Heterosis in compositional, physical, and wet-milling characteristics of hybrids from exotic introgressed by adapted inbred lines in corn

by

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CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

This thesis is divided into five chapters: 1) a general literature review, 2) to 4) three manuscripts, and 5) general conclusions. Chapter 1 includes six topics: i) corn production in the United States of America, ii) wet milling of corn, iii) factors affecting the wet-milling characteristics of corn, iv) use of genetic diversity and the Germplasm Enhancement of Maize (GEM) project, v) use of Near-infrared spectroscopy, and vi) heterosis for the wet-milling characteristics of corn. Chapter 2 reports the compositional, physical, and wet-milling characteristics of ten lines from the GEM project and five adapted inbred lines. Chapters 3 and 4 include information about the expression of heterosis in the compositional, physical, and wet milling characteristics for the F_1 and F_2 generations of hybrids from crosses among exotic introgressed and adapted inbred lines, respectively. Chapter 5 reports general conclusions.

Literature Review

Corn production in the United States

Corn or maize (*Zea mays* L.) is the most important crop in the United States of America. The USA is the world leader in corn production since in the cycle 2005/06 it produced 282 311 thousand metric tons, which accounts for 40% of the total world production (USDA, 2007a). Corn production has increased over time, as higher yields followed improvements in technology (seed varieties, fertilizer, pesticides, and machinery) and in production practices (reduced tillage, irrigation, crop rotation, and pest management systems). In 2006, 70.6 million acres were harvested for grain with average yields of 149.1 bushels (bu) per acre and a total volume production of 10.5 billion bu (USDA, 2007b). The crop is grown in most US states, but production is concentrated in the Corn Belt (including Iowa, Illinois, Indiana, eastern portions of South Dakota and Nebraska, western Kentucky and Ohio, and the northern two-thirds of Missouri), with 82% of the production. Iowa and Illinois, the top corn-producing states, typically account for slightly more than one third of the crop. In fact, Iowa leads the states with 20% of the total national production, which is 8.5% of the total world production (USDA, 2007a).

Wet milling of corn

According to Hallauer (1987) the four major uses of maize are for livestock feed, human consumption, industrial purposes, and seed. Globally, 67% of the maize is used for livestock feed, 25% for human consumption and industrial purposes, and the balance is either used for seed or is lost in wastage. Use of corn to produce food and industrial products has been increasing (Dijkhuizen et al, 1998; Singh et al, 2001a; Butzen and Hobbs, 2002). In 2006, 33% of the corn production was processed to produce ethanol, sweeteners (high fructose corn syrup, glucose, and dextrose), starch, and other fermentation products (USDA, 2007c).

Although most ethanol for fuel is now produced through dry grind technology (Sharma et al, 2006), starch and fermented products are primarily obtained from the wet milling processing of corn. Corn wet milling is a process (Figure 1) that separates the corn kernels into its main fractions of starch, gluten, germ, and fiber by using chemical, biochemical, and mechanical operations (Singh et al, 1997). Companies that wet mill not only separate corn kernel fractions but also convert them to final products. Because of this, the wet-milling processing of corn is known as the “corn-refining” industry (Johnson, 2000; Butzen and Hobbs, 2002).

Several industrial, pilot-plant, and laboratory procedures have been developed over the last 50 years to extract starch from the corn kernels. Pilot-plant scale procedures with a sample size varying from 10 to 100 kg of grain have been designed and wet-milling studies that could be extrapolated to the industrial level have been carried out. Anderson (1957) developed a pilot plant with a 100 kg capacity to study the wet-milling processing of cereal grains and to get estimations of the investment and operation costs for the whole process to design a wet milling plant. Rubens (1990) describes a 75-kg capacity pilot plant constructed specifically to study the wet milling of corn and concluded that the separation of starch and gluten fractions needed to be improved. Singh et al (1997) used a smaller pilot plant to process 10 kg samples of corn and obtained lower starch and higher gluten yields in relation to the 1-kg and 100-g laboratory procedures used in the same study with the same hybrids. They attributed these differences to the difficulty to separate starch from the fiber and gluten fractions.

Over the last 10 years laboratory wet-milling procedures have been used to determine the wet-milling characteristics of new corn hybrids and inbred lines in advanced or early breeding programs. These types of procedures use a smaller sample size of corn that varies from 1 kg to 10 g of cleaned corn kernels. Eckhoff et al (1993) developed a 1-kg laboratory procedure designed to increase the reproducibility and accuracy of product yields and obtained fraction yields comparable to those of the wet-milling industry. Steinke and Johnson

(1991) and Steinke et al (1991) used 300-g samples of corn to study the effect of multiple enzymes on different steeping conditions of corn. Eckhoff et al (1996) developed the first 100-g laboratory wet-milling procedure to reduce sample size and labor time required to determine the wet-milling properties of corn samples. Singh et al (1997) made some modifications and improved this procedure, which is now widely used because it produces fraction yields values that are comparable to the industry levels and reduces the economic cost and labor required to analyze a large number of corn samples. Dowd (2003) has proposed some improvements to this laboratory-scale corn wet-milling procedure to better model the industrial process. Vignaux et al (2006) used a 10-g laboratory-scale procedure to determine the wet-milling properties of corn samples when the amount of grain is a limiting factor for the use of larger bench-scale procedures. This new procedure can be particularly useful in early corn breeding programs such as for when transgenic lines have been developed for pharmaceutical protein production or some other special use. A very small scale procedure for starch extraction (2 to 10 corn kernels) was optimized by Ji et al (2004) but used either sedimentation or centrifugation to isolate the starch fraction. This is not a bench-scale procedure but can be used when small amounts of starch are required to study, for example, its thermal or viscosity properties.

Factors affecting the wet-milling characteristics of corn

Several studies have been conducted to evaluate differences in the wet-milling properties of corn samples. Genetics is one of the most important factors that determine wet-milling differences among corn hybrids. As indicated by Eckhoff (1995), genetic differences among corn hybrids, and the associated differences in the wet-milling characteristics and fraction yields, can negatively affect production costs and economic gains of the wet-milling industry. Zehr et al (1995) compared the wet-milling properties among inbred lines and their hybrids and reported enough precision in the evaluation of fraction yields to detect differences among genotypes. The wet-milling characteristics of waxy (Singh et al, 1996), high oil (Raush, et al, 1999), dent and flint (Haros and Suarez, 1998), and selected yellow dent corn hybrids (Singh et al, 1998) have also been reported.

The effects of grain moisture content at harvest and drying conditions have been studied (Weller et al, 1988; Weller et al, 1989; Mistry et al, 1993; Haros and Suarez, 1998; Haros et al, 2003) and it was found that starch yield and starch recovery decrease as both harvest moisture and drying air temperature increased. Storage time and storage temperature were studied by Singh et al (1998) and they found no significant differences for starch yield of hybrids in relation to storage time but starch yields of samples stored at $4^{o}C$ were higher than the starch yields of the same hybrids stored at room temperature.

As steeping is the first and one of the most important steps of the wet-milling process, the effect of different steeping procedures and conditions have been widely studied (Steinke and Johnson, 1991; Steinke et al, 1991; Yang et al, 2005; Cabrales et al, 2006). Results indicate that starch yield is affected by the sulfur dioxide (SO_2) source and that use of enzymes and SO_2 during steeping can facilitate the separation of wet-milling fractions. However, it has also been demonstrated that the addition of enzymes to the steeping solution is not an effective practice to reduce the steeping time or the SO_2 concentration (Johnston and Singh, 2001). The effect of alternative milling techniques on the yield of recovered fractions has also been studied (Neryng and Reilly, 1984; Singh et al, 2001c).

Finally, the relationship between physical and compositional characteristics and the wet-milling properties of corn samples were analyzed by Fox et al (1992) and Singh et al (2001a). Both studies found that starch yield and starch recovery is favored by high starch and low protein concentrations in the corn samples and that some physical characteristics like test weight and absolute density are also correlated with the wet-milling efficiency of corn.

Use of genetic diversity and the Germplasm Enhancement of Maize (GEM) project

Hybrids with high grain yield and higher starch, protein, or oil content are available to growers. However, these types of hybrids are the result of crossing adapted inbred lines and rarely have corn lines from exotic germplasm been crossed with elite inbreds to develop new and useful breeding sources (Singh et al, 2001a). Breeders use valuable sources of genetic variation to develop new lines and hybrids (Salhuana and Smith, 1998). Despite its utility as sources of variation for crop improvement, exotic germplasm, [all sources whether domestic, temperate, or tropical that is unadapted to the breeder’s target environment (Goodman, 1985)], has seldom been used in advanced breeding programs. In fact, less than 1% of the U.S. corn germplasm base had an exotic origin in 1984 (Goodman, 1985), increasing to almost 3% in 1996 (Goodman, 1999). Because researchers realized that exotic materials in breeding programs are valuable to obtain useful breeding lines, the Germplasm Enhancement of Maize project (GEM), a coordinated and cooperative effort among public and private sectors, was developed to improve and expand the germplasm base of corn used to develop new hybrids with better agronomic performance and value added characteristics (Pollak, 2003). GEM followed the Latin American Maize Project (LAMP), a coordinated international project to evaluate maize genetic resources (Salhuana et al, 1991). In GEM, the highest-yielding accessions from LAMP were crossed to a proprietary inbred line to make a 50% exotic breeding cross and then crossed to another adapted inbred line of the same heterotic group from another company to produce a new breeding cross with 25% exotic genetic background. These materials are yield tested as testcrosses and the best ones are used to develop new inbred lines (Salhuana et al, 1998). The complete GEM breeding protocol to develop S_3 lines or S_2 synthetics is shown by Pollak (2003).

Use of Near-Infrared spectroscopy

Near-infrared (NIR) spectroscopy is an analytical technology that is now widely used in agriculture because it is nondestructive, measurements are rapid, and instruments are simple to use. Every organic compound has a unique NIR composite spectrum, determined by its chemical composition, that can be read by using a spectrophotometer and that provides positive identification of different materials. If the spectra of two samples are very similar it indicates that the two samples have similar physical and chemical composition. The amount of material present can be determined from the peak intensity of the spectrum (Workman and Shenk, 2004). According to Delwiche (2004), introduction of whole grain analyzers has replaced ground grain instruments due to: i) sample preparation is simple because the grind step is not necessary; ii) precision has improved because the source of variation represented by the grinders is eliminated; and iii) precision improvement due to the elimination of human variation during the packing of the sample and in the particle size caused by differences in the hardness of grain samples. The same author points out that larger sample amounts are needed to determine chemical compositions and that could be a disadvantage in early stages of a breeding program. Several studies have been conducted to correlate easily measured compositional characteristics to fraction yields from wet milling. Fox et al (1992) studied the relationship of grain proximate composition to the wet-milling properties of corn samples and reported that hybrids with lower protein content yielded more starch. Wehling et al (1993) attempted to predict starch yield from corn samples by using NIR reflectance spectroscopy and reported that correlation coefficients for several calibrations ranged from 0.8 to 0.9 but that reproducibility of the laboratory wet-milling procedure was a limiting factor. Dijkhizen et al (1998) used also NIR reflectance spectroscopy and correlated it to the values of the wet-milling fractions when the 100-g laboratory procedure was used. They reported that starch content from NIR reflectance and starch yield were highly correlated ($r = 0.8$) as were protein content and gluten yield ($r = 0.72$).

Near-infrared transmittance (NIT) technology can be useful in a breeding program because it can be used as a tool to predict the wet-milling properties of new corn hybrids based on compositional values for starch, protein, and oil and by analyzing the correlation between this group of variables and the recovered fractions from wet milling. Singh et al (2001a) studied the compositional, physical, and wet-milling properties of accessions used in the GEM project and found a highly positive and a highly negative correlation between starch yield and starch and protein content, respectively. Paulsen et al (2003) developed NIT calibrations for extractable starch based on the 100-g wet-milling procedure values and concluded that NIT can be an easy method to determine the quality of corn samples, specifically extractable starch, as raw materials for the wet-milling industry. Singh et al (2005) conducted a site-specific study of corn starch, protein, oil, and extractable starch variation using NIT spectroscopy and reported that starch yield was positively correlated with starch content and negatively correlated with protein content.

Heterosis for the wet-milling characteristics of corn

The heterosis concept was developed through observations in work with hybrid corn. Shull (1952) defined heterosis as “increased vigor, size, fruitfulness, speed of development, resistance to disease and insect pests, or to climatic rigors of any kind, manifested by crossbred organisms as compared with corresponding inbreds, as the specific result of unlikeness in the constitution of the uniting parental gametes”.

Heterosis can be defined as the difference between the hybrid performance and the mean value of the inbred parents (Falconer and Mackay, 1996), which is known as mid-parent heterosis. However, the highest value of the best parent for a trait of interest, or high-parent heterosis, is also used mainly for self-pollinated crops where breeders are interested in finding a hybrid with better performance than either of the parents (Lamkey and Edwards, 1999). Heterosis values are normally expressed as a percentage relative to the reference value. 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 (2001b) studied the expression of heterosis by the cross of ten GEM accessions (exotic populations used in GEM) to the public inbred lines B73 and Mo17. They reported higher levels of protein and a reduction of the starch contents of the hybrids, in relation to the mean value of the parents, which led to the expression of poor wet-milling properties. However, these studies have focused on the expression of heterosis in the F_1 generation of hybrids. No studies have considered the expression of heterosis by the F_2 generation or in hybrid grain. This is important because hybrid grain is the raw material for plants that process corn through the wet-milling procedure. Additionally, there are no studies focused on the analysis of heterosis expressed by adapted inbred breeding lines introgressed with exotic germplasm that could provide additional value added characteristics for corn breeding.

General Objectives

Use of genetic diversity and screening of exotic germplasm is one of the most important axes of any breeding program focused on the development of improved genotypes for a particular final use and for a predefined target environment. Based on wet milling information from a GEM cooperator, ten GEM lines were selected to start a research project with the following general objectives: i) to determine the compositional, physical, and wet-milling characteristics of ten exotic lines from the GEM project, three commercial inbred lines used as testers, and the resulting F_1 and F_2 generations of hybrid seed, and ii) to determine the expression of mid-parent and high-parent heterosis for the compositional, physical, and wet-milling characteristics for the same group of corn samples.

Information from this study will help to recognize exotic gemplasm as a valuable source of value-added characteristics that can be introgressed into elite adapted breeding lines and contribute to enhance the genetic diversity used to produce the corn hybrids grown in the United States or any other part of the world. Our results will enhance genetic diversity in corn used for wet milling and can also be valuable for the seed industry, plant breeders, farmers, and the corn processing industry.

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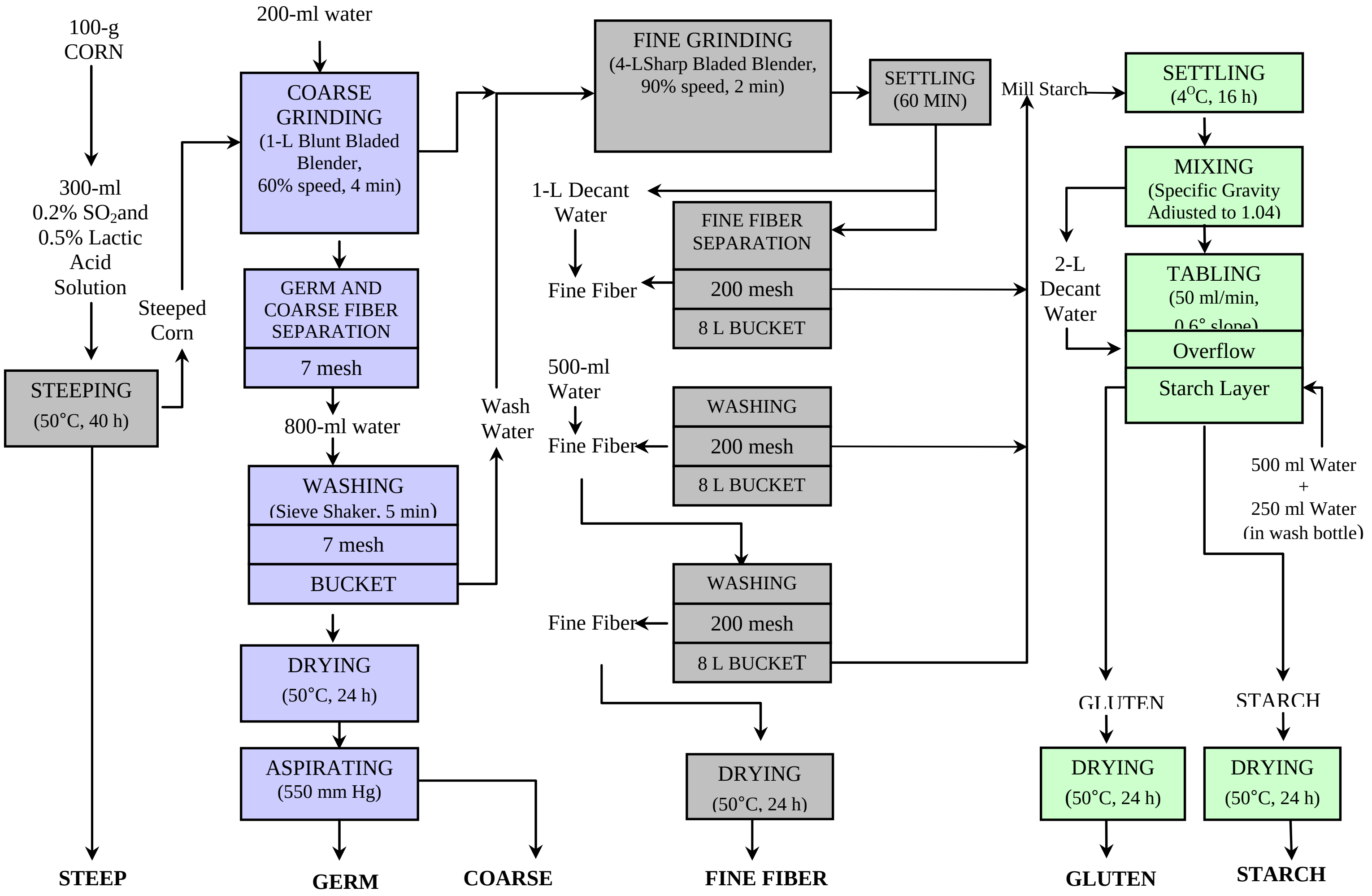
Figure 1. The 100-g modified wet-milling procedure (Adapted from Vignaux et al, 2006)



CHAPTER 2. WET-MILLING CHARACTERISTICS OF TEN LINES FROM THE GERMPLASM ENHANCEMENT OF MAIZE PROJECT AND FIVE CORN BELT LINES

Abstract

Corn (*Zea Mays* L.) is the main crop in the United States and starch is the most important product derived from corn kernels. Use of corn by the processing industry has steadily increased and hybrids with high grain yield and higher starch, protein, or oil content are available to growers. However, utilization of exotic germplasm in maize breeding programs does not represent more than 3% of the genetic base currently in use to produce corn hybrids grown in the United States. In addition to yield trial evaluations to determine the agronomic performance of new corn cultivars, it is necessary to determine the physical, compositional, and milling characteristics that could provide added value for processing and increase the usefulness of new inbred lines and hybrids. The present study was conducted to determine whether Corn Belt lines introgressed with exotic materials from Argentina, Chile, Uruguay, Cuba, and Florida have appropriate wet-milling characteristics. Ten lines from the Germplasm Enhancement of Maize project with different starch content yields, three commercial inbred lines, and two public inbred lines (B73 and Mo17) were analyzed using both Near-Infrared Transmitance (NIT) and a 100-g wet-milling procedure. Statistical differences ($P<0.05$) were found for yield of the wet-milled fractions (starch, fiber, gluten, and germ). AR227 and CU562, two lines of exotic origin, had similar or better starch yield and starch recovery than B73 and the other adapted inbred lines, which indicates that these lines can potentially be used to improve the proportion of extractable starch present in the kernels of their hybrids. Residual protein levels in the starch and gluten fractions were in the range of 0.26-0.32% and 38-45%, respectively. The starch yield of exotic corn lines from wet milling correlated positively with starch content and was negatively correlated with protein content of the corn kernels. Oil content in the germ varied from 50 to 60%. Our results indicate that the use of exotic germplasm to improve the wet-milling characteristics in a corn breeding program may enhance genetic diversity available for breeding.

