Agronomic Performance of Soybean Hybrids from Single, Three-Way, Four-Way, and Five-Way Crosses, and Backcross Populations

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Male-sterility systems combined with insect-mediated cross-pollination in soybean [Glycine max (L.) Merr.] have been shown to produce large quantities of hybrid seed, which can be useful for studying heterosis and agronomic performance in different population types. The procedure was used to obtain large quantities of hybrid seed for replicated yield trials. The objective of our study was to evaluate agronomic performance of soybean single, three-way, four-way, and five-way crosses, and three backcross generations (BC1, BC2, and BC3) to estimate heterosis for yield and other agronomic traits. Parental genotypes were male-sterile, female-fertile lines, selected for excellent insect pollinator attraction, crossed to a group of male parents selected for their agronomic performance. In 2003, F1 seed of single crosses were evaluated in replicated experiments at two locations in Iowa and

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one location in Indiana. In 2004, three-way crosses and BC$_1$ populations were evaluated at one location in Iowa. In 2005, hybrid seed of single, three-way, and four-way crosses, BC$_1$, and BC$_2$ populations of eight families were planted at one location in Iowa. In 2006, the five-way crosses and BC$_3$ populations were planted at one location in Iowa. In all experiments, hybrids and parents were evaluated. Results showed a range in mid-parent heterosis values (MPH) for yield from $-35\%$ to $+42\%$, and in high-parent heterosis (HPH) from $-44\%$ to $+42\%$. For yield, backcross populations had higher heterosis estimates than the other population types. For seed protein content, mean MPH ranged from $-1\%$ to $+7\%$, and mean HPH from $-4\%$ to $+7\%$. For oil content, mean MPH ranged from $-10\%$ to $+6\%$, and mean HPH from $-13\%$ to $+3\%$. Negative heterosis values were observed, indicating that a large number of different parental combinations need to be evaluated to identify the superior heterotic combinations. These evaluations will determine the feasibility of hybrid soybean production for commercial purposes.

**KEYWORDS** soybean, Glycine max, heterosis, male-sterility, insect-mediated cross-pollination, agronomic performance, single crosses, three-way crosses, four-way crosses, five-way crosses, backcrosses

**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 the superior yield over traditionally bred cultivars. Hybrid superiority over their parental inbred lines, heterosis, or hybrid vigor is expressed when parents of the hybrid have different alleles at a locus, and there is some level of dominance among the alleles (Falconer & Mackay, 1996).

Soybean is an autogamous species in which current commercial cultivars are inbred lines. Heterosis, however, has been reported in soybean. A summary of 14 experiments conducted since 1930, in which a total of 456 different crosses were evaluated, indicated that average mid-parent heterosis (MPH) values for yield grain ranged from $+14\%$ to $+46\%$, and high-parent heterosis (HPH) values ranged from $+4\%$ to $+34\%$ (Palmer et al., 2001). The majority of the experiments were conducted using space-planted $F_1$ plants, and population types were single crosses.
Other experiments, in which larger quantities of hybrid seed were available, were conducted in replicated plots. In these cases, average yield MPH estimates for 2, 27, and 7 hybrid combinations were +26%, +3%, and +4%, respectively (Brim & Cockerham, 1961; Nelson & Bernard, 1984; Lewers, 1996). In China, Sun et al. (1999) summarized data from a comprehensive heterosis test program in which 846 combinations out of 1,123 showed positive MPH estimates. Of those, 248 had a mean HPH value of +20%. In this evaluation, production of F1 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 F1 generation of two parental combinations derived from crosses between current soybean cultivars. The average yield of one cross was +16% greater than the highest-yielding parent, and the average yield of the other cross was +5% greater than the highest-yielding parent. Hybrid lines evaluated were derived from single-cross populations developed by hand pollination.

In spite of these results, commercial exploitation of heterosis in soybean has not yet been realized. Difficulties in obtaining the large quantities of hybrid seeds 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 plantings. Hybrids have been mostly evaluated in single-row plots, in some cases using spaced F1 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 mostly 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; e.g., hybrids in which more than two parents are crossed, i.e. three-, four-, and five-way crosses, and even backcross lines.

Soybean populations developed by three-way, four-way, and backcrosses have been used with the objective of line development for yield improvement, but not as a means to determine heterosis. Thorne and Fehr (1970), in the comparison of single and three-way crosses, reported that three-way cross-population means usually were significantly higher than single crosses for yield, seed protein and oil contents. Gianzio and Voss (1994) compared lines from single and three-way crosses, and backcross populations, and observed that populations developed by backcrossing had the highest yield followed by three-way and single-cross populations. Cober and Voldeng (2000) evaluated lines from backcross and single-cross populations and found that average seed yield and protein content of backcross lines were no different than lines from the single crosses. No heterosis estimates were provided in these studies.
The study reported herein refers to evaluations of single, three-way and backcross populations conducted in 2003 and 2004, and to the evaluation of complex crosses and additional backcross generations conducted in 2005 and 2006. The objective was to evaluate agronomic performance of soybean single, three-way, four-way, and five-way crosses, and three backcross generations (BC1, BC2, and BC3) to estimate heterosis for yield and other agronomic traits.

