Agronomic performance of soybean [*Glycine max* (L.) Merr.] **hybrids**

by

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CHAPTER 1. GENERAL INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most important crop species cultivated worldwide. It is a source of oil and protein and is used for livestock feed, human food, and for industrial purposes. The major producers are the United States, which accounts for 40% of world production, Brazil with 24%, Argentina with 19%, and China with 8%, (FAO Statistics, 2006). Research goals are related to increases in grain yield with desirable seed quality characteristics such as high protein and oil content. Soybean breeding programs have been focused on the development of inbred lines, which represents the totality of cultivars available in the market. However, increase in soybean yields may be possible through the development of hybrids (Palmer et al., 2001).

Hybrids in Agriculture

Many important field crops such as maize, sorghum, sunflower, and rice are commercially planted as hybrids, as well as many horticultural crops and floriculture. The importance of hybrids is due to the expression of hybrid vigor, known as heterosis. This usually refers to the increase in size, yield, rate of growth, etc. of F_1 plants over the performance of the parents (Duvick, 1999). Heterosis can be expressed when the parents of a hybrid have different alleles at a locus and there is some level of dominance among those alleles (Falconer and Mackay, 1996). The two classical explanations for heterosis are dominance and overdominance. According to Birchler et al. (2003) these explanations are usually framed in classical genetic terms and may be inadequate to address the underlying molecular events that result in heterosis. From a molecular point of view, regulatory interactions producing novel effects on target genes under hybrid conditions could be correlated with heterosis. However, the underlying basis of heterosis remains elusive (Birchler, et al., 2003).

Hybrid development is one of the most important breakthroughs in agriculture, mainly because of the increase of yield in open-pollinated species. Estimates of the global annual added yield in maize, sorghum, sunflower, and rice, are 10%, 19%, 30%, and 4%, respectively, compared to the average of open-pollinated varieties (Duvick, 1999).

In spite of the success of hybrid production in open-pollinated plants, in most self-pollinated species it is not widely used, probably due to complexities associated with floral structure. There are, however, some successful examples of commercial hybrid production in self-pollinated species. Hybrid rice in China is used in more than 50% of the area planted to rice. Rice hybrids have the potential of yielding 15-20% more than the best inbred variety. Cytoplasmic-genetic male-sterility systems have been widely used for developing hybrid rice (Virmani 1999). Also, hybrid wheat has shown consistent yield increases in many countries around the world (Jordaan et al., 1999). In wheat, the most common male-sterility system used is the chemical hybridizing agent (CHA), described as a compound that has the property of affecting male sterility (Cisar and Cooper, 2002). In the 1980s, the first hybrid pigeonpea cultivar was released for commercial plantings in India and had a yield of 25% to 30% over commercial checks (Saxena et al., 2005). In this crop, a cytoplasmic-nuclear male sterility system is used for hybrid seed production. This is the first example of hybrid production in a self-pollinated legume species. The success of hybrid production in the above self-pollinated species, and mainly in pigeonpea, is encouraging to the soybean community.

Hybrids in Soybean

According to Palmer et al. (2001) there are five components, which are crucial for the successful development of commercial hybrid soybean:

1. Parental combinations that produce heterosis levels superior to the best pure-line cultivars.
2. A stable male-sterile, female-fertile sterility system.
3. A selection system to obtain 100% female (pod parent) plants that set seed normally and can be harvested mechanically.
4. An efficient pollen transfer mechanism from pollen parent to pod parent.
5. An economical level of seed increase for the seedsman and growers that ultimately benefits the consumer.

Regarding the first requirement for hybrid seed soybean production, several studies have showed that heterosis levels, above the high parent, are possible. Palmer et al. (2001) summarized the results of fourteen experiments in heterosis reported since 1930 for a total of 456 different crosses. The average value of mid-parent heterosis (MPH) ranged from +14% to +46%, and average value for HPH ranged from +4% to +34%. Most of the studies, however, were done with spaced hybrid plants.

In other experiments, where more hybrid seed was available, yield tests were done in replicated plots. Average yield MPH percentages for 2, 27, and 7 hybrid combinations were +26%, +3%, and +4% respectively (Brim and Cockerham, 1961; Nelson and Bernard, 1984; Lewers, 1996). In 1997, Cerna et al. (1997) found HPH values of 16 F_1 crosses ranging from -17% to +97%. In the study of Manjarrez-Sandoval et al. (1997), HPH ranged from +0.8% to

+15% in 24 hybrid combinations. Sun et al. (1999) summarized data collected from a comprehensive heterosis test program in China, in which 846 combinations from a total of 1123 combinations showed positive MPH. A total of 248 combinations, out of the 846, showed a mean HPH of +20%. Traditional hand pollination was used for the production of F_1 hybrid seed in China. Heterosis evaluations were done at six institutes in China, with two replications of single-row plots.

In recent studies, Pandini et al. (2002) evaluated 30 F_1 hybrids in single rows, finding that MPH ranged from -6 to +132% and HPH from -44 to +72%. Burton and Brownie (2006) evaluated the F_1 generation of two combinations derived from crosses between current cultivars. The average yield of one cross was 16 % greater than that of the highest-yielding parent and the average yield of the other cross was 5 % greater than that of the highest-yielding parent. Ortiz-Perez et al. (2007) evaluated heterosis for yield and agronomic traits from single-crosses, three-way crosses, and backcrosses (BC_1F_1). MPH values for yield varied from -59% to +37% for single-crosses, -14% to +16% for 3-way crosses, and -7% to +42% for BC_1F_1 crosses. HPH varied from -66% to +17% for single-crosses, -25% to -5% for three- way crosses, and -16% to +42% for BC_1F_1 crosses.

Results of these studies suggest that significant yield increases are possible for some F_1 hybrid combinations, but the release of commercial hybrids remains a challenge. In China, however, the first hybrid soybean cultivar was released in 2002 (Palmer et al, 2003).

In order to meet the second requirement for soybean hybrid production, a stable male-sterile, female-fertile sterility system is necessary. Mutations affecting male cell and organ development have generated male-sterile, female-fertile lines that can be used as female parents for hybrid seed production (Palmer, 2000). All the nuclear male sterility mutations in soybean are stable, except the partial male-sterile (*msp*) mutant (Palmer et al., 2004), and possibly the *ms8* mutation (unpublished data). Cytoplasmic-genetic male sterile systems (CMS) are available in soybean in China (Sun et al., 2000), although not in the United States.

The third requirement, is a selection system to obtain 100% female plants for hybrid seed production fields, because female rows will be segregating for the nuclear *ms* mutations. Then both, male-sterile and male-fertile plants will be present in the commercial fields. Thus, a method to identify male-sterile, female-fertile plants is necessary in soybean for hybrid seed production. Any selection system employed with the soybean nuclear male-sterile genes, such as seed size differential (Carter et al., 1984), linkage between genes controlling the green cotyledon trait and the *Ms5* locus (Burton and Carter, 1983), the linkage between *W1* flower color locus, and the *Ms6* locus (Lewers et al., 1996, 1998a, 1998b; Lewers and Palmer, 1997), may be suitable for commercial hybrid seed production. Other option for a precise selection method of male-sterile female-fertile plants in commercial fields, was described by Stine and Eby (2002) by using the linkage of the nuclear Midwest Oilseed (MWO) male-sterile, female-fertile trait with a chemical resistance locus. All the successful commercial hybrid field crops use CMS with nuclear restoration. This may become the preferred method in soybean.

After a stable male-sterile system is identified, it is necessary to find the means to transfer pollen from the male to the female parent. In soybean, manual cross-pollination to produce large quantities of hybrid seed is difficult, time consuming, and expensive. The small size of the soybean flowers, the low success rate and the few seeds obtained per hybrid pod contribute to the difficulty of manually producing large quantities of hybrid seed (Fehr, 1991).

Insect cross-pollination of male-sterile soybean plants facilitates the production of hybrid seed (Lewers et al., 1996; Ortiz-Perez et al., 2007; Nelson and Bernard, 1984). Pollinator insects used in hybrid soybean seed production are commonly used for commercial pollination in other crops, i.e. honey bees, *Aphis mellifera*, and alfalfa leaf cutter bees, *Megachile rotundata* F. It is also possible, that some wild native bees could be more efficient pollinators in soybeans than the species mentioned above (Ortiz-Perez et al., 2007). In the studies to be reported here, the insects mainly used as pollinators for hybrid seed production in Chile were alfalfa leaf cutter bees. In Texas, another location that has been used to produce hybrid soybean seed, the pollinator insects were mainly native bees, primarily from families *Megachilidae*, *Halictidae*, *Anthophoridae*, and *Andrenidae* (Ortiz-Perez et al., 2007). Soybean programs that rely on insect pollination for hybrid seed production will have to monitor parent plants for insect preferences simultaneously with selection of the same parents for agronomic traits (Palmer et al., 2001).

In soybean, heterosis has been observed. In some cases, the better hybrids yielded between 10% to 20% more than the higher-yielding parent (Palmer et al., 2001). However, heterosis studies in soybean are not numerous because of the limitations in producing large quantities of hybrid seed. Many of the studies in hybrid soybean have been conducted in single rows with spaced plants, conditions that are different from commercial fields (Palmer et al, 2001). Results of the studies, therefore, can not be extrapolated to production in commercial fields due to the dissimilarity of conditions.

Duvick (1999) suggested the theory that gain in yield and performance, achieved with the improvement of hybrids in crops such as maize, sorghum, and sunflower, might have been similar for open-pollinated varieties, if more attention had been devoted to their improvement, starting with the then existing superior varieties. The opposite could be true for soybean. Most breeding efforts have been devoted to the improvement of inbred lines and little attention has been given to hybrid development. Thus, more research in areas such as pollinator-insect attraction, and selection of parental lines for general and specific combining ability are necessary, before hybrid soybeans could become a commercial reality.

In our study, all hybrid seeds were produced using genetic male-sterility genes and insect pollination with the objective to have enough seeds for replicated tests. Since the goal of hybrid breeding is to identify and then reliably reproduce superior hybrid genotypes (Duvick, 1999), the general objective of this study was to evaluate soybean hybrids from different parental combinations.

The thesis is organized in two chapters/studies, each with specific objectives. Objectives for the first study were: (1) to evaluate the agronomic performance of soybean single-cross, F_1 hybrids, and (2) to estimate the heterosis for yield, and other agronomic characteristics of single-cross F_1 hybrids at two locations in Iowa. The second study, had as objectives (1) the evaluation of yield in hybrid soybean populations developed by single-crosses, three-way crosses, four-way crosses, five-way crosses, and backcrosses (BC_1, BC_2 and BC_3), and (2) to estimate the heterosis for yield, and other agronomic characteristics for each of the hybrid populations.

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CHAPTER 2. EVALUATION OF SOYBEAN [*Glycine max* (L.) Merr.] F_1 HYBRIDS

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ABSTRACT

In soybean [*Glycine max* (L.) Merr.], manual cross-pollination is difficult and time consuming, and not conducive as an economical way to produce large quantities of hybrid seed. Male-sterility systems identified in soybean, combined with insect-mediated cross-pollination, have been shown to produce large quantities of hybrid seed that can be useful for agronomic performance studies. This procedure was used in this study to produce hybrid seed for the conduct of replicated yield trials. The objectives were: (1) to evaluate the agronomic performance of soybean F_1 hybrids, and (2) to estimate the heterosis for yield, and other agronomic characteristics of F_1 hybrids at two locations in Iowa. Parental genotypes were two male-sterile, female-fertile lines with the *ms3* and *ms9* mutations, and a group of six male-parent lines. Three of them were high-yielding public cultivars and the other three were high-yielding public breeding lines with greater than 50% 'exotic' germplasm. In 2005, twelve F_1 hybrid combinations were evaluated along with parent lines and high-yielding checks. Over locations, checks and male parent lines showed the highest yields. Mid-parent heterosis values (MPH) ranged from -29% to +32%, and high-parent heterosis (HPH) from -23% to +1%. In 2006, eleven F_1 hybrid combinations were evaluated for yield along with the parent lines and commercial checks. In 2006 and over locations, checks and male parents had the highest yields. MPH values ranged from -53% to -21%, and HPH from -66% to -35%.

Seed size and seed protein content showed HPH for some parent combinations. For traits related with vegetative growth, such as height and lodging, positive values of MPH and HPH were found.

INTRODUCTION

Heterosis, the measure of the average superiority of a hybrid over its parental inbred lines, is an important factor in the development of hybrid cultivars. Heterosis can be expressed when the parents of a hybrid have different alleles at a locus and there is some level of dominance among those alleles (Falconer and Mackay, 1996).

Heterosis, F_1 hybrid vigor, in soybean [*Glycine max* (L.) Merr.] does exist. Burton (1987) attributed heterosis in soybean to additive by additive epistatic interactions. In a more recent study, Burton and Brownie (2006) listed additional possible causes of heterosis in soybean, as to " include gene complementation or interaction of duplicate favorable loci in repulsion, linked dominant alleles that are inherited as a unit, a greater number of dominant alleles in the F_1 than either parent separately, multiple dosage-dependant regulatory loci, and/or overdominance".

Heterosis observed in soybean has indicated that in some cases, the better hybrids yielded 10% to 20% more than the higher-yielding parent (Palmer et al., 2001). However, heterosis studies in soybean are not numerous because of the limitation in producing large quantities of hybrid seed. Many of the studies of hybrid soybean have been conducted in single rows with spaced plants (Palmer et al, 2001). These results can not be extrapolated to production fields due to the dissimilarity of conditions.

According to Palmer et al. (2001) five components are crucial for the successful development of commercial hybrid soybean. The first is that parent combinations must produce heterosis levels superior to the best pure-line cultivars. The second is that a stable male-sterile, female-fertile sterility system is needed. The third is that a selection system to obtain 100% female (pod parent) plants that set seed normally and can be harvested mechanically is necessary. The fourth refers to the pollen transfer mechanism. This has to be efficient, to transfer pollen from the male to pod parent. The fifth is that an economical level of seed increase for the seedsman and growers has to be in place, which also ultimately benefits the consumer.

Palmer et al. (2001) summarized the results of fourteen heterosis experiments reported since 1930 for a total of 456 different crosses. The average value of mid-parent heterosis (MPH) ranged from +14% to +46%, and average value for HPH ranged from +4% to +34%. Most of the studies, however, were done with spaced hybrid plants.

In other experiments, where more hybrid seed was available, yield tests were done in replicated plots. Average yield MPH percentages for 2, 27, and 7 hybrid combinations were +26%, +3%, and +4% respectively (Brim and Cockerham, 1961; Nelson and Bernard, 1984; Lewers, 1996). Sun et al. (1999) summarized data collected from a comprehensive heterosis test program in China, in which 846 combinations from 1123 showed positive MPH. Two hundred forty eight combinations, out of the 846, showed a mean HPH of +20%. Traditional hand pollination was used for the production of F_1 hybrid seed, and the evaluation of heterosis was done at six research institutes in China, planted in one row plots with two replications.

Ortiz-Perez et al. (2007) evaluated heterosis for yield and agronomic traits from single-cross, three-way crosses, and backcrosses (BC_1F_1). MPH values for yield varied from -59% to +37% for single-crosses, -14% to +16% for 3-way crosses, and -7% to +42% for BC_1F_1. HPH varied from -66% to +17% for single-crosses, -25% to -5% for three- way crosses, and -16% to +42% for BC_1F_1 crosses. Burton and Brownie (2006) evaluated the F_1 generation of two combinations derived from crosses between current soybean cultivars. The average yield of one cross was 16 % greater than that of the highest-yielding parent and the average yield of the other cross was 5 % greater than that of the highest-yielding parent. Results of these studies suggest that significant yield increases are possible for some combinations, but the release of commercial hybrids remains a challenge. In China, however, the first hybrid soybean cultivar was released in 2002 (Palmer et al, 2003).

In the United States, hybrid soybean could increase yield by making use of heterosis in different genetic combinations, in which case it would contribute to growers' profits. Several aspects of the hybrid seed production scheme, however, need to be evaluated before hybrids can become a commercial reality. One is to find a feasible system of F_1 hybrid seed production. For the study to be reported here, an efficient hybrid seed production system was used. During the course of their work, Ortiz et al., (2007) conducted selection within female parent genotypes for insect attractiveness, using as criteria an increase in the number of out-crossed pods. The authors observed important changes in seed production based on this criterion. For the study reported herein, only female plants derived from the most productive individuals were used to produce the hybrid seed. Another important difference between the two studies is that for the study reported here, the male parents used were current high-yielding public cultivars, as opposed to the old public cultivars used by Ortiz et al. (2007).

