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## Abstract

Recurrent selection is a method for population improvement which has been used in soybean [*Glycine max* (L.) Merrill] to modify traits such as grain yield, seed-protein content, seed-oil content, tolerance to iron-deficiency chlorosis, and seed size. Nuclear male-sterility with insect-mediated cross-pollination has been successfully used in recurrent selection schemes in soybean. However, little attention has been given to selection to increase the agronomic performance of male-sterile plants per se. The objective of this study was to evaluate the response of male-sterile lines segregating for male-sterile alleles *ms2*, *ms3*, *ms6*, *ms8*, and *ms9* to phenotypic recurrent selection for increased seed-set after 3 cycles, using a selected group of male parents. Bees halictidae, anthophoridae, andrenidae, and megachilidae were utilized as the pollinator vector. The results indicated that recurrent selection in a favorable environment was successful to increase the number of seeds per male-sterile plant. Although a differential response was observed among populations, the seed-set observed would justify the use of some specific male-sterile selections as female parents in a hybrid soybean seed production system.

## Keywords

Male sterility, Recurrent selection, Seed-set, Soybean

## Disciplines

Agronomy and Crop Sciences | Plant Biology | Plant Breeding and Genetics

## Comments

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# Insect-mediated cross-pollination in soybean [*Glycine max* (L.) Merrill]: II. Phenotypic recurrent selection

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tolerance to iron-deficiency chlorosis, and seed size. Nuclear male-sterility with insect-mediated cross-pollination has been successfully used in recurrent selection schemes in soybean. However, little attention has been given to selection to increase the agronomic performance of male-sterile plants per se. The objective of this study was to evaluate the response of male-sterile lines segregating for male-sterile alleles *ms2*, *ms3*, *ms6*, *ms8*, and *ms9* to phenotypic recurrent selection for increased seed-set after 3 cycles, using a selected group of male parents. Bees halictidae, anthophoridae, andrenidae, and megachilidae were utilized as the pollinator vector. The results indicated that recurrent selection in a favorable environment was successful to increase the number of seeds per male-sterile plant. Although a differential response was observed among populations, the seed-set observed would justify the use of some specific male-sterile selections as female parents in a hybrid soybean seed production system.

**Keywords** Male sterility · Recurrent selection · Seed-set · Soybean

## Abbreviations

RS	Recurrent selection
RCBD	Randomized complete block design
BC	Backcross
LSD	Least significant difference
CV	Coefficient of variation

## Introduction

Recurrent selection (RS) is a method of population improvement which was designed to improve population performance. This is accomplished by increasing the frequency of favorable alleles for traits quantitatively inherited, and without decreasing the genetic variability for continued selection (Hallauer 1985; Fehr 1991; Lewers and Palmer 1997). RS includes the systematic selection of desirable individuals from a population followed by recombination of the selected individuals to form a new population. Phenotypic recurrent selection is practiced on an individual plant or the progeny of the plant in single or replicated plots. Thus, improved populations or lines can be used as cultivars per se, as parents of hybrids, inbred lines, clonal cultivars or parents of a synthetic cultivar (Fehr 1991).

In soybean [*Glycine max* (L.) Merrill], RS schemes have been used to improve traits such as grain yield (Kenworthy and Brim 1979; Sumarno and Fehr 1982; Piper and Fehr 1987; Holbrook et al. 1989; Burton et al. 1990; Werner and Wilcox 1990); seed-protein content (Brim and Burton 1979; Miller and Fehr 1979; Holbrook et al. 1989; Xu and Wilcox 1992); seed-oil content (Burton and Brim 1981) tolerance to iron-deficiency chlorosis (Prohaska and Fehr 1981), and seed size (Tinius et al. 1991; 1993).

The most used methods for RS in soybean are mass selection and  $S_n$  (self pollinated to the  $n$ th generation) (Lewers and Palmer 1997). Fehr and Ortiz (1975) observed the greatest genetic gain per year for yield in soybean using  $S_1$  testing compared to  $S_4$  and half-sib family selection. Carter et al. (1982) conducted six cycles of selection for increased seed-protein content in soybean with two populations, reporting an increase of 1.2 and 3.3% units, respectively.

Nuclear male sterility with insect-mediated cross-pollination has been successfully used in recurrent selection schemes in soybean. Wilson et al. (1981) implemented three cycles of mass recurrent selection for increasing the seed oleic acid content in a population derived from  $F_{3,4}$  progeny of cross-pollination between two exotic lines segregating for the *ms1* male-sterile allele. Burton and Brim (1981) reported an increase in seed-oil content using high-oil-content lines as male parents insect-mediated pollinated to male-sterile plants segregating for the *ms1* allele. Werner and Wilcox (1990) and Xu and Wilcox (1992)

used a type of mass selection ( $S_0$  recurrent selection) to modify grain yield, maturity, and seed-protein content where intermating was insect-mediated and facilitated by using the *ms2* male-sterile allele. Thus, RS strategies in soybean have been used to improve traits directly related to agronomic performance in lines that will be likely used as cultivars. However, selection to increase the seed-set on male-sterile plants per se has received minimal attention. Graybosch and Palmer (1988) used *ms1ms1*, *ms2ms2*, and *ms3ms3* male-sterile plants to evaluate seed-set using alfalfa leaf cutter bees from families halictidae, anthophoridae, andrenidae, and megachilidae and honey bees as pollinators. Seed-set on male-sterile plants as a percentage of male-fertile plants ranged from 1.6% to 32.6% across two planting dates. Roumet and Magnier (1993) evaluated seed-set on male-sterile plants segregating for the *ms2* allele in caged plots containing alfalfa leaf cutter bees to facilitate pollination. The seed-set observed on male-sterile plants represented 60% of their fertile counterpart.