MATERIALS AND METHODS

Single, three-way, four-way, five-way crosses, and backcross generations (BC1, BC2, and BC3) of eight different families were evaluated (Table 1). A family was formed by each original single-cross hybrid developed by crossing seven nuclear male-sterile, female-fertile lines as female parent to 11 lines as male parents. Complex or different types of population structures were formed from this starting point, identified hereinafter as a family. In all field experiments, the dominant male-fertile, female-fertile sibling of each homozygous recessive male-sterile, female-fertile parent was planted in the same field along with hybrids to calculate heterosis estimates. The male parents also were evaluated.

Plant Materials

Female parents had excellent insect pollinator attraction and good agronomic characteristics previously selected by Ortiz-Perez et al. (2007). Genetic lines segregating for nuclear male-sterile mutations were used as female parents; ms2 (A00-39 and A00-41; Cervantes-Martinez et al., 2007), ms3 (T284H; Chaudhari & Davis, 1977), ms6 (T295H; Skorupska & Palmer, 1989), ms8 (T358H), and ms9 (T359H; Palmer, 2000). Male parents were

<table>
<thead>
<tr>
<th>TABLE 1 Parentage of Each Hybrid Generation (Single Cross, BC1, BC2, BC3, Three-, Four-, and Five-Way Crosses)</th>
</tr>
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<tbody>
<tr>
<td>Generation</td>
</tr>
<tr>
<td>Single cross</td>
</tr>
<tr>
<td>BC1</td>
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<td>BC2</td>
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<td>BC3</td>
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<td>Three-way cross</td>
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<tr>
<td>Four-way cross</td>
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<tr>
<td>Five-way cross</td>
</tr>
</tbody>
</table>

⁺⁺♂ = Female parent.
⁺⁺⁺RP = recurrent parent = male parent.
selected on the basis of excellent agronomic characteristics. Every year, a subset of the hybrid generations was tested. Field-test performances were conducted in several years. In 2003, single-cross populations were planted. The BC₁ and three-way cross populations were evaluated in 2004. In 2005, F₁ hybrid seed of single, three-way, four-way, BC₁, and BC₂ crosses were evaluated. The BC₃ and five-way crosses were evaluated in 2006.

Hybrid Crosses from Complex Population Structures

Hybrid seed production was accomplished in several steps. In all cases when cross-pollination was required, insect-mediated cross-pollination was used. Initial single-cross combinations between the male-sterile, female-fertile, and the male parent lines were defined as a full-sib family. There were eight such full-sib families. From each of the original families, different hybrid populations were developed. F₁ hybrid seed of single crosses were obtained in Plainview, Texas, and some in Massai, Chile. From each location a seed sample was saved for yield trials. A second sample was planted for one generation of selfing at the off-season nursery near Isabela, Puerto Rico, where 40 to 60 F₂ plants were harvested individually. F₂ seed was planted for a second generation of selfing at the same nursery location. One set of male-sterile, female-fertile F₂:₃ plants was crossed to the recurrent male parent to produce the first backcross generation, BC₁. The other set of male-sterile, female-fertile F₂:₃ plants was crossed with a high-yielding cultivar to produce three-way crosses. Crosses were done in Plainview, Texas, using native insect pollinators. Seeds of the BC₁F₁ and three-way crosses were used for yield trials in Iowa (Table 2).

Samples of hybrid seeds were sent to the off-season nursery in Isabela, Puerto Rico where both, BC₁F₁ and three-way crosses underwent a generation of selfing. Male-sterile BC₁F₂ plants were crossed to the recurrent parent to produce the second backcross generation, BC₂. Male-sterile three-way cross plants were crossed to a high yielding cultivar to produce the four-way crosses, in Plainview, Texas. As mentioned, remnant seeds from each of the population types were saved to conduct yield trials in Iowa and for selfing in Isabela, Puerto Rico. After selfing, male-sterile BC₂F₂ plants were crossed to the recurrent parent to produce the third backcross generation, BC₃. Male-sterile four-way cross plants were crossed to a high yielding cultivar to produce the five-way crosses (Table 2).

Six-row plots were used for insect-mediated cross-pollination to produce hybrid seeds. Rows one and six were planted with the male parent; and rows two to five, with the segregating male-sterile line, used as female parent. Rows, spaced 76 cm apart, were 4.8 m long. Planting density was 14 seeds/m. Separation between neighboring plots of different families was 1.2 m. Each plot was replicated three times in a randomized complete-block design.
TABLE 2 Timetable for the Development of Single Cross, BC1, BC2, BC3, Three-way, Four-way, and Five-way Cross Hybrid Soybean Seed Used for the Conduct of Yield and Agronomic Performance Trials

