

Increasing Kernel Density for Two Inbred Lines of Maize

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ABSTRACT

Improving grain quality of maize (*Zea mays* L.), including endosperm hardness and density, is often a breeding objective. Dense seed is preferred by dry millers and for alkaline processing, and can command a price premium at market. This study attempted to increase kernel density in a backcrossing program for two inbreds of maize using two selection techniques, specific gravity of kernels and the percentage of sinking kernels in a salt solution (or sinkers). Two inbreds, B73G and A632, were crossed with synthetics exhibiting apparent high kernel density, and several generations of backcrossing and self-pollination followed. Examples of mean comparisons of backcross-derived inbreds with the recurrent parents, B73G and A632 are as follows: B73G—Specific gravity, 1.251 and 1.206; Sinkers, 62.3 and 14.9%; and A632—Specific gravity, 1.266 and 1.250; Sinkers 45.4 and 29.1%. Both the specific gravity and sinkers techniques were successful for increasing kernel density during backcrossing.

THE TERM SEED QUALITY is a broad concept with many connotations, depending on the end-use of the seed (Okoruwa and Kling, 1996; Rooney et al., 2004). Eckhoff and Paulsen (1996) have discussed many of the factors, including endosperm hardness and density, relating to grain quality for different end-uses. Hard, dense seed is often equated to seed quality and has significance for harvesting, handling, and other considerations (Watson, 1987). It is preferred by dry millers (Wu and Bergquist, 1991) and often commands a 10% market premium (Hill et al., 1991; Hahn et al., 2000; U.S. Grains Council, 2001). In dry milling, profits largely depend on the amount of large “flaking” grits that are produced since flaking grits are the highest value endosperm product; larger grits result from denser kernels. Dense-kernelled maize is the preferred type for alkaline-cooking processes for making masa, tortilla chips, and snack foods as well. There is even limited evidence that dense kernels result in higher yields of ethanol (Murthy et al., 2004a, 2004b).

Dense seed is usually hard, with vitreous endosperm (Mestres et al., 1991), and the degree is often determined or inferred by visual examination. In contrast to visual examination, this research was designed to select for kernel density by two quantitative determinations, namely, specific gravity and sinkers (i.e., the percentage of sinking kernels in a salt solution). Initial success with these two techniques was reported by Bergquist and Thompson (1992). In that paper the term “floaters” referred to the percentage of floating kernels, which is the complement of “sinkers” used in this research. The objective of

this research was to evaluate these two techniques, specific gravity and sinkers, for their effectiveness in increasing the kernel density of inbred lines B73G and A632 by backcrossing.

MATERIALS AND METHODS

B73G Enhancement

The first objective of this work was to increase the kernel density of the experimental inbred line B73G (Koester et al., 1993). This line has an expected relationship of 94% with B73, sheds pollen about 1 wk earlier, but lacks satisfactory yield consistency. It was crossed with two sources of apparent higher kernel density as judged by visual examination. These were TG, a cross of a composite of Mexican races with Gaspé Flint, and the synthetic B73G.SYN8.SQB (abbreviated herein as SYN). Information relating to this synthetic has been reported by Bergquist and Thompson (1992).

A backcrossing program was followed, with B73G as the recurrent parent, to increase its kernel density. Over 100 backcrossed ears were evaluated from each source for each generation with about 25% selected for the next generation. There were four backcross generations for the TG source and three for the SYN source. After the backcrossing phase, selection of individual ears for kernel density continued for several generations of self-pollination. For convenience of handling, two subgroups were formed for the TG group and three for the SYN group.

A632 Enhancement

A second objective was to increase the kernel density of the inbred A632. It was crossed with the sources TG and SYN and with a third source, SYN9.SQA (abbreviated herein as 9A). This synthetic was constituted from a cross of a North Carolina (SYN9) and a Missouri synthetic (MoSQA). Both synthetics had been through several cycles of selection for stalk strength. There were three backcrosses for each of the three sources, with A632 as the recurrent parent. Bulk selection was used for this group. Five families were grown for each source and about 10 ears were bulked for each family. From this bulked backcross seed, kernel density selections were made for the next generation. After the backcrossing phase, four self-pollinated generations were produced for each of the five families for each source. About 10 ears were bulked for each family during this phase.

Field Experiments

Backcross inbreds, that is, progeny from the last backcrossing, and the recurrent parent, B73G, were grown in two states, South Dakota (91 entries) and North Carolina (40 entries), and compared for kernel density (Table 1). The backcross inbreds were from the S₄ and S₅ generations after the third and fourth backcrosses from the two sources TG and SYN, respectively. Means from both sources were combined in the results (Table 1) and are for self-pollinated seed.

