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Abstract

Currently, there is no economical way to produce large quantities of F₁ hybrid soybean seed in the USA. One of the fundamental requirements for hybrid seed production is the availability of a stable male-sterile, female-fertile system. However, the more challenging barrier is the efficient transfer of pollen from the male parent to the female parent. This could potentially be achieved through pollinator insects. Our observations suggested that seed set on male-sterile, female-fertile plants is a good indicator of insect attraction. The objective of this study was to evaluate seed set among male-sterile, female-fertile lines segregating for male-sterile, female-fertile *ms6* allele by using *Megachile rotundata* as pollinator vector. Thirty-four pairs of near-isogenic lines, the *ms6 w1w1* donor parent, and its two isogenic lines *W1w1* and *w1w1* segregating for male-sterile (*ms6*) allele were used. The *W1* locus controls flower color and hypocotyle pigmentation. Seed set was evaluated on field-grown plants in 2001–2003 near Ames, IA. Although the observed seed set was not commercially acceptable, our results indicated significant differences in seed set among lines. This suggests that preferential attraction of pollinators occurred, and selection among male-sterile, female-fertile lines could be used to obtain female parents suitable to produce larger amounts of hybrid soybean seed. In addition, the effect of flower color on seed set was statistically significant. White-flowered lines (*w1w1*) produced more seed set compared to purple-flowered lines (*W1W1*). Lastly, the important effect of year suggested that the effect of environmental conditions on seed set among lines segregating for male sterility was of paramount importance to plant–pollinator interactions. This needs to be assessed in order to establish an efficient hybrid soybean program

Keywords

Soybean, Hybrids, Male-sterile lines, Pollinators

Disciplines

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

Comments

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Evaluation of insect-mediated seed set among soybean lines segregating for male sterility at the *ms6* locus[☆]

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Abstract

Currently, there is no economical way to produce large quantities of F₁ hybrid soybean seed in the USA. One of the fundamental requirements for hybrid seed production is the availability of a stable male-sterile, female-fertile system. However, the more challenging barrier is the efficient transfer of pollen from the male parent to the female parent. This could potentially be achieved through pollinator insects. Our observations suggested that seed set on male-sterile, female-fertile plants is a good indicator of insect attraction. The objective of this study was to evaluate seed set among male-sterile, female-fertile lines segregating for male-sterile, female-fertile *ms6* allele by using *Megachile rotundata* as pollinator vector. Thirty-four pairs of near-isogenic lines, the *ms6 w1w1* donor parent, and its two isogenic lines *WIWI* and *w1w1* segregating for male-sterile (*ms6*) allele were used. The *WI* locus controls flower color and hypocotyle pigmentation. Seed set was evaluated on field-grown plants in 2001–2003 near Ames, IA. Although the observed seed set was not commercially acceptable, our results indicated significant differences in seed set among lines. This suggests that preferential attraction of pollinators occurred, and selection among male-sterile, female-fertile lines could be used to obtain female parents suitable to produce larger amounts of hybrid soybean seed. In addition, the effect of flower color on seed set was statistically significant. White-flowered lines (*w1w1*) produced more seed set compared to purple-flowered lines (*WIWI*). Lastly, the important effect of year suggested that the effect of environmental conditions on seed set among lines segregating for male sterility was of paramount importance to plant–pollinator interactions. This needs to be assessed in order to establish an efficient hybrid soybean program.

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1. Introduction

Since soybean [*Glycine max* (L.) Merrill] is a self-pollinated species, hybrids for commercial production have received little attention. One reason is that manual cross-pollination to produce large quantities of hybrid soybean seed is difficult and time-consuming. The low success rate, and the few seeds obtained per pod have contributed to the difficulty of producing large quantities of hybrid seed. Commercial success of F₁ hybrid soybean will require the efficient transfer of pollen from the male parent to the female parent (Palmer et al., 2001). The use of nuclear male sterility and cytoplasmic-genetic male

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sterility in soybean has raised the real possibility that F₁ hybrid soybean could be produced commercially. Mutations that selectively eliminate male reproductive function and leave female function unimpaired have potential application in hybrid seed production (Horner and Palmer, 1995).