The objectives of the study were therefore; (1) to evaluate agronomic performance of soybean F_1 hybrids, and (2) to estimate heterosis for yield, and other agronomic characteristics of the F_1 hybrids at two locations in Iowa.

MATERIALS AND METHODS

Twenty-two soybean genotypes were evaluated. Twelve F_1 hybrids were developed by crossing two nuclear male-sterile lines as female parents to six male parents (Tables 1 and 2). For each female parent, the homozygous dominant male-fertile, female-fertile sibling was planted in the same field with the hybrids. The six male parents and two checks also were evaluated.

Plant materials

Female parents had excellent insect pollinator attraction and good agronomic characteristics. Lines segregating for nuclear male-sterile mutations were used as female parents; *ms*3*ms3* (T284H) (Chaudhari and Davis, 1977), and *ms9ms9* (T359H) (Palmer, 2000). The six male parent lines were three high yielding public cultivars, K1547 (Kansas State University, Manhattan, KS), IA2052 (Iowa State University, Ames, IA), IA2050 (Iowa State University, Ames, IA), and three high yielding public breeding lines with greater than 50% ‘exotic’ germplasm, LG00-6182, LG00-6193, and LG01-4756 from the USDA-ARS at Urbana, IL. Breeding lines LG00-6182 and LG00-6193 were selections from the same two-parent mating. Male parents were chosen because of high yield, acceptable plant height, and maturity adapted to Iowa. Twelve F_1 hybrid combinations were developed by insect-mediated cross-pollination between two female lines with six male lines. Three cultivars currently used in commercial plantings were used as checks, DSR Exp.202b developed by Dairyland Seed Co., Inc., Otterbein, IN, and two public cultivars, Apex (Cooper et al., 2003), and IA2068 (Iowa State University, Ames, IA), (Table 2).

F_1 seed production

Hybrid seed was produced in a full-season nursery in Chile, South America. Each plot for hybrid seed production had six rows. Rows one and six were planted with the male parents; rows two to five were planted with the segregating male-sterile line. Each row was 4.8 m long, spaced 76 cm between rows and 1.2 m among plots, planted with 14 seeds per m. Each plot was replicated three times for each of the 12 hybrid combinations in a randomized complete block design, where the two male-sterile, female-fertile lines (*ms3ms3* and *ms9ms9*) were crossed with each of the six selected males.

At flowering, male-sterile plants were identified and labeled; fertile siblings were removed as a pollen source. This procedure was done in the middle rows (2-5) where the segregating male-sterile lines were planted for each hybrid combination. Insect vectors transferred pollen from the male-parent rows to the male-sterile, female-fertile plants. Alfalfa leaf cutter bees (*Megachile rotundata* F.) were used as the insect pollinator species. Each plot was bulk harvested and hybrid seed for each combination was sent to Iowa for planting in summers 2005 and 2006 (Tables 1 and 2).

Field testing

Four-row plots of F_1 hybrids, parents, and checks were planted in replicated plots. Each row was 5.2 m long with a spacing of 0.76 m between rows. Two locations near Ames, and one location near Gilbert, IA were used for the experiment. In 2005, checks and parental lines were evaluated at all locations; hybrid combinations also were evaluated but not at all locations (Table 2). In 2006, checks and parental lines, except the homozygous dominant male-fertile, female-fertile *Ms9Ms9* were evaluated at two locations near Ames, IA. In 2006, the hybrid combination *ms9* x IA2052 was not evaluated due to lack of seed, the other hybrid combinations were evaluated but not at all locations. A randomized complete-block design with two replications was used at each location and year. However, since all hybrids were not evaluated at all locations, the design was unbalanced.

Traits evaluated were seed yield, seed protein content, seed oil content, lodging, plant height, maturity date, and seed size. Maturity date was recorded as the number of days from planting until 95% of the pods in the two middle rows were brown. Plant height and lodging were recorded at harvest. Plant height was measured in centimeters for two plants from each of the two middle rows. Lodging was a visual observation of the whole plot; it was recorded on a 1 to 5 scale with 1 being all plants upright, 3 being at 45^o, and 5 being all plants prostrate. The two middle rows were harvested at maturity and the seed was used to determine yield, seed size and seed composition. Seed samples of 25 g were sent to the USDA National Center for Agricultural Utilization Research (NCAUR), Peoria, IL to determine seed protein content and seed oil content using near infrared transmittance.

Statistical analysis

For all genotypes, a mixed model analysis of variance was performed separately for agronomic variables for each year. Mixed models are an important approach for modeling which take into account more complicated data structures in a flexible way (Brown and

Prescott, 2004). Because of the unbalanced design, the data were analyzed with PROC MIXED of SAS v. 9.1 (SAS Institute, 2003). In the model, genotype was a fixed effect and location, genotype by location, and replications within location were considered random effects. Least Square Means (LSMEANS) were used to estimate the performance of hybrids, parents, and checks for each year. Least Square Difference (LSD) test to separate the means was performed. When the means all have the same standard error, it is relatively easy to rank the means. However, in this study the experimental design structure was unbalanced, creating unequal standard errors for each pairwise comparison. The LSD estimation was done using the highest standard errors of the pairwise mean comparisons in order to perform a more conservative test.

The performance of hybrids relative to their parents can be measured as mid-parent heterosis, and high-parent heterosis (Fehr, 1991). Mid-parent heterosis was determined as:

$$\text{Mid-parent heterosis (\%)} = \frac{F_1 - MP}{MP} \times 100$$

Where F_1 = performance of the hybrid and MP= average performance of the parents. The ESTIMATE statement of SAS v. 9.1 (SAS Institute, 2003) was used to determine the difference between the mean of each hybrid with the average of its parents.

High-parent heterosis was determined as:

$$\text{High-parent heterosis (\%)} = \frac{F_1 - HP}{HP} \times 100$$

Where F_1 = performance of hybrid and HP = performance of best parent.

The LSMEANS statement with a PDIFF option, which requests that differences of the LS-means be displayed, was used to estimate the difference between the hybrid and the parent with the best performance for a specific trait. Multiple comparison tests were done with the Bonferroni method. A BON option of SAS v. 9.1 (SAS Institute, 2003) was used to estimate adjusted p-values for multiple comparisons.

RESULTS

For all traits, the combined analysis of variance for the single-cross populations, parents, and checks, indicated no significant differences among genotypes, except for seed size (Appendix I, Table 1). Significant differences between years were detected only for seed oil content and a significant interaction for genotype and year was observed for all traits.

In 2005, the analysis of variance for the single-cross populations, parents, and checks, indicated significant differences among genotypes for all traits (Appendix I, Table 2). There was a significant interaction between genotypes and locations for all traits, except seed protein and oil contents. This indicates protein and oil contents across genotypes were similar at both locations in 2005. In 2006, the analysis of variance for the single-cross populations, parents, and checks, indicated significant differences among genotypes (Appendix I, Table 3). A significant interaction between genotypes and locations was observed only for seed protein content. As in the combined analysis of variance, all significant differences were at the $P<0.05$ level.

Orthogonal contrasts were performed to detect differences among and between groups of genotypes every year (Appendix I, Tables 4 and 5). In general, and considering both years and all traits, the contrast within lines (parents and checks), and between hybrids *vs* lines showed significant ($P \leq 0.05$) differences. For yield and seed size in both years, and also for seed oil content in 2006, the group of hybrids with *ms3ms3* as female parent, herein referred as *ms3* group, was significantly different from the group of hybrids with *ms9ms9* as female parent, herein referred as *ms9* group. Significant differences within the group of hybrids were observed for seed size in both years and for yield only in 2006 (Appendix I, Table 4).

Agronomic performance of parent lines, checks, and hybrid

Male parents had the best yield performance in both years (Table 3). In 2005, average yield of checks was 2985 kg/ha, and 3096 kg/ha in 2006. The highest yielding check was IA2068 in both years, 3576 kg/ha in 2005, and 3999 kg/ha in 2006. Average yield of male parents was 2797 kg/ha in 2005, and 3297 kg/ha in 2006. In 2005, the highest yielding male parent was IA2050, and LG01-4756 was the highest yielding male parent in 2006. Average yield of female parents was 1897 kg/ha in 2005, and 1696 kg/ha in 2006. Among female parents, the homozygous dominant male-fertile, female-fertile *Ms9Ms9,* was the highest yielding in both years.

The average yield of hybrids of the *ms3* group was 2418 kg/ha in 2005 and 1262 kg/ha in 2006 (Table 3). In this group, the highest yielding hybrid was *ms3* x K1547 in 2005, and *ms3* x IA2050 in 2006. In the *ms9* group, the average yield was 1920 kg/ha in 2005, and 1903 kg/ha in 2006. The highest yielding hybrid was *ms9* x IA2052 in 2005 and *ms9* x LG01-4756 in 2006. The best yielding hybrids involved crosses with the highest yielding male parents IA2050 and LG01-4756, which suggests additive gene action for yield expression.

Hybrids of the *ms9* group showed average seed size larger than that of parents and checks for both years (Table 3). Female parents and hybrids had the highest content of seed protein, and oil content that was lower than checks and male parents. Hybrids with high seed protein content had low yields, and the correlation values were negative (Appendix I, Tables 6 and 7).

In both years, average maturity of female parents and hybrids was later than for male parents and checks (Table 4). In general, and on average, hybrids were taller than their parents, particularly in 2006. Lodging was similar among parents and hybrids in 2005, although hybrids had higher lodging scores than their parents in 2006 (Table 4).

Heterosis in hybrids

Hybrids of the *ms3* group had positive mid-parent heterosis (MPH) for yield in 2005, with the exception of just one combination (Table 5). In 2005, average MPH value of the *ms3* group was +13%. In this group, the hybrids with the highest values were *ms3* x IA2052 and *ms3* x K1547, both showing +32% MPH. Also in 2005, average MPH for hybrids of the *ms9* group was -24%. In 2006, yield MPH with both female parents was negative and ranged from -53% to -21%. The trend in high positive values of MPH for hybrids of the *ms3* group observed in 2005, and the negative values observed in 2006 may be explained in part by the poor performance of the female parent lines in 2006.

In 2005, the two hybrids with the highest positive MPH for yield also showed positive HPH, although the estimates were not significantly different from zero (Table 5). In general, all HPH values were negative or not different from zero, irrespective of the female parent used in the hybrid combination. The average HPH value for hybrids of the *ms3* group was -13%, and -31% for hybrids of the *ms9* group. Similar results were observed in 2006, average HPH for hybrids of the *ms3* group was -62%, and -42% for hybrids of the *ms9* group. For seed size and in 2005, mostly positive MPH and some positive HPH values were observed (Table 5). In 2006, no consistent trend in both heterosis estimates was observed.

In general, and considering both years, MPH ranged from -4% to +23%, and HPH from -15% to +21%. Similar variable results were observed for seed protein content heterosis estimates (Table 6). For this trait in 2005, hybrids of the *ms9* group had an average MPH of +3% and HPH of +1%. In 2006, hybrids of the *ms3* group had an average MPH of +4% and HPH of +1% in 2006. For seed oil content, the majority of the hybrids had negative MPH and HPH estimates.

In this study, hybrids which showed negative MPH and HPH values for yield, also had negative values for oil content. In both years, yield and oil content had a positive association, ($r=0.78$ in 2005, and $r=0.84$ in 2006). This correlation coefficients were significantly different from zero at the $P<0.05$ level.

For maturity, lodging, and plant height, hybrids showed varying heterosis values depending on the year (Table 7). In some cases the values were higher than the corresponding traits of their parents, reflected in positive estimates of MPH and HPH. In others, values were negative. For maturity, considering both years and both female parents, MPH ranged from -6% to +6%, and HPH from -7% to +4%. For lodging, MPH ranged from -24% to +101%, and HPH from -29% to +58%. For plant height, MPH ranged from -8% to +28%, and HPH from -14% to +23%. In both years, lodging and height had a positive association, ($r=0.73$ in 2005, and $r=0.62$ in 2006), significantly different from zero at the $P<0.05$ level.

DISCUSSION

The study was conducted to evaluate F_1 hybrid performance and to estimate MPH and HPH for seed yield and agronomic traits. Results indicated that depending on the year and parent combinations, there were some hybrids that performed better than the mid-parent value, although none of the hybrids were better than the best parent of the cross. Estimates of MPH and HPH were in general variable across years, locations, and parent combinations.

Two female lines with different male-sterile alleles were used as parents, which allowed evaluation of performance of male- sterile sources in hybrid combinations. A result of the comparison was the finding of significant differences for yield between hybrids of the *ms3* group and the *ms9* group, although it is important to indicate that the male-sterile alleles used in the study were introgressed into different genetic backgrounds. Since soybean does have numerous different alleles for male sterility (Palmer et al., 2004), it would be important for future studies to include several of the alleles, introgressed into the same genetic background. In the study reported herein, two interpretations are possible, i) an interaction between male-sterile alleles with their respective genetic background, or ii) differences in the combining ability between the two male-sterile alleles.

In general, cultivars, checks, and male parent lines, had yield performances superior to hybrids. A possible explanation for these results is that the homozygous dominant male-fertile, female-fertile siblings of each *ms* mutant had poor yield performance. The male-sterile alleles were introgressed into old genetic lines, in which yield was not considered as a prime factor for selection. The low yielding background of the female lines may have had a negative effect in F_1 hybrid performance, and on heterosis. It is possible, that if the male-sterile genes used in the study would have been introgressed into modern higher yielding genetic backgrounds, hybrids may have had better performance and consequently positive values for heterosis. As previously stated by Burton and Brownie (2006) “heterosis should be predictive of good parental combinations”.

Variability in hybrid performance and of estimates for MPH and HPH observed in the study are similar to previous reports. Values of HPH and MPH heterosis for yield differ among different studies. Heterosis values for yield in studies done in spaced plants were reported by Weber et al. (1970), who evaluated 85 crosses, with an average HPH of +13%, and an average MPH of +25%. Chaundhary and Singh (1974) observed average HPH for seed yield of +26%. Heterosis values for yield in studies done under normal plant density, have been reported by several authors. Cerna et al. (1997) found that HPH values of 16 F_1 crosses ranged from -17% to +97%, and MPH ranged from +7% to +102%. In the study of Manjarrez-Sandoval et al. (1997), HPH ranged from +1% to +15% in 24 hybrid combinations; average MPH was 7%. In the study of Nelson and Bernard (1984), HPH from 5 of 27 hybrid combinations ranged from +13% to +19%, and average MPH was +8% . In 1996, Lewers et al. (1996) found the average HPH for 36 test crosses was +7%. In more recent studies, Pandini et al. (2002) evaluated 30 F_1 hybrids, which had HPH ranging from -44 to +72%, and MPH from -6 to +132%. In 2006, Burton and Brownie (2006) reported average HPH of +16% and +5% in two crosses. In the study of Ortiz-Perez et al. (2007), HPH in single crosses varied from -41% to +11%, and MPH varied from -34% to +15%.

Significant positive MPH and HPH values for height, lodging, seed size, and seed protein content were found in several crosses. The observations for plant height and lodging indicate that heterosis was present mainly in traits associated with vegetative growth. These findings are in agreement with published information (Nelson and Bernard, 1984), and according to Lewers et al. (1998) it is possible that vegetative heterosis may increase early lodging and pod abortion, in turn reducing grain yield.

An increase in seed size is commonly observed on F_1 plants normally grown in soybean crossing blocks (Cianzio and Palmer, personal communication). We found the same pattern for heterosis for this trait. The positive heterosis observed for seed protein content may be the result of the inverse correlations widely reported in the literature between seed yield and seed protein content and seed protein and seed oil content. Negative correlations between seed protein and seed oil, and seed protein and yield also were detected in the study reported here, and by Burton (1987). This, however, is contrary to results reported by Cober and Voldeng (2000), who did not find pleiotropic effect of low seed yield and high seed protein content in the two populations they evaluated. Different genotypes and maturity groups were used in the two studies, and this could be a possible explanation for the diversity in the observations reported.

