GENETICS UNDERLYING FLAKING GRIT YIELD FROM DRY MILLED MAIZE GRAIN

BY

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THESIS

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ABSTRACT

A survey of U.S. corn germplasm was conducted to explore genetics underlying flaking grit yield (FGY) and evaluate the relationship between FGY and agronomic performance, with an aim to devise breeding strategies to simultaneously improve FGY and agronomic performance in the future. The survey used a broad-based set of inbreds that represent parentage of heterotic subgroups utilized in current commercial maize hybrids. The 12 inbreds were crossed in a diallel design without reciprocals to create 66 test hybrids which were evaluated for agronomic, dry milling, ear, and kernel properties over 3 years. The overall mean among test hybrids for FGY was 29.4 g grits/100g corn dry basis with a range from 24.0 to 36.0 g grits/100g corn dry basis. Narrow and broad sense heritability estimates for FGY were 0.53 and 0.65, respectively, indicating the importance of additive gene action for this trait. The general combining ability (GCA) variance for FGY was twice the size of the specific combining ability variance, indicating the importance of GCA. Heterosis is not a factor in expression of FGY. In most cases, FGY was not correlated with agronomic, ear, and kernel traits, but FGY was phenotypically correlated ($p < 0.05$) with both grain yield ($r = -0.50$) and test weight ($r = 0.52$). Grain yield and FGY were genetically correlated with an $r = -0.43$. A moderate amount (31%) of the variation in flaking grits per acre (FGA) was explained by multiple regression of several traits including grain yield as well as simple physical kernel characters: test weight, kernel depth, 100 kernel volume.

Overall, the results of this study indicated presence of genetic variation for FGY in U.S. maize germplasm which could be exploited to develop new corn hybrids

with improved FGY and FGA.   Furthermore, performance for FGA may be predicted

to some extent based on performance for some select agronomic and kernel

characteristics. However, there is a need to explore additional options for improving

predictions of FGA. Kernel characteristics such as kernel density and breakage

susceptibility, implicated in previous reports to be highly correlated with FGY, may

represent potential candidate traits for such a study.

ACKNOWLEDGMENTS

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INTRODUCTION

In 2010, 1.58% of the total U.S. corn harvest, or 197 million bushels (5,003.8 million kilograms) of the 12,446 million bushels (316,128.4 million kilograms) of corn grain produced, was used in cereals and other food products (USDA Economic Research Service, 2012). *Per capita* maize consumption is approximately 160 pounds a year in the U.S. (Farmers Feed US, 2012), this highlights that corn is an important human dietary component.

A corn kernel is comprised of three major components: endosperm, bran, and germ (Wolf et al., 1952) (Figure 1). Seebauer et al. (2010) indicated that genetic factors play a large role in determining kernel composition. The endosperm, which comprises over 80% of a corn kernel, is made up of hard, translucent and soft, floury endosperm. The bran or pericarp is a thin outer layer covering the corn kernel and the germ contains the embryo.

Dry milling is a process used to mechanically isolate kernel components. Specifically, dry milling separates germ and bran from the endosperm based on differences in physical properties and chemical composition. This separation is facilitated by the lack of continuity among tissues and structural distinctions of each kernel component (Wolf et al., 1952). Separating major kernel components is critical for shelf-life and nutritional quality of endosperm products. Endosperm which has germ attached has a higher fat content and can become rancid from the oil in the germ. The commercial dry milling process is comprised of four key steps: tempering, degerminating, aspiration, and density separation. Tempering involves a controlled addition of moisture to the corn kernel, which allows for the separation of the

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endosperm from the bran and germ. Moisture distribution inside the corn kernel plays a vital role with regard to milling action (Mehra and Eckhoff, 1997). The optimum milling moisture content for the bran and endosperm are not equal (Butcher and Stenvert, 1973). The tempering process creates a moisture gradient in the corn kernel so that the bran and germ are at higher moisture than the endosperm. The objective is to make the bran and germ tough and resilient to fragmentation without softening the endosperm (Butcher and Stenvert, 1973). Degerminating is the mechanical process of breaking the corn kernel into the various fractions, separating endosperm particles from bran and germ. Bran is recovered by aspiration while the germ is recovered by density differences using gravity tables. Screening sorts various endosperm fractions and recovers the flaking grits (largest endosperm particles) and other various products of the endosperm, e.g., smaller grits, meal and flour. Flaking grits result from endosperm particles that are one half to one third that of the whole corn kernel (Caldwell et al., 2000) and are of value to breakfast cereals because they are the main ingredient in corn flakes.

Flaking grits are the most valued product of the dry milling process. Flaking grit yield (FGY) is important to dry millers, but is challenging as a breeding target. As a processing trait, FGY becomes measurable considerably downstream of grain harvest and results from endosperm particle that pass through a 3 mesh screen (6.73 mm openings) but are retained in a 5 mesh screen (4.00 mm openings). Furthermore, until recently, it has been difficult to measure due to lack of small-scale capacity for dry milling. In the past, FGY was estimated indirectly by assessing certain grain quality traits such as: test weight (Blandino et al., 2010; Kirleis and Stroshine, 1990;

Lee et al., 2007; Pan et al., 1996; Paulsen and Hill, 1985; Pomeranz and

Czuchajowska, 1987), kernel hardness (Blandino et al., 2010; Kirleis and Stroshine,

1990; Lee et al., 2007; Litchfield and Shove, 1990; Narváez-González et al., 2006),

kernel density (Kirleis and Stroshine, 1990), breakage susceptibility (Paulsen and Hill,

1985), and protein content (Dorsey-Redding et al., 1991; Jackson et al., 1988; Lee et

al., 2007; Lee et al., 2005; Philippeau et al., 2000; Yuan and Flores, 1996). Mestres

and Matencio (1996) reported that proteins were the only endosperm component

linked to hardness and milling characteristics of maize kernels. That is, hard

endosperm results in larger endosperm particles, which is a key factor in FGY.

Differences in kernel structure among hybrids account for the variation among hybrids

in dry milling yield (Rausch et al., 2009). The protein matrix, located in the

endosperm, is not as mechanically strong in soft endosperm as in hard endosperm

(Tester et al., 2004). The protein matrix acts as a supporting material among starch

granules to provide rigidity (Narváez-González et al., 2006). Narváez-González et al.

(2006) concluded that starch granules in the hard endosperm were polygonal, while

the soft endosperm were spherical. The polygonal shape results from the starch

granules being tightly packed (Robutti et al., 1974) and drawn closely together by

adhesive forces between protein and starch (Narváez-González et al., 2006).

Kernel hardness has been associated with amylose starch content (Dombrink-

Kurtzman and Knutson, 1997) and can be measured by near-infrared spectroscopy

(Robutti et al., 2000). This process can also be used to estimate kernel density and

hardness (Dorsey-Redding et al., 1991). Prediction of FGY has been achieved with

high correlation ($R^2 = 0.91$) using a milling evaluation factor (MEF), which is based

on the relationship between FGY and two grain quality traits, kernel density and test weight (Kirleis and Stroshine, 1990; Pan et al., 1996; Wehling et al., 1996). Lee et al. (2007) reported a model (R^2 = 0.52) using test weight, protein content, pycnometer density, time to grind in the Stenvert Hardness Tester, and kernel size distribution to predict FGY, but suggested a need to explore alternative rules and procedures that would enable breeders, producers, and processors to more confidently predict maize dry milling quality.

Until recently, FGY could be estimated directly only on a pilot scale which demands a large grain sample of 40 kg (Mehra and Eckhoff, 1997); a small scale procedure for evaluating breeding lines and multiple hybrid combination testing was not available. In 2009, a small scale procedure which utilizes a horizontal drum degerminator that allows for the processing of 1 kg samples was developed at the University of Illinois at Urbana-Champaign (Rausch et al., 2009). This process is ideal for comparing numerous lines and hybrids and has facilitated exploration of the genetics underlying FGY (Rausch et al., 2009). Estimates of FGY from the small scale procedure are representative of FGY obtained in industry (K.D. Rausch, 2012, personal communication). However, fat content in the flaking grit fractions from the small scale dry milling procedure were higher than for commercial flaking grit fractions (Rausch et al., 2009).

Extensive effort in research and development has gone into improving agronomic performance of modern corn hybrids, resulting in a nearly four-fold increase in direct investment since the 1970s (Duvick and Cassman, 1999). In addition to grain yield, other key traits that are being improved to benefit growers are

seedling vigor, standability, stay green, plant height, ear height, and test weight. Dry milled maize to produce breakfast cereals for human consumption is a relatively small market. Although a relatively small percentage of the annual maize crop is used directly for human consumption, the "corn cereal pipeline" is an excellent example of an agricultural production system that provides primary ingredients for dietary staples. It is a complex pipeline involving groups with different interests and expertise including seed companies, farmers, dry millers, food companies, and consumers (Figure 2). Since multiple segments of the agricultural food chain are involved, benefits need to be maximized for each. This study focused on the front end of the cereal pipeline, centering on characteristics that are beneficial for farmers and dry millers that can be improved through plant breeding. If hybrids were bred for superior FGY, then the cost of flaking grits might be decreased. This decrease could ultimately be passed along to the consumer. Thus, we sought to explore the relationship of FGY to agronomic performance with the aim of finding ways to enhance both simultaneously.

The purpose of this study was to conduct a survey evaluating FGY in U.S. germplasm. Specifically, the objectives were to (i) characterize genetics underlying FGY, namely levels of performance, amount of genetic variation, heritability, type of gene action involved, general and specific combining ability, and level of heterosis, (ii) evaluate the phenotypic and genetic relationships between FGY and agronomic performance including grain yield, and (iii) devise breeding strategies to simultaneously increase FGY and agronomic performance.

MATERIALS AND METHODS

Plant Materials

To survey U.S. corn germplasm, we used a broad-based set of lines that represent parentage of heterotic subgroups used in current commercial maize hybrids. After creating a proprietary pedigree database of 685 maize inbred lines for which Plant Variety Protection (PVP) patent protection had expired, Mikel and Dudley (2006) identified a core set of 12 elite inbreds based on the number of times each was used as an ancestor line. This set of 12 inbreds represents a wide array of genetic diversity (Table 1): public lines B73 and Mo17, parents of a widely adapted hybrid grown extensively in the U.S. in the 1970s and 1980s, and ten proprietary inbreds LH1, PHG39, PHJ40, LH123HT, LH82, PH207, PHG35, PHG47, PHG84, and PHZ51. The latter were originally developed by Pioneer Hi-Bred International, Inc. or Holdens. The inbreds were crossed in a diallel breeding scheme (all possible crosses without reciprocals) to create 66 test hybrids for this research.

Seed of the 12 inbred lines was acquired from the USDA North Central Regional Plant Introduction Station in Ames, Iowa. Starting in 2008, inbreds were grown at the University of Illinois Crop Sciences Research and Education Center and crossed each year prior to the year of testing to produce seed of the 66 test hybrids for field trials. Iowa Stiff Stalk Synthetic (SSS) and the Non Stiff Stalk (NSS) represent the female and male heterotic groups, respectively, used extensively in U.S. commercial corn germplasm. Test hybrids included 32 SSS × NSS hybrids, 28 NSS × NSS hybrids, and 6 SSS × SSS hybrids. Intrapool crosses (NSS × NSS, SSS × SSS) were included to facilitate the genetic analysis. Field research trials were conducted in

2009, 2010, and 2011 at the University of Illinois Crop Sciences Research and Education Center in Urbana, Illinois.

Experimental Design

The 66 test hybrids, 6 commercial hybrid checks, and 12 parental inbreds were grown in 2009, 2010, and 2011 in Urbana, IL in a split plot incomplete block design with three replications per environment. Generations were split to prevent inter-plot effects due to differences in plant height and vigor, with 2 blocks of inbreds and 12 blocks of hybrids, each containing 6 entries per block. Entries were assigned randomly to blocks within generation using CycDesigN (Whittaker et al., 1999), which helps to balance distances between any pair of entries across replications within an environment for very large experiments such as this. The experiment was machine planted with 35 kernels per row, which was later thinned back to 30 plants. Plots consisted of 4 rows and were 17.5 feet (5.334 m) long, 10 feet (3.048 m) wide with 2.5 feet (1 m) alleys, corresponding to a final plant density of approximately 30,000 plants per acre (74,000 plants per hectare), which is appropriate to hybrids of this timeframe. Data was collected from the center two rows only to minimize effects of maturity and plant height from adjacent plots.