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Population development</th>
<th>Testing of hybrids (F1 seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plainview, TX, and Massai, Chile</td>
<td>2002</td>
<td>Single-crosses (F1)</td>
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</tr>
<tr>
<td>Isabela, PR</td>
<td>2002</td>
<td>Selfing of the F1</td>
<td></td>
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<tr>
<td>Ames and Gilbert, IA, and Otterbein, IN</td>
<td>2003</td>
<td>Selfing of the F2</td>
<td>Agronomic evaluation of single crosses</td>
</tr>
<tr>
<td>Isabela, PR</td>
<td>2003</td>
<td>Selfing of the F2</td>
<td></td>
</tr>
<tr>
<td>Plainview, TX</td>
<td>2004</td>
<td>BC1F1 : Cross between F2/3 male-sterile plants and recurrent parent</td>
<td>Three-way : Cross between F2/3 male-sterile plants and DSR Exp.202b</td>
</tr>
<tr>
<td>Isabela, PR</td>
<td>2004</td>
<td>Selfing of the BC1F1 crosses</td>
<td>Selfing of the three-way crosses</td>
</tr>
<tr>
<td>Ames, IA</td>
<td>2004</td>
<td>Selfing of the BC1F1 crosses</td>
<td></td>
</tr>
<tr>
<td>Plainview, TX</td>
<td>2004</td>
<td>BC1F1 : Cross between BC1F2 male-sterile plants and recurrent parent</td>
<td>Four-way : Cross between Three-way male-sterile plants and GH 4190</td>
</tr>
<tr>
<td>Isabela, PR</td>
<td>2005</td>
<td>Selfing of the BC2F1 crosses</td>
<td>Selfing of the Four-way crosses</td>
</tr>
<tr>
<td>Ames, IA</td>
<td>2005</td>
<td>Selfing of the BC2F1 crosses</td>
<td></td>
</tr>
<tr>
<td>Plainview, TX</td>
<td>2005</td>
<td>BC1F1 : Cross between BC1F2 male-sterile plants and recurrent parent</td>
<td>Five-way : Cross between Four-way male-sterile plants and DSR Exp.202c</td>
</tr>
<tr>
<td>Ames, IA</td>
<td>2006</td>
<td>BC1F1 : Cross between BC1F2 male-sterile plants and recurrent parent</td>
<td>Five-way : Cross between Four-way male-sterile plants and DSR Exp.202c</td>
</tr>
</tbody>
</table>

Agronomic evaluation of Five-way crosses and BC1F1 crosses
At flowering, male-sterile plants were identified and labeled, and fertile siblings were removed from the middle rows (2–5). The procedure to obtain hybrid seed has been described (Ortiz-Perez et al., 2007; Perez, Cianzio, & Palmer, forthcoming) and a similar procedure was used for the study. Native bees from families Megachilidae, Halictidae, Anthophoridae, and Andrenidae were observed to carry out the pollinations. In soybean, pollinators visit the nearest flower within rows, carrying most of the pollen within rows rather than between rows (Boerma & Moradshahi, 1975; Chang & Kiang, 1987). Each plot was harvested in bulk and the number of plants per bulk ranged from 15 to 30 depending on the number of hybrid seeds per plant. The F₁ hybrid seed for each combination, along with the parental lines, were planted in summers 2003, 2004, 2005, and 2006 in Iowa for agronomic performance evaluations (Table 2).

Field Testing

Hybrids and parents were planted in a randomized complete-block design with two replications. Plots were four rows, each 5.2 m long and spaced 76 cm between rows. In 2003, the F₁ of single-cross populations were planted at two locations near Ames, Iowa, and at one location in Otterbein, Indiana. In 2004, three-way and BC₁ populations were planted at one location near Ames, Iowa. In 2005, single crosses, three-way crosses, four-way crosses, BC₁, and BC₂ populations were evaluated at one location near Ames, Iowa. Not included in the planting were BC₁ and BC₂ seeds of family A00-72 (ms₈) × A00-68, and F₁ single-cross hybrids of families A00-41 (ms₂) × A00-73, A00-63 (ms₂) × Wells, A00-73 (ms₉) × Raiden, A94-(20 × –19) (ms₆) × A00-39, and A00-72 (ms₈) × A00-68 due to shortage of hybrid seed. In 2006, five-way crosses and BC₃ seeds from all families were evaluated, except the BC₃ family of A00-72 (ms₈) × A00-68. Planting of a second location was not possible due to limited seed production.

Seed yield, seed protein and oil contents, lodging, plant height, maturity date, and seed size were evaluated. Maturity was recorded when 95% of the pods in the two middle rows were brown (stage 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 for two plants in each of the two middle rows, from the soil to the top of the plant. Lodging was a visual observation of the whole plot recorded on a 1 to 5 scale with 1 being all plants upright, 3 being plants at 45°, and 5 being prostrate. The two middle rows were harvested to estimate seed yield. Seed size also was recorded. Seed samples of 25 g were sent to the USDA National Center for Agricultural Utilization Research (NCAUR), Peoria, Illinois, to determine seed protein and seed oil content via near-infrared transmittance.
Statistical Analysis

An analysis of variance was performed separately for each agronomic variable and year, using PROC GLM of SAS (SAS Institute, 2003). Least square means (LSMEANS) were used to estimate performance of hybrids and parents for each year, and the least square difference (LSD) test was used to separate means of each trait.

