes only 40 markers (Gepts, 1988), and there are insufficient natural markers in common bean that are favorable for efficient mapping. In 1979, a program was initiated to induce marker mutations in common bean to provide additional material for mapping and to produce new variability that could be exploited in basic research and plant breeding. One useful type of mutation for which I am searching is genie male sterility. There now is not a single male sterility mutant in common bean that is generally available for exploitation. There is only one previous report of a stable genie male sterility in common bean (Mutschler and Bliss, 1980), and this source has a complex inheritance that is not yet fully understood (Ashraf and Bassett, 1986; Bassett, 1989). Although there is no genie male-sterile (with complete ovule fertility) available to bean breeders and geneticists, there is a close substitute, the indehiscent anther (*ian*) character (Bassett, 1989; Wyatt, 1984). This mutant prevents pollen from being shed and effectively emasculates the plant, but no practical exploitation of *ian* has yet been reported.

This paper presents evidence that a marked male sterile mutant gene was induced in common bean and has potential for exploitation in basic research and applied breeding programs.

Methods and Materials

Origin of materials. Three dry bean breeding lines, 182-1, B-128, and B-351, were used in a program to induce marker mutations. These lines have small, black seeds and half-runner habit and were developed and released by the Tropical Agricultural Research Station, Mayaguez, Puerto Rico. Dry seed of these lines were treated with 200 Gy of gamma radiation and planted in the field in 1982 to produce M₂ seed. Mature seed was harvested from individual M₁ plants and planted in more than 700 separate M₂ field plots in 1983. Most M₂ plots had ≈100 plants, except where seed was limiting. These populations were observed for mutant plants at weekly intervals. Four plots segregated for the spindly branch marker mutant, viz., 3-99, 3-

100, 3-146, and 3-296. The spindly branch mutants from 3-99, 3-100, and 3-146 have been reported previously (Awuma and Bassett, 1988; Bassett, 1990) and have been given the gene symbols *sb*, *sb-2*, *sb-3*, respectively. The spindly branch mutant from plot 3-296 is the subject of this paper.

Bumblebee pollination. One of the difficulties of searching for male sterility mutants in M₂ populations at Gainesville, Fla., is the presence of large populations of bumblebees that efficiently pollinate bean. Segregants for male sterility are so thoroughly pollinated that one cannot rely on barren or late-maturing plants as indicators of male sterility. When a marked male-sterile mutant occurs, its progeny "revert" to normal when grown in the greenhouse, i.e., the presumed M₃ progeny test is in fact an F₁ progeny derived from outcrossing in the field. Such materials must be tested again in the field in M₄ or F₂, as the case may be. If the line segregates again for the mutant, it is a prime suspect for a marked male-sterile, i.e., a pleiotropic mutant that controls the expression of male sterility and a morphological marker character.

Inheritance tests. Plot 3-296 was derived from breeding line 182-1. Seeds were harvested from the single plant in 3-296 showing the spindly branch phenotype. In Fall 1983, nine plants were grown in the greenhouse from seed of this mutant. All nine plants had completely normal phenotype, indicating that cross-pollination had occurred and, perhaps, the mutant selection was also male-sterile. Putative F₂ seed were harvested and bulked for progeny testing. The F₂ seed were planted in the field in Spring 1984 in plot 4-519, which segregated for spindly branch plants. The plot was covered with netting to exclude bumblebees. The flowers of spindly branch plants were examined for anther development and pollen shed. At maturity, the spindly branch plants were examined for pods developed from self-pollination, and a search was made among nonspindly plants for barren plants.

Spindly branch segregants in plot 4-519 also were backcrossed by hand to 182-1. The F₁ progeny were grown in the greenhouse, and F₂ seed were planted in the field in Spring 1985 in plot 5-1, which was covered with netting to exclude bumblebees. About 3 weeks after flowering, plants were classified into fertile and male-sterile segregants on the basis of pod set, i.e., fertile plants had a full pod set and male-sterile plants, although flowering profusely, did not set any pods. Flowers on the barren plants were examined for anther development and

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pollen shed. The plot was also searched for barren, nonspindly branch plants and fertile, spindly branch plants, hereafter referred to as recombinant phenotypes.