Phenotypic Evaluation

Entries were evaluated for numerous traits related to agronomic performance such as dry milling performance, ear characteristics, and kernel characteristics (Table 2). Agronomic traits measured include seedling vigor, days to pollen shedding, days to silking, plant height, ear height, staygreen, percent barren plants, and grain yield. Included in the dry milling traits that were measured are test weight, FGY, and flaking

grits per acre (FGA). Cob length, cob width, fill length, ear width, number of kernel rows, kernels per row, hundred kernel weight, and hundred kernel volume are the ear traits that were collected. Kernel traits that were evaluated include kernel length, kernel width, and kernel depth. Ear and kernel characteristics are considered to be grain yield component traits as well as potential dry milling yield predictors.

Seedling vigor was recorded as a visual rating on a scale from 1 (less vigorous) through 10 (most vigorous). Days to pollen shedding was recorded when 50% of the plants in the plot were shedding pollen. When 50% of the plants in the plot had exposed silks, days to silking was recorded. To obtain days to silking and pollen shed, the planting date was recorded then the days were added up from planting to either pollen shed or exposed silks. Anthesis silking interval was computed by subtracting days to silking from days to pollen shedding. Also, plant height was recorded as the distance from the ground to the base of the flag leaf in centimeters. Likewise, ear height was recorded as distance from the ground to the base of the ear shank in centimeters. Staygreen was recorded by visually rating the percent of the plot which remained green; 1 (less that 10%) to 10 (100% still green). Percent of plants per plot without an ear was recorded as percent barren.

Prior to harvest, five ears were hand collected at random from the center of the plots so that ear and kernel traits could be measured. Cob length was recorded in millimeters and was measured from the base of the ear to the tip of the ear. Cob width was recorded after the grain had been removed and was recorded from the center of the ear to measure the diameter of the cob. Fill length was measured from the base of the ear to where to the grain stopped at the tip of the ear. Ear width was taken in the

center of the ear with grain to record the diameter of the ear. The number of kernel

rows was a count of the number of kernels rows occupying the circumference of the

ear. Kernels per row were taken as a count in one row of the number of kernels in that

row. Hundred kernel weight was a count from a seed counter of one hundred intact

kernels and weighed. Hundred kernel volume was a measure of the volume for the

same 100 kernels weighed and was recorded using a graduated cylinder. Kernel

length was an average of ten kernels measured from tip cap to crown. The same ten

kernels then had kernel width recorded, measured by lining up crowns of kernels.

Kernel depth was measured on the same ten kernels by laying germ face down and

stacking the kernels. All three kernel measurements were taken with two sets of 10

kernels, as an average of the two means. Cob size was calculated by multiplying cob

width $\times$ cob length $\times$ π. Ear size was calculated by multiplying ear width $\times$ fill length

$\times$ π. Kernel size was calculated by multiplying kernel length $\times$ kernel width $\times$ kernel

depth. Sphericity was calculated by the following equation (Pomeranz et al., 1984):

$$\text{Sphericity} = \frac{(\text{Kernel Length} \times \text{Kernel Width} \times \text{Kernel Depth})^{\frac{1}{3}}}{\text{Kernel Length}}$$

Sphericity ranges from 0 (flat kernel) to 1 (spherical kernel) to calculate a

dimensionless measure.

All plots were hand harvested to facilitate collection of the large grain sample

required for the dry milling process. Harvested plots were forced air dried at $\approx$ 120

degrees Fahrenheit until grain moisture content reached approximately 15%. Plots

were shelled using a single ear sheller (SCS-2, Agriculex, Guelph, Ontario, Canada).

During shelling, grain moisture and test weight were recorded using an electronic

moisture tester (mini GAC plus, Dickey-John, Auburn, IL). The five ears collected

earlier were added back into a bulk grain sample so that total grain yield could be

calculated for each plot. Grain yield estimates were adjusted to 15% moisture content

for plot comparison. Two 1 kg grain subsamples from each plot were used for dry

milling.

Dry Milling Evaluation

The dry milling procedure utilized to estimate FGY was adapted from Rausch

et al. (2009) (Figure 3).

Pre-screening

Corn samples were tested for moisture content to ensure that they were within

the optimal range for dry milling (12-15% moisture content). At high moisture levels

the endosperm loses its friability, but while at low moisture levels the bran becomes

brittle and is easily broken (Butcher and Stenvert, 1973). If corn moistures were less

than or equal to 12%, the corn samples were pre tempered for one week at 33°F to

prevent germination from the addition of water. Water was added to samples as

needed to bring the grain moisture level to 14%. The amount of water (mL) required

for pre-tempering was calculated according to the following equation (Rausch et al.,

2009):

$$\text{Water to add (mL)} = \left\{ \left[\frac{1 - \left(\frac{MC}{100}\right)}{\left[\left(1 - \left(\frac{MC}{100}\right)\right) - \left(\frac{14 - MC}{100}\right)\right]} \right] \times WT \right\} - WT$$

where

MC = initial moisture content (%) and WT = initial grain sample weight (g).

After one week of pre-tempering, samples were allowed one day to adjust to room temperature before moisture was rechecked to ensure that samples were above 12%. If samples were over 15% moisture, they were exposed to air flow at room temperature to allow for slow drying. Once adjusted to 12-15% moisture content, samples were screened on a No. A-P 12/64 inch (4.76 mm) round hole screen to remove broken corn and foreign material (BCFM). In addition, kernels with symptoms of disease or signs of insect damage were manually removed to ensure the highest quality of grain was dry milled.

Tempering

A single step tempering method (Rausch et al., 2009) using room temperature tap water to increase the initial moisture content of the sample by 8.5% was performed. The amount of water (mL) required for tempering each sample was determined according to the equation (Rausch et al., 2009):

$$\text{Water to add (mL)} = \left\{ \left[\frac{1 - \left(\frac{TMC}{100} \right)}{1 - \left(\frac{TMC}{100 - 0.085} \right)} \right] \times TWT \right\} - TWT$$

where

TMC = moisture content prior to tempering (%) and TWT = grain sample weight prior to tempering (g).

Corn samples were tempered for 18 min at room temperature using sealed 4 L plastic cylindrical bottles that rotated on horizontal axes continuously at 0.5 rpm to mix the samples.

Degerminating

Tempered corn was passed through a horizontal drum degerminator (Rausch et al., 2009) and then conditioned in a convection oven at 49°C for 2 hours, reducing the moisture content of grain sample that was increased during the tempering step. After conditioning, samples were cooled for 1 hour before being sieved in a sifter box (Rausch et al., 2009) over a 5 mesh screen (4.0 mm openings), which created streams that passed over (+5) or through (-5) the screen. The +5 fractions were roller milled. Roller milling crushed endosperm, flatted germ, and left pericarp unchanged so the flaking grits could be screened out. The +5 sample was passed through the roller mill four times, then sieved in a sifter box over a 10 mesh screen (1.68 mm openings), creating streams that passed over (+10) or through (-10). Material initially retained on the 5 mesh screen (+5) and passed through the 10 mesh screen (-10) after roller milling was recovered as the flaking grit fraction.

Yield Determination

Flaking grit fractions were converted to FGY estimates on a dry basis to standardize across samples. A plot mean was taken for the two subsamples dry milled in each plot. Flaking grit fractions were expressed as the proportion of original grain sample mass on a dry basis (g /100g db) and are referred to as fraction yields. To calculate FGY, dry basis determinations were made on the flaking grit fraction using a standard convention oven method (AACC International, 2000). Then FGA (lbs/ac) was calculated as grain yield (bu/ac) × FGY (g/100g db).

Extending FGY to a "per acre" basis, we devised a new trait, FGA. This could be considered similar to measures by the dairy industry to rank silage corn hybrids by

the estimated amount of milk produced from dairy cattle feeding on the particular

silage corn hybrid (Schwab et al., 2003). In addition, FGA is more representative of a

hybrid's true productivity for FGY.

Statistical Analyses

Statistical analyses were conducted with SAS software, Version 9.2, using

MIXED, MEANS, and CORR procedures (SAS Institute, 2008). All statistical

analyses were conducted using plot means. An analysis of variance (ANOVA) was

performed to estimate generation least square means and heterosis. Single year

analyses were conducted to test for homogeneity of errors before combining data

across environments. Shapiro-Wilk's test was conducted to test the null hypothesis,

i.e., that the population was normally distributed ($p < 0.05$).

The mixed model for single environment analysis was:

$$y_{ijkl} = \mu + r_i + c_j + b_{k(ij)} + g_{l(k)} + rg_{il(k)} + \varepsilon_{ijkl}$$

where

y_{ijkl} = phenotypic observation of the $ijkl^{\text{th}}$ genotype
μ = general mean
r_i = random effect of ith replication
c_j = fixed effect of jth generation
$b_{k(ij)}$ = random effect of kth block nested within i^{th} replication and j^{th} generation
$g_{l(k)}$ = random effect of lth genotype nested within k^{th} generation
$rg_{il(k)}$ = random interaction of ith replication and m^{th} genotype nested within k^{th} generation
ε_{ijkl} = error of $ijkl^{\text{th}}$ observation

The linear mixed model for the combined analysis across environments was:

$$y_{ijklm} = \mu + e_i + r_{j(i)} + c_k + b_{l(ijk)} + g_{m(k)} + eg_{im(k)} + rg_{mj(ik)} + \varepsilon_{ijklm}$$

where

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y_{ijklm} = phenotypic observation of the $ijklm^{th}$ genotype

μ = general mean

e_i = fixed effect of ith environment

$r_{j(i)}$ = random effect of jth replication nested within i^{th} environment

c_k = fixed effect of kth generation

$b_{l(ijk)}$ = random effect of lth block nested within i^{th} environment, j^{th} replication, and k^{th} generation

$g_{m(k)}$ = random effect of mth genotype nested within k^{th} generation

$eg_{im(k)}$ = random interaction of ith environment and m^{th} genotype nested within k^{th} generation

$rg_{mj(ik)}$ = random interaction of jth replication and m^{th} genotype nested within i^{th} environment and k^{th} generation

ε_{ijklm} = error of $ijklm^{th}$ observation

General combining ability (GCA) and specific combining ability (SCA), as well as heritability estimates, were calculated using only the 66 test hybrids. Variance components were estimated using the linear mixed model:

$$y_{ijkl} = \mu + e_k + r_{l(k)} + g_i + g_j + s_{ij} + ge_{ik} + ge_{jk} + se_{ijk} + \varepsilon_{ijkl}$$

where

μ = general mean

e_k = fixed effect of kth environment

$r_{l(k)}$ = random effect of lth replication nested within k^{th} environment

g_i or g_j = random GCA effect of ith or j^{th} parent

s_{ij} = random SCA effect of the cross between i^{th} and j^{th} parent

ge_{ik} or ge_{jk} = random interaction effect between GCA of ith or j^{th} parent with k^{th} environment

se_{ijk} = random interaction effect between SCA of the cross between i^{th} and j^{th} parent with k^{th} environment

e_{ijkl} = random error of $ijkl^{th}$ observation

Using the variance components obtained from the linear mixed model analysis, estimates of broad sense heritability and narrow sense heritability were calculated as outlined in Zare et al. (2011) in keeping with Teklewold and Becker (2005).

