Physical, Compositional, and Wet Milling Characteristics of Grain from Crosses of Corn Inbreds with Exotic and Nonexotic Background

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ABSTRACT

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Corn breeders have developed hybrids with enhanced compositional characteristics, but exotic germplasm represents little of the germplasm base used to produce these hybrids. Effects of the exotic germplasm on physical, compositional, and wet-milling properties as well as the proximate composition of recovered fractions need to be determined before these materials are of value to the corn processing industry. Ten lines from the Germplasm Enhancement of Maize (GEM) project with exotic germplasm introgressed from Argentina, Chile, Uruguay, Cuba, and Florida were crossed to three adapted inbred lines (testers) and grain from the resulting 30 hybrids were evaluated for physical, compositional, and wet-milling characteristics and the expression of heterosis in these variables. The B73xMo17 adapted public hybrid was used as control. Grain obtained by self-pollination of the hybrid plants was analyzed using near-infrared transmittance (NIT) technology and a 100-g wet-milling procedure. There was great variation among physical, compositional, and wet-milling characteristics, and some of the experimental hybrids with exotic origin had better starch yield and starch recovery than B73xMo17, which suggests that wet-milling characteristics of U.S. hybrids can be improved by breeding with exotic germplasm. In particular, GEM breeding crosses AR16035:S19, CH05015:N15, CUBA117:S1520, and FS8B(T):N1802 could be valuable germplasm sources to produce inbreds with good milling properties. Testers varied in ability to produce hybrids with good milling properties, indicating that choice of tester is an important factor when evaluating this end use. Although general trait trends for mid- and high-parent heterosis were revealed, individual variation among hybrids and testers was large for most traits. This demonstrates the importance of analyzing individual hybrids that are intended for the wet-milling industry when breeding with exotic introgressed lines.

Corn breeders have developed highly productive hybrids with enhanced concentrations of starch, protein, or oil depending on their use. However, despite the economical importance of corn to the United States, hybrids largely come from one race, the Corn Belt dent (Duvick et al 2004), and <1% of the U.S. corn germplasm base had an exotic origin in 1984 (Goodman 1985), increasing to ≈3% in 1996 (Goodman 1999). This narrow germplasm base increases the risk of genetic vulnerability to insect and disease damage, affects the overall agronomic performance, reduces the odds of finding new materials with value-added characteristics, and limits our ability to face new challenges arising from climate change. The Germplasm Enhancement of Maize project (GEM), a coordinated and cooperative effort among public and private sectors, was started to improve and enhance the germplasm base of corn used to produce new hybrids in the United States. This project develops new breeding lines from crosses of adapted and exotic germplasm that are available to corn breeding programs (Pollak 2003). Exotic germplasm used in GEM was identified as high in yield by the Latin American Maize Project (LAMP), the first coordinated international project for the evaluation of corn collections (Salhuana et al 1991).

Although most ethanol is now produced by dry-grind technology (Sharma et al 2006), corn wet milling is the largest commercial source of purified starch. Corn wet milling separates corn kernels into main fractions of starch, gluten, germ, and fiber using chemical, biochemical, and mechanical operations (Singh et al 1997). Screening germplasm is the first step in developing new hybrids with more appropriate characteristics for this use. Breeding for changes in the chemical composition of the corn kernels has proven effective (Dudley and Lambert 2004), but breeding for fraction yields during the wet-milling process needs to be supported with reliable methods of estimation. The 100-g wet-milling procedure developed by Eckhoff et al (1996) and modified by Singh et al (1997) can be a useful laboratory-level procedure because it yields fraction values that are comparable to industry and reduces the cost and labor required to evaluate corn samples.

To determine the value of new corn hybrids, it is necessary to have a point of reference between performances of the hybrid with the inbred lines used as parents. A useful reference parameter is heterosis. Heterosis is the difference between the hybrid performance and the mean value of the inbred parents, or alternatively, the highest value of the best parent, for a trait of interest normally expressed as a percentage. A few studies have documented the presence of heterosis for the physical, compositional, and wet-milling characteristics of inbred lines and their hybrids in corn. Zehr et al (1995) studied 15 adapted inbred lines and 20 related hybrids and reported significant divergence of hybrids from mid-parent values for wet-milling fraction values. Singh et al (2001a) studied the expression of heterosis by crossing 10 exotic populations with the public inbred lines B73 and Mo17 and reported higher levels of protein and reduced starch contents of the hybrids relative to the inbred lines, which led to the expression of poor wet-milling properties of the hybrids, especially for low values of starch yield and starch recovery. However, these studies focused on the expression of heterosis in the F_1 (or seed) generation of hybrids but did not consider the expression of heterosis in the F_2 (or grain) generation. Knowing heterosis for the grain generation is important because that is the raw material for plants that process corn through the wet-milling procedure. Additionally, no studies have focused on the analysis of heterosis expressed by adapted inbred lines introgressed with exotic germplasm that are potential breeding lines to provide improved value-added characteristics for corn breeders.

Ten lines with exotic introgressed genetic background from the GEM project were crossed to three adapted inbred lines used as testers to 1) determine the physical, compositional, and wet-milling characteristics of hybrid grain from the self-pollination of hybrids made from crosses between exotic introgressed and adapted inbred lines, and 2) determine the expression of mid-parent and high-parent heterosis for the same characteristics and hybrids. We have previously reported characteristics of the 10 lines (Taboada-Gaytan et al 2009) and the F_1 seed generation (Taboada-Gaytan et al 2010) and will compare our results with those of the preceding generations.

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MATERIALS AND METHODS

Genetic Materials

As described in our previous studies (Taboada-Gaytan et al 2009, 2010), 10 GEM lines of five germplasm sources, each source represented by a relatively high and low starch yield line from a wet-milling screening done by the private GEM cooperator Cerestar (Hammond, IN; now acquired by Cargill, Minneapolis, MN) were selected. The lines had 50% or 25% exotic genetic background from Argentina, Chile, Cuba, Florida, and Uruguay and were grouped according to starch yield from wet milling as high-starch exotic lines (HSEL): AR16035:S19-285-1-B (AR285), CH05015: N15-182-1-B (CH182), CUBA117:S1520-562-1-B (CU562), FS8B(T): N1802-35-1-B (FS35), and UR13085:N0215-11-1-B (UR11); and low-starch exotic lines (LSEL):AR16035:S19-227-1-B (AR227), CH05015:N15 -143-1-B (CH143), CUBA117: S1520-153-1-B (CU153), FS8B(T):N1802-32-1-B (FS32), and UR13085:N0215-14-1-B (UR14). Breeding and development of GEM lines is described in Pollak (2003). Each line was crossed to three commercial inbred lines used as testers (Tester 1 with low starch and high protein content that produces commercial hybrids with low starch content and low starch yield; Tester 2 the Bt version of Tester 1; and Tester 3 with higher starch and lower protein contents than Testers 1 and 2 and that produces commerical hybrids with high starch content and high starch yield). Tester seed was provided by Golden Harvest Seeds (Clinton, IL, now acquired by Syngenta, Wilmington, DE). In this study, we present the characteristics of the F_2 generation (hybrid grain) obtained from self-pollination of the F_1 generation of hybrids. The hybrid grain obtained from self-pollinating the cross of public inbred lines B73 and Mo17 was included as a check. Physical, compositional, and wet-milling characteristics of the GEM lines, testers, B73 and Mo17 are described in Taboada-Gaytan et al (2009) and of the F_1 generation (hybrid seed) in Taboada-Gaytan et al (2010).

Sample Preparation

The F_1 generation of hybrids was produced in Clinton, IL, in the summer of 2003 and advanced to the F_2 by self-pollination during the summer of 2004 in Ames, IA. At harvest, all the self-pollinated ears from a row (3.8 m long and 76 cm wide) were hand-harvested and dried to ≈10% moisture content by circulating warm air at 38°C for 72 hr. Diseased ears and those that set few kernels were discarded. Grain from normal ears was bulked after shelling and stored at 4°C until needed. Two replicates per hybrid of bulked grain were used to determine physical, compositional, and wet-milling characteristics. Samples (100-g) were randomly selected then hand-picked to remove any foreign material and cracked or broken kernels.