Hybrid performance relative to parents was measured as mid-parent heterosis (MPH) and high-parent heterosis (HPH) (Fehr, 1991). MPH was determined as:

\[ \text{MPH} (\%) = \frac{(H - MP)}{MP} \times 100 \]

Where \( H \) = performance of the hybrid population (single cross, three-way, four-way, BC\(_1\), or BC\(_2\)) and MP = mean performance of the parents. MP for the three-way crosses was estimated as \( MP = \frac{[(P1 + P2)/2] + P3}{2} \); for the four-way crosses, \( MP = \frac{[\frac{[(P1 + P2)/2] + P3}{2}] + P4}{2} \); and \( MP = \frac{[\frac{[(P1 + P2)/2] + P3}{2}] + P4}{2} + P5}{2} \) for the five-way crosses. For the backcrosses, the MP was estimated as \( MP = \frac{[(P1 + P2)/2] + P2}{2} \) for the BC\(_1\) crosses; \( MP = \frac{[\frac{[(P1 + P2)/2] + P2}{2}] + P2}{2} \) for the BC\(_2\) crosses, and \( MP = \frac{[\frac{[(P1 + P2)/2] + P2}{2}] + P2}{2} + P2}{2} \) for the BC\(_3\) crosses. P1 and P2 are the parental lines used in the initial single crosses; P2 also is the recurrent parent used in the backcross populations. P3, P4, and P5 are the additional parents used to create the three-, four-, and five-way populations.

High-parent heterosis was determined as:

\[ \text{HPH} (\%) = \frac{(H - HP)}{HP} \times 100 \]

Where \( H_1 \) = performance of hybrid, and HP = performance of ‘high’ or best parent.

For MPH, the ESTIMATE statement of SAS v. 9.1 (SAS Institute, 2003) was used to determine differences between mean of each hybrid with that of its parents. For the HPH, the LSMEANS statement with a PDIFF option was used to estimate the difference between the hybrid and the parent with the best performance. Multiple-comparison tests were done with the Bonferroni method. The BON option of SAS v. 9.1 (SAS Institute, 2003) was used to estimate adjusted p-values for multiple comparisons.

RESULTS

Single-Cross, Three-Way, Four-Way, BC\(_1\), and BC\(_2\) Populations

In 2003, 2004, and 2005, significant (\( P \leq 0.05 \)) differences in grain yield between genotypes were observed (data not shown). In 2005, single,
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Three-way, and four-way crosses, BC₁, and BC₂ populations were evaluated, and therefore comparisons were made between hybrid populations (Figure 1). Yield of single-cross populations of families A00-39 (ms₂) × Corsoy 79 and A00-68 (ms₃) × A00-41 was lower than in the more complex population structures, i.e., three-way, four-way, BC₁, and BC₂ populations. Two of the families with single-cross populations evaluated had the same female parent with the ms₂ mutant allele. In the family A00-39 (ms₂) × Hark, yield of the single-cross population was higher than in the more complex population structures, in contrast to results observed for the family A00-39 (ms₂) × Corsoy 79. This difference could indicate that the ms₂ allele may not have pleiotropic effects on the yield of the single-cross hybrids, and/or that non-additive gene action might be present among the parents of the cross.

**FIGURE 1** Yield mean values of parents, single-cross (F₁), Three-way crosses,¹ Four-way crosses,² BC₁,³ and BC₂⁴ populations of three families at one location near Ames, Iowa, in 2005.

P₁ = Parent in the initial single cross; P₂ = Parent in the initial single cross and recurrent parent; P₃ = Additional parent in the Three-way cross and Four-way cross; P₄ = Additional parent in the Four-way cross; ¹ Three-way cross = single cross × DSR Exp.202b; ² Four-way cross = (single cross × DSR Exp.202b) × GH 4190; ³ BC₁ = single cross × recurrent parent; ⁴ BC₂ = BC₁ × recurrent parent.
Additionally, it was observed that in families A00-39 (ms2) × Corsoy 79 and A00-63 (ms2) × Wells, the BC1 population yielded significantly (P ≤ 0.05) higher than the BC2. In the family A94-(20 × −19) (ms6) × A00-39, the opposite was true—the BC2 generation had significantly (P ≤ 0.05) higher yield than the BC1. Considering three- vs. four-way crosses, five of the nine families had lower yields in the three-way than in the four-way population structures. Significant (P ≤ 0.05) differences were observed in families A00-39 (ms2) × Hark and A00-41 (ms2) × A00-73, and suggest that it might be possible to identify particular parental combinations and population structures in which superior yields may be obtained.

