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Impact of early seed quality selection on maize inbreds and hybrids

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Abstract
Seed composition, genetic background, and environment influence seed quality. Plant breeders selecting for improved seed composition seldom select their inbreds for improved seed quality traits. The standard germination test evaluates seed viability, but it often overestimates field performance. Therefore, seed vigor tests are used to predict seed germination under stressful environments. There is little information on the possible genetic improvement of seed selected for both, improved seed composition and vigor. The objectives of this study were 1) to evaluate the seed quality attributes of a group of maize (Zea mays L.) inbreds selected for high protein content; 2) to assess whether early selection improves the seed quality and decreases the phenotypic variability of seed vigor in a group of inbreds; and 3) to calculate the breeding parameters of general (GCA) and specific combining ability (SCA). During summer 2002 and 2003, related sets of inbred lines were grown in replicated nurseries near Clinton, IL, and Ames, IA. Seed from each inbred was produced by self pollination. Some of the inbred lines grown in 2002 and all grown in 2003 were high-protein white lines that also had been selected for germination cold tolerance and high post-accelerated aging field emergence. In 2002, the mean percentage of standard germination test, saturated cold test, accelerated aging test, soak test, and fast green test for the group of selected high-protein white inbreds were significantly ($P \leq 0.05$) higher than the corresponding average values of the yellow inbred checks. There was genetic variability for seed quality in these sets of high protein white inbreds even after a very intense selection process for improved seed quality traits. GCA effects for seed quality were more important than the SCA effects, indicating that the additive effect of the inbreds was more important than the dominant effect to the final seed quality of the hybrids. Selecting inbreds for high seed quality early in the breeding program is beneficial and important for improving germination and field performance.

Keywords
Maize, Seed quality, Seed composition, Inbred selection

Disciplines
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IMPACT OF EARLY SEED QUALITY SELECTION ON MAIZE INBREDS AND HYBRIDS

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ABSTRACT - Seed composition, genetic background, and environment influence seed quality. Plant breeders selecting for improved seed composition seldom select their inbreds for improved seed quality traits. The standard germination test evaluates seed viability, but it often overestimates field performance. Therefore, seed vigor tests are used to predict seed germination under stressful environments. There is little information on the possible genetic improvement of seed selected for both, improved seed composition and vigor. The objectives of this study were 1) to evaluate the seed quality attributes of a group of maize (*Zea mays* L.) inbreds selected for high protein content; 2) to assess whether early selection improves the seed quality and decreases the phenotypic variability of seed vigor in a group of inbreds; and 3) to calculate the breeding parameters of general