

Evaluation of maize (*Zea mays* L) inbred lines for yield component traits and kernel morphology

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Abstract

The economical production of the seed of any hybrid maize (*Zea mays* L) variety is dependent on the parental characteristics of the inbred line used as the seed parent. Grain yield, thousand-kernel weight, ear length and kernel morphology are traits that are important in determining the suitability of an inbred line for use as a seed parent in a seed production field. In 2009, replicated field trials at Janesville, WI and Stanton, MN included 27 maize inbred lines obtained from the North Central Regional Plant Introduction Station in Ames, IA. Inbred line evaluations included measurements of grain yield, thousand-kernel weight, and ear length. Separation of the harvested seed into the different size classifications based on kernel morphology allowed further characterization of inbred lines. Significant differences existed among inbred lines for grain yield, thousand-kernel weight, ear length and kernel morphology. Among this group of inbred lines, ear length appeared to make a greater contribution to grain yield than thousand-kernel weight. Correlation analysis indicated that a strong negative relationship exists between kernel morphology types classified as “large rounds” and “medium flats”, with the latter group being the more desirable of the two. The results of this study suggest that plant breeders may want to consider evaluating inbred parents for their kernel morphology before beginning a breeding project or before selecting an inbred to serve as the seed parent in a production field.

Keywords: *Zea mays* L, inbred, yield components, kernel morphology, hybrid seed production

Introduction

The commercial success of any maize (*Zea mays* L) hybrid variety is dependent on many factors including the parental characteristics of the inbred lines involved in the cross. Hybrid varieties that are expensive to produce in a seed production field have limited commercial potential regardless of their grain yield potential. Any trait that contributes to the stable production of large quantities of hybrid seed in a seed production field will be of interest to plant breeders. For inbred lines that serve as the seed parent in a production field, the evaluation of the grain yield and yield components of the inbred line *per se* is a way of estimating the profitability of commercial seed production for a maize hybrid.

A number of studies have evaluated the relationship between grain yield and yield components (Love and Wentz 1917; Guo et al, 2008; Peng et al, 2011). Bekavac et al (2008) found significant correlations between maize inbred *per se* and testcross performance for stay green, anthesis-silking interval, stalk water content and grain moisture. Rafiq et al (2010) evaluated 10 maize inbred lines in testcross combinations and as lines *per se*. Based on genotypic correlation coefficients, they found a significant correlation between 100-kernel weight and grain yield. Ross et al (2006) examined phenotypic correlations among yield components in Iowa Long-Ear Synthetic. In that

study, ear length correlated positively with grain yield on a plant basis and kernel weight.

Although studies of inbred lines for traits such as cob diameter, ear length, 100-kernel weight, kernel row number, and grain yield exist in the literature, there is a lack of published research on the sizing of the seed obtained from seed parents in a commercial hybrid seed field. In the seed industry, sizing refers to the process where harvested seed is separated into different seed lots based on the width, thickness and length of the kernel (Wych, 1988). Sizing involves passing seed through a series of screens of varying sizes to separate a seed lot into fractions with similar kernel morphology. The objective of sizing is to produce a seed lot with acceptable uniformity. Uniformity of the seed lot plays a role in the packaging and acceptability of the seed for sale to producers for planting. Seed lots with variable seed sizes can introduce stand establishment issues upon planting which can lead to yield loss if the variability is great enough (Nielsen, 1996).

The objective of this research was to evaluate a sample of inbred lines for their parental characteristics including the sizing of the seed into different fractions based on kernel morphology. The results of this research will assist in understanding the relationship between yield components, kernel morphology and the *per se* grain yield of the inbred lines.

Materials and Methods

Inbred lines

This study included 27 maize inbred lines (Table 1) obtained from the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. These inbred lines, previously protected by a plant variety protection (PVP) certificate and/or an intellectual property protection patent, had been involved in commercial hybrid seed production. It is unknown if these inbreds were used as the seed parent or the pollen parent in a production field during their period of PVP or patent protection. Upon the expiration of this protection, these lines became publicly available through the NCRPIS for breeding projects or research studies. Upon request of inbred lines with expired PVP certificates or patents, the NCRPIS sent 15 kernels to the requestor. A seed increase for each inbred line occurred at Syngenta research nurseries located at Seward, Nebraska or Stanton, Minnesota.