In 2003, average grain yield for single-cross hybrids was 1,811 kg/ha across the three locations (Table 3). The single-cross population of the family A00-39 (ms2) × Corsoy 79 had the highest mean yield value. The highest yielding parent was Corsoy 79. In 2004, the mean yield of three-way and BC1 populations across families was 2,660 kg/ha. In 2005, the mean yield of single, three-way, and four-way crosses, BC1 and BC2 hybrid populations across families was 2,598 kg/ha. The family A00-63 (ms2) × Wells had the highest mean yield of all complex hybrid populations in 2004 (three-way and BC1) and also in 2005 (three-way, four-way, BC1, and BC2 crosses). For the three years, cultivars used as male parents had the highest mean yield among all genotypes. In 2004 and 2005, the highest yielding male parents were used to develop the three- and four-way crosses (Table 3).

Hybrid populations across all families had higher seed protein and lower oil contents than their parent lines in 2003, 2004, and 2005 (Tables 4 and 5). Also, hybrids had larger seed size than the corresponding parents (Table 6). Lodging, however, was similar among all genotypes, although hybrids were taller and later maturing than parents (Table 6).

Across years and locations, positive and negative heterosis values for grain yield were observed. In 2003, MPH values for single crosses ranged from −34 to +15%, whereas the range for HPH was from −41% to +11%. The highest average MPH value was for the single cross of the family A00-41 (ms2) × A00-73 (Table 3). In 2004, positive and negative MPH values were observed for grain yield of the three-way cross and BC1 populations. In the three-way populations, the HPH values were all negative, possibly due to the high yield of the male parent used in the three-way crosses (Table 3). Positive HPH values were observed for BC1 populations. The BC1 population of the family A00-63 (ms2) × Wells showed a HPH value of +42%, the highest HPH across all hybrid populations and years. In 2005, MPH values ranged from −35% to +17%, and HPH from −44% to +13%. Positive MPH values were found in the BC1 populations of families A00-39 (ms2) × Corsoy 79 and A00-63 (ms3) × Wells. Positive HPH values observed in the BC1 population of family A00-63 (ms3) × Wells were in agreement with the results observed in 2004 (Table 3).
### TABLE 3

Mean Values for Yield, for Parents, Single-Cross, Three-Way Crosses, Four-Way Crosses, BC₁, and BC₂ Populations, and Mid-Parent Heterosis (MPH) and High-Parent Heterosis (HPH) for Hybrid Populations in Each Family, at Two Locations in Iowa and One Location in Indiana in 2003; and at One Location in Iowa in 2004, and in 2005

<table>
<thead>
<tr>
<th>Family</th>
<th>Yield (kg ha⁻¹) 2003</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Yield (kg ha⁻¹) 2004</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Yield (kg ha⁻¹) 2005</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
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<tbody>
<tr>
<td>A00-39 (Ms2ₜ)</td>
<td>1991</td>
<td>2263</td>
<td>3162</td>
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<tr>
<td>Corsoy 79</td>
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(Continued)
For seed protein content, heterosis values had a narrower range than for grain yield for MPH and HPH. Across years and hybrid populations, mean MPH values ranged from –1% to +7%, and mean HPH ranged from –4% to +7% (Table 4). Positive and negative MPH and HPH values also were observed for seed oil content in all hybrid populations. Mean MPH values ranged from –10% to +6%, and mean HPH from –13% to +3% (Table 5). For seed size, MPH values ranged from –10% to +22%, and HPH from –24% to +21% (Table 6). Positive MPH values were observed for height, lodging, and maturity, with means over all hybrid populations across families being +9% for height, +15% for lodging, and +4% for maturity. The range for height HPH values was –20% to +15%; for lodging, –28% to +7%; and for maturity, +1% to +5% (Table 6).

Five-Way and BC₃ Crosses
As mentioned, five-way and BC₃ crosses only were evaluated in 2006. For all traits, the combined analysis of variance for parents, BC₃, and five-way
TABLE 4 Mean Values for Seed Protein Content, for Parents, Single-cross, Three-way crosses\(^a\), Four-way Crosses\(^b\), BC\(_1\)\(^c\), and BC\(_2\)\(^d\) Populations, and Mid-Parent Heterosis (MPH) and High-Parent Heterosis (HPH) for Hybrid Populations in Each Family, at Two Locations in Iowa and One Location in Indiana in 2003\(^{†}\); and at One Location in Iowa in 2004\(^{††}\) and in 2005

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<th>MPH (%)</th>
<th>HPH (%)</th>
<th>2004 Seed protein (g kg(^{-1}))</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
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(Continued)
cross populations indicated significant differences (P ≤ 0.05) among genotypes (data not shown). As previously observed, the male parent cultivars had the highest yield among all genotypes, with a mean yield of 2,606 kg/ha (Table 7). The highest yielding male parent was DSR Exp.202c, which was used to develop the five-way crosses.

Mean yield of all hybrid populations across families was 2,453 kg/ha, with A00-39 (ms2) × Corsoy being the highest yielding family (Table 7). Similar to the less complex population structures, hybrid seed size across families was larger than for parents (Table 8). Also, seed protein content was higher in hybrids, whereas seed oil content was lower than parent lines. Hybrids were taller and later maturing across all families.

