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Keywords

Soybean, Male sterility, Pollinator, Seed-set

Disciplines

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

Comments

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Insect-mediated seed-set evaluation of 21 soybean lines segregating for male sterility at 10 different loci

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Abstract The first requirement to establish a successful hybrid soybean program is the availability of a stable male-sterile, female-fertile system. Male sterility has been an important tool in soybean breeding programs to improve traits such as yield, seed-protein and seed-oil content, and seed size. However, improvement of seed-set per se on male-sterile plants has not been an important breeding objective. The evaluation of the out-crossing potential of the available male-sterile, female-fertile soybean lines is crucial to determine the future of hybrid soybean. The objective of this study was to evaluate seed-set among 21 soybean lines segregating for male

sterility at 10 different loci using *Megachile rotundata* as insect pollinator. Seed-set was evaluated in field conditions in 2001, 2002, and 2003 near Ames, Iowa. Our results indicated significant differences in seed-set among male-sterile lines. The effects of maturity group, pubescence color, the locus and/or the allele, and the genetic background of the lines segregating for male sterility were significant. Differences for seed-set among lines with independent mutational events at the same locus, (i.e. different alleles), also were significant. These results imply that interaction effects with the genetic background of the lines for traits related to fertility/sterility and insect-pollinator attraction and reward are important. Flower color had little effect on seed-set in the evaluated lines. The effect of year was very important on the performance of the lines for seed-set. This suggested that environmental conditions that favor plant–pollinator relationships need to be determined in order to increase insect-mediated cross-pollination to develop an efficient hybrid soybean program.

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Keywords Soybean · Male sterility · Pollinator · Seed-set

Abbreviations

MG Maturity group
CV Coefficient of variation
LSD Least significant difference

Introduction

Soybean, *Glycine max* (L.) Merr., is a self-pollinated legume crop. Breeding efforts to increase grain yield and other agronomic traits have been conducted by use of pure-line development programs. Hybridization is the first step, and it has been utilized to produce pure-line cultivars, but the use of hybrids per se has been impractical. Manual cross-pollination is difficult, and time-consuming. The low success rate of manual cross-pollination also is a hindrance to produce large quantities of hybrid seed (Fehr 1991).

When nuclear male sterility was discovered in soybean, it was viewed as a potential useful tool for intermating large numbers of plants. Cytoplasmic-nuclear male sterility systems are known in soybean and one system has been developed to produce large quantities of F₁ hybrid seed, making commercial production a real possibility (Sun et al. 2000).

Since the success of F₁ hybrid soybean production requires the efficient transfer of pollen from the male parent to the female parent, insect pollen vectors are the most practical means by which pollen can be transferred. In soybean, however, little attention has been paid to the prevalence of pollinating insects as cross-pollination vectors. Outcrossing-rates in the cultivated soybean species, *Glycine max* have been reported as less than 1% (Weber and Hanson 1961; Weber and Fehr 1967; Boerma and Moradshahi 1975). For *G. soja*, the wild annual species, the mean outcrossing rate estimate was 13%, ranging from 9.3 to 19% among four populations in Japan (Fujita et al. 1997). Wild perennial species such as *G. argyrea*, and *G. clandestina*, have both self-fertilized cleistogamous flowers and chasmogamous flowers on the same plant. Insect pollinators visit the chasmogamous flowers and the outcrossing rates varied from zero to complete outcrossing, with an average of 40% (Schoen and Brown 1991). *Glycine clandestina* is a closely related perennial species to *G. argyrea* (Hymowitz 2004). Schoen and Brown (1991) sampled two populations of *G. clandestina* (1500 and 750 m elevation). In the 1500 m population, approximately 60% of

the chasmogamous flowers were the result of self-pollinations. This contrasts to the zero self-pollination of chasmogamous flowers with the 750 m population. This large variation in outcrossing rates of the perennial species was considered to be related to the contrast in the environmental conditions for insect-mediated cross pollination, and to some extent to a greater reward available to pollinators (Schoen and Brown 1991). Such rewards include nectar, pollen, aroma, floral display, etc., incentives that benefit the pollinator while carrying out the cross-pollination.