Experimental design

In 2009, replicated field trials at two locations at Janesville, WI and one location at Stanton, MN included the 27 inbreds increased previously. Entries were planted according to a randomized complete block design with four replications except for the non irrigated trial at Janesville which consisted of three replications. Plots consisted of a single, 3.75 m long row with 75 cm between rows. The planting density

for the irrigated trials was 72,000 plants per hectare. On the non-irrigated field at Janesville, the planting density was 99,000 plants per hectare.

At physiological maturity, all ears on all plants in all plots were hand harvested and dried to a uniform moisture content of approximately 15%. Calculation of ear length (EL) involved measuring each primary ear from its base to its tip. Based on these measurements the standard deviation for the primary ear length (SDEL) of each inbred was determined. Shelling of entries with an AEC Model SH31 small batch sheller allowed the calculation of grain yield per plot (GYPP). The weight of 1,000 kernels from each grain sample (TKW) was determined for each entry. Separation of kernels based on kernel morphology occurred using a 0.52 cm x 1.9 cm oblong sieve. Kernels that remained on top of the sieve were "rounds" and the seed passing through the sieve were "flats". Separation of round and flat kernels into different size categories occurred using the set of screens: 0.95 cm, 0.83 cm, 0.67 cm, and 0.60 cm. The kernels that passed through the 0.95 cm screen but not the 0.83 cm screen are either large rounds (LR) or large flats (LF). The kernels that passed through the 0.83 cm screen but not the 0.67 cm screen are either medium rounds (MR) or medium flats (MF). Kernels that passed through the 0.67 cm screen but not the 0.60 cm screen are either small rounds (SR) or small flats (SF). Excluded from analysis were kernels that

Table 1 - Inbred lines obtained from the North Central Regional Plant Introduction Station in Ames, Iowa and evaluated in 2009 at Janesville, Wisconsin and Stanton, Minnesota.

Inbred Name	Company	PVP No.	NCRPIS No.
FAPW	DeKalb-Pfizer Genetics	8200152	PI600958
DMDF-13D	DeKalb-Pfizer Genetics	8200151	PI600956
B47	Pioneer Hi-Bred International, Inc.	8300141	PI601009
G50	Pioneer Hi-Bred International, Inc.	8300143	PI601006
207	Pioneer Hi-Bred International, Inc.	8300144	PI601005
G80	Pioneer Hi-Bred International, Inc.	8400128	PI601037
6103	Asgrow Seed Company	8500005	PI601159
IB014	DeKalb-Pfizer Genetics	8500123	PI601208
PHG29	Pioneer Hi-Bred International, Inc.	8600047	PI601270
PHG84	Pioneer Hi-Bred International, Inc.	8600130	PI601320
PHJ40	Pioneer Hi-Bred International, Inc.	8600133	PI601321
PHG72	Pioneer Hi-Bred International, Inc.	8600134	PI601319
PHG47	Pioneer Hi-Bred International, Inc.	8600131	PI601318
PHZ51	Pioneer Hi-Bred International, Inc.	8600132	PI601322
PHG86	Pioneer Hi-Bred International, Inc.	8700170	PI601442
IBO2	DeKalb-Pfizer Genetics	8700197	PI601457
PHN11	Pioneer Hi-Bred International, Inc.	8800037	PI601497
PHK76	Pioneer Hi-Bred International, Inc.	8800036	PI601496
PHK42	Pioneer Hi-Bred International, Inc.	8800035	PI601495
PHT77	Pioneer Hi-Bred International, Inc.	8800038	PI601499
NS501	DowElanco	8800149	PI601583
MYOQ603	DowElanco	8800150	PI601584
11430	Cargill, Inc.	8800177	PI601558
2MA22	DeKalb-Pfizer Genetics	8800193	PI601560
MBST	DeKalb-Pfizer Genetics	8800194	PI601566
PHR47	Pioneer Hi-Bred International, Inc.	8800213	PI601572
PHH93	Pioneer Hi-Bred International, Inc.	8800216	PI601567

were too large to pass through the 0.95 cm screen or kernels that were so small that they passed through the 0.60 cm screen. Upon the completion of the sizing process, the percentage by weight of each kernel type was calculated.

The model for analyses of variance included environments, replications within environments, genotypes and the genotype by environment interaction. An analysis using means over replications allowed the generation of entry least square means. For each trait, inbred means were the average value calculated from all of the replications at all locations. Calculation of correlation coefficients involved trait values paired by inbred.