Backcross inbreds and the recurrent parent, B73G, were compared for kernel density in tester crosses and as inbreds per se in South Dakota (Table 2). The tester was the hybrid

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Table 1. Means for specific gravity and sinkers for self-pollinated seed from the B73G group in two states, South Dakota and North Carolina, and the concentrations of the salt solutions.

Inbreds	No.	Specific gravity	Sinkers	Salt solution specific gravity
		g cm ⁻³	%	g cm ⁻³
Brookings County, SD, 2001				
B73G backcross inbreds	91	1.281	48.9	1.290
B73G	7	1.245	11.2	1.290
	<i>t</i>	4.34**	5.35**	
Wake County, NC, 2002				
B73G backcross inbreds	40	1.218	67.8	1.225
B73G	6	1.169	13.3	1.225
	<i>t</i>	5.08**	15.5**	
Both states				
B73G backcross inbreds	35	1.251	62.3	1.289
B73G	6	1.206	14.9	1.289
	<i>t</i>	6.48**	16.07**	

** Significant at the 0.01 probability level.

DeKalb 493. The inbreds and the tester were grown in paired rows and reciprocal crosses were made with an average of four ears each. Inbred and tester plants were also self-pollinated. The backcross inbreds were from the S₃ and S₄ generations after the third and fourth backcrosses from the two sources, TG and SYN, respectively. Means from both sources were combined in the results (Table 2).

Backcross inbreds and the recurrent parent, B73G, were compared for kernel density with F₂ seed from three hybrids from crosses with the inbred A654. This inbred has shown satisfactory hybrid performance with B73 type lines. Two hybrids were with backcross inbreds and one with the recurrent parent. These three hybrids were grown in a randomized complete block design with nine replications in 2000 in South Dakota. Plants were self- or sib-pollinated and, on average, six ears were harvested and bulked for each row. Seeds were graded with round hole screens in 0.40-mm increments. The grade fraction with the greatest weight for each row was used for specific gravity and sinker evaluation.

Yield Trials

Backcross inbreds and the recurrent parent, B73G, were compared in two yield tests as hybrid crosses with the single cross FR615 × FR697. Each test was an 8 by 8 lattice of two replications grown at three locations in the coastal plain area of North Carolina in 2004. Plots were two rows of 25 plants per

Table 2. Means for specific gravity and sinkers for kernels of inbreds and tester crosses of the B73G group and concentration of salt solutions (Brookings County, South Dakota, 2000).

Cross or inbred	No.	Specific gravity	Sinkers	Salt solution specific gravity
		g cm ⁻³	%	g cm ⁻³
Inbreds as seed parent, F₁ seed				
B73G backcross inbreds × tester	50	1.254	54.7	1.240
B73G × tester	6	1.201	18.3	1.240
	<i>t</i>	8.36**	3.81**	
Tester as seed parent, F₁ seed				
Tester × B73G backcross inbreds	96	1.190	48.3	1.193
Tester × B73G	5	1.179	28.0	1.193
	<i>t</i>	1.39	3.17**	
Tester per se, F₂ seed				
Tester per se	50	1.184	44.5	1.189
Inbreds per se, selfed seed				
B73G backcross inbreds	413	1.254	50.6	1.259
B73G	25	1.184	5.1	1.259
	<i>t</i>	13.2**	24.7**	

** Significant at the 0.01 probability level.

row. The first test consisted of 50 backcross inbreds, 11 entries of the recurrent parent, and three commercial hybrids. The second included 53 additional backcross inbreds, seven entries of the recurrent parent, and four hybrids. Commercial hybrids were included to complete the lattice designs. Means from both tests were combined for *t* test calculations between the backcross inbreds and the recurrent parent.

A632 Enhancement

Backcross inbreds and the recurrent parent, A632, were compared for kernel density for self-pollinated seed in two evaluations (Table 3). The first evaluation included 90 observations as averaged for three generations, two repetitions, three sources, and five families. The initial seed came from the third backcross. Five families were selected from each of three sources: TG, SYN, and 9A. Seed for each family was divided into two parts to give two repetitions for each. The families were grown and self-pollinated for three successive years, 2001 through 2003, to give the S₁, S₂, and S₃ generations after the third backcross. These were produced by bulking about 10 self-pollinated ears for each family each year and selecting for kernel density.

The second evaluation, conducted in 2004 in South Dakota (Table 3), included six families from the backcross group and two entries of the recurrent parent, A632, grown in a randomized block design with five replications. The six families consisted of two S₄ families from the third backcross from each of the three sources: TG, SYN, and 9A. Kernel density means were calculated from bulked seed from about 10 self-pollinated ears for each row.