Attractiveness to insect pollinators in soybean has been minimally manipulated and selected for. Erickson et al. (1978), however, showed that honey bee populations can be established to forage in soybean fields to increase yields. However, no studies have been done on the value of using gregarious pollinators such as wild bees on soybeans to increase yields. One example of a solitary insect species used for commercial pollination of crops is the alfalfa leaf cutter bee, *Megachile rotundata* F. It is extensively employed for the production of alfalfa seed. Roumet and Magnier (1993) evaluated seed-set on male-sterile, female-fertile soybean plants using alfalfa leaf cutter as pollinator in caged plots. The seed-set observed on the sterile plants represented 60% of the normal seed-set of their fertile counterparts. This indicated that this pollinator also can be used as an agent of soybean cross-pollination.

Bradner (1969) stated that all of the breeding components have been described for the production of hybrid soybean seed, except that a suitable vector to transfer pollen has not been found. Parental lines might be selected for aroma (volatiles) or attractiveness, that when incorporated into a hybrid seed production program, would attract and reward pollinating insects.

Seed-set on male-sterile, female-fertile plants has been reported as a good indicator of insect-pollinator attraction (Graybosch and Palmer 1988; Lewers and Palmer 1997; Lewers et al. 1996, 1998; Ortiz-Perez et al. 2004, 2006). In soybean, male sterility has been used primarily to

generate random mating populations, or to facilitate hybridization in pure line development programs (Lewers and Palmer 1997). The feasibility of the use of male-sterile lines as female parents in hybrid soybean programs has received little interest. Thus, few studies have been reported that evaluate seed-set on male-sterile soybean plants in replicated years and environments. Evaluation of the out-crossing capability of the available male-sterile, female-fertile soybean lines is crucial to determine the future potential for hybrid soybean. The objective of this study was to evaluate seed-set among 21 soybean lines segregating for male sterility at 10 male-sterile loci using *Megachile rotundata* as pollinator during three growing seasons near Ames, Iowa.

Materials and methods

Genetic material

Twenty-one soybean lines from the USDA Soybean Genetic Collection representing 10 different loci were evaluated. Mutant lines segregating at the *ms1*, *ms2*, *ms3*, *ms4*, *ms6*, *ms7*, *ms8*, *ms9*, MWO *ms*, and a genetically uncharacterized line (A00-74), were evaluated for out-crossed seed-set. The lines included four near-isolines, cvs. Williams, Wells, Beeson, and Clark derived from T259 (*ms2ms2*). Some of the lines had independent mutational events at the *ms1*, *ms2*, *ms3*, and *ms6* loci (Table 1). Only lines within maturity groups I–IV were tested. Genetic type T277 (*ms5ms5*), maturity group V was not included.

Table 1 Male-sterile, female-fertile lines, designation, and references, evaluated for seed-set at Ames, Iowa in 2001, 2002, and 2003

Mutant	Designation ^a	Maturity group	Flower color	Pubescence color	References
<i>ms1</i> (Urbana)	T266H	IV	P	T	Boerma and Cooper (1978), Palmer et al. (1978)
<i>ms1</i> (North Carolina)	L74-03 Clark (6) × T260H	IV	P	T	Bernard et al. (1991)
<i>ms2</i> (Eldorado)	L75-0570 Wells (6) × T259H	II	P	G	Bernard et al. (1991)
<i>ms2</i> (Eldorado)	L79-1308 Clark (6) × T259H	IV	P	T	Bernard et al. (1991)
<i>ms2</i> (Eldorado)	L75-0587 Beeson (6) × T259H	II	P	G	Bernard et al. (1991)
<i>ms2</i> (Eldorado)	L74-01 Williams (6) × T259H	III	W	T	Bernard et al. (1991)
<i>ms2</i> (Ames 1)	T360H	I	P	G	Palmer (2000)
<i>ms2</i> (Ames 2)	T375H	II	P	G	Cervantes-Martínez et al. (2005)
<i>ms2</i>	A00-41 ^b	II	P	G	Cervantes-Martínez et al. (2005)
<i>ms2</i>	A00-55 ^c	II	P	G	Cervantes-Martínez et al. (2005)
<i>ms3</i> (Flanagan)	T284H	IV	P	G	Chaudhari and Davis (1977), Graybosch and Palmer (1987)
<i>ms3</i> (Washington)	T273H	IV	P	T	Palmer et al. (1980)
<i>ms3</i> (Plainview)	T291H	III	P	G	Skorupska and Palmer (1990)
<i>ms4</i>	T274H	I	P	T	Delannay and Palmer (1982)
<i>ms6</i> (Ames 1)	T295H	II	W	T	Skorupska and Palmer (1989), Palmer and Skorupska (1990)
<i>ms6</i> (Ames 2)	T354H	I	P + W	T	Ilarlsan et al. (1999)
<i>ms7</i>	T357H	II	P	G	Palmer (2000)
<i>ms8</i>	T358H	II	W	G	Palmer (2000)
<i>ms9</i>	T359H	II	P	G	Palmer (2000)
A00-70	MW0 ^d	III	P	T	Jin et al. (1997)
A00-74	A00-74 ^e	III	P	T	Palmer (unpublished)