Results and Discussion

Analysis of variance indicated significant differences for GYPP, TKW, EL and SDEL, and the various seed size categories among the inbred lines studied. The inbred means calculated for each trait indicated considerable diversity among the set of inbred lines included in this study (Table 2). The low GYPP, low TKW, high SDEL and the high percentage of LR of some inbreds would have made their use as a seed parent problematic. For example, PHG84 had a very low seed yield and a very high SDEL suggesting that this line lacked stability in the set of environments

selected for this study. PHK42 and B47 are interesting lines, both having a high GYPP and a lower SDEL suggesting that these lines would be useful as seed parents barring a defect not detected in this study. PHG72 has a high GYPP and a very high percentage of MF, a phenotype considered as favorable for seed processing, and uniformity of planting. Inbreds with this type of phenotype could be a source of useful germplasm in a line improvement program. Inbreds such as IBO2 having a low GYPP and high percentage of LR would be less suitable as seed parents in production. Low yielding seed parents in a production field will make production of seed of a hybrid maize variety more expensive. Although the actual production role of each inbred in this study is unknown, it is likely that at least some of these inbreds did not serve as a seed parent because of low GYPP and high LR.

Correlations among the traits studied indicated the existence of several statistically significant relationships (Table 3). A highly significant and positive correlation exists between GYPP and EL. The correlation between GYPP and TKW was not significant. This suggests that EL is a more important yield component than TKW in contributing to final grain yield among this group of inbred lines. This conclusion is in agreement with Ross et al (2006) where it was determined that kernel weight was not associated with ear length or grain yield on a plant basis in the F_{2:3}

Table 2 - Trait means for 27 inbreds grown in Janesville, WI and Stanton, MN in 2009.

Inbred Name	Yield Component Traits				Kernel Morphology Categories as % of GYPP					
	GYPP (g)	TKW (g)	EL (cm)	SDEL (cm)	LR	MR	SR	LF	MF	SF
PHK42	1687	231	13.4	0.98	19.3	29.4	3.2	26.1	20.8	1.1
B47	1601	257	14.6	1.78	24.1	24.8	0.9	32.8	16.2	1.1
FAPW	1564	235	14.6	2.67	13.1	40.3	1.6	10.5	32.1	2.4
PHG86	1521	233	14.1	1.80	6.1	44.4	3.7	3.2	36.8	5.8
11430	1515	247	12.9	2.02	20.4	37.4	1.6	10.9	28.0	1.6
PHT77	1484	221	12.9	1.54	9.9	17.9	1.7	30.9	35.5	4.0
PHG72	1467	196	12.5	1.39	1.9	11.4	2.1	8.5	64.6	11.5
PHH93	1418	261	12.6	1.87	23.7	32.2	1.7	19.7	21.3	1.4
PHN11	1370	226	12.6	0.97	18.8	37.3	2.1	16.8	22.0	2.9
PHG29	1354	230	13.3	1.05	21.1	27.4	2.1	23.9	23.1	2.4
MBST	1291	228	13.5	1.84	16.9	24.7	0.5	31.7	25.0	1.0
IB014	1273	219	13.1	1.85	24.8	30.4	2.2	14.4	24.4	3.8
207	1259	230	12.7	1.57	26.8	25.3	1.7	24.0	19.6	2.6
MYOQ603	1186	220	12.1	1.04	12.7	26.9	2.7	20.9	34.3	2.5
PHJ40	1183	233	12.8	1.59	19.1	38.1	1.8	12.4	27.1	1.4
G80	1180	267	13.7	2.77	28.9	33.4	1.1	15.1	20.1	1.4
PHK76	1175	268	13.3	2.61	23.3	20.3	0.4	36.8	17.8	1.3
PHR47	1144	262	11.7	2.78	27.9	25.5	0.5	28.8	16.2	1.2
PHZ51	1128	248	12.7	3.01	25.2	31.1	1.2	18.4	22.6	1.5
G50	1029	219	14.2	1.41	13.1	41.1	2.6	11.6	29.4	2.2
NS501	966	237	11.4	1.41	25.1	21.7	1.1	22.3	28.0	1.8
PHG47	943	184	11.4	1.24	3.1	32.7	7.5	1.2	37.1	18.3
D MDF-13D	905	240	13.1	2.47	27.7	40.6	0.3	15.4	15.6	0.4
6103	815	215	11.4	2.37	14.1	54.4	1.1	3.7	25.0	1.5
IBO2	744	271	11.9	1.09	35.7	33.1	0.9	20.4	8.8	1.1
2MA22	715	199	10.8	2.57	25.3	21.9	0.2	33.0	18.9	0.6
PHG84	294	181	11.5	3.49	16.7	66.3	1.1	3.2	10.3	2.3
LSD(0.05)	332	30	1.1	1.1	11.5	7.9	1.7	7.7	10.6	2.1