Insect pollen vectors may be the preferred means by which pollen can be transferred between parents. There are over 3000 species of bees in North America. Most are solitary bees, but a well-known minority of them is social, meaning that they live together in colonies and cooperate in colony tasks. Both solitary and social species are important in crop pollination, but the social species are more easily managed. The honey bee, *Apis mellifera* (L.) represents the best example of social organization. In this species, the colony is the basic reproductive unit, which contains a single long-lived reproductive queen, a large number of non-reproductive workers, and a few males (drones). The workers tend the brood from the time the egg is laid until it is a mature larva, feeding it small quantities of food at intervals during each day. Even more significant in terms of social development is the complex division of labor made possible through the development of communication and the constant food inter-exchange and the stimulation they provide through body contact. In contrast, solitary bees occur in groups or colonies, in which each female builds and provisions her own nest without assistance from other bees (Stephen et al., 1969). One solitary insect species used for commercial pollination of crops is the alfalfa leaf cutter bee *Megachile rotundata* (F.), which is extensively employed for the production of alfalfa seed (Pedersen et al., 1972). Although soybean is an autogamous species, it possesses most characteristics of an entomophilus plant species (Juliano, 1976; Erickson and Garment, 1979; Arroyo, 1981; Erickson, 1983; Delaplane and Mayer, 2000; Horner et al., 2003). Pollinator activity has been reported in soybean (Bradner, 1969); however, traits influencing preferential pollination on male-sterile, female-fertile lines that could be used as female parents in a hybrid system have not been clearly elucidated.

Palmer and Lewers (1998) developed 34 pairs of soybean lines by backcrossing the *ms6* and *w1* alleles to 34 recurrent parents. The crosses produced near-isogenic lines cosegregating for the *ms6* allele (male sterility) and *w1* (white flower) alleles in coupling phase or white-flowered lines segregating for the *ms6* allele. Neither seed set, nor the attractiveness to pollinator insects has been evaluated on these near-isogenic lines. Previous studies indicated that seed set on male-sterile, female-fertile plants is a good indicator of insect pollinator attraction (Lewers et al., 1996, 1998; Lewers and Palmer, 1997; Ortiz-Perez et al., 2004). The objective of this study was to evaluate seed set among male-sterile, female-fertile lines segregating for the *ms6* allele using *M. rotundata* as pollinator during three growing seasons near Ames, Iowa.

2. Materials and methods

2.1. Plant material

Thirty-four pairs of soybean lines were developed by backcrossing the *ms6* and *w1* alleles to 34 recurrent parents (Palmer and Lewers, 1998). The lines were developed with the *ms6* donor cytoplasm and the recurrent parent cytoplasm. Each pair of near-isogenic lines consisted of one line developed with the recurrent parent used as male and the donor parent used as female (donor parent cytoplasm), whereas the other line was developed with the donor parent used as male and the recurrent parent as female (recurrent parent cytoplasm). The two near-isogenic lines differed in their cytoplasm source. Genetic Type T295 was the donor of the *ms6* allele (Table 1). It is a white-flowered male-sterile, female-fertile soybean mutant registered as GS-1, PI533601 (Palmer and Skorupska, 1990). The *Ms6* locus affects pollen production; *Ms6* plants are fertile, whereas *ms6ms6* plants are female-fertile and completely male-sterile due to tapetal abnormalities. Male sterility is inherited as a single-recessive gene (Skorupska and Palmer, 1989).

Seventy-one male-sterile lines were evaluated in this study (34 pairs, the donor parent, and its two isogenic lines). Nine pairs were white-flowered, segregating at the *Ms6* locus (*Ms6ms6 w1w1*), and 25 pairs were purple-flowered cosegregating at the *Ms6* and *W1* loci in coupling phase (*Ms6ms6 W1w1*). Nine of the recurrent parents were high-yielding accessions introduced into the United States from China, Japan, and the former USSR; five lines were ancestors of modern soybean cultivars in maturity group II and contributed more than 55% of the northern gene pool for cultivars released between 1971 and 1981 (Delannay et al., 1983); seven lines were important modern cultivars which represented five different public breeding programs, and 13 lines were commercial cultivars or breeding lines from eight different companies. All 34 near-isogenic pairs were similar to their respective recurrent parent for pubescence color, growth habit, pod-wall color, seed-coat color, and maturity.

2.2. Field studies

The near-isogenic male-sterile lines, the donor parent, Genetic type T295H (*Ms6ms6w1w1*), and its isogenic lines *Ms6ms6W1W1* and *Ms6ms6W1w1* were grown at the Bruner farm near Ames, IA, in the summers of 2001–2003. The experiments were conducted in fields with a Clarion-Nicollet loam soil type. 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. A randomized complete block design (RCBD) was used with 4 replications/male-sterile line. Plants were not thinned. At flowering, alfalfa leaf cutter bees (*M. rotundata*) domiciles were placed in alley-ways surrounding the plots. Each domicile

Table 1
Male-sterile lines and donor parents evaluated for seed set (flower color, pubescence color, and origin of the recurrent parents are listed)