^a T numbers refer to Genetic Types, L numbers refer to near-isogenic lines derived from the line used as female parent in the listed cross

^b Sister line of T375, from a heterozygous plant

^c Sister line of T375, sib crosses on a male-sterile plant

^d Male-sterile, female-fertile mutant line from Midwest Oilseeds, Adel, Iowa

^e From T278M

Three lines were evaluated from maturity group I, nine from maturity group II, four from maturity group III, and five from maturity group IV. Four lines were white-flowered, 17 were purple-flowered. Ten lines were tawny pubescence; 11 had gray pubescence (Table 1).

Field evaluation

A randomized complete block design (RCBD) with four replications per male-sterile line was used. The lines were grown at the Bruner farm near Ames, IA, in the summers of 2001, 2002, and 2003. Each year the lines were grown in four-row plots, 1.8 m long, spaced 76 cm between rows. Twenty seeds per meter were planted. Plants were not thinned. At flowering, alfalfa leaf cutter bee (*Megachile rotundata*) domiciles were placed in alley-ways surrounding the plots. Each domicile contained about 300 bee pupae. One domicile per 14 soybean plots was used. Flower and pubescence color were recorded. Once flowering ended, the domiciles were removed. At maturity, male-sterile plants were identified (male-sterile plants produce fewer pods compared to male-fertile plants; most sterile plant stems stay green). The number of male-sterile plants and number of seeds/male-sterile plant were recorded. Seed-set on the male-sterile plants was assumed to be produced with pollen from the fertile sibling plants within each segregating row or adjacent sibling rows. For each line, 40 male-fertile plants within segregating families were single-plant threshed and progeny tested. These segregating progenies provided the male-sterile plants that were used for evaluation the second season. Again, 40 male-fertile plants within segregating families were single-plant threshed. They were progeny tested and evaluated the third season. Soil temperature, air temperature, rainfall, relative humidity, and wind speed records were kept throughout the growing season and obtained from the IEM (Iowa Environmental Mesonet). No irrigation was applied.

Statistical analysis

Data were subjected to Analysis of Variance with PROC GLM of SAS (SAS 2003). A RCBD was used where the linear model was:

$$Y_{ijk} = \mu + Y_i + B(i)j + L_k + YL_{ik} + BL(i)jk$$

where: μ = the general mean; Y_i = the effect of the i th year; $B(i)j$ = the effect of the j th block nested in the i th year; L_k = the effect of the k th male-sterile line; YL_{ik} = the interaction effect between the i th year and the k th male-sterile line; $BL(i)jk$ = the interaction effect between the j th block nested in the i th year and the k th male-sterile.

Replications and years were treated as random factors; male-sterile lines, as fixed factors. The mean number of seeds/male-sterile plant across lines was compared for the three years. Orthogonal contrasts were computed for flower color, pubescence color, maturity group, and male-sterile locus. Comparisons among independent mutations at the same locus also were made. Effects were considered significant in all statistical calculations if $P < 0.05$.

Results and discussion

Environment and seed-set

The combined analysis of variance showed significant differences in seed-set for the effects of male-sterile line and year (Table 2). Significant interactions for year \times male-sterile line also were observed, and then individual analyses were conducted for each year. The mean seed yield for male-sterile lines was 6.16 seeds/male-sterile plant in 2001, 10.08 in 2002, and 9.16 in 2003.