Physical Characteristics

Variables measured to determine the physical characteristics of the experimental corn hybrids were test weight, 1,000-kernel weight, and absolute density. Kernel absolute density (g/cm^3) was estimated by using a grain analyzer (Foss Infratec 1241, Tecator, Hoganas, Sweden). Test weight was determined by following the Federal Grain Inspection Services procedures (FGIS 1988) then values were converted to kilograms by hectoliter (k/hL). The 1,000-kernel weight was measured using an electronic counter (model 850-2, International Marketing and Design, San Antonio, TX) to count the kernels then weighing them in a precision top-loading balance (OHAUS Explorer Pro Model EP4102C, Pine Brook, NJ).

Compositional Characteristics

Moisture, starch, protein, and oil contents of bulked whole kernels from each hybrid were estimated with near-infrared transmittance (NIT) technology using a Foss Infratec 1241 grain analyzer. NIT equipment was calibrated and standardized by the Grain Quality Laboratory (GQL) at Iowa State University; the GQL supplies a major portion of the corn and soybean NIT calibration databases of the USDA Federal Grain Inspection Service. The GQL bases its starch calibration on the wet-chemistry reference method of the Corn Refiners Association (CRA 2006). The CRA method is used by the corn wet-milling industry and is the official reference for trade determined by USDA-FGIS. The method was designed and is maintained by the wet-milling industry to be reflective of the starch yield from a traditional wet mill and was originally established to balance a wet-mill process.

Wet-Milling Characteristics

Two samples from each hybrid were analyzed using the 100-g modified wet-milling procedure of Singh et al (1997), slightly modified to improve reproducibility as described in Taboada-Gaytan et al (2009). Moisture contents of recovered starch, gluten, fiber, and germ were determined in triplicate by Method 14.004 (AOAC 1984) and used to calculate fraction yields on a dry basis. Whole kernel moisture was estimated with three replicates by Approved Method 44-15A (AACC International 2010) to calculate total solids recovery.

Composition of Recovered Fractions

Recovered starch fractions were analyzed for protein contents with the macro-Kjeldahl method of Method A-18 (CRA 2006). Recovered gluten fractions were analyzed for protein contents by Method 993.13 (AOAC 2003) using a combustion nitrogen analyzer RapidN III (Elementar Americas, Mt. Laurel, NJ) with a protein factor of 6.25. Oil contents in the germs were quantified as crude free-fat content according to the Methods 14-084 and 14-085 (AOAC 1984) using the Goldfisch procedure. Recovered fractions composition values were expressed as percentage on a dry basis (% db).

Statistical Analyses

Groups of lines and testers were considered as fixed effects and lines within groups as random factors. Proc GLM and Proc CORR (SAS Institute, Cary, NC) were used to determine statistical differences among different values and Pearson correlation coefficients. Statistical differences among groups of lines, lines within groups, and testers were determined using multiple mean comparisons with least significant differences (LSD) to estimate differences among groups, lines, and testers. Groups were tested by lines within group mean square, lines, and the interaction effect of line by tester were tested by the error mean square, and tester and the interaction effect of group by tester were tested by the line × tester mean square. All test significance were at $P = 0.05$ unless otherwise noted.

Calculation of Heterosis

The performance of a hybrid in relation to its parents was expressed as mid-parent or high-parent heterosis. Calculations were made by using formulas for each.

Mid-parent heterosis is the performance of a hybrid compared with the average performance of its parents: (%) = [(Hybrid – MP)/ MP] × 100. High-parent heterosis is a comparison of the performance of the hybrid with that of the best parent in the cross: % = [(Hybrid – HP)/HP] × 100, where MP = average performance of parents per se, given by (parent1 + parent2)/2; HP = performance of the best parent.

RESULTS AND DISCUSSION

Analysis of Variance

There were significant differences for all physical, compositional, and wet-milling variables in this study (Tables I and II). No differences appeared between groups of lines (HSEL and LSEL) and the interaction effect of group by tester, which indicated that the physical, compositional, and wet-milling characteristics of the parental lines are not strictly reflected in the F_2 or hybrid grain generation. Due to the lack of differences for the effect of groups of lines and the interaction effect of group × tester, discussion of results will be based on the effect of the different hybrids over all lines. The effect of lines was significant for all the variables under study, which means that corn lines introgressed with exotic germplasm can produce hybrids with either good or inferior specific properties and thus wide genetic diversity can be exploited to improve wet-milling parameters of new hybrids through breeding. The effect of tester was significant for test weight, absolute density, and kernel size. Testers produced hybrids with different starch and protein concentrations, showing a differential effect of the male Corn Belt parent on the compositional characteristics of the hybrids produced, but oil concentration was not affected by male parent. If the hybrids are grouped by tester, there were statistical differences ($P < 0.01$) for starch, gluten, fiber, and germ yield, which means that each tester produced progeny with different wet-milling characteristics. The interaction of female line by tester was highly significant for all variables under study, indicating differences in combining ability of lines and testers. It is better to test hybrids advanced to the F_2 generation than inbred lines because hybrid grain provides more accurate parameters of the millability of new breeding materials. This would facilitate the selection of useful parents in a corn breeding program focused on improvement of the wet-milling efficiency of new corn hybrids. However, compared to evaluating inbred lines, this would also add two breeding generations (making and self-pollinating the F_1) or one year (assuming use of a winter nursery) to the evaluation process.

Physical Characteristics

The physical properties of corn kernels can be influenced by moisture content, hybrid type, environmental conditions of production in different years, and postharvest management practices (Watson 1987). Test weight measures density or weight per unit of volume of a grain at a standardized moisture level of 15.5% (Harper 2003). Test weight of the hybrids was 73.3–80.5 with a mean of 77.6 k/hL (Table III). Similar values were reported by Li et al (1996), but our values were, in general, higher than those reported in some other studies where values of 66.8–79.5 k/hL were found (Fox et al 1992; Singh et al 1997; Raush et al 1999; Vignaux et al 2006). F_2 values were slightly lower than F_1 values (72.98–84.06 with a mean of 79.94 k/hr) (Taboada-Gaytan et al 2010) but because production of the F_1 and hybrids used in other studies were in different environments, the values need to be compared cautiously. The B73xMo17 F_2 had a value of 73.8 k/hL. According to Watson (1987), the range for dent corn hybrids was 65–75 k/hL, but starch yield and other wet-milling characteristics are not significantly affected if test weight does not drop below 61.7 k/hL (Watson 1987). In this study, higher test weight values may be influenced by drying the grain samples to ≈10% moisture content and test weight can slightly increase as corn is dried. More likely, our experimental hybrids have high test weight from contribution of exotic germplasm.

The 1,000-kernel weight of the hybrids had great variation at 305.3–433.2 g with a mean value of 377.2 g. The F_1 had lower values (232.6 × 388.4 g with a mean value of 294.0 g), but a wider range of 156 g vs. 128 g for the F_2 (Taboada-Gaytan et al 2010). The B73xMo17 hybrid had a value of 394.4 g. Similar values were reported by Fox et al (1992) when studying the wet-milling properties of 27 hybrids with a wide range of compositional and physical characteristics and Zehr et al (1995), who reported a mean of 359 g for 1,000-kernel weight for a group of hybrids representing U.S. germplasm groups. Singh et al (1997) found a range of 266.2–378.4 g for 1,000-kernel weight for one waxy and three regular yellow dent corn hybrids.

TABLE I
Analysis of Variance for Physical and Compositional Characteristics of Hybrid Grain from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers[a]

Source	df	**Mean Square** Values		
Physical Characteristics		**Test Wt**[b]	**TKW**[c]	**Abs Density**[d]
Group	1	130ns	4245.87ns	0.0001ns
Line (group)	8	13.48***	2912.41***	0.0010***
Tester	2	17.91**	5982.45*	0.0017**
Group × Tester	2	6.89ns	210.56ns	0.0003ns
Line × Tester (group)	16	2.52***	1555.91***	0.0002***
Compositional characteristics %db		**Starch**	**Protein**	**Oil**
Group	1	0.38ns	0.01ns	0.47ns
Line (group)	8	0.47***	0.88***	0.15***
Tester	2	6.89***	7.10**	0.21ns
Group × Tester	2	0.10ns	0.01ns	0.09ns
Line × Tester (group)	16	0.52***	0.80***	0.07**

[a] *, **, *** Statistically significant at 0.05, 0.01, 0.001 probability levels; ns, not significant.

[b] Test weight (K/hL).