generation. These results differ somewhat from those of Rafiq et al (2010), who found highly significant and positive correlations between ear length and grain yield per plant, and 100-kernel weight and grain yield per plant. In their study, ear length and 100-kernel weight were important yield components contributing to grain yield.

Gonzalo et al (2010) examined planting density response in a maize population of recombinant inbred lines and found quantitative trait loci (QTL) related to the barrenness expressed by some genotypes. In this current study, a higher SDEL was associated with a lower GYPP. The lower GYPP of inbred lines with a higher SDEL could be an indication of the lines relative stability in the face of abiotic stresses or of the planting densities used in this experiment. Inbred lines with a high EL and a low SDEL would be desirable to plant breeders because the grain yield stability would make hybrid seed production more reliable and predictable for any given inbred line. Since no significant correlation existed between EL and SDEL among this group of inbreds, breeders should be able to incorporate both an increased EL and a decreased SDEL in a new seed parent inbred. Upon the assumption that SDEL is an indicator of stress tolerance or a measure of stability across environments, the negative correlation between GYPP and SDEL suggests that the best seed parents will also be the most stable across environments.

Correlations between GYPP and the percentage of the various seed size classifications indicated that the only statistically significant relationship existed with MR and MF. The correlation between GYPP and the percentage of MR or MF indicated that high yielding lines tended to have a higher percentage of MF and a lower percentage of MR than lower yielding lines. The percentage of SR and SF in the seed lots was usually less than 5% combined for most inbreds and did not contribute significantly to final seed yield. PHG47 was an exception to this with a high combined percentage of SR and SF. The combined size of the SF and SR classifications explains the low TKW observed with this inbred. Seed parents with a low

TKW can pose a problem in the packaging of the hybrid seed for sale to producers. Typically, producers purchase hybrid maize varieties for planting in unit bags consisting of 80,000 kernels. If the TKW of the seed parent is low, this leads to a unit bag of a lower weight. This is a concern to producers because of the planter adjustments that are necessary to accommodate a smaller seed size and the perception that small kernels are less viable (Nielsen, 1996).

Correlations between TKW and the different seed size classifications were significant for most combinations except MR. Not surprisingly, a positive correlation was found between TKW and both LR and LF kernel types. Typically, inbreds with a high percentage of LR are not desirable as seed parents because of the planting issues arising from this type of kernel morphology. In addition to the planting issue, LR seed is also in greater danger of mechanical damage that can occur during the seed conditioning process at the production plant. This is due to the exposure of the embryo, which is greater for a kernel with LR morphology than a flat morphology (Nielsen, 1996). Prior to starting a new breeding project, a plant breeder may want to characterize potential parents involved in the cross for their kernel morphology. If the breeding objective is to develop new and improved seed inbred parents, a plant breeder may want to avoid breeding projects where both inbred parents involved have a high LR. However, the separation of a seed lot into the different seed classifications can be a time consuming process, requiring appropriate screens and a seed lot of sufficient size to allow the correct characterization of an inbred line. This process can become particularly cumbersome if the program demands the characterization of thousands of inbred lines in a short period. To make the selection process more efficient in terms of time and resources, a plant breeder may want to establish a range in TKW, a trait more easily measured, below or above which the breeder would not recommend the use of a particular inbred as a seed parent.

For this sample of inbreds, the correlation between EL and the various seed size classifications

Table 3 - Correlation coefficients among yield component traits and kernel morphology classifications involving 27 maize inbred lines.