Although insect-mediated cross-pollination has been used to produce seed on male-sterile plants, traits influencing preferential pollination on male-sterile, female-fertile soybean lines have not been clearly elucidated. Previous reports have shown that seed-set on male-sterile lines is a strong indicator of pollinator preference (Graybosch and Palmer 1988; Lewers and Palmer 1997; Ortiz-Perez et al. 2004, 2006a, b). Preferential pollination observed through seed-set suggested that selection on male-sterile plants for high seed-set can be attained. Thus selected male-sterile, female-fertile lines could be suitable to produce large amounts of hybrid soybean seed. The objective of this study was to evaluate the response to phenotypic recurrent selection for increased seed-set on male-sterile, female-fertile soybean lines segregating for male-sterile alleles *ms2*, *ms3*, *ms6*, *ms8*, and *ms9*, using several bee species as pollination vectors.

## Materials and methods

### Cycle 0

#### *Plant materials and field evaluation*

Male-sterile mutant lines segregating for single recessive nuclear genes were selected from a group

of male-sterile lines evaluated in a three-year experiment at Ames, IA (Ortiz-Perez et al. 2006b). From that study, high and low seed-set male-sterile lines were selected. The selected female parents were male-sterile, female-fertile lines from maturity groups II and IV, segregating for *ms2* in two different genetic backgrounds (L75-0587, Bernard et al. 1991; and T375H, Cervantes-Martinez et al. 2005), *ms3* (T284H) (Chaudhari and Davis 1977), *ms6* (T295H) (Skorupska and Palmer 1989), *ms8* (T358H) (Palmer 2000), and *ms9* (T359H) (Palmer 2000) (Table 1). These lines were field-grown and used as female parents to be insect-mediated cross-pollinated by eight male-fertile, female-fertile soybean lines, used as male parents. The male parents were chosen based on either their attractiveness to pollinator insects and/or on agronomic characteristics. Testing a number of males and females was because we wanted to evaluate “attractive” females in combination with “attractive” males, but also poor females combined with good and poor males, trying to determine the nature of the pollination traits inheritance. Eight single-cross combinations were established (Table 1). Seed-set on male-sterile, female-fertile lines was evaluated using a randomized complete block design (RCBD) with five replications and eight entries. Each entry was the combination of one segregating male-sterile, female-fertile line (female parent) and one male-fertile, female-fertile line (male parent). Each plot was six rows, the first and the sixth rows were male-fertile lines (males) and the four center rows were segregating male-sterile lines (female parents). Each 4.8-m long row was spaced 76-cm within rows, 1.2-m between plots, and 14 seeds per meter were planted. Irrigation was applied when necessary.

**Table 1** Mean values for pod number and seed number/soybean male-sterile plant in presence of alfalfa leaf cutter bees and native pollinators for two-way cross-populations (Cycle 0. Texas 2002)

<sup>a</sup> Near-isogenic line of T375

<sup>b</sup> Near-isogenic line of cv. Beeson. The *ms2* allele from T259

<sup>c</sup> Near-isogenic line of PI 429077. The *ms6* allele from T295

Population	Mean pod number/ male-sterile plant	Mean seed number/ male-sterile plant
A00-39 <i>ms2</i> (T375) × Corsoy 79	45	91
A00-39 <i>ms2</i> (T375) × Hark	50	102
A00-41 <i>ms2</i> <sup>a</sup> × A00-73 <i>Ms9</i>	52	105
A00-63 <i>ms2</i> (Beeson) <sup>b</sup> × Wells	33	67
A00-68 <i>ms3</i> (T284) × A00-41 <i>Ms2</i>	35	74
A00-72 <i>ms8</i> (T358) × A00-68 <i>Ms3</i>	35	72
A00-73 <i>ms9</i> (T359) × Raiden	60	120
A94-20 × 19 ( <i>ms6</i> ) <sup>c</sup> × A00-39 <i>Ms2</i>	29	58
Mean	43	81
LSD ( $\alpha = 0.05$ )	8	17
CV	28	30

The eight single-cross combinations were cross-pollinated using alfalfa leaf cutter bees (*Megachile rotundata*) as pollinators in summer 2002 at Plainview, Texas. At the beginning of flowering R1 (Fehr et al. 1971), alfalfa leaf cutter bee pupae were placed in a container in the base of a ‘bee board’, which consisted of a wooden board with 10.6-cm by 10.6-cm wood cylinders long, with closely-spaced holes 0.47 cm in diameter, and 8.89 cm deep. Approximately 10,000 pupae were placed on the bee board, which was placed in the middle of the experimental plots. The bees emerged as adults 2 days later.