[c] 1,000 kernel weight (g).

[d] Absolute density (g/cm^3).

TABLE II
Analysis of Variance for Selected Wet-Milling Characteristics of Hybrid Grain from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers[a]

		Mean Square Values of Wet-Milling Characteristics (% db)			
Source	**df**	**Starch**	**Gluten**	**Fiber**	**Germ**
Group	1	2.46ns	0.01ns	1.84ns	2.14ns
Line (group)	8	9.36***	5.68***	3.76***	4.64***
Tester	2	36.19**	20.36**	4.12**	4.45**
Group × Tester	2	0.01ns	2.00ns	0.46ns	0.92ns
Line × Tester (group)	16	4.32***	2.63***	0.48**	0.51ns

[a] **, *** Statistically significant at 0.01 and 0.001 probability levels; ns, not significant.

Absolute density, a measure of weight per unit of volume, had a mean of 1.28 g/cm^3 and was similar to the values reported by previous studies (Fox et al 1992; Zehr et al 1995; Raush et al 1999) and to the F$_1$ with a mean of 1.305 g/cm^3 (Taboada-Gaytan et al 2010). Absolute density in corn varies at 1.18–1.40 g/cm^3 (Rooney et al 2004). Kernel density is affected by moisture content and can affect the yields of the wet-milling fractions of a corn hybrid because absolute density is primarily determined by the kernel hardness, which is an index of the relative proportion of horny to floury endosperm (Correa et al 2002). Starch from floury endosperm is easier to recover than starch from horny endosperm (Watson 1987).

Compositional Characteristics

Starch, protein, and oil contents were statistically different at $P < 0.01$ (Table I). The mean starch content of the experimental hybrids was 70.2% db; slightly higher than that of B73xMo17 which was 69.6% (Table III). These values are higher than those for the F$_1$ seed with a mean of 68.2 and 68.7% for B73xMo17 (Taboada-Gaytan et al 2010). Yellow dent corn hybrids grown in the United States contain 65–70% starch (Rooney et al 2004). Tester 3 produced hybrids with significantly higher starch contents, with a mean of 70.9% db (Table IV) than Testers 1 and 2 (means of 69.8 and 70.0% db, respectively). All hybrids with exotic germplasm and starch contents of >71% db had Tester 3 as the male parent (Table III). These results were similar to those reported for yellow dent corn hybrids (Dowd 2003; Vignaux et al 2006) but lower than the starch contents of some commercial hybrids used in other studies where starch contents were 71.8–76.1% (Singh et al 1997, 2005).

The protein contents of the experimental hybrids ranged from 7.8% for FS32xTester 3 to 11.05% for UR11xTester 1 with a mean of 9.9% (Table III). B73xMo17 had a protein content of 11.1%. As expected, Testers 1 and 2 produced hybrids with higher protein contents (10.3 and 10.2%, respectively) than hybrids where Tester 3 was the male parent (9.2%). These values were within the range of protein content reported for yellow dent hybrids cultivated in the United States of 8–10% (Rooney et al 2004). The protein contents of hybrids with exotic introgressed background were higher than values reported for several yellow corn dent hybrids (Fox et al 1992; Singh et al 1997, 2005; Vignaux et al 2006) but lower than values reported for the F$_1$ (mean 11.95%) reported by Taboada-Gaytan et al (2010).

The F$_1$ of CH143xTester 2 had a very high protein content of 14.1% in that study, and the F$_2$ was also among the highest in protein content. Values >10% were considered high and it was a factor that contributed to lower than anticipated starch recovery from wet milling because protein has negative correlation with starch yield.

Oil contents in the corn kernels were 4.0–5.3% with a mean of 4.54% db for the experimental hybrids, while the control hybrid contained 4.4% (Table III). These values were slightly higher than the values of normal yellow dent corn hybrids at 3–5% (Orthoefer and Eastman 2004) but can reach values of 8% for high-oil corn hybrids (Raush et al 1999) and similar to the values reported for the F$_1$ (Taboada-Gaytan et al 2010).

The information related to compositional characteristics of the experimental hybrids used in this study indicates that genetic background affects variation in the main components of corn kernels.

TABLE III
Physical and Compositional Characteristics of Hybrid Grain from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers[a]

		Physical Characteristics			Compositional Characteristics (%)		
Line	Tester	Test Wt[b]	TKW[c]	Abs. Density[d]	Starch	Protein	Oil
AR227	1	80.3 ± 0.28	330.7 ± 0.16	1.291 ± 0.00	70.7 ± 0.14	9.3 ± 0.00	4.7 ± 0.07
	2	79.0 ± 0.29	398.5 ± 2.08	1.290 ± 0.00	70.6 ± 0.00	9.7 ± 0.07	4.4 ± 0.00
	3	78.3 ± 0.01	399.3 ± 2.21	1.280 ± 0.00	70.3 ± 0.14	9.9 ± 0.07	4.4 ± 0.07
AR285	1	80.2 ± 0.41	365.8 ± 1.63	1.297 ± 0.00	69.9 ± 0.28	10.3 ± 0.07	4.5 ± 0.07
	2	80.4 ± 0.06	376.8 ± 0.47	1.299 ± 0.00	69.9 ± 0.07	10.1 ± 0.07	4.8 ± 0.14
	3	79.0 ± 0.06	411.8 ± 3.44	1.285 ± 0.00	70.5 ± 0.07	9.6 ± 0.00	4.5 ± 0.14
CH143	1	78.0 ± 0.06	311.4 ± 3.21	1.305 ± 0.00	69.3 ± 0.07	11.0 ± 0.07	4.6 ± 0.14
	2	77.9 ± 0.05	306.9 ± 2.28	1.303 ± 0.00	69.5 ± 0.00	10.8 ± 0.07	4.7 ± 0.07
	3	74.9 ± 0.16	355.5 ± 0.86	1.267 ± 0.01	71.2 ± 0.21	9.0 ± 0.07	4.7 ± 0.21
CH182	1	78.7 ± 0.37	374.9 ± 2.33	1.305 ± 0.00	69.7 ± 0.07	11.0 ± 0.07	4.2 ± 0.07
	2	78.6 ± 0.06	356.8 ± 1.11	1.302 ± 0.00	70.0 ± 0.21	10.5 ± 0.21	4.4 ± 0.14
	3	78.3 ± 0.25	359.3 ± 2.38	1.387 ± 0.00	70.9 ± 0.14	8.9 ± 0.07	4.7 ± 0.28
CU153	1	80.0 ± 0.00	364.4 ± 1.85	1.292 ± 0.00	69.6 ± 0.21	10.3 ± 0.07	4.8 ± 0.07
	2	79.9 ± 0.11	377.1 ± 3.12	1.278 ± 0.00	69.4 ± 0.28	10.3 ± 0.14	5.0 ± 0.00
	3	76.9 ± 0.57	338.4 ± 3.45	1.270 ± 0.00	71.2 ± 0.42	8.8 ± 0.21	4.6 ± 0.21
CU562	1	78.4 ± 0.42	375.4 ± 1.72	1.280 ± 0.00	69.9 ± 0.00	10.1 ± 0.00	4.7 ± 0.07
	2	78.6 ± 0.11	395.1 ± 0.69	1.280 ± 0.00	70.3 ± 0.35	9.7 ± 0.14	4.6 ± 0.14
	3	74.4 ± 0.44	424.7 ± 2.18	1.247 ± 0.00	70.5 ± 0.07	9.9 ± 0.14	4.4 ± 0.07
FS32	1	78.7 ± 0.15	388.7 ± 0.84	1.279 ± 0.00	69.4 ± 0.07	10.4 ± 0.14	4.8 ± 0.07
	2	76.8 ± 0.17	418.4 ± 0.16	1.272 ± 0.01	69.9 ± 0.07	10.1 ± 0.07	4.9 ± 0.28
	3	73.4 ± 0.15	381.1 ± 0.04	1.236 ± 0.00	71.9 ± 0.07	7.9 ± 0.07	4.6 ± 0.14
FS35	1	76.1 ± 0.03	325.7 ± 1.40	1.275 ± 0.00	70.7 ± 0.35	9.4 ± 0.14	4.7 ± 0.14
	2	76.2 ± 0.18	432.5 ± 1.05	1.269 ± 0.00	70.2 ± 0.14	10.2 ± 0.07	4.5 ± 0.00
	3	76.3 ± 0.16	403.8 ± 0.85	1.277 ± 0.00	71.5 ± 0.07	8.6 ± 0.00	4.4 ± 0.14
UR11	1	75.0 ± 0.08	388.3 ± 3.89	1.268 ± 0.00	69.7 ± 0.14	11.1 ± 0.07	4.0 ± 0.00
	2	75.3 ± 0.43	371.8 ± 5.70	1.272 ± 0.00	70.1 ± 0.07	10.5 ± 0.00	4.2 ± 0.14
	3	76.7 ± 0.42	421.3 ± 1.84	1.276 ± 0.00	71.3 ± 0.21	9.4 ± 0.07	4.3 ± 0.07
UR14	1	78.9 ± 0.40	357.7 ± 1.48	1.301 ± 0.00	69.6 ± 0.00	10.6 ± 0.00	4.5 ± 0.07
	2	76.3 ± 0.38	376.6 ± 2.72	1.281 ± 0.01	69.9 ± 0.00	10.3 ± 0.07	5.0 ± 0.49
	3	77.3 ± 0.06	427.2 ± 2.16	1.287 ± 0.00	70.0 ± 0.00	10.5 ± 0.00	4.2 ± 0.07
B73xMo17		73.8	394.4	1.265	69.6	11.1	4.4