In soybean, most gene action reported for economically important traits is additive, and heritability estimates are low (Brim, and Cockerham, 1961; Burton, 1987). In the study reported here, deviations from the mid-parent value were found for yield, seed size, seed protein content, seed oil content, maturity, height, and lodging, which may indicate epistatic effects, as suggested by Thorne and Fehr (1970). In soybean, heterosis effects are not yet well understood and several explanations have been proposed by Burton and Brownie (2006); such as "i) gene complementation or interaction of duplicate favorable loci in repulsion, ii) linked dominant alleles inherited as a unit, iii) a greater number of dominant alleles in the F_1 than in either parent separately, iv) multiple dosage-dependant regulatory loci, v) and/or overdominance". If heterosis in soybean is governed by any of the mentioned phenomenon, it is necessary that complementarity exists between both parent lines to obtain yield heterosis in the hybrid, or in other words, that both parents need to possess dominant genes at different loci controlling seed yield (Pandini et al., 2002).

In the study reported here, limited numbers of combinations were evaluated and results can not be extrapolated to other crosses, however, an insight on the complexities and difficulties about the evaluation of heterosis, and the possibility of establishing hybrid soybean as a commercial entity has been gained. Understanding heterosis in soybean and identification of general and specific combining effects among parents will be necessary and will require extensive studies, conducted with different sources of male sterility in common high-yielding and different genetic backgrounds, conducted over a wide range of environments. The hybrid seed to conduct these experiments can be obtained using insect-mediated pollinations which has proven to be an efficient method to produce large quantities of hybrid seed. Once this information is collected, it will also be necessary to devise predictive systems to identify genotypes with good combining ability. Results from the study reported here, previous experiments and those proposed will contribute to determine the economics and the feasibility of commercial hybrid soybean as a means to increase seed yield.

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TABLE 1. Time table to produce the F_1 hybrid soybean seeds used to conduct yield and agronomic performance trials.

Location	Year	Procedure
Massai, Chile	Nov. 2004 –April 2005	F_1 Hybrid seed production
Ames and Gilbert, IA	May. 2005 – Oct. 2005	Yield trials
Massai, Chile	Nov. 2005 –April 2006	F_1 Hybrid seed production
Ames, and Gilbert, IA	May. 2006 – Oct. 2006	Yield trials

TABLE 2. Soybean hybrids, parents and checks evaluated in 2005 and 2006 at two locations near Ames, IA (Loc 1 and Loc 2), and one location near Gilbert, IA (Loc 3).

Lines	**2005**			**2006**	
	Loc 1	**Loc 2**	**Loc 3**	**Loc 1**	**Loc 2**
Female parents[a]					
Ms3Ms3	X	X	X	X	X
Ms9Ms9	X	X	X	X	
Male parents					
IA2050	X	X	X	X	X
IA2052	X	X	X	X	X
K1547	X	X	X	X	X
LG00-6182	X	X	X	X	X
LG00-6193	X	X	X	X	X
LG01-4756	X	X	X	X	X
Hybrids					
ms3 x IA2050	X	X		X	X
ms3 x IA2052	X			X	X
ms3 x K1547	X			X	
ms3 x LG00-6182	X	X		X	
*m*s3 x LG00-6193	X	X		X	X
ms3 x LG01-4756	X			X	X
*m*s9 x IA2050	X	X	X	X	
*m*s9 x IA2052	X	X	X		
ms9 x K1547	X	X	X	X	
ms9 x LG00-6182	X	X		X	
ms9 x LG00-6193	X	X		X	
ms9 x LG01-4756	X	X		X	
Checks					
IA2068	X	X	X	X	X
Apex	X	X	X	X	X
DSR Exp.202b	X	X	X	X	X

[a] Fertile sibling of male-sterile, female-fertile parents.

TABLE 3. Yield, seed size, seed protein, and seed oil content of F_1 hybrids, parents and checks in 2005 and 2006, in replicated tests averaged over locations.[a]

Classification	Yield (Kg ha^{-1})		Seed size (mg seed^{-1})		Protein (g Kg^{-1})		Oil (g Kg^{-1})	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**
Female parents[b]								
Ms3Ms3	1504	1459	133	124	428	432	183	163
Ms9Ms9	2290	1932	154	159	420	440	187	164
Mean	1897	1696	143	141	424	436	185	164
Male parents								
IA2050	3142	3149	152	156	405	410	199	191
IA2052	2805	3447	141	143	408	424	201	188
K1547	2813	3391	148	149	394	396	206	183
LG00-6182	2399	3073	144	147	407	398	192	182
LG00-6193	2913	3105	166	168	412	409	190	180
LG01-4756	2710	3616	162	166	410	406	199	174
Mean	2797	3297	152	155	406	407	198	183
Hybrids								
ms3 x IA2050	2433	1410	149	135	409	428	191	165
ms3 x IA2052	2840	1155	146	139	408	438	195	158
ms3 x K1547	2849	1137	144	138	405	435	194	160
ms3 x LG00-6182	2139	1279	146	149	428	436	185	161
ms3 x LG00-6193	1662	1223	143	142	429	440	184	157
ms3 x LG01-4756	2587	1370	143	140	418	434	187	159
Mean	2418	1262	145	141	416	435	189	160
ms9 x IA2050	1948	1840	171	183	425	435	185	166
ms9 x IA2052	2049	ND[c]	165	ND	425	ND	186	ND
ms9 x K1547	2027	1636	185	165	426	435	187	167
ms9 x LG00-6182	1817	1985	163	172	418	418	190	177
ms9 x LG00-6193	1904	1932	171	170	422	414	189	181
ms9 x LG01-4756	1775	2120	162	172	425	431	190	166
Mean	1920	1903	169	172	423	427	188	172
Checks								
Apex	2799	2565	143	132	390	372	206	193
DSR Exp.202b	2580	2724	145	140	410	415	198	190
IA2068	3576	3999	131	132	377	389	204	193
Mean	2985	3096	139	135	392	392	202	192
LSD[d]	**805**	**785**	**29**	**17**	**29**	**37**	**14**	**21**

[a] Two replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

[b] Fertile siblings of male-sterile, female-fertile parents.

[c] ND = No data.

[d] LSD estimation was done using the highest standard errors of the pairwise mean comparisons.

TABLE 4. Height, lodging, and maturity of F_1 hybrids, parents and checks in 2005 and 2006, in replicated tests averaged over locations.[a]

Classification	Height (cm)		Lodging[b] (score)		Maturity[c] (days)	
	2005	2006	2005	2006	2005	2006
Female parents[d]						
Ms3Ms3	97	111	3	3	138	146
Ms9Ms9	91	88	2	3	133	142
Mean	94	100	2	3	136	144
Male parents						
IA2050	76	81	2	2	129	139
IA2052	88	99	2	2	132	138
K1547	78	89	1	1	136	144
LG00-6182	102	116	2	2	133	146
LG00-6193	105	120	2	3	134	143
LG01-4756	92	98	2	2	138	144
Mean	90	101	2	2	134	142
Hybrids						
ms3 x IA2050	98	110	2	3	135	148
ms3 x IA2052	101	112	2	3	129	150
ms3 x K1547	103	108	3	3	130	136
ms3 x LG00-6182	98	115	2	3	139	149
ms3 x LG00-6193	98	106	2	4	135	149
ms3 x LG01-4756	103	114	2	4	134	149
Mean	100	111	2	3	134	146
ms9 x IA2050	96	109	2	4	133	148
ms9 x IA2052	94	ND[e]	2	ND	133	ND
ms9 x K1547	89	104	2	3	133	147
ms9 x LG00-6182	101	111	2	3	135	148
ms9 x LG00-6193	92	103	2	2	134	148
ms9 x LG01-4756	93	107	2	3	135	148
Mean	94	107	2	3	134	148
Checks						
Apex	48	48	1	1	136	141
DSR Exp.202b	69	72	1	2	129	138
IA2068	78	89	2	4	131	135
Mean	65	70	1	2	132	138
LSD	**13**	**24**	**1.4**	**1.6**	**8**	**9**

[a] Two replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

[b] Lodging score: 1 = upright, 5 = prostrate.

[c] Maturity = days from planting to stage R8

[d] Fertile siblings of male-sterile, female-fertile parents.

[e] ND = No data.

TABLE 5. Mid-parent heterosis (MPH), high-parent heterosis (HPH), and average heterosis for each *ms* hybrid group for yield and seed size in 2005 and 2006, in replicated tests averaged over locations.[a]

Hybrids	Yield				Seed size			
	2005		2006		2005		2006	
	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
ms3 x IA2050	+5	-23	-39*	-55*	+5	-2	-3	-13*
ms3 x IA2052	+32	+1	-53*	-66*	+7	+4	+4	-3
ms3 x K1547	+32	+1	-53*	-66*	+2	-3	+1	-7
ms3 x LG00-6182	+10	-11	-44*	-58*	+5	+2	+10*	+2
ms3 x LG00-6193	-25	-43	-46*	-61*	-4	-14	-3	-15*
ms3 x LG01-4756	+23	-5	-40*	-62*	-3	-12	-4	-15*
Mean	+13	-13	-46	-62	+2	-4	+1	-9
ms9 x IA2050	-28*	-38	-28*	-42	+12*	+11	+16*	+15
ms9 x IA2052	-20	-27	ND[b]	ND	+12*	+8	ND	ND
ms9 x K1547	-21*	-28	-39*	-52*	+23*	+21*	+7	+4
ms9 x LG00-6182	-22	-24	-21	-35	+9	+6	+13*	+8
ms9 x LG00-6193	-27*	-35	-23	-38	+7	+3	+4	+1
ms9 x LG01-4756	-29*	-35	-24*	-41	+2	0	+6	+4
Mean	-24	-31	-27	-42	+11	+8	+9	+6

*P-value ≤ 0.05

[a] Two replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

[b] ND = No data.

TABLE 6. Mid-parent heterosis (MPH), high-parent heterosis (HPH), and average heterosis for each *ms* hybrid group for seed protein and seed oil content in 2005 and 2006, in replicated tests averaged over locations.[a]

Hybrids	Seed protein				Seed oil			
	2005		2006		2005		2006	
	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
ms3 x IA2050	-2	-5	+2	-1	0	-4	-7*	-14
ms3 x IA2052	-2	-5	+2	+1	+1	-3	-10*	-16*
ms3 x K1547	-1	-5	+5*	0	0	-6	-8*	-13
ms3 x LG00-6182	+2	0	+5*	+1	-1	-4	-6*	-12
ms3 x LG00-6193	+2	0	+4	+2	-2	-3	-9*	-13
ms3 x LG01-4756	0	-2	+3	0	-2	-6	-7*	-8
Mean	0	-3	+4	+1	-1	-4	-8	-13
ms9 x IA2050	+3	+1	+2	-1	-4*	-7	-6*	-13
ms9 x IA2052	+3	+1	ND[b]	ND	-4*	-8	ND	ND
ms9 x K1547	+5*	+1	+4	-1	-5*	-9	-4	-9
ms9 x LG00-6182	+1	0	0	-5	0	-1	+2	-3
ms9 x LG00-6193	+2	+1	-2	-6	0	0	+5	0
ms9 x LG01-4756	+2	+1	+2	-2	-2	-5	-2	-4
Mean	+3	+1	+1	-3	-2	-5	-1	-6

*P-value ≤ 0.05

[a] Two replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

[b] ND = No data.

TABLE 7. Mid-parent heterosis (MPH), high-parent heterosis (HPH), and average heterosis for each *ms* hybrid group for maturity, lodging, and height in 2005 and 2006, in replicated tests averaged over locations.[a]

Hybrids	Maturity				Lodging				Height			
	2005		2006		2005		2006		2005		2006	
	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
ms3 x IA2050	+1	-3	+4*	+1	-16	-35	+24*	-9	+14*	+1	+14*	-1
ms3 x IA2052	-5	-7	+5*	+3	-18	-27	+30*	0	+9	+4	+6	0
ms3 x K1547	-5*	-6	-6*	-7	+51*	+9	+47*	-4	+17*	+6	+8	-3
ms3 x LG00-6182	+2	0	+2	+2	0	-17	+11*	-6	-1	-4	+1	-1
ms3 x LG00-6193	-1	-2	+3*	+2	-3	-17	+20*	+15	-3	-7	-8	-11
ms3 x LG01-4756	-3	-3	+3*	+2	-5	-18	+12*	+8	+9	+6	-2	+2
Mean	-2	-3	+2	0	+1	-17	+24	+1	+7	+1	+3	-2
ms9 x IA2050	+2	0	+6*	+4	+44*	+34	+101*	+58	+15*	+5	+28	+23
ms9 x IA2052	+1	0	ND[y]	ND	-6	-15	ND	ND	+5	+3	ND	ND
ms9 x K1547	-1	-2	+3	+2	+28	+10	+73*	+20	+5	-2	+17	+17
ms9 x LG00-6182	+2	+1	+3	+1	+21	+18	+28	+19	+5	-1	+8	-5
ms9 x LG00-6193	0	0	+4	+4	-4	-10	-24	-29	-6	-13	-1	-14
ms9 x LG01-4756	-1	-2	+3	+2	+16	+8	+35	+19	+2	+1	+15	+9
Mean	0	0	+4	+3	+16	+7	+43	+17	+4	-1	+14	+6

*P-value ≤ 0.05

[a] Two replications at two locations near Ames, and one location near Gilbert, IA in 2005; two replications near Ames, IA in 2006.

[b] ND = No data.

APPENDIX I

TABLE 1. Combined analysis of variance for yield, seed size, seed protein, and seed oil content, maturity, height, and lodging for soybean checks, parents and F_1 hybrids in 2005 and 2006 at Ames, and Gilbert, IA.

Source of variation	Df	Mean squares						
		Yield	Seed size	Protein	Oil	Maturity	Lodging	Height
Genotype	22	2481259	10.88*	10.16	5.09	69	2.089	1297
Year	1	11965484	0.51	65.49	139.12*	3325	8.679	1687
Year*genotype	21	1754844*	2.89*	9.91*	2.74*	38*	2.024*	638*
Replication (location)	3	538137*	0.65	2.44	0.40	42	0.464	958
Location(year)	1	1654054*	4.94	12.06*	1.97	204	1.262*	79
Residual	142	176422	1.18	1.98	0.51	11	0.303	43

* P-value ≤ 0.05

TABLE 2. Analysis of variance for yield, seed size, seed protein, and seed oil content, maturity, height, and lodging for soybean checks, parents and F_1 hybrids in 2005 at Ames, and Gilbert, IA.

Source of variation	df	Mean squares						
		Yield	Seed size	Protein	Oil	Maturity	Lodging	Height
Genotype	22	1461319*	10.14*	10.27*	2.97*	37.67*	1.10*	1058.24*
Location	2	19266959*	1.78	74.99*	10.58*	695.82*	5.06	272.72
Location*genotype	32	276644*	2.14*	2.10	0.51	17.93*	0.47*	43.06*
Replication (location)	3	744948*	0.65	2.10	0.42	32.02*	1.01*	99.76*
Residual	62	166425	1.32	2.40	0.53	5.79	0.16	25.69

* P-value ≤ 0.05

TABLE 3. Analysis of variance for yield, seed size, seed protein, and seed oil content, maturity, height, and lodging for soybean checks, parents and F_1 hybrids in 2006 at Ames, IA.

Source of variation	df	Mean squares						
		Yield	Seed size	Protein	Oil	Maturity	Lodging	Height
Genotype	21	3042434*	6.388*	12.255*	5.703*	69.272*	2.931*	1070.837*
Location	1	1196557*	1.976	24.761*	3.186	158.334	1.288	349.012
Location*genotype	14	133301	0.381	2.424*	0.724	8.977	0.243	95.863
Replication (location)	2	13438	0.113	1.734	0.317	22.660	0.346	14.084
Residual	32	104783	0.359	0.922	0.387	15.292	0.385	56.140

* P-value ≤ 0.05

TABLE 4. Contrasts within and between groups of genotypes for yield, seed size, seed protein, and seed oil content, maturity, height, and lodging for soybean checks, parents and hybrids in 2005 at Ames, and Gilbert, IA.