Introduction

Corn or maize (*Zea mays* L.) is the most important crop in the United States and starch is the most abundant component of the corn kernels. Use of corn to produce food and industrial products has been increasing (Dijkhuizen et al, 1998; Singh et al, 2001; Butzen and

Hobbs, 2002). In 2006, 33% of the corn production was processed to produce ethanol, sweeteners (high fructose corn syrup, glucose, and dextrose), starch, and other fermentation products (USDA, 2007).

Hybrids with high grain yield and higher starch, protein, or oil contents are available to growers. These hybrids are the result of crossing adapted inbred lines and rarely have corn lines from exotic germplasm been crossed with elite inbreds to develop new and useful breeding lines (Singh et al, 2001). Breeders need sources of genetic variation to develop new lines and hybrids (Salhuana and Smith, 1998). Despite its utility as source of variation for crop improvement, exotic germplasm has seldom been used in advanced breeding programs. In fact, less than 1% of the U.S. corn germplasm base had an exotic origin in 1984 (Goodman, 1985), only increasing to nearly 3% in 1996 (Goodman, 1999). Because researchers realized that exotic materials in breeding programs are valuable to obtain useful breeding lines, the Germplasm Enhancement of Maize project (GEM), a coordinated and cooperative effort among public and private sectors, was developed to improve and expand the germplasm base of corn used to develop new hybrids with better agronomic performance and value-added characteristics (Pollak, 2003). GEM followed the Latin American Maize Project (LAMP), a coordinated international project to evaluate maize genetic resources (Salhuana et al, 1991). In GEM, the highest-yielding accessions from LAMP were crossed to a proprietary inbred line to make a 50% exotic breeding cross and then crossed to another adapted inbred line of the same heterotic group to produce a new breeding cross with 25% exotic genetic background. These materials were yield tested as testcrosses and the best ones used to develop new inbred lines (Salhuana et al, 1998). The GEM breeding protocol for developing S_3 lines is described by Pollak (2003).

There are few published papers that have reported studies about physical, compositional, and wet-milling characteristics of inbred lines and their hybrids. Zehr et al (1995) evaluated wet-milling characteristics on 15 Corn Belt inbred lines and 20 related hybrids. They observed significant divergence of hybrids from the average value of both parents and attributed the lower values for germ and fiber and the higher values of gluten and filtrate solids to the bigger kernel size of the hybrids. A positive correlation between starch yield and starch content and a negative correlation between grain hardness and starch yield were also reported. Singh et al (2001) studied the compositional, physical, and wet-milling properties of 49 accessions used in the GEM project, two commercial hybrids, and the B73 and Mo17 public inbred lines. They reported lower starch and higher protein contents for GEM accessions than for either commercial hybrids or Corn Belt lines. Absolute densities were also higher for GEM accessions. Their conclusion was that low values for absolute densities and test weights as well as greater starch and lower protein contents are desirable characteristics that would improve the wet-milling properties of a corn sample.

Based on wet-milling information from the GEM cooperator Cerestar (Hammond, IN), 10 GEM lines were selected to study the wet-milling properties of elite inbred lines introgressed with exotic germplasm. This work can be considered as a continuation of Singh et al (2001) in order to know the effect of adapted germplasm in the improvement of the wet-milling characteristics of GEM accessions after making them available in Corn Belt breeding programs by crossing them to proprietary inbreds and pedigree selection to make S_3 exotic introgressed inbred lines. The objectives of this project were i) to determine the physical, compositional, and the wet-milling characteristics of 10 exotic lines from the GEM project, three commercial inbred lines, and two public inbred lines used as checks, and ii) to determine whether GEM lines had better wet-milling properties compared to the accessions . Our hypothesis was that there are exotic corn lines from the GEM project with similar or better wet-milling characteristics than the commercial and public inbred lines.

Materials and Methods

Genetic Materials

GEM lines originally underwent pedigree selection from exotic accessions by adapted line(s) breeding crosses, selected for grain yield as testcrosses of S_2 lines crossed to commercial testers, and then evaluated for wet-milling properties by the private cooperator Cerestar (Hammond, IN, now acquired by Cargill, Minneapolis, MN). Ten corn lines from the GEM project were used in this study. The lines were selected on the basis of starch yield after laboratory wet milling by Cerestar. The highest and lowest starch-yielding lines for each of five germplasm sources were chosen. The lines had 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). Three commercial inbred lines (Line 1, Line 2, and Line 3) that provide to hybrid progeny different starch contents were also included. Seed of commercial lines was provided by Golden Harvest Seeds, Inc. (Clinton, IL, now acquired by Syngenta, Wilmington, DE). B73 and

Mo17, two formerly widely used public inbred lines, were included as checks. The commercial and public inbred lines were classified into a third group as adapted germplasm.

Sample Preparation

Seed of the lines was produced in Ames, IA, in the summer of 2003 by self pollination. All the ears from a two-row plot (3.8 m long and 76 cm between rows) were hand harvested and allowed to dry to approximately 10% moisture content by circulating warm air at 38^{o}C (100^{o}F) for 72 h. Seed from all ears was bulked after shelling and stored at 4^{o}C until used. The 100 g samples were hand picked and any foreign material and cracked or broken kernels were removed. Two replications per sample were used to determine the physical, compositional, and wet-milling characteristics of the corn lines in this study.

Physical Characteristics

Three variables were quantified to determine the physical characteristics of the corn lines: test weight, 1000-kernel weight, and absolute density. Test weight is a measure of the weight per unit of volume of a grain at a standardized moisture level. In U.S. grain trading transactions this is normally expressed in pounds per bushel. Standards and procedures to estimate test weights are provided by the USDA Grain Inspection, Packers and Stockyards Administration (GIPSA). In this case, test weight was determined by following the Federal Grain Inspection Services procedures (FGIS, 1988) and expressed as kilograms per hectoliter (Kg/hL). The 1000-kernel weight (g) was measured by using an electronic counter (Electronic Counter Model 850-2, International Marketing and Design Corp., San Antonio, TX) to count the kernels and then weighing them in a precision top-loading balance (OHAUS

Explorer Pro Model EP4102C, Pine Brook, NJ). Absolute density is a measurement of the volume of a specified weight of kernels and is expressed in grams per cubic centimeter. Absolute density (g/cm^3) was estimated by using a FOSS Infratec 1241 Grain Analyzer (Tecator, Hoganas, Sweden).

Compositional Characteristics

Moisture, starch, protein, and oil contents of bulked whole kernels from each line were estimated with Near-infrared Transmittance (NIT) technology by using a FOSS Infratec™ 1241 Grain Analyzer (Tecator, Hoganas, Sweden). 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 NIR calibration databases of the USDA Federal Grain Inspection service.

Wet-Milling Characteristics

Lines were analyzed in the laboratory by using the 100-g modified wet-milling procedure of Singh et al (1997). This procedure yields starch, gluten, fine and coarse fiber, germ, and steepwater fractions. The procedure was slightly modified to improve reproducibility as follows. The 100-g samples were placed in a 500-ml flask and steeped in a 300 ml of a solution containing 0.5% of lactic acid (Lactic Acid 85%, Certified A.C.S., Fisher Scientific) and 0.2% of sulfur dioxide (Sodium Bisulfite Certified A.C.S., Fisher Scientific). The corn was steeped in a single batch process in a water bath at 50^{o}C for 40 h. The starch slurry resulting after the fine grinding and fine fiber separation was placed in a 4-L glass beaker and allowed to settle overnight at 4^{o}C. Starch and gluten was separated the following morning by using the tabling procedure as described by Eckhoff et al (1996). The suspension of starch and gluten flows over an inclined table (2.44 m long and 5.08 cm wide), which allows the starch to settle and the gluten particles, some water-soluble proteins, and water to overflow to the end of the table and be collected in a plastic container. Moisture contents of the recovered fractions were determined in triplicate by using the AOAC Method 14.004 (AOAC, 1984). The whole kernel moisture was estimated with three replications by following the AACC Method 44-15A (AACC, 2000) and used to calculate the total solids recovery on a dry basis.

Composition of Recovered Fractions

Protein contents of the starch fractions were determined by using the macro-Kjeldahl method, the procedure used by the Corn Refiners Association (Method A-18 CRA, 2006). Protein contents in the recovered gluten fractions were determined by using the AOAC Method 993.13 (AOAC, 2003) and a combustion nitrogen analyzer RapidN III from Elementar Americas, Inc. (Mt. Laurel, NJ) with a protein factor of 6.25. Oil contents in the germ fractions were quantified as crude free fat content according to the AOAC Method 14-084 and 14-085 (AOAC, 1984) by using the Goldfisch procedure.

Statistical Analysis

The procedures Proc GLM and Proc CORR of SAS (SAS Institute, Cary, NC) were used to determine statistical differences and correlations among different values, respectively. A linear model was used where lines were considered as a fixed factor. Statistical differences among groups of lines were determined by using contrasts from the GLM procedure of SAS.

In order to do the statistical comparison of groups of lines, lines were considered as a nested factor within groups. Multiple mean comparisons with least significant differences at $P<0.05$ were carried out to rank groups of lines for compositional and wet-milling characteristics.

Result and Discussion

Physical Characteristics

Significant differences for test weight, 1000-kernel weight, and absolute density were found (Table 1). Test weights of the GEM lines ranged from 73.5 to 80.4 kg/hL and they were significantly higher than those of B73, Mo17, and the commercial inbred lines used in this study. Our results were in agreement with Singh et al (2001), in which test weights ranged from 65.5 to 85.3 with a mean of 79.3 kg/hL for 51 exotic accessions used in GEM. The 1000-k weight of the 10 exotic lines ranged from 255.6 to 341.1 g with a mean value of 297.6 g; these values were similar to those of the adapted lines (mean of 293.4 g). A range from 240 to 399 g and a mean of 308.5 g was reported by Singh et al (2001). Zehr et al (1995) reported a mean of 268 g when studying the wet-milling properties of 15 inbred lines representatives of the germplasm groups used to produce hybrid seed in the United States. Absolute density had a mean value of 1.301 and 1.272 (g/cm^3) for the exotic and adapted lines, respectively. Zehr et al (1995) and Singh et al (2001) both reported means of 1.32 for adapted inbred lines and exotic GEM accessions, respectively. The relative proportion of vitreous to floury endosperm represents the kernel hardness (Correa et al, 2002), which is the main determinant of kernel density. As a consequence, kernel density can be a determining factor of the wet-milling properties of a corn line or hybrid because starch from a softer endosperm is easier to recover during the wet processing of corn due to weaker protein matrices around starch granules (Watson, 1987). Because the inbreds' seed is produced by self pollination, these differences in physical properties can be attributed to genetic differences among lines but also to differences in pollination techniques, especially if several people are doing the pollinations. However, the physical differences found among exotic, adapted inbred lines, and GEM accessions, can also be attributed to differences in growing location, because differences in growing environment (variation in soil type, fertility, climate) can affect the physical properties and the quality of the corn kernels (Watson, 1987; Singh et al, 2005). This wide variation found in both exotic inbred lines and GEM accessions shows the great genetic diversity present in exotic corn that can be exploited in advanced breeding programs.

Compositional Characteristics

Starch, protein, and oil contents of the lines (Table 1) were statistically different ($P<0.05$). Starch contents of the GEM lines were slightly higher than for the adapted lines with a mean of 69.7 and 69.0% db for each group, respectively. Some lines with exotic background in the HSEL group had starch contents above 70% db (AR285, FS35, and UR11). The HSEL group had a mean starch content of 70.2% db and was statistically superior to the LSEL and adapted groups. These results differ with those reported by Singh et al (2001), where they found that the GEM accessions contained less starch (mean of 67.7% db) than the two Corn Belt inbreds and the two commercial hybrids used as controls.

Protein and oil contents did not vary greatly. The mean protein content was 11.6, 11.9, and 11.5% db for lines with exotic and adapted origins, respectively (Table 3). Zehr et al (1995) considered similar values as high and attributed the lower than anticipated starch recovery from wet milling to the negative correlation between protein content and extractable starch (Fox et al, 1992; Singh et al, 2005). The mean oil contents of 4.2, 4.6, and 4.2% db for HSEL, LSEL, and adapted inbred lines, respectively (Table 3), were lower than values reported by Singh et al (2001) who found a mean of 5.2% db.

Wet-milling Characteristics

The wet-milling properties of the materials in the present study are shown in Table 2. Statistical differences ($P<0.05$) were found among lines for all variables evaluated. Starch yield is the most important fraction from the wet-milling process (Singh and Eckhoff, 1996) and indicates millability, or ease with which kernel components are separated by wet milling (Curtis et al, 1988). The starch yields of the lines varied significantly with means of 61.4, 60.8, and 59.6% db for the HSEL, LSEL, and adapted groups of lines, respectively (Table 3). These values were higher than those found for a group of accessions used in the GEM project where a mean of 54.3% db was reported (Singh et al, 2001). However, the starch yield of the exotic lines should be compared with caution to those obtained when milling dent corn hybrids where starch yields are normally higher (Weller et al, 1988; Singh and Eckhoff, 1996; Singh et al 1997; Dowd, 2003, Vignaux et al, 2006). The higher starch yields of dent hybrids can be caused by having lower protein contents and, probably, to higher proportions of soft to hard endosperm in the corn kernels as indicated by the lower absolute densities reported for these types of hybrids. The mean starch yield for the adapted inbreds was 59.6% db; this value is higher than the mean value of 56.2% db reported by Zehr et al (1995) for a group of adapted inbred lines. GEM lines AR227 and CU562 had similar or better starch recovery than the best public inbred line B73 (Table 2), which is evidence that potential to improve wet-milling characteristics of hybrids grown in the United States can be found in exotic germplasm. Additionally, accessions from Argentina have been ranked in the 15% highest grain yielding accessions and, even though they lacked flowering synchrony and had poor stalk strength, they showed an excellent grain yield potential (Salhuana et al, 1998). Lines introgressed with the accession CUBA117 have good yield potential when testcrossed to an elite adapted inbred line and then evaluated for agronomic performance in yield trials (Pollak, 2003).

Gluten yields ranged from 15.0 to 19.5 with a mean of 17.3 and 17.1% db for the HSEL and LSEL groups of lines, respectively, which were not statistically different; the group of adapted inbreds varied from 13.55 to 18.93 with mean of 16.28% db (Table 3). The values are higher than those reported for GEM accessions (Singh et al, 2001), adapted lines (Zehr, 1995), or commercial dent hybrids (Fox et al, 1992; Singh et al, 1997; Dowd, 2003; Vignaux et al, 2006). This poor starch-gluten separation can be attributed to high protein content of these lines and to the presence of a protein matrix surrounding the starch granules, which makes the release of starch granules more difficult (Watson, 1984).

Fiber yield was lower than values reported by Singh et al (2001) and Zehr et al (1995) for similar materials and adapted inbred lines, respectively. There were significant differences for fiber yield among groups of lines (Table 3). Germ and steepwater yields were similar to these two studies (Table 2).

Starch recovery (SR) is the result of dividing starch yield by the starch content and provides an excellent indicator of the millability of any corn material because it represents the extractable starch obtained from wet milling. In our study, SR varied from 83.0 to 92.1% with a mean of 87.7% for both groups of exotic inbred lines (Tables 2 and 3). These values were higher and had less variation than those reported by Singh et al (2001), which indicates that the procedure had a good reproducibility but also represents the positive effect of crossing exotic accessions to adapted germplasm. SR for the adapted inbred lines varied from 81.5% for Line 1 to 92.2% for B73; the overall mean was 86.3%, similar to the mean value reported by Zehr et al (1995) of 84.8% for a set of adapted inbred lines. Total solids recovery (TSR) varied from 99.1 to 99.9% and is similar to the values reported for the industry of 99.6 to 100% (Singh and Eckhoff, 1996).

Composition of Recovered Fractions

The compositions of some recovered fractions are shown in Table 4. The protein contents of the starch fractions ranged from 0.26 to 0.31%. According to Vignaux et al (2006), the typical level of residual protein in commercial starch is 0.3% but it ranges from 0.27 to 0.32% (Watson, 1984). Singh et al (2001) reported an average of 1.05% of protein content in starch from GEM accessions and attributed it to poor starch-gluten separation that can be a characteristic of lines, adapted or with exotic origin, that have a high level of protein content in the corn kernels.

Protein contents of gluten samples ranged from 38.3 to 44.5% for the exotic lines. These values were lower than typical industry samples of around 66% (Dowd, 2003) but were similar to those reported by Singh et al (2001) of 42.4%. This low protein content has been attributed to the difficulty to separate starch from the gluten fractions, which results in higher gluten yields with a high concentration of starch and, as a consequence, lower protein contents in the gluten samples. Gluten obtained when using the tabling method to make the starch-gluten separation rarely contains more than 50% protein content (Watson, 1984).

The oil content in the germ fraction averaged 56.0% and ranged from 49.5 to 60.2% for the 10 GEM lines. The commercial lines had a mean of 63.7%. These values were high compared to those reported for high-oil corn hybrids of 52.5 to 57.1% (Raush et al, 1999). However, we assume they are correct because random samples were rerun, including the commercial lines, and the results were the same. Vignaux et al (2006) reported values ranging from 47.9 to 54.7% for several commercial corn dent hybrids.

Single Factor Correlations

Correlation coefficients of the physical and compositional characteristics with the wet-milling properties of the materials under study (Table 5) varied but followed the same pattern reported in previous studies (Fox et al, 1992; Zehr et al, 1995; Singh et al, 2001). As expected, starch yield was positively correlated with starch content and was negatively correlated with protein content in the corn kernels. This is important because now it is possible to easily screen new inbreed lines and hybrids for compositional characteristics during early stages of breeding programs using NIT technology and then make the selection decision based on this information if materials with high extractable starch are desired. Gluten yield had a significant negative correlation with starch yield (data not shown) and this variable was also highly correlated with absolute density and compositional protein of the corn kernels; the first correlation indicates that corn cultivars with lower absolute densities and lower protein content would be preferred if high starch yield is the primary consideration in selection.

Conclusions

Groups of lines were statistically different and the HSEL group showed higher starch content, starch yield, and starch recovery than the LSEL group, which means that they had better millability and more appropriate wet milling characteristics for the corn processing industry. AR16035:S19-227-1-B and CUBA117:S1520-562-1-B had similar wet-milling characteristics, related to starch yield, than the Corn Belt inbred line B73. These exotic lines were developed and selected for high grain yield as testcrosses in yield trials, which is evidence that there is potential in exotic germplasm to improve the agronomic performance and the wet-milling characteristics of hybrids grown in the United States.

GEM lines in this study had higher starch yields and starch recoveries than the group of accessions used in GEM that were evaluated by Singh et al (2001). This improvement of the millability of GEM lines may be an effect of the adapted germplasm given by an increase of the starch and a reduction of the protein contents, in relation to the values of the eccessions, through breeding by pedigree selection.

There was a positive correlation between starch content and starch yield and a negative correlation between protein content and starch yield. This indicated that genotypes with high starch and low protein contents will produce higher and purer starch yields. NIT technology may be used as a predictive tool to screen early progeny for high starch yield and save time and costs in a corn breeding program directed at improving the wet-milling efficiency in corn given by the extractable starch available in the corn kernels.

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Table 1. Physical and compositional characteristics of ten corn lines introgressed with exotic germplasm from the GEM project and five adapted Corn Belt Lines.