[a] *, **, *** Statistically significant at 0.05, 0.01, 0.001 probability levels; ns, not significant.

[b] Test weight (K/hL).

[c] 1,000 kernel weight (g).

[d] Absolute density (g/cm^3).

Wet-Milling Characteristics

Hybrids with exotic germplasm showed statistical differences for starch, gluten, fiber, and germ yields (Table II). Genetic variability among hybrids can affect production costs and economic gains of the wet-milling industry, so evaluating new hybrids before use by industry is important. Starch yield is the most important recovered fraction from the wet milling of corn (Singh and Eckhoff 1996) and is a millability indicator, the ease with which kernel components are separated (Weller et al 1988). Starch recovery is the main parameter of the millability and, as a consequence, of the quality of the hybrids for the wet-milling industry.

Starch yields of the experimental hybrids were 58.1–65.3 (Table IV) with a mean of 62.4% db. These values are higher than the F_1 (Taboada-Gaytan et al 2010) and similar to the mean starch yield of B73xMo17 which was 62.0% db. Several hybrids with exotic origin (including AR285xTester3, CH182xTester 3, FS32xTester 3, and CU562xTester 2) had better millability than B73xMo17, which shows that potential to improve wet-milling characteristics of hybrids grown in the United States through breeding with exotic germplasm. These crosses were also high in starch yield when F_1 seed was analyzed (Taboada-Gaytan et al 2010). As expected, crosses with Tester 3 had higher starch yields (Tables IV and V) except with the CU562 line, in which case Tester 2 gave higher starch yields.

In the tabling method used in most laboratory-scale wet-milling procedures (Singh and Eckhoff 1996; Dowd 2003), starch is heavier than gluten and settles in the first two-thirds of the table, while the gluten remains suspended in the water and is washed to the table end. Gluten yield was 9.6–16.6 (Table IV) with a mean of 14.1% db. Although our results are higher than those reported in other laboratory studies using exotic accessions (Singh et al 2001), adapted lines (Zehr 1995), high-oil corn hybrids (Rausch et al 1999), and commercial yellow dent corn hybrids (Singh et al 1998; Dowd 2003; Yang et al 2005; Vignaux et al 2006), they are lower than the F_1 that had a mean of 17.36% db (Taboada-Gaytan et al 2010) and comparable to our results with B73xMo17 (mean gluten yield of 15.3% db).

Protein contents of the experimental hybrids were within the range of typical commercial hybrids, so it is unlikely that our gluten results were due to the protein levels, unless some protein factor from the exotic germplasm still contributed to poor release of the starch granules. Identification of such a factor is outside the scope of our research. It is more likely that the high gluten yields were due to our laboratory wet-milling method because the gluten yield of B73xMo17 was also high.

Fiber yield was 11.3–15.4 with a mean of 12.6% db. B73xMo17 had a fiber yield of 12.2% db. These values were higher than those reported in previously cited studies, but similar to those we reported for the F_1 (Taboada-Gaytan et al 2010).

Germ yields were 2.9–6.8 with a mean of 5.1% db, also similar to those of the F_1. The lines that produced higher germ yields were CH143, FS32, and FS35 when crossed to any of the three testers, which indicated that these lines have good germ separation during the first grind of the wet-milling procedure. CH143 and FS35 also produced higher germ yields when crossed to the three testers and measured as F_1 (Taboada-Gaytan et al 2010). B73xMo17 had a germ yield of 6.6% db. These values were similar to those reported by other laboratory studies (Singh et al 1998; Dowd 2003; Yang et al 2005; Vignaux et al 2006). Our results indicated that, even if some compositional or physical characteristics from exotic germplasm affected the wet-milling properties of the materials under study, the germ yield of these experimental hybrids was not affected. However, germ yield can be significantly affected by alternative milling techniques such as gaseous SO_2 processing, alkali wet milling, and intermittent milling and dynamic steeping (Singh et al 2001).

TABLE IV
Wet-Milling Fraction Yields of Hybrid Grain from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

		Wet-Milling Characteristics (% db)					
Line	Tester	Starch	Gluten	Fiber	Germ	Steep Water	SR[a]
AR227	1	63.6 ± 0.21	14.4 ± 0.23	11.6 ± 0.14	4.5 ± 0.04	5.6 ± 0.04	89.9 ± 0.12
	2	62.8 ± 0.04	15.9 ± 0.18	12.0 ± 0.58	2.9 ± 0.67	5.8 ± 0.02	89.0 ± 0.06
	3	62.8 ± 0.06	14.5 ± 0.33	11.4 ± 0.20	5.4 ± 0.23	5.7 ± 0.13	89.3 ± 0.26
AR285	1	63.0 ± 0.17	14.0 ± 0.12	12.3 ± 0.08	4.7 ± 0.22	5.3 ± 0.08	90.1 ± 0.61
	2	63.1 ± 0.00	14.8 ± 0.66	12.2 ± 0.52	4.0 ± 1.13	5.5 ± 0.07	90.3 ± 0.09
	3	64.2 ± 0.07	14.6 ± 0.25	11.6 ± 0.01	3.9 ± 0.10	5.4 ± 0.04	91.1 ± 0.19
CH143	1	60.4 ± 0.17	14.9 ± 0.85	12.0 ± 1.05	6.3 ± 0.11	5.8 ± 0.08	87.2 ± 0.16
	2	59.4 ± 0.41	15.6 ± 1.65	12.5 ± 0.30	5.9 ± 1.43	6.0 ± 0.13	85.5 ± 0.59
	3	63.9 ± 0.23	12.0 ± 0.23	11.3 ± 0.35	6.5 ± 0.42	5.8 ± 0.05	89.9 ± 0.59
CH182	1	59.9 ± 0.19	16.6 ± 0.26	13.1 ± 0.47	4.6 ± 0.03	5.3 ± 0.00	86.0 ± 0.37
	2	62.2 ± 0.23	16.2 ± 0.46	12.9 ± 0.09	2.9 ± 0.67	5.5 ± 0.00	88.9 ± 0.61
	3	65.0 ± 0.14	12.6 ± 0.25	11.9 ± 0.15	4.7 ± 0.17	5.5 ± 0.14	91.7 ± 0.38
CU153	1	61.5 ± 0.11	15.2 ± 0.00	12.6 ± 0.23	4.4 ± 0.57	5.7 ± 0.01	88.4 ± 0.11
	2	62.6 ± 0.54	14.6 ± 0.69	12.1 ± 0.19	5.2 ± 0.30	5.6 ± 0.08	90.2 ± 1.15
	3	64.6 ± 0.05	12.4 ± 0.58	12.6 ± 0.16	4.9 ± 0.63	5.1 ± 0.18	90.8 ± 0.47
CU562	1	64.4 ± 0.26	12.8 ± 1.37	11.9 ± 0.25	5.6 ± 0.16	5.4 ± 0.11	92.2 ± 0.37
	2	65.3 ± 0.38	14.3 ± 0.29	11.6 ± 0.93	4.4 ± 1.71	5.5 ± 0.08	92.9 ± 0.07
	3	63.4 ± 0.34	14.1 ± 0.88	12.0 ± 0.22	5.0 ± 0.77	5.2 ± 0.01	90.0 ± 0.39
FS32	1	59.8 ± 0.18	14.1 ± 0.88	13.3 ± 0.10	6.8 ± 0.79	5.6 ± 0.06	86.2 ± 0.17
	2	61.8 ± 0.12	13.6 ± 0.11	13.0 ± 0.08	5.9 ± 0.20	5.7 ± 0.06	88.5 ± 0.08
	3	65.2 ± 0.27	9.6 ± 0.05	12.8 ± 0.18	6.4 ± 0.02	5.4 ± 0.09	90.7 ± 0.28
FS35	1	62.3 ± 0.48	12.9 ± 0.06	12.8 ± 0.04	6.3 ± 0.35	5.4 ± 0.08	88.2 ± 1.12
	2	61.2 ± 0.10	14.5 ± 0.24	13.4 ± 0.01	5.2 ± 0.42	5.1 ± 0.02	87.2 ± 0.32
	3	64.9 ± 0.19	11.7 ± 0.14	11.9 ± 0.01	6.0 ± 0.21	5.1 ± 0.03	90.8 ± 0.35
UR11	1	58.2 ± 0.22	15.3 ± 0.12	15.4 ± 0.12	5.7 ± 0.03	5.2 ± 0.00	83.4 ± 0.14
	2	59.0 ± 0.27	15.1 ± 0.24	14.9 ± 0.08	5.1 ± 0.48	5.3 ± 0.03	84.2 ± 0.30
	3	63.3 ± 0.35	13.2 ± 0.54	12.8 ± 0.64	5.3 ± 0.34	5.3 ± 0.01	88.8 ± 0.23
UR14	1	60.8 ± 0.11	15.4 ± 0.00	13.3 ± 0.25	4.2 ± 0.08	5.7 ± 0.22	87.4 ± 0.16
	2	61.9 ± 0.47	15.1 ± 0.36	13.1 ± 0.29	3.9 ± 0.46	5.3 ± 0.10	88.6 ± 0.67
	3	62.1 ± 0.08	14.4 ± 0.34	12.0 ± 0.02	5.5 ± 0.49	5.4 ± 0.04	88.7 ± 0.11
B73xMo17		62.0	15.3	12.2	6.6	4.7	89.1