At flowering R2 (Fehr et al. 1971), the male-sterile plants were identified by their lack of pollen production and labeled. Male-fertile plants tend to have earlier flowering compared to their male-sterile siblings, so they were removed at the first flower. The remaining male-sterile plants were checked out for pollen presence three times during the flowering period, at least 10 flowers/male-sterile plant. At maturity the number of pods per male-sterile plant and number of seed per male-sterile plant were recorded. Selection for high seed-set within each cross-combination was made. Fifteen percent selection intensity was applied resulting in 56 selected plants in total or seven plants per combination.

## Cycle 1

### *Plant material and field evaluation*

F<sub>1</sub> seed from male-sterile plants selected in Texas 2002 was grown in an off-season nursery in Fall

2002, at Isabela, Puerto Rico. A composite of 25 hybrid seeds per cross-combination was grown under natural photoperiod. At harvest, each plant was threshed individually. Fifty  $F_2$  seeds from three single-plants within each cross-combination were selected and planted in the same off-season location in January 2003 with controlled photoperiod (exposure to continuous light for 15 days after emergence, to 14.5 h days for an additional 35 days, and to natural day length until maturity). Only fertile  $F_2$  plants were harvested. The progeny of individual fertile plants was considered as a family, so we selected 24 plants out of the 50  $F_2$  plants, so 24  $F_{2:3}$  families per cross-combination were obtained. The  $F_{2:3}$  derived families from each cross-combination were field-grown in summer 2003 at Plainview, Texas, where they were insect-mediated cross-pollinated to their eight male-fertile, female-fertile recurrent parents ( $BC_1$  crosses) and to a common parent, DSR Exp. 202b (three-way crosses) (Table 3). A RCBD with six replications and 16 entries was used. Each plot was constituted in the same way as cycle 0. Irrigation was applied when necessary. At flowering, male-sterile plants were identified as in cycle 0. Only solitary native bees from the families halictidae, anthophoridae, andrenidae, and megachilidae were carrying out the pollinations.

At maturity the number of pods and number of seeds per male-sterile plant were recorded. Selection for the highest seed-set in male-sterile plants within each segregating  $F_{2:3}$  derived families was made. Fifteen percent selection intensity was applied resulting in 128 selected plants, 8 plants per combination. Thus, three-way and  $BC_1F_1$  seed were produced from selected high-seed-set male-sterile plants, and used to start cycle 2.

## Cycle 2

### *Plant materials and field evaluation*

Twenty seeds from each single-plant selection from Texas 2003 were grown in an off-season nursery in January 2004, at Isabela, Puerto Rico under controlled photoperiod. Selection for the three-way and  $BC_1F_1$  plants with the highest seed-set was made. At harvest, each plant was threshed individually. Three-way- $F_2$  and  $BC_1F_2$  seeds were produced and 24 three-

way  $F_2$  and  $BC_1F_2$  derived families were selected. The selected 24 three-way- $F_2$  and  $BC_1F_2$  derived families were grown in the field in summer 2004 at Plainview, Texas, where they were insect-mediated cross-pollinated to their recurrent parents to produce  $BC_2F_1$  seed, and to a common parent (DSR Exp. 202c) to produce four-way- $F_1$  seed (Table 3). The male parents were commercial lines with agronomic characteristics along with traits favorable to pollination. Each plot was constituted in the same manner as for cycles 0, and 1. Irrigation was applied when necessary. At flowering, the process to identify male-sterile plants was the same as for cycles 0 and 1. The bee families carrying out the pollinations were the same as for cycle 1. At maturity, plant height, number of pods, and number of seed per male-sterile plant were recorded. Only plants with greater than 80 pods and that were 50 cm or taller were selected for harvest.

## Cycle 3

### *Plant materials and field evaluation*

Twenty seeds from each single-plant selection from Texas 2004 were grown in an off-season nursery in January 2005, at Isabela, Puerto Rico, under controlled photoperiod. Selection for the four-way and  $BC_2F_1$  plants with the highest seed-set was made. At harvest, each plant was threshed individually. Four-way- $F_2$  and  $BC_2F_2$  seeds were produced. Only four four-way  $F_2$  and  $BC_2F_2$  derived families were selected. The selected four four-way  $F_2$  and  $BC_2F_2$  derived families were grown in the field in summer 2005 at Plainview, Texas, where they were insect-mediated cross-pollinated to their recurrent parents to produce  $BC_3F_1$  seed, and to a common parent (GH 4190) to produce five-way- $F_1$  seed. The fact that selected individuals were selected and then intermated, makes this strategy slightly different to the traditional recurrent selection scheme, we could consider it as early generation selection with complex crosses. In cycle 3, the fertile female parents from each cross-combination also were evaluated for seed-set. This was made to compare the seed-set of advanced populations versus their fertile female parents. To make the most accurate comparison, each fertile female parent plot was thinned

according to the spacing observed in their male-sterile, female-fertile counterpart. Irrigation was applied when necessary. A RCBD with two replications per family and 22 entries was used. These 22 entries corresponded to each BC<sub>2</sub> and four-way crosses, plus their 7 female-fertile parents. At flowering, the process to identify male-sterile plants was the same as for cycles 0 and 1. The bee species carrying out the pollinations were the same as for cycles 1 and 2. At maturity, plant height, and number of seed per male-sterile plant were recorded. Only plants with greater than 80 pods and 50 cm or taller were selected for harvest.