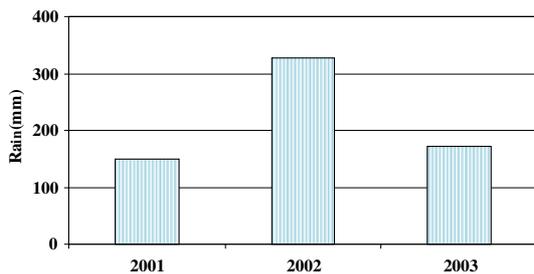
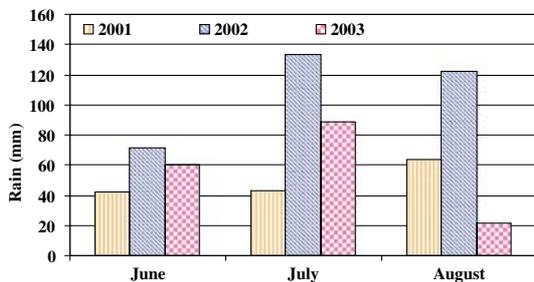
In 2001, the lowest seed-set was observed which could be as result of adverse climatic conditions that year. Warmer nights during the critical period of flowering (which occurred between the first week of July and the first week of August) were recorded in 2001. Relative humidity in 2001 was higher and fluctuated more during the critical flowering period compared to 2002 and 2003. Variation in total rainfall was observed across years (Fig. 1). In 2001, total rainfall from July through August was less compared to 2002 and 2003, especially at the flowering period, recorded in July, (Fig. 2). These conditions could account for the lower seed-set across male-sterile

Table 2 Combined analysis of variance for seed-set on male-sterile lines evaluated at Ames, Iowa in 2001, 2002, and 2003

Source of variation	Degrees of freedom	Mean squares
Year	2	354.78*
Male-sterile line	20	748.49*
Block \times male-sterile line	60	7.39NS
Year \times male-sterile line	40	65.84*

* Significant at the 0.001 probability level

NS = not significant

**Fig. 1** Total rainfall from June 1 to August 31 at Ames, Iowa in 2001, 2002, and 2003**Fig. 2** Total monthly rainfall from June 1 to August 31 at Ames, Iowa in 2001, 2002, and 2003

lines for 2001. Large differences in soil temperature, and wind speed records across years were not observed.

Male-sterile line performance

The effect of line on seed-set was significant (Table 2). The male-sterile lines that presented the highest mean values across years were *ms9* (T359H), *ms2* (T375H), *ms3* (T284H), and *ms2* (A00-41) (Table 3). The lowest means were observed in A00-74 (from T278M), *ms3* (T273H), *ms7* (T357H), and *ms2* (L75-0587) (Table 3).

Year \times male-sterile line interaction was present (Table 2); however, the ranking across years for the higher and lower yielding lines had little variation (data not shown).

Graybosch and Palmer (1988) reported from 1.3 to 126.6 seeds/male-sterile plant in lines carrying the male-sterile mutations *ms1* (T266H), *ms2* (L79-1308), *ms2* (L74-01), *ms2* (T259H), and *ms3* (T273H) using two planting dates and honey bees and alfalfa leaf cutter bees as pollinators. Carter et al. (1983) reported from 11 to 97 pods/male-sterile plant using *ms2* (L74-01) with two planting dates, and honey bees as pollinator. Palmer et al. (1983) reported from 11.6 to 28.3 seeds/male-sterile plants using *ms2* (T259H) and *ms3* (T273H) with native pollinators in two different environments.

Compared to our results, all these previous reports mentioned higher seed-set. In these studies, two common factors were the rouging of the male-fertile siblings within segregating rows, and the use of unrelated male-fertile pure lines as the pollen donor. In our study, male-fertile sibling plants were not rouged, we assumed that the pollen source would be male-fertile sibling plants. Since the male-fertile siblings were not rouged, spacing between plants was denser at flowering time when bees carried out the cross-pollination. The overall lower seed-set in our study also could be a result of lower male-parent insect attraction. The mean seed-set observed was similar to that reported by Lewers et al. (1996) for one of the spatial patterns tested in lines cosegregating for the *ms6* and *w1* alleles. The experiments were conducted at the same location as the present study, but honey bees were used as pollinator. For that spatial pattern, rouging of fertile plants was not made. In the present experiment, the mean number of seeds/male-sterile plant across male-sterile lines and years was 8.46, whereas the highest yielding line had 35.36 seeds/male-sterile plant. Ortiz-Perez et al. (2006) reported 2.23 seeds/male-sterile plant across male-sterile lines segregating for the *ms6* allele, their highest yielding line had 9.47 seeds/male-sterile plant. Both experiments were grown at the same location and the same years.