Line	Physical Characteristics			Compositional Characteristics		
	TestWt[1]	1000kWt[2]	ADen[3]	Starch	Protein	Oil
AR227	80.41a[4]	282.37f	1.295e	69.80d	10.95g	4.70b
AR285	79.14b	273.91h	1.305cd	70.25c	11.50e	4.35d
CH143	78.33c	269.14i	1.319b	68.30h	12.80a	4.45cd
CH182	80.19a	255.63k	1.330a	69.85d	11.85d	4.00f
CU153	77.69d	267.76i	1.303d	68.85g	12.35b	4.75b
CU562	76.33e	285.65e	1.272fg	69.05g	12.35b	4.55c
FS32	75.60f	331.64b	1.267g	69.35ef	11.50e	5.05a
FS35	79.03b	340.58a	1.322b	70.80b	11.05fg	4.35d
UR11	73.55h	341.14a	1.309c	71.05a	11.40e	3.85g
UR14	74.56g	328.63b	1.291e	69.55e	12.05c	4.20e
Line 1	76.36e	261.15j	1.295e	67.60j	12.75a	4.00f
Line 2	76.25e	291.17d	1.291e	68.00i	11.90cd	4.35d
Line 3	75.25f	295.20c	1.260h	70.35c	10.20h	3.90fg
B73	73.26h	278.49g	1.246i	69.30f	11.20f	4.40d
Mo17	74.44g	341.13a	1.274f	69.80d	11.45e	4.15e
LSD	1.21	3.22	0.005	0.23	0.17	0.14

[1] Test weight (Kg/hL), [2] 1000 kernels weight (g), [3] Absolute Density (g/cm^3)

[4] Values with different letters in the same column are statistically different at $P<0.05$.

Table 2. Wet-milling fraction yields of ten corn lines introgressed with exotic germplasm from the GEM project and five adapted Corn Belt Lines.

Line	Wet Milling Characteristics (%db)						
	Starch	Gluten	Fiber	Germ	SteepW[1]	SR[2]	TSR[3]
AR227	64.25a[4]	14.98e	10.06k	5.20de	5.22de	92.05a	99.70abcd
AR285	61.61ef	17.87b	11.03gh	4.00ghi	5.29d	87.70c	99.80abc
CH143	56.40i	19.50a	10.69hi	7.54a	5.51c	82.58ef	99.64bcd
CH182	62.48cd	17.36bc	10.08k	4.53efgh	5.10efg	89.45b	99.54cd
CU153	60.98fg	17.22bcd	10.87hi	5.01def	5.33d	88.56bc	99.40de
CU562	63.14bc	16.88bcd	10.54ij	4.19fghi	5.05fg	91.43a	99.79abc
FS32	60.67g	16.73cd	10.18jk	6.51bc	5.78b	87.49c	99.87ab
FS35	59.08h	17.47bc	12.57e	5.69cd	4.97g	83.44e	99.77abc
UR11	60.90fg	16.77cd	13.56c	3.60i	5.10efg	85.71d	99.91ab
UR14	61.91de	16.84cd	11.37fg	4.71efg	5.06fg	89.02b	99.88ab
Line 1	55.09j	18.93a	15.70a	3.88ghi	5.85b	81.49f	99.43d
Line 2	56.48i	17.74bc	15.24b	3.67hi	6.05a	83.06e	99.11e
Line 3	62.71c	13.55f	13.03d	5.21de	5.00fg	89.14b	99.49cd
B73	63.86ab	14.97e	9.60l	6.45bc	5.10efg	92.15a	99.96a
Mo17	59.83h	16.22d	11.67f	6.77ab	5.12ef	85.71d	99.61bcd
LSD	0.75	1.02	0.40	0.87	0.14	1.16	0.32

[1] Steepwater, [2] Starch recovery, and [3] Total solids recovery.

[4] Values with different letters in the same column are statistically different at $P<0.05$.

Table 3. Compositional characteristics and wet-milling fraction yields of groups of corn lines with exotic and adapted origin.

Group of Lines	Group of Variables							
	Composition (% db)			Wet-milling Yields (% db)				
	Starch	Protein	Oil	Starch	Gluten	Fiber	Germ	SR
HSEL	70.20a[1]	11.63b	4.22b	61.44a	17.27a	11.55b	4.40c	87.55a
LSEL	69.17b	11.93a	4.63a	60.84b	17.05a	10.63c	5.79a	87.94a
Adapted	69.01c	11.50c	4.16b	59.59c	16.28b	13.05a	5.20b	86.31b

HSEL = High starch exotic lines, LSEL = Low starch exotic lines, and SR = Starch recovery.

[1] Values with different letters in the same column are statistically different at $P<0.05$.

Table 4. Composition of recovered fractions from the wet milling of ten corn lines introgressed with exotic germplasm from the GEM project and five adapted Corn Belt Lines.

Line	Recovered Fractions (% db)		
	PStarch[1]	PGluten[2]	Oil[3]
AR227	0.308a[4]	44.49bc	60.23b
AR285	0.259bc	40.68ef	58.81bc
CH143	0.278ab	38.70fg	59.01bc
CH182	0.267abc	38.29g	51.44gh
CU153	0.280ab	41.17de	56.41de
CU562	0.279ab	43.30bcd	58.75bc
FS32	0.275ab	40.51ef	49.49h
FS35	0.298ab	39.07efg	54.37ef
UR11	0.292ab	40.23efg	57.72cd
UR14	0.300ab	42.91cd	53.40fg
Line 1	0.280ab	40.55ef	63.80a
Line 2	0.279ab	39.93efg	63.44a
Line 3	0.230c	45.17b	63.77a
B73	0.292ab	47.96a	50.17h
Mo17	0.289ab	43.46bc	47.16i
LSD	0.04	2.17	2.18

[1] and [2], protein content in starch and gluten samples, respectively. [3] Oil content in the germ fraction.
[4] Values with different letters in the same column are statistically different at $P<0.05$.

Table 5. Correlation coefficients between physical, compositional, and wet-milling characteristics of ten corn lines introgressed with exotic germplasm from the GEM project and five adapted Corn Belt lines.

Fraction Yields	Physical Characteristics			Compositional Characteristics		
	TestWt	1000kWt	ADen	Starch	Protein	Oil
Starch	-0.01	0.03	-0.34	0.58*	-0.55*	0.14
Gluten	0.31	-0.22	0.65*	-0.49	0.83**	0.05
Fiber	-0.23	0.12	0.11	-0.24	0.09	-0.53*
Germ	-0.06	0.16	-0.22	-0.03	-0.09	0.38
SteepW	0.03	-0.24	0.01	-0.75*	0.45	0.29
SR	-0.01	-0.15	-0.44	0.32	-0.38	0.26
TSR	-0.24	0.41	-0.18	0.55*	-0.23	0.13

TestWt = Test weight, 1000kWt = One-thousand kernel weight, ADen = Absolute density, SteepW = Steepwater yield, SR = Starch recovery, and TSR = Total solids recovery.

* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

CHAPTER 3. PHYSICAL, COMPOSITIONAL, WET-MILLING CHARACTERISTICS AND HETEROSIS OF THE F_1 GENERATION OF HYBRIDS FROM EXOTIC INTROGRESSED BY ADAPTED INBRED LINES IN CORN

Abstract

Corn (*Zea Mays* L.) is one of the most important cereal crops in the world and the main crop in the United States. Starch is the most abundant component of the corn kernels and the most valuable fraction recovered by wet milling of corn. The amount wet milled from a sample is an indication of the millability or ease with which kernel components are separated. New hybrids with high grain yield and higher starch, protein, or oil contents have been developed and are available to corn growers. However, these hybrids are the result of crossing adapted inbred lines and rarely have corn lines from exotic germplasm been crossed with elite inbreds to develop new and useful breeding lines. Ten inbred lines with exotic origin (Argentina, Chile, Cuba, Florida, and Uruguay) from the Germplasm Enhancement of Maize project were grouped as high starch and low starch exotic lines (HSEL and LSEL) according to the starch yield produced and were crossed to three commercial adapted inbred lines used as testers. The B73xMo17 public hybrid was used as a control. The F_1 generation of these 30 experimental hybrids was analyzed using both Near-Infrared Transmittance (NIT) and a 100-g wet-milling procedure. There was no significant difference between groups of hybrids from high and low starch lines, but the effect of lines and testers, as well as the interaction effect of lines by tester (hybrids), were statistically significant and produced great variation among physical, compositional, and wet-milling characteristics of the experimental hybrids. Exotic inbred lines from Argentina and Cuba had better wet-milling characteristics when crossed to Tester 3. Negative heterosis for starch content and positive heterosis for protein content was expressed, which led to negative heterosis values for starch yield and starch recovery and poor wet-milling properties of the F_1 generation of hybrids with exotic germplasm. NIT may be used as a predictive tool to screen early progeny for high starch and low protein contents, which are associated with higher starch yield and starch recovery, to save time and costs in a corn breeding program directed to the improvement of the wet-milling efficiency of corn.

Introduction

Corn or maize (*Zea mays* L) ranks along with wheat (*Triticum* spp.) and rice (*Oryza* spp.) as the three most important cereal crops in the world. Corn is the main crop produced in the United States because it is widely grown for use in food and livestock feed. In 2006, corn production was estimated at 10.5 billion bushels (bu) with average yields of 149.1 bu per acre (USDA, 2007). According to Hallauer (1987) the four major uses of maize are for livestock feed, human consumption, industrial purposes, and seed. Globally, 67% of the maize is used for livestock feed, 25% for human consumption and industrial purposes, and the balance for seed or is lost in wastage. Industrial use of corn to produce ethanol, sweeteners (high fructose corn syrup, glucose, and dextrose), starch, and other fermentation products has increased during the last decade (Dijkhuizen et al, 1998; Singh et al, 2001a; Butzen and Hobbs, 2002) and will increase further in coming years.

Corn wet-milling is the largest commercial source of starch for food and industrial products. This is a process that separates the corn kernels into its main fractions of starch, gluten protein, germ, and fiber by using chemical, biochemical, and mechanical operations (Singh et al, 1997). In the past, determining wet-milling properties was a very expensive, tedious and time-consuming procedure that limited the laboratory evaluation of a large number of samples from new inbred lines or experimental hybrids. However, over the last 10 years laboratory procedures have been developed to determine the wet-milling characteristics of new corn hybrids and inbred lines in advanced breeding programs. A 100-g wet-milling procedure developed by Eckhoff et al (1996) and modified by Singh et al (1997) is now a widely used laboratory-level procedure because it has enabled the classification of a large number of corn samples for millability and reduced the economic cost and labor required.

Corn improvement around the world has been associated with a reduction in the germplasm base as new lines have been derived by intercrossing existing elite inbred lines (Goodman, 1999). Due to the risk that this would lead to a narrowing of the germplasm used to produce the corn hybrids grown in the United States, it is necessary to increase our understanding of the production potential present in exotic genetic resources as an option to improve yields and value-added characteristics. The Germplasm Enhancement of Maize project (GEM), a coordinated and cooperative effort among public and private sectors, was initiated to improve and enlarge the germplasm base of corn used to develop new hybrids with better agronomic performance and value-added characteristics (Pollak, 2003) by taking advantage of the genetic diversity present in the exotic germplasm. The GEM project followed the Latin American Maize Project (LAMP), the first coordinated international project for the evaluation of a major world crop (Salhuana et al, 1991). The breeding protocol followed in GEM to produce exotic introgressed breeding lines is presented by Pollak (2003).

Heterosis, also known as hybrid vigor, is the difference between the hybrid performance and either the mean of the inbred parents or the highest value of the best parent for a trait of interest. There are few studies that have documented the presence of heterosis for the physical, compositional, and wet-milling characteristics of inbred lines and their progeny in corn. Zehr et al (1995) studied 15 inbred lines and 20 related hybrids and reported significant divergence of hybrids from mid-parent values for wet-milling fraction yields. Singh et al (2001b) crossed ten GEM accessions to the public inbred lines B73 and Mo17. Crossing to the Corn Belt lines increased the protein and reduced the starch contents of the hybrids compared to the adapted lines, which led to the expression of poor wet-milling properties of the hybrids, especially low values for starch yield and starch recovery.

Based on wet-milling information from the GEM project, 10 lines with exotic introgressed genetic background were selected and crossed to three adapted inbred lines to i) study the physical, compositional, and wet-milling characteristics of hybrids from crosses among exotic introgressed and adapted inbred lines, and ii) determine the mid-parent and high-parent heterosis for these traits.

Materials and Methods

Genetic Materials

Ten corn lines from the GEM project were selected on the basis of starch yield after laboratory wet-milling done by the private cooperator Cerestar (Hammond, IN, now acquired by Cargill, Minneapolis, MN). The highest and the lowest starch-yielding lines for each of five different germplasm sources were chosen. The lines had 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). These lines were crossed with three commercial inbred lines used as testers (Tester 1, Tester 2, and Tester 3) with different compositional characteristics. These inbreds usually produce hybrids with differences in their compositional properties and, as a consequence, with potentially different yields of the main fractions recovered after wet milling. Tester 1 was an inbred line with low starch and high protein contents expected to produce hybrids with low starch content and low starch yield; Tester 2 was the Bt version of Tester 1; and Tester 3 was an inbred line that had higher starch and lower protein contents and was thus expected to produce hybrids with high starch content and starch yield. Seed of the testers was provided by Golden Harvest Seeds, Inc. (Clinton, IL, now acquired by Syngenta, Wilmington, DE). In this study, the wet-milling characteristics of the hybrids are presented. The hybrid from the B73 by Mo17 cross, two formerly widely used public inbred lines, was included as a check.

Sample Preparation

Seed of the hybrids was produced in Ames, IA, in the summer of 2004 by hand pollination. Four rows (3.8 m long and 76 cm wide) were planted for each exotic line with two rows of each tester planted adjacent to them and as many crosses as possible were made. A completely random design with one replication was used to arrange the exotic inbred lines in the field. At harvest, ears that set few kernels or that presented disease symptoms were discarded. All normal ears from each cross were hand harvested and dried to approximately 10% moisture content by circulating warm air at 38^{o}C (100^{o}F) for 72 h. Seed from all ears was bulked after shelling and stored at 4^{o}C until used. The 100-g samples were hand picked and any foreign material and cracked or broken kernels were removed. Two replications per sample of bulked seed were used to determine the physical, compositional, and wet-milling characteristics of each hybrid.

Physical Characteristics

The variables measured to determine the physical characteristics of the experimental corn hybrids were test weight, 1000-kernel weight, and absolute density. Test weight is a measure of the weight per unit of volume of a grain at a standardized moisture level and in the U.S. grain trading business is normally expressed in pounds per bushel. Standards and procedures to estimate test weights are provided by the USDA Grain Inspection, Packers and

Stockyards Administration (GIPSA). In our study, test weight was determined by following the Federal Grain Inspection Services procedures (FGIS, 1988) and expressed as kilograms per hectoliter (Kg/hL). 1000-Kernel weight provides a measure of the size of the corn kernels; this variable was determined by using an electronic counter (Electronic Counter Model 850-2, International Marketing and Design Corp., San Antonio, TX) to count the kernels and a precision top-loading balance (OHAUS Explorer Pro Model EP4102C, Pine Brook, NJ) to determine the total weight of the sample, which was expressed in g. Absolute density is a measurement of the volume of a specified weight of kernels and is expressed in g/cm^3; this variable was estimated by using a FOSS Infratec 1241 Grain Analyzer (Tecator, Hoganas, Sweden).

Compositional Characteristics

Moisture, starch, protein, and oil contents of bulked whole kernels from each hybrid were estimated with Near-infrared Transmittance (NIT) technology by using a FOSS Infratec™ 1241 Grain Analyzer (Tecator, Hoganas, Sweden). 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 NIR calibration databases of the USDA Federal Grain Inspection service.

Wet-milling Characteristics

Two samples from each line were analyzed in the laboratory by using a 100-g modified wet-milling procedure (Singh et al, 1997). This procedure determines starch, gluten, fine and coarse fiber, germ, and steepwater fractions. The procedure was slightly modified to improve reproducibility as described in Chapter 2. Moisture contents of the four main recovered fractions (starch, gluten, fiber, and germ) were determined in triplicate by using the AOAC Method 14.004 (AOAC, 1984) and the values were used to calculate the final fraction yields on a dry basis. The whole kernel moisture was estimated with three replications by following the AACC Method 44-15A (AACC, 2000) and the values were used to calculate the total solids recovery on a dry basis.

Composition of Recovered Fractions

Protein contents of the starch fractions were determined by using the macro-Kjeldahl method and the Corn Refiners Association Method A-18 (CRA, 2006). Protein contents in the recovered gluten fractions were determined with the AOAC Method 993.13 (AOAC, 2003) by using a combustion nitrogen analyzer RapidN III from Elementar Americas, Inc. (Mt. Laurel, NJ) and a protein factor of 6.25. Oil content in the germ was quantified as crude free fat content according to the AOAC Method 14-084 and 14-085 (AOAC, 1984) by using the Goldfisch procedure. All compositional values of the recovered fractions were expressed as percentage on a dry basis (% db).

Statistical Analysis

The following linear model was used for the statistical analysis of the information by using a completely random design:

$$\boldsymbol{Y_{ijkl} = \mu + G_i + L_{(i)j} + T_k + GT_{ik} + LT_{(i)jk} + E_{(ijkl)}}$$

Where:

Y_{ijkl} = Response observed for the ijklth experimental unit.

μ = Overall mean.

G_i = Effect of the ith group of exotic corn lines.

$L_{(i)j}$ = Effect of the jth line nested in the ith group.

T_k = Effect of the kth tester.

GT_{ik} = Interaction effect of the ith group of exotic corn lines with the kth tester.

$LT_{(i)jk}$ = Interaction effect of the jth line with the kth tester.

$E_{(ijkl)}$ = Effect associated with experimental error.

Groups of lines and testers were considered as fixed and lines within groups as random factors. Proc GLM of SAS (SAS Institute, Cary, NC) was used to determine statistical differences among different values. Pearson correlation coefficients were calculated among the line by tester interaction mean over replications, which represent the value for each hybrid, by using PROC CORR of SAS. Statistical differences among groups of lines, lines within groups, and testers were determined using least significant differences (LSD) to estimate differences among groups, lines, and testers. Groups were tested by lines within groups, lines and the interaction effect of line by tester were tested by the error, and tester and the interaction effect of group by tester were tested by the line by tester expected mean square.

Calculation of Heterosis

The performance of a hybrid in relation to its parents can be expressed as mid-parent or high-parent heterosis. Calculations were made by using the following formulas:

a) Mid-parent heterosis is the performance of a hybrid compared with the average performance of its parents.

$$\text{Mid-parent heterosis (\%)} = \frac{F1 - MP}{MP} \times 100$$

b) High-parent heterosis is a comparison of the performance of the hybrid with that of the best parent in the cross.

$$\text{High-parent heterosis (\%)} = \frac{F1 - HP}{HP} \times 100$$

Where:

F1 = Performance of hybrid.

MP = Average performance of parents per se, given by (parent1 + parent2)/2.

HP = Performance of the best parent.

Result and Discussion

Analysis of Variance

Significant differences ($P<0.01$) among all variables were found but not for all sources of variation (Tables 1 and 2). The sources of variation represented by groups of lines (HSEL and LSEL) and the interaction effect of group by tester were not statistically significant for all the physical, compositional, and wet-milling characteristics. Line had significant effects ($P<0.01$) for all variables in the study, which mean that the exotic corn lines produced hybrids with specific physical, compositional, and wet-milling properties. Different testers produced hybrids with different seed sizes but similar test weights and absolute densities and significant differences in the starch, protein, and oil contents. When the hybrids were grouped by tester, the wet-milling variables were statistically different, which mean that each tester produced progeny with different starch, gluten, and fiber yields. No differences were observed for the effect of tester on germ yield. The interaction of line by tester, which represents the specific properties of the different hybrids produced, was highly significant for all the variables in the study, indicating differences in combining ability for lines and testers. It is important to point out that for wet-milling purposes it is better to test hybrids than lines because in this way a larger range of differences for the recovered fractions will be expressed and facilitate the selection of parents in a corn breeding program focused on improving the wet-milling efficiency.

Physical Characteristics

Test weight is a measure of density or weight per unit of volume of a grain at the standardized moisture level of 15.5% (Harper, 2003). Test weights of the hybrids ranged from 73.0 to 84.1 with a mean of 79.9 k/hL (Table 3). Test weight of the B73xMo17 F_1 hybrid was 80.9 k/hL. Singh et al (2001a) reported similar values with a mean of 79.3 k/hL for a total of 51 exotic accessions used in the GEM project. In our study, test weight values may be higher than the 73 k/hL of dent corn hybrids (Watson, 1987), because we used samples dried to approximately 10% moisture content and as corn becomes drier test weight slightly increases.