I	TKW	EL	SDEL	LR	MR	SR	LF	MF	SF
GYPP	0.34 ^{NS}	0.65 ^{**}	-0.39 [*]	-0.22 ^{NS}	-0.45 [*]	0.14 ^{NS}	0.23 ^{NS}	0.40 [*]	0.04 ^{NS}
TKW	1	0.39 [*]	0.06 ^{NS}	0.66 ^{**}	-0.25 ^{NS}	-0.44 [*]	0.44 [*]	-0.42 [*]	-0.54 ^{**}
EL		1	-0.06 ^{NS}	-0.07 ^{NS}	-0.02 ^{NS}	-0.02 ^{NS}	0.08 ^{NS}	0.06 ^{NS}	-0.18 ^{NS}
SDEL			1	0.22 ^{NS}	0.37 ^{NS}	-0.47 [*]	-0.06 ^{NS}	-0.34 ^{NS}	-0.29 ^{NS}
LR				1	-0.10 ^{NS}	-0.61 ^{**}	0.47 [*]	-0.80 ^{**}	-0.68 ^{**}
MR					1	0.09 ^{NS}	-0.68 ^{**}	-0.31 ^{NS}	-0.12 ^{NS}
SR						1	-0.52 ^{**}	0.46 [*]	0.82 ^{**}
LF							1	-0.35 ^{NS}	-0.49 ^{**}
MF								1	0.66 ^{**}
SF									1

NS - Not significant at the 0.05 probability level; *, ** significant at the 0.05 and 0.01 probability levels, respectively.

was not significant. This suggests that it may be possible for plant breeders to combine an increased EL with a more desirable MF kernel morphology in an inbred line.

Significant correlations among the different seed size classifications exist, in part, because the percentage of each classification for any inbred must sum to 100%. The highly significant negative correlation between LR and MF is interesting since it involves the two kernel types of greatest interest to plant breeders. Based on the relationship between the two classifications, plant breeders need only determine the proportion of LR or MF in any grain sample and do not require data on the proportion of all the size classifications measured in this study. In most cases, the proportion of LR seed will be smaller than the proportion of MF seed in a grain sample, especially for inbreds developed for use as seed parents. By designing the kernel sizing process so that only the proportion of the LR classification is determined from the total grain sample, plant breeders may be able rapidly evaluate the size of LR as a proportion of the total grain sample. By culling those lines with a high LR, plant breeders can be confident that the lines selected will have an increased proportion of MF. This strategy could be particularly effective for the rapid evaluation of thousands of inbred lines for kernel morphology.

Although measurements of TKW, LR or MF could be useful in developing improved inbred lines for use as seed parents, there is great value in identifying quantitative trait loci (QTL) for these traits. The use of molecular markers to identify genotypes with desirable kernel morphology would reduce the cost of inbred development since it would eliminate the need to calculate TKW or the proportion of any seed size classification. QTL analysis of two maize mapping populations involving Chinese foundation inbred lines identified several QTLs associated with yield component and kernel related traits (Peng et al, 2011). Traits examined in that study included grain yield per plant, 100-kernel weight, kernel number per plant, kernel density, kernel length and kernel width. Several QTL associated with kernel traits appeared to be stable across environments indicating their utility in marker assisted breeding. Work by Ross et al (2006) identified QTL for ear length, grain yield, kernel weight and kernel depth. Results from that study showed a repulsion linkage for QTL between ear length and grain yield on chromosome 5, explaining in part the difficulty in increasing seed yield by selection for increased ear length. QTL studies involving kernel morphology traits such as the proportion of LR in a grain sample would provide information useful to plant breeders developing new seed parent inbred lines. The results of this present study suggest that sufficient diversity exists among this group of inbred lines such that a follow up study involving a population of inbred lines developed from a cross between inbreds contrast-

ing for a yield component trait or kernel morphology class, might lead to the discovery of marker associations for traits such as TKW or LR. The value of these associations increases if they remain stable over environments.

The inferences made in this study are somewhat limited by the size of the sample of inbreds studied. Because of the importance of kernel morphology and seed parent traits to the economic production of hybrid maize varieties, further research is required to increase our understanding of the relationships among these traits and their genetic control. However, the results of this study suggest that plant breeders may be able to improve the breeding efficiency of their programs by evaluating inbreds for traits that are important in seed parents. Significant diversity for these traits existed in just this limited sample of inbred lines and it was possible to identify inbreds possessing a favorable seed parent phenotype. Although the development of the inbreds evaluated in this study took place more than twenty years ago, this germplasm may still be useful in a current breeding program if the focus is on developing improved seed parents.

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