Further breeding work was done with the new male-sterile mutant to transfer it to other genetic backgrounds, e.g., 'Sprite' and 'Triumph' snap beans and dry bean breeding line 5-593. During 1985-87, several thousand segregating progeny were planted in plots protected with netting to determine whether the two recombinant phenotypes would appear, i.e., male-sterile plants with normal branching and spindly branch plants with male fertility. Hereafter, the spindly branch male-sterile character will be indicated with the abbreviation SBMS.

A testcross was made by crossing an SBMS segregant in 7-574 with a heterozygote F_1 from 6-9 SBMS $\times$ 5-593. Line 7-574 was derived from a cross of an SBMS segregant in plot 4-519 by 'Sprite'. An F_3 SBMS segregant from this cross was crossed and backcrossed to 5-593, and the BC_1 - F_2 was designated plot 7-574. The pedigree of 6-9 also began with a cross of a SBMS segregant in 4-519 by 'Sprite', but the F_2 SBMS segregant from this cross was crossed with 'Triumph'. The F_2 from this cross was designated 6-9.

Allelism tests. Allelism tests were made by crossing breeding line 7-175, which was segregating for the SBMS character from 3-296, with the other spindly branch mutants, 3-99, 3-100, and 3-146. The F_1 progeny were grown in the greenhouse and the F_2 were grown in the field in 1988. These populations were classified for normal, spindly fertile, and spindly male-sterile segregants, and they were searched for any recombinant phenotypes that might appear at low frequency as described above.

Results and Discussion

Inheritance tests. The nine progeny plants from the M_2 spindly branch mutant (from 3-296) were grown in the greenhouse in Fall 1983, and all had a normal phenotype. Seed from a bulk harvest of all nine progeny plants were planted in the field in Spring 1984 in plot 4-519. This material segregated for normal and spindly branch plants. An examination of the flowers on spindly branch segregants in 4-519 revealed that there was no pollen shed and that the anthers were shriveled. Crosses made with pollen from 182-1 onto spindly branch segregants resulted in normal seed development and well-filled pods. Furthermore, no pods were observed on spindly branch plants that were not the product of hand pollination. From the above experience, it was inferred that the original M_2 plant was not only a spindly branch mutant but also a mutant for male sterility. Insects had cross-pollinated this plant, producing " M_3 " seed that were really F_1 and, therefore, had normal phenotype. The " M_4 " population 4-519 was really an F_2 , which segregated for the recessive SBMS phenotype.

The F_1 from the cross 4-519 SBMS $\times$ 182-1 produced only fertile nonspindly (normal) plants. The F_2 from this cross segregated for normal and SBMS plants, and no recombinant phenotypes were observed. The observed segregation in F_2 for normal and SBMS plants gave a good fit to a 3:1 ratio (Table 1). This

Table 1. Segregation for the spindly branch male-sterile mutant sb^{ms} in the F_2 population 5-1 (from the cross $sb^{ms} \times$ 182-1) and in a testcross population, 9-402 [$7-574 sb^{ms} \times F_1$ 6-9 (+/ sb^{ms})].

Population	Phenotypic classes		Ratio tested	χ^2 value	P value
	Normal	sb^{ms}			
5-1	597	180	3:1	1.39	0.24
9-402	39	33	1:1	0.50	0.48

result is consistent with the hypothesis that a single recessive gene controls the SBMS phenotype, although tight linkage between genes for two mutants, one for spindly branch and the other for male sterility, has not been ruled out. Therefore, I propose the gene symbol sb^{ms} for the spindly branch male-sterile character, based on allelism test results to be described below.

The single recessive gene hypothesis was subsequently tested in a testcross in which sb^{ms} was crossed with heterozygous plants from the cross $sb^{ms} \times$ 5-593. Progeny from the testcross segregated for normal, sb^{ms} , and crippled seedling plants; the latter class probably due to the dosage-dependent lethality genes, $D1-1$ and $D1-2$, reported by Shii et al. (1980). The crippled seedling problem arose from crossing Andean snap bean cultivars with MesoAmerican breeding lines. Out of a total of 113 testcross plants, 41 were crippled seedlings. The remaining 72 plants segregated for 39 normal and 33 sb^{ms} plants, which gave a satisfactory fit to a 1:1 segregation ratio (Table 1). These results support the hypothesis that a single recessive factor controls the SBMS character, contingent upon additional testing for the possibility of tight linkage between two mutant loci, one for spindly branch and one for male sterility.