The 1000-kernel weights of the hybrids ranged from 232.6 to 388.4 g with a mean value of 294.0 g. This wide range (156 g) shows that kernel sizes of the hybrids had great variation. Similar variation for this trait was reported by Singh et al (2001a) who obtained lower values for a set of GEM accessions than for the commercial dent hybrids used as controls and thus found greater fiber and lower starch yields for the GEM germplasm, which was attributed to greater ratios of surface area to mass. The hybrid of B73xMo17 had a value of 262.7 g. Zehr et al (1995) reported a mean of 359 g for a group of hybrids representative of the germplasm groups used in the United States, which indicates that inbred lines adapted to the Corn Belt produce hybrids with bigger seed size than inbred lines that are in the process of selection for adaptation to this area.

Absolute densities had a mean of 1.305 g/cm^3 for the experimental hybrids. This value was similar to the absolute density reported in Chapter 2 for the exotic introgressed lines used as female parents and higher than the value for the adapted inbred lines used as the male parents (1.301 and 1.272 g/cm^3, respectively). Zehr et al (1995) reported a mean value of 1.34 g/cm^3 for F_1 hybrids, which was significantly higher than the value of the parental lines. Singh et al (2001a) reported a mean value of 1.32 g/cm^3 and did not find a combination of reduced absolute density and low protein content in this set of accessions, which favors wet-milling efficiency.

Compositional Characteristics

Starch, protein, and oil contents (Table 1) were statistically significant for the lines and line by tester interaction. The mean starch content of the hybrids with exotic germplasm (68.2% db, Table 3), was slightly lower than the starch content of the B73xMo17 hybrid (68.7%). However, some hybrids with exotic background had starch contents of or above 70% db (FS35 and UR11). These results are similar to those reported for some yellow dent corn hybrids (Fox et al, 1992; Zehr et al, 1995) and high-oil corn hybrids (Raush et al, 1999) but lower than the starch contents of some of the commercial hybrids used in other studies (Singh et al, 1997; Singh et al, 2005; Vignaux et al, 2006).

The mean protein content of the experimental hybrids was 12.0%; however, the CH143 line produced hybrids with higher compositional protein (14.1% when crossed to Tester 2). The B73xMo17 hybrid had a protein content of 11.4%. These values are considered high. Higher protein contents can contribute to lower starch content and starch recovery from wet milling due to negative correlation between the protein content of the corn kernels and the starch yield during wet milling (Fox et al, 1992; Singh et al, 2001a).

Mean oil contents (4.6 and 4.4%) for the experimental hybrids and the hybrid used as control were slightly higher than the 4.0% value of normal yellow dent corn hybrids (Rooney et al, 2004) but lower than the oil content of 8% that can be present in high-oil corn hybrids (Raush et al, 1999). The compositional characteristics of the materials used in this study indicated that great variation can be found as a result of the wide genetic diversity associated with the different genetic background of the experimental hybrids tested.

Wet-milling Characteristics

Statistical differences ($P<0.01$) among hybrids was found for all wet-milling variables evaluated (Table 2). The wet-milling properties of the materials in the study are shown in Table 4. Variability of the corn hybrids and their different wet-milling characteristics is a factor that can negatively affect production costs and economic gains of the wet-milling industry (Eckhoff, 1995). Because of this, it is important to test any new hybrid to determine its wet-milling properties before using it as raw material in wet milling, which prefers uniform hybrids that give good starch yields. Starch is the most important recovered fraction (Singh and Eckhoff, 1996) from the wet-milling process and represents an indicator of the millability, or ease with which kernel components are separated (Weller et al, 1988). Starch yield and starch recovery (represented by the extractable fraction of the total compositional starch) have been considered to be the most important parameters of the millability of the hybrids. Starch yields of the experimental hybrids varied from 53.4 to 62.4 with a mean of

58.7% db. These values are lower than those reported when milling yellow dent corn hybrids (Weller et al, 1988; Singh and Eckhoff, 1996; Singh et al, 1997; Dowd, 2003, Vignaux et al, 2006). However, it needs to be pointed out that the corn samples used in this study were the F_1 generation of experimental hybrids that have smaller kernel sizes and higher protein contents because seeds are produced on inbred ears (Table 1), which makes starch separation more difficult. On the other hand, corn samples used in the cited reports corresponded to grain of yellow dent hybrids that normally have bigger kernel sizes, higher starch contents, and lower protein contents, which facilitates the starch separation and, as a consequence, produce higher starch yields. The mean starch yield of the B73xMo17 hybrid was 64.4% db and was superior to all the experimental hybrids. Nevertheless, we previously found (Chapter 2) that some exotic lines had similar or better millability than the public inbred line B73, which is evidence that potential to improve wet-milling characteristics of hybrids grown in the United States can be found in this exotic germplasm.

Tabling is used in most of the laboratory-scale wet-milling procedures (Singh and Eckhoff, 1996; Dowd, 2003) to separate the starch from the gluten fractions. This separation is based on the principle of particle density differences. Starch is heavier than the 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 ranged from 14.5 to 20.3 with a mean of 17.4% db. These results are higher than those reported for adapted inbred lines and their hybrids (Zehr et al, 1995) and GEM accessions (Singh et al, 2001a). This poor starch-gluten separation can be attributed to the high protein contents of these hybrids, which makes the release of the starch granules more difficult (Watson, 1984).

Fiber yield ranged from 10.6 to 16.6 and a mean of 12.8% db. These values are high and may be the result of the greater proportion of surface area to mass associated with the small kernels of the F_1 generation of the hybrids studied (Singh et al, 2001a).

Germ yield varied from 4.4 to 7.2 with a mean of 5.3% db. The line that produced the highest germ yields was CH143 when crossed with any of the three testers, which indicated that this particular line had good germ separation characteristics during the first grind of the wet-milling procedure used. The B73xMo17 hybrid had a germ yield of 6.8% db. These values are similar to those reported by other studies (Fox et al, 1992; Zehr et al, 1995; Singh et al, 2001a) which shows that even when some compositional or physical characteristics of the corn kernels affect the wet-milling properties of the materials in the study, the germ yield of these experimental hybrids is not affected by these factors. Higher germ yields have been reported for high-oil corn hybrids (Raush et al, 1999). Steepwater yields were similar to those reported by Fox et al (1992) and Zehr et al (1995).

Starch recovery (SR) is calculated by dividing starch yield by the starch content. Because starch is the most important product obtained from wet milling, this variable is an excellent indicator of the millability of any corn material. In our study, SR varied from 70.0 to 91.3% with a mean of 86.1 %. These results are similar to values obtained by Fox et al (1992), Zehr et al (1995), and Dowd (2003) but lower than those reported by Singh et al (1997). SR for the B73xMo17 hybrid was 93.8%. Total solids recovery (TSR) varied from 99.3 to 100.0 and was similar to the values reported for the industry (99.6 to 100%). TSR of 98% are generally achievable if the samples are carefully milled (Singh and Eckhoff, 1996).

Composition of Recovered Fractions

The composition of some recovered fractions is shown in Table 5. The protein contents of the starch samples varied from 0.22 to 0.32% with a mean of 0.27% db. According to Vignaux et al (2006), the typical level of residual protein in commercial starch is 0.3% but it can range from 0.27 to 0.32% (Watson, 1984). Singh et al (2001a) reported an average of 1.05% protein content in starch recovered from the GEM accessions and attributed it to the poor starch-gluten separation that can be a characteristic of germplasm, with either adapted or exotic origin, that has a high level of protein content in the corn kernels.

Protein content of gluten samples ranged from 31.9 to 45.8% db. These values are lower than achieved by industry (66%) (Dowd, 2003) and can be attributed to the difficulty in separating starch from the gluten fractions, which results in lower starch yield, higher gluten yield with a high concentration of starch and, as a consequence, lower protein content in the gluten samples (Singh et al, 2001a). However, gluten obtained when using the tabling method to acieve the starch-gluten separation rarely contains more than 50% protein content (Watson, 1984).

Mean oil content in the germ fraction was 60.9 % and ranged from 56.1 to 64.8% db. Despite the fact that these values seem high, according to contents reported for high-oil corn hybrids (52.5 to 57.1%) (Raush et al, 1999), we verified our results by rerunning some samples, including the testers, following in detail the recommended procedure (AOAC, 1984), and the results (data not shown) were very similar to those reported here. The B73 x Mo17 hybrid contained 52.2% db oil and was the lowest of all hybrids evaluated.

Compositional and Wet-milling Characteristics of Experimental Hybrids Grouped by Tester

When the experimental hybrids were grouped by tester, statistical differences ($P<0.05$) were found among compositional and wet-milling properties (Table 6). Testers 1 and 2, two Bt isolines, produced hybrids with similar wet-milling characteristics for all variables evaluated. There were no significant differences between groups of hybrids where either Tester 1 or Tester 2 were the male parent. These similarities were the result of the similar values for Testers 1 and 2 for starch and protein contents, which produced statistically identical results for starch yields and starch recovery for both groups of experimental hybrids.

Tester 3 produced hybrids that had superior starch yields and starch recoveries, as a consequence of the higher starch and lower protein concentrations in comparison to Testers 1 and 2. This indicated that this inbred line, when used as the male parent, produces progeny that would be preferred by the wet-milling industry because its hybrids have better millability.

According to Singh and Eckhoff (1996), a low coefficient of variation or standard deviation of the recovered fraction yields after replicated wet milling of corn samples is an indicator of the reproducibility of the procedure. Coefficients of variance for the recovered fractions were similar to values obtained by Dowd (2003) and lower than those reported by Eckhoff et al (1996) and Singh et al (1997), which show that the reproducibility of our procedure is similar to or better than the procedure used in other laboratories.

Heterosis for the Physical, Compositional and Wet-milling Characteristics

Mid-parent Heterosis

Mid-parent heterosis values for the physical, compositional, and wet-milling properties of the two groups of experimental hybrids are shown in Table 7. The values to make the calculations were taken from Chapter 2 for the parental lines 1 and Tables 3 and 4 for the hybrids. There was positive mid-parent heterosis with similar values for test weight, 1000-kernel weight, and absolute density for both HSEL and LSEL hybrids, which indicated that the F_1 generation maintained similar physical characteristics to those of their exotic or adapted parents.

The compositional properties of the hybrids with exotic germplasm, although without significant differences between groups, had differences in the mean value of their inbred parents. Starch contents had a negative mid-parent heterosis value (-1.01 and -1.71 for HSEL and LSEL hybrids, respectively), while the value for protein content was positive in both groups of hybrids (0.31 and 4.70 for HSEL and LSEL hybrids, respectively). There was positive mid-parent heterosis for oil content in both groups of hybrids, which means that hybrids from exotic introgressed lines can be valuable for wet-millers since germ oil is a high-value coproduct from wet milling.

Starch yield had negative mid-parent heterosis values for both groups of hybrids (-0.71 and -2.43 for HSEL and LSEL groups, respectively); gluten and germ yields showed positive heterosis and fiber, steepwater, and starch recoveries had variable values depending upon the group of hybrids. The combination of lower starch and higher protein contents of the hybrids in comparison to the mean value of their inbred parents can be the cause of the poor wet-milling properties of the materials in the present study since any of the hybrids had superior starch yield than the mean value of the parents. Nevertheless, the protein content of the LSEL group of hybrids was higher than the protein content of the HSEL hybrids, which, in agreement with Fox et al (1992), points to the compositional protein as one of the most determinant factors of the wet-milling efficiency of corn hybrids. Singh et al (2001b) reported a positive mean value for starch content and a negative mean value for protein content when examining 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. The B73xMo17 F_1 hybrid had better wet-milling characteristics than any of the experimental hybrids since it had positive values for starch and germ yield and for starch recovery and negative values for gluten and fiber yield.

High-parent Heterosis

High-parent heterosis values for physical, compositional, and wet-milling properties of the two groups of experimental hybrids are shown in Table 8. In the case of gluten, fiber, and steepwater yields, three fractions where high values are not desirable in the wet-milling industry, heterosis was calculated using the parent with the lower value; if the heterosis value expressed by the hybrid is negative, then those hybrids can be considered as materials with appropriate wet milling properties. The average values for test weight and absolute density followed the same pattern as the mid-parent heterosis values, with positive values for both variables. The 1000-kernel weight had negative values, which indicates that hybrids had smaller kernel size than the parental line with the bigger seed size from each cross. The LSEL hybrids had the lower values for this variable (-4.8%) which mean that this group of hybrids had, on average, smaller seed size than the HSEL hybrids. Singh et al (2001b) 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.

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

Starch yield and starch recovery, the two main parameters of the millability of the hybrids, had negative high-parent heterosis values, which indicates that, in general, the hybrids with exotic germplasm had poor wet-milling efficiency. However, hybrids that had Tester 3 as a male parent produced higher starch yield and better starch recovery than hybrids of Tester 1 or Tester 2 as the male parent. Singh et al (2001b) reported that GEM accessions that were crossed to Mo17 had higher starch and lower protein contents and higher starch yields and starch recoveries than when the same group of accessions was crossed to B73. They concluded that hybrids with Mo17 had better wet-milling characteristics than B73 crosses.

Correlation Coefficients

Correlation coefficients among the physical, compositional, and wet-milling characteristics of the experimental hybrids are shown in Table 9. The physical properties of the corn kernels had a direct effect on the compositional characteristics but they did not have a marked effect on the wet-milling properties of the experimental hybrids. There was a significant positive correlation between starch content and starch yield and a negative strong correlation between protein content and starch yield, which indicate that hybrids with high starch and low protein content will produce higher starch yields. This high correlation between compositional values obtained from NIT and the wet-milling yield of important fractions indicated that NIT technology may have value to predict the extractable starch of experimental hybrids in early stages of a corn breeding program. In general, correlations varied largely but followed the same pattern reported in previous studies (Weller et al, 1988; Fox et al, 1992; Zehr et al, 1995; Dijkhizen et al, 1998; Singh et al, 2001a).

Conclusions

There was great variation among physical, compositional, and wet-milling characteristics among the experimental hybrids from the crosses between exotic inbred lines and commercial adapted lines used as testers. This suggested that genetic diversity was present and that potential to improve wet-milling characteristics of hybrids grown in the United States can be found in exotic germplasm.

Even though hybrids from the HSEL group of lines had slightly higher starch yields and starch recoveries than hybrids from the LSEL group of lines, they were statistically similar as groups. However, there were statistical differences among hybrids, without considering groups of lines, which indicates that some particular sources, such as CUBA117, may produce hybrids with appropriate wet-milling characteristics.

Testers produced groups of hybrids that were statistically different. Tester 3 produced hybrids with higher starch content, higher starch yield, and better starch recovery than Testers 1 and 2, which indicates that inbred lines that give to its progeny high starch content may be preferable if hybrids with high starch yield are required.

Negative heterosis for starch content and positive heterosis for protein content were expressed, which led to negative heterosis values for starch yield and starch recovery and poor wet-milling properties of the F_1 generation of hybrids with exotic germplasm.

There was a positive correlation between starch content and starch yield and between protein content and gluten yield and a strong negative correlation between protein content and starch yield. This means that genotypes with high starch and low protein contents will produce higher starch yields and starch recoveries.

NIT technology may be used as a predictive tool to screen early progeny for high starch and lower protein content and identify genotypes with, in theory, high extractable starch values and save time and costs in a corn breeding program directed to the improvement of the wet-milling efficiency in corn.

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Table 1. Analysis of variance for the physical and compositional characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Source	df	Mean Squares		
		Physical Characteristics		
		TestWt	1000KWt	ADen
Group	1	0.02ns	1077.55ns	0.0003ns
Line (Group)	8	40.10***	7562.47***	0.0014***
Tester	2	3.80ns	8533.98***	0.0004ns
Group*Tester	2	0.61ns	967.80ns	0.0001ns
Line*Tester (Group)	16	3.23***	592.77***	0.0001***
		Compositional Characteristics		
		StarchC	ProtC	OilC
Group	1	15.10ns	7.00ns	0.37ns
Line (Group)	8	4.38***	1.44***	0.39***
Tester	2	3.02**	2.47*	1.21***
Group*Tester	2	1.44ns	0.86ns	0.03ns
Line*Tester (Group)	16	0.44***	0.62***	0.10***

TestWt = Test weight (K/hL); 1000KWt = One thousand-kernels weight (g); ADen = Absolute density (g/cm^3); StarchC, ProtC, and OilC = Starch, protein, and oil content (% db).

*, **, ***, and ns = Statistically significant at 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 2. Analysis of variance for the wet-milling characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Source	df	Mean Squares			
		Wet Milling Characteristics (% db)			
		Starch	Gluten	Fiber	Germ
Group	1	25.96ns	5.95ns	0.25ns	2.82ns
Line (Group)	8	15.77***	4.76***	6.90***	2.15***
Tester	2	78.52***	16.99**	19.94***	0.28ns
Group*Tester	2	12.98ns	2.39ns	2.80ns	0.01ns
Line*Tester (Group)	16	3.60***	1.83***	0.88***	0.46**

, *, and ns = Statistically significant at 0.01 and 0.001 probability levels and not significant, respectively.