### Statistical analysis

For cycle 0, analyses of variance were performed using PROC GLM of SAS (SAS Institute Inc., Cary, NC 2003). Replications were treated as random factors and two-way crosses, as fixed factors. A RCBD was used where the linear model was:

$$Y_{ijk} = \mu + C_i + B_j + CB_{ij} + E_{ijk}$$

where:  $\mu$  = the general mean;  $C_i$  = the effect of the  $i$ th two-way cross;  $B_j$  = effect of the  $j$ th block;  $CB_{ij}$  = the interaction effect of the  $i$ th two-way cross and the  $j$ th block, and  $E_{ijk}$  = experimental error associated with the  $ijk$  observation.

For cycles 1, 2, and 3, analyses of variance were performed using PROC GLM of SAS (SAS Institute Inc., Cary, NC 2003). Replications and families within crosses were treated as random factors, and crosses, as fixed factors. A RCBD was used where the linear model was:

$$Y_{ijkl} = \mu + C_i + F_{(i)j} + B_k + CB_{ik} + FB_{(i)jk} + E_{ijkl}$$

where:  $\mu$  = the general mean;  $C_i$  = the effect of the  $i$ th cross;  $F_{(i)j}$  = effect of the  $j$ th family nested in the  $i$ th cross;  $B_k$  = effect of the  $k$ th block;  $CB_{ik}$  = the interaction effect of the  $i$ th cross and the  $k$ th block,  $FB_{(i)jk}$  = the interaction effect of the  $j$ th family nested in the  $i$ th cross and the  $k$ th block, and  $E_{ijkl}$  = experimental error associated with the  $ijkl$  observation.

Each cycle was analyzed separately to determine differences among cross-combinations for the evaluated traits. A combined analysis using a regression model was conducted where the cycles were

considered as independent variables. The number of seed and number of pods were considered as dependent variables.

The regression model was:

$$Y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$$

where:  $Y_i$  = predicted response of the  $i$ th cycle;  $\beta_0$  = intercept;  $\beta_1$  = regression slope,  $x_i$  = the value of the independent variable,  $\varepsilon_i$  = random error related to the  $i$ th observation.

## Results and discussion

### Cycle 0

The combined analysis of variance for seed-set and pod production showed significant differences among single-cross combinations. The two-way cross that had the highest mean values for seed-set was A00-73 *ms9* × Raiden (120 seeds/male-sterile plant). The lowest values were observed in the crosses A94-20 × 19 (*ms6*) × A00-39 *Ms2*, and A00-63 *ms2* (Beeson) × Wells which averaged 58, and 66 seeds/male-sterile plant, respectively (Table 1). For pod number, differences among two-way crosses also were observed. However, the rank order for the crosses for number of pods/male-sterile plants was the same as for seed-set, indicating that number of pods (rather than number of seed/pod) influenced the total seed-set/per each male-sterile plant. The highest seed-set observed for the two-way cross A00-73 *ms9* × Raiden was consistent with the highest seed-set observed when male-sterile plants from A00-73 *ms9* were evaluated in a previous study (Ortiz-Perez et al. 2006b). The same trend was observed for the lowest seed-set, the two-way cross A00-63 *ms2* (Beeson) × Wells. That is, these latter lines presented the lowest seed-set both (Ortiz-Perez et al. 2006b). Graybosch and Palmer (1988), in contrast, found that *ms2* carrying lines presented higher number of seed/male-sterile plant when compared to male-sterile lines carrying the *ms1* and *ms3* alleles, they used plant introductions and commercial lines as male parents; honey bees and alfalfa leaf cutter bees were the pollinators.

The male-sterile lines used as females had previously been evaluated and selected for high and low seed-set (Ortiz-Perez et al. 2006b). Thus the rank



observed for seed-set among male-sterile lines was not totally unexpected, however, the relative differences in seed-set between studies for the high seed-set was of interest, in the present study the seed-set observed across all the female lines was superior to the reported by Ortiz-Perez et al. 2006b for the same lines. Differences in floral abortion among the male-sterile lines could cause a difference in seed-set, although failure in fertilization has been reported to be negligible in soybean flower abortion (Abernethy et al. 1977). It is possible that asynchrony factors such as differences in flowering duration between the female and the adjacent male parent could cause differences in seed-set among the two-way crosses. However, according to Suso et al. (2006), differences in out-crossing rate caused by asynchrony among parents did not explain most of the variation observed in inter-crossed faba bean [*Vicia faba* (L.)] accessions. They attributed such variation to differences in floral traits.

### Cycle 1

Significant differences for seed-set among crosses and among families from the same cross were observed (Table 2). The mean for number of seed/male-sterile plant for  $F_{2:3}$  segregating families using a common male parent (three-way crosses) was significantly different from that observed  $F_{2:3}$  families using their recurrent male parent ( $BC_1$  crosses), suggesting that the male parent had a major effect on seed-set. The cross A00-73 *ms9* × Raiden  $BC_1$  (Raiden was the recurrent parent) presented the highest seed-set among the  $BC_1$  crosses. For the three-way crosses, (A00-39 *ms2* × Hark) × DSR Exp. 202b had the highest mean for seed-set (Table 3).