Source of variation	df[a]	df[b]	P-value of the difference						
			Yield	Seed size	Protein	Oil	Maturity	Lodging	Height
Within hybrids	11	32	0.14654	0.00108	0.48486	0.69074	0.49378	0.68837	0.12738
Between *ms3* and *ms9* hybrids	6	32	0.00510	0.00001	0.12570	0.46740	0.75440	0.26880	0.00690
Within lines (parents and checks)	10	32	0.00003	0.00310	0.00001	0.00000	0.00261	0.00272	0.00000
Between hybrids and lines	1	32	0.00004	0.00095	0.00005	0.00000	0.81135	0.00873	0.00000

[a] df of the genotypes involved in the contrast
[b] df of the residual

TABLE 5. Contrasts among and between groups of genotypes for yield, seed size, seed protein, and seed oil content, maturity, height, and lodging for soybean checks, parents and F_1 hybrids in 2006 at Ames, IA.

Source of variation	df[a]	df[b]	P-value of the difference						
			Yield	Seed size	Protein	Oil	Maturity	Lodging	Height
Within hybrids	10	14	0.03432	0.00000	0.82792	0.19604	0.01957	0.24702	0.92647
Between *ms3* and *ms9* hybrids	1	14	0.00015	0.00000	0.24731	0.00762	0.42412	0.58135	0.29963
Within lines (parents and checks)	10	14	0.00001	0.00000	0.00221	0.00194	0.00199	0.00000	0.00000
Between hybrids and lines	1	14	0.00000	0.00016	0.00006	0.00000	0.00002	0.00001	0.00001

[a] df of the genotypes involved in the contrast
[b] df of the residual

TABLE 6. Correlation matrix for yield, seed size, seed protein and seed oil contents, maturity, height in 2005 at Ames, and Gilbert, IA.

	Yield	Seed size	Protein	Oil	Maturity	Height	Lodging
Yield	1.00	-0.36	-0.86**	0.78**	-0.52	-0.36	-0.31*
Seed size		1.00	0.47**	-0.35*	0.09	0.19*	-0.08
Protein			1.00	-0.90**	0.35*	0.55*	0.41
Oil				1.00	-0.28	-0.69**	-0.55
Maturity					1.00	0.08	0.12*
Height						1.00	0.73**
Lodging							1.00

* P-value ≤ 0.05
** P-value ≤ 0.01

TABLE 7. Correlation matrix for yield, seed size, seed protein and seed oil contents, maturity, height in 2006 at Ames, IA.

	Yield	Seed size	Protein	Oil	Maturity	Height	Lodging
Yield	1.00	0.07	-0.75**	0.84**	-0.57**	-0.40**	-0.46**
Seed size		1.00	0.15	-0.02	0.33	0.24	0.07
Protein			1.00	-0.84**	0.44**	0.59**	0.55**
Oil				1.00	-0.61**	-0.63**	-0.60**
Maturity					1.00	0.57**	0.32*
Height						1.00	0.62**
Lodging							1.00

* P-value ≤ 0.05
** P-value ≤ 0.01

FIGURE 1. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for yield of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

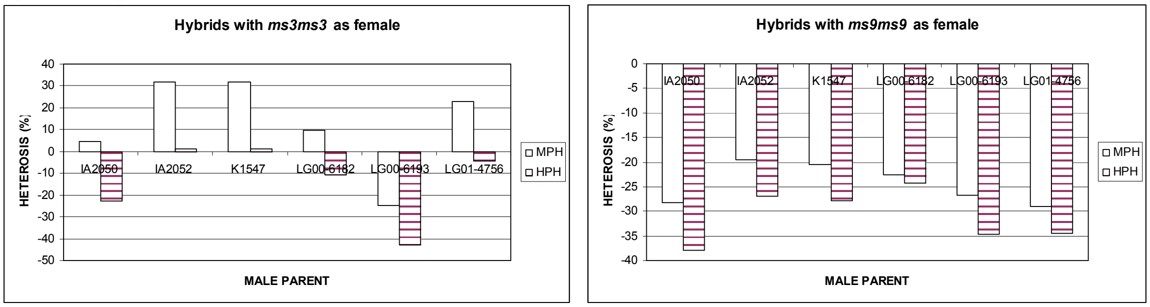


FIGURE 2. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for yield of hybrids grouped by female parent at Ames, IA in 2006.

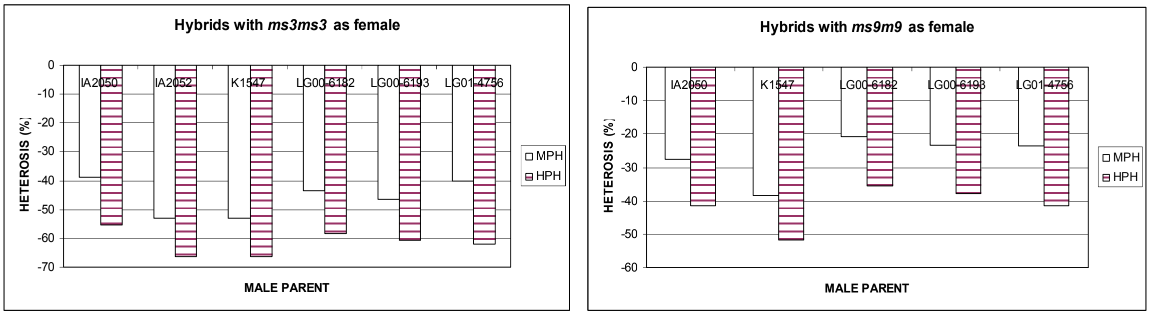


FIGURE 3. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for seed size of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

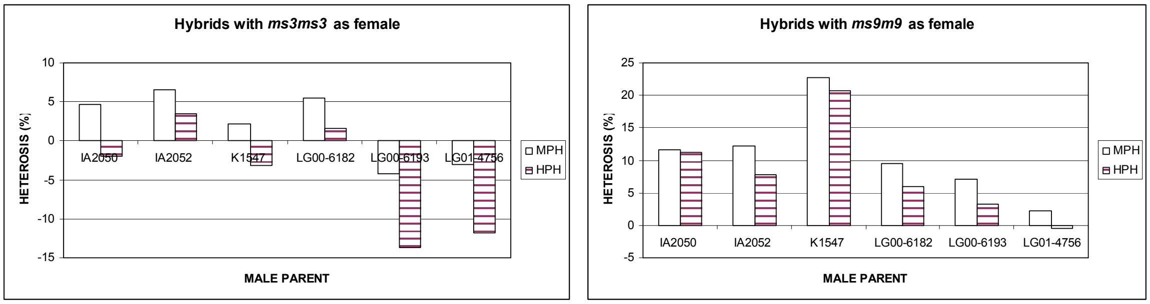


FIGURE 4. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for seed size of hybrids grouped by female parent at Ames, IA in 2006.

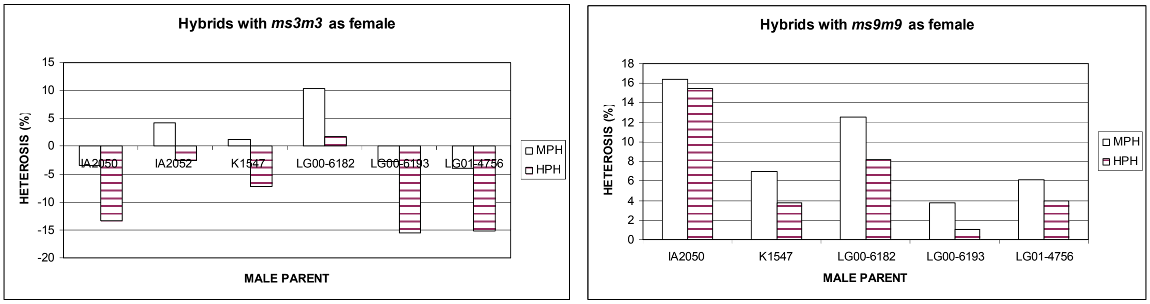


FIGURE 5. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for seed protein content of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

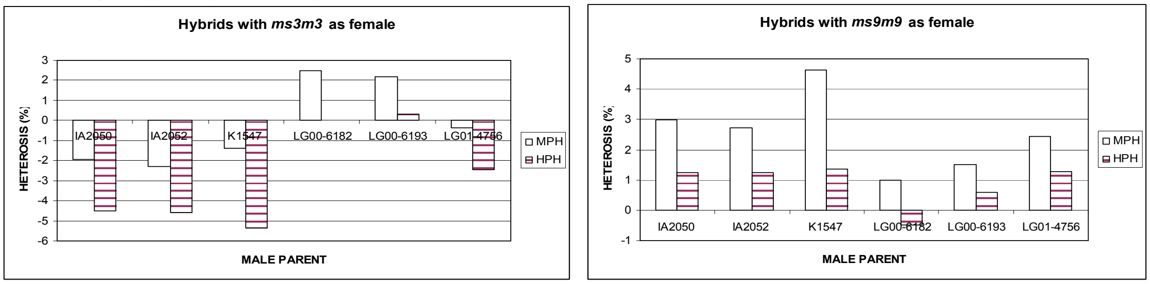


FIGURE 6. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for seed protein of hybrids grouped by female parent at Ames, IA in 2006.

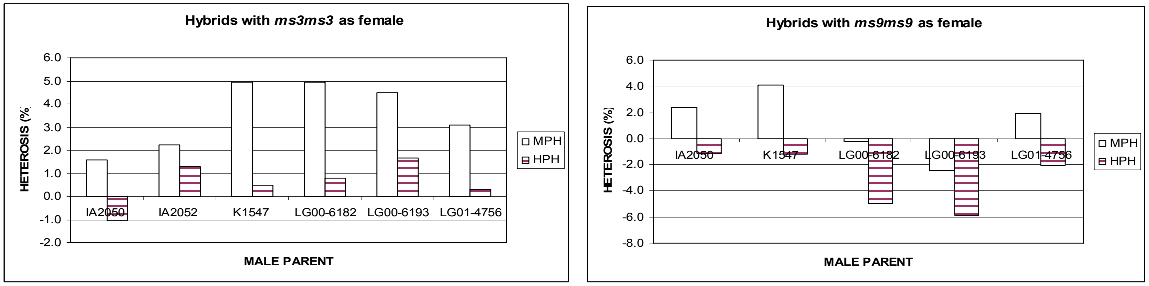


FIGURE 7. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for seed oil content of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

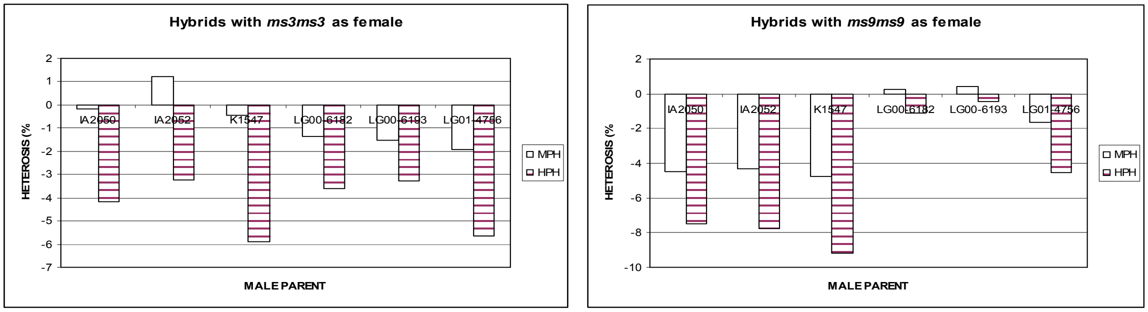


FIGURE 8. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for seed oil of hybrids grouped by female parent at Ames, IA in 2006.

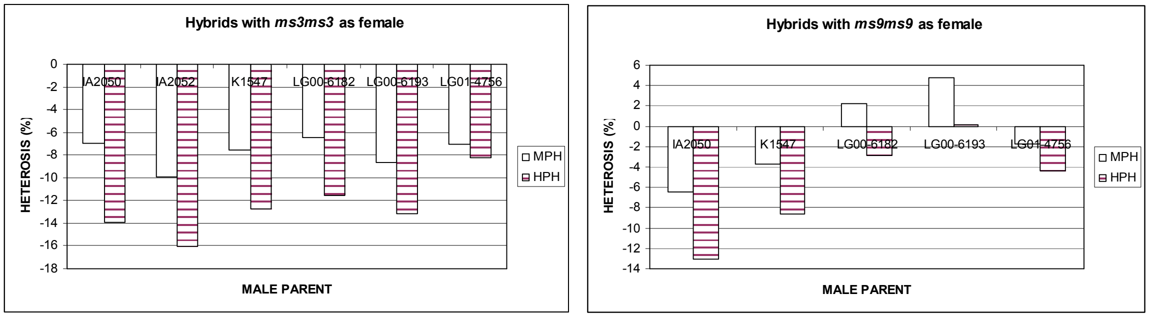


FIGURE 9. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for maturity of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

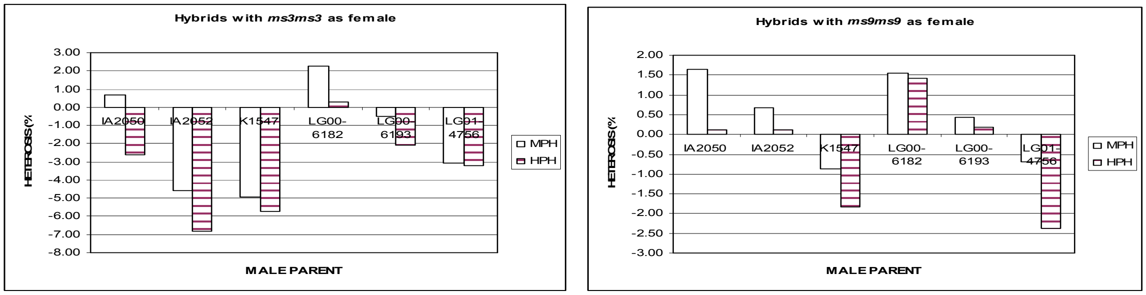


FIGURE 10. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) values for maturity of hybrids grouped by female parent at Ames, IA in 2006.

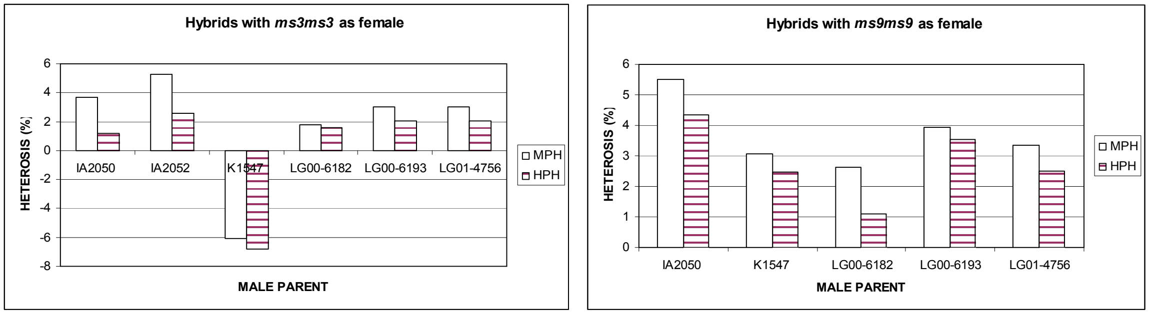


FIGURE 11. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for lodging of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

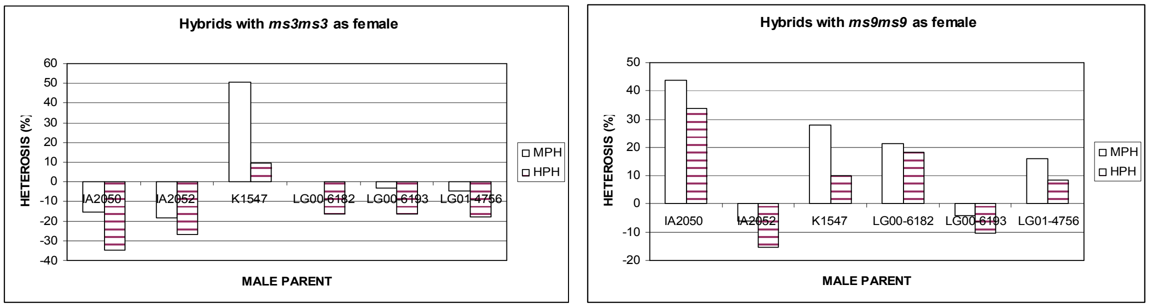


FIGURE 12. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for lodging of hybrids grouped by female parent at Ames, IA in 2006.