Table 3. Physical and compositional characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Hybrid	Physical Characteristics			Compositional Characteristics (% db)		
	TestWt[1]	1000kWt[2]	ADen[3]	Starch	Protein	Oil
AR227T1	79.0 ± 0.00	270.8 ± 4.02	1.298 ± 0.00	67.3 ± 0.28	12.4 ± 0.07	4.5 ± 0.14
AR227T2	84.1 ± 0.14	271.1 ± 4.42	1.330 ± 0.00	66.7 ± 0.14	12.5 ± 0.21	5.2 ± 0.07
AR227T3	81.9 ± 0.16	304.3 ± 5.23	1.318 ± 0.00	68.1 ± 0.21	11.8 ± 0.14	4.4 ± 0.00
AR285T1	81.8 ± 0.11	312.2 ± 0.56	1.302 ± 0.00	68.0 ± 0.07	12.0 ± 0.07	4.8 ± 0.07
AR285T2	82.0 ± 0.13	286.4 ± 3.26	1.316 ± 0.00	68.4 ± 0.00	11.6 ± 0.07	4.7 ± 0.14
AR285T3	81.0 ± 0.09	324.6 ± 2.58	1.319 ± 0.01	68.5 ± 0.00	11.8 ± 0.14	4.3 ± 0.07
CH143T1	80.8 ± 0.18	237.9 ± 0.84	1.322 ± 0.00	66.8 ± 0.14	13.0 ± 0.00	4.7 ± 0.07
CH143T2	80.3 ± 0.20	232.6 ± 2.03	1.330 ± 0.00	65.7 ± 0.07	14.1 ± 0.00	4.9 ± 0.07
CH143T3	79.3 ± 0.14	273.5 ± 0.04	1.322 ± 0.00	66.9 ± 0.14	12.7 ± 0.00	4.9 ± 0.07
CH182T1	82.2 ± 0.71	273.9 ± 3.37	1.328 ± 0.00	68.0 ± 0.14	12.3 ± 0.07	4.2 ± 0.07
CH182T2	82.6 ± 0.11	278.3 ± 0.89	1.322 ± 0.00	68.1 ± 0.07	12.3 ± 0.07	4.6 ± 0.07
CH182T3	82.6 ± 0.16	324.6 ± 1.22	1.325 ± 0.00	68.5 ± 0.07	11.7 ± 0.07	4.3 ± 0.00
CU153T1	82.6 ± 0.16	271.4 ± 1.99	1.306 ± 0.00	68.0 ± 0.07	11.2 ± 0.00	5.3 ± 0.07
CU153T2	82.9 ± 0.33	282.8 ± 3.20	1.317 ± 0.00	66.8 ± 0.07	12.6 ± 0.07	5.5 ± 0.07
CU153T3	81.9 ± 0.06	283.0 ± 0.37	1.312 ± 0.00	67.4 ± 0.21	12.2 ± 0.07	4.7 ± 0.07
CU562T1	79.9 ± 0.58	247.5 ± 1.50	1.284 ± 0.01	67.1 ± 0.07	12.5 ± 0.07	4.7 ± 0.28
CU562T2	80.3 ± 0.03	265.7 ± 1.43	1.309 ± 0.00	68.8 ± 0.07	11.1 ± 0.14	5.3 ± 0.00
CU562T3	81.9 ± 0.11	335.7 ± 1.21	1.299 ± 0.00	68.4 ± 0.07	11.9 ± 0.07	4.4 ± 0.07
FS32T1	78.6 ± 0.24	357.0 ± 1.51	1.284 ± 0.00	68.1 ± 0.07	13.0 ± 0.21	4.6 ± 0.21
FS32T2	78.8 ± 0.11	350.2 ± 2.64	1.295 ± 0.00	68.5 ± 0.07	12.1 ± 0.07	4.6 ± 0.00
FS32T3	79.0 ± 0.17	370.4 ± 1.46	1.289 ± 0.00	69.8 ± 0.07	10.6 ± 0.07	4.5 ± 0.07
FS35T1	79.2 ± 0.08	299.5 ± 6.22	1.291 ± 0.00	69.3 ± 0.07	11.0 ± 0.14	4.7 ± 0.07
FS35T2	80.0 ± 0.12	337.2 ± 0.45	1.298 ± 0.00	68.8 ± 0.14	11.7 ± 0.21	5.0 ± 0.35
FS35T3	79.9 ± 0.11	388.4 ± 2.04	1.300 ± 0.00	70.0 ± 0.42	10.6 ± 0.07	4.3 ± 0.35
UR11T1	76.4 ± 0.28	248.9 ± 2.90	1.294 ± 0.00	69.5 ± 0.00	11.9 ± 0.07	4.6 ± 0.00
UR11T2	76.1 ± 0.18	282.5 ± 1.80	1.281 ± 0.00	69.3 ± 0.14	11.5 ± 0.00	4.5 ± 0.07
UR11T3	73.0 ± 0.18	267.8 ± 4.76	1.275 ± 0.00	70.1 ± 0.14	10.8 ± 0.00	4.0 ± 0.21
UR14T1	78.2 ± 0.18	276.8 ± 1.61	1.300 ± 0.00	69.1 ± 0.07	12.1 ± 0.00	4.6 ± 0.07
UR14T2	77.1 ± 0.06	260.1 ± 1.46	1.302 ± 0.00	67.9 ± 0.07	12.7 ± 0.00	5.4 ± 0.07
UR14T3	75.0 ± 0.29	304.1 ± 1.07	1.286 ± 0.00	68.8 ± 0.00	11.7 ± 0.00	4.0 ± 0.00
B73xMo17	80.93	262.71	1.310	68.7	11.4	4.4

[1]Test weight (k/hL), [2]1000 kernels weight (g), [3]Absolute Density (g/cm^3)

Table 4. Wet-milling characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Hybrid	Wet-milling Characteristics (% db)					
	Starch	Gluten	Fiber	Germ	SteepW[1]	SR[2]
AR227T1	60.20 ± 0.45	16.02 ± 0.35	11.67 ± 0.86	6.13 ± 0.03	5.72 ± 0.03	89.45 ± 1.04
AR227T2	59.53 ± 0.23	16.84 ± 0.39	11.58 ± 0.21	5.72 ± 0.16	5.71 ± 0.01	89.26 ± 0.52
AR227T3	60.12 ± 0.08	17.46 ± 0.26	11.47 ± 0.23	4.67 ± 0.57	5.64 ± 0.05	88.34 ± 0.40
AR285T1	58.56 ± 0.30	17.38 ± 0.35	12.74 ± 0.54	5.07 ± 0.38	5.62 ± 0.06	86.17 ± 0.54
AR285T2	60.37 ± 0.48	16.96 ± 0.13	12.15 ± 0.48	4.36 ± 0.18	5.61 ± 0.09	88.26 ± 0.71
AR285T3	60.46 ± 0.16	16.75 ± 0.14	11.55 ± 0.07	5.44 ± 0.03	5.22 ± 0.12	88.26 ± 0.23
CH143T1	54.47 ± 0.25	19.70 ± 0.45	13.83 ± 0.06	6.11 ± 0.11	5.37 ± 0.04	81.54 ± 0.21
CH143T2	53.36 ± 0.16	20.26 ± 0.28	13.86 ± 0.32	6.73 ± 0.57	5.65 ± 0.03	81.28 ± 0.16
CH143T3	58.33 ± 0.19	17.14 ± 0.07	11.53 ± 0.20	7.16 ± 0.33	5.45 ± 0.06	87.18 ± 0.47
CH182T1	56.23 ± 0.18	19.99 ± 0.14	13.10 ± 0.56	4.75 ± 0.75	5.31 ± 0.04	82.69 ± 0.44
CH182T2	60.79 ± 0.11	18.31 ± 0.33	10.64 ± 0.23	5.00 ± 0.08	5.10 ± 0.03	89.33 ± 0.25
CH182T3	61.76 ± 0.19	16.41 ± 0.23	11.22 ± 0.07	4.69 ± 0.31	5.86 ± 0.42	90.22 ± 0.18
CU153T1	57.80 ± 0.11	18.41 ± 0.01	13.32 ± 0.37	4.42 ± 0.12	5.66 ± 0.11	85.05 ± 0.24
CU153T2	55.44 ± 0.17	19.16 ± 0.41	14.60 ± 0.13	4.37 ± 0.39	6.01 ± 0.21	83.06 ± 0.16
CU153T3	59.50 ± 0.25	17.23 ± 0.49	11.77 ± 0.02	5.79 ± 0.28	5.31 ± 0.11	88.35 ± 0.66
CU562T1	59.43 ± 0.05	17.52 ± 0.81	12.89 ± 0.35	4.54 ± 0.60	5.38 ± 0.15	88.65 ± 0.02
CU562T2	61.80 ± 0.26	15.85 ± 0.30	11.76 ± 0.18	4.96 ± 0.18	5.20 ± 0.12	89.89 ± 0.47
CU562T3	62.39 ± 0.13	16.44 ± 0.55	10.99 ± 0.16	4.65 ± 0.40	5.03 ± 0.04	91.29 ± 0.09
FS32T1	55.08 ± 0.02	19.20 ± 0.20	14.27 ± 0.25	5.57 ± 0.26	5.71 ± 0.08	80.93 ± 0.11
FS32T2	56.80 ± 0.23	18.26 ± 0.54	14.14 ± 0.12	5.20 ± 0.09	5.54 ± 0.01	82.97 ± 0.25
FS32T3	61.91 ± 0.23	14.93 ± 0.01	12.17 ± 0.30	5.55 ± 0.48	5.04 ± 0.03	88.76 ± 0.25
FS35T1	57.05 ± 0.32	16.83 ± 0.02	14.00 ± 0.35	6.03 ± 0.01	5.56 ± 0.08	82.38 ± 0.37
FS35T2	57.78 ± 0.08	16.86 ± 0.52	13.95 ± 0.03	5.86 ± 0.51	5.51 ± 0.09	83.98 ± 0.29
FS35T3	61.96 ± 0.30	14.54 ± 0.06	11.82 ± 0.05	6.06 ± 0.49	4.97 ± 0.06	88.52 ± 0.96
UR11T1	54.21 ± 0.35	18.00 ± 0.37	16.61 ± 0.47	5.14 ± 0.04	5.80 ± 0.05	78.00 ± 0.50
UR11T2	56.92 ± 0.23	17.50 ± 0.45	15.00 ± 0.07	4.91 ± 0.17	5.61 ± 0.04	82.13 ± 0.15
UR11T3	60.48 ± 0.13	16.38 ± 0.09	12.87 ± 0.34	5.20 ± 0.01	4.70 ± 0.01	86.27 ± 0.37
UR14T1	58.42 ± 0.30	16.80 ± 0.11	13.51 ± 0.21	5.39 ± 0.00	5.45 ± 0.06	84.61 ± 0.52
UR14T2	57.18 ± 0.13	17.99 ± 1.05	13.99 ± 0.51	5.07 ± 0.63	5.22 ± 0.11	84.27 ± 0.29
UR14T3	62.32 ± 0.13	15.76 ± 0.38	11.53 ± 0.09	5.29 ± 0.47	4.73 ± 0.13	90.58 ± 0.18
B73xMo17	64.37	14.06	9.09	6.76	5.20	93.76

[1]Steepwater and [2]Starch recovery.

Table 5. Composition of recovered fractions from the wet-milling of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Hybrid	PStarch	PGluten	Oil
AR227T1	0.305 ± 0.02	45.22 ± 0.15	62.32 ± 1.16
AR227T2	0.315 ± 0.02	43.73 ± 1.68	64.83 ± 0.11
AR227T3	0.275 ± 0.02	40.08 ± 0.26	62.48 ± 0.05
AR285T1	0.290 ± 0.01	40.35 ± 0.47	63.34 ± 0.71
AR285T2	0.290 ± 0.01	42.86 ± 0.08	63.69 ± 0.32
AR285T3	0.260 ± 0.00	44.42 ± 0.14	61.47 ± 0.07
CH143T1	0.285 ± 0.01	38.28± 0.88	61.72 ± 1.15
CH143T2	0.285 ± 0.01	41.00 ± 2.53	62.19 ± 0.11
CH143T3	0.220 ± 0.01	42.24 ± 0.73	58.01 ± 1.01
CH182T1	0.245 ± 0.01	31.88 ± 1.64	61.64 ± 0.16
CH182T2	0.250 ± 0.00	39.03 ± 0.23	60.51 ± 1.91
CH182T3	0.235 ± 0.04	39.95 ± 2.16	58.61 ± 0.54
CU153T1	0.270 ± 0.01	36.16 ± 0.08	64.06 ± 0.03
CU153T2	0.265 ± 0.01	36.87 ± 0.33	64.11 ± 0.08
CU153T3	0.230 ± 0.00	40.33 ± 0.75	62.12 ± 0.10
CU562T1	0.295 ± 0.01	42.59 ± 0.91	62.67 ± 0.31
CU562T2	0.270 ± 0.01	42.46 ± 1.78	63.68 ± 0.38
CU562T3	0.260 ± 0.00	41.89 ± 0.32	61.23 ± 0.30
FS32T1	0.285 ± 0.02	37.94 ± 0.98	60.29 ± 0.01
FS32T2	0.295 ± 0.01	37.29 ± 1.31	57.76 ± 0.18
FS32T3	0.240 ± 0.01	40.25 ± 0.65	58.47 ± 0.95
FS35T1	0.235 ± 0.01	37.11 ± 0.78	58.22 ± 0.15
FS35T2	0.280 ± 0.00	38.48 ± 2.30	60.58 ± 0.05
FS35T3	0.235 ± 0.01	41.08 ± 0.08	57.09 ± 1.20
UR11T1	0.245 ± 0.01	34.87 ± 2.18	61.86 ± 0.36
UR11T2	0.270 ± 0.01	35.42 ± 0.90	60.88 ± 0.04
UR11T3	0.240 ± 0.00	41.04 ± 0.95	58.82 ± 0.59
UR14T1	0.290 ± 0.01	39.94 ± 1.82	58.86 ± 0.22
UR14T2	0.280 ± 0.01	41.70 ± 1.03	59.67 ± 0.30
UR14T3	0.280 ± 0.01	45.82 ± 1.31	56.14 ± 0.16
B73xMo17	0.260	48.01	52.21

PStarch, PGluten, and Oil = Protein content in starch and gluten samples and oil content of the germ, respectively.

Table 6. Mean values and coefficients of variation for the compositional and wet-milling characteristics of groups of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Variable	Tester	Mean (% db)	CV	Grouping
Starch Content LSD = 0.44	1	68.09	0.20	B
	2	67.87		B
	3	68.63		A
Protein Content LSD = 0.53	1	12.11	0.82	A
	2	12.19		A
	3	11.55		B
Starch LSD = 1.27	1	57.14	0.40	B
	2	57.99		B
	3	60.92		A
Starch Recovery LSD = 1.74	1	83.94	0.51	B
	2	85.44		B
	3	88.78		A
Gluten LSD = 0.91	1	17.98	2.26	A
	2	17.80		A
	3	16.30		B
Fiber LSD = 0.63	1	13.59	2.54	A
	2	13.16		A
	3	11.69		B
Germ LSD = 0.45	1	5.31	6.76	A
	2	5.21		A
	3	5.45		A
Steepwater LSD = 0.24	1	5.56	2.09	A
	2	5.51		A
	3	5.16		B

Table 7. Mid-parent heterosis for physical, compositional, and wet-milling characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Hybrid	Physical			Compositional			Wet-milling					
	TestWt	1000k	ADen	Starch	Protein	Oil	Starch	Gluten	Fiber	Germ	SteepW	SR
AR285T1	5.21	16.70	0.15	-1.34	-1.03	14.97	0.36	-5.54	-4.68	28.68	0.90	1.86
AR285T2	5.54	1.37	1.39	-1.05	-0.85	8.05	2.24	-4.75	-7.50	13.69	-1.06	3.37
AR285T3	4.93	14.07	2.85	-2.56	8.76	4.24	-2.73	6.62	-3.99	18.13	1.46	-0.18
CH182T1	5.01	6.00	1.18	-1.05	0.00	5.00	-4.35	10.17	1.63	12.96	-3.01	-3.25
CH182T2	5.60	1.79	0.88	-1.20	3.58	10.18	2.20	4.33	-15.96	21.95	-8.52	3.57
CH182T3	6.28	17.86	2.32	-2.28	6.12	8.86	-1.33	6.18	-2.90	-3.70	16.04	1.04
CU562T1	4.66	-9.47	0.04	-1.79	-0.40	9.94	0.53	-2.15	-1.75	12.52	-1.28	2.53
CU562T2	5.26	-7.87	2.15	0.40	-8.45	19.10	3.33	-8.43	-8.77	26.21	-6.31	3.03
CU562T3	8.06	15.59	2.61	-1.87	5.54	4.14	-0.85	8.05	-6.75	-1.06	0.10	1.11
FS35T1	1.94	-0.45	-1.34	0.14	-7.56	12.57	-0.06	-7.53	-0.96	26.02	2.77	-0.10
FS35T2	3.04	6.75	-0.65	-0.86	1.96	14.94	0.00	-4.23	0.32	25.21	0.00	0.88
FS35T3	3.58	22.18	0.70	-0.81	-0.24	4.24	1.75	-6.25	-7.66	11.19	-0.30	2.58
UR11T1	1.93	-17.35	-0.61	0.25	-1.45	17.20	-6.53	0.84	13.53	37.43	5.94	-6.70
UR11T2	1.60	-10.65	-1.46	-0.32	-1.29	9.76	-3.02	1.42	4.17	35.08	0.63	-2.67
UR11T3	-1.88	-15.83	-0.74	-0.85	0.00	3.23	-2.14	8.05	-3.20	18.05	-6.93	-1.32
Mean	**4.05**	**2.71**	**0.63**	**-1.01**	**0.31**	**9.76**	**-0.71**	**0.45**	**-2.96**	**18.82**	**0.03**	**0.38**

Table 7. Continued

AR227T1	0.78	-0.35	0.23	-2.04	4.64	3.45	0.89	-5.51	-9.39	35.02	3.34	3.09
AR227T2	7.37	-5.46	2.86	-3.19	9.41	14.92	-1.38	2.93	-8.46	28.97	1.33	1.95
AR227T3	5.23	5.37	3.17	-2.82	11.58	2.33	-5.29	22.40	-0.65	-10.28	10.37	-2.49
CH143T1	4.47	-10.28	1.15	-1.69	1.76	11.24	-2.29	2.52	4.81	7.01	-5.46	-0.60
CH143T2	3.89	-16.97	1.92	-3.60	14.17	11.36	-5.46	8.81	6.90	20.07	-2.25	-1.86
CH143T3	3.27	-3.07	2.52	-3.50	10.43	17.37	-2.06	3.72	-2.78	12.31	3.71	1.54
CU153T1	7.24	2.63	0.54	-0.33	-10.76	21.14	-0.40	1.85	0.26	-0.56	1.25	0.03
CU153T2	7.70	1.19	1.54	-2.37	3.92	20.88	-5.60	9.61	11.83	0.69	5.62	-3.20
CU153T3	7.10	0.54	2.38	-3.16	8.20	8.67	-3.79	11.99	-1.51	13.31	2.81	-0.56
FS32T1	3.45	20.45	0.23	-0.55	7.22	1.66	-4.84	7.68	10.28	7.22	-1.81	-4.21
FS32T2	3.79	12.46	1.25	-0.25	3.42	-2.13	-3.03	5.95	11.25	2.16	-6.34	-2.70
FS32T3	4.74	18.18	2.02	-0.07	-2.30	0.56	0.36	-1.39	4.87	-5.29	-6.49	0.50
UR14T1	3.63	-6.13	0.54	0.77	-2.42	12.20	-0.14	-6.07	-0.18	25.49	-0.09	-0.76
UR14T2	2.25	-16.07	0.85	-1.27	6.05	26.32	-3.40	4.05	5.15	21.00	-6.03	-2.06
UR14T3	0.13	-2.51	0.82	-1.64	5.17	-1.23	0.02	3.72	-5.49	6.65	-5.96	1.68
Mean	**4.34**	**0.00**	**1.47**	**-1.71**	**4.70**	**9.92**	**-2.43**	**4.82**	**1.79**	**10.92**	**-0.40**	**-0.64**
B73xMo17	9.59	-15.20	3.97	-1.22	0.66	2.92	4.08	-9.84	-14.53	2.27	1.76	5.43

TestWt = Test weight, 1000k = One-thousand kernel weight, ADen = Absolute density, SteepW = Steepwater yield, and SR = Starch recovery.
* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

Table 8. High-parent heterosis for physical, compositional, and wet-milling characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Hybrid	Physical			Compositional			Wet-milling					
	TestWt	1000k	ADen	Starch	Protein	Oil	Starch	Gluten	Fiber	Germ	SteepW	SR
AR285T1	3.36	13.98	-0.23	-3.20	-5.88	10.34	-4.95	-2.74	15.50	26.75	6.24	-1.74
AR285T2	3.61	-1.64	0.84	-2.63	-2.52	8.05	-2.01	-4.40	10.15	9.00	6.05	0.64
AR285T3	2.35	9.96	1.07	-2.63	2.61	-1.15	-3.59	23.62	4.71	4.41	4.40	-0.99
CH182T1	2.51	4.88	-0.15	-2.65	-3.53	5.00	-10.00	15.15	29.96	4.86	4.12	-7.56
CH182T2	3.01	-4.42	-0.60	-2.51	3.36	5.75	-2.70	5.47	5.56	10.38	0.00	-0.13
CH182T3	3.01	9.96	-0.38	-2.63	-1.27	7.50	-1.51	21.11	11.31	-9.98	17.20	0.86
CU562T1	4.64	-13.36	-0.85	-2.82	-1.96	3.30	-5.88	3.79	22.30	8.35	6.53	-3.04
CU562T2	5.20	-8.75	1.39	-0.36	-10.12	16.48	-2.12	-6.10	11.57	18.38	2.97	-1.68
CU562T3	7.30	13.72	2.12	-2.77	-3.64	-3.30	-1.19	21.33	4.27	-10.75	0.60	-0.15
FS35T1	0.22	-12.06	-2.34	-2.12	-13.73	8.05	-3.44	-3.66	11.38	5.98	11.87	-1.27
FS35T2	1.23	-0.99	-1.82	-2.82	-1.68	14.94	-2.20	-3.49	10.98	2.99	10.87	0.65
FS35T3	1.10	14.04	-1.66	-1.13	-4.07	-1.15	-1.20	7.31	-5.97	6.50	0.00	-0.70
UR11T1	0.05	-27.04	-1.15	-2.18	-6.67	15.00	-10.99	7.33	22.49	32.47	13.73	-9.00
UR11T2	-0.20	-17.19	-2.14	-2.46	-3.36	3.45	-6.54	4.35	10.62	33.79	10.00	-4.18
UR11T3	-2.99	-21.50	-2.60	-1.34	-5.26	2.56	-3.56	20.89	-1.23	-0.19	-6.00	-3.22
Mean	**2.29**	**-2.69**	**-0.57**	**-2.28**	**-3.85**	**6.32**	**-4.13**	**7.33**	**10.91**	**9.53**	**5.91**	**-2.10**