[a] Starch recovery.

Steepwater yield had a mean of 5.5%. This was in agreement with previously reported values (Dowd 2003; Vignaux et al 2006) and within the range reported by the wet-milling industry, which produces steepwater with 5–10% solids (Johnson and May 2003).

Starch recovery (SR) results from dividing starch yield by the starch content is expressed as %db. In our study, SR was 83.4–92.9% (Table IV) with a mean of 88.9%. These results were higher than values obtained by Fox et al (1992) and Zehr et al (1995), similar to those reported by Dowd (2003), but lower than those obtained by Weller et al (1988) or Singh et al (1997). Vignaux et al (2006) reported excellent SR values of 91.3–99.3% db when the 100-g wet-milling procedure was used. SR for B73xMo17 was 89.1%. UR11xTester 1 had the lowest SR for both F_1 and F_2 and CU562 crossed to all tester lines had high SR for both seed and grain generations (Taboada-Gaytan et al 2010).

Total solids recovery (TSR) was 99.3–99.9 (data not shown because of the narrow range of values) and was similar to values reported by Singh et al (1998), Dowd (2003), Yang et al (2005), and Vignaux et al (2006). TSR values for the industry were 99.6–100%, although 98% is generally achievable if the samples are carefully milled (Singh and Eckhoff 1996).

Compositions of Recovered Fractions

Recovered fractions compositions are shown in Table VI. The protein content of the starch samples was 0.19–0.27% with a mean of 0.23% db for the experimental hybrids, while B73xMo17 had a protein content of 0.31%. In the wet-milling industry, starch from the primary centrifuges contains 3–5% protein but after purification, the washed starch should contain 0.30–0.35% total residual protein and 0.01% soluble protein (Johnson and May 2003; Orthoefer and Eastman 2004). The typical level of residual protein in commercial starch is 0.30% at 0.27–0.32% (Watson 1984; Vignaux et al 2006). Values were 0.26–0.50% in recent studies at the laboratory-scale level (Raush et al 1999; Dowd et al 2003; Vignaux et al 2006). Singh et al (2001b) reported an average protein content of 1.05% in starch recovered from GEM accessions and attributed it to the poor starch-gluten separation. Protein content of gluten samples was 36.1–47.0 with a mean of 40.3%. B73xMo17 had 44.1% protein content. These values were lower than typical industry samples, which is sold as corn gluten meal for animal feed and contains a minimum of 60% protein content (Johnson and May 2003), although values of ≈66% can also be obtained (Dowd 2003). Our low values can be explained by the difficulty to separate starch from the gluten fractions, which resulted in higher gluten yields with a high concentration of starch and, as a consequence, lower protein content in the gluten samples. This can also explain the lower than expected starch yields for some of the hybrids evaluated. However, gluten obtained when using the tabling method to make the starch-gluten separation rarely contains >50% protein content (Watson 1984).

The mean oil content in the germ fraction was 59.0% of 55.8–62.5%. These values were higher than those reported for the wet-milling industry of 42–50% db (Johnson and May 2003; Orthoefer and Eastman 2004) or for some high-oil corn hybrids, whose germ oil yields can be 52.5–57.1% (Raush et al 1999).

TABLE V
Mean Values and Coefficients of Variation (CV) for Selected Compositional and Wet-Milling Characteristics of Groups of Corn Hybrids from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Tester Lines

Variable	Tester	Mean (% db)	CV	Grouping
Starch Content			0.25	
LSD = 0.48				
	1	69.83		B
	2	69.96		B
	3	70.90		A
Protein Content			0.96	
LSD = 0.60				
	1	10.33		A
	2	10.19		A
	3	9.23		B
Starch Yield			0.41	
LSD = 1.39				
	1	61.38		B
	2	61.93		B
	3	63.93		A
Starch Recovery			0.50	
LSD = 1.53				
	1	87.89		B
	2	88.52		B
	3	90.17		A
Gluten			3.85	
LSD = 1.09				
	1	14.53		A
	2	14.79		A
	3	12.93		B
Fiber			2.90	
LSD = 0.47				
	1	12.83		A
	2	12.79		A
	3	12.03		B
Germ LSD = 0.48			11.82	
	1	5.33		A
	2	4.53		B
	3	5.36		A
Steepwater			1.58	
LSD = 0.15				
	1	5.50		AB
	2	5.53		A
	3	5.38		B

TABLE VI
Composition of Recovered Fractions from Wet-Milling of Hybrid Grain from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

		Recovered Fractions[a] (%db)		
Line	Tester	PStarch	PGluten	OGerm
AR227	1	0.27 ± 0.02	39.0 ± 0.20	60.6 ± 0.88
	2	0.19 ± 0.01	36.1 ± 0.76	60.5 ± 0.41
	3	0.21 ± 0.00	39.4 ± 2.43	59.1 ± 0.98
AR285	1	0.24 ± 0.00	40.2 ± 0.59	60.7 ± 0.13
	2	0.23 ± 0.01	39.5 ± 0.81	61.0 ± 0.40
	3	0.23 ± 0.00	40.7 ± 1.68	57.7 ± 0.27
CH143	1	0.23 ± 0.00	42.0 ± 2.93	61.2 ± 0.77
	2	0.24 ± 0.00	39.6 ± 4.45	61.4 ± 0.40
	3	0.19 ± 0.00	43.3 ± 0.72	59.3 ± 0.21
CH182	1	0.25 ± 0.05	38.1 ± 1.39	58.5 ± 0.08
	2	0.24 ± 0.00	37.3 ± 1.46	59.1 ± 0.12
	3	0.22 ± 0.02	40.3 ± 0.22	58.0 ± 0.54
CU153	1	0.24 ± 0.00	36.6 ± 0.25	61.7 ± 0.08
	2	0.24 ± 0.00	38.8 ± 0.50	61.7 ± 0.21
	3	0.21 ± 0.01	38.6 ± 0.75	58.8 ± 0.04
CU562	1	0.23 ± 0.01	42.1 ± 1.87	61.9 ± 0.25
	2	0.22 ± 0.02	40.3 ± 2.84	62.5 ± 0.52
	3	0.23 ± 0.01	41.2 ± 0.87	57.8 ± 0.23
FS32	1	0.25 ± 0.02	42.4 ± 0.83	55.8 ± 0.28
	2	0.25 ± 0.00	41.1 ± 1.00	57.7 ± 0.54
	3	0.19 ± 0.00	47.0 ± 4.15	56.1 ± 0.17
FS35	1	0.23 ± 0.03	41.7 ± 1.67	55.9 ± 1.16
	2	0.26 ± 0.03	40.7 ± 0.14	57.2 ± 0.56
	3	0.23 ± 0.00	43.8 ± 0.84	56.2 ± 0.54
UR11	1	0.23 ± 0.02	38.8 ± 0.47	56.6 ± 0.99
	2	0.24 ± 0.01	38.7 ± 0.69	60.5 ± 0.54
	3	0.23 ± 0.02	40.5 ± 1.74	58.8 ± 0.04
UR14	1	0.25 ± 0.02	38.8 ± 0.25	59.0 ± 0.33
	2	0.25 ± 0.00	39.7 ± 0.57	59.1 ± 0.76
	3	0.24 ± 0.02	41.7 ± 1.36	55.9 ± 1.38
B73xMo17	0.31	44.1	49.4	