For yield, MPH values ranged from −35% to +42%, and HPH from −38% to +3% (Table 7). Positive MPH values were observed in the BC3 populations of families A00-39 (ms2) × Hark, A00-41 (ms2) × A00-73, and A00-73 (ms9) × Raiden. HPH values were detected in the BC3 population of the family A00-73 (ms9) × Raiden.

TABLE 4 (Continued)

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<td>HPH</td>
</tr>
<tr>
<td></td>
<td>protein</td>
<td>(%)</td>
<td>(%)</td>
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<td>A00-68f</td>
<td>381</td>
<td>414</td>
<td>419</td>
</tr>
<tr>
<td>Single cross</td>
<td>411</td>
<td>+5</td>
<td>+3</td>
</tr>
<tr>
<td>Three-way</td>
<td>411</td>
<td>+2</td>
<td>−1</td>
</tr>
<tr>
<td>Four-way</td>
<td>415</td>
<td>+3*</td>
<td>−1</td>
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<td>Additional parents of Three-waya and Four-wayb crosses</td>
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<tr>
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<td>399</td>
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<td>GH 4190b</td>
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<td>Overall mean value</td>
<td>388</td>
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<td>+1</td>
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<td>LSD</td>
<td>22</td>
<td>23</td>
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1Single crosses evaluated in 2003, Ortiz-Perez et al., 2007; 1three-way and BC1 populations evaluated in 2004, Ortiz-Perez et al., 2007; 1three-way cross = single cross × DSR Exp.202b; 1four-way cross = (single cross × DSR Exp.202b) × GH 4190; 1BC1 = single cross × recurrent parent; 1BC2 = BC1 × recurrent parent; 1dominant male-fertile, female-fertile sibling of the homozygous recessive male-sterile, female-fertile parent; 1Male-fertile recurrent parent; *P-value ≤ 0.05.
### TABLE 5

Mean Values for Seed Oil Content, for Parents, Single-Cross, Three-way Crosses\(^a\), Four-way Crosses\(^b\), BC\(_1\)^, and BC\(_2\)^, Populations, and Mid-Parent Heterosis (MPH) and High-Parent Heterosis (HPH) for Hybrid Populations in Each Family, at Two Locations in Iowa and One location in Indiana in 2003\(^†\); and at One Location in Iowa in 2004\(^††\) and in 2005

<table>
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<tr>
<th>Family</th>
<th>2003(^†) Seed oil (gkg(^{-1}))</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>2004(^††) Seed oil (gkg(^{-1}))</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>2005 Seed oil (gkg(^{-1}))</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
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<td>202</td>
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<td>197</td>
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<tr>
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</tr>
<tr>
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<td>-3</td>
<td>182</td>
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<td>-5</td>
<td>179</td>
<td>-10(^*)</td>
<td>-11(^*)</td>
</tr>
<tr>
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<td>+1</td>
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<td>-3(^*)</td>
</tr>
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<td>186</td>
<td>199</td>
<td>BC(_2)</td>
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<td>179</td>
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<td>-10(^*)</td>
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<td>-13</td>
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<td>-4</td>
<td>185</td>
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<td>-8(^*)</td>
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<td>-4</td>
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<td>Raiden(^f)</td>
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<td>-2</td>
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<td>-8(^*)</td>
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<td>+2</td>
<td>193</td>
<td>+6(^*)</td>
<td>+3</td>
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(Continued)
For seed size, MPH values ranged from −4% to +30%, and HPH from −20% to +28% (Table 8). Seed protein content of MPH values ranged from −3% to +3%, and of HPH from −6% to +2%. For seed oil content, the range was from −8% to +7% for MPH values, and −11% to +5% for HPH. For height, MPH values ranged from −20% to +62%, and HPH from −22% to +28%. And for maturity, MPH range was +2% to +11%, and −1% to +9% for HPH.

DISCUSSION

The objective of the study was to evaluate agronomic performance of soybean hybrids developed from complex crosses to determine heterosis expression of economically important traits. To develop the complex population structures, families were formed using the F1 single-cross hybrids crossed to different male parents or backcrossed to the highest-yielding parent of the single cross as recurrent parent. In some crosses, and due to limited seed production, not all hybrid types could be evaluated in a common
TABLE 6 Mean Values for Seed Size, Days to Maturity, Plant Height, and Lodging for Parents, Single-Cross, Three-way Crosses\(^c\), Four-way Crosses\(^d\), BC\(_1\)\(^e\) and BC\(_2\)\(^e\) Populations, and Mid-Parent Heterosis (MPH) and High-Parent Heterosis (HPH) for Hybrid Populations in Each Family, at One Location Near Ames, Iowa, in 2005