Table 8. Continued

AR227T1	-1.75	-4.45	0.23	-3.58	-2.75	-4.26	-6.30	6.94	16.00	17.88	9.58	-2.82
AR227T2	4.59	-6.89	2.70	-4.44	5.04	10.64	-7.35	12.42	15.11	10.00	9.39	-3.03
AR227T3	1.85	3.08	1.78	-3.20	7.76	-6.38	-6.43	28.86	14.02	-10.36	12.80	-4.03
CH143T1	3.15	-11.61	0.23	-2.20	1.56	5.62	-3.42	4.07	29.37	-18.97	-2.54	-1.16
CH143T2	2.52	-20.12	0.83	-3.81	10.16	10.11	-5.52	14.21	29.65	-10.74	2.54	-2.14
CH143T3	1.24	-7.35	0.23	-4.90	-0.78	10.11	-6.98	26.49	7.86	-5.04	9.00	-2.20
CU153T1	6.32	1.36	0.23	-1.23	-12.16	11.58	-5.21	6.91	22.54	-11.78	6.19	-3.96
CU153T2	6.71	-2.87	1.07	-2.98	2.02	15.79	-9.08	11.27	34.31	-12.77	12.76	-6.21
CU153T3	5.42	-4.13	0.69	-4.19	-1.21	-1.05	-5.12	27.16	8.28	11.13	6.20	-0.89
FS32T1	2.93	7.65	-0.85	-1.80	1.96	-8.91	-9.21	14.76	40.18	-14.44	-1.21	-7.50
FS32T2	3.34	5.60	0.31	-1.23	1.68	-8.91	-6.38	9.15	38.90	-20.12	-4.15	-5.17
FS32T3	4.50	11.69	1.74	-0.78	-7.83	-10.89	-1.28	10.18	19.55	-14.75	0.80	-0.43
UR14T1	2.41	-15.77	0.39	-0.65	-5.10	9.52	-5.64	-0.24	18.82	14.44	7.71	-4.95
UR14T2	1.11	-20.85	0.85	-2.37	5.39	24.14	-7.64	6.83	23.04	7.64	3.16	-5.34
UR14T3	-0.33	-7.46	-0.39	-2.20	-2.90	-4.76	-0.62	16.31	1.41	1.54	-5.40	1.62
Mean	**2.93**	**-4.81**	**0.67**	**-2.64**	**0.19**	**3.49**	**-5.75**	**13.02**	**21.27**	**-3.76**	**4.46**	**-3.21**
B73xMo17	8.72	-22.99	2.83	-1.58	-0.44	0.00	0.80	-6.08	-5.31	-0.15	1.96	1.75

TestWt = Test weight, 1000k = One-thousand kernel weight, ADen = Absolute density, SteepW = Steepwater yield, and SR = Starch recovery.
* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

Table 9. Correlation coefficients among physical, compositional, and wet-milling characteristics of corn hybrids from crosses of inbred lines introgressed with exotic germplasm and commercial adapted testers.

Factor	TsWt	kkWt	ADen	StaC	ProC	OilC	StaY	GluY	FibY	Germ	SWY	SR	TSR
TsWt	1.00	0.05	0.75**	-0.51**	0.20	0.50**	0.07	0.22	-0.38*	-0.18	-0.42*	0.27	-0.32
kkWt		1.00	-0.28	0.47**	-0.44*	-0.25	0.38*	-0.42*	-0.21	-0.06	-0.17	0.23	0.05
ADen			1.00	-0.61**	0.44*	0.32	-0.11	0.37*	-0.34	0.12	0.33	0.11	-0.32
StaC				1.00	-0.85**	-0.48**	0.39*	-0.60**	0.08	-0.22	-0.44*	0.05	0.03
ProC					1.00	0.22	-0.60**	0.74**	0.18	0.29	0.39*	-0.33	0.20
OilC						1.00	-0.31	0.26	0.20	0.01	0.57**	-0.16	0.12
StaY							1.00	-0.83**	-0.83**	-0.21	-0.53**	0.94**	-0.33
GluY								1.00	0.50**	-0.05	0.43*	-0.68**	0.27
FibY									1.00	-0.02	0.40*	-0.93**	0.42*
Germ										1.00	-0.07	-0.68**	0.05
SWY											1.00	-0.41*	0.09
SR												1.00	-0.37*
TSR													1.00

TsWt = Test weight, kkWt = One-thousand kernel weight, ADen = Absolute density, StaC = Starch content, ProC = Protein content, OilC = Oil content, StaY = Starch yield, GluY = Gluten yield, FibY = Fiber yield, Germ = Germ yield, SWY = Steepwater yield, SR = Starch recovery, and TSR = Total solids recovery.

* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

CHAPTER 4. HETEROSIS IN PHYSICAL, COMPOSITIONAL, AND WET-MILLING CHARACTERISTICS OF HYBRID GRAIN FROM CROSSES BETWEEN EXOTIC INTROGRESSED BY ADAPTED INBRED LINES IN CORN

Abstract

Kernels from corn (*Zea Mays* L.) are one of the most economical sources of metabolizable energy used in livestock feeding and starch used for the processing industry for food and industrial products. Starch is the most important fraction from the wet-milling process and indicates the ease with which kernel components are separated. Corn breeders have developed new hybrids with enhanced compositional characteristics that are available to growers but corn lines from exotic germplasm represent only 3% of the germplasm base used to produce the hybrids grown in the United States. Studies to determine the physical, compositional, and wet-milling properties of new hybrids, as well as the proximate composition of recovered fractions, need to be conducted at the laboratory level before these materials are of value to the corn processing industry. Ten lines from the Germplasm Enhancement of Maize project with exotic germplasm introgressed from Argentina, Chile, Uruguay, Cuba, and Florida were crossed to three adapted inbred lines and the resulting thirty hybrids were evaluated to determine their physical, compositional, and wet-milling characteristics and the expression of heterosis in these variables. The B73 x Mo17 hybrid was used as control. The F_2 or grain generation of these 30 experimental hybrids, obtained by self-pollination of the F_1, was analyzed using both Near-Infrared Transmitance (NIT) technology and a 100-g wet-milling procedure. There was a great variation among physical, compositional, and wet-milling characteristics and some of the experimental hybrids with exotic origin, such as AR285, CH182, and FS32 by Tester 3 and CU562 by Tester 2, had better starch yield and starch recovery than B73xMo17, which suggests that genetic diversity is present and that potential to improve wet-milling characteristics of hybrids grown in the United States through breeding can be found in exotic germplasm.

Introduction

Corn (*Zea mays* L.) is widely grown throughout the world due to its high value as a source of carbohydrates. Corn is one of the most economical sources of metabolizable energy used in livestock feeding and starch used for the processing industry as raw material for food and industrial products (Johnson, 2000).

Corn breeders have focused on developing highly productive hybrids with enhanced concentrations of starch, protein, or oil in comparison to elite hybrids currently used. Despite its economical importance, however, production of corn hybrids grown in the United States uses only 5 % of the total corn germplasm available in the world (Goodman, 1985). This narrow germplasm base increases the risk of genetic vulnerability to insect and disease damage, affect the overall agronomic performance, and reduce the odds of finding new materials with value-added characteristics. The major source of germplasm for the hybrids grown in the U.S. is one race, the Corn Belt dent (Duvick et al, 2004). Regardless of its utility as a source of variation for crop improvement, exotic germplasm has seldom been used in advanced breeding programs. In fact, less than 1% of the U.S. corn germplasm base had an exotic origin in 1984 (Goodman, 1985), which increased to almost 3% in 1996 (Goodman, 1999).

The Germplasm Enhancement of Maize project (GEM), a coordinated and cooperative effort among public and private sectors, was developed to improve and enhance the germplasm base of corn used to produce new hybrids in the United States. This project works with exotic germplasm to introgress to elite inbred lines characteristics of better agronomic performance and value-added properties (Pollak, 2003). GEM started after the Latin American Maize Project (LAMP), the first coordinated international project for the evaluation of corn collections (Salhuana et al, 1991). The final objective in GEM is to develop new breeding lines with exotic introgressed characteristics and make them available to other corn breeding programs. The complete breeding protocol used in GEM is reported by pollak (2003).

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 the corn kernels into its main fractions of starch, gluten, germ, and fiber by using chemical, biochemical, and mechanical operations (Singh et al, 1997). The wet-milling industry is known as the “corn-refining” industry because it converts corn kernel fractions in processed products (Johnson, 2000; Butzen and Hobbs, 2002).

As corn is the raw material for the wet-milling industry, screening germplasm is important to develop new hybrids with the more appropriate characteristics for this particular use. Because the aim of a plant breeding program is to develop improved genotypes for a predefined target environment and for a specific use, many new hybrids are produced continuously as result of the breeding effort in both the public and private sectors. Breeding for changes in the chemical composition of the corn kernels has proven be 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) is now a widely used laboratory-level procedure because it has yielded fraction values that are comparable to industry and reduced the cost and labor required to analyze a large number of corn samples.

In order to determine the agronomic value of new corn hybrids, it is necessary to have a point of reference between the performance of the progeny in relation to the inbred lines used as parents. One of these parameters is heterosis. Heterosis is the difference between the hybrid performance and the mean value of the inbred parents, or the highest value of the best parent for a trait of interest, and is normally expressed as a percentage relative to the reference value. 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 ten GEM accessions with the public inbred lines B73 and Mo17 and reported higher levels of protein and reduced starch contents of the hybrids, which led to the expression of poor wet-milling properties of the hybrids, especially low values of starch yield and starch recovery. However, these studies focused on the expression of heterosis in the F_1 generation of hybrids. No studies have considered the expression of heterosis in the F_2 generation or in hybrid grain. This is important because hybrid grain 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 provide additional value-added characteristics for corn breeding.

Ten lines with exotic introgressed genetic background from the GEM project were crossed to three adapted inbred lines, used as testers to: i) determine the physical, compositional, and wet-milling characteristics of hybrid grain from the self pollination of hybrids from crosses among exotic introgressed and adapted inbred lines, and ii) determine the expression of mid-parent and high-parent heterosis for the same characteristics and hybrids.

Materials and Methods

Genetic Materials

Ten corn lines from the GEM project were selected on the basis of starch yield after laboratory wet milling done by the private cooperator Cerestar (Hammond, IN, now acquired by Cargill, Minneapolis, MN). The highest and the lowest starch-yielding lines for each of the five different germplasm sources were chosen. The lines had 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). These lines were crossed to three commercial inbred lines used as testers (Tester 1, Tester 2, and Tester 3) that had different physical and compositional characteristics. Testers were expected to produce hybrids with differences in physical and compositional properties and, as a consequence, different yields of the fractions recovered after laboratory wet milling of the corn kernels. Tester 1 was an inbred line that had low starch and high protein content and was expected to produce hybrids with low starch content and low starch yield. Tester 2 was the Bt version of Tester 1. Tester 3 was an inbred line with higher starch and lower protein contents than Testers 1 and 2 and was expected to produce hybrids with high starch content and high starch yield. Seed of the testers was provided by Golden Harvest Seeds, Inc. (Clinton, IL, now acquired by Syngenta, Wilmington, DE). In this paper we present the wet-milling characteristics of the F_2 generation (hybrid grain) obtained from self pollination of the F_1 generation of hybrids from the cross among exotic and adapted inbred lines. The hybrid grain from the cross of B73 x Mo17 was included as a check.

Sample Preparation

The F_1 generation of hybrids was produced in Clinton, IL, in the summer of 2003 and then advanced to the F_2 by self-pollination during the summer of 2004 in Ames, IA, under a completely random design with one replication. At harvest, all the self-pollinated ears from a row (3.8 m long and 76 cm wide) were hand-harvested and dried to approximately 10% moisture content by circulating warm air at $38^{o}C$ for 72 h; ears that set few kernels or had disease symptoms were discarded. Grain from normal ears was bulked after shelling and stored at $4^{o}C$ until used. The 100-g samples were hand-picked and any foreign material and cracked or broken kernels were removed. Two replications per sample of bulked seed were used to determine physical, compositional, and wet-milling characteristics of each hybrid.

Physical Characteristics

The variables measured to determine the physical characteristics of the experimental corn hybrids were test weight, 1000-kernel weight, and absolute density. A description of the variables and the procedures used to measure them is presented in Chapter 3 of this thesis.

Compositional Characteristics

Moisture, starch, protein, and oil contents of bulked whole kernels from each hybrid were estimated with Near-infrared Transmittance (NIT) technology by using a FOSS Infratec™ 1241 Grain Analyzer (Tecator, Hoganas, Sweden). 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 NIR calibration databases of the USDA Federal Grain Inspection Service.

Wet-milling Characteristics

Two samples from each line were analyzed by using the 100-g modified wet-milling procedure of Singh et al (1997). This procedure yields starch, gluten, fine and coarse fiber, germ, and steepwater fractions. The procedure was slightly modified to improve reproducibility as described in Chapter 2 of this thesis. Moisture contents of the four recovered fractions (starch, gluten, fiber, and germ) were determined in triplicate by using the AOAC Method 14.004 (AOAC, 1984) and used to calculate fraction yields on a dry basis. The whole kernel moisture was estimated with three replications by following the AACC Method 44-15A (AACC, 2000) and used to calculate total solids recovery.

Composition of Recovered Fractions

Protein contents of the starch fractions were determined by using the macro-Kjeldahl method; the procedure used was the Corn Refiners Association Method A-18 (CRA, 2006). Protein content in the recovered gluten fractions was determined with the AOAC Method 993.13 (AOAC, 2003) by using a combustion nitrogen analyzer RapidN III from Elementar Americas, Inc. (Mt. Laurel, NJ) and a protein factor of 6.25. Oil contents in the germs were quantified as crude free fat content according to the AOAC Method 14-084 and 14-085 (AOAC, 1984) by using the Goldfisch procedure. All the values for the compositions of the recovered fractions were expressed as percentage on a dry basis (% db).

Statistical Analysis

The following linear model was used for the statistical analysis of the data by using a completely random design:

$$\boldsymbol{Y_{ijkl} = \mu + G_i + L_{(i)j} + T_k + GT_{ik} + LT_{(i)jk} + E_{(ijkl)}}$$

Where:

Y_{ijkl} = Response observed for the ijklth experimental unit.
μ = Overall mean.
G_i = Effect of the ith group of exotic corn lines.
$L_{(i)j}$ = Effect of the jth line nested in the ith group.
T_k = Effect of the kth tester.
GT_{ik} = Interaction effect of the ith group of exotic corn lines with the kth tester.
$LT_{(i)jk}$ = Interaction effect of the jth line with the kth tester.
$E_{(ijkl)}$ = Effect associated to experimental error.

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

Calculation of Heterosis

The performance of a hybrid in relation to its parents can be expressed as mid-parent or high-parent heterosis. Calculations were made by using the following formulas:

c) Mid-parent heterosis is the performance of a hybrid compared with the average performance of its parents.

$$\text{Mid-parent heterosis (\%)} = \frac{F1 - MP}{MP} \times 100$$

d) High-parent heterosis is a comparison of the performance of the hybrid with that of the best parent in the cross.

$$\text{High-parent heterosis (\%)} = \frac{F1 - HP}{HP} \times 100$$

where:

F1 = Performance of hybrid.

MP = Average performance of parents per se, given by (parent1 + parent2)/2.

HP = Performance of the best parent.

Result and Discussion

Analysis of Variance

Significant differences were found for all physical, compositional, and wet-milling variables in this study (Tables 1 and 2). There were no differences 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 generation or hybrid grain. Due to the lack of differences between the effect of groups of lines and the interaction effect of group x tester, discussion of results will be based on the effect of the different hybrids produced as a whole. The effect of lines was significant for all the variables under study, which means that the exotic corn lines will produce hybrids with specific properties and represents the wide genetic diversity that can be exploited to improve the wet-milling parameters of new hybrids through breeding. The effect of tester was significant for test weight and absolute density and at $P<0.05$ for kernel size. Testers produced hybrids with different starch and protein concentrations, showing a differential effect of the pollen source on the compositional characteristics of the hybrids produced. Oil concentration was not affected by the source of pollen. If the hybrids are grouped by tester, statistical differences ($P<0.01$) were found for starch, gluten, fiber, and germ yield, which means that each tester produces progeny with different wet milling characteristics. The interaction of line by tester, which represents the specific properties of the different hybrids produced, was highly significant for all the variables under study, indicating differences in combining ability for lines and testers. As indicated in Chapter 2, it is important to point out that, for wet milling purposes, it is better to test hybrids that are advanced to the F_2 generation than inbred lines because hybrid grain will provide more accurate parameters of the millability of the new materials. This would facilitate the selection of parents in a corn breeding program focused on the improvement of the wet milling efficiency of new corn hybrids.

Physical Characteristics

The physical properties of corn kernels can be influenced by moisture content, hybrid type, environmental conditions of production in different years, and post-harvest management practices (Watson, 1987). Test weight is a measure of the 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 ranged from 73.3 to 80.5 with a mean of 77.6 k/hL (Table 3). 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 from 66.8 to 79.5 k/hL were found (Fox et al, 1992; Singh et al, 1997; Raush et al, 1999; Vignaux et al, 2006). The B73xMo17 F_2 hybrid had a value of 73.8 k/hL. According to Watson (1987), the range for dent corn hybrids varies from 65 to 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 the present study, test weight values may be higher because we used samples dried to approximately 10% moisture content and as corn is dried, test weight can slightly increase. The 1000-k weight of the hybrids had great variation, ranging from 305.3 to 433.2 g with a mean value of 377.2 g. 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. Zehr et al (1995) reported a mean of 359 g for 1000-k weight for a group of hybrids representing the germplasm groups used in the United States. Singh et al (1997) found a range from 266.2 to 378.4 g for 1000-k weight for one waxy and three regular yellow dent corn hybrids. 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). Absolute density in corn varies from 1.18 to 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 mainly 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 and protein contents of the hybrids (Table 1) were statistically different at P<0.01; oil content showed statistical differences at P<0.01. The mean starch content of the hybrids with exotic material introgressed was 70.2% db; slightly higher than that of B73xMo17, which was 69.6% (Table 3). Yellow dent corn hybrids grown in the United States contain from 65 to 70% starch (Rooney et al, 2004). Tester 3 produced hybrids with significantly higher starch contents, with a mean of 70.9% db, than Testers 1 and 2 (means of 69.8 and 70.0% db, respectively). All hybrids with exotic background that had starch contents of >71% db had Tester 3 as the male parent. 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 ranged from 71.8 to 76.1% (Singh et al, 1997; Singh et al, 2005).

The protein contents of the experimental hybrids ranged from 7.8% for FS32 x Tester 3 to 11.05% for UR11 x Tester 1 with a mean of 9.9%. B73xMo17 had a protein content of 11.1% (Table 3). As expected, Testers 1 and 2 produced groups of 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 to 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; Singh et al, 2005; Vignaux et al, 2006). Values >10% were considered high and it was a factor that contributed to lower than anticipated starch recovery from wet-milling because protein content has been negatively correlated with starch yield.

Oil contents in the corn kernels ranged from 4.0 to 5.3% with a mean of 4.54% db for the experimental hybrids; the control hybrid contained 4.4% db oil. These values were slightly higher than the values of normal yellow dent corn hybrids, which can vary from 3 to 5% (Orthoefer and Eastman, 2004), but can reach values of 8% for high-oil corn hybrids (Raush et al, 1999). The information related to compositional characteristics of the experimental hybrids used in this study indicates that the genetic background affects the variation in the main components of corn kernels.

Wet-milling Characteristics

Hybrids with exotic germplasm showed statistical differences for starch, gluten, fiber, and germ yields (Table 2). Variability among corn hybrids is a factor that can negatively affect production costs and economic gains of the wet-milling industry because of the inherent different wet-milling characteristics and fraction yields associated to genetic differences among corn hybrids (Eckhoff, 1995). For this reason, it is important to evaluate new corn hybrids to determine their wet-milling characteristics before their use by industry. Industry is primarily interested in uniform and good milling hybrids. Starch yield is the most important recovered fraction from the wet milling of corn (Singh and Eckhoff, 1996) and is an indicator of millability, 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 used in the wet-milling industry.