[a] PStarch and PGluten, protein content in starch and gluten samples, respectively; OGerm, oil content of germ.

Wet-Milling Characteristics of Experimental Hybrids

Statistical differences ($P < 0.05$) were found for compositional and wet-milling characteristics when the experimental hybrids were grouped by tester (Table V). Testers 1 and 2 are isolines, genetically identical except for the genetically modified Bt gene, that produced hybrids with statistically identical results for all variables in the study except for germ yield. This indicates that the Bt gene did not significantly affect the wet-milling properties of the experimental hybrids in the study. Tester 3 produced hybrids that had statistically superior starch yield and starch recovery to those hybrids produced with Testers 1 and 2 (Table III), indicating that the right adapted parental inbred can overcome deficiencies of an exotic introgressed parental inbred to make a hybrid better suited for wet milling.

A low coefficient of variation or standard deviation of the recovered fractions yields after replicated wet milling of corn samples is an indicator of the reproducibility of the procedure (Singh and Eckhoff 1996). Coefficients of variance for the recovered fractions were similar to the values obtained by Dowd (2003) and lower than those reported by Eckhoff et al (1996) and Singh et al (1997), which indicated that our reproducibility was acceptable.

Mid-Parent Heterosis for Physical, Compositional, and Wet-Milling Characteristics

Mid-parent heterosis values for the physical, compositional, and wet-milling properties of the HSEL and LSEL groups of experimental hybrids are shown in Tables VII and VIII using data reported in Taboada-Gaytan et al (2009) for parental lines and from Tables III and IV for hybrids. There was positive mid-parent heterosis for test weight and 1,000-kernel weight and a negative value for absolute density for both HSEL and LSEL groups. It was evident that a bigger kernel size for the hybrid grain was an expression of hybrid vigor in relation to the parental lines. Negative values for absolute densities can be caused by higher concentrations of soft starch in the corn kernels, which explains the higher starch yields obtained when milling hybrid grain than when milling parental lines. However, individual hybrid values varied between positive and negative values for test weight and absolute density.

Compositional characteristics of the hybrid grain had no significant differences between groups (Table I) and mean heterosis values were similar for the two groups except that LSEL lines had higher positive heterosis for starch. Starch concentration values were all positive and oil values were mostly positive, while values for protein content were negative in both groups of hybrids. Positive mid-parent heterosis for oil means that exotic introgressed corn lines can be valuable for wet-millers because germ oil is a high-value coproduct from the wet-milling of corn.

Starch yield had positive mid-parent heterosis values for both groups of hybrids; gluten and fiber yields showed negative heterosis values, which means that starch-gluten separation is more efficient for the hybrid grain than for the parental lines and that

TABLE VII
Mid-Parent Heterosis for Physical and Compositional Characteristics of Corn Hybrids from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

	Physical			Compositional		
Hybrid	Test Wt[a]	TKW[b]	Abs. Density[c]	Starch	Protein	Oil
Hybrids with high starch exotic lines						
AR285xT1[d]	3.2	36.7	–0.2	1.4	–15.1	7.8
AR285xT2	3.5	33.4	0.1	1.1	–13.7	10.3
AR285xT3	2.3	44.7	0.2	0.3	–11.5	9.1
CH182xT1	0.5	45.1	–0.6	1.4	–10.6	5.0
CH182xT2	0.5	30.5	–0.7	1.6	–11.6	5.4
CH182xT3	0.8	30.5	7.1	1.1	–19.3	19.0
CU562xT1	2.7	37.3	–0.3	2.3	–19.5	9.9
CU562xT2	3.0	37.0	–0.1	2.6	–20.0	3.4
CU562xT3	–1.4	46.2	–1.5	1.2	–12.2	4.1
FS35xT1	–2.1	8.3	–2.6	2.2	–21.0	12.6
FS35xT2	–1.9	36.9	–2.9	1.2	–11.1	5.8
FS35xT3	–1.1	27.0	–1.1	1.3	–19.1	6.7
UR11xT1	0.1	28.9	–2.6	0.5	–8.1	1.9
UR11xT2	0.5	17.6	–2.2	0.8	–9.9	2.4
UR11xT3	3.1	32.4	–0.7	0.9	–13.0	11.0
Mean	0.9	32.8	–0.5	1.3	–14.4	7.6
Hybrids with low starch exotic lines						
AR227xT1	2.4	21.7	–0.3	2.9	–21.5	8.1
AR227xT2	0.9	39.0	–0.2	2.5	–15.1	–2.8
AR227xT3	0.6	38.3	0.2	0.3	–6.4	2.3
CH143xT1	0.9	17.5	–0.2	2.0	–13.9	8.9
CH143xT2	0.8	9.5	–0.2	2.0	–12.6	6.8
CH143xT3	–2.5	26.0	–1.8	2.7	–21.7	12.6
CU153xT1	3.7	37.8	–0.5	2.0	–17.9	9.7
CU153xT2	3.8	34.9	–1.5	1.4	–15.1	9.9
CU153xT3	0.6	20.2	–0.9	2.3	–22.0	6.4
FS32xT1	3.6	31.1	–0.2	1.4	–14.2	6.1
FS32xT2	1.2	34.4	–0.6	1.8	–13.7	4.3
FS32xT3	–2.7	21. 6	–2.2	2.9	–27.2	2.8
UR14xT1	4.6	21.3	0.6	1.5	–14.5	9.8
UR14xT2	1.2	21.5	–0.8	1.6	–14.0	17.0
UR14xT3	3.2	37.0	0.9	0.1	–5.6	3.7
Mean	1.5	27.5	–0.5	1.8	–15.7	7.0
B73xMo17	–0.1	27.3	0.4	0.1	–2.0	2.9

[a] Test weight (K/hL).
[b] 1,000 kernel weight (g).
[c] Absolute density (g/cm^3).
[d] Tester.

hybrid corn kernels have a lower surface to mass ratio that produced lower fiber yields. Germ yield had positive heterosis for both groups of hybrids and can be a consequence of the bigger kernel size of the hybrid grain.

Steepwater solids had variable values depending on the group of hybrids. Starch recovery followed the same pattern as starch yield for both groups of hybrids. The combination of higher starch and lower protein contents of the hybrids, in comparison to the mean value of the inbred parents, may be the cause of the better wet-milling properties of the materials in the study because all the hybrids had higher starch yields than the mean value of the parents.

The mean protein content of the LSEL group of hybrids was higher than the mean protein content of the HSEL hybrids, which, in agreement with Fox et al (1992), points to the protein content as one of the primary factors of the wet-milling efficiency of the corn hybrids because starch yield and starch recovery were higher for the HSEL group of hybrids. Singh et al (2001a) reported a positive mean value for starch content and a negative mean value for protein content for the mid-parent heterosis of a group of 10 GEM accessions crossed to Mo17 as the male parent, which had as a result a 6.9% positive mid-parent heterosis for the starch yield of the F_1 generation of hybrids.

Regardless of the general trends discussed above, individual variation among hybrids and testers was large for most traits. Again, this demonstrates the importance of analyzing individual hybrids that are intended for the wet-milling industry when using exotic introgressed lines.