Line	Flower color	Pubescence color	Source of pedigree information for the recurrent parent
Ancestors of modern cultivars			
A.K. Harrow	White	Gray	Selection from FC30761 from China
Mandarin (Ottawa)	Purple	Gray	Selection from PI36653 from China
Mandarin	Purple	Gray	PI36653 from China
Manchu	Purple	Tawny	PI30593 from China
Richland	Purple	Gray	PI70502-2 from China, no. 8225
Plant introductions from the former USSR			
PI297544	White	Gray	USSR, no. II-2-269, Primorszkaja 529
PI370059	Purple	Gray	USSR, no. 5622
PI384474	Purple	Gray	USSR, no. 4987, VNIIMK7
Plant introductions from China			
PI427099	White	Gray	China, Jilin no. 3
PI91167	Purple	Gray	China, no.6622
PI261474	White	Gray	China, Kohoju
Plant introductions from Japan			
PI227333	White	Gray	Japan, Ohozyu
PI416941	Purple	Gray	Japan, no. H-020063, Houten Hakuby
PI417076	Purple	Gray	Japan, no. H-020169, Koushurei 224
Public lines			
Hack	White	Gray	Nickell et al., 1985
Hardin	Purple	Gray	Fehr et al., 1983
<i>Ms6ms6WIW1</i>	Purple	Gray	Palmer and Skorupska, 1990
<i>Ms6ms6WIw1</i>	Purple	Gray	Palmer and Skorupska, 1990
T295H (<i>Ms6ms6w1w1</i>)	White	Gray	Palmer and Skorupska, 1990
BSR 101	Purple	Gray	Tachibana et al., 1987
Corsoy 79	Purple	Gray	Bernard and Cremeens, 1988
Elgin	Purple	Tawny	Fehr and Bahrenfus, 1984
Century	Purple	Tawny	Wilcox et al., 1980
Hoyt	Purple	Tawny	Cooper et al., 1991
Private lines			
82-378	White	Gray	Land O' Lakes, Inc.
82-165	White	Tawny	Land O' Lakes, Inc.
CX155	Purple	Gray	DeKalb Genetics Corp.
Glenn	Purple	Gray	ProfiSeed Inc.
A3307	White	Tawny	Asgrow Seed Co.
AX2858	Purple	Tawny	Asgrow Seed Co.
AG-020	Purple	Tawny	Asgrow Seed Co.
J201	Purple	Gray	Mycogen Seeds
S1346	Purple	Gray	Novartis Seeds Inc.
G3197	Purple	Gray	Novartis Seeds Inc.
P596-13	Purple	Gray	Pioneer Hi-Bred, Int'l.
P42257	Purple	Gray	Pioneer Hi-Bred, Int'l.
P301002	Purple	Tawny	Pioneer Hi-Bred, Int'l.

contained about 300 bee pupae. One domicile per 14 soybean plots was used. Flower and pubescence color were recorded. After flowering, the domiciles were removed. At maturity, male-sterile plants were identified visually within segregating rows (male-sterile plants produced fewer pods compared to male-fertile plants and stems remain green in most sterile plants). The number of male-sterile plants and number of seeds/male-sterile plant were recorded. The pollen source for the male-sterile plants was assumed to come 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 the next season. The segregating progeny provided the male-sterile plants that were used for evaluation. Again 40 male-fertile plants within segregating families were single-plant threshed. They were progeny tested the next season. Soil temperature, air temperature, rainfall, relative humidity, and wind speed records were obtained throughout from the IEM (Iowa environmental mesonet). No irrigation was applied. The same procedure was followed in summers 2002 and 2003.

2.3. 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:

$$Y_{ijkl} = \mu + Y_i + B_{(i)j} + L_k + YL_{ik} + BL_{(i)jk} + C_l + YC_{il} \\ + BC_{(i)jl} + LC_{kl} + YLC_{ikl} + BLC_{(i)jkl}$$

where μ is 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 line; C_l the effect of the l th cytoplasm source; YC_{il} the interaction effect between the i th year and the l th cytoplasm source; $BC_{(i)jl}$ the interaction effect between the j th block nested in the i th year and the l th cytoplasm source; LC_{kl} the interaction effect between the k th male-sterile line and the l th cytoplasm source; YLC_{ikl} the interaction effect between the i th year, the k th male-sterile line, and the l th cytoplasm source and $BLC_{(i)jkl}$ is the interaction effect between the j th block nested in the i th year, the k th male-sterile line, and the l th cytoplasm source.

Replications and years were treated as random factors; male-sterile lines and cytoplasm source as fixed factors. The mean number of seeds per male-sterile plant across lines was compared for the 3 years. Selected orthogonal contrasts were computed for flower color, pubescence color, and origin of the recurrent parents. Effects were considered significant in all statistical calculations, if $P < 0.05$. For the donor parent T295H (*Ms6ms6w1w1*), orthogonal contrasts for flower color were computed by comparisons with its isogenic lines *Ms6ms6W1w1* and *Ms6ms6WIW1*.