Although the general mean for seed-set in three-way crosses (89 seed/male-sterile plant) and  $BC_1$  crosses (75 seed/male-sterile plant) was very similar to that observed in cycle 0 (81 seed/male-sterile plant), most important was the large variation among  $F_{2:3}$  families from the same cross (Table 3). Such large variation was observed mainly for some of the crosses that yielded the highest seed-set. This variation was not observed in crosses that presented the lowest seed-set (Fig. 1).

**Table 2** Mean squares for seed number/soybean male-sterile plant for three-way,  $BC_1$ , four-way,  $BC_2$ , five-way, and  $BC_3$  crosses (Texas, 2003, 2004, and 2005)

	Source of variation	Mean squares
2003		
Cycle 1		
Three-way crosses	Cross	49,512***
	Family (cross)	8,127***
$BC_1$ crosses	Cross	26,378***
	Family (cross)	10,194***
2004		
Cycle 2		
Four-way crosses	Cross	49,236**
	Family (cross)	19,919***
$BC_2$ crosses	Cross	27,363***
	Family (cross)	16,407***
2005		
Cycle 3		
Five-way crosses	Cross	15,647*
	Family (cross)	12,577.69NS
$BC_3$ crosses	Cross	27,696**
	Family (cross)	10,122.70**

\*, \*\*, \*\*\* Significant at the 0.05, 0.01, and 0.001 probability levels respectively; NS = Not significant

$BC_1$  and three-way crosses involving A00-63 *ms2* (Beeson) and Wells as parents were observed in the lower rank for seed-set (Table 3). This also was observed in their two-way cross in cycle 0, which might imply a very low frequency of genes (alleles) in those lines for traits involved in insect-mediated cross-pollination.

Once all families were quantified for seed-set, the data fitted a segregation ratio that suggested as few as two recessive genes involved in pollinator preference (data not presented). The results also suggest that both parents contributed genes (or alleles) that were different for pollinator attraction or reward, irrespective of whether the recurrent parent or a common male parent was used.

### Cycle 2

Male-sterile plants from four-way crosses had a higher mean for seed-set compared to  $BC_2$  crosses (Table 3). Variation among families from the same



**Table 3** Mean values for seed number/male-sterile soybean plant and maximum and minimum mean for family within three-way, four-way, five-way, BC<sub>1</sub>, BC<sub>2</sub>, and BC<sub>3</sub> crosses (Texas 2003, 2004, and 2005)

Population	2003 Cycle 1 common male 1			2004 Cycle 2 common male 2			2005 Cycle 3 common male 3		
	Three-way cross			Four-way cross			Five-way cross		
	Plant mean	Family mean		Plant mean	Family mean		Plant mean	Family mean	
		Max	Min		Max	Min		Max	Min
Female parent									
(A00-39 <i>ms2</i> × Corsoy 79)	94	174	61	305	465	244	168	190	142
(A00-39 <i>ms2</i> × Hark)	123	316	57	175	330	6	164	246	68
(A00-41 <i>ms2</i> × A00-73 <i>Ms9</i> )	119	195	27	223	337	94	217	227	88
[A00-63 <i>ms2</i> (Beeson) × Wells]	40	84	8	263	297	228	137	151	126
(A00-68 <i>ms3</i> × A00-41 <i>Ms2</i> )	96	187	49	184	263	78	234	252	224
(A00-72 <i>ms8</i> × A00-68 <i>Ms3</i> )	102	159	68	241	329	147	181	238	152
(A00-73 <i>ms9</i> × Raiden)	107	173	6	156	266	38	242	251	172
[A94-20 × 19 ( <i>ms6</i> ) × A00-39 <i>Ms2</i> ]	48	104	24	169	232	63	124	177	104
Mean	89			190			183		
LSD ( $\alpha = 0.05$ )	25			40			57		
CV	60			61			50		
	BC <sub>1</sub>			BC <sub>2</sub>			BC <sub>3</sub>		
(A00-39 <i>ms2</i> × Corsoy 79)	84	198	17	141	201	49	91	161	45
(A00-39 <i>ms2</i> × Hark)	90	219	33	198	271	73	150	214	122
(A00-41 <i>ms2</i> × A00-73 <i>Ms9</i> )	79	142	35	169	269	3	136	165	24
[A00-63 <i>ms2</i> (Beeson) × Wells]	49	95	18	72	140	36	99	108	87
(A00-68 <i>ms3</i> × A00-41 <i>Ms2</i> )	82	176	28	205	482	75	232	313	144
(A00-72 <i>ms8</i> × A00-68 <i>Ms3</i> )	34	45	26	ND	ND		ND	ND	
(A00-73 <i>ms9</i> × Raiden)	100	234	13	135	241	67	217	261	180
[A94-20 × 19 ( <i>ms6</i> ) × A00-39 <i>Ms2</i> ]	82	137	33	131	245	10	170	190	134
Mean	75			150			145		
LSD ( $\alpha = 0.05$ )	15			33			36		
CV	59			51			40		

ND = No data

cross also was observed, and was very extreme in some crosses (Table 4). The crosses A00-41 *ms2* × A00-73 *Ms9* (BC<sub>2</sub>), and A94-20 × 19 (*ms6*) × A00-39 *Ms2* (BC<sub>2</sub>) showed the most extreme values for the highest family mean as compared to the lowest family mean (Table 3), although less intra-family variation for number of seeds was observed.