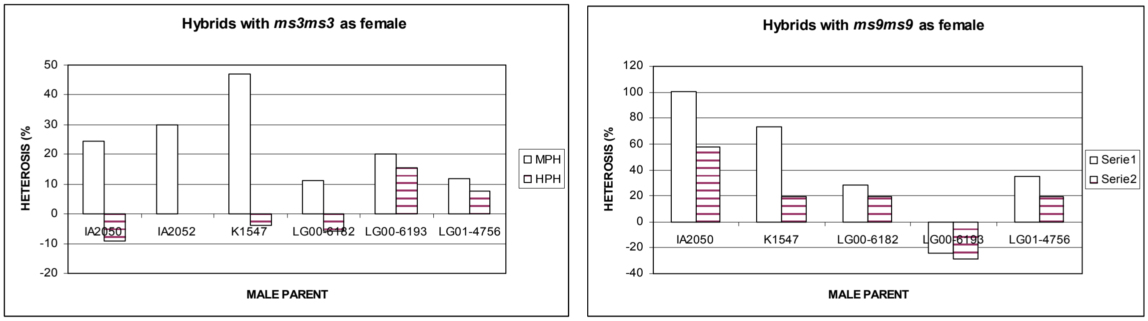


FIGURE 13. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for height of hybrids grouped by female parent at Ames, and Gilbert, IA in 2005.

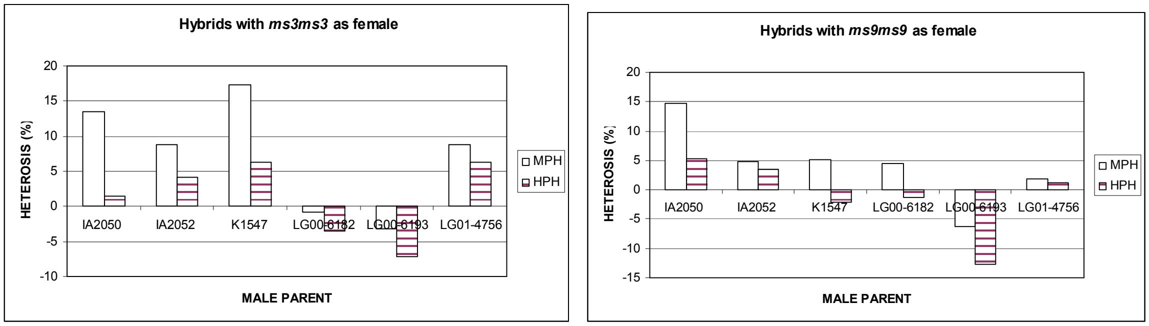
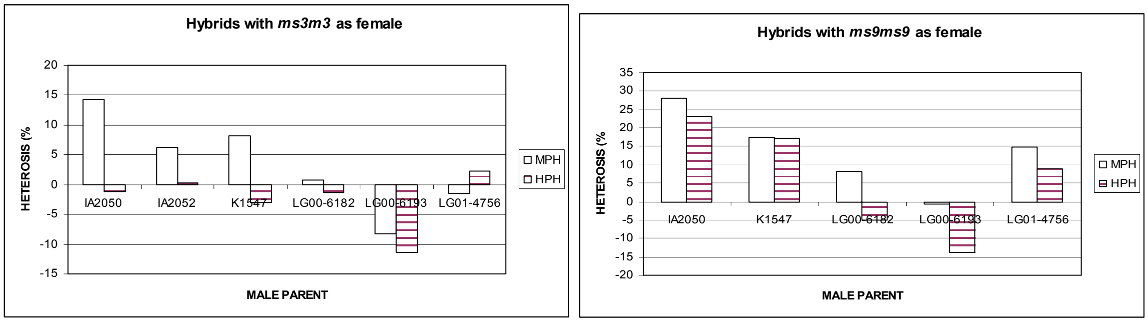


FIGURE 14. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for height of hybrids grouped by female parent at Ames, IA in 2006.



CHAPTER 3. AGRONOMIC PERFORMANCE OF SOYBEAN [*Glycine max* (L.) Merr.] LINES FROM F_1, 3-WAY CROSSES, 4-WAY CROSSES, 5-WAY CROSSES, BC_1, BC_2, and BC_3 HYBRIDS

A paper to be submitted to *Journal of Crop Improvement*

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ABSTRACT

Male sterility systems identified in soybean [*Glycine max* (L.) Merr.], combined with insect-mediated cross-pollination have been shown to produce large quantities of hybrid seed that can be useful for the identification of heterotic patterns in soybean. This procedure was used in this study to produce hybrid seed for the conduct of replicated yield trials. The objectives were: (1) to evaluate yield in hybrid soybean populations developed by single-crosses, three-way crosses, four-way crosses, five-way crosses, and backcrosses (BC_1, BC_2, and BC_3), and (2) to estimate heterosis for yield, and other agronomic characteristics of the hybrid populations. Parental genotypes were male-sterile lines and a group of male parents selected for their agronomic performance. In 2005, F_1, 3-way and 4-way crosses, BC_1, and BC_2 populations of eight F_1 single-cross families were evaluated in replicated experiments at one location near Ames, IA. Consecutively, 5-way crosses and BC_3 populations were evaluated in 2006. Mid-parent heterosis values (MPH) of yield ranged from -35% to +17% . High-parent heterosis (HPH) of yield ranged for single-crosses from -35% to -1%; for 3-way crosses from -31% to -4 %, for 4-way crosses from -44% to -26%, for 5-way crosses from -38% to -20%,

and BC_1 crosses from -34% to + 13%, BC_2 crosses from -21% to -8%, and BC_3 crosses from -22% to +3%. Backcross populations had higher heterosis for yield. The few positive MPH and HPH values obtained, suggest that many cross combinations need to be evaluated before heterotic combinations can be identified that will determine the feasibility of commercial hybrid soybean production.

INTRODUCTION

Commercial hybrid development of crop species has been one of the major breakthroughs in agricultural history. In cross pollinated crops, such as maize, sorghum, pearl millet, rapeseed, onion, and tomato, hybrids are extensively planted for commercial production because of their superior yield over traditionally bred cultivars. Hybrid superiority over their parental inbred lines, heterosis or hybrid vigor, is expressed when the parents of a hybrid have different alleles at a locus and there is some level of dominance among those alleles (Falconer and Mackay, 1996).

Soybean is an autogamous species in which most of the current soybean commercial cultivars are inbred lines. Heterosis, however, has been reported in soybean, i.e. some hybrids yielded 10% to 20% more than the higher-yielding parent (Palmer et al., 2001). A summary of 14 experiments conducted to measure heterosis since 1930, in which a total of 456 different crosses were evaluated, indicated that average mid-parent heterosis (MPH) for yield ranged from +14% to +46%, and average high parent heterosis (HPH) from +4% to +34% (Palmer et al., 2001). The majority of these experiments were conducted using spaced F_1 plants.

In other experiments, where more hybrid seed was available, yield tests were done in replicated plots. In these cases, average yield MPH percentages for 2, 27, and 7 hybrid combinations were +26%, +3%, and +4% respectively (Brim and Cockerham, 1961; Nelson and Bernard, 1984; Lewers, 1996). In China, Sun et al. (1999) summarized data from a comprehensive heterosis test program, in which 846 combinations out of 1123 showed positive MPH estimates. Of those, 248 out of 846, showed a mean HPH of +20%. In this evaluation, production of F_1 hybrid seed was done by hand pollination, and six institutes participated, by planting two replications of each experiment using one row plots. Burton and Brownie (2006) evaluated the F_1 generations of two combinations derived from crosses between current soybean cultivars. The average yield of one cross was 16 % greater than that of the highest-yielding parent and the average yield of the other cross was 5 % greater than the highest-yielding parent.

In spite of these positive results, commercial exploitation of heterosis in soybean, however, has not been realized. Difficulties in obtaining large quantities of hybrid seeds, which would be required for production fields, have acted as a deterrent. Additionally, heterosis studies in soybean are somewhat limited, particularly when experimental conditions are compared to commercial fields. Hybrids mostly have been evaluated in single-row plots, in some cases even using spaced F_1 plants. These planting conditions, so distinct from commercial soybean production preclude extrapolation of results to commercial fields (Palmer et al, 2001).

Hybrid production and heterosis evaluation in previous studies have been done with single-cross hybrids, probably assuming that hybrid vigor would mainly result from specific combining ability between the two parents. There are, however, other cross schemes in soybean that could be considered. For example, hybrids in which more than two parents are crossed, i.e. 3-way crosses, 4-way crosses, and 5-way crosses, and even backcross lines.

In soybean, 3-way, 4-way and backcross populations have been used with the objective of population and line development for yield improvement. Thorne and Fehr (1970) compared single- and three-way crosses as a means to develop high-protein lines with high-yield. The authors reported that three-way cross populations means usually were significantly higher than single-crosses for yield, seed protein, seed oil, and seed protein plus seed oil content. Lawrence and Frey (1974) compared several backcross generations, BC_1F_2, BC_2F_2, BC_3F_2, BC_4F_2, and BC_5F_2 generations, from the cross of adapted *Avena sativa* with exotic *Avena sterilis*. The authors reported that the BC_1F_2, BC_2F_2, BC_3F_2, and BC_4F_2 generations were better than the BC_5F_2 generation for the selection of high-yielding transgressive segregants..

Studies also have been conducted in which single- , and 3-way crosses were compared to backcrosses. Cober and Voldeng (2000) evaluated backcrosses and single-crosses with the objective of developing high-yield, high-protein lines. They found that the average seed yield and protein content of backcrosses were no different than single-crosses. Cianzio and Voss (1994) compared single-, three-way, and backcross populations for the selection of high-yielding soybean cultivars with improved Fe-efficiency. The populations developed by backcrossing had the highest yield followed by the three-way crosses and the single-cross population. Heterosis evaluations, however, were not considered in the soybean lines derived from these more complex population structures. In other crop species, backcrosses were done as part of the development of hybrid cultivars, a method known as convergent improvement (Fehr, 1991). The method is used to improve inbred line performance of parents that will be later used in hybrid combinations. The method mainly has been used in maize (Sprague et al., 1958; Richey, 1927; Lonnquist et al., 1979).

In soybean, a previous study conducted by Ortiz-Perez et al. (2007), in which heterosis for yield and agronomic traits was evaluated for single-, three-way and backcrosses (BC_1F_1) reported that yield MPH values ranged from -59% to +37% in single-crosses, -14% to +16% for 3-way crosses, and -7% to +42% for BC_1F_1. HPH estimates ranged from -66% to +17% for single-crosses, -25% to -5% for three- way crosses, and -15% to +41% for BC_1F_1 crosses. In this study only the BC_1 generation was considered, along with comparisons of single- *vs* 3-way crosses.

The study to be reported here refers to a more comprehensive evaluation of complex crosses and additional backcross generations than reported by Ortiz et al., (2007). The objectives of the study were to evaluate seed yield and agronomic performance of soybean single-crosses, 3-way crosses, 4-way crosses, 5-way crosses, and backcross generations BC_1, BC_2, and BC_3, and to estimate heterosis for the same traits. Due to shortage of seed availability, only one location in Iowa was used.

MATERIALS AND METHODS

Backcrosses (BC_1, BC_2, and BC_3), and 3-, 4-, 5-way crosses of eight families were evaluated. Hybrids were developed by crossing seven nuclear male-sterile lines as female parents to eleven lines as male parents. For each female parent, the homozygous dominant male-fertile, female-fertile sibling was planted in the same field with hybrids in order to determine heterosis values. Male parents and commercial checks also were evaluated (Table 1).

Plant materials

Female parents had excellent insect pollinator attraction and good agronomic characteristics. Segregating lines for nuclear male-sterile mutations were used as female parents. These lines were, *ms*2 (A00-39 and A00-41) (Cervantes-Martinez et al., 2005), *ms*3 (T284H) (Chaudhari and Davis, 1977), *ms*6 (T295H) (Skorupska and Palmer, 1989), *ms*8 (T358H) (Palmer, 2000), and *ms*9 (T359H) (Palmer, 2000). Male parents were chosen mainly because of their attractiveness to pollinator insects and excellent agronomic characteristics (Table 1). Every year, a subset of the hybrid generations was tested. In 2005, F_1 hybrids, 3-way crosses, 4-way crosses, BC_1, and BC_2 were evaluated. In 2006, BC_3 and 5-way crosses were evaluated (Table 1).

Hybrid crosses

The process for hybrid seed production had several steps because of the nature of the crosses. Initial single-cross combinations between the male-sterile, female-fertile lines and the male parent lines would define a full-sib family, herein referred as family. From eight families, different hybrid populations were developed depending on seed availability (Tables 1 and 2). F_1 hybrids were produced in Texas. This F_1 seed was saved for yield trials, and a sample of F_1 seeds was used for selfing at the off-season nursery near Isabela, Puerto Rico, where 40 to 60 F_2 plants were harvested individually. The F_2 had a generation of selfing and one set of male-sterile, female-fertile $F_{2:3}$ plants was crossed to the recurrent male parent to produce the first backcross generation, BC_1. The other set of male-sterile $F_{2:3}$ plants was crossed with a high yielding cultivar to produce the 3-way crosses. Seeds of the BC_1F_1 and 3-way crosses were used for yield trials. Samples of the hybrid seeds were sent to the off-season nursery near Isabela where both, BC_1F_1 and 3-way crosses, underwent a generation of selfing. Male-sterile BC_1F_2 plants were crossed to the recurrent parent to produce the second backcross generation, BC_2. Male-sterile 3-way crosses plants were crossed to a high yielding cultivar to produce the 4-way crosses. Again, seeds were saved for yield trials and for selfing near Isabela. After selfing, male-sterile BC_2F_2 plants were crossed to the recurrent parent to produce the third backcross generation, BC_3. Male-sterile 4-way cross plants were crossed to a high yielding cultivar to produce the 5-way crosses (Table 3).

Hybrid seed production

The hybrid seed was produced in Plainview, TX. Each plot for hybrid seed production had six rows, rows one and six were planted with the male parents; rows two to five with the segregating male-sterile line. Each row was 4.8 m long, spaced 76 cm between rows and 1.2 m among plots, planted with 14 seeds per m.

At flowering, male-sterile plants were identified and labeled, and the fertile siblings were removed to avoid their use as pollen source. This procedure was done in the middle rows (2-5) where the segregating male-sterile lines were planted for each hybrid combination. Insect vectors transferred pollen from the male-parent rows to the male-sterile, female-fertile plants. Native bees from families *Megachilidae, Halictidae, Anthophoridae*, and *Andrenidae* were observed to carry out the pollinations (Ortiz-Perez et al., 2007). Each plot was bulk harvested and hybrid seed for each combination was planted in summer 2005 and 2006 in Iowa (Tables 1 and 3). F_1, 3-way, 4-way crosses, BC_1, and BC_2 hybrid populations were tested in 2005, and 5-way crosses and BC_3 populations in 2006.

Field testing

Hybrids, parents, and checks were planted in a randomized complete-block design with two replications at one location near Ames, IA. Plots were four rows, each row was 5.2 m long and spaced 0.76 m between rows. In 2005, backcrosses BC_1 and BC_2 of family 6, and F_1 hybrids of families 3, 4, 6, 7, and 8 were not included in the test due to lack of adequate hybrid seed. In 2006, 5-way crosses and BC_3 from all families were evaluated, except for the BC_3 of family 6 which could not be planted because BC_1 and BC_2 generations were not obtained due to lack of adequate hybrid seed (Tables 1 and 3).

Traits evaluated were yield, seed protein content, seed oil content, lodging, plant height, maturity date, and seed size. Maturity date was recorded when 95% of the pods in the two middle rows were brown R8 (Fehr et al., 1971). Days from planting to maturity were calculated. Plant height and lodging were recorded prior to harvest. Plant height was measured in centimeters for two plants in each of the two middle rows. Lodging was a visual observation of the whole plot; it was recorded on a 1 to 5 scale with 1 being upright, 3 being at a 45^{o}, and 5 being prostrate. The two middle rows were harvested at maturity and the seed was used to estimate yield, and seed size. Seed samples of 25 g were sent to the USDA National Center for Agricultural Utilization Research (NCAUR), Peoria, IL to determine seed protein content and seed oil content using near infrared transmittance.

Statistical analysis

For all genotypes, a mixed model analysis of variance was performed separately for each agronomic variable each year, using SAS PROC MIXED (SAS Institute, 2003). In the model, genotypes were fixed and replications were considered random effects. Least Square

Means (LSMEANS) were used to estimate the performance of hybrids, parents, and checks for each year. Least Square Difference (LSD) test to separate the means was performed.