<table>
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<tr>
<th>Family</th>
<th>Seed size (mg seed(^{-1}))</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Maturity (days)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Height (cm)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Lodging (score)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
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<td>137</td>
<td>116</td>
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<td>116</td>
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<td>+1</td>
<td>101</td>
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<td>+4(^*)</td>
<td>115</td>
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<td>−28</td>
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<td>+8(^*)</td>
<td>143</td>
<td>+3(^*)</td>
<td>+1</td>
<td>105</td>
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<td>−12</td>
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<td>−22</td>
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<td>−7</td>
<td>139</td>
<td>+4(^*)</td>
<td>+1</td>
<td>110</td>
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<td>+71</td>
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<td>−4</td>
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<td>105</td>
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<td>4</td>
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<td>+3(^*)</td>
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<th>HPH</th>
<th>Maturity (days)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Height (cm)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Lodging (score)</th>
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<th>HPH (%)</th>
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<tr>
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<td><strong>109</strong></td>
<td><strong>17</strong></td>
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</table>

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$^a$Three-way cross = single cross × DSR Exp.202b.

$^b$Four-way cross = (single cross × DSR Exp.202b) × GH 4190.

$^c$BC$_1$ = single cross × recurrent parent.

$^d$BC$_2$ = BC$_1$ × recurrent parent.

$^e$Dominant male-fertile, female-fertile sibling of the homozygous recessive male-sterile, female-fertile parent.

$^f$Male-fertile recurrent parent.

$^*$P-value ≤ 0.05.
TABLE 7 Mean Values for Yield, Seed Protein and Seed Oil Content, for Parents, Five-Way Crosses, and BC₃ Populations, and Mid-Parent Heterosis (MPH) and High-Parent Heterosis (HPH) for Hybrid Populations in Each Family at One Location Near Ames, Iowa, in 2006

<table>
<thead>
<tr>
<th>Family</th>
<th>Yield (kg ha⁻¹)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Seed protein (g kg⁻¹)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Seed oil (g kg⁻¹)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
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<td>427</td>
<td>183</td>
<td>173</td>
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<td>-10*</td>
<td>170</td>
<td>-6*</td>
<td>-7*</td>
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<tr>
<td>Corsoy 79d</td>
<td>3030</td>
<td>426</td>
<td>180</td>
<td>431</td>
<td>+1</td>
<td>0</td>
<td>173</td>
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<td>-10*</td>
</tr>
<tr>
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<td>2978</td>
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<td>170</td>
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<td>-7*</td>
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<td>-7*</td>
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<tr>
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<td>183</td>
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<td>-8*</td>
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<td>438</td>
<td>158</td>
<td>417</td>
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<td>-6*</td>
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<td>166</td>
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</table>

Additional parents of Five-way cross

- DSR Exp.202b⁵
- GH 4190⁶
- DSR Exp.202a

Overall mean value

| LSD  | 497  | 12  | 9   |

*Five-way cross = ((single cross × DSR Exp.202b) × GH 4190) × DSR Exp.202c.
BC₃ = BC₂ × recurrent parent.
Dominant male-fertile, female-fertile sibling of the homozygous recessive male-sterile, female-fertile parent.
Male-fertile recurrent parent.
P-value ≤ 0.05.
TABLE 8 Mean Values for Seed Size, Days to Maturity, and Plant Height for Parents, Five-Way Crosses,\textsuperscript{a} and BC\textsubscript{3}\textsuperscript{b} Populations, and Mid-Parent Heterosis (MPH) and High-Parent Heterosis (HPH) for Hybrid Populations in Each Family at One Location Near Ames, Iowa, in 2006

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<tr>
<th>Family</th>
<th>Seed size (mg seed(^{-1}))</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Maturity(^c) (days)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
<th>Height (cm)</th>
<th>MPH (%)</th>
<th>HPH (%)</th>
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<td>+10*</td>
<td>+9*</td>
<td>+9*</td>
<td>107</td>
<td>+62*</td>
</tr>
<tr>
<td>A00-39\textsuperscript{d}</td>
<td>152</td>
<td>161</td>
<td>104</td>
<td>145</td>
<td>125</td>
<td>107</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Five-way</td>
<td>141</td>
<td>141</td>
<td>159</td>
<td>0</td>
<td>+7*</td>
<td>+2</td>
<td>+2</td>
<td>118</td>
<td>+20*</td>
</tr>
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<td>BC\textsubscript{3}</td>
<td>162</td>
<td>162</td>
<td>161</td>
<td>-4</td>
<td>-5</td>
<td>+10*</td>
<td>0</td>
<td>116</td>
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<tr>
<td>A00-72 (Ms(8^c))</td>
<td>134</td>
<td>134</td>
<td>149</td>
<td>0</td>
<td>-18*</td>
<td>-1</td>
<td>+20*</td>
<td>+4</td>
<td>75</td>
</tr>
<tr>
<td>A00-68\textsuperscript{d}</td>
<td>125</td>
<td>125</td>
<td>137</td>
<td>0</td>
<td>-18*</td>
<td>-1</td>
<td>+20*</td>
<td>+4</td>
<td>75</td>
</tr>
<tr>
<td>A00-39\textsuperscript{d}</td>
<td>157</td>
<td>157</td>
<td>161</td>
<td>+14*</td>
<td>+10*</td>
<td>+9*</td>
<td>+9*</td>
<td>117</td>
<td>+19*</td>
</tr>
<tr>
<td>Five-way</td>
<td>152</td>
<td>152</td>
<td>152</td>
<td>+14*</td>
<td>+10*</td>
<td>+9*</td>
<td>+9*</td>
<td>117</td>
<td>+19*</td>
</tr>
</tbody>
</table>