Starch yields of the experimental hybrids varied from 58.1 to 65.3 with a mean of 62.4% db. These values were similar to those reported when milling normal yellow dent corn hybrids (Singh and Eckhoff, 1996; Singh et al 1997; Dowd, 2003; Vignaux et al, 2006) and higher than the starch yield reported for high-oil (Raush et al, 1999) or waxy hybrids (Singh et al, 1996). However, even when some experimental hybrids have starch yields of >65%, higher yields for yellow dent hybrids have been reported at the laboratory level (Eckhoff et al, 1996; Singh et al, 1998; Yang et al, 2005). The starch yield average for the wet milling industry is around 67.5% db (Johnson and May, 2003). The mean starch yield of the B73xMo17 hybrid was 62.0% db. Several hybrids with exotic origin (AR285xTester3, CH182xTester 3, FS32xTester 3, and CU562xTester 2) had better millability than the B73xMo17 hybrid, which shows that potential to improve wet-milling characteristics of hybrids grown in the United States through plant breeding can be found in exotic germplasm.

Starch and gluten separation was made using the tabling method, which is used in most of the laboratory-scale wet-milling procedures (Singh and Eckhoff, 1996; Dowd, 2003). This fraction separation is based on the principle of particle density differences. Starch is heavier than the 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 varied from 9.6 to 16.6 with a mean of 14.1% db. B73xMo17 had a gluten yield of 15.3% db. The results are higher than those reported with exotic accessions (Singh et al, 2001), adapted lines (Zehr, 1995), high-oil corn hybrids (Raush et al, 1999), and commercial yellow dent corn hybrids (Singh et al, 1998; Dowd, 2003; Yang et al, 2005; Vignaux et al, 2006). The gluten yield is higher than the typical industry yield of 5% (Johnson and May, 2003). This poor starch-gluten separation can be caused by the high protein content of the hybrids evaluated, which makes more difficult the release of the starch granules during the wet milling process (Watson, 1984).

Fiber yield ranged from 11.3 to 15.4 with a mean of 12.6% db. The B73xMo17 hybrid had a fiber yield of 12.2% db. These values were higher than those reported in previously cited studies and for the normal industry fiber yield of 11.5% (Johnson and May, 2003).

Germ yields varied from 2.9 to 6.8 with a mean of 5.1% db. 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. The B73xMo17 hybrid had a germ yield of 6.6% db. These values were similar to those reported by other studies (Singh et al, 1998; Dowd, 2003; Yang et al, 2005; Vignaux et al, 2006), but lower than the 7.5% reported by the wet-milling industry (Johnson and May, 2003), for some yellow dent corn hybrids (Weller et al, 1989), or the germ yield reported for high-oil corn hybrids (Raush et al, 1999). Our results indicated that, even when some compositional or physical characteristics of the corn kernels affect 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, 2001c).

Steepwater yield had a mean of 5.5% and 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). Nevertheless, our results were higher than the values obtained by Singh et al (1998) and Yang et al (2005) and can be directly influenced by the relatively high protein contents of the experimental hybrids in the study because the higher the protein content, the higher the steepwater yield (Watson, 1984).

Starch recovery (SR) results from dividing starch yield by the starch content and is expressed as percentage on a dry basis value. In our study, SR varied from 83.4 to 92.9% 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 ranging from 91.3 to 99.3% db when the 100-g g wet-milling procedure was used. SR for the B73xMo17 hybrid was 89.1%.

Total Solids Recovery (TSR) varied from 99.3 to 99.9 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 vary from 99.6 to 100%, although 98% is generally achievable if the samples are carefully milled (Singh and Eckhoff, 1996).

Compositions of Recovered Fractions

The compositions of some recovered fractions are shown in Table 5. The protein contents of the starch samples varied from 0.19 to 0.27% with a mean of 0.23% db for the experimental hybrids. The B73 x Mo17 starch sample 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 o 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% ranging from 0.27 to 0.32% (Vignaux et al 2006; Watson, 1984). Values ranging from 0.26 to 0.50% have been found 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 of 1.05% of protein content in starch recovered from

GEM accessions and attributed it to the poor starch-gluten separation. Protein contents of gluten samples ranged from 36.1 to 47.0 with a mean of 40.3%. The B73xMo17 hybrid 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 around 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 (Singh et al, 2001b). 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 more than 50% protein content (Watson, 1984).

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

Wet-Milling Characteristics of Experimental Hybrids Grouped by Tester

Statistical differences ($P<0.05$) were found when the experimental hybrids were grouped by tester and their compositional and wet-milling characteristics analyzed (Table 6). Testers 1 and 2 are isolines, genetically identical but differing only by the Bt gene, that produced hybrids with statistically identical results for all variables in the study (except for germ yield). These two testers produced hybrids with similar starch yield and starch recovery, which can be attributed to the similar values found for the groups of hybrids produced by

Testers 1 and 2 in physical and compositional characteristics (Table 3). This indicates that the Bt gene did not significantly affect the wet-milling properties of the experimental hybrids in the study. Hybrids from Tester 2 (the Bt version of Tester 1) had higher starch and lower protein contents than hybrids where Tester 1 was the male parent, which resulted in slightly higher starch yield and starch recovery values for these experimental hybrids than those hybrids from Tester 1. Tester 3 produced hybrids that had statistically superior starch yield and starch recovery than hybrids produced with Testers 1 and 2, as a consequence of the higher starch and lower protein concentrations in its hybrids (Table 3). These results indicated that this inbred line, when used as the male parent, will produce hybrids that would be better suited for wet milling due to their better millability.

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 the reproducibility of our procedure was similar to or better than the procedure used in other laboratories.

Heterosis for the Physical, Compositional and Wet-milling Characteristics

Mid-parent Heterosis

Mid-parent heterosis values for the physical, compositional, and wet-milling properties of the two groups of experimental hybrids are shown in Table 7. The values to make the calculations were taken from Chapter 2 in this thesis for the parental lines and from Tables 3 and 4 in this Chapter. There was positive mid-parent heterosis with similar values for test weight and 1000-kernel weight and a negative value for absolute density for both HSEL and LSEL hybrids. It was evident that a bigger kernel size for the hybrid grain (32.8% and 27.5% for the HSEL and LSEL groups of hybrids, respectively) 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 can explain the higher starch yields that can be obtained when milling hybrid grain than when milling the parental lines.

The compositional characteristics of the hybrid grain with exotic germplasm, although without significant differences between groups, had differences with the mean value of their inbred parents. Starch concentration had a positive mid-parent heterosis value (1.3 and 1.8% for HSEL and LSEL hybrids, respectively); the values for protein content were negative in both groups of hybrids (-14.4 and -15.7% for HSEL and LSEL hybrids, respectively). There was positive mid-parent heterosis with similar values for oil contents in both groups of hybrids, which means that exotic introgressed corn lines can be valuable for wet-millers since germ oil is a high-value coproduct from the wet-milling processing of corn.

Starch yield had positive mid-parent heterosis values for both groups of hybrids (4.8 and 4.7 for HSEL and LSEL groups, respectively); 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 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 upon 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 their inbred parents, may be the cause of the better wet-milling properties of the materials in the study since 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 since 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.

High-parent Heterosis

High-parent heterosis values for the physical, compositional, and wet-milling properties of the two groups of experimental hybrids are shown in Table 8. As stated in Chapter 3, in the case of gluten, fiber, and steepwater solids yields, high values 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 can be considered as materials with appropriate wet milling properties. The average values for test weight and absolute density were lower for the HSEL hybrids than the values for the LSEL hybrids, which indicated that kernels from the first group were softer and produced higher starch yields. The 1000-kernel weight had positive values, which indicates that hybrids had bigger kernel size than the line with the bigger seed size from both parents. The LSEL hybrids showed lower values for this variable (mean of 21.5%) which means that this group of hybrids has, on average, smaller seed size than the HSEL hybrids. Singh et al (2001a) reported negative high-parent heterosis values for this variable when ten 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 hybrids with exotic germplasm introgressed have better wet-milling efficiency. Hybrids that had Tester 3 as a male parent produced higher starch yield and better starch recovery values than hybrids where either Tester 1 or 2 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.

Correlation Coefficients

Correlation coefficients among the physical, compositional, and wet-milling characteristics of the materials in the study are shown in Table 9. The physical properties of the corn kernels have a direct effect on the compositional characteristics but they did not have a marked direct effect on the wet-milling properties of the experimental hybrids. 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). Something important to point out is 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 to separate 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; 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, can be used to screen samples for extractable starch in experimental hybrids in early stages of a corn breeding program. In general, correlations showed variation but followed the same pattern reported in previous studies (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 x adapted inbred lines in corn. This suggests that the genetic diversity and production potential present in exotic germplasm can be used to improve the wet-milling characteristics of hybrids grown in the United States.

Hybrids AR285xTester 3, CH182xTester 3, FS32 and FS35xTester 3, and CU562xTester 3 had starch yields of 65% db and starch recoveries higher than 90%, which indicates that the original accessions (AR16035, CH05015, CUBA117, and FS8B(T)) used in GEM can be a valuable source of value-added characteristics if a breeding program to increase the extractable starch of corn hybrids incorporate breeding lines from these accessions as part of their germplasm base.

Tester 3 produced hybrids that were statistically superior for their 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 if hybrids with high starch yield are required by the wet-milling industry.

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 germplasm in relation 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.

Near Infrared Transmittance estimations are rapid and nondestructive, which is advantageous for corn breeders during early stages of selection. NIT technology may be applicable to maize breeding programs as a predictive tool to screen early progeny for high starch content and extractable starch. Application of this technology can save time and costs in a corn breeding program directed to the improvement of the millability of corn hybrids to be used as raw material by the wet-milling industry.

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Table 1. Analysis of variance for the physical and compositional characteristics of hybrid grain from crosses of exotic introgressed inbred lines and commercial adapted testers.

Source	df	Mean Squares		
		Physical Characteristics		
		TestWt	1000KWt	ADen
Group	1	1.30ns	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		
		StarchC	ProtC	OilC
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**

TestWt = Test weight (K/hL); 1000KWt = One thousand-kernels weight (g); ADen = Absolute density (g/cm^3); StarchC, ProtC, and OilC = Starch, protein, and oil content (% db).

*, **, ***, and ns = Statistically significant at 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 2. Analysis of variance for the wet-milling characteristics of hybrid grain from crosses of exotic introgressed inbred lines and commercial adapted testers.

Source	df	Mean Squares			
		Wet Milling Characteristics (% db)			
		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

, *, and ns = Statistically significant at 0.01 and 0.001 probability levels and not significant, respectively.

Table 3. Physical and compositional characteristics of hybrid grain from crosses of exotic introgressed inbred lines and commercial adapted testers.

Hybrid	Physical Characteristics			Compositional Characteristics (% db)		
	TestWt[1]	1000kWt[2]	ADen[3]	Starch	Protein	Oil
AR227T1	80.3 ± 0.28	330.7 ± 0.16	1.291 ± 0.00	70.7 ± 0.14	9.3 ± 0.00	4.7 ± 0.07
AR227T2	79.0 ± 0.29	398.5 ± 2.08	1.290 ± 0.00	70.6 ± 0.00	9.7 ± 0.07	4.4 ± 0.00
AR227T3	78.3 ± 0.01	399.3 ± 2.21	1.280 ± 0.00	70.3 ± 0.14	9.9 ± 0.07	4.4 ± 0.07
AR285T1	80.2 ± 0.41	365.8 ± 1.63	1.297 ± 0.00	69.9 ± 0.28	10.3 ± 0.07	4.5 ± 0.07
AR285T2	80.4 ± 0.06	376.8 ± 0.47	1.299 ± 0.00	69.9 ± 0.07	10.1 ± 0.07	4.8 ± 0.14
AR285T3	79.0 ± 0.06	411.8 ± 3.44	1.285 ± 0.00	70.5 ± 0.07	9.6 ± 0.00	4.5 ± 0.14
CH143T1	78.0 ± 0.06	311.4 ± 3.21	1.305 ± 0.00	69.3 ± 0.07	11.0 ± 0.07	4.6 ± 0.14
CH143T2	77.9 ± 0.05	306.9 ± 2.28	1.303 ± 0.00	69.5 ± 0.00	10.8 ± 0.07	4.7 ± 0.07
CH143T3	74.9 ± 0.16	355.5 ± 0.86	1.267 ± 0.01	71.2 ± 0.21	9.0 ± 0.07	4.7 ± 0.21
CH182T1	78.7 ± 0.37	374.9 ± 2.33	1.305 ± 0.00	69.7 ± 0.07	11.0 ± 0.07	4.2 ± 0.07
CH182T2	78.6 ± 0.06	356.8 ± 1.11	1.302 ± 0.00	70.0 ± 0.21	10.5 ± 0.21	4.4 ± 0.14
CH182T3	78.3 ± 0.25	359.3 ± 2.38	1.387 ± 0.00	70.9 ± 0.14	8.9 ± 0.07	4.7 ± 0.28
CU153T1	80.0 ± 0.00	364.4 ± 1.85	1.292 ± 0.00	69.6 ± 0.21	10.3 ± 0.07	4.8 ± 0.07
CU153T2	79.9 ± 0.11	377.1 ± 3.12	1.278 ± 0.00	69.4 ± 0.28	10.3 ± 0.14	5.0 ± 0.00
CU153T3	76.9 ± 0.57	338.4 ± 3.45	1.270 ± 0.00	71.2 ± 0.42	8.8 ± 0.21	4.6 ± 0.21
CU562T1	78.4 ± 0.42	375.4 ± 1.72	1.280 ± 0.00	69.9 ± 0.00	10.1 ± 0.00	4.7 ± 0.07
CU562T2	78.6 ± 0.11	395.1 ± 0.69	1.280 ± 0.00	70.3 ± 0.35	9.7 ± 0.14	4.6 ± 0.14
CU562T3	74.7 ± 0.44	424.7 ± 2.18	1.247 ± 0.00	70.5 ± 0.07	9.9 ± 0.14	4.4 ± 0.07
FS32T1	78.7 ± 0.15	388.7 ± 0.84	1.279 ± 0.00	69.4 ± 0.07	10.4 ± 0.14	4.8 ± 0.07
FS32T2	76.8 ± 0.17	418.4 ± 0.16	1.272 ± 0.01	69.9 ± 0.07	10.1 ± 0.07	4.9 ± 0.28
FS32T3	73.4 ± 0.15	381.1 ± 0.04	1.236 ± 0.00	71.9 ± 0.07	7.9 ± 0.07	4.6 ± 0.14
FS35T1	76.1 ± 0.03	325.7 ± 1.40	1.275 ± 0.00	70.7 ± 0.35	9.4 ± 0.14	4.7 ± 0.14
FS35T2	76.2 ± 0.18	432.5 ± 1.05	1.269 ± 0.00	70.2 ± 0.14	10.2 ± 0.07	4.6 ± 0.00
FS35T3	76.3 ± 0.16	403.8 ± 0.85	1.277 ± 0.00	71.5 ± 0.07	8.6 ± 0.00	4.4 ± 0.14
UR11T1	75.0 ± 0.08	388.3 ± 3.89	1.268 ± 0.00	69.7 ± 0.14	11.1 ± 0.07	4.0 ± 0.00
UR11T2	75.3 ± 0.43	371.8 ± 5.70	1.272 ± 0.00	70.1 ± 0.07	10.5 ± 0.00	4.2 ± 0.14
UR11T3	76.7 ± 0.42	421.3 ± 1.84	1.276 ± 0.00	71.3 ± 0.21	9.4 ± 0.07	4.3 ± 0.07
UR14T1	78.9 ± 0.40	357.7 ± 1.48	1.301 ± 0.00	69.6 ± 0.00	10.6 ± 0.00	4.5 ± 0.07
UR14T2	76.3 ± 0.38	376.6 ± 2.72	1.281 ± 0.01	69.9 ± 0.00	10.3 ± 0.07	5.0 ± 0.49
UR14T3	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

[1] Test weight (k/hL), [2] 1000 kernels weight (g), [3] Absolute Density (g/cm^3)

Table 4. Wet-milling fraction yields of hybrid grain from crosses of exotic introgressed inbred lines and commercial adapted testers.

Hybrid	Wet-milling Fraction Yields (% db)					
	Starch	Gluten	Fiber	Germ	SteepW[1]	SR[2]
AR227T1	63.58 ± 0.21	14.39 ± 0.23	11.56 ± 0.14	4.47 ± 0.04	5.63 ± 0.04	89.93± 0.12
AR227T2	62.84 ± 0.04	15.92 ± 0.18	12.01 ± 0.58	2.89 ± 0.67	5.83 ± 0.02	89.01 ± 0.06
AR227T3	62.76 ± 0.06	14.50 ± 0.33	11.40 ± 0.20	5.42 ± 0.23	5.66 ± 0.13	89.28 ± 0.26
AR285T1	62.98 ± 0.17	14.01 ± 0.12	12.34 ± 0.08	4.70 ± 0.22	5.25 ± 0.08	90.10 ± 0.61
AR285T2	63.05 ± 0.00	14.77 ± 0.66	12.21 ± 0.52	3.96 ± 1.13	5.51 ± 0.07	90.27 ± 0.09
AR285T3	64.19 ± 0.07	14.61 ± 0.25	11.64 ± 0.01	3.93 ± 0.10	5.36 ± 0.04	91.12 ± 0.19
CH143T1	60.36 ± 0.17	14.85 ± 0.85	12.02 ± 1.05	6.31 ± 0.11	5.79 ± 0.08	87.16 ± 0.16
CH143T2	59.44 ± 0.41	15.55 ± 1.65	12.52 ± 0.30	5.90 ± 1.43	6.00 ± 0.13	85.53 ± 0.59
CH143T3	63.94 ± 0.23	11.95 ± 0.23	11.34 ± 0.35	6.48 ± 0.42	5.78 ± 0.05	89.87 ± 0.59
CH182T1	59.88 ± 0.19	16.58 ± 0.26	13.12 ± 0.47	4.56 ± 0.03	5.28 ± 0.00	85.98 ± 0.37
CH182T2	62.19 ± 0.23	16.21 ± 0.46	12.85 ± 0.09	2.87 ± 0.67	5.54 ± 0.00	88.90 ± 0.61
CH182T3	65.00 ± 0.14	12.60 ± 0.25	11.88 ± 0.15	4.66 ± 0.17	5.50 ± 0.14	91.68 ± 0.38
CU153T1	61.48 ± 0.11	15.15 ± 0.00	12.62 ± 0.23	4.66 ± 0.57	5.74 ± 0.01	88.39 ± 0.11
CU153T2	62.59 ± 0.54	14.55 ± 0.69	12.05 ± 0.19	5.18 ± 0.30	5.59 ± 0.08	90.19 ± 1.15
CU153T3	64.64 ± 0.05	12.42 ± 0.58	12.55 ± 0.16	4.89 ± 0.63	5.12 ± 0.18	90.78 ± 0.47
CU562T1	64.44 ± 0.26	12.76 ± 1.37	11.87 ± 0.25	5.55 ± 0.16	5.44 ± 0.11	92.19 ± 0.37
CU562T2	65.29 ± 0.38	14.25 ± 0.29	11.91 ± 0.93	4.39 ± 1.71	5.52 ± 0.08	92.94 ± 0.07
CU562T3	63.41 ± 0.34	14.09 ± 0.88	12.00 ± 0.22	4.96 ± 0.77	5.15 ± 0.01	90.01 ± 0.39
FS32T1	59.77 ± 0.18	14.09 ± 0.88	13.30 ± 0.10	6.79 ± 0.79	5.63 ± 0.06	86.18 ± 0.17
FS32T2	61.80 ± 0.12	13.55 ± 0.11	12.99 ± 0.08	5.91 ± 0.20	5.66 ± 0.06	88.47 ± 0.08
FS32T3	65.17 ± 0.27	9.64 ± 0.05	12.83 ± 0.18	6.41 ± 0.02	5.43 ± 0.09	90.70 ± 0.28
FS35T1	62.32 ± 0.48	12.89 ± 0.06	12.77 ± 0.04	6.31 ± 0.35	5.35 ± 0.08	88.22 ± 1.12
FS35T2	61.21 ± 0.10	14.49 ± 0.24	13.44 ± 0.01	5.16 ± 0.42	5.08 ± 0.02	87.20 ± 0.32
FS35T3	64.86 ± 0.19	11.69 ± 0.14	11.86 ± 0.01	6.04 ± 0.21	5.11 ± 0.03	90.77 ± 0.35
UR11T1	58.15 ± 0.22	15.29 ± 0.12	15.38 ± 0.12	5.73 ± 0.03	5.21 ± 0.00	83.42 ± 0.14
UR11T2	58.97 ± 0.27	15.06 ± 0.24	14.87 ± 0.08	5.11 ± 0.48	5.25 ± 0.03	84.18 ± 0.30
UR11T3	63.29 ± 0.35	13.21 ± 0.54	12.77 ± 0.64	5.32 ± 0.34	5.31 ± 0.01	88.83 ± 0.23
UR14T1	60.83 ± 0.11	15.42 ± 0.00	13.34 ± 0.25	4.20 ± 0.08	5.68 ± 0.22	87.39 ± 0.16
UR14T2	61.90 ± 0.47	15.07 ± 0.36	13.10 ± 0.29	3.92 ± 0.46	5.33 ± 0.10	88.56 ± 0.67
UR14T3	62.08 ± 0.08	14.41 ± 0.34	12.02 ± 0.02	5.51 ± 0.49	5.41 ± 0.04	88.68 ± 0.11
B73xMo17	62.00	15.31	12.18	6.55	4.74	89.09

[1]Steepwater and [2] Starch recovery.