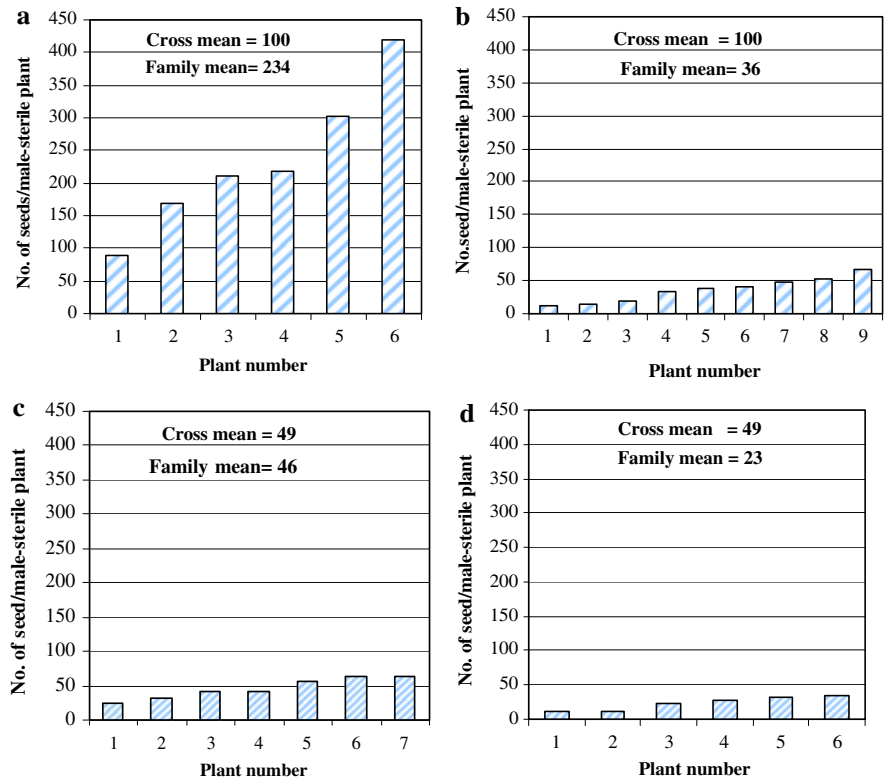
For some four-way crosses, variation for seed-set among families was evident, but not that extreme (Fig. 2). The crosses [A94-20 × 19 (*ms6*) × A00-39 *Ms2* × DSR Exp. 202b] × GH 4190, (A00-68 *ms3* × A00-41 *Ms2* × DSR Exp. 202b] × GH 4190, and [(A00-41 *ms2* × A00-73 *Ms9*) × DSR

Exp. 202b] × GH 4190 presented 3-fold variation for the highest family mean as compared to the lowest family mean (Table 3).

### Cycle 3

The seed-set in the F<sub>2</sub> families using a common male parent, (five-way crosses) were again significantly different from those observed in the F<sub>2</sub> families using their recurrent male parent (BC<sub>3</sub> crosses), and presented a higher mean for seed-set compared to the latter (Table 3).

**Fig. 1** Mean values for seed number per soybean male-sterile plant for two families from the BC<sub>1</sub> cross [A00-73 *ms9* (T359) × Raiden] (1a, and b), and from the BC<sub>1</sub> cross [A00-63 *ms2* (Beeson) × Wells] (1c, and d). Texas 2003



**Table 4** Percentage gain for number of seed/soybean male-sterile plant among cycles for backcross populations (Texas 2002, 2003, 2004, and 2005)

Population	Cycle 0	BC <sub>1</sub>	BC <sub>2</sub>	BC <sub>3</sub>	
	Mean no. seed/ male-sterile plant	% relative to cycle 0	% relative to cycle 1	% relative to cycle 2	Mean no. seed/ male-sterile plant
(A00-39 <i>ms2</i> (T375) × Corsoy 79)	91	-8	+68	-35	91
(A00-39 <i>ms2</i> (T375) × Hark)	102	-11	+120	-24	150
(A00-41 <i>ms2</i> <sup>a</sup> × A00-73 <i>Ms9</i> )	105	-25	+114	-19	136
[A00-63 <i>ms2</i> (Beeson) × Wells]	67	-26	+44	+39	99
[A00-68 <i>ms3</i> (T284) × A00-41 <i>Ms2</i> ]	74	+11	+149	+14	232
[A00-72 <i>ms8</i> (T358) × A00-68 <i>Ms3</i> ]	72	-52	ND	ND	ND
[A00-73 <i>ms9</i> (T359) × Raiden]	120	-16	+35	+60	217
[A94-20 × 19 ( <i>ms6</i> ) × A00-39 <i>Ms2</i> ]	58	+41	+59	+13	170