The performance of the hybrids relative to their parents can be measured as high-parent heterosis (HPH), and mid-parent heterosis (MPH), (Fehr, 1991). Mid-parent heterosis was determined as:

$$\text{Mid-parent heterosis (\%)} = \frac{F_1 - MP}{MP} \times 100$$

Where F_1 = performance of hybrid and MP= average performance of parents. The ESTIMATE statement of SAS v. 9.1 (SAS Institute, 2003) was used to determine the difference between the mean of each hybrid with the average of its parents.

High-parent heterosis was determined as:

$$\text{High-parent heterosis (\%)} = \frac{F_1 - HP}{HP} \times 100$$

Where F_1 = performance of hybrid, and HP = performance of best parent.

The LSMEANS statement with a PDIFF option was used to estimate the difference between the hybrid and the parent with the best performance for a specific trait. Multiple comparison tests were done with the Bonferroni method. A BON option of SAS v. 9.1 (SAS Institute, 2003) was used to estimate adjusted p-values for multiple comparisons.

RESULTS

F_1, three-way, four-way crosses, BC_1, and BC_2 crosses

For all traits, the combined analysis of variance for checks, parents, F_1, BC_1, BC_2, 3-, and 4-way cross populations, indicated significant differences ($P \leq 0.05$) among genotypes (Appendix I, Table 1). Orthogonal contrasts were performed to detect differences between parents and each hybrid population (Appendix I, Table 3). Significant differences ($P \leq 0.05$)

were found for parents and hybrid populations for most traits, except lodging. For all traits, lines in backcross generations (BC_1 and BC_2) were not significantly different from lines in 3- and 4-way cross populations.

Differences among hybrid populations for each family were calculated (Table 4). The F_1 populations of only three families were evaluated. In two of the three families, yield was lower in the F_1 population compared with the more complex hybrid populations. For the family A00-39 (*ms2*) x Corsoy 79, yield was lower in the F_1 population, however, in the family A00-39 (*ms2*) x Hark, the F_1 population was superior to the other populations. This finding could indicate that the *ms2* mutation may not have pleiotropic effects on the yield performance of F_1 hybrids, although non-additive gene action might be present.

In two out of three families, the BC_1 population yielded significantly ($P \leq 0.05$) higher than the BC_2 population (Table 4). In the third family, the opposite was true, the BC_2 generation yielded significantly ($P \leq 0.05$) higher than the $BC_{1.}$

Parent lines, checks, and hybrid populations

Checks and male parents had the best yield performance (Table 5). Average yield of checks was 3959 kg/ha, with the highest yielding check being IA2068, with a yield of 4634 kg/ha. Average yield of parents was 2919 kg/ha, with the highest yielding male parent being GH 4190, which was the male parent used in the 4-way crosses.

Average yield of all hybrids was 2598 kg/ha. Average yield of complex hybrid populations (3-way, 4-way crosses, BC_1, and BC_2 crosses) of the family A00-63 (*ms2*) x Wells showed the highest values (Table 5). Since female parents of four families had the *ms2* mutation, hybrid populations derived of these four families are referred as the *ms2* group.

Average performance of each *ms* group (*ms2, ms3, ms6, ms8*, and *ms9*), were estimated for all traits. The *ms9* group had the highest yield (Table 6).

Hybrids across all groups had larger seed, than checks and parents (Table 5). Similarly, seed protein content was higher for hybrids, although seed oil content was higher for checks and parent lines. Average lodging was similar for all genotypes in the study. Hybrids were taller and later maturing across all groups.

Heterosis in hybrids

For yield, mid-parent heterosis (MPH) estimates ranged from -35% to +17% and high-parent heterosis (HPH) from -44% to +13% (Table 7). Positive MPH was found in the BC_1 populations of families A00-39 (*ms2*) x Corsoy 79 and A00-63 (*ms3*) x A00-41. HPH was found in the BC_1 population of family A00-63 (*ms3*) x A00-41.

For seed size, MPH values ranged from -10% to +22%, and HPH from -24% to +21% (Table 7). For seed protein content, the average MPH was +3%, and the average HPH was +1%. Averages for seed oil content were -3% for MPH, and -5% for HPH. Positive MPH values were observed for height, lodging, and maturity, where MPH values averaged for all hybrid populations across families +9% for height, +15% for lodging, and +4% for maturity. Ranges for height HPH were -20 % to +15%; for lodging -28% to +7%; and for maturity, +1% to +5% (Table 8).

Five-way crosses and BC_3 crosses

For all traits, the combined analysis of variance (ANOVA) for checks, parents, BC_3, and 5-way cross populations, indicated significant differences ($P \leq 0.05$) among genotypes

(Appendix I, Table 2). Orthogonal contrasts to detect differences between parents and each hybrid population indicated significant ($P \leq 0.05$) differences for parents and hybrids for all traits, except for seed protein and oil content (Appendix I, Table 4). Significant ($P \leq 0.05$) differences between the BC_3 and 5-way crosses were detected for all traits, except days to maturity and plant height.

Parent lines, checks, and hybrid populations

Checks and male-parent lines had the best yield performance (Table 9). Average yield of checks was 3765 kg/ha, and for parents was 2606 kg/ha. The highest yielding check was IA2068 with a yield of 4635 kg/ha, while the highest yielding male parent was DSR Exp.202c. This was the male parent used in the 5-way crosses.

Average yield of all hybrids across families was 2453 kg/ha (Table 9). Average yield of the 5-way crosses and BC_3 of the family A00-39 (*ms2*) x Corsoy had the highest yield. Across all groups of hybrids, the *ms2* group had the highest yield (Table 10).

Seed size of hybrids across all families, was larger than that of checks and parents (Table 9). Similarly, seed protein content was higher for all hybrids, however, seed oil content was higher in checks and parent lines. Hybrids were taller and later maturing across all families.

Heterosis in hybrids

For yield, mid-parent heterosis (MPH) values ranged from -30% to +10% and high-parent heterosis (HPH) from -38% to +3% (Table 11). Positive MPH was observed in the

BC_3 populations of families A00-39 (*ms2*) x Hark, A00-41 (*ms2*) x A00-73, and A00-73 (*ms9*) x Raiden. HPH was detected in the BC_3 population of family A00-73 (*ms9*) x Raiden.

For seed size, MPH ranged from -4% to +30%, and HPH from -20% to +28% (Table 11). For seed protein content, MPH ranged from -3% to +3%, and HPH from -6% to +2%. For seed oil content, MPH ranged from -8% to +7%, and HPH from -11% to +5%. For height, MPH ranged from -20% to +62%, and HPH from -22% to +28%. For maturity, MPH ranged from +2% to +11%, and HPH from -1% to +9%.

DISCUSSION

In the study, performance of soybean hybrids obtained from complex crosses, and heterosis estimates were determined. Families were formed by using the F_1 single-cross hybrids crossed to other male parents or backcrossed using the highest-yielding parent of the single-cross as recurrent parent. Due to scarcity of seed, and to the procedure used for developing the complex populations, populations could not be evaluated in a common environment, therefore genotypic effects and heterosis are confounded with environment. In spite of this limitation, trends were observed in all population types and the discussion will focus on them.

The study has shown that complex population structures may be used to develop hybrids that would exhibit heterosis. However, before more definite conclusions might be drawn, different hybrid population structures will have to be tested in common environments, i.e. locations and years. Also, due to the poor performance of the female parent, it is suggested that male-sterile genes be introgressed in high-yielding lines, in order for both parents of the cross to contribute alleles with positive effects in the final expression of traits.

In general and as reported before (Chapter 2, unpublished), F_1 hybrids of single-crosses were outyielded by checks and some of the male parents, possibly due to the low yielding performance of the male-sterile, female-fertile parent lines used in the original F_1 hybrids. However, hybrids from more complex population structures had better yields than the single-cross hybrids. These observations indicate that use of additional high yielding parents to form the more complex populations had favorable impacts on yield and agronomic traits. These findings may suggest that each of the parents contributed genes with positive effects to the final expression of traits, i.e. F_1's had lower yield than the BC_1, BC_2, 3-way cross, and 4-way cross populations. The superior performance of 3- and 4-way populations compared to F_1's may be explained by the genetic contributions of the high yielding lines used in the more complex crosses. Further support for this assumption may be obtained from the comparison between 3-way *vs* 4-way crosses. Both population types were evaluated during the same year, and the 4-ways had higher yields than the 3-way crosses. This may be due to the fact that the male parent of the 4-way cross, GH 4190, was the highest yielding parent that year. The result may suggest additive gene action for yield as it has been indicated by Brim and Cockerham (1961), who reported the majority of economically important traits in soybeans were controlled by genes with additive effects

Results from the comparison of F_1 hybrids to backcross populations also were similar to comparisons previously discussed. A trend for higher yield in the backcross populations (BC_1 and BC_2) was observed compared to F_1's, which is in agreement with Ortiz-Perez et al. (2007) in soybean, and with Lawrence and Frey (1974) in oat. Lawrence and Frey (1974) suggested backcrossing may restore desired genetic complexes already present in adapted genotypes, which could have been disrupted during F_1 hybrid development.

Individual differences within groups of male-sterile genes also were observed in this study. For the *ms2* gene, the F_1 hybrids of A00-39 (*ms2*) x Corsoy 79, had the lowest yield of all hybrids. The cross A00-39 (*ms2*) x Hark, however, had F_1's that were superior to all other hybrid populations. This could be an indication that the *ms2* mutation does not have a pleiotropic effect on yield performance of F_1 hybrids. It may also suggest specific combining ability since F_1 hybrids of the same female parent yielded differently depending on the male parent used in the cross.

Also observed in these hybrid populations was the general trend of low yield values associated with low seed oil and high seed protein content, which is in agreement with negative correlations reported (Burton, 1987). Hybrids were taller than parents and checks, although lodging was similar for all hybrid populations. These observations agree with Nelson and Bernard (1984), who indicated that hybrids had superior vegetative growth. Additionally, hybrids had larger seeds than parents.

Heterosis estimates in this study were different from previously reported (Chapter I). Estimates for each trait ranged widely with some being positive and high. For yield and across families, positive and negative heterosis values were obtained, which were within the range reported in the literature (Burton and Brownie, 2006; Cerna et al., 1997; Chaundhary and Singh, 1974; Lewers et al., 1996; Manjarrez-Sandoval et al., 1997; Nelson and Bernard, 1984; Ortiz-Perez et al., 2007; Pandini et al., 2002; Weber et al., 1970). Positive significant heterosis for height, lodging, seed size, and seed protein content also were observed in several crosses, which suggest heterosis was present mainly in traits associated with vegetative growth as reported by Nelson and Bernard (1984), and Lewers et al. (1998). A possible explanation for some of the HPH values was provided by Ortiz-Perez et al. (2007), who mentioned that the large difference among parent lines may explain the negative and low heterosis estimates. This could also be the explanation for this study, since some of the parent lines used were the same in both studies.

In the study reported here, deviations of the mid-parent value were detected, that could indicate epistatic effects as suggested by Thorne and Fehr (1970). Another important finding was that HPH for yield was observed in BC_1 and BC_3 populations, in agreement with Ortiz-Perez et al. (2007). The observation that backcross populations had positive heterosis estimates, indicates that additive x additive epistasis could be present (Lamkey and Edwards, 1999; Leininger and Frey, 1962; cited by Lawrence and Frey, 1970).

The results also indicated that other parental combinations, more complex than the classic single-crosses could contribute to heterosis in soybean, i.e. backcross populations which were the parental combination showing higher heterosis for yield. Similar results also were found by Ortiz-Perez et al. (2007). One concern related to the use of backcross and more complex crosses is that more seasons would be needed for hybrid seed production than with single-crosses.

The trends observed in this study may open up new possibilities for the use of heterosis in commercial soybean production. However, before a final conclusion may be drawn, extensive evaluations of single-cross combinations, along with more complex crosses may have to be conducted. It will also be necessary to select for higher yielding female parent lines. And lastly, an efficient seed production scheme will have to be devised to simultaneously evaluate all hybrids in the same environments, i.e. locations and years.

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TABLE 1. Hybrid populations (F_1, BC_1, BC_2, BC_3, 3-way, 4-way, and 5-way), parents and checks evaluated in 2005 and in 2006 at one location near Ames, IA.

Genotypes		Year of evaluation	
		2005	2006
Parents			
A00-39 (*Ms2Ms2*)		X	X
A00-41 (*Ms2Ms2*)		X	X
A00-63 (*Ms2Ms2*)		X	X
A00-68 (*Ms3Ms3*)		X	X
A00-72 (*Ms8Ms8*)		X	X
A00-73 (*Ms9Ms9*)		X	X
A94-(20 x -19) (*Ms6Ms6*)		X	X
Wells		X	X
Corsoy 79		X	X
DSR Exp.202b		X	X
GH 4190		X	X
Hark		X	X
Raiden		X	X
DSR Exp.202c			X
Hybrids			
Family[a]	**Population**		
A00-39 (*ms2*) x Corsoy 79	F_1	X	
	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X
A00-39 (*ms2*) x Hark	F_1	X	
	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X
A00-41 (*ms2*) x A00-73	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X

TABLE 1. (Continued)

Hybrids		Year of evaluation	
		2005	2006
Family	Population		
A00-63 (*ms2*) x Wells	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X
A00-68 (*ms3*) x A00-41	F_1	X	
	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X
A00-73 (*ms9*) x Raiden	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X
A94-(20 x -19) (*ms6*) x A00-39	3-way	X	
	4-way	X	
	5-way		X
	BC_1	X	
	BC_2	X	
	BC_3		X
A00-72 (*ms8*) x A00-68	3-way	X	
	4-way	X	
	5-way		X
Checks			
IA2068		X	X
GH 3919		X	
Apex			X

[a] Family = Initial single-cross combination between the male-sterile, female-fertile lines and the male parent line.

TABLE 2. Pedigree of each hybrid generation (F_1, BC_1, BC_2, BC_3, 3-, 4-, and 5-way crosses).

Generation	Pedigree
F_1	♀[+] x RP[++]
BC_1	F_1 x RP
BC_2	BC_1 x RP
BC_3	BC_2 x RP
3-way cross	F_1 x DSR Exp.202b
4-way cross	3-way cross x GH 4190
5-way cross	4-way cross x DSR Exp.202c

[+] ♀ = Female parent

[++] RP = Recurrent parent = Male parent

TABLE 3. Time table to produce the F_1, BC_1, BC_2, BC_3, 3-way, 4-way, and 5-way cross hybrid soybean seeds used to conduct yield and agronomic performance trials.