Table 5. Composition of recovered fractions from the wet-milling of hybrid grain from crosses of exotic introgressed inbred lines and commercial adapted testers.

Hybrid	PStarch	PGluten	Oil
AR227T1	0.270 ± 0.02	39.00 ± 0.20	60.63 ± 0.88
AR227T2	0.194 ± 0.01	36.12 ± 0.76	60.51 ± 0.41
AR227T3	0.211 ± 0.00	39.35 ± 2.43	59.07 ± 0.98
AR285T1	0.235 ± 0.00	40.15 ± 0.59	60.73 ± 0.13
AR285T2	0.230 ± 0.01	39.49 ± 0.81	61.03 ± 0.40
AR285T3	0.234 ± 0.00	40.68 ± 1.68	57.70 ± 0.27
CH143T1	0.232 ± 0.00	41.97 ± 2.93	61.19 ± 0.77
CH143T2	0.236 ± 0.00	39.63 ± 4.45	61.36 ± 0.40
CH143T3	0.187 ± 0.00	43.28 ± 0.72	59.26 ± 0.21
CH182T1	0.245 ± 0.05	38.05 ± 1.39	58.53 ± 0.08
CH182T2	0.237 ± 0.00	37.32 ± 1.46	59.14 ± 0.12
CH182T3	0.223 ± 0.02	40.26 ± 0.22	58.00 ± 0.54
CU153T1	0.239 ± 0.00	36.55 ± 0.25	61.66 ± 0.08
CU153T2	0.236 ± 0.00	38.75 ± 0.50	61.66 ± 0.21
CU153T3	0.208 ± 0.01	38.61 ± 0.75	58.82 ± 0.04
CU562T1	0.231 ± 0.01	42.06 ± 1.87	61.90 ± 0.25
CU562T2	0.216 ± 0.02	40.30 ± 2.84	62.51 ± 0.52
CU562T3	0.230 ± 0.01	41.22 ± 0.87	57.83 ± 0.23
FS32T1	0.252 ± 0.02	42.36 ± 0.83	55.81 ± 0.28
FS32T2	0.248 ± 0.00	41.10 ± 1.00	57.72 ± 0.54
FS32T3	0.189 ± 0.00	47.00 ± 4.15	56.07 ± 0.17
FS35T1	0.229 ± 0.03	41.74 ± 1.67	55.88 ± 1.16
FS35T2	0.256 ± 0.03	40.70 ± 0.14	57.17 ± 0.56
FS35T3	0.233 ± 0.00	43.83 ± 0.84	56.16 ± 0.54
UR11T1	0.225 ± 0.02	38.84 ± 0.47	56.56 ± 0.99
UR11T2	0.242 ± 0.01	38.68 ± 0.69	60.53 ± 0.54
UR11T3	0.228 ± 0.02	40.48 ± 1.74	58.77 ± 0.04
UR14T1	0.246 ± 0.02	38.82 ± 0.25	59.04 ± 0.33
UR14T2	0.248 ± 0.00	39.70 ± 0.57	59.14 ± 0.76
UR14T3	0.236 ± 0.02	41.68 ± 1.36	55.86 ± 1.38
B73xMo17	0.312	44.09	49.41

Starch, PGluten, and Oil = Protein content in starch and gluten samples and oil content of the germ, respectively.

Table 6. Mean values and coefficients of variation (CV) for the compositional and wet-milling characteristics of groups of corn hybrids from crosses of exotic introgressed inbred lines and commercial adapted testers.

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

Table 7. Mid-parent heterosis for physical, compositional, and wet-milling characteristics of corn hybrids from crosses of exotic introgressed inbred lines and commercial adapted testers.

Hybrid	Physical			Compositional			Wet-milling					
	TestWt	1000k	ADen	Starch	Protein	Oil	Starch	Gluten	Fiber	Germ	SteepW	SR
AR285T1	3.15	36.73	-0.23	1.41	-15.05	7.78	7.93	-23.86	-7.67	19.29	-5.75	6.51
AR285T2	3.48	33.36	0.08	1.12	-13.68	10.34	6.78	-17.05	-7.04	3.26	-2.82	5.73
AR285T3	2.34	44.72	0.19	0.28	-11.52	9.09	3.27	-7.00	-3.24	-14.66	4.18	3.05
CH182T1	0.54	45.09	-0.57	1.42	-10.57	5.00	1.86	-8.62	1.78	8.44	-3.56	0.60
CH182T2	0.49	30.50	-0.65	1.56	-11.58	5.39	4.56	-7.64	1.50	-30.00	-0.63	3.07
CH182T3	0.75	30.46	7.10	1.14	-19.27	18.99	3.84	-18.47	2.81	-4.31	8.91	2.67
CU562T1	2.69	37.31	-0.27	2.31	-19.52	9.94	9.01	-28.73	-9.53	37.55	-0.18	6.63
CU562T2	3.03	36.99	-0.12	2.59	-20.00	3.37	9.16	-17.68	-7.60	11.70	-0.54	6.53
CU562T3	-1.44	46.23	-1.50	1.15	-12.20	4.14	0.77	-7.39	1.82	5.53	2.49	-0.30
FS35T1	-2.05	8.25	-2.56	2.17	-21.01	12.57	9.17	-29.18	-9.66	31.87	-1.11	6.98
FS35T2	-1.85	36.92	-2.87	1.15	-11.11	5.75	5.94	-17.69	-3.34	10.26	-7.80	4.74
FS35T3	-1.09	27.03	-1.08	1.31	-19.06	6.67	6.51	-24.63	-7.34	10.83	2.51	5.19
UR11T1	0.06	28.94	-2.61	0.54	-8.07	1.91	0.27	-14.34	5.13	53.21	-4.84	-0.22
UR11T2	0.53	17.60	-2.15	0.83	-9.87	2.44	0.48	-12.72	3.26	40.58	-5.83	-0.24
UR11T3	3.09	32.41	-0.66	0.85	-12.96	10.97	2.40	-12.86	-3.95	20.77	5.15	1.61
Mean	**0.91**	**32.84**	**-0.53**	**1.32**	**-14.36**	**7.62**	**4.80**	**-16.52**	**-2.87**	**13.62**	**-0.66**	**3.50**

Table 7. Continued

AR227T1	2.44	21.69	-0.31	2.91	-21.52	8.05	6.55	-15.13	-10.25	-1.54	1.72	3.64
AR227T2	0.86	38.96	-0.23	2.47	-15.10	-2.76	4.10	-2.69	-5.06	-34.84	3.46	1.66
AR227T3	0.60	38.27	0.20	0.32	-6.38	2.33	-1.13	1.65	-1.26	4.13	10.76	-1.45
CH143T1	0.85	17.45	-0.15	1.99	-13.89	8.88	8.28	-22.72	-8.90	10.51	1.94	6.25
CH143T2	0.79	9.55	-0.15	1.98	-12.55	6.82	5.32	-16.49	-3.43	5.26	3.81	3.27
CH143T3	-2.46	25.99	-1.74	2.70	-21.74	12.57	7.36	-27.69	-4.38	1.65	9.99	4.67
CU153T1	3.86	37.79	-0.54	2.02	-17.93	9.71	5.94	-16.18	-5.01	4.84	2.68	3.96
CU153T2	3.81	34.94	-1.46	1.42	-15.05	9.89	6.57	-16.76	-7.70	19.35	-1.76	5.10
CU153T3	0.56	20.22	-0.90	2.30	-21.95	6.36	4.52	-19.27	5.02	-4.31	-0.87	2.17
FS32T1	3.58	31.14	-0.16	1.35	-14.23	6.08	3.27	-20.98	2.78	30.70	-3.18	2.00
FS32T2	1.15	34.36	-0.55	1.78	-13.68	4.26	5.51	-21.38	2.20	16.11	-4.31	3.75
FS32T3	-2.68	21.59	-2.18	2.93	-27.19	2.79	5.64	-36.33	10.56	9.39	0.74	2.70
UR14T1	4.56	21.30	0.62	1.49	-14.52	9.76	3.98	-13.78	-1.44	-2.21	4.12	2.50
UR14T2	1.19	21.52	-0.77	1.64	-13.99	16.96	4.57	-12.84	-1.54	-6.44	-4.05	2.93
UR14T3	3.20	36.96	0.90	0.07	-5.62	3.70	-0.37	-5.17	-1.48	11.09	7.55	-0.45
Mean	**1.49**	**27.45**	**-0.50**	**1.83**	**-15.69**	**7.03**	**4.67**	**-16.38**	**-1.99**	**4.25**	**2.17**	**2.85**
B73xMo17	-0.07	27.30	0.40	0.07	-1.99	2.92	0.25	-1.83	14.53	-0.91	-7.24	0.18

TestWt = Test weight, 1000k = One-thousand kernel weight, ADen = Absolute density, SteepW = Steepwater yield, and SR = Starch recovery.
* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

Table 8. High-parent heterosis for physical, compositional, and wet-milling characteristics of corn hybrids from crosses of exotic introgressed inbred lines and commercial adapted testers.

Hybrid	Physical			Compositional			Wet-milling					
	TestWt	1000k	ADen	Starch	Protein	Oil	Starch	Gluten	Fiber	Germ	SteepW	SR
AR285T1	1.34	33.55	-0.61	-0.50	-19.22	3.45	2.22	-21.60	11.88	17.50	-0.76	2.74
AR285T2	1.59	29.41	-0.46	-0.50	-15.13	10.34	2.34	-16.74	10.70	-1.00	4.16	2.93
AR285T3	-0.18	39.50	-1.53	0.21	-16.52	3.45	2.36	7.82	5.53	-24.57	7.20	2.22
CH182T1	-1.86	43.56	-1.88	-0.21	-13.73	5.00	-4.16	-4.49	30.16	0.66	3.53	-3.88
CH182T2	-1.98	22.54	-2.11	0.21	-11.76	1.15	-0.46	-6.62	27.48	-36.64	8.63	-0.61
CH182T3	-2.36	21.71	4.29	0.78	-24.89	17.50	3.65	-7.01	17.86	-10.56	10.00	2.49
CU562T1	2.67	31.42	-1.16	1.23	-20.78	3.30	2.06	-24.41	12.62	32.46	7.72	0.83
CU562T2	2.97	35.69	-0.85	1.81	-21.46	1.10	3.41	-15.58	13.00	4.77	9.31	1.65
CU562T3	-2.14	43.87	-1.97	0.21	-19.84	-3.30	0.43	3.99	13.85	-4.80	3.00	-1.55
FS35T1	-3.71	-4.37	-3.56	-0.14	-26.27	8.05	5.48	-26.22	1.59	10.90	7.65	5.73
FS35T2	-3.58	26.99	-4.01	-0.85	-14.29	5.75	3.61	-17.06	6.92	-9.31	2.21	4.51
FS35T3	-3.45	18.56	-3.40	0.99	-22.17	1.15	3.43	-13.73	-5.65	6.15	2.82	1.83
UR11T1	-1.78	13.82	-3.13	-1.90	-12.94	0.00	-4.52	-8.83	13.42	47.68	2.16	-2.67
UR11T2	-1.25	8.99	-2.83	-1.34	-11.76	-3.45	-3.17	-10.20	9.66	39.24	2.94	-1.79
UR11T3	1.93	23.50	-2.52	0.35	-17.54	10.26	0.92	-2.51	-2.00	2.11	6.20	-0.35
Mean	**-0.79**	**25.92**	**-1.72**	**0.02**	**-17.89**	**4.25**	**1.17**	**-10.88**	**11.13**	**4.97**	**5.12**	**0.94**

Table 8. Continued

AR227T1	-0.14	16.76	-0.31	1.29	-27.06	0.00	-1.04	-3.94	14.91	-14.04	7.85	-2.30
AR227T2	-1.75	36.86	-0.39	1.15	-18.49	-6.38	-2.19	6.28	19.38	-44.42	11.69	-3.30
AR227T3	-2.62	35.26	-1.16	-0.07	-9.59	-6.38	-2.32	7.01	13.32	4.03	13.20	-3.01
CH143T1	-0.42	15.70	-1.06	1.46	-14.06	3.37	7.02	-21.55	12.44	-16.31	5.08	5.65
CH143T2	-0.55	5.40	-1.21	1.76	-15.63	5.62	5.24	-12.34	17.12	-21.75	8.89	2.97
CH143T3	-4.38	20.43	-3.94	1.21	-29.69	5.62	1.96	-11.81	6.08	-14.06	15.60	0.82
CU153T1	2.97	36.09	-0.84	1.09	-19.22	1.05	0.82	-12.02	16.10	-6.99	7.69	-0.19
CU153T2	2.84	29.51	-1.92	0.80	-16.60	5.26	2.64	-15.51	10.86	3.39	4.88	1.84
CU153T3	-1.02	14.63	-2.53	1.21	-28.74	-3.16	3.08	-8.34	15.46	-6.14	2.40	1.84
FS32T1	3.06	17.21	-1.24	0.07	-18.43	-4.95	-1.48	-15.78	30.65	4.30	-2.60	-1.50
FS32T2	0.72	26.16	-1.47	0.79	-15.13	-2.97	1.86	-19.01	27.60	-9.22	-2.08	1.12
FS32T3	-2.91	14.91	-2.45	2.20	-31.30	-8.91	3.92	-28.86	26.03	-1.54	8.60	1.75
UR14T1	3.33	8.85	0.46	0.07	-16.86	7.14	-1.74	-8.43	17.33	-10.83	12.25	-1.83
UR14T2	0.07	14.60	-0.77	0.50	-14.52	14.94	-0.02	-10.51	15.22	-16.77	5.34	-0.52
UR14T3	2.72	29.99	-0.31	-0.50	-12.86	0.00	-1.00	6.35	5.72	5.76	8.20	-0.52
Mean	**0.13**	**21.49**	**-1.28**	**0.87**	**-19.21**	**0.68**	**1.12**	**-9.90**	**16.55**	**-9.64**	**7.13**	**0.19**
B73xMo17	-0.86	15.62	-0.71	-0.29	-3.06	0.00	-2.91	2.27	26.88	-3.25	-7.06	-3.32

TestWt = Test weight, 1000k = One-thousand kernel weight, ADen = Absolute density, SteepW = Steepwater yield, and SR = Starch recovery.
* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

Table 9. Correlation coefficients among physical, compositional, and wet-milling characteristics of corn hybrids from crosses of exotic introgressed inbred lines and commercial adapted testers.

Factor	TsWt	kkWt	ADen	StaC	ProC	OilC	StaY	GluY	FibY	Germ	SWY	SR	TSR
TsWt	1.00	-0.21	0.77**	-0.49**	0.32	0.27	0.02	0.46*	-0.38*	-0.46*	0.48**	0.22	0.10
kkWt		1.00	-0.42*	0.16	-0.06	-0.27	0.16	-0.07	0.05	-0.07	-0.41*	0.13	0.30
ADen			1.00	-0.57**	0.55**	0.01	-0.31	0.65**	-0.14	-0.41*	0.48**	-0.16	-0.25
StaC				1.00	-0.93**	-0.13	0.71**	-0.73**	-0.29	0.07	-0.41*	0.48**	0.09
ProC					1.00	-0.19	-0.81**	0.83**	0.42*	-0.11	0.25	-0.64**	-0.17
OilC						1.00	0.26	-0.22	-0.33	0.06	0.36*	0.37*	0.15
StaY							1.00	-0.69**	-0.72**	-0.16	-0.19	0.96**	0.35
GluY								1.00	0.28	-0.51**	0.29	-0.57**	-0.21
FibY									1.00	0.11	-0.27	-0.77**	-0.21
Germ										1.00	-0.06	-0.23	0.05
SWY											1.00	-0.07	0.04
SR												1.00	0.40*
TSR													1.00

TsWt = Test weight, kkWt = One-thousand kernel weight, ADen = Absolute density, StaC = Starch content, ProC = Protein content, OilC = Oil content, StaY = Starch yield, GluY = Gluten yield, FibY = Fiber yield, Germ = Germ yield, SWY = Steepwater yield, SR = Starch recovery, and TSR = Total solids recovery.

* and ** indicate statistical significance at 0.05 and 0.01 probability levels, respectively.

CHAPTER 5. GENERAL CONCLUSIONS

Groups of lines were statistically different and the HSEL group showed higher starch content, starch yield, and starch recovery than the LSEL group, which means that they have better wet-milling characteristics for the corn processing industry. AR16035:S19-227-1-B and CUBA117:S1520-562-1-B had similar wet-milling characteristics, related to starch yield, than the Corn Belt inbred line B73. These exotic lines were developed and selected for high grain yield as testcrosses in yield trials, which is evidence of the potential present in exotic germplasm to improve the agronomic performance and the wet-milling characteristics of hybrids grown in the United States.

There was great variation among physical, compositional, and wet-milling characteristics of the experimental hybrids from the crosses between exotic inbred lines and commercial adapted lines used as testers in both the F_1 and the F_2 generations. This suggests that genetic diversity is present and that exotic germplasm is a valuable source to improve the wet-milling characteristics of corn hybrids used by the wet-milling industry.

Testers produced groups of hybrids that were statistically different. Tester 3 produced hybrids with higher starch contents, higher starch yields, and better starch recovery than Testers 1 and 2, which indicates that inbred lines that give to its progeny high starch and low protein content produce hybrids appropriate for wet milling.

Negative heterosis for starch content and positive heterosis for protein content were expressed, which led to negative heterosis values for starch yield and starch recovery and poor wet milling properties of the F_1 generation of hybrids with exotic germplasm. Positive mid-parent and high-parent heterosis for starch content and negative values for protein content were expressed by the F_2 generation or hybrid grain. This led to positive heterosis values for starch yield and starch recovery and better wet-milling properties for some hybrid grain samples with exotic germplasm than B73xMo17.

There was a significant positive correlation between starch content and starch yield and starch recovery and a highly significant negative correlation between protein content and starch content, starch yield, and starch recovery. This information indicates that genotypes with high starch and low protein contents will produce hybrids with better millability.

Because Near Infrared Transmittance (NIT) estimations are rapid and nondestructive, which is advantageous for corn breeders during early stages of selection, NIT technology may be applicable to maize breeding programs as a predictive tool to screen early progeny for compositional chemical characteristics, such as high starch and low protein contents, and then predict their wet-milling properties. Application of this technology can save time and costs in a corn breeding program directed to the improvement of the millability of corn hybrids to be used as raw material by the wet-milling industry.