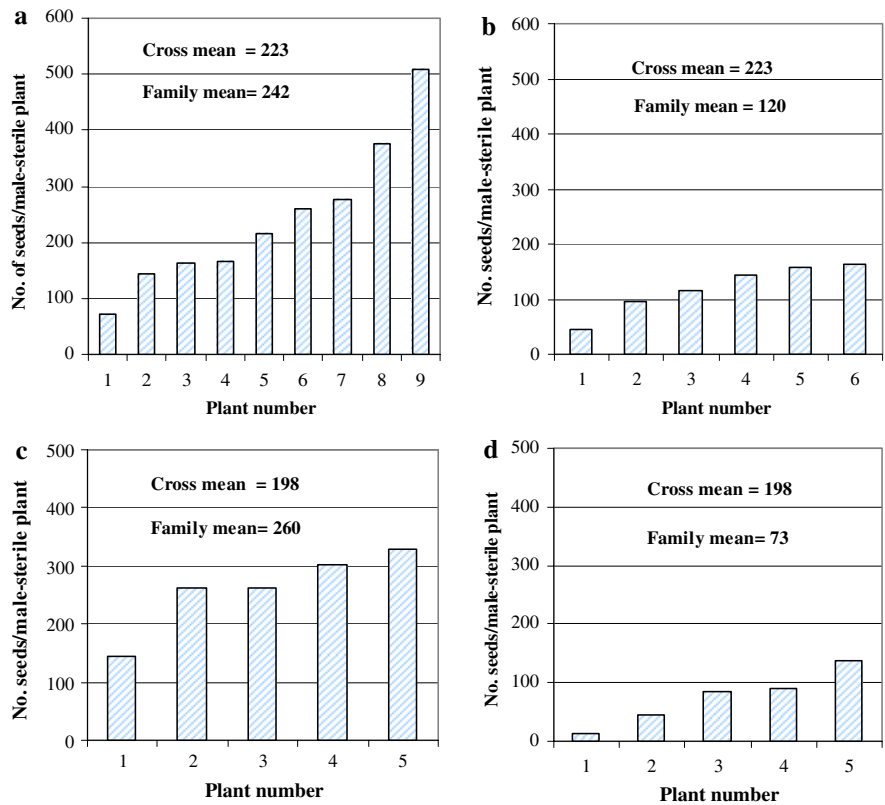
<sup>a</sup> Near-isogenic line of T375

ND = No data

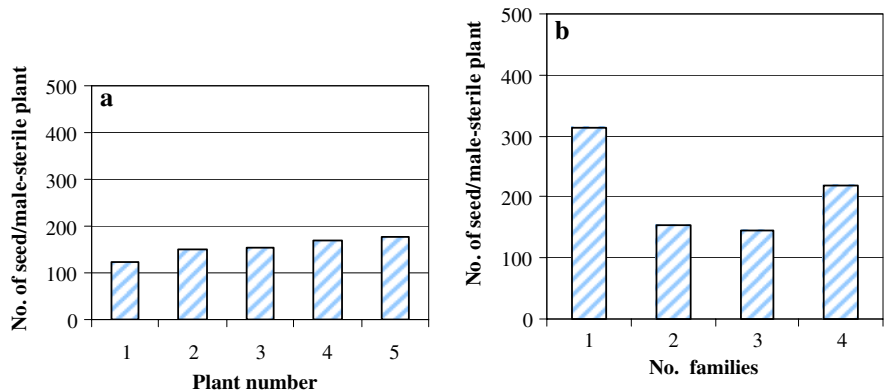
The five-way cross [(A00-73 *ms9* × Raiden) × DSR Exp. 202b × GH 4190] × DSR Exp. 202c had the highest seed-set. Among the BC<sub>3</sub> crosses, A00-68 *ms3* × A00-41 *Ms2* had the highest mean for seed-set (Table 3). Variation among

families from the same cross also was observed for both, five-way crosses and BC<sub>3</sub> crosses. Less intra-family variation for number of seed/male-sterile plant was observed compared to the populations evaluated in the previous cycles (Fig. 3). For the BC<sub>3</sub> crosses

**Fig. 2** Mean values for seed number/male-sterile plant for two families from the four-way cross [A00-41 *ms2* × A00-73 *Ms9* × DSR Exp. 202b] × GH 4190 (2a, and b), and the BC<sub>2</sub> cross A00-39 *ms2* (T375) × Hark (2c, and d). Texas 2004



**Fig. 3** Mean values for seed number per male-sterile plant for a family from the BC<sub>3</sub> A00-68 *ms3* × A00-41 cross (3a), and variation for seed-set among families from the same cross (3b). Texas 2005

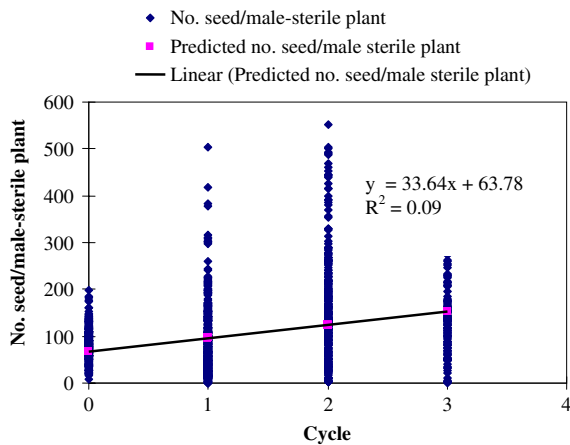


this was expected, since the additive genetic variability within lines is reduced to a half of that present in the preceding generation (Fehr 1991).

The fact that male-sterile plants from three-way crosses, four-way crosses, and five-way crosses out-yielded the BC crosses in each cycle was of special interest. For the three-, four-, and five-way crosses a different male parent was used with the assumption was that adding new genetic material to the original cross-combinations could cause a greater change in

the mean for seed-set compared to the BC populations. If that assumption was correct, then we could expect that these male parents would bring a different assortment of alleles (since the commercial lines used as male parents were unrelated) for traits related to insect-mediated-cross-pollination. For the BC crosses, the same male parent was used to create the each new population.