A factor that could influence the higher seed-set observed in the present experiment could be

Table 3 Mean values for seed-set on male-sterile lines across years at Ames, Iowa in 2001, 2002, and 2003

Lines	Mean no. seed/male-sterile plant			
	2001	2002	2003	Mean
<i>ms1</i> (Urbana)	4.77	5.13	8.77	6.22
<i>ms1</i> (North Carolina)	0.63	2.58	3.31	2.17
<i>ms2</i> (Eldorado)	0.49	3.75	2.53	2.26
<i>ms2</i> (Eldorado)	1.35	8.05	5.82	5.07
<i>ms2</i> (Eldorado)	0.44	2.94	0.66	1.35
<i>ms2</i> (Eldorado)	2.48	3.88	1.09	2.48
<i>ms2</i> (Ames 1)	0.98	8.16	2.50	3.88
<i>ms2</i> (Ames 2)	11.00	32.21	17.27	20.16
<i>ms2</i> (A00-41)	17.66	20.28	18.43	18.79
<i>ms2</i> (A00-55)	10.65	22.69	17.43	16.92
<i>ms3</i> (Flanagan)	18.70	14.72	23.63	19.02
<i>ms3</i> (Washington)	2.13	1.83	2.03	1.99
<i>ms3</i> (Plainview)	3.85	16.16	13.85	11.29
<i>ms4</i>	6.02	11.85	6.03	7.97
<i>ms6</i> (Ames 1)	0.83	11.91	3.50	5.41
<i>ms6</i> (Ames 2)	0.76	8.37	3.24	4.12
<i>ms7</i> (A00-71)	2.02	2.62	1.28	1.97
<i>ms8</i> (A00-72)	13.01	9.98	22.07	15.02
<i>ms9</i> (A00-73)	27.49	20.03	35.36	27.63
A00-70	3.73	4.05	3.25	3.67
A00-74	0.24	0.60	0.28	0.37
CV	29.62	26.89	35.02	
LSD	2.57	3.83	4.53	

the threshold preference of nectar quality and volume in soybean nectaries in the group of lines evaluated. Mean nectar sugar content in soybean has been reported between 37 and 45% (Erickson 1975; Kettle and Taylor 1979). Whether a differential sugar content/volume in the lines influenced pollinator visitation and outcrossing in the present study was not evaluated. Other factors that could influence a differential seed-set could be related to floral traits per se. Suso et al. (2005) reported that outcrossing and yield in *Vicia faba* could be improved by artificial selection on pre-mating traits such as floral design and display. They observed that most of the variation for outcrossing was associated with variation in floral display size, where selection for plants that produce more inflorescences with fewer flowers might be considered.

Flower color

Little variation in seed-set between purple-flowered and white-flowered lines was observed. Orthogonal contrasts showed significant differences among

white- and purple-flowered lines only for 2002 (Table 4), where purple-flowered lines produced more seed-set than white ones (Fig. 3). Ortiz-Perez et al. (2006), however, reported a significant effect of flower color on seed-set in the male-sterile lines (*ms6*) they evaluated. White-flowered lines had higher seed-set compared to purple-flowered lines.

In our experiment, the association between flower color and seed-set could not be clearly established. The effect of flower color also may be confounded by the effects of nectar quality and quantity, volatiles, etc. in pollinator reward; variables that were not determined in this study.