**Additional parents of Five-way\(\textsuperscript{a}\) crosses**

| DSR Exp.202b\textsuperscript{a} | 142 | 142 | 75 |
| GH 4190\textsuperscript{a} | 138 | 154 | 114 |
| DSR Exp.202c\textsuperscript{a} | 137 | 145 | 94 |

**Overall mean values**

| LSD | 15 |

\(^a\)Five-way cross = ((single cross × DSR Exp.202b) × GH 4190) × DSR Exp.202c.

\(^b\)BC\textsubscript{3} = BC\textsubscript{2} × recurrent parent.

\(^c\)Dominant male-fertile, female-fertile sibling of the homozygous recessive male-sterile, female-fertile parent.

\(^d\)Male-fertile recurrent parent.

\*P-value ≤ 0.05.
environment. Also some of the complex cross evaluations were only conducted during one year. Genotypic effects and heterosis estimates for some of the populations are therefore 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. In the complex populations, the use of additional high-yielding parents had favorable impacts on yield and agronomic traits. These observations suggest that each parent contributed genes with positive effects, and that probably additive gene action is present for the traits evaluated. The observation that hybrids from more complex populations had higher yields than those of the single crosses also supports the importance of the contribution of favorable alleles from each additional parent. In future evaluations, it will also be very important to introgress the male-sterile genes into high-yielding genetic backgrounds. With both parents of the single cross being high-yielding lines, it will be possible for each to contribute alleles with positive effects. These observations agree with previous reports in which additive gene action has been mentioned as responsible for the expression of grain yield (Brim & Cockerham, 1961). Burton and Brownie (2006), nevertheless, suggested several possible explanations for the genetic basis of heterosis in soybean; such as gene complementation; linked dominant alleles inherited as a unit, a greater number of dominant alleles in the F1 than in the parents, and multiple dosage-dependant regulatory loci and/or overdominance. The simultaneous evaluations of genotypes in common environments will facilitate identification of desirable parental combinations.

Individual differences in male-sterile gene performance, depending on the male parent used in the single cross, were observed in our study. For the ms2 mutation, the single-cross hybrids of A00-39 (ms2) × Corsoy 79 had the lowest yield. The cross A00-39 (ms2) × Hark, however, had single-cross populations that were superior to all other hybrid populations (Figure 1). This could be an indication that it might be possible to identify male-sterile genes showing specific combining ability effects since single-cross populations of the same female parent yielded differently depending on the male parent used in the cross.

Poor yielding hybrid populations also had low seed oil and high protein contents, in agreement with correlations previously reported (Miller & Fehr, 1979; Brim & Burton, 1979; Burton, 1987). Hybrids had larger seed size than the parents. These results have been similarly observed when hybrids were developed using male-sterility systems and insect-mediated cross-pollination (Nelson & Bernard, 1984; Ortiz-Perez, 2005), and also when hybrids were produced by hand-pollination (Manjarrez-Sandoval et al., 1997; Burton & Brownie, 2006). In general, hybrids were taller; however, lodging was similar for all populations. The observations also
agree with previous reports, which indicated that hybrids had more vegetative growth, i.e., vegetative heterosis (Nelson & Bernard, 1984; Lewers, 1996).

Heterosis in this study was variable, as it also has been in previous reports. Across families, heterosis estimates for grain yield were within reported ranges (Weber, Empig, & Thorne, 1970; Chaudhari & Singh, 1974; Nelson & Bernard, 1984; Cerna et al., 1997; Manjarrez-Sandoval et al., 1997; Lewers et al., 1998; Pandini, Natal, & Celis de Almeida Lopes, 2002; Burton & Brownie, 2006; Ortiz-Perez et al., 2007). Deviations from the mid-parent value were detected in the BC1 and BC3 populations, as well as in HPH values for yield. The observations may suggest epistatic effects, possibly interaction of additive × additive, as previously observed by Leininger and Frey (1962), Thorne and Fehr (1970), and Lamkey and Edwards (1999).

Results also indicated that other parental combinations, more complex than the classic single crosses, could contribute to heterosis expression in soybean, i.e., backcross populations (parental combination that showed higher heterosis for yield) and four-way crosses. An important consideration for the use of backcross and more complex crosses as a means to exploit heterosis is that more seasons would be required for hybrid seed production than with single crosses. Use of these population structures could impose time and labor frames that may increase cost of seed production for commercial plantings. The trends observed in this study open up new possibilities for heterosis use in commercial soybean production. Before definite conclusions may be drawn, however, replicated evaluations in common environments of single-cross combinations, along with more complex crosses, need to be conducted.

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