3. Results and discussion

3.1. Environmental conditions

The combined analysis showed significant differences in seed set for the effects of lines and years (Table 2). No

Table 2

Combined analysis of variance for seed set on male-sterile lines in Ames, Iowa for the 2001–2003 growing seasons

Source of variation	Degrees of freedom	Mean squares
Year	2	545.12***
Male-sterile line	36	49.60***
Year \times male-sterile line	72	12.96***
Cytoplasm source	1	0.36 NS
Male-sterile line \times cytoplasm source	33	2.08 NS
Year \times male-sterile line \times cytoplasm source	66	1.76**

NS = not significant.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

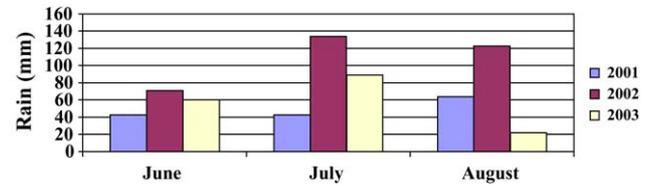


Fig. 1. Total accumulated rainfall from 1 June to 31 August in Ames, Iowa for the 2001–2003 growing seasons.

significant effect for cytoplasm source was observed, suggesting that differences in seed set were not related to the effects of the cytoplasm genome. Significant interactions for year \times male-sterile line, and year \times male-sterile line \times cytoplasm source were observed, so individual analyses were conducted for each year. The seed yield across male-sterile lines was 0.72 seeds/male-sterile plant in 2001, 3.48 seeds/male-sterile plant in 2002, and 2.44 seeds/male-sterile plant for 2003. The lowest seed set was observed in 2001, which could be due to climatic conditions. A clear pattern regarding differences in soil temperature, and wind speed records across years was not observed; however, air temperature fluctuation was slightly less in 2001, compared to 2002 and 2003. Warmer nights were observed during the critical period of flowering that was recorded between the first week of July and the first week of August (Fig. 1). Frantz et al. (2004) reported that night respiration increased 4%°C in soybean which caused leaf mass to decrease significantly. Considerable reduction of leaf area along with increasing seed growth rate could limit the total photosynthate available for seed growth (Seddigh and Jolliff, 1984), which could result in more seed abortions. The proportion of soybean flowers developing into mature pods ranges from 20 to 70% depending upon variety (Van Schaik and Probst, 1958), and environmental factors such as soil moisture (Swen, 1933), temperature, and photoperiod (Van Schaik and Probst, 1958).

Relative humidity in 2001 was higher and fluctuated more during the critical flowering period compared to 2002 and 2003. A large variation in total rainfall was observed across years. In 2001, total rainfall from July to August was less than one-half compared to 2002 (Fig. 1), the most favorable year for seed set. Water stress imposed during flowering reduces photosynthesis and the amount of photosynthetic assimilates allocated to floral organs, and thus might also increase the rate of flower abortion (Raper and Kramer, 1987). Since the plots were not irrigated, rainfall was considered a key factor affecting the plant's growth and development. The poor performance of the lines in 2001, the year with the smallest amount of accumulated rain, seems to support this observation.

3.2. Seed set on male-sterile lines

The effect of line on seed set was significant (Table 2). The male-sterile lines that presented the highest mean values

Table 3
Ranking of male-sterile lines across years for seed set and mean values in Ames, Iowa for the 2001–2003 growing seasons