Location	Year	Procedure
Plainview, TX, and Massai, Chile	2002	Single-crosses (F_1)
Isabela, PR	2002 (Fall)	Selfing of the F_1
Ames and Gilbert, IA, and Otterbein, IN	2003	Yield tests of single-crosses
Isabela, PR	2003	Selfing of the F_2
Plainview, TX	2004 (Spring)	BC_1F_1 : Cross between $F_{2:3}$ male-sterile plants and recurrent parent 3-way : Cross between $F_{2:3}$ male-sterile plants and DSR Exp.202b
Isabela, PR	2004	Selfing of the BC_1F_1 Selfing of the 3-way crosses
Ames, IA	2004	Yield test of BC_1F_1 and 3-way crosses
Plainview, TX	2004	BC_2F_1 : Cross between BC_1F_2 male-sterile plants and recurrent parent 4-way : Cross between 3-way male-sterile plants and GH 4190
Isabela, PR	2005	Selfing of the BC_2F_1 Selfing of the 4-way crosses
Ames, IA	2005	Yield tests of F_1, 3-way crosses, 4-way crosses, BC_1F_1, and BC_2F_1
Plainview, TX	2005	BC_3F_1 : Cross between BC_2F_2 male-sterile plants and recurrent parent 5-way : Cross between 4-way male-sterile plants and DSR Exp.202c
Ames, IA	2006	Yield tests of 5-way crosses and BC_3F_1

TABLE 4. Estimates of the differences among F$_1$, 3-way crosses^a, 4-way crosses^b, BC$_1$^{c} and BC$_2$^{d} populations within families at one location near Ames, IA in 2005

Family^e	Comparison	Yield	Seed size	Protein	oil	Maturity	Height	Lodging
A00-39 (*ms2*) x Corsoy 79	F$_1$ - 3-way	-109	-16*	-14*	15*	-8*	-15	1
	F$_1$ - 4-way	-404	8	-16*	5	-7*	-4	0
	F$_1$ - BC$_1$	-974*	-4	7	-5	-4*	-10	0
	F$_1$ - BC$_2$	-486*	3	-2	0	-4*	-24*	1
	3-way - 4-way	-295	24*	-2	-9*	1	11	0
	BC$_1$ - BC$_2$	488*	7	-8	5	0	-14	0
A00-39 (*ms2*) x Hark	F$_1$ - 3-way	923*	10	-3	3	-4*	15	1
	F$_1$ - 4-way	100	-2	4	-5	-3*	3	1
	F$_1$ - BC1	695*	-1	-4	-1	-2*	10	1
	F$_1$ - BC2	391	2	-13*	7*	-3*	13	1
	3-way - 4-way	-823*	-12*	7	-8*	1	-12	0
	BC$_1$ - BC$_2$	-304	3	-10	8*	-1	3	-1
A00-68 (*ms3*) x A00-41	F$_1$ - 3-way	-168	-9	6	-1	1	3	1
	F$_1$ - 4-way	-77	-2	24*	-13*	0	-9	-1
	F$_1$ - BC$_1$	-427	-7	10	-4	1	-3	-1
	F$_1$ - BC$_2$	-144	-17*	10	-5	1	-12	-1
	3-way - 4-way	91	6	19*	-12*	-1	-12	-1*
	BC$_1$ - BC$_2$	282	-9*	0	-1	-1	-10	0
A00-41 (*ms2*) x A00-73	3-way - 4-way	-547*	-3	-14*	7*	-4*	-32*	0
	BC$_1$ - BC$_2$	314	5	-4	3	-1	-1	1
A00-63 (*ms2*) x Wells	3-way - 4-way	-313	-9	15*	-3	-3*	-15	-1
	BC$_1$ - BC$_2$	688*	6	-2	0	-2*	-19*	0
A00-73 (*ms9*) x Raiden	3-way - 4-way	168	-14*	-8	7*	-5*	-17*	0
	BC$_1$ - BC$_2$	38	-8	-18*	11*	0	-18*	0
A94-(20 x-19) (*ms6*) x A00-39	3-way - 4-way	375	-1	-19*	3	-2*	-18*	0
	BC$_1$ - BC$_2$	-805*	-2	-8	4	1	-25*	1
A00-72 (*ms8*) x A00-68	3-way - 4-way	-432	-1	-3	1	-1	3	1

[a] 3-way cross = F$_1$ family x DSR Exp.202b

[b] 4-way cross = 3-way cross x GH 4190

[c] BC$_1$ = F$_1$ family x male parent

[d] BC$_2$ = BC$_1$ x male parent

[e] Family = Initial single-cross combination between the male-sterile, female-fertile lines and the male parent line

* P-value ≤ 0.05

TABLE 5. Mean values for yield, seed size, seed protein and seed oil content, days to maturity, plant height, and lodging for checks, parents, F_1, 3-way crosses[a], 4-way crosses[b], BC_1[c], and BC_2[d] populations at one location near Ames, IA in 2005.

	Population	Yield (Kg ha^{-1})	Seed size (mg seed^{-1})	Protein (g Kg^{-1})	Oil (g Kg^{-1})	Maturity[e] (days)	Height (cm)	Lodging[f] (score)
Parents								
A00-39 (*Ms2Ms2*)		3162	156	394	197	137	116	4
A00-41 (*Ms2Ms2*)		2892	131	403	199	133	114	4
A00-68 (*Ms3Ms3*)		2728	127	419	183	140	114	3
A00-72 (*Ms8Ms8*)		2756	133	394	193	130	110	4
A00-73 (*Ms9Ms9*)		2968	149	411	187	135	102	2
A94-(20 x -19) (*Ms6Ms6*)		2274	126	389	182	134	93	4
A00-63 (*Ms2Ms2*)		2924	177	393	195	133	110	3
Wells		2791	146	414	189	135	103	2
Corsoy 79		2806	127	400	200	132	115	5
DSR Exp.202b		3158	138	399	202	135	79	1
GH 4190		4085	142	406	193	141	119	3
Hark		3084	149	403	197	135	105	3
Raiden		2321	165	424	181	136	67	4
Mean		2919	144	404	192	135	104	3
Family[g]								
A00-39 (*ms2*) x Corsoy 79	F_1	2063	153	406	194	136	101	4
	3-way	2172	169	420	179	143	115	3
	4-way	2467	146	422	189	143	105	4
	BC_1	3037	157	399	199	139	110	4
	BC_2	2549	151	408	194	139	124	3
Mean		2457	155	411	191	140	111	3
A00-39 (*ms2*) x Hark	F_1	3122	159	412	191	138	117	4
	3-way	2198	149	414	188	142	102	4
	4-way	3021	160	408	196	141	114	4
	BC_1	2426	160	415	193	140	107	3
	BC_2	2730	157	425	184	141	104	4
Mean		2700	157	415	190	140	109	4
A00-41 (*ms2*) x A00-73	3-way	2305	151	405	197	139	98	3
	4-way	2852	154	419	190	143	130	3
	BC_1	2848	154	416	188	141	104	4
	BC_2	2534	149	419	185	142	105	3
Mean		2635	152	415	190	141	109	3
A00-63 (*ms2*) x Wells	3-way	2330	135	424	185	140	103	3
	4-way	2643	144	409	188	142	117	3
	BC_1	3295	155	411	188	141	107	3
	BC_2	2607	148	412	188	142	126	3
Mean		2719	145	414	187	141	113	3

TABLE 5. (continued).

	Population	Yield (Kg ha^{-1})	Seed size (mg seed^{-1})	Protein (g Kg^{-1})	Oil (g Kg^{-1})	Maturity[e] (days)	Height (cm)	Lodging[f] (score)
A00-68 (*ms3*) x A00-41	F_1	2355	143	434	179	143	109	3
	3-way	2524	152	428	180	142	106	3
	4-way	2433	145	409	192	143	118	4
	BC_1	2782	150	423	183	142	112	4
	BC_2	2500	160	424	184	142	121	4
Mean		2519	150	424	184	142	113	3
A00-73 (*ms9*) x Raiden	3-way	3046	155	406	192	137	106	3
	4-way	2877	169	414	185	142	123	3
	BC_1	2386	145	413	193	141	101	3
	BC_2	2348	153	431	182	141	119	3
Mean		2664	155	416	188	140	112	3
A94-(20 x -19) (*ms6*) x A00-39	3-way	2654	153	410	187	140	107	3
	4-way	2279	154	429	184	142	126	3
	BC_1	2098	161	412	190	141	93	4
	BC_2	2904	163	420	185	140	117	3
Mean		2484	158	418	186	141	111	3
A00-72 (*ms8*) x A00-68	3-way	2453	151	412	191	140	110	4
	4-way	2885	152	415	190	140	107	3
Mean		2669	151	414	190	140	109	3
Overall mean of hybrids		**2598**	**153**	**416**	**188**	**141**	**111**	**3**
Checks								
IA2068		4634	126	375	205	129	95	3
GH 3919		3285	156	408	194	141	105	3
Mean		3959	141	392	200	135	100	3
LSD		**714**	**10**	**14**	**6**	**19**	**17**	**18**

[a] 3-way cross = F_1 family x DSR Exp.202b

[b] 4-way cross = 3-way cross x GH 4190

[c] BC_1 = F_1 family x male parent

[d] BC_2 = BC_1 x male parent

[e] Maturity = days from planting

[f] Lodging score: 1 = upright, 5 = prostrate.

[g] Family = Initial single-cross combination between the male-sterile, female-fertile lines and the male parent line

TABLE 6. Mean values for yield, seed size, seed protein and oil content, days to maturity, plant height, and lodging for F_1, 3-way crosses[a], 4-way crosses[b], BC_1[c], and BC_2[d] populations for each *ms* hybrid group at one location near Ames, IA in 2005.

Hybrid group	Population	Yield ($Kg\ ha^{-1}$)	Seed size ($mg\ seed^{-1}$)	Protein ($g\ Kg^{-1}$)	Oil ($g\ Kg^{-1}$)	Maturity[e] (days)	Height (cm)	Lodging[f] (score)
m2	F_1	2592	156	409	193	137	109	4
	3-way	2251	151	416	187	141	104	3
	4-way	2746	151	414	191	142	116	3
	BC_1	2902	156	410	192	140	107	3
	BC_2	2605	151	416	188	141	115	3
Mean		2619	153	413	190	140	110	3
m3	F_1	2355	143	434	179	143	109	3
	3-way	2524	152	428	180	142	106	3
	4-way	2433	145	409	192	143	118	4
	BC_1	2782	150	423	183	142	112	4
	BC_2	2500	160	424	184	142	121	4
Mean		2519	150	424	184	142	113	3
m9	3-way	3046	155	406	192	137	106	3
	4-way	2877	169	414	185	142	123	3
	BC_1	2386	145	413	193	141	101	3
	BC_2	2348	153	431	182	141	119	3
Mean		2664	155	416	188	140	112	3
m6	3-way	2654	153	410	187	140	107	3
	4-way	2279	154	429	184	142	126	3
	BC_1	2098	161	412	190	141	93	4
	BC_2	2904	163	420	185	140	117	3
Mean		2484	158	418	187	141	111	3
m8	3-way	2453	151	412	191	140	110	4
	4-way	2885	152	415	190	140	107	3
Mean		2669	151	414	190	140	109	3
Overall mean		**2484**	**158**	**418**	**186**	**141**	**111**	**3**

[a] 3-way cross = F_1 family x DSR Exp.202b
[b] 4-way cross = 3-way cross x GH 4190
[c] BC_1 = F_1 family x male parent
[d] BC_2 = BC_1 x male parent
[e] Maturity = days from planting
[f] Lodging score: 1 = upright, 5 = prostrate.

TABLE 7. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for yield, seed size, seed protein and seed oil content for F_1, 3-way crosses[a], 4-way crosses[b], BC_1[c] and BC_2[d] populations at one location near Ames, IA in 2005.

Family	Population	Yield		Seed size		Protein		Oil	
		MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
A00-39 (*ms2*) x Corsoy 79	F_1	-31*	-35*	+8*	-2	+2*	+2	-2	-3
	3-way	-29*	-31*	+21*	+8	+5*	+5	-10*	-11*
	4-way	-31*	-40*	+3	-7	+5*	+4	-4*	-6*
	BC_1	+5	-4	+17*	+1	0	0	0	0
	BC_2	-11	-19	+15*	-4	+2	+2	-3*	-3
A00-39 (*ms2*) x Hark	F_1	0	-1	+4	1	+3*	+2	-3*	-3
	3-way	-30*	-30	+2	-5	+4*	+3	-6*	-7*
	4-way	-16*	-26*	+12*	+3	+1	0	0	-3
	BC_1	-22*	-23	+6*	+2	+4*	+3	-2	-2
	BC_2	-12	-14	+4	0	+6*	+5	-7*	-7*
A00-41 (*ms2*) x A00-73	3-way	-24*	-27	+9*	+1	0	-1	0	-3
	4-way	-20*	-30*	+10*	+4	+3*	+2	-3*	-6
	BC_1	-3	-4	+7*	+4	+2	+1	-1	-5
	BC_2	-14	-15	+2	0	+2	+2	-2	-7*
A00-63 (*ms2*) x Wells	3-way	-23*	-26	-10*	-24*	+6*	+2	-6*	-8*
	4-way	-25*	-35*	-2	-19*	+1	-1	-4*	-7*
	BC_1	+17*	+13	+1	-13*	+1	-1	-1	-4
	BC_2	-7	-11	-1	-16*	0	0	-1	-4
A00-68 (*ms3*) x A00-41	F_1	-16*	-19	+11*	+9	+5*	+3	-6*	-10*
	3-way	-15*	-20	+14*	+10	+6*	+6*	-8*	-11*
	4-way	-31*	-40*	+5	+2	+1	-2	-1	-5
	BC_1	-2	-4	+15*	+14	+4*	+1	-6*	-8*
	BC_2	-13	-14	+22*	+21*	+5*	+1	-7*	-8*
A00-73 (*ms9*) x Raiden	3-way	+5	-4	+5	-6	-1	-4	0	-5
	4-way	-18*	-30*	+16*	+2	+2	-2	-4*	-8*
	BC_1	-4	-20	-10*	-12	-2	-3	+6*	+3
	BC_2	-2	-21	-6*	-7	+2	+2	0	-3
A94-(20 x -19) (*ms6*) x A00-39	3-way	-10	-16	+9*	-2	+4*	+3	-5*	-8*
	4-way	-35*	-44*	+9*	-1	+7*	+6	-5*	-9*
	BC_1	-29*	-34*	+8*	+3	+5*	+5	-2*	-4
	BC_2	-5	-8	+7*	+4	+7*	+7*	-5*	-6
A00-72 (*ms8*) x A00-68	3-way	-17*	-22	+13*	+10	+2	-2	-2	-5
	4-way	-18*	-29*	+10*	+7	+3*	-1	-2	-6
Mean		**-15**	**-21**	**+7**	**0**	**+3**	**+1**	**-3**	**-5**

[a] 3-way cross = F_1 family x DSR Exp.202b

[b] 4-way cross = 3-way cross x GH 4190

[c] BC_1 = F_1 family x male parent

[d] BC_2 = BC_1 x male parent

*P-value ≤ 0.05

TABLE 8. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for days to maturity, plant height, and lodging, for F_1, 3-way crosses[a], 4-way crosses[b], BC_1[c] and BC_2[d] populations at one location near Ames, IA in 2005.

Family	Population	Maturity		Height		Lodging	
		MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
A00-39 (*ms2*) x Corsoy 79	F_1	+1	-1	-13*	-13	-9	-17
	3-way	+6*	+4*	+18*	-1	+21	-28
	4-way	+3*	+1	-3	-12	+29	-22
	BC_1	+4*	+1	-5	-5	-19	-22
	BC_2	+5*	+1	+8	+7	-26*	-28
A00-39 (*ms2*) x Hark	F_1	+2*	+1	+6	1	+19	+7
	3-way	+5*	+3*	+8	-12	+51*	-7
	4-way	+2*	0	+7	-4	+38*	-7
	BC_1	+3*	+2	-1	-8	-6	-20
	BC_2	+4*	+3*	-2	-10	+13	-7
A00-41 (*ms2*) x A00-73	3-way	+3*	+3*	+5	-14	+37	-25
	4-way	+4*	+1	+22*	+9	+32	-19
	BC_1	+5*	+4*	-1	-9	+30	-13
	BC_2	+5*	+5*	+2	-8	+22	-25
A00-63 (*ms2*) x Wells	3-way	+4*	+3*	+11	-6	+29	-17
	4-way	+3*	+1	+11	-1	+28	0
	BC_1	+4*	+4*	+3	-2	+13	-8
	BC_2	+5*	+5*	+22*	+15	+17	-8
A00-68 (*ms3*) x A00-41	F_1	+4*	+2	-5	-5	-8	-25
	3-way	+5*	+1	+10	-7	+11	-37
	4-way	+3*	+1	+9	-1	+50*	-6
	BC_1	+5*	+1	-2	-2	-3	-13
	BC_2	+6*	+1	+6	+6	-8	-13
A00-73 (*ms9*) x Raiden	3-way	+1*	+1	+29*	+4	+26	-31
	4-way	+3*	0	+23*	+4	+22	-25
	BC_1	+4*	+4*	+33*	0	-9	-19
	BC_2	+4*	+4*	+65*	+17	-14	-19
A94-(20 x -19) (*ms6*) x A00-39	3-way	+4*	+2	+17*	-7	+33	-13
	4-way	+3*	+1	+19*	+6	+25	-13
	BC_1	+3*	+3*	-16*	-20	-5	-7
	BC_2	+2*	+2	+4	+1	-19	-20
A00-72 (*ms8*) x A00-68	3-way	+4*	0	+15*	-4	+60*	-7
	4-way	+2*	-1	0	-9	+22	-20
Mean		**+4**	**+2**	**+9**	**-3**	**+15**	**-16**

[a] 3-way cross = F_1 family x DSR Exp.202b

[b] 4-way cross = 3-way cross x GH 4190

[c] BC_1 = F_1 family x male parent

[d] BC_2 = BC_1 x male parent

*P-value ≤ 0.05

TABLE 9. Mean values for yield, seed size, seed protein content, seed oil content, days to maturity, and plant height for checks, parents, 5-way crosses[a], and BC$_3$[b] populations at one location near Ames, IA in 2006.