Test Weight Comparisons

Correlation coefficients for commercial maize were calculated for test weight of grain vs. specific gravity and vs. sinkers. Thirty samples of maize were collected from the Cooperative Elevator, Sinai, SD. Ten samples had been processed through a drier and provided correlations on a dry weight basis. Twenty samples were collected as maize grain was delivered to the elevator and provided correlations on a fresh weight basis.

Pollen Effect

To examine the effect of pollen source on kernel density, plants of 11 adapted hybrids (eight commercial and three experimental) were pollinated with two types of pollen. The two types were the hybrid plant's own pollen and pollen from random plants of the two high kernel-density lines, which were the enhanced progeny from the two inbreds, A632 and B73G. Five ears were harvested and bulked from each hybrid for each pollen type. The hybrids were grown in Brookings County, South Dakota in 1999.

Table 3. Means for specific gravity and sinkers for self-pollinated seed of the A632 group (Brookings County, South Dakota).

Inbreds	No.	Specific gravity	Sinkers
		g cm ⁻³	%
Three generations combined, 2001–2003			
A632 backcross inbreds	90	1.266	45.4
A632	24	1.250	29.1
	<i>t</i>	3.59**	4.59**
One year, five replications, 2004			
A632 backcross inbreds	6	1.230	53.0
A632	2	1.220	41.0
	<i>F</i> (1 df)	6.65**	10.32**

** Significant at the 0.01 probability level.

Density Determinations

The two kernel density measurements, specific gravity and sinkers, were calculated on dry weight basis with one exception: when test weight was correlated with kernel density on a fresh weight basis. Otherwise, seeds were dried as normally practiced. In most cases, a rough grading of the seed was done to remove the largest and smallest seed. Kernel samples for the specific gravity technique were drawn by volume and averaged 20 to 30 g. These samples were weighed first in air and again when suspended in a mesh basket submerged in tap water plus a wetting agent to maximize contact of seeds with water. The formula:

$$\text{weight in air}/(\text{weight in air} - \text{weight in water})$$

was used to give specific gravity as a density measure of grams per cubic centimeters.

For the sinkers technique, kernel samples were also drawn by volume and averaged 30 to 50 g, depending on seed availability. These samples were placed in a salt solution of NaNO_3 and tap water plus a wetting agent. The concentration of the salt solution was calibrated for specific gravity by a hydrometer and was chosen to give about 50% sinking kernels for a group or subgroup. In general, the concentration of the salt solutions was within the range of -0.01 to $+0.02$ of the specific gravity of the kernel sample averages. Sinking kernels were retrieved, washed by dipping in water, dried, and weighed. The weights of sinking kernels were converted to percentage for each sample to give a density measure related to the salt concentration. Both specific gravity and sinkers means were considered when making selections, but the final choice of seed for the next generation was based on the sinkers.

All field plantings in South Dakota were single row plots at a rate of about 43 700 plants ha^{-1} . In North Carolina single row plots were used for inbred plantings and two-row plots for yield tests, both at the rate of about 45 000 plants ha^{-1} .

RESULTS

B73G Results

Comparisons show that backcross inbreds had greater kernel density than the recurrent parent, B73G, in each of two states, South Dakota and North Carolina, and for both states combined (Table 1). Combined means for the backcross inbreds and for the recurrent parent were as follows, respectively: Specific gravity, 1.251 and 1.206; Sinkers, 62.3 and 14.9%. Means were significantly different ($P < 0.01$). These comparisons, for two diverse environments, show that these two selection techniques were effective for increasing kernel density during backcrossing. These inbreds in South Dakota would be considered full season, whereas in North Carolina they would be early maturing.

The backcross inbreds had greater kernel density than the recurrent parent, B73G, in crosses with the tester and also as inbreds per se (Table 2). Differences were significant by t tests ($P < 0.01$). In crosses, means for specific gravity were closely related to that of the seed parent. When the backcross inbreds were seed parents, means were close to that of the inbreds per se and, conversely, when the tester was the seed parent, means were similar to that of the tester. Means for the backcross inbreds as seed parent in tester crosses and as inbreds per

se were as follows, respectively, for specific gravity: 1.254 and 1.254. When the tester was the seed parent, means for the tester crosses and for the tester per se were, respectively, 1.190 and 1.184. However, the backcross-inbred crosses had comparatively greater kernel density than did the recurrent-parent crosses regardless of whether the seed parents were the inbreds or the tester.

Comparisons for sinkers, as were made in Table 2 for specific gravity, would not be appropriate because different salt concentrations were used for the various subgroups. However, within subgroups, means for sinkers were significantly greater for the backcross inbreds ($P < 0.01$) than those for the recurrent parent.

Kernel density evaluations were made for F_2 seed from three hybrids, two for backcross inbreds and one for the recurrent parent, B73G, in crosses with the inbred A654. Comparative means were as follows: Specific gravity, 1.264 and 1.257; Sinkers, 70.9 and 55.1%. The difference between means for specific gravity were not significant by F -test (1 df) but were for sinkers ($P < 0.01$).