Lines	Flower color	Mean no. of seed/male-sterile plant/season			
		2001	2002	2003	Mean
82–165	White	4.13	6.96	5.09	5.39
PI297544	White	1.21	9.45	5.18	5.28
A.K. Harrow	White	1.46	9.47	3.52	4.82
Hack	White	2.31	5.22	6.36	4.63
PI91167	Purple	0.86	8.47	4.2	4.51
PI261474	White	0.59	7.33	4.83	4.25
PI227333	White	0.68	8.17	3.02	3.96
Hardin	Purple	0.56	3.72	5.32	3.20
PI370059	Purple	0.29	4.85	4.31	3.15
WIWI (isoline of T295H)	Purple	0.46	6.28	2.48	3.07
BSR 101	Purple	0.34	4.9	3.89	3.04
Corsoy 79	Purple	1	3.2	3.78	2.66
CX155	Purple	0.96	4.1	2.28	2.45
G3197	Purple	0.95	3.09	2.98	2.34
Mandarin	Purple	0.45	2.88	3.57	2.30
Elgin	Purple	0.86	2.57	2.79	2.07
A3307	White	0.39	3.03	1.77	1.73
PI417076	Purple	0.11	2.74	2.33	1.73
Glenn	Purple	0.77	2.15	2.16	1.69
PI427099	White	0.49	3.18	1.07	1.58
S1346	Purple	0.49	2.34	1.8	1.54
WIWI (isoline of T295H)	Purple	0.63	2.05	1.61	1.43
Century	Purple	0.54	1.95	1.74	1.41
Mandarin (Ottawa)	Purple	0.47	1.88	1.46	1.27
82–378	White	0.48	2.57	0.74	1.26
PI416941	Purple	0.05	2.46	1.25	1.25
T295H (<i>w1w1</i>)	White	0.43	1.84	1.34	1.20
P422-57	Purple	0.82	1.82	0.94	1.19
P3010-02	Purple	0.73	1.66	0.73	1.04
J201	Purple	0.39	1.89	0.65	0.98
AG-020	Purple	0.57	1.51	0.79	0.96
Manchu	Purple	0.35	1.55	0.77	0.89
PI384474	Purple	0.08	1.25	1.33	0.89
Richland	Purple	0.18	0.84	1.51	0.84
P596-13	Purple	0.58	0.71	1.09	0.79
AX2858	Purple	0.12	0.55	0.23	0.30
Hoyt	Purple	0.38	0.14	0.29	0.27
Mean		0.72	3.48	2.44	
Maximum		4.12	9.47	6.36	
Minimum		0.05	0.14	0.23	
LSD ($\alpha = 0.05$)		0.42	1.11	1.18	

were 82–165 from Land O' Lakes, PI297544, an accession from the former USSR, A.K. Harrow, an ancestor of modern cultivars, and Hack, a public line (Table 3). The lowest means were observed in Hoyt, a public line, AX2858 from Asgrow Seed Co., P596-13 from Pioneer Hi-Bred International, and Richland, an ancestor of modern cultivars from China (Table 3). In general, the mean number of seeds/male-sterile plant observed across male-sterile lines was low; considerably less than that reported by Lewers et al. (1996) for lines cosegregating for the *ms6* allele (male sterility) and *w1* (white flower) alleles. These authors recorded 28.6, 18.2, and 9.3 seeds/male-sterile plant for the

three plant spatial patterns they tested to evaluate seed set by using honey bees as a pollinator. In our experiment, the mean across lines was only 2.23 seeds/male-sterile plant. This difference could be due to differences in the methods used. Lewers et al. (1996) rouged the male-fertile sibling plants at the first trifoliolate stage. In our experiment, male-fertile sibling plants were not rouged, and spacing between plants was denser at flowering time when bees were carrying out the pollination. As a result of the higher plant density, any moisture stress would be magnified resulting in a reduction in seed set on the male-sterile plants. Previous studies showed that the incidence of cross-pollination by insects in soybean is greatest among plants in close proximity and decreases with increased distance from the pollen source (Boerma and Moradshahi, 1975; Caviness, 1966; Jaycox, 1970; Nelson and Bernard, 1979; Handel, 1983; De Jong et al., 1993).

Another important aspect in this comparison between experiments is the pollinator used. In our experiment, the alfalfa leaf cutter bee was used as the pollinator. Leaf cutter bee preferences related to plant spatial patterns in soybean have not been determined. According to Chiang and Kiang (1987), it is possible to increase the rate of outcrossing and seed yield in soybeans by attracting bee visitation using a high density stand with each plant surrounded by a different genotype. In alfalfa, leaf cutter bees forage mainly on flowers exposed to the sun, missing the many shaded flowers in a dense stand (Pedersen et al., 1972). This could help to explain the poor seed set observed across male-sterile lines in years 2001–2003, when compared to the less dense plantings of Lewers et al. (1996). Another factor could be the threshold preference of nectar quality and volume content in soybean nectaries for alfalfa leaf cutter bees. For honey bees, response thresholds change with quality of sugar offered (10–50%), and the roles of foragers. Bees returning with pollen have response thresholds lower than those returning with nectar (Pankiw et al., 2001). Mean nectar sugar content in soybean has been reported between 37 and 45% (Erickson, 1975; Kettle and Taylor, 1979).