During 1985-87, 5157 F_2 progeny (data not shown) from crosses of $sb^{ms} \times$ normal were observed for possible recombinant segregants on the assumption that the sb^{ms} character is really the product of two closely linked genes, one for spindly branch and the other for male sterility. No recombinant were found, providing additional support for the hypothesis that a single, pleiotropic gene controls SBMS. If close linkage exists between two mutant loci, the recombination frequency is very low.

Allelism tests. Allelism tests of sb^{ms} with $sb-2$ and $sb-3$ gave F_1 progeny that were normal, and the F_2 segregated in a 9 normal : 3 spindly fertile : 4 SBMS ratio (Table 2). Clearly, these sb mutants are not allelic with the SBMS locus. The allelism test with sb gave F_1 progeny that were fertile spindly branch, and the F_2 segregated in a 3 spindly fertile : 1 SBMS ratio (Table 2). From these results one can infer that SBMS is a new allele at the sb locus and that the order of dominance is $Sb > sb > sb^{ms}$.

Female fertility. The number of seeds per pod and the number of pods per plant in SBMS segregants appear to be equal to normal segregants, provided that pollination by either insects or man is adequate and competition with nonSB plants is minimized by increasing in-row plant spacing to 20 cm or more. No quantitative data were taken on these yield components.

When sb^{ms} was crossed to various other genetic backgrounds and carried through several segregating generations, there was no loss of recessivity of the male sterility, i.e., no seeds developed from self-pollination due to "leakiness" of the male sterility in a new genetic background. No partial restoration of fertility was observed.

Discussion of applications. There have been previous reports of marked male sterility mutants in other plant species, such as lima bean (*Phaseolus lunatus* L.) (Allard, 1953) and watermelon [*Citrullus lanatus* (Thunb.) Mansf.] (Watts, 1962). Whether such mutants are exploited for practical breeding, as was the case in watermelon (Watts, 1967), depends on many factors, such as the availability of efficient pollinating insects and great practical advantage in comparison to more conventional breeding procedures.

The spindly branch (SB) character is not easily detected in early seedling development, but as more nodes and branches are developed, segregating populations can be classified easily a few days before flowering. Thus, it is feasible to rogue out

Table 2. Allelism tests of *sb^{ms}* with the three spindly branch mimic mutants: *sb*, *sb-2*, and *sb-3*.

Cross	Generation	Phenotypic segregation			Ratio tested	χ^2 value	P value
		Normal	<i>sb</i> (fertile)	<i>sb^{ms}</i>			
<i>sb^{ms}</i> x <i>sb</i> (3-99)	F ₁	0	56	0	3:1	0.44	0.51
	F ₂	0	376	134			
<i>sb^{ms}</i> x <i>sb-2</i> (3-100)	F ₁	36	0	0	9:3:4	1.43	0.49
	F ₂	292	84	124			
<i>sb^{ms}</i> x <i>sb-3</i> (3-146)	F ₁	26	0	0	9:3:4	0.87	0.65
	F ₂	296	95	142			

all fertile segregants in a row to produce an all male-sterile row for experimental F₂ seed production.

SB produces thinner and more numerous branches, which is a structural change that persists after senescence. Even after all the foliage has dropped, the SB plants can be readily distinguished from normal, provided that the plants are spaced ≈ 20 cm or more in the row. Most marked male-steriles are easily identified at flowering, but it is rare to have a marker that is also obvious at full senescence. The latter characteristic allows a breeder to enter a mature, fully defoliated field and select for male-sterile segregants without the need for previous tagging.

The *sb^{ms}* gene can be used in the various recurrent selection schemes for plant improvement. In material segregating for SBMS, it is possible to make controlled crosses between selected plants in the field with great efficiency, or one can allow insects to make random crosses between fertile and male-sterile plants in the field. Recurrent selection might be achieved by alternating between mass selection among nonSBMS plants one year and allowing open-pollination among the self progeny the next year. Seed could be harvested from SBMS plants after open-pollination; this seed would produce the next generation, which would again be subjected to mass selection. This procedure would be particularly appropriate where the most desirable environment for mass selection does not have adequate populations of bumblebees and carpenter bees to permit effective open-pollination. Several cycles of this procedure could produce a "landrace"-type cultivar that is highly uniform for seed color (and pattern), size, and shape; highly uniform for disease resistance; moderately uniform for plant habit; and highly variable for genes not made uniform by mass selection. Such cultivars might be more stable in their performance over years and be more widely adapted geographically.

Seed of the *sb^{ms}* mutant is available from M.J.B. upon request.

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