Pubescence color

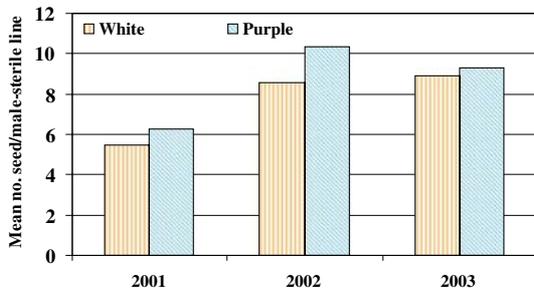
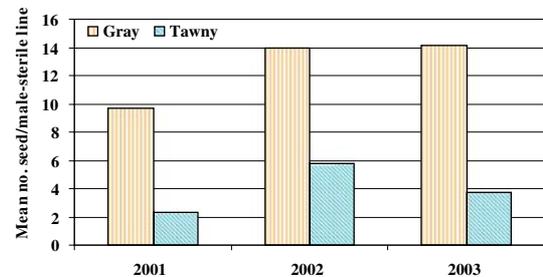
Variation in seed-set for pubescence color was observed (Fig. 4). Orthogonal contrasts showed significant differences among lines with gray pubescence and tawny pubescence (Table 4). This is in agreement with that results of Ortiz-Perez et al. (2006), who observed higher seed-set in gray pubescence *ms6* lines compared to tawny pubescence *ms6* lines grown in the same field and

Table 4 Mean values for seed-set and statistical significance for flower color and pubescence color contrasts for male-sterile lines evaluated at Ames, Iowa in 2001, 2002, and 2003

Trait	Mean no. seed/male-sterile line		
	2001	2002	2003
Flower color			
Purple-flowered lines	6.27	10.33	9.27
White-flowered lines	5.44	8.59	8.88
Contrast			
Purple-flowered lines versus white-flowered lines	NS	*	NS
Pubescence color			
Tawny-pubescence lines	2.29	5.82	3.73
Gray-pubescence lines	9.66	13.96	14.09
Contrast			
Tawny-pubescence lines versus gray-pubescence lines	**	**	**

*,** Significant at the 0.05, and 0.001 probability levels respectively

NS = Not significant

**Fig. 3** Flower color effect on mean number of seed/male-sterile line at Ames, Iowa in 2001, 2002, and 2003**Fig. 4** Pubescence color effect on mean number of seed/male-sterile line at Ames, Iowa in 2001, 2002, and 2003

years as the present study. The influence of pubescence color on seed-set could be considered as an indirect effect on the plant's microclimate. The location where the lines were tested did not present a cool-season climate where tawny lines could possibly perform better (Morrison et al. 1994).

Maturity group

When the lines were grouped by maturity group, orthogonal contrasts showed significant differences for seed-set among lines (Table 5). Lines from MG I were originally found at the Bruner Farm, where the present experiments were conducted. However, as a maturity group, lines from MG I had one of lowest seed-set (Table 5). Across years, lines from maturity group II presented

higher seed-set when compared to lines from maturity groups I, III, and IV. Lines from MG II are adapted in the area where the experiments were conducted. One factor that could favor lines from MG II for seed-set was that insect activity and out-crossing could be higher at the time these lines were flowering compared to the earlier flowering MG I lines. It is possible that lines from MG IV presented a higher seed-set when compared to MG I and III only because of flower availability for the pollinators. However, the higher seed-set observed for MG II and MG IV lines also could reflect pollinator preference, not only flower availability in time and space. Each MG was comprised of at least three lines segregating for male sterility at a different locus, thus comparisons could be confounded by possible interactions with maturity group, genetic

Table 5 Mean values for seed-set and statistical significance for maturity group (MG) contrasts for male-sterile lines evaluated at Ames, Iowa in 2001, 2002, and 2003

	Mean no. seed/male-sterile line		
	2001	2002	2003
Maturity group			
MG I	2.58	9.46	3.92
MG II	9.28	14.04	13.17
MG III	2.57	6.04	4.61
MG IV	5.51	6.46	8.70
Contrast			
MG I vs. MG II	**	**	**
MG I vs. MG III	NS	*	NS
MG I vs. MG IV	**	*	**
MG II vs. MG III	**	**	**
MG II vs. MG IV	**	**	**
MG III vs. MG IV	**	NS	*

*** Significant at the 0.01 and 0.001 probability levels respectively
NS = Not significant

background of the lines, and the male-sterile locus, which could affect the performance of the lines.