Plant-pollinator interaction is a very complex process. The effectiveness of pollination in plants is determined largely by three factors: (1) number of pollinators that visit the plant; (2) number of flowers each pollinator probes during its visit to the plant; and (3) effectiveness of the pollinator transferring appropriate pollen at each flower (Faegri and Van Der Pill, 1971; Cresswell and Galen, 1999). The 34 pairs of near-isogenic lines were selected as a random sample of high-yielding accessions from China, Japan, the former USSR, public sources, and from private companies. Their attractiveness to pollinators had not been evaluated previously. The fact that their fertile recurrent parent was high-yielding did not necessarily correspond with high seed set in converted male-sterile near-isogenic lines. One reason could be that fertile soybean plants do not benefit from insect pollination (Rubis, 1970), whereas sterile plants need to be attractive to pollinators in order to produce

seed set. However, according to Erickson (1975) a significant yield increase in cv. Corsoy and in cv. Hark was observed as result of pollination activity of honey bees. Yet, interactions between plants and pollinators are influenced also by both biotic factors (floral structure, timing of anthesis, quantity and quality of floral rewards, presence of others pollinator species) (Erickson, 1975; Corbet, 1990), and abiotic factors (i.e. temperature, wind, solar radiation) (Corbet, 1990). Year \times male-sterile line interaction was present (Table 2); however, ranking of the lines for seed set was similar across years for the 10 highest-yielding lines and the 10 lowest-yielding lines.

Selected orthogonal contrasts were estimated for flower color, pubescence color, and origin of the recurrent parent. For the latter, lines were sub-grouped into ancestors, modern cultivars, public lines, private lines, and accessions from China, the former USSR, and Japan.

3.3. Recurrent parent origin

Differences in seed set between new cultivars and ancestors of modern cultivars were significant only in 2002, which was the best year for seed set (Table 4). Lines from private companies yielded statistically significant less seed set when compared to public, Chinese, former USSR, and Japanese lines. Differences in seed set between Chinese and Japanese cultivars were not significant in any year (Table 4). Plant introductions or accessions introduced from Asia, selections from these introductions, or natural crosses that arose from these introductions, share a common gene pool (Sleper and Shannon, 2003). It was not unexpected that male-sterile lines with recurrent parents from the same geographical area would perform similarly. Although the

Table 4

Statistical significance of orthogonal contrasts for the origin of recurrent parents in seed set for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001–2003 growing seasons

Contrast	Mean no. of seed/male-sterile plant/season		
	2001	2002	2003
Modern cultivars vs. ancestors	NS	*	NS
Private vs. ancestors	NS	***	**
Public vs. ancestors	NS	**	***
Private vs. public	NS	**	***
Private vs. Chinese	***	***	***
Private vs. USSR	**	***	***
Private vs. Japanese	***	***	*
Chinese vs. USSR	NS	***	**
Chinese vs. Japanese	NS	NS	NS
Chinese vs. public	***	***	**
USSR vs. Japanese	*	*	***
USSR vs. public	**	***	NS
Japanese vs. public	***	***	***

NS = not significant.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 5

Statistical significance and means for seed set for flower color and pubescence color contrasts for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001–2003 growing seasons

Contrast	Mean no. of seed/male-sterile plant/season		
	2001	2002	2003
Flower color			
Purple-flowered lines vs. white-flowered lines	***	***	***
Mean no. of seed/male-sterile plant			
Purple-flowered lines	0.51	2.22	1.73
White-flowered lines	1.66	5.74	3.38
Pubescence color			
Tawny-pubescence lines vs. gray-pubescence lines	**	***	***
Mean no. of seed/male-sterile plant			
Tawny-pubescence lines	0.9	2.21	1.58
Gray pubescence lines	0.67	3.91	2.73

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

private lines as a group were not high seed set lines, the line that presented the highest seed set in this study, 82–165, was one released by a private company (Land' O Lakes Inc., Table 1). 82–165 is a white-flowered line with indeterminate growth habit, tawny pubescence, and plant height of 81 cm. Hoyt, the lowest seed set line, was an F₅ derived line from Harcor \times Elf (Cooper et al., 1991), has determinate growth habit, purple flowers, tawny pubescence, and plant height averaged 50 cm.

3.4. Flower color

Variation in seed set between purple-flowered and white-flowered lines was observed. Orthogonal contrasts showed significant differences among white- and purple-flowered lines across years (Table 5). White-flowered lines outyielded purple ones (Fig. 2), but the variation in seed set across years for white-flowered lines was higher compared to purple-flowered lines (data not shown). Our results differed from the report by Gay et al. (1999) who found that overall,

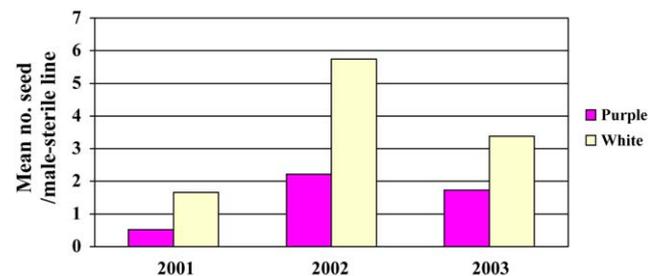


Fig. 2. Flower color effect on mean number of seed per male-sterile line for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001–2003 growing seasons.