High-Parent Heterosis for Physical, Compositional, and Wet-Milling Characteristics

High-parent heterosis values for physical, compositional, and wet-milling properties of the two groups of experimental hybrids are shown in Tables IX and X. The average values for test weight and absolute density were lower for HSEL hybrids than those for LSEL hybrids, indicating that kernels from the first group were softer and produced higher starch yields. The 1,000-kernel weight had positive values, which signifies that hybrids had larger kernel size than the larger kernelled parental line. LSEL hybrids showed lower values for this variable, which means that this group of hybrids has, on average, smaller seed size than HSEL hybrids. Singh et al (2001a) reported negative high-parent heterosis values for this variable when 10 GEM accessions were crossed to the public inbred line B73 (–6.2%), but positive values when the same accessions were crossed to Mo17 (8.0%).

Values for the compositional characteristics of the experimental hybrids followed the same trend for starch and oil contents for the mid-parent heterosis with positive values for both groups of hybrids. Protein content heterosis had negative values and was, on average, lower for the HSEL than for the LSEL group of hybrids.

Starch yield and starch recovery, the two main indicators of the millability of corn, had positive high-parent heterosis values, higher than those of B73xMo17, which indicates that some experimental hybrids have better wet-milling efficiency than the adapted hybrid when compared to the most efficient parental line. Hybrids with Tester 3 as a male parent produced higher starch yield and had better starch recovery values than hybrids where either Tester 1 or 2

TABLE VIII
Mid-Parent Heterosis for Wet-Milling Characteristics of Corn Hybrids from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

	Wet-Milling Characteristics					
Hybrid	**Starch**	**Gluten**	**Fiber**	**Germ**	**Steep Water**	**SR[a]**
Hybrids with high starch exotic lines						
AR285xT1[b]	7.9	–23.9	–7.7	19.3	–5.8	6.5
AR285xT2	6.8	–17.1	–7.0	3.3	–2.8	5.7
AR285xT3	3.3	–7.0	–3.2	–14.7	4.2	3.1
CH182xT1	1.9	–8.6	1.8	8.4	–3.6	0.6
CH182xT2	4.6	–7.6	1.5	–30.0	–0.6	3.1
CH182xT3	3.8	–18.5	2.8	–4.3	8.9	2.7
CU562xT1	9.0	–28.7	–9.5	37.6	–0.2	6.6
CU562xT2	9.2	–17.7	–7.6	11.7	–0.5	6.5
CU562xT3	0.8	–7.4	1.8	5.5	2.5	–0.3
FS35xT1	9.2	–29.2	–9.7	31.9	–1.1	7.0
FS35xT2	5.9	–17.7	–3.3	10.3	–7.8	4.7
FS35xT3	6.5	–24.6	–7.3	10.8	2.5	5.2
UR11xT1	0.3	–14.3	5.1	53.2	–4.8	–0.2
UR11xT2	0.5	–12.7	3.3	40.6	–5.8	–0.2
UR11xT3	2.4	–12.9	–4.0	20.8	5.2	1.6
Mean	4.8	–16.5	–2.9	13.6	–0.7	3.5
Hybrids with low starch exotic lines						
AR227xT1	6.6	–15.1	–10.3	–1.5	1.7	3.6
AR227xT2	4.1	–3.0	–5.1	–34.8	3.5	1.7
AR227xT3	–1.1	1.7	–1.3	4.1	10.8	–1.5
CH143xT1	8.3	–22.7	–8.9	10.5	1.9	6.3
CH143xT2	5.3	–16.5	–3.4	5.3	3.8	3.3
CH143xT3	7.4	–27.7	–4.4	1.7	10.0	4.7
CU153xT1	5.9	–16.2	–5.0	4.8	2.7	4.0
CU153xT2	6.6	–16.8	–7.7	19.4	–1.8	5.1
CU153xT3	4.5	–19.3	5.0	–4.3	–0.9	2.2
FS32xT1	3.3	–21.0	2.8	30.7	–3.2	2.0
FS32xT2	5.5	–21.4	2.2	16.1	–4.3	3.8
FS32xT3	5.6	–36.3	10.6	9.4	0.7	2.7
UR14xT1	4.0	–13.8	–1.4	–2.2	4.1	2.5
UR14xT2	4.6	–12.8	–1.5	–6.4	–4.0	2.9
UR14xT3	–0.4	–5.2	–1.5	11.1	7.6	–0.5
Mean	4.7	–16.4	–2.0	4.3	2.2	2.9
B73xMo17	0.3	–1.8	14.5	–0.9	–7.2	0.2

[a] Starch recovery.

[b] Tester.

were the male parents. Singh et al (2001a) reported that GEM accessions that were crossed to Mo17 had higher starch and lower protein contents and higher starch yields and starch recoveries than the same group of accessions crossed with B73. Because high values for gluten, fiber, and steepwater solids yields are not desirable in the wet-milling industry, heterosis for these variables was calculated taking as reference the parent with the lower value. If the heterosis value expressed by the hybrid grain is negative, then those hybrids have good wet-milling properties. Many hybrids in both groups had high positive values for fiber and steepwater solids yield, but large negative values for gluten. Just as for mid-parent heterosis, individual hybrids had wide variation in values for high-parent heterosis for most traits.

Correlation Coefficients

Correlation coefficients among the physical, compositional, and wet-milling characteristics show that physical properties of the corn kernels have a direct effect on compositional characteristics but they did not have a marked direct effect on the wet-milling properties of experimental hybrids (Table XI). Test weight and absolute density had highly significant negative correlation with starch content, which indicates that the harder the corn kernels, the lower the starch concentration and, as a consequence, the lower the starch yield because this variable was significantly correlated with starch content ($r = 0.71$). It is important to point out the strong negative correlation between protein content and starch content ($r = -0.93$) and protein content and starch yield ($r = -0.81$) which, in agreement with Fox et al (1992), shows that protein content is a determinant factor of the amount of starch that can be stored in the corn kernels and then extracted through the wet-milling process. Gluten yield was significantly correlated with protein content ($r = 0.83$), starch content ($r = -0.73$), and starch yield ($r = -0.69$) and can be explained by the difficulty in separating the starch from the gluten fraction as the levels of protein content are increased. Starch recovery was negatively affected by protein content and gluten and fiber yield, while starch yield had a highly significant correlation with starch recovery ($r = 0.96$). The high correlation between compositional information obtained from NIT technology and wet-milling yield of some important fractions such as starch yield and starch recovery indicates that NIT should be investigated for screening samples for extractable starch in experimental hybrids in early stages of a corn breeding program when large numbers of genotypes need to be evaluated. In general, correlations followed the same pattern reported previously (Weller et al 1988; Fox et al 1992; Zehr et al 1995; Dijkhizen et al 1998; Singh et al 2001b).

CONCLUSIONS

There was variation for the physical, compositional, and wet-milling characteristics of the F_2 grain from experimental hybrids from crosses between exotic-by-adapted inbred lines in corn. This

TABLE IX
High-Parent Heterosis for Physical and Compositional Characteristics of Corn Hybrids from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