Broad sense heritability:

$$H^2 = \frac{2\sigma^2_{GCA} + \sigma^2_{SCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \left(\frac{2\sigma^2_{GCA \times E}}{Env}\right) + \left(\frac{\sigma^2_{SCA \times E}}{Env}\right) + \left(\frac{\sigma^2_{error}}{Env * Rep}\right)}$$

Narrow sense heritability:

$$h^2 = \frac{2\sigma^2_{GCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \left(\frac{2\sigma^2_{GCA \times E}}{Env}\right) + \left(\frac{\sigma^2_{SCA \times E}}{Env}\right) + \left(\frac{\sigma^2_{error}}{Env * Rep}\right)}$$

where

Env = environment, Rep = replication, and GCA×E and SCA×E are interactions of

GCA and SCA with the environment.

Estimated variance components for additive genetic component were used to

calculate genetic correlations. Genetic correlations between pairs of traits were

calculated using the equation presented by Bernardo (2010):

$$\text{Genetic correlation: } r_A = \frac{cov_{XY}}{\sqrt{var_X \times var_Y}}$$

where

cov_{XY} = covariance between X and Y, var_X = additive genetic variance of X, and

var_Y = additive genetic variance of Y.

To compute cov_{XY}, a dummy variable was created as Z = X+Y (Isik, 2009) and the

following equation was used to solve for cov_{XY} (Bernardo, 2010; Isik, 2009).

$$var (X + Y) = var_X + var_Y + 2cov_{XY}$$

where

var (X + Y) = additive genetic variance of X and Y summed.

Variances were estimated using PROC VARCOMP (SAS Institute, 2008) for X, Y, and Z.

In addition, Pearson correlation coefficients (r) were computed for all pairs of traits. In order to determine the usefulness of grain yield, ear and kernel characteristics for indirect selection to improve FGY, a multiple regression analysis was performed using PROC REG (SAS Institute, 2008). Stepwise regression with forward elimination was used to generate a prediction model for FGA, considering agronomic traits including grain yield, test weight, cob length, cob width, fill length, ear width, number of kernel rows, kernels per row, 100 kernel weight, 100 kernel volume, kernel length, kernel width, kernel depth, and kernel size. With this method, the single most significant predictor was added to the prediction model in a single iteration. The model was tested and non-significant variables were deleted. The process continued until no new significant variables could be added to the model and all variables included in the model were significant. A stepwise selection and elimination criteria of alpha level of $p \leq 0.05$ was used in building the regression model. Mallows' C_p criterion (Kutner et al., 2004) was used to assess the fit of the regression model. The Mallows' C_p value should be close to the number of predictor variables present in the regression model to avoid over fitting. In the model, the residual sum of squares will decrease as more variables are added to the model without actually improving predictability. Low Mallows' C_p values and high adjusted R^2 values were used to assess the predictability of the model. The model was trained using the 2009 and 2010 data and then validated using the 2011 data to evaluate lack

of fit (Kutner et al., 2004). Lack of fit challenges the null hypothesis such that if $p \leq$

0.05, then the prediction model is considered to be biased.

RESULTS AND DISCUSSION

Trait Performance

Flaking Grit Yield

Means and standard deviations are presented for the 27 traits collected from the test hybrids, commercial check hybrids, and parental inbreds (Table 3). The overall mean for FGY for test hybrids was 29.4 g/100g db, compared with an overall mean of 27.3 g/100g db for the commercial check hybrids (Table 3). Means for FGY ranged from 24.0 to 36.0 g/100g db among test hybrids and 24.5 to 31.4 g/100g db among the commercial checks hybrids. PHJ40×LH123HT had the highest FGY and LH1×PHG47 had the lowest FGY (Table 4). Thirteen of the test hybrids out yielded the best commercial check for FGY (Table 4). Rausch et al. (2009) reported an overall FGY mean of 39.16 g/100g db and a range of 29.6 to 47.0 g/100g db for 11 commercial hybrids that were presumed to contain a wide range of milling properties. Results from this study show FGY similar to Rausch et al. (2009); however the upper spectrum of performance was higher in the Rausch et al. study: 47.0 g/100g db versus 36.0 g/100g db in this study using parents of diverse heterotic subgroups. Blandino et al. (2010) reported FGY means that ranged from 40.4 to 60.0 g/100g for 13 hybrids used for food processing in North Italy. However, Blandino et al. (2010) only performed dry milling on typical, flat-shaped, whole kernels from the middle part of the ear to reduce variability in kernel size. FGY appears to be higher with grain of higher test weight (Blandino et al., 2010; Kirleis and Stroshine, 1990; Lee et al., 2007; Pan et al., 1996; Paulsen and Hill, 1985; Pomeranz and Czuchajowska, 1987). Many of the hybrids tested by Rausch et al. (2009) and Blandino et al. (2010) had test

weights greater than 60 lb/bu (75 kg/hL), which is not necessarily typical of all yellow dent hybrids.

Parental inbreds means for FGY ranged from 24.3 to 39.2 g/100g db with a mean of 31.9 g/100g db (Table 3). Inbred performance for FGY was superior to the test hybrids FGY on average. Blandino et al. (2010) reported correlations of FGY with kernel length (r = -0.60), FGY with kernel width (r = -0.13), and FGY with kernel depth (r = 0.46). Comparing the overall means between the test hybrids and parental inbreds (Table 3), we found that the parental inbreds on average have decreased kernel width and kernel length but increased kernel depth over the test hybrids. These findings are in agreement with Blandino et al. (2010) and highlight that grain quality parameters are essential in maximizing value in outputs from the dry milling process.

Grain Yield

In general, the test hybrids performed well for grain yield with a mean of 171.1 bu/ac (10.7 Mg/ha) (Table 3). B73×PHG47, a SSS×NSS hybrid, had the highest grain yield with a mean of 199.3 bu/ac (12.5 Mg/ha) and PHG84×PHZ51, a NSS×NSS hybrid, had the lowest grain yield with a mean of 135.7 bu/ac (8.5 Mg/ha) (Table 4). Surprisingly, many of the NSS×NSS hybrids were above the overall grain yield average of 171.1 bu/ac (10.7 Mg/ha) for the test hybrids. This reflects that different heterotic patterns have been utilized between companies. For example, public records on the parentage of ex-PVP inbreds suggests that Mo17 was not utilized in developing the NSS heterotic group at Pioneer Hi-bred International (M.A. Mikel, 2012, personal communication). Grain yield means among the commercial hybrid checks ranged

from 211.7 to 222.8 bu/ac (13.2 to 14.0 Mg/ha), with an overall mean of 215.3 bu/ac (13.5 Mg/ha) (Table 3). As expected, all of the commercial check hybrids out yielded the test hybrids for grain yield (Table 4), demonstrating the impact of plant breeding over the past decades.

Flaking Grits per Acre

Test hybrids produced FGA ranges from 2155.5 to 3450.0 lbs/ac (2.4 to 3.9 Mg/ha), with an overall mean of 2768.6 lbs/ac (3.1 Mg/ha). PHJ40×LH123HT had the highest FGY (Table 4) but, in contrast, B73×PHG84 had the highest ranking for FGA (Table 5) among test hybrids. The commercial check hybrids produced means that ranged from 2841.5 to 3702.2 lbs/ac (3.1 to 4.1 Mg/ha) for FGA with an overall mean of 3222.7 lbs/ac (3.6 Mg/ha) (Table 3). Check1 produced more FGA than any of the test hybrids (Table 5). These results suggest that, hybrids with high FGY are not necessarily indicative of those with high FGA. Likewise, hybrids with high FGY tended to be below average in grain yield. With FGA, a balance between grain yield and FGY is realized. Table 6 shows the top 5 ranking hybrids out of the 32 SSS×NSS. For example, B73×PHG84 which ranked 11 for grain yield and ranked 4 for FGY (Table 4) was top ranked among test hybrids for FGA. Table 6 shows hybrids were able to achieve a mean of ≈ 3200 lbs/ac of flaking grits in various ways, highlighting the balance of grain yield and FGY represented by the trait, FGA. As such, the dry miller could buy a high grain yielding hybrid with a lower FGY and handle more grain or provide a premium for a lower grain yielding hybrid with superior FGY and handle less grain to reach the desired pounds of flaking grits.

Agronomics

Comparing performances among the test hybrids and commercial check hybrids, we found that mean performance for seedling vigor, anthesis silking interval, staygreen, percent barren plants, fill length, ear width, kernels per row, rows, 100 kernel volume and weight, and kernel size was greater for commercial check hybrids. This could be attributed to plant breeding improvements for better stress tolerance and general plant health. Additionally, we noticed that plant height was greater for commercial check hybrids, but ear height was the same. Breeders might be indirectly selecting lines with larger upper canopies, thus allowing more light to enter the canopy for photosynthesis and, ultimately, conversion to kernel mass increasing grain yield.

Kernel Characteristics

Evaluation of test hybrids revealed physical kernel traits in ranges typical for yellow dent corn (Table 3). Test weight, 100 kernel volume, 100 kernel weight, kernel width, kernel length, kernel depth, kernel size, and sphericity varied among test hybrid, with mean ranges of 54.6-59.3 lb/bu (68.3-74.1 kg/hL), 31.5-49.7 mL, 21.6-34.5 g, 6.7-9.2 mm, 10.7-12.8 mm, 3.9-4.7 mm, 297.7-482.2 mm^3, and 0.6-0.7, respectively (Table 3). PHJ40×LH123HT and B73×PHG39 had the highest test weight (Table 5). Many authors have reported that an increase in test weight has been associated with increased FGY. Two-thirds of the test hybrids had test weights above the commercial standard of 56 lb/bu (70 kg/hL). Overall means for kernel width and kernel depth were similar for test hybrids and commercial check hybrids but kernel length was larger for commercial check hybrids (Table 3). Overall test weight mean was similar between the test hybrids and commercial check hybrids.

Heritability and Gene Action

Narrow and broad sense heritabilities were estimated for each of the 27 traits collected (Table 7). Narrow sense heritability reflects the additive gene action controlling phenotypic expression whereas broad sense heritability reflects both additive and non-additive gene action underlying phenotypic expression (Falconer and Mackay, 1996). The difference between broad sense and narrow sense provides a measure of the non-additive gene action involved, including dominance, over-dominance, and epistasis. Narrow sense heritability estimates were considered different than zero since variance components of GCA were significant ($p \leq 0.10$).

Narrow sense heritabilities for all traits ranged from 0.04 to 0.93 for the test hybrids (Table 7). Moderate narrow sense heritabilities ($0.40 \leq h^2 \leq 0.80$) were estimated for grain yield, test weight, FGY, and FGA. The highest narrow sense heritabilities (>0.90) were seen for plant height, ear height, days to silking, days to pollen shedding, and number of rows. Narrow sense heritability estimates for plant and ear height, days to silking and pollen shed, and number of kernel rows were similar to Flint-Garcia et al. (2009) results. Our results agreed with Smalley et al. (2004), for grain yield, ear height, and plant height narrow sense heritability estimates. Hallauer et al. (2010) also found the same results for grain yield narrow sense heritability. If selection was performed on traits with a low to moderate heritability estimates, then a slower rate of progress would be expected as compared with a trait of high heritability (Bernardo, 2010).

Broad sense heritability among the test hybrids ranged from 0.23 to 0.96 (Table 7). Results show moderate estimates ($0.40 \leq H^2 \leq 0.80$) of broad sense

heritability for grain yield, test weight, FGY, and FGA. However, Saleh et al. (2002) reported a moderate broad sense heritability for grain yield which is similar to our findings. Results show high estimates of broad sense heritability (>0.90) for plant height, ear height, days to silking, days to pollen shed, number of kernel rows, kernel width, kernel size, and sphericity. Our results agree with Saleh et al. (1994) and Cardinal et al. (2001), who reported a high broad sense heritability estimate for days to pollen shed. Flint-Garcia et al. (2009) reported high broad sense heritabilities estimates for days to pollen and silking, plant and ear height which is consistent with our findings. Generally, when high broad sense heritability estimates are obtained, nongenetic factors have an insignificant impact relative to genetic factors (Bernardo, 2010).