Yield tests provided hybrid comparisons of the backcross inbreds with the recurrent parent B73G. There were 103 backcross inbreds and 18 entries for B73G in topcrosses with the tester $\text{FR615} \times \text{FR697}$. Differences were not significant for the agronomic characters measured. The means were as follows, respectively: yield, 6.60 and 6.82 Mg ha^{-1} ; kernel moisture, 15.9 and 15.8 cg g^{-1} ; erect plants, 80 and 78%; ear height, 88 and 87 cm; and tassel date, 55 and 55 d. These means are for two tests combined. In individual tests, there was evidence that the backcross inbreds were slightly lower than the recurrent parent for yield and slightly higher for kernel moisture as indicated by t tests ($P < 0.05$).

A632 Results

Kernel density means for the A632 group (Table 3) are for self-pollinated seed from the bulk method of selection. Means in the first part of this table are for backcross inbreds averaged for 90 observations (three generations, two repetitions, three sources, and five families). Comparative means with those of the recurrent parent, A632, are as follows, respectively: Specific gravity, 1.266 and 1.250; Sinkers, 45.4 and 29.1%. These means were significantly different by t tests ($P < 0.01$).

Also presented are means for six S_4 families (two from each of three sources) that were derived from six of the families reported in the first part of the table (Table 3). All seeds were from self-pollinated plants. Comparative means with those of the recurrent parent, A632, which was included twice in each replication, are as follows, respectively: Specific gravity, 1.230 and 1.220; Sinkers, 53.0 and 41.0%. Means were significantly different by F -tests (1 df; $P < 0.01$). These comparisons show that the bulk method of selection was effective for increasing kernel density by backcrossing.

Test Weight Correlations

Correlation coefficients were calculated for test weight of grain vs. specific gravity and vs. sinkers. Ten samples on

a dry weight basis (14.0% moisture) gave the following correlations, respectively: Specific gravity vs. Test weight, 0.751 ($P < 0.05$) and Sinkers vs. Test weight, 0.812 ($P < 0.01$). Twenty samples on a fresh weight basis (22.8% moisture) gave the following corresponding correlations, respectively: 0.331 (NS) and 0.475 ($P < 0.05$). Although not calculated herein, density based on the volume of test weight samples would be less than the density of the kernels per se because of space between kernels. For example, a volume of 35 239 cm³ (1 bushel) weighing 25 401 g (56 lbs.) would have a density of 0.721 g cm⁻³.

Pollen Effect

A pollen affect was noted for kernel density when pollinations were with two types of pollen. Kernel density of seed from pollinations of 11 hybrids with pollen from high kernel density plants was compared with seed produced by self- or sib-pollination of the hybrids. Means were as follows, respectively: Specific gravity, 1.256 and 1.244; Sinkers, 81.7 and 64.8%. Differences were not significant by *t* test for specific gravity ($P > 0.05$) but were for sinkers ($P < 0.01$).

DISCUSSION

The specific gravity and sinkers techniques, as selection criteria during backcrossing and inbreeding, were successful for increasing kernel density of two inbreds of maize, B73G and A632. Success was shown for kernel density for three types of seed: Self-pollinated seed of inbreds, F₁ seed of tester crosses, and F₂ seed of hybrids. Differences for kernel density between backcross inbreds and the recurrent parent were evident when averaged for two states (B73G group) and for three generations (A632 group).

Specific gravity is a measure of the collective density of all kernels in a sample. The sinkers technique identifies the kernels with highest density, which then can be saved for the next generation. The level of selection can be set by varying the concentration of the salt solution. Both techniques were used for making selections. Specific gravity served as an initial screening and as a guide for determining the concentration of the salt solutions. The sinkers technique was the major criterion for selection because it was more consistent in defining differences and because it identified the seeds for the next generation. The goal was to have an average of 50% sinking kernels among the samples of a group so that the samples would be arrayed around that mean. In some instances, the concentration of the salt solution was misjudged and it was necessary to reevaluate with an adjusted concentration.

A limited attempt to relate specific gravity and sinkers with test weight of grain gave positive results. Both gave

good correlations on a dry weight basis, but low correlations on a fresh weight basis (22.8% moisture). Yield tests did not show significant differences for grain yield, kernel moisture, erect plants, ear height, or days to tasseling between backcross inbreds and the recurrent parent in tester crosses for two tests combined.

The specific gravity technique was less consistent than sinkers for seed produced on hybrid plants. One reason could be the relatively small sample size used for the specific gravity technique. An improvement in this technique would be needed if it were to be used extensively for seeds produced on hybrid plants, otherwise sinkers would be the preferred technique.

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