	Physical			Compositional		
Hybrid	Test Wt[a]	TKW[b]	Abs Density[c]	Starch	Protein	Oil
Hybrids with high starch exotic lines						
AR285xT1[d]	1.3	33.6	–0.6	–0.5	–19.2	3.5
AR285xT2	1.6	29.4	–0.5	–0.5	–15.1	10.3
AR285xT3	–0.2	39.5	–1.5	0.2	–16.5	3.5
CH182xT1	–1.9	43.6	–1.9	–0.2	–13.7	5.0
CH182xT2	–2.0	22.5	–2.1	0.2	–11.8	1.2
CH182xT3	–2.4	21.7	4.3	0.8	–24.9	17.5
CU562xT1	2.7	31.4	–1.2	1.2	–20.8	3.3
CU562xT2	3.0	35.7	–0.9	1.8	–21.5	1.1
CU562xT3	–2.1	43.9	–2.0	0.2	–19.8	–3.3
FS35xT1	–3.7	–4.4	–3.6	–0.1	–26.3	8.1
FS35xT2	–3.6	27.0	–4.0	–0.9	–14.3	5.8
FS35xT3	–3.5	18.6	–3.4	1.0	–22.2	1.2
UR11xT1	–1.8	13.8	–3.1	–1.9	–13.0	0.0
UR11xT2	–1.3	9.0	–2.8	–1.3	–11.8	–3.5
UR11xT3	1.9	23.5	–2.5	0.4	–17.5	10.3
Mean	–0.8	25.9	–1.7	0.0	–17.9	4.3
Hybrids with low starch exotic lines						
AR227xT1	–0.1	16.8	–0.3	1.3	–27.1	0.0
AR227xT2	–1.8	36.9	–0.4	1.2	–18.5	–6.4
AR227xT3	–2.6	35.3	–1.2	–0.1	–9.6	–6.4
CH143xT1	–0.4	15.7	–1.1	1.5	–14.1	3.4
CH143xT2	–0.6	5.4	–1.2	1.8	–15.6	5.6
CH143xT3	–4.4	20.4	–3.9	1.2	–29.7	5.6
CU153xT1	3.0	36.1	–0.8	1.1	–19.2	1.1
CU153xT2	2.8	29.5	–1.9	0.8	–16.6	5.3
CU153xT3	–1.0	14.6	–2.5	1.2	–28.7	–3.2
FS32xT1	3.1	17.2	–1.2	0.1	–18.4	–5.0
FS32xT2	0.7	26.2	–1.5	0.8	–15.1	–3.0
FS32xT3	–2.9	14.9	–2.5	2.2	–31.3	–8.9
UR14xT1	3.3	8.9	0.5	0.1	–16.9	7.1
UR14xT2	0.1	14.6	–0.8	0.5	–14.5	14.9
UR14xT3	2.7	30.0	–0.3	–0.5	–12.9	0.0
Mean	0.1	21.5	–1.3	0.9	–19.2	0.7
B73xMo17	–0.9	15.6	–0.7	–0.3	–3.1	0.0

[a] Test weight (K/hL).
[b] 1,000 kernel weight (g).
[c] Absolute density (g/cm^3).
[d] Tester.

suggests that the genetic diversity and production potential present in exotic germplasm can be a useful resource to improve the wet-milling characteristics of hybrids grown in the United States.

Hybrids AR285xTester 3, CH182xTester 3, FS32 and FS35x Tester 3, and CU562xTester 3 had starch yields of 65% db and starch recoveries >90%, which indicates that not only these parental lines but the original breeding crosses (AR16035:S19, CH05015:N15, CUBA117:S1520, and FS8B(T):N1802) used in GEM can be valuable germplasm sources in a breeding program to increase the extractable starch of corn hybrids. These breeding crosses have the potential to produce inbreds with better milling properties as hybrids than the inbreds used in this study. They are also potential candidates for evaluating potential as ethanol raw ingredients using dry-grind technology.

TABLE X
High-Parent Heterosis for Wet-Milling Characteristics of Corn Hybrids from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

	Wet-Milling Characteristics					
Hybrid	Starch	Gluten	Fiber	Germ	Steep Water	SR[a]
Hybrids with high starch exotic lines						
AR285xT1[b]	2.2	–21.6	11.9	17.5	–0.8	2.7
AR285xT2	2.3	–16.7	10.7	–1.0	4.2	2.9
AR285xT3	2.4	7.8	5.5	–24.6	7.2	2.2
CH182xT1	–4.2	–4.5	30.2	0.7	3.5	–3.9
CH182xT2	–0.5	–6.6	27.5	–36.6	8.6	–0.6
CH182xT3	3.7	–7.0	17.9	–10.5	10.0	2.5
CU562xT1	2.1	–24.4	12.6	32.5	7.7	0.8
CU562xT2	3.4	–15.6	13.0	4.8	9.3	1.7
CU562xT3	0.4	4.0	13.9	–4.8	3.0	–1.6
FS35xT1	5.5	–26.2	1.6	10.9	7.7	5.7
FS35xT2	3.6	–17.1	6.9	–9.3	2.2	4.5
FS35xT3	3.4	–13.7	–5.7	6.2	2.8	1.8
UR11xT1	–4.5	–8.8	13.4	47.7	2.2	–2.7
UR11xT2	–3.2	–10.2	9.7	39.2	2.9	–1.8
UR11xT3	0.9	–2.5	–2.0	2.1	6.2	–0.4
Mean	1.2	–10.9	11.1	5.0	5.1	0.9
Hybrids with low starch exotic lines						
AR227xT1	–1.0	–3.9	14.9	–14.0	7.9	–2.3
AR227xT2	–2.2	6.3	19.4	–44.4	11.7	–3.3
AR227xT3	–2.3	7.0	13.3	4.0	13.2	–3.0
CH143xT1	7.0	–21.6	12.4	–16.3	5.1	5.7
CH143xT2	5.2	–12.3	17.1	–21.8	8.9	3.0
CH143xT3	2.0	–11.8	6.1	–14.1	15.6	0.8
CU153xT1	0.8	–12.0	16.1	–7.0	7.7	–0.2
CU153xT2	2.6	–15.5	10.9	3.4	4.9	1.8
CU153xT3	3.1	–8.3	15.5	–6.1	2.4	1.8
FS32xT1	–1.5	–15.8	30.7	4.3	–2.6	–1.5
FS32xT2	1.9	–19.0	27.6	–9.2	–2.1	1.1
FS32xT3	3.9	–28.9	26.0	–1.5	8.6	1.8
UR14xT1	–1.7	–8.4	17.3	–10.8	12.3	–1.8
UR14xT2	–0.0	–10.5	15.2	–16.8	5.3	–0.5
UR14xT3	–1.0	6.4	5.7	5.8	8.2	–0.5
Mean	1.1	–9.9	16.6	–9.6	7.1	0.2
B73xMo17	–2.9	2.3	26.9	–3.3	–7.1	–3.3

[a] Starch recovery.
[b] Tester.

TABLE XI
Correlation Coefficients Among Physical, Compositional, and Wet-Milling Characteristics of Corn Hybrids from Crosses of Exotic Introgressed Inbred Lines and Commercial Adapted Testers

Factor	Test Wt[a]	TKW[b]	Abs Den[c]	Starchd	Protein	Oil	Starch[e]	Gluten	Fiber	Germ	Steep W[f]	SR[g]
Test Wt[a]	1.0	–0.2	0.8**	–0.5**	0.3	0.3	0.0	0.5*	–0.4*	–0.5*	0.5**	0.2
TKW[b]		1.0	–0.4*	0.2	–0.1	–0.3	0.2	–0.1	0.1	–0.1	–0.4*	0.1
Abs Den[c]			1.0	–0.6**	0.6**	0.0	–0.3	0.7**	–0.1	–0.4*	0.5**	–0.2
Starch[d]				1.0	–0.9**	–0.1	0.7**	–0.7**	–0.3	0. 1	–0.4*	0.5**
Protein					1.0	–0.2	–0.8**	0.8**	0.4*	–0.1	0.3	–0.6**
Oil						1.0	0.3	–0.2	–0.3	0. 1	0.4*	0.4*
Starch[e]							1.0	–0.7**	–0.7**	–0.2	–0.2	1.0**
Gluten								1.0	0.3	–0.5**	0.3	–0.6**
Fiber									1.0	0.1	–0.3	–0.8**
Germ										1.0	–0.1	–0.2
Steep W[f]											1.0	–0.1
SR[g]												1.0

[a] Test weight.
[b] TKW
[c] Absolute density.
[d] compositional starch.
[e] Starch from wet milling.
[f] Steep water.
[g] Starch recovery.

Tester 3 produced hybrids that were statistically superior for wet-milling properties than hybrids produced with Testers 1 and 2. Tester 3 produced hybrids with higher starch and lower protein content, higher starch yield, and better starch recovery, which indicated that inbred lines that produce hybrids with high starch and lower protein content may be preferable for the wet-milling industry, especially when crossing to exotic introgressed inbreds.

Positive mid-parent and high-parent heterosis for starch content and negative values for protein content were expressed by both groups of hybrids. This led to positive heterosis values for starch yield and starch recovery and better wet-milling properties for some hybrid grain samples with exotic introgressed germplasm in comparison to B73xMo17 hybrid grain.

There was a positive correlation between starch content and starch yield and starch recovery and a strong negative correlation between protein content and starch yield and starch recovery. This indicated that genotypes with high starch and low protein contents produce hybrids with better millability.

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