	Population	Yield (Kg ha^{-1})	Seed size (mg seed^{-1})	Protein (g Kg^{-1})	Oil (g Kg^{-1})	Maturity[c] (days)	Height (cm)
Parents							
A00-39 (*Ms2Ms2*)		2698	171	427	183	145	117
A00-41 (*Ms2Ms2*)		1902	123	438	158	155	112
A00-63 (*Ms2Ms2*)		2762	180	438	174	142	100
A00-68 (*Ms3Ms3*)		1895	125	437	160	151	113
A00-72 (*Ms8Ms8*)		1976	134	432	165	149	102
A00-73 (*Ms9Ms9*)		2521	145	443	165	145	97
A94-(20 x -19) (*Ms6Ms6*)		1935	152	436	166	161	104
Wells		2749	156	439	174	145	102
Corsoy 79		3030	127	426	180	146	138
DSR Exp.202b		3205	142	418	193	142	75
DSR Exp.202c		3734	137	430	184	145	94
GH 4190		3229	138	423	172	154	114
Hark		2754	155	443	181	145	107
Raiden		2087	154	433	169	145	64
Mean		**2606**	**146**	**433**	**173**	**148**	**103**
Family[d]							
A00-39 (*ms2*) x Corsoy 79	5-way	2978	156	431	173	156	108
	BC$_3$	2428	152	434	170	154	109
Mean		2703	154	432	172	155	109
A00-39 (*ms2*) x Hark	5-way	2434	160	421	174	161	109
	BC$_3$	2808	161	441	168	158	129
Mean		2621	161	431	171	159	119
A00-41 (*ms2*) x A00-73	5-way	2339	154	417	176	161	135
	BC$_3$	2593	151	440	165	161	108
Mean		2466	153	429	170	161	121
A00-63 (*ms2*) x Wells	5-way	2383	144	423	177	162	114
	BC$_3$	2157	152	434	169	156	130
Mean		2270	148	429	173	159	122
A00-68 (*ms3*) x A00-41	5-way	2710	150	432	176	158	108
	BC$_3$	1839	160	432	169	157	130
Mean		2274	155	432	172	158	119
A00-73 (*ms9*) x Raiden	5-way	2578	159	423	178	161	116
	BC$_3$	2320	175	433	171	158	107
Mean		2449	167	428	174	160	111

TABLE 9. (continued).

	Population	Yield (Kg ha^{-1})	Seed size (mg seed^{-1})	Protein (g Kg^{-1})	Oil (g Kg^{-1})	Maturity^{c} (days)	Height (cm)
A94-(20 x -19) (*ms6*) x A00-39	5-way	2790	141	427	174	159	118
	BC$_3$	2106	162	431	168	161	116
Mean		2448	152	429	171	160	117
A00-72 (*ms8*) x A00-68	5-way	2333	157	440	171	161	117
Overall mean of families		**2453**	**156**	**431**	**172**	**159**	**117**
Checks							
IA2068		4635	132	399	189	142	104
Apex		2895	156	376	203	147	44
Mean		3765	144	387	196	145	74
LSD		**497**	**15**	**12**	**9**	**7**	**15**

[a] 5-way cross = 4-way cross x DSR Exp.202c

[b] BC$_3$ = BC$_2$ x male parent

[c] Maturity = days from planting

[d] Family = Initial single-cross combination between the male-sterile, female-fertile lines and the male parent line

TABLE 10. Mean values for yield, seed size, seed protein and oil content, days to maturity, and plant height for checks, parents, 5-way crosses[a], and BC_3[b] populations for each *ms* hybrid group at one location near Ames, IA in 2006.

Hybrid group	Population	Yield (Kg ha^{-1})	Seed size (mg seed^{-1})	Protein (g Kg^{-1})	Oil (g Kg^{-1})	Maturity (days)	Height (cm)
ms2	5-way	2534	154	423	175	160	116
	BC_3	2497	154	437	168	157	119
Mean		**2515**	**154**	**430**	**172**	**158**	**118**
ms3	5-way	2710	150	432	176	158	108
	BC_3	1839	160	432	169	157	130
Mean		**2274**	**155**	**432**	**172**	**158**	**119**
ms9	5-way	2578	159	423	178	161	116
	BC_3	2320	175	433	171	158	107
Mean		**2449**	**167**	**428**	**174**	**160**	**111**
ms6	5-way	2790	141	427	174	159	118
	BC_3	2106	162	431	168	161	116
Mean		**2448**	**152**	**429**	**171**	**160**	**117**
ms8	5-way	2333	157	440	171	161	117

[a] 5-way cross = 4-way cross x DSR Exp.202c

[b] $BC_3 = BC_2$ x male parent

TABLE 11. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) for yield, seed size, seed protein and seed oil content, days to maturity, and plant height for 5-way crosses[a], and BC_3[b] populations at one location near Ames, IA in 2006.

Family	Population	Yield		Seed size		Protein		Oil		Maturity		Height	
		MPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	MPH (%)	HPH (%)	HPH (%)	MPH (%)	HPH (%)	MPH (%)	HPH (%)
A00-39 (*ms2*) x Corsoy 79	5-way	-13*	-20	+12*	-9	+1	+7	-22*	0	-5*	-10*	+6*	+1
	BC_3	-19*	-20	+17*	-11	+2	-20*	-21	+2	-6*	-7	+6*	+6
A00-39 (*ms2*) x Hark	5-way	-29*	-35*	+13*	-7	-1	+11	-7	-5*	-4*	-10*	+10*	+5
	BC_3	+2	+2	+3	-6	0	+19*	+10	0	-7*	-8	+9*	+9
A00-41 (*ms2*) x A00-73	5-way	-30*	-37*	+12*	+6	-3*	+38*	+19	-6*	-2	-9*	+9*	+4
	BC_3	+4	+3	+5	+4	0	+10	-4	0	0	0	+11*	+4
A00-63 (*ms2*) x Wells	5-way	-30*	-36*	+2	-20*	-1	+17*	0	-4	-2	-8	+10*	+5
	BC_3	-22*	-22	-3	-16	-1	+28*	+28*	-1	-3	-3	+8*	+8
A00-68 (*ms3*) x A00-41	5-way	-18*	-27*	+10*	+6	+1	+9	-5	-1	-2	-9*	+7*	+2
	BC_3	-3	-3	+30*	+28*	-1	+15*	+15	-1	+7*	+5	+2	+2
A00-73 (*ms9*) x Raiden	5-way	-23*	-31*	+14*	+3	-1	+22*	+2	-4	-1	-8	+10*	+5
	BC_3	+10	-8	+14*	+14	0	+62*	+10	-2	+1	+1	+9*	+9*
A94-(20 x -19) (*ms6*) x A00-39	5-way	-17*	-25	0	-18	0	+20*	+1	-2	-4*	-10*	+7*	-1
	BC_3	-21*	-22	-4	-5	+1	0	-1	-1	-8*	-8	+10*	0
A00-72 (*ms8*) x A00-68	5-way	-30*	-38*	+14*	+10	+3*	+19*	+3	+1	-5*	-11*	+9*	+5
Overall mean		**-16**	**-21**	**+9**	**-1**	**0**	**+17**	**+2**	**-2**	**-3**	**-6**	**+8**	**+4**

[a] 5-way cross = 4-way cross x DSR Exp.202c

[b] $BC_3 = BC_2$ x male parent

*P-value ≤ 0.05

APPENDIX I

TABLE 1. Analysis of variance for yield, seed size, seed protein and seed oil content, days to maturity, plant height, and lodging for checks, parents, F_1, 3-way crosses[a], 4-way crosses[b], BC_1[c] and BC_2[d] populations at one location near Ames, IA in 2005.

Source of variation	df	Mean squares						
		Yield	Seed size	Protein	Oil	Maturity	Height	Lodging
Genotype	47	448067*	2.595*	2.683*	0.769*	26.211*	261.499*	0.617*
Replication	1	3486957*	0.002	6.567*	0.040	15.844*	1127.853*	19.260*
Residual	47	54789	0.267	0.353	0.091	0.567	65.745	0.282

[a] 3-way cross = F_1 family x DSR Exp.202b

[b] 4-way cross = 3-way cross x GH 4190

[c] BC_1 = F_1 family x male parent

[d] BC_2 = BC_1 x male parent

* P-value ≤ 0.05

TABLE 2. Combined analysis of variance for yield, seed size, seed protein and seed oil content, days to maturity, plants height, and lodging for checks, parents, 5-way crosses[a] and BC_3[b] populations at one location near Ames, IA in 2006.

Source of variation	df	Mean squares					
		Yield	Seed size	Protein	Oil	Maturity	Height
Genotype	30	712130*	4.001*	5.502*	2.419*	104.083*	1010.424*
Replication	1	7253	0.441	0.354	0.530	33.470	68.646
Residual	34	59795	0.580	0.303	0.156	10.427	49.129

[a] 5-way cross = 4-way cross x DSR Exp.202c

[b] BC_3 = BC_2 x male parent

* P-value ≤ 0.05

TABLE 3. Contrasts within and between groups of genotypes for yield, seed size, seed protein and seed oil content, days to maturity, plant height, and lodging for parents, F_1, 3-way crosses[a], 4-way crosses[b], BC_1[c] and BC_2[d] populations at one location near Ames, IA in 2005.

Contrast	**P-value of the difference**						
	Yield	**Seed size**	**Protein**	**Oil**	**Maturity**	**Height**	**Lodging**
Parents vs. Hybrids	0.000	0.000	0.000	0.000	0.000	0.000	0.367
Parents vs. F_1	0.000	0.001	0.000	0.004	0.000	0.175	0.058
Parents vs. Backcrosses (BC_1 and BC_2)	0.000	0.000	0.000	0.000	0.000	0.002	0.423
Parents vs. BC_1	0.006	0.000	0.000	0.125	0.000	0.628	0.338
Parents vs. BC_2	0.000	0.000	0.000	0.000	0.000	0.000	0.721
Parents vs. 3-, and 4-way crosses	0.000	0.000	0.000	0.000	0.000	0.001	0.772
Parents vs 3-way crosses	0.000	0.000	0.000	0.000	0.000	0.402	0.620
Parents vs 4-way crosses	0.375	0.000	0.002	0.250	0.000	0.000	0.898
Backcrosses (BC_1 and BC_2) vs. 3-, and 4-way crosses	0.223	0.107	0.536	0.935	0.126	0.727	0.583
BC_1 vs. BC_2	0.264	0.906	0.003	0.000	0.139	0.000	0.596
3-way crosses vs 4-way crosses	0.010	0.485	0.775	0.100	0.000	0.000	0.189
F_1 vs. BC1	0.116	0.248	0.132	0.092	0.000	0.349	0.256
F_1 vs. BC2	0.472	0.286	0.365	0.207	0.000	0.047	0.125
F_1 vs. 3-way crosses	0.638	0.999	0.452	0.662	0.000	0.462	0.035
F_1 vs. 4-way crosses	0.139	0.606	0.588	0.429	0.000	0.030	0.240

[a] 3-way cross = F_1 family x DSR Exp.202b
[b] 4-way cross = 3-way cross x GH 4190
[c] BC_1 = F_1 family x male parent
[d] BC_2 = BC_1 x male parent

TABLE 4. Contrasts within and between groups of genotypes for yield, seed size, seed protein and seed oil content, days to maturity, and plant height for parents, 5-way crosses[a] and BC_3[b] populations at one location near Ames, IA in 2006.

Contrasts	**P-value of the difference**					
	Yield	**Seed size**	**Protein**	**Oil**	**Maturity**	**Height**
Parents vs. Hybrids	0.022	0.000	0.092	0.275	0.000	0.000
Parents vs. BC_3	0.001	0.000	0.251	0.002	0.000	0.000
Parents vs 5-way	0.627	0.006	0.001	0.166	0.000	0.000
BC_3 vs 5-way	0.009	0.027	0.000	0.000	0.115	0.269

[a] 5-way cross = 4-way cross x DSR Exp.202c
[b] BC_3 = BC_2 x male parent

CHAPTER 4. GENERAL CONCLUSIONS

The first study evaluated yield and agronomic performance of soybean F_1 hybrids and estimated heterosis for yield, and other agronomic characteristics. The male-sterile alleles used in the study were introgressed into different genetic backgrounds. Thus, the results reported in this study have two possible interpretations, i) an interaction between male-sterile alleles with their respective genetic background, or ii) differences in the combining ability between the two male-sterile alleles. Additional studies including several of the male-sterility alleles, introgressed in a common genetic background may clarify these effects.

Some hybrids performed better than the mid-parent value, although none of the hybrids was better than the best parent of the cross. The low yielding background of the female lines may have had an effect in F_1 hybrid performance, and on heterosis. It is possible that if the male-sterile genes used in the study would have been introgressed in modern higher yielding genetic backgrounds, hybrids may have had better performance and consequently positive values for heterosis.

The second study evaluated yield in hybrid soybean populations developed by single-crosses, 3-way crosses, 4-way crosses, 5 -way crosses, and backcrosses (BC_1, BC_2, and BC_3), and estimated heterosis for yield, and other agronomic characteristics of the hybrid populations. Heterosis results in this study were different from the previous study, although the evaluation was done in only one year. Heterosis estimates had wide ranges for each of the traits, with some of the values being positive and high in percentage. As in the first study, F_1 hybrids of single-crosses were in general outyielded by checks and some of the male parents, possibly due to the low yielding performance of the male-sterile, female-fertile parent lines used in the original F_1 hybrids. However, hybrids from more complex population structures had better yields than the single-cross F_1 hybrids. These observations indicate that use of additional high yielding parents in the more complex crosses had a favorable impact on yield and agronomic traits, suggesting that each of the parents contributed genes to the cross with positive effects on their final expression. The results also indicated a trend for higher yield values in the backcross populations (BC_1 and BC_2) compared to the F_1. These observations agree with results obtained by Ortiz-Perez et al. (2007) in soybean, and by Lawrence and Frey (1974) in oat. A possible explanation to these observations was provided by Lawrence and Frey (1974). The authors suggested backcrossing may restore desired genetic complexes already present in adapted genotypes. If this is assumed correct, the opposite may be said, that is, genetic complexes may have been disrupted during formation of the F_1 hybrid.

Individual differences within families formed by the male-sterile genes also were observed in this study. For the *ms2* gene, the F_1 hybrids of A00-39 (*ms2*) x Corsoy 79, had the lowest yield of all other hybrids. However, in the cross A00-39 (*ms2*) x Hark, the F_1 was superior to all other hybrid populations. The result could indicate that the *ms2* mutation does not have a pleiotropic effect on the yield performance of F_1 hybrids. It also suggests specific combining ability since F_1 hybrids of the same female parent had different yields depending on the male parent used.

In both studies, the observations for plant height and lodging indicated that heterosis was present mainly in traits associated with vegetative growth. These findings are in agreement with published information (Nelson and Bernard, 1984), and according to Lewers et al. (1998) it is possible that vegetative heterosis may increase early lodging and pod abortion, in turn reducing grain yield. The positive heterosis values observed for seed protein content may be the result of the inverse correlations widely reported in the literature between seed yield and protein content, and protein and oil content. The deviations from the mid-parent value, found for all traits under study, may indicate epistatic effects, as suggested by Thorne and Fehr (1970).

The hybrid seed to conduct these experiments was obtained using insect-mediated pollinations which has been proven to be an efficient method to produce large quantities of hybrid seed. Once more information is collected, it will also be necessary to devise predictive systems to identify genotypes with good combining ability. Limited numbers of combinations were evaluated and results can not be extrapolated to other crosses, however, an insight on the complexities and difficulties about the evaluation of heterosis, and the possibility of establishing hybrid soybean as a commercial entity has been gained.

Duvick (1999) suggested the theory that gain in yield and performance, achieved with the improvement of hybrids in crops such as maize, sorghum, and sunflower, might have been similar for open-pollinated varieties, if more attention had been devoted to their improvement, starting with the then existing superior varieties. The opposite could be true for soybean. Most breeding efforts have been devoted to the improvement of inbred lines and little attention has been given to hybrid development. Thus, more research in areas such as pollinator-insect attraction, and selection of parental lines for general and specific combining ability are necessary, before hybrid soybeans become a commercial reality.