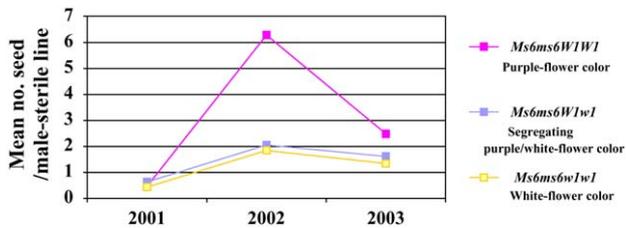


Fig. 3. Mean comparison for seed set among the donor parent Genetic Type T295H *Ms6ms6w1w1*[§] and its isogenic lines *Ms6ms6W1w1*[§] and *Ms6ms6W1W1* in Ames, Iowa for the 2001–2003 growing seasons. [§] Close genetic linkage between the *Ms6* and *W1* loci ($3.14 \pm 0.80\%$ recombination).

purple-flowered soybean lines did not significantly differ from white-flowered lines for yield, seed protein, and seed weight when evaluated as F_{2:5} families segregating for purple, pink, and white flower color.

When the donor parent, Genetic Type T295H *Ms6ms6w1w1* (white flower color) was compared with its isogenic line *Ms6ms6W1w1* (in which selfed progeny segregate for flower color producing plants with white flower color and plants with purple flower color), and *Ms6ms6W1W1* (in which selfed progeny produce only purple flower color plants), the results did not support the general observation that white-flowered lines had more seed set. *Ms6ms6W1W1* (isogenic line purple flower color) had higher seed set (Fig. 3) compared to *Ms6ms6W1w1* (segregating white/purple flower color) and the donor parent T295H *Ms6ms6w1w1* (white flower color). White and purple flower color in soybean are controlled by a single gene with purple (*W1*) being dominant (Bernard and Weiss, 1973). Homozygous recessive *w1* alleles produce pink flower color in soybean when in the presence of the non-allelic gene *W1* by modifying the expression of purple pigmentation (Stephens and Nickell, 1992).

One possible explanation for this difference is that there was a differential interaction of the *w1* and *W1* alleles with the genetic background of the donor line that favored the dominant homozygous condition *W1W1* over the heterozygous *W1w1* and the homozygous recessive *w1w1*. Skorupska and Palmer (1989) reported a pleiotropic effect of the *ms6* allele in flower size in families from Genetic type T295H *Ms6ms6w1w1*. Male-sterile plants (*ms6ms6w1w1*) had a smaller size flower when compared to fertile, the purple flower *Ms6_W1W1* plants. Since there is a close genetic linkage between the *Ms6* and *W1* loci (3.14 ± 0.80 recombination value) more than 92% of *w1w1* white plants are expected to be male-sterile (Palmer et al., 1998).

The seed set observed in our experiment for Genetic Type T295H *Ms6ms6w1w1* was not significantly different from *Ms6ms6W1w1* (data not shown). This could be explained by the fact that in selfed progeny from the *Ms6ms6W1w1* isogenic line, male-sterile plants are likely to have white flower color because of the close coupling phase linkage of the *ms6* and *w1* loci. Whether a possible interaction between white flower color and small flower size influenced the seed

set through pollinator rewards (nectar quality and volume, volatiles, etc.) remains to be tested. An alternative explanation for the higher seed set observed in the white-flowered near-isogenic lines is that differences were related to the genetic background of the lines regardless of the effect of the white flower color.

The highest lines for seed set were quite diverse, but the common factor was that they were white-flowered lines, while the lowest seed set lines were purple-flowered lines (Table 3). Robacker et al. (1983) stated that environmental conditions which promote greater flower production, larger flower size, more intensely colored flowers, and higher nectar secretion promote greater honey bee attractiveness. In soybean, honey bees seem to be equally attracted to white- and purple-flowered lines (Jaycox, 1970; Mason, 1979). Even though pollinator preference among petal color variants has been reported in several plant species (Levin, 1972; Waser and Price, 1981; Brown and Clegg, 1984; Schoen and Clegg, 1985; Epperson and Clegg, 1987; Stanton et al., 1986, 1989; Levin and Brack, 1995), the literature presents contradicting reports depending on pollinator species and frequency of polymorphic phenotypes. Stanton et al. (1989) tested the effect of petal color on mating patterns in radish, *Raphanus raphanistrum* (L.). They reported that the cabbage white butterfly *Pieris rapae*, the most common pollinator, visited yellow flowers 50% more when compared to white flowers. Epperson and Clegg (1987) evaluated visitation patterns by pollinators in an *Ipomoea purpurea* population presenting white and purple color flowers. They reported that bumble bees, the most common pollinator, showed no preference when white-flower-plants were common, but discriminated against the white-flower color petal when it was rare. Our findings about the preference of alfalfa leaf cutter bees for male-sterile plants presenting white-color flower (such preference observed through a higher seed set) were unexpected, since the literature reports indicate pollinator preference toward colored flowers.