Male-sterile locus and allele

Differences in seed-set were observed when the lines were grouped by male-sterile locus (data not shown). Contrasts among lines with independent mutational events at the same locus also showed significant differences for seed-set (Table 6). Regardless of their different genetic background, the four near-isogenic lines segregating for *ms2* (T259H), and the *ms2* (T360H) performed similarly, [i.e. low seed-set; significant differences were not observed among them (data not shown)]. Significant differences were not observed among the two lines segregating for *ms6* in either year.

Palmer et al. (1983) reported that male parents, female parent background, and environments seemed to have little effect on cross-pollination and seed-set onto *ms2ms2* (T259) plants. However, in the present study, the seed-set for lines segregating for *ms2* (T375H) was quite different. These lines presented some of the highest seed-set values (Table 3). The *ms2* (T375H) line and its sister lines (A00-41 and A00-55) presented similar high values for seed-set across years. These results could reflect differences in pollinator preference caused by the mutations *per se*, or as result of interaction of line background by mutation. This interaction was observed among lines with independent

mutational events at the same locus. The effect of the mutations *per se* was observed for the four lines segregating for the *ms2* (T259H), and the *ms6* loci. It is possible that for these loci, a strong pleiotropic effect of the mutation on some traits (attractiveness to pollinators, floral attributes, etc.) was the key component for the performance of the lines. However, the interaction effect of genetic background by mutation in the performance of the lines was observed in most cases. The literature presents contradicting results for seed-set for the same lines evaluated in different environments (see Lewers and Palmer 1997). This emphasizes the strong interaction present not only for plant–environment, but also pollinator–plant–environment, since seed production on male-sterile plants is affected by pollinator species, preference and activity.

Conclusions

The objective of this study was to evaluate seed-set in the available collection of lines segregating for nuclear male sterility in soybean. Preferential cross-pollination among male-sterile lines as measured through seed-set was observed. Selection of the highest yielding male-sterile lines followed by crossing, and further evaluation in replicated environments to improve the observed seed-set should be possible. Such lines could be used as female parents in hybrid soybean programs. The effects of maturity group, pubescence

Table 6 Mean values for seed-set and statistical significance for independent mutational events at the same locus contrasts for male-sterile lines evaluated at Ames, Iowa in 2001, 2002, and 2003

Male-sterile locus	Mean no. seed/male-sterile line		
	2001	2002	2003
<i>ms1</i>			
T266H	4.77	5.13	8.77
T260H ^a	0.63	2.58	3.31
<i>ms2</i>			
T259H ^b	1.19	4.65	2.52
T360H	0.98	8.16	2.50
T375H ^c	13.10	25.06	17.71
<i>ms3</i>			
T284H	18.70	14.72	23.63
T273H	2.13	1.83	2.03
T291H	3.85	16.16	13.85
<i>ms6</i>			
T295H	0.83	11.91	3.50
T354H	0.76	8.37	3.24
Contrasts			
<i>ms1</i> T266H vs. <i>ms1</i> T260H ^a	**	NS	*
<i>ms2</i> T259H ^b vs. <i>ms2</i> T360H	NS	*	NS
<i>ms2</i> T259H ^b vs. <i>ms2</i> T375H ^c	***	***	***
<i>ms2</i> T360H vs. <i>ms2</i> T375H ^c	***	***	***
<i>ms3</i> T284H vs. <i>ms3</i> T273H	***	***	***
<i>ms3</i> T284H vs. <i>ms3</i> T291H	***	NS	***
<i>ms3</i> T273H vs. <i>ms3</i> T291H	NS	***	***
<i>ms6</i> T295H vs. <i>ms6</i> T354H	NS	NS	NS

*, **, *** Significant at the 0.01 and 0.001 probability levels respectively

NS = Not significant

^a T260 *ms1* (North Carolina) is a cv. Clark near-isogenic line

^b Average seed-set of L74-01, L75-0570, L75-0587, and L79-1308 near-isolines

^c Average seed-set of T375H, and sister lines A00-41 and A00-55

color, and the male-sterile loci also were significant. We did not observe significant differences for seed-set among the lines that could be attributed to flower color. Differences for seed-set among lines representing independent mutational events at the same locus also were significant, which implies the importance of interaction effects with the genetic background of the lines for the traits related to fertility and pollinator preference. The effect of year was very important on the seed-set performance of the lines. This suggested that environmental conditions that favor plant–pollinator relationships need to be identified in order to establish an efficient hybrid soybean seed production system.

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