Maturity differences among lines could increase the potential to respond differentially to selection



**Fig. 4** Predicted and observed response to selection for seed number in soybean male-sterile plants after three selection cycles. Texas 2002, 2003, 2004, and 2005

regardless any floral or pollination trait, however in a previous 3-years study where we selected the parents from, we did not record any segregation for maturity genes, which was expected if we had combinations such as *Ele2* × *e1E2* for example. A weak positive correlation among plant height and seed-set was observed (data not presented), suggested that maturity and plant height were not the main factor affecting seed-set on the male-sterile plants.

The mean values for the selective cycles indicated a linear increase for number of seed/male-sterile plant with recurrent selection from cycle 0 to cycle 3 for the BC strategy (Fig. 4). Estimators of the regression parameters were significant for the effects of cycle on number of seed/male-sterile plant, although the determination coefficient was very low, indicating a weak prediction of the model (Fig. 4).

The cross-combinations responded differently to selection. We observed a negative change from cycle 0 to cycle 1 in most of the cross-combinations (Table 4), which could be related to the fact that from cycle 0 to cycle 1, the evaluated lines were  $F_{2,3}$  derived families. After two selfing generations, variability for the traits associated with pollinator could play a factor in the decrease of seed-set, differences in environmental conditions between cycle 0 and cycle 1 should be considered as well. Selection for pollinator attractiveness was not practiced in those two selfing generations. The best year for all populations was 2004 where cycle 2 was evaluated. For that year, a record rainfall was

observed, which likely had favorably affected the overall seed-set of the lines. Seed-set on male-sterile plants as a percentage of male-fertile plants has been reported to range from 1.6% to 32.6% up to 60% (Graybosch and Palmer 1988; Roumet and Magnier 1993) of their fertile counterpart in open field conditions and caged plots. In the present study, the seed-set observed in male-sterile plants after three selection cycles in some of the crosses was higher than these reports (Table 5). Both, the BC strategy and adding a new male parent each cycle were effective to increase the mean seed-set across populations. Environmental conditions favorable to both plants and pollinators played a key role. BC populations showed a lower mean compared to the populations where a new male parent was added each cycle, suggesting the effect of the male on the performance of the male-sterile lines. Heterozygosity in the traits involved in cross-pollination and seed-set is a factor to consider for both strategies. More homogeneous lines were observed in the BC strategy compared to the populations with a background where five parents are expected to segregate for those traits. The results we observed in our study suggested the improvement for seed-set among male-sterile populations, through selection. Comparison of the advanced generations vs. their female-fertile parents gave us some valuable insights about such improvement, however, evaluation of all the generations in replicated environments needs to be conducted. Currently BC<sub>3</sub> derived lines are being selfed to derive homozygous lines to be tested for seed-set in replicated environments.

## Conclusions

The results of this study indicated that phenotypic recurrent selection in a favorable environment was successful to increase the number of seed/male-sterile soybean plant. Mean seed-set per family as high as 313 seeds per male-sterile plant was observed after just three selection cycles. A differential response was observed among the cross-combinations, suggesting variability for those traits among the parental lines. Continuous selection within low-seed populations failed to increase seed-set on male-sterile, female-fertile plants. Evaluation of the selected lines in relation to their fertile-female parental line

**Table 5** Seed-set from fertile female soybean parents-derived BC<sub>3</sub> crosses compared in percent relative to their fertile female parent (Texas 2005)

Fertile female parent	Mean no. seed/ fertile-female line	Fertile female parents- derived BC <sub>3</sub> crosses	Mean no. seed/ male-sterile line	% seed-set relative to fertile female parent
A00-39 <i>Ms2</i>	217	A00-39 <i>ms2</i> × Corsoy 79	91	42
A00-39 <i>Ms2</i>	217	A00-39 <i>ms2</i> × Hark	150	69
A00-41 <i>Ms2</i>	219	A00-41 <i>ms2</i> × A00-73 <i>Ms9</i>	136	42
A00-63 <i>Ms2</i> (Beeson)	231	A00-63 <i>ms2</i> (Beeson) × Wells	99	43
A00-68 <i>Ms3</i>	287	A00-68 <i>ms3</i> × A00-41 <i>Ms2</i>	232	80
A00-73 <i>Ms9</i>	384	A00-73 <i>ms9</i> × Raiden	217	56
A94-20 × 19 ( <i>Ms6</i> )	281	A94-20 × 19 ( <i>ms6</i> ) × A00-39 <i>Ms2</i>	170	60
Mean	244		146	
LSD	90		36	
CV	50		40	

(percent of seed-set on male-sterile plants compared to fertile normal plants from the same background) indicated from 42% to 80% of the normal seed-set observed in their fertile female parent, which is higher than previous reports in the literature. The pollen transfer from the male parent to the female parent has been the most challenging barrier to the development of an efficient hybrid system in soybean. In this study bee pollinators were used to produce large quantities of hybrid seed on male-sterile plants. Thus the seed-set observed would justify the use of some of these selections as female parents in a hybrid soybean seed production system.

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