White-flowered, male-sterile lines showed two-fold seed set compared to purple-flowered lines (Table 5), even when white-flowered lines accounted for only 29% out of the total number of male-sterile lines tested. Severson (1983) reported significant differences between purple and white soybean varieties for fructose and glucose content, nectar volume and total carbohydrate content per flower. White-flowered varieties had a more uniform carbohydrate content per flower throughout the day than did purple-flowered lines. Alfalfa-leaf-cutter-bee color preference has not been reported in soybean. In alfalfa, Goplen (1970) observed that leaf cutter bees preferred purple flowers to yellow flowers to a degree that influenced pod and seed set. One factor that could account for the variation observed among white- and purple-flowered lines is differences in the ultraviolet patterning. White and purple flowers of soybean lines were observed under UV light (265 nm range) but no visible pattern was observed that could serve as a guide for

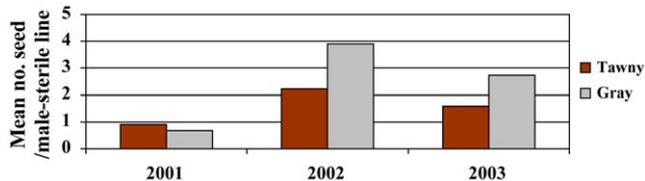


Fig. 4. Pubescence color effect on mean number of seed per male-sterile line for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001–2003 growing seasons.

pollinators (unpublished data, 2005). Whether those white-flowered lines have traits associated with nectar quality and/or volume, or volatile production, remain to be tested.

Flower color affected cross-pollination in our studies, but it is difficult to separate the effect of flower color with the genetic background of the near-isogenic lines. When the effect of flower color in seed set was compared in a common genetic background (T295H and its isogenic lines), higher seed set was observed in purple-flowered plants (Fig. 3). The effect of 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.

3.5. 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 5). One effect of trichome color is to regulate temperature. In soybean, pubescence color may influence the microclimate of the canopy and, consequently, yield. Morrison et al. (1994) evaluated yield, stability, and general adaptability in soybean lines differing in pubescence color. They reported that seed yield in tawny lines was higher in years or locations receiving < 2600 - CHU (Corn Heat Units) of accumulated temperature compared to gray lines. The effect of dark trichomes is related with improvement in heat absorption during the day. In our study, lines with gray pubescence had more seed set compared to lines with tawny pubescence. The advantage observed for gray lines could be related to the fact that the location and the years where the lines were tested did not present a cool-season climate where tawny lines possibly would perform better. Although gray lines performed better than tawny ones (Table 5), the role of pubescence color and its effect on seed set in male-sterile lines remains elusive. The influence of pubescence color on seed set could be indirect. It might affect leaf-surface reflectance, and modify the microclimate of the plants by altering the canopy heat load (Morrison et al., 1994), and not be directly related to pollination reward.

3.6. Cytoplasm source

The cytoplasm source effect was not significant among male-sterile lines. The effect of the interaction line ×

cytoplasm source was not significant, only the triple interaction; year × line × cytoplasm source was significant (Table 2). This could be as result of differential seed set observed among cytoplasm sources only in the year when the highest seed set was observed (2002).

4. Summary

This study evaluated the feasibility of hybrid soybean seed production on a group of soybean lines segregating for male sterility at the *ms6* locus. Since a large number of lines were tested, some factors involved directly in pollinator attraction and/or reward could not be evaluated. Preferential pollination was present among male-sterile lines observed through seed set, suggesting that selection among male-sterile, female-fertile lines can be made in order to obtain female parents suitable to produce hybrid soybean seed. The effect of flower color in seed set was significant. White-flowered lines had more seed set than purple-flowered lines, which was unexpected. Since the seed set observed was not suitable for commercialization, more research needs to be conducted on male-sterile lines to determine which traits are involved in insect cross-pollination. The important effect of year on seed set suggested that the influence of environmental conditions on seed set among male-sterile lines was of paramount importance to plant–pollinator interactions and needs to be assessed in order to establish an efficient hybrid soybean program.

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