We observed FGY estimates at 0.53 for narrow sense heritability and 0.65 for broad sense heritability (Table 7). Narrow and broad sense heritability was estimated at 0.43 and 0.49 for FGA, respectively. The margin between broad sense and narrow sense heritability for FGY and FGA were small, which indicates that FGY and FGA were controlled mainly by additive genetic variances. For FGA, variance components for GCA and SCA with the environment are significant (P < 0.01) and larger than GCA and SCA variances, suggesting that multiple site testing will be important in breeding for FGA improvement. Furthermore, based on the additive nature of these traits, breeding strategies to improve both parents of prospective new hybrids should be considered.

Test weight has a narrow sense heritability of 0.62 and a broad sense heritability of 0.80, indicating that this trait is moderately controlled by additive gene

action, but is also influenced by non-additive gene action. In contrast, heritabilities for grain yield were estimated at 0.70 for broad sense and 0.43 for narrow sense (Table 4). The difference for grain yield between broad and narrow sense heritability is larger than for any trait other collected, confirming that grain yield is largely subject to non-additive gene action.

General and Specific Combining Ability

The diallel design has been used successfully by quantitative geneticists to gain a better understanding of the nature of gene action involved in quantitative traits (Gardner and Eberhart, 1966). In particular, the diallel design facilitates estimates of GCA and SCA. For test hybrids, variance components were partitioned into GCA and SCA and their interactions with environment (Table 7). Most variance components of FGY, grain yield, test weight, and FGA were significant ($P < 0.1$), exceptions being SCA variance for FGA and GCA with environment for grain yield. Variance estimates can be compared within a trait; however, the absolute values cannot be compared across traits due to the differences in the units of measure. The relative importance of GCA and SCA can be expressed as a ratio of GCA/SCA or interactions of GCA/SCA with environment and these ratios can be compared across traits to assess the relative importance of additive and non-additive gene action.

Variance of GCA for FGY was more than double the SCA variance component, indicating a strong additive component. The magnitude of the variance for interactions of SCA with environment was greater than SCA while GCA with environment was similar to GCA, suggesting that FGY is influenced by non-additive component with the environment interaction. For grain yield, GCA variance was

smaller than the SCA variance indicating the importance of non-additive gene action and supports use of large breeding programs to assemble favorable gene combinations that maximize hybrid grain yield. The variance of GCA with environment for grain yield is not significant, indicating that the environment does not influence expression of the additive component in grain yield. However, the variance of SCA with environment is larger than the main SCA variance, indicating that grain yield is largely influenced by environment. Thus, in a breeding program to improve yield performance, hybrids need to be tested over multiple sites to accurately assess grain yield and the hybrids' interaction with the environment for the expression of non-additive effects. The variance of GCA for test weight was larger than the SCA variance indicating additive gene action. The interaction of both GCA and SCA with the environment was lower than GCA or SCA, respectively, suggesting that the environment does not lead to changes in hybrid rank. Variance for FGA GCA was more than triple the SCA variance component indicating predominantly additive gene action. The interaction of the variance of GCA and SCA with the environment for FGA was substantially higher than the corresponding GCA and SCA variances. Because the environmental interaction was low with FGY, we infer that environmental influences are associated more with the grain yield factor.

Heterosis

Heterosis or hybrid vigor refers to the phenomenon involving hybrid offspring that exhibit phenotypic performance that is superior to the mean performance of the corresponding inbred parents (Falconer and Mackay, 1996). Effects of heterosis on trait expression are important to understand so that any positive effects can be

exploited in hybrid development. The hypotheses for the genetic causes of heterosis are dominance, overdominance, and epistasis (Reif et al., 2005).

Heterosis was estimated and found to be significant ($p < 0.01$) for all traits except FGY, kernel depth, and sphericity (Table 3). A negative heterosis value was calculated for FGY, indicating heterosis will not be useful when breeding for FGY. This finding reflects that, on average, the parental inbreds had higher FGY than test hybrids which was observed (Table 3). Heterosis was a large factor in grain yield. Munaro et al. (2011) reported that grain yield had high level of heterosis compared to other traits assessed in their study. Heterosis has been extensively studied in maize because of its substantial expression in grain yield (Reif et al., 2005). Test weight displayed a very low amount of heterosis whereas FGA displayed a moderate amount heterosis. Cob size and ear size are derived traits which could explain the large heterosis values relative to other traits. Standard errors were low except for cob size, ear size, and kernel size which were derived traits. Results suggest that FGY is not significantly influenced by dominance, overdominance, or epistatic effects. However, grain yield and test weight are. Furthermore, heterosis is a factor in FGA, apparently due to the influence of grain yield in this trait.

Correlations of FGY with Agronomic Performance and Yield Components

Phenotypic Correlations

Pearson correlation coefficients were calculated to assess the phenotypic relationship between each trait collected with grain yield, test weight, FGY, and FGA (Table 8). Performance for FGY was negatively correlated ($p < 0.01$) with grain yield ($r = -0.50$) and positively correlated with test weight ($r = 0.52$); however, in both

cases, the relationships were not strong. Most importantly, this provides the first

insight to the relationship between FGY and grain yield.   Pan et al. (1996) reported

that FGY was highly correlated with test weight (r = 0.91). Dorsey-Redding et al.

(1991) suggested that test weight is not a precise indicator of any specific grain quality

trait. Blandino et al. (2010) reported that test weight was significantly correlated with

FGY (r = 0.84) using thirteen commercial hybrids cultivated in Northern Italy.   Lee et

al. (2007) found FGY to be significantly correlated (P < .01) with test weight (r =

0.42) after evaluating 114 commercial hybrids grown in the U.S. corn belt. Other

correlations between performances for traits and FGY ranged from $-0.65 \leq r_p \leq 0.44$

(Table 8) for days to silking and FGA, respectively. In addition, FGY was

significantly (P < 0.01) correlated with kernel width, kernel length, kernel depth,

sphericity, kernels per row, ear width, cob width, cob size but all had weak

relationships (r < 0.50). For example, the correlation between FGY and kernel length

was r = -0.22 (Table 8); thus, the R^2 value is 0.05, revealing that 95% of the variation

was unexplained.

Trait correlations can be valuable and exploited for trait improvement by

indirect selection for a correlated trait (Chen and Lübberstedt, 2010). However,

selections that only focus on one trait may actually have negative effects on other

traits of interest. For example, the negative relationship between FGY and grain yield

could result in reduced agronomic performance if selection was only for FGY.

However, Duarte et al. (2005) reported that since correlations were low (r ≤ 0.30)

among grain yield and grain quality parameters, the author suggests that high grain

yield and good grain quality can be produced simultaneously.

Genetic Correlations

Additive genetic correlations were calculated to assess the genetic relationship among grain yield, test weight, FGY, and FGA (Table 9). Genetic correlations can arise from two causes, pleiotropy and linkage. Pleiotropy occurs when one gene influences phenotypic expression of multiple traits. Linkage occurs when genes for one trait are co-located on a chromosome (i.e. closely linked) with genes influencing the other trait. Both sets tend to be inherited together and are not easily separated. The genetic correlation between grain yield and FGY was estimated at r = -0.43. This finding suggests that simultaneous improvement of both grain yield and FGY may be difficult because selection for favorable genes for one trait may have a negative impact for the other trait. This result is taken with caution though as genetic correlations are comprised of three estimated variance components and standard errors associated with genetic correlations are very large (Bernardo, 2010).

Predicting Performance

The trait FGA is a measure of productivity and dry milling value on a 'per acre' basis. However, its measurement requires estimation of FGY, which is laborious and considerably downstream of harvest. We investigated the potential to predict FGA based on variables collected at/near harvest. This work follows the example set in breeding for improved, silage corn. There, a model has been constructed that estimates energy intake of dairy cattle from corn silage that can be used to estimate milk production per acre based on the specific corn silage line in the diet (Schwab et al., 2003). This model is useful for corn breeders developing silage hybrids as it enables ranking of hybrids for estimated milk production. The model used to predict

the production of milk on a per acre basis for corn silage outlined in Schwab et al.

(2003) and reported by Undersander et al. (1993) was:

$$\text{Milk per acre} = \text{formlk} \times \frac{\text{yld}}{\text{DMI}}$$

where

$$\text{formlk} = \big((88.9 - (0.779 \times \text{ADF})) \times 0.0245\big) - 0.12$$

ADF $\quad$ = acid detergent fiber, sample collected at harvest
yld $\quad$ = biomass yield, pound per acre
DMI $\quad$ = dry matter intake, pound per day

This model provides the ability to rapidly collect samples at harvest that can be

scanned for ADF by near infra-red reflectance spectroscopy shortly after harvest to

estimated milk production from dairy cattle.

Results of stepwise regression analysis showed that significant ($p < 0.05$)

independent variables in the selected model for the prediction of FGA were grain

yield, test weight, kernel depth, and 100 kernel volume. The model was constructed

using the first two years of data and validated using the third year of data. The test for

lack of fit was not significant at $p \leq 0.05$, indicating that the model fits and is not

biasing our prediction. However, this model accounts for only 31% of the variability

among the test hybrids for FGA ($p < 0.05$):

$$\text{FGA} = -4190.3 + 11.7\,(\text{grain yield}) + 88.6\,(\text{test weight})$$

$$- 295.8\,(\text{kernel depth}) + 27.8\,(\text{100 kernel volume})$$

A large negative intercept indicates that the variation present among test hybrids is

large resulting in a model that is not able to accurately predict FGA. Adding other

physical grain property variables did not improve the predictability of the model. Any

additional variables in the model actually increased the Mallow C_p's value which indicates a decrease in the fit of the regression model.

The regression analysis was performed to identify a simple, non-destructive method to facilitate indirect selection for FGA that would help breeders, processors, and producers to efficiently identify sources of germplasm suitable for producing flaking grits that are processed for human consumption. Paulsen and Hill (1985) created a prediction equation ($R^2 = 0.82$) for FGY that utilized test weight and breakage susceptibility. Kirleis and Stroshine (1990) found that kernel density was highly correlated ($R^2 = 0.77$) with a Milling Evaluation Factor (MEF). Kernel density and test weight was highly correlated ($R^2 = 0.91$) with a MEF (Kirleis and Stroshine, 1990). The MEF terminology is calculated by combining the amount of endosperm that remains on the 3.5 mesh wire, 5 mesh wire, and 7 mesh wire (Kirleis and Stroshine, 1990). Lee et al. (2007) reported a model to predict FGY that used test weight, protein content, pycnometer density, time to grind in Stenvert Hardness Tester, and kernel size distribution; it explained 52% of the variation for FGY. The models reported by Paulsen and Hill (1985), Kirleis and Stroshine (1990), and Lee et al. (2007) require specialized equipment that can be costly, time consuming, and labor intensive and grain samples that can be destroyed to obtain measurements to predict FGY. Our model was based on physical kernel traits that could rapidly be collected soon after harvest that did not require specialized equipment to predict flaking grit productivity. Furthermore, we sought to predict FGA rather than FGY. Unfortunately, physical kernel characteristics alone will not provide enough detail to accurately predict the yield of FGA. A better assessment of kernel quality is needed.

There is a need to explore methods involving simple and easy-to-use techniques to identify hybrids best suited for FGA. Ideally, the methods would enable quick turnaround for prompt decision making so that the breeder can make line selections in time for winter nursery. We explored the relationship between FGY and agronomic performance which has not previous been investigated. Comparing many of the dry milling traits was not within the scope of this project.

The FGA model provides the first insight to improving both grain yield and FGY simultaneously. Based on finding of Kirleis and Stroshine (1990) and Paulsen and Hill (1985), we suggest that including breakage susceptibility and/or kernel density in the regression model may improve the prediction of FGA. As better grain quality predictor variables are identified the model can be revised to accommodate the variables and improve the prediction of FGA.

Breeding Strategies

Breeding corn hybrids for improved FGA requires a process that can be implemented effectively and efficiently to maximize response to selection and accelerate market launch. Breeding strategies will focus on increasing FGA as both grain yield and FGY need to be increased simultaneously. A selection index could be used that included FGA and also large-effect quantitative trait loci (QTL) for FGY if identified. Molecular markers could be used to guide selection and to break the negative relationship between grain yield and FGY, that is, select for chromosomal regions that positively influence one trait and not negatively impact the other. Thus, agronomic performance and dry milling yield could be increased simultaneously. In

addition, doubled haploid technology could be used to speed development of inbred lines.

A suitable breeding population could be created using the inbred parents from top yielding hybrids for FGA. From this study, B73 and PHJ40 as well as LH82 and PHG84 would be suitable parents to create female and male breeding populations, respectively. It is important to note that improvement for FGA needs to be improved on both the SSS and NSS heterotic patterns, since additive gene action has been associated with FGA. Improving both parents for FGA will result in hybrids with improved FGA. Intra-pool crosses (B73×PHJ40 or LH82×PHG84) could be made to create F2 families from which recombinant inbred lines (RILs) could be produced through pedigree-selection, single seed descent (as outlined in Sleper and Poehlman (2006)), or doubled haploid technology. After the creation of the RILs, interpool (SSS×NSS) crosses could be performed to generate testcross hybrids which could be grown in multiple environments to measure agronomic performance and produce grain to measure FGA. QTL mapping on the testcross hybrids could be used to identify chromosomal regions associated with performance for FGY or FGA and/or other traits. Selections could be made among the RILs or another cycle of selection could be initiated to make further genetic gains. The aim would be the development of inbreds with improved FGA and agronomic performance.

CONCLUSIONS

This survey of U.S. germplasm indicated that genetic diversity is present for FGY which could be exploited to improve performance for this trait in new corn hybrids. Heterosis was not present for FGY. This was supported by the small differential between narrow sense heritability (0.53) and broad sense heritability (0.65), suggesting that FGY is primarily controlled by additive gene action. Furthermore, variance component estimates suggest GCA is more prominent than SCA. FGY has a negative phenotypic correlation with grain yield and positive phenotypic correlation with test weight. In addition, a negative genetic correlation was observed between grain yield and FGY, suggesting linkage or pleiotrophy exists between these traits. Performance for FGA may be predicted to some extent based on simple physical kernel characteristics such as test weight, kernel depth, 100 kernel volume along with grain yield, which together explained 31% of the variation for FGA. However, further work is needed to improve the predictability of FGA. A next step would be to evaluate other kernel characteristics such as kernel density and breakage susceptibility as possibilities to obtain better predictability of FGA.

REFERENCES

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. The Association: St. Paul, MN.

Bernardo, R. 2010. Breeding for quantitative traits in plants. 2 ed. Stemma Press, Woodbury, MN.

Blandino, M., M.C. Mancini, A. Peila, L. Rolle, F. Vanara, and A. Reyneri. 2010. Determination of maize kernel hardness: comparison of different laboratory tests to predict dry-milling performance. J. Sci. Food Agric. 90:1870-1878.

Butcher, J., and N.L. Stenvert. 1973. Conditioning studies on Australian wheat. I. The effect of conditioning on milling behaviour. J. Sci. Food Agric. 24:1055-1066.

Caldwell, E.F., R.B. Fast, and J.M. Faubion. 2000. Manufacturing technology of ready-to-eat cereals, in: R. B. Fast and E. F. Caldwell (Eds.), Breakfast cereals and how they are made, American Association of Cereal Chemists, Inc., St. Paul, MN. pp. 19.

Cardinal, A.J., M. Lee, N. Sharopova, W.L. Woodman-Clikeman, and M.J. Long. 2001. Genetic mapping and analysis of quantitative trait loci for resistance to stalk tunneling by the European corn borer in maize. Crop Sci. 41:835-845.

Chen, Y., and T. Lübberstedt. 2010. Molecular basis of trait correlations. Trends Plant Sci. 15:454-461.

Dombrink-Kurtzman, M.A., and C.A. Knutson. 1997. A study of maize endosperm hardness in relation to amylose content and susceptibility to damage. Cereal Chem. 74:776-780.

Dorsey-Redding, C., C.R. Hurburgh, L.A. Johnson, and S.R. Fox. 1991. Relationships among maize quality factors. Cereal Chem. 68:602-605.

Duarte, A.P., S.C. Mason, D.S. Jackson, and J. de C. Kiehl. 2005. Grain quality of Brazilian maize genotypes as influenced by nitrogen level. Crop Sci. 45:1958-1964.

Duvick, D.N., and K.G. Cassman. 1999. Post–green revolution trends in yield potential of temperate maize in the North-Central United States. Crop Sci. 39:1622-1630.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. 4 ed. Longman, Essex, England.

Farmers Feed US. 2012. Indiana corn farmer. www.farmersfeedus.org/in/corn/10.

Flint-Garcia, S.A., A.L. Bodnar, and M.P. Scott. 2009. Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. Theor. Appl. Genet. 119:1129-1142.

Gardner, C.O., and S.A. Eberhart. 1966. Analysis and interpretation of the variety cross diallel and related populations. Biometrics 22:439-452.

Hallauer, A.R., M.J. Carena, and J.B. Miranda Filho. 2010. Selection: experimental results, in: J. Prohens, et al. (Eds.), Quantitative genetics in maize breeding, Springer, New York, NY. pp. 367.

Isik, F. 2009. FOR 728: quantitative forest genetics methods. http://www4.ncsu.edu/~fisik/FOR728LectureNotes9%20-%20Genetic%20Correlations.pdf.

Jackson, D.S., L.W. Rooney, O.R. Kunze, and R.D. Waniska. 1988. Alkaline processing properties of stress-cracked and broken corn (Zea mays L.). Cereal Chem. 65:133-137.

Kirleis, A.W., and R.L. Stroshine. 1990. Effects of hardness and drying air temperature on breakage susceptibility and dry-milling characteristics of yellow dent corn. Cereal Chem. 67:523-528.

Kutner, M.H., C.J. Nachtsheim, and J. Neter. 2004. Applied linear regression models, McGraw-Hill/Irwin, New York, NK.

Lee, K.-M., T.J. Herrman, S.R. Bean, D.S. Jackson, and J. Lingenfelser. 2007. Classification of dry-milled maize grit yield groups using quadratic discriminant analysis and decision tree algorithm. Cereal Chem. 84:152-161.

Lee, K.-M., T.J. Herrman, J. Lingenfelser, and D.S. Jackson. 2005. Classification and prediction of maize hardness-associated properties using multivariate statistical analyses. J. Cereal Sci. 41:85-93.

Litchfield, J.B., and G.C. Shove. 1990. Dry milling of U.S. hard-endosperm corn in Japan: product yield and corn properties. Appl. Eng. Agric. 6:629-634.

Mehra, S.K., and S.R. Eckhoff. 1997. Single-stage short-duration tempering of corn for dry-milling. Cereal Chem. 74:484-488.

Mestres, C., and F. Matencio. 1996. Biochemical basis of kernel milling characteristics and endosperm vitreousness of maize. J. Cereal Sci. 24:283-290.

Mikel, M.A., and J.W. Dudley. 2006. Evolution of North American dent corn from public to proprietary germplasm. Crop Sci. 46:1193-1205.

Munaro, E.M., K.E. D'Andrea, M.E. Otegui, A.G. Cirilo, and G.H. Eyhérabide. 2011. Heterotic response for grain yield and ecophysiological related traits to nitrogen availability in maize. Crop Sci. 51:1172-1187.

Narváez-González, E.D., J. de Dios Figueroa-Cárdenas, S. Taba, E.C. Tostado, R.Á.M. Peniche, and F.R. Sánchez. 2006. Relationships between the microstructure, physical features, and chemical composition of different maize accessions from Latin America. Cereal Chem. 83:595-604.

Pan, Z., S.R. Eckhoff, M.R. Paulsen, and J.B. Litchfield. 1996. Physical properties and dry-milling characteristics of six selected high-oil maize hybrids. Cereal Chem. 73:517-520.

Paulsen, M.R., and L.D. Hill. 1985. Corn quality factors affecting dry milling performance. J. Agric. Eng. Res. 31:255-263.

Philippeau, C., J. Landry, and B. Michalet-Doreau. 2000. Influence of the protein distribution of maize endosperm on ruminal starch degradability. J. Sci. Food Agric. 80:404-408.

Pomeranz, Y., and Z. Czuchajowska. 1987. Laboratory tests to predict the commerical yield of flaking or large grits in dry corn milling. J. Food Sci. 52:830.

Pomeranz, Y., C.R. Martin, D.D. Traylor, and F.S. Lai. 1984. Corn hardness determination. Cereal Chem. 61:147-150.

Rausch, K.D., L.E. Pruiett, P. Wang, L. Xu, R.L. Belyea, and M.E. Tumbleson. 2009. Laboratory measurement of yield and composition of dry-milled corn fractions using a shortened, single-stage tempering procedure. Cereal Chem. 86:434-438.

Reif, J.C., A.R. Hallauer, and A.E. Melchinger. 2005. Heterosis and heterotic patterns in maize. Maydica 50:215-223.

Robutti, J., F. Borras, M. Ferrer, M. Percibaldi, and C.A. Knutson. 2000. Evaluation of quality factors in Argentine maize races. Cereal Chem. 77:24-26.

Robutti, J.L., R.C. Hoseney, and C.E. Wassom. 1974. Modified opaque-2 corn endosperms. II. Structure viewed with scanning electron microscope. Cereal Chem. 51:173-180.

Saleh, G., M.R. Yusop, and Y.T. Chai. 1994. Heritability and response to recurrent selection in two sweet corn varieties. Pertanika J. Tropical Agric. Sci. 17:185-190.

Saleh, G.B., D. Abdullah, and A.R. Anuar. 2002. Performance, heterosis and heritability in selected tropical maize single, double and three-way cross hybrids. J. Agric. Sci. 138:21-28.

SAS Institute. 2008. The SAS system for Windows: Version 9.2. SAS Inst., Cary, NC.

Schwab, E.C., R.D. Shaver, J.G. Lauer, and J.G. Coors. 2003. Estimating silage energy value and milk yield to rank corn hybrids. Anim. Feed Sci. Technol. 109:1-18.

Seebauer, J.R., G.W. Singletary, P.M. Krumpelman, M.L. Ruffo, and F.E. Below. 2010. Relationship of source and sink in determining kernel composition of maize. J. Exp. Bot. 61:511-519.

Sleper, D.A., and J.M. Poehlman. 2006. Breeding self-pollinated crops, Breeding field crops Blackwell publishing, Ames, IA. pp. 141-146.

Smalley, M.D., J.L. Daub, and A.R. Hallauer. 2004. Estimation of heritability in maize by parent-offspring regression. Maydica 49:221-229.

Tester, R.F., J. Karkalas, and X. Qi. 2004. Starch—composition, fine structure and architecture. J. Cereal Sci. 39:151-165.

Undersander, D.J., W.T. Howard, and R.D. Shaver. 1993. Milk per acre spreadsheet for combining yield and quality into a single term. J. Prod. Agric 6:231-235.

USDA Economic Research Service. 2012. Yearbook Tables. www.ers.usda.gov.

Wehling, R.L., D.S. Jackson, and B.R. Hamaker. 1996. Prediction of corn dry-milling quality by near-infrared spectroscopy. Cereal Chem. 73:543-546.

Whittaker, D., E.R. Williams, and J.A. John. 1999. CycDesign: A package for the computer generation of experimental designs, Version 1.2. CSIRO Forestry and Forest Products, and The University of Waikato, Canberra and Hamilton. Canberra, Australia.

Wolf, M.J., C.L. Buzan, M.M. MacMasters, and C.E. Rist. 1952. Structure of the mature corn kernel. I. Gross anatomy and structural relationships. Cereal Chem. 29:321-333.

Yuan, J., and R.A. Flores. 1996. Laboratory dry-milling performance of white corn: effect of physical and chemical corn characteristics. Cereal Chem. 73:574-578.

FIGURES

Figure 1. Major components of a corn kernel: endosperm, germ, and bran as well as tip cap.

Figure 2. Five groups with interests in the complex "corn cereal pipeline".

- Seed Companies
  - Increased grain yield
  - Improved agronomic performance

- Farmers
  - Maximized grain yield
  - Agronomic performance

- Dry Millers
  - Superior flaking grit yield
  - Low fat flaking grits

- Food Companies
  - Quality of food products
  - Added nutritional value
  - Consumer health

- Consumers
  - Good valued product
  - Nutritionally healthy product
  - Product quality
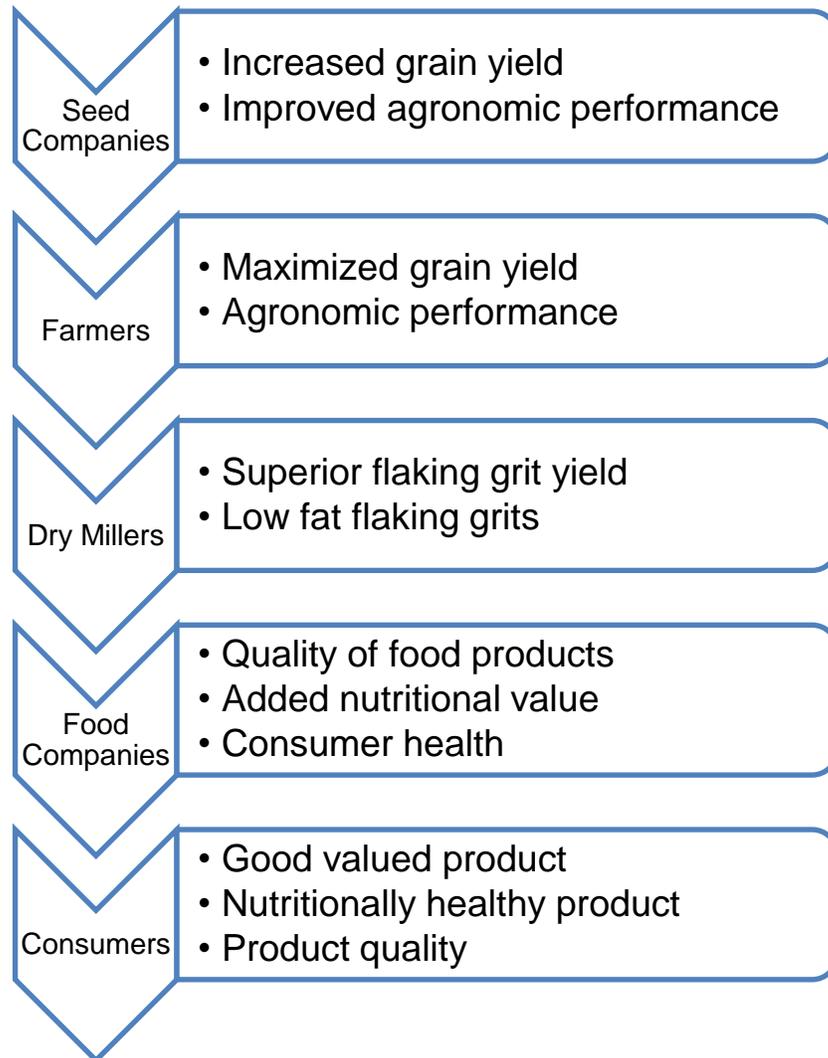
Figure 3. Small scale dry milling procedure adapted from Rausch et al. (2009).
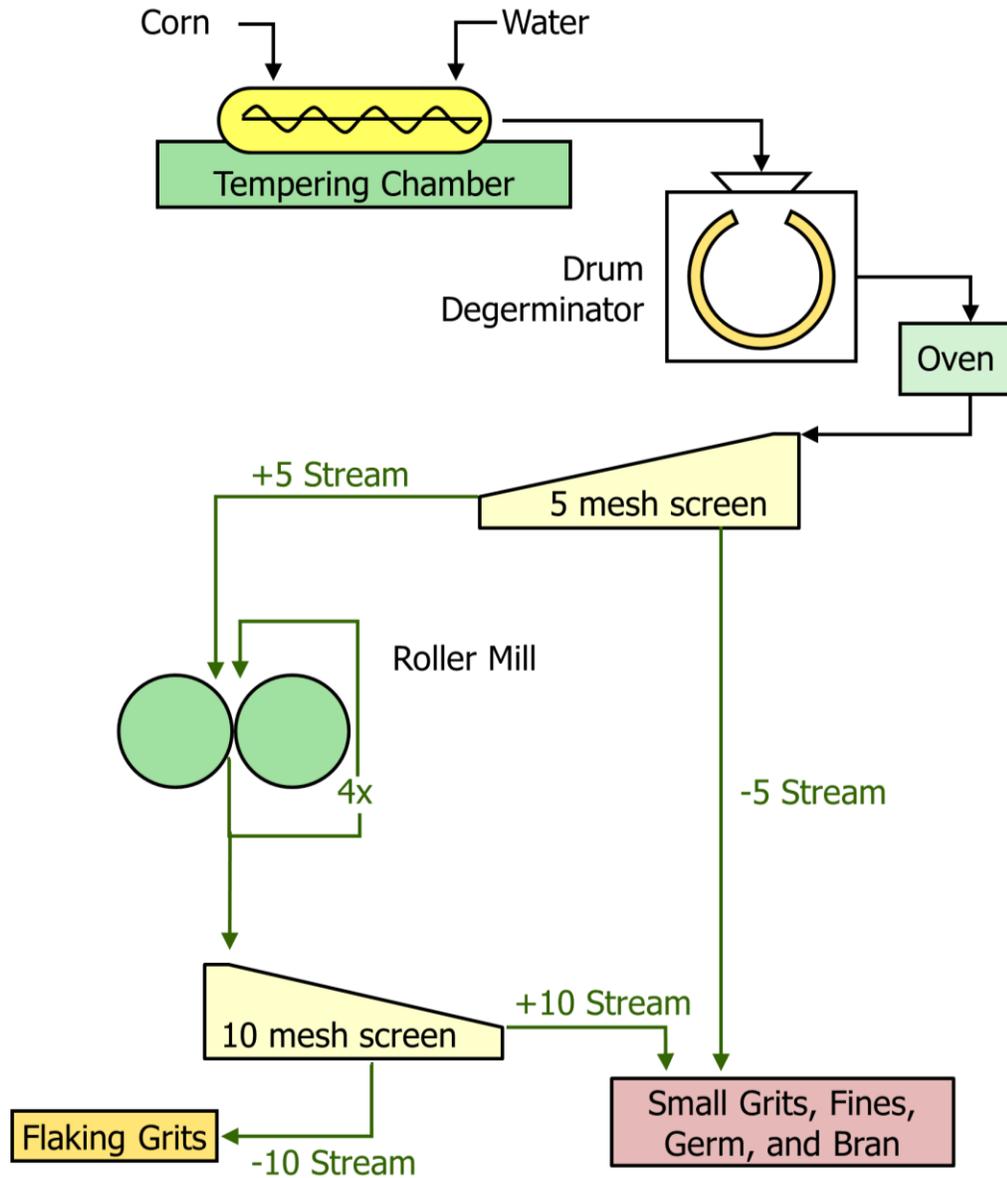
TABLES

Table 1. Descriptions of the 12 inbreds which served as parents of evaluated hybrids. F and M groups represent the female and male heterotic groups, respectively, utilized in developing corn hybrids in the U.S.

Line	Group	Assignee	Background
B73	F	None (Public)	Iowa Stiff Stalk Synthetic
LH1	F	Holden Foundation Seeds	Iowa Stiff Stalk Synthetic; B37 type
PHG39	F	Pioneer Hi-Bred International	Maiz Amargo/Iowa Stiff Stalk Synthetic; B37/B14 type
PHJ40	F	Pioneer Hi-Bred International	Iowa Stiff Stalk Synthetic
LH123HT	M	Holden Foundation Seeds	Pioneer Hybrid 3535
LH82	M	Holden Foundation Seeds	Krug /W153
Mo17	M	None (Public)	Lancaster
PH207	M	Pioneer Hi-Bred International	Iodent/Long Ear OPV/Minn13
PHG35	M	Pioneer Hi-Bred International	Oh07-Midland/Iodent/Linstrom Long Ear/Minn13
PHG47	M	Pioneer Hi-Bred International	Oh43/Iodent*Wf9/MKSDTA C10 Synthetic
PHG84	M	Pioneer Hi-Bred International	Oh07-Midland/Minn13/Iodent/Reid YD/Osterland YD/Lancaster/Pioneer Female Composite OPV
PHZ51	M	Pioneer Hi-Bred International	Minn13/Iodent/Reid YD/Osterland YD/Lancaster/South US Land Race Synthetic/Funks G4949/ Midland

Table 2. Trait class, description, and unit of measure for each trait collected and evaluated.

Class	Trait	Description
Agronomic	Seedling vigor	Rating with scale 1 to 10, 10 = best, 1 worst
	Plant height	Distance from ground to node with flag leaf
	Ear height	Distance from ground to node with ear
	Days to silking	Days from planting to 50% silk emergence
	Days to pollen shed	Days from planting to 50% pollen shed
	Anthesis silking interval	Days to pollen minus days to silk
	Staygreen	Rating scale 1 to 10, 10 = 100% green, 1=10% green
	Percent barren plants	Percent of plants in the plot without an ear
	Grain yield	Weight of harvested grain per acre, adjusted to 15.5% moisture
Dry milling	Test weight	Weight of one bushel volume of grain; related to grain quality
	Flaking grit yield	Total flaking grit yield
	Flaking grits per acre	Grain yield × flaking grit yield
Ear	Cob length	Length of the entire ear
	Cob width	Width of cob at median length
	Cob size	π × cob width × cob length
	Fill length	Length of the ear with kernels
	Ear width	Width of ear at median length
	Ear size	π × ear width × fill length
	Rows	Number of kernel rows
	Kernels per row	Number of kernels per row
	Hundred kernel volume	Volume of 100 kernels
	Hundred kernel weight	Weight of 100 kernels
Kernel	Kernel width	Mean distance across kernel; average of 10 kernels; 2 replications
	Kernel length	Mean distance from kernel tip cap to crown; average of 10 kernels; 2 replications
	Kernel depth	Mean distance across the side; average of 10 kernels; 2 replications
	Kernel size	Kernel length × kernel width × kernel depth; 2 replications
	Sphericity	(kernel size)^(1/3))/kernel length; range from 0 to 1, 0 = flat 1 = round

Table 3. Overall mean, standard deviation, and range for the 66 test hybrids, 6 commercial check hybrids, 12 parental inbreds, as well as the level of heterosis and heterosis SE for each trait.

Trait	Unit	Test hybrids (N=66)				Commercial check hybrids (N=6)			
		Mean	Std	Min	Max	Mean	Std	Min	Max
Seedling vigor	rating	5.2	0.4	3.8	6.2	5.3	0.4	4.9	6.0
Plant height	cm	224.6	21.0	175.0	268.9	233.4	9.0	223.0	247.2
Ear height	cm	105.8	12.1	78.3	132.8	105.2	5.7	95.4	113.0
Days to silking	days	64.2	2.5	57.9	68.7	63.0	1.1	61.9	64.4
Days to pollen shed	days	63.2	2.3	57.6	67.0	62.9	1.1	61.6	64.0
Anthesis silking interval	days	-0.9	0.6	-2.6	0.2	-0.1	0.2	-0.4	0.1
Staygreen	rating	3.0	1.1	1.1	5.8	4.0	0.5	3.3	4.8
Percent barren plants	%	1.3	0.9	0.2	4.6	0.5	0.3	0.2	0.9
Grain yield	bu/ac	171.1	13.6	135.7	199.3	215.3	4.1	211.7	222.8
Test weight	lb/bu	57.1	1.1	54.6	59.3	57.0	1.8	54.7	59.0
Flaking grit yield	g/100g db	29.4	2.6	24.0	36.0	27.3	2.8	24.5	31.4
Flaking grits per acre	lbs/ac	2768.6	289.1	2155.5	3450.0	3222.7	332.7	2841.5	3702.2
Cob length	mm	172.1	11.0	150.1	205.0	175.3	10.4	162.2	185.5
Cob width	mm	27.3	1.0	24.5	29.2	27.6	0.5	27.1	28.4
Cob size	mm*3	14772.8	833.6	12927.6	16744.9	15196.7	746.7	13991.2	15912.3
Fill length	mm	153.9	11.9	132.8	190.4	162.3	10.4	146.7	171.7
Ear width	mm	43.8	1.8	39.4	47.3	45.5	1.1	43.7	46.8
Ear size	mm*3	21186.0	1367.7	18276.4	24698.7	23239.0	1260.1	21459.5	24361.9
Rows	count	15.2	1.6	11.7	19.0	15.9	0.9	14.7	17.2
Kernel per row	count	34.6	2.4	29.5	40.8	36.2	2.4	33.2	39.7
Hundred kernel volume	mL	40.7	3.7	31.5	49.7	44.1	3.0	40.7	48.9
Hundred kernel weight	g	28.3	2.8	21.6	34.5	30.5	1.6	28.6	33.1
Kernel width	mm	7.9	0.5	6.7	9.2	7.8	0.3	7.6	8.2
Kernel length	mm	11.7	0.5	10.7	12.8	12.6	0.6	12.0	13.5
Kernel depth	mm	4.3	0.2	3.9	4.7	4.3	0.2	4.0	4.6
Kernel size	mm*3	394.2	38.7	297.7	482.2	425.8	31.2	401.1	482.4
Sphericity		0.6	0.0	0.6	0.7	0.6	0.0	0.6	0.6

Table 3. Continued.

Trait	Unit	Parental inbreds (N=12)				Heterosis	Heterosis SE
		Mean	Std	Min	Max		
Seedling vigor	rating	4.6	0.7	2.9	5.7	0.6†	0.09
Plant height	cm	161.4	30.0	123.8	206.9	63.2†	1.47
Ear height	cm	73.6	15.0	46.1	93.1	32.2†	1.05
Days to silking	days	69.3	5.1	60.0	75.9	5.1†	0.18
Days to pollen shed	days	66.9	4.4	58.4	72.4	3.7†	0.12
Anthesis silking interval	days	-2.4	1.3	-4.7	-0.8	1.4†	0.02
Staygreen	rating	2.1	1.6	0.7	5.3	0.9†	0.12
Percent barren plants	%	3.9	3.3	0.2	9.4	2.6†	0.00
Grain yield	bu/ac	66.9	18.0	37.4	98.8	104.2†	3.88
Test weight	lb/bu	56.7	1.7	52.4	59.6	0.4†	0.06
Flaking grit yield	g/100g db	31.9	3.9	24.3	39.2	-2.5	0.41
Flaking grits per acre	lbs/ac	1170.4	423.3	637.6	2157.8	28.5†	1.43
Cob length	mm	135.8	14.6	114.4	165.7	36.3†	1.16
Cob width	mm	26.1	1.8	21.6	28.9	1.2†	0.07
Cob size	mm^3	11130.0	942.8	9201.3	12590.4	3628.4†	107.86
Fill length	mm	120.2	12.7	97.6	148.1	33.6†	1.48
Ear width	mm	38.2	2.4	34.7	42.9	5.5†	0.30
Ear size	mm^3	14429.3	1326.6	12657.8	16510.0	6736.2†	325.61
Rows	count	14.1	2.8	10.1	21.2	1.1†	0.05
Kernel per row	count	23.0	2.4	18.2	27.0	11.5†	0.00
Hundred kernel volume	mL	34.7	5.4	26.1	45.0	6.0†	0.59
Hundred kernel weight	g	24.0	4.0	18.0	31.6	4.3†	0.47
Kernel width	mm	7.5	0.6	6.5	8.4	0.4†	0.06
Kernel length	mm	9.8	0.7	9.0	10.7	2.0†	0.03
Kernel depth	mm	4.7	0.5	4.0	6.0	-0.05	0.03
Kernel size	mm^3	348.1	50.9	268.9	433.3	46.0†	5.19
Sphericity		0.7	0.1	0.6	0.8	-0.1	0.00

† Traits significant at p < 0.01

Table 4. Hybrid and inbred means for flaking grit yield (FGY) and grain yield in rank order.

Rank	Genotype	Flaking grit yield (FGY) (g/100g db)	Genotype	Grain yield (bu/ac)
1	PHJ40×LH123HT	36.0	Check4	222.8
2	PHJ40×PHG84	34.7	Check2	216.5
3	PHJ40×LH82	34.5	Check3	214.4
4	B73×PHG84	34.0	Check1	214.1
5	LH82×PHG35	33.2	Check6	212.1
6	LH82×PHZ51	32.3	Check5	211.7
7	PH207×PHG84	32.1	B73×PHG47	199.3
8	LH82×PHG84	31.9	PHG39×MO17	197.9
9	LH82×PH207	31.8	PHG39×PHG47	197.5
10	PHG84×PHZ51	31.8	PHG39×PHZ51	194.0
11	LH123HT×LH82	31.6	B73×MO17	192.0
12	PHJ40×MO17	31.5	B73×LH82	189.1
13	PHJ40×PHG35	31.5	MO17×PHG47	188.8
14	Check1	31.4	LH82×PHG47	187.9
15	LH123HT×PHG84	31.3	B73×LH123HT	186.5
16	PHG35×PHG84	31.1	B73×PHG39	185.1
17	LH1×LH82	31.0	PHG39×PHG84	184.2
18	LH123HT×MO17	30.9	PHG39×LH82	183.6
19	B73×PHJ40	30.9	LH123HT×LH82	183.4
20	PHG39×PHJ40	30.8	LH1×LH82	182.8
21	PHG39×LH82	30.8	PHG47×PHZ51	182.6
22	PHJ40×PHZ51	30.7	B73×PHG84	182.5
23	B73×LH123HT	30.6	PHG39×LH123HT	181.8
24	LH82×PHG47	30.5	PHG39×PHJ40	178.4
25	B73×PHG35	30.4	PHG47×PHG84	178.3
26	LH123HT×PHG35	30.3	MO17×PHG84	178.3
27	B73×LH82	30.3	LH82×PHZ51	177.2
28	LH82×MO17	30.1	PHG35×PHG47	175.3
29	PHJ40×PHG47	30.1	LH82×MO17	175.1
30	MO17×PHG84	30.0	MO17×PH207	175.0
31	PHJ40×PH207	30.0	LH123HT×PH207	174.7
32	LH123HT×PHZ51	30.0	LH123HT×PHG84	174.1
33	LH123HT×PHG47	30.0	LH123HT×PHG35	173.5
34	PH207×PHG35	29.9	PHJ40×PHG47	173.3
35	LH1×PHG35	29.7	B73×PHG35	172.8
36	PHG35×PHZ51	29.6	PH207×PHG47	172.8

Table 4. Continued.

Rank	Genotype	Flaking grit yield (FGY) (g/100g db)	Genotype	Grain yield (bu/ac)
37	B73×PHG39	29.5	B73×PHZ51	172.4
38	B73×PH207	29.5	PHG35×PHG84	171.8
39	LH1×PHJ40	29.4	LH123HT×PHZ51	170.4
40	PHG47×PHG84	29.3	LH123HT×PHG47	170.1
41	Check2	29.3	MO17×PHG35	169.4
42	LH123HT×PH207	29.1	PHJ40×LH82	169.4
43	LH1×PHG39	29.1	LH1×PHZ51	169.2
44	PHG39×PHG84	29.0	LH1×MO17	169.0
45	PHG39×PHG35	28.6	PH207×PHG84	168.9
46	MO17×PHZ51	28.5	B73×PHJ40	168.8
47	LH1×PHG84	28.3	PHJ40×PHG84	168.6
48	Check5	28.3	LH82×PHG35	168.5
49	B73×LH1	28.3	PHG39×PH207	168.4
50	PH207×PHZ51	28.2	PHG35×PHZ51	166.7
51	MO17×PH207	28.0	LH1×LH123HT	165.8
52	PHG39×LH123HT	27.9	LH1×PHG47	165.0
53	LH1×PHZ51	27.7	LH82×PHG84	165.0
54	B73×PHZ51	27.7	PHJ40×PH207	164.6
55	PHG39×PH207	27.6	LH1×PHG84	164.4
56	MO17×PHG35	27.3	LH1×PHJ40	163.9
57	PHG39×MO17	27.3	MO17×PHZ51	163.1
58	LH1×PH207	26.9	LH82×PH207	163.1
59	PHG35×PHG47	26.5	LH123HT×MO17	161.4
60	PHG39×PHG47	26.4	PHJ40×MO17	160.4
61	LH1×LH123HT	26.2	PH207×PHZ51	158.8
62	PHG47×PHZ51	26.0	PHJ40×PHZ51	157.5
63	Check6	25.6	PHG39×PHG35	157.2
64	LH1×MO17	25.2	PHJ40×LH123HT	155.8
65	PH207×PHG47	25.0	PH207×PHG35	155.6
66	B73×PHG47	25.0	LH1×PH207	154.1
67	PHG39×PHZ51	25.0	B73×LH1	151.9
68	Check4	24.7	B73×PH207	146.8
69	Check3	24.5	LH1×PHG35	146.1
70	B73×MO17	24.1	PHJ40×PHG35	144.9
71	MO17×PHG47	24.0	LH1×PHG39	141.9
72	LH1×PHG47	24.0	PHG84×PHZ51	135.7

Table 4. Continued.

Rank	Genotype	Flaking grit yield (FGY) (g/100g db)	Genotype	Grain yield (bu/ac)
P1	LH82	39.2	LH82	98.8
P2	PHG35	35.6	LH123HT	80.9
P3	PHJ40	34.3	B73	79.4
P4	MO17	33.8	MO17	76.4
P5	PHG39	33.0	PH207	74.9
P6	LH123HT	32.4	PHG35	74.1
P7	LH1	32.0	PHJ40	68.8
P8	PHG84	31.9	PHG47	64.5
P9	B73	29.4	PHZ51	57.1
P10	PHG47	29.1	LH1	51.8
P11	PHZ51	27.4	PHG84	38.7
P12	PH207	24.3	PHG39	37.4

Table 5. Hybrid and inbred means for flaking grits per acre (FGA) and test weight in rank order.

Rank	Genotype	Flaking grits per acre (FGA) (lbs/ac)	Genotype	Test weight (lb/bu)
1	Check1	3702.2	PHJ40×PHG84	59.3
2	Check2	3479.3	B73×PHG39	59.3
3	B73×PHG84	3450.2	PHJ40×LH123HT	59.0
4	Check5	3319.1	Check1	59.0
5	PHJ40×LH82	3265.4	PHG39×LH82	58.9
6	PHJ40×PHG84	3249.7	PHG39×LH123HT	58.8
7	LH123HT×LH82	3209.4	B73×LH1	58.7
8	LH82×PHG47	3186.4	Check2	58.7
9	B73×LH82	3180.8	PHG39×PHJ40	58.7
10	LH1×LH82	3174.1	B73×PHG84	58.5
11	LH82×PHZ51	3161.8	PHJ40×PHZ51	58.4
12	B73×LH123HT	3150.0	PHG39×PHG84	58.4
13	LH82×PHG35	3135.4	LH1×PHG39	58.4
14	PHG39×LH82	3120.9	LH1×PHG84	58.3
15	PHJ40×LH123HT	3089.5	PHG39×MO17	58.2
16	Check4	3022.9	PHG39×PH207	58.1
17	PHG39×PHJ40	3016.2	PHJ40×PHG35	58.0
18	PH207×PHG84	2993.2	PH207×PHG84	57.9
19	B73×PHG39	2984.8	PHG39×PHG47	57.9
20	LH123HT×PHG84	2975.8	PHG35×PHG84	57.9
21	Check6	2971.4	LH1×LH82	57.8
22	PHG39×PHG84	2962.4	Check5	57.8
23	PHG39×MO17	2960.2	LH1×MO17	57.7
24	MO17×PHG84	2928.2	PHJ40×LH82	57.7
25	B73×PHJ40	2921.5	PHG39×PHG35	57.7
26	B73×PHG35	2915.9	LH1×LH123HT	57.6
27	LH82×PH207	2903.0	LH1×PHZ51	57.6
28	LH82×PHG84	2898.6	LH1×PHJ40	57.6
29	LH82×MO17	2898.0	B73×LH123HT	57.6
30	PHG47×PHG84	2892.4	PHG39×PHZ51	57.5
31	LH123HT×PHG35	2864.4	PHG84×PHZ51	57.4
32	PHJ40×PHG47	2860.5	LH1×PH207	57.4
33	LH123HT×PHZ51	2844.8	MO17×PHG84	57.3
34	Check3	2841.4	B73×PHJ40	57.3

Table 5. Continued.

Rank	Genotype	Flaking grits per acre (FGA) (lbs/ac)	Genotype	Test weight (lb/bu)
35	PHG35×PHG84	2835.8	LH123HT×LH82	57.2
36	PHG39×PHG47	2831.4	LH82×PHG84	57.2
37	LH123HT×PHG47	2815.7	B73×PHG35	57.2
38	PHJ40×MO17	2791.0	LH123HT×PHG84	57.1
39	LH123HT×PH207	2789.4	LH123HT×PHG47	57.1
40	PHG39×LH123HT	2764.7	LH1×PHG35	57.0
41	PHJ40×PH207	2761.4	LH123HT×PHZ51	56.9
42	PHG35×PHZ51	2754.1	LH82×PHZ51	56.8
43	B73×PHG47	2751.3	LH123HT×MO17	56.8
44	LH123HT×MO17	2694.2	B73×PHZ51	56.7
45	PHJ40×PHZ51	2685.2	PHJ40×PH207	56.7
46	MO17×PH207	2679.6	PHJ40×MO17	56.7
47	PHG39×PHZ51	2668.4	PH207×PHZ51	56.7
48	B73×PHZ51	2649.9	B73×PH207	56.7
49	LH1×PHJ40	2645.4	PHJ40×PHG47	56.6
50	PHG47×PHZ51	2625.8	LH82×PHG35	56.6
51	PH207×PHG35	2588.9	LH82×MO17	56.6
52	LH1×PHG84	2588.3	MO17×PHZ51	56.5
53	MO17×PHZ51	2565.9	B73×LH82	56.4
54	B73×MO17	2562.0	LH82×PHG47	56.4
55	LH1×PHZ51	2554.2	Check3	56.3
56	PHG35×PHG47	2554.2	LH1×PHG47	56.2
57	PHJ40×PHG35	2552.5	PHG47×PHZ51	56.2
58	PHG39×PH207	2538.5	MO17×PHG35	55.9
59	MO17×PHG35	2504.9	LH82×PH207	55.9
60	PH207×PHZ51	2484.2	MO17×PH207	55.8
61	MO17×PHG47	2478.0	PHG35×PHZ51	55.8
62	PHG39×PHG35	2452.2	LH123HT×PH207	55.7
63	PH207×PHG47	2399.0	Check6	55.6
64	B73×PH207	2398.5	PHG35×PHG47	55.5
65	PHG84×PHZ51	2398.5	LH123HT×PHG35	55.5
66	LH1×LH123HT	2389.5	B73×MO17	55.3
67	LH1×PHG35	2372.7	PH207×PHG35	55.3
68	B73×LH1	2364.9	PHG47×PHG84	55.2
69	LH1×MO17	2334.1	MO17×PHG47	55.1
70	LH1×PH207	2284.8	PH207×PHG47	54.8
71	LH1×PHG39	2270.8	Check4	54.7
72	LH1×PHG47	2155.4	B73×PHG47	54.6

Table 5. Continued.

Rank	Genotype	Flaking grits per acre (FGA) (lbs/ac)	Genotype	Test weight (lb/bu)
P1	LH82	2157.7	LH1	59.6
P2	PHG35	1458.2	LH82	58.3
P3	LH123HT	1423.5	LH123HT	57.5
P4	MO17	1370.9	PHJ40	57.2
P5	PHJ40	1302.0	PHG39	57.0
P6	B73	1279.0	PHG84	57.0
P7	PHG47	1016.4	PHG35	57.0
P8	PH207	995.1	PHZ51	56.6
P9	LH1	883.7	PHG47	56.2
P10	PHZ51	868.0	B73	55.8
P11	PHG84	652.4	MO17	55.6
P12	PHG39	637.8	PH207	52.4

Table 6. Top 5 performing F×M hybrids for key traits with overall hybrid mean and ranking.

| Genotype | Genotype mean | | | Rank by trait | | |
	Grain yield (bu/ac)	Flaking grit yield (g/100g db)	Flaking grits per acre (lbs/ac)	Grain yield	Flaking grit yield	Flaking grits per acre
Grain yield						
B73XPHG47	199.3	25.0	2749.6	1	29	17
PHG39XMO17	197.9	27.3	2962.4	2	24	10
PHG39XPHG47	197.5	26.4	2833.6	3	26	13
PHG39XPHZ51	194.0	25.0	2671.2	4	30	19
B73XMO17	192.0	24.1	2564.8	5	31	22
Flaking grit yield						
PHJ40XLH123HT	155.8	36.0	3091.2	28	1	8
PHJ40XPHG84	168.6	34.7	3248.0	19	2	3
PHJ40XLH82	169.4	34.5	3264.8	16	3	2
B73XPHG84	182.5	34.0	3449.6	11	4	1
PHJ40XMO17	160.4	31.5	2788.8	25	5	14
Flaking grits per acre						
B73XPHG84	182.5	34.0	3449.6	11	4	1
PHJ40XLH82	169.4	34.5	3264.8	16	3	2
PHJ40XPHG84	168.6	34.7	3248.0	19	2	3
B73XLH82	189.1	30.3	3180.8	6	12	4
LH1XLH82	182.8	31.0	3175.2	10	7	5

Table 7. Narrow (h^2) and broad (H^2) sense heritabilities and variance component estimates for all traits based on the 66 test hybrids across all environments.

Trait	h^2	H^2	$H^2 - h^2$	Sources of variation				
				σ^2_{GCA}	σ^2_{SCA}	$\sigma^2_{GCA \times E}$	$\sigma^2_{SCA \times E}$	σ^2_{error}
Seedling vigor	0.45	0.48	0.03	0.044**	0.006	0.012	0.087**	0.566***
Plant height	0.90	0.96	0.06	230.570**	30.632***	2.394	25.289***	97.322***
Ear height	0.90	0.94	0.04	75.574**	8.119***	0.693	3.193	70.197***
Days to silking	0.93	0.93	0	3.505**	0.025	0.452***	0.313***	0.947***
Days to pollen shed	0.93	0.94	0.01	2.991**	0.086**	0.341***	0.177***	0.714***
Anthesis silking interval	0.52	0.65	0.13	0.091**	0.044*	0.037**	0.044	0.752***
Staygreen	0.82	0.89	0.07	0.608**	0.100**	0.083**	0.113**	0.705***
Percent barren plants	0.04	0.23	0.19	0.020	0.164	0.264**	0.147	4.097***
Grain yield	0.43	0.70	0.27	43.158**	53.170***	6.330	73.749***	286.920***
Test weight	0.62	0.80	0.18	0.460**	0.267***	0.184***	0.179***	0.965***
Flaking grit yield	0.53	0.65	0.12	2.045*	0.909***	2.406***	1.793***	4.357***
Flaking grits per acre	0.43	0.49	0.06	6.411*	1.816	11.003***	7.965***	46.082***

* Significant at the 0.10 probability level
** Significant at the 0.05 probability level
*** Significant at the 0.01 probability level

Table 7. Continued.

Trait	h^2	H^2	$H^2 - h^2$	Sources of variation				
				σ^2_{GCA}	σ^2_{SCA}	$\sigma^2_{GCA \times E}$	$\sigma^2_{SCA \times E}$	σ^2_{error}
Cob length	0.76	0.82	0.06	52.419**	9.001**	18.607***	3.376	99.821***
Cob width	0.62	0.69	0.07	0.323**	0.070	0.056*	0	2.625***
Cob size	0.51	0.61	0.10	200540.000**	77961.000	128095.000**	3824.810	1974559.00***
Fill length	0.79	0.83	0.04	65.028**	7.083*	17.442***	8.292	122.780***
Ear width	0.52	0.68	0.16	0.950**	0.591**	0.398**	0	8.161***
Ear size	0.58	0.60	0.02	611140.000**	43165.000	186164.000*	193711.000	6019521.00***
Rows	0.91	0.95	0.04	1.390**	0.135***	0.098***	0.097***	0.507***
Kernel per row	0.70	0.79	0.09	2.339**	0.572*	0.487**	0.901**	7.110***
Hundred kernel volume	0.76	0.84	0.08	6.088**	1.299**	1.500***	2.331***	6.312***
Hundred kernel weight	0.77	0.85	0.08	3.550**	0.721***	0.914***	1.038***	3.661***
Kernel width	0.89	0.94	0.05	0.142**	0.015**	0.004*	0.007	0.132***
Kernel length	0.65	0.85	0.20	0.078**	0.048***	0.015**	0.020**	0.180***
Kernel depth	0.75	0.82	0.07	0.017**	0.003*	0.003**	0.001	0.049***
Kernel size	0.84	0.90	0.06	730.090**	100.520**	74.425**	68.666	899.050***
Sphericity	0.82	0.92	0.10	0.0004**	0.0001***	0.00003**	0.00005**	0.0004***

* Significant at the 0.10 probability level
** Significant at the 0.05 probability level
*** Significant at the 0.01 probability level

Table 8. Pearson correlation coefficients (r values) for the relationship between phenotypic performance for all traits with grain yield, test weight, flaking grit yield (FGY), and flaking grits per acre (FGA) among the 66 test hybrids.

Trait	Grain yield	Test weight	Flaking grit yield (FGY)	Flaking grits per acre (FGA)
Seedling vigor	-0.06	0.09**	-0.04	-0.11***
Plant height	0.22***	-0.10**	-0.06	0.17***
Ear height	0.16***	-0.01	-0.04	0.12***
Days to silking	0.53***	-0.30***	-0.65***	-0.08**
Days to pollen shed	0.54***	-0.29***	-0.63***	-0.06
Anthesis silking interval	-0.15***	0.11***	0.32***	0.16***
Staygreen	0.36***	-0.05	-0.24***	0.15***
Percent barren plants	-0.16***	0.13***	-0.06	-0.23***
Grain yield	1	-0.42***	-0.50***	0.55***
Test weight	-0.42***	1	0.52***	0.04
Flaking grit yield	-0.50***	0.52***	1	0.44***
Flaking grits per acre	0.55***	0.04	0.44***	1
Cob length	0.10**	0.10**	0.08	0.15***
Cob width	-0.12***	-0.04	0.30***	0.20***
Cob size	-0.02	0.04	0.27***	0.24***
Fill length	0.24***	-0.06	-0.08	0.16***
Ear width	0.09**	-0.16***	0.14***	0.26***
Ear size	0.22***	-0.14***	0.04	0.27***
Rows	0.18***	-0.14***	0.02	0.21***
Kernel per row	0.38***	-0.24***	-0.16***	0.25***
Hundred kernel volume	0.13***	-0.09**	0.10***	0.26***
Hundred kernel weight	0.11**	0.05	0.14***	0.26***
Kernel width	-0.14***	0.14***	0.13***	-0.04
Kernel length	0.40***	-0.30***	-0.22***	0.21***
Kernel depth	0.06	-0.01	-0.10**	-0.03
Kernel size	0.13***	-0.06	-0.07	0.06
Sphericity	-0.33***	0.26***	0.17***	-0.18***

* Significant at the 0.10 probability level
** Significant at the 0.05 probability level
*** Significant at the 0.01 probability level

Table 9. Genetic correlations among key traits for 66 test hybrids expressed as Pearson correlation coefficients (r values).

	Test weight	Flaking grit yield (FGY)	Flaking grits per acre (FGA)
Grain yield	-0.21	-0.43	0.72
Test weight	1	0.29	0
Flaking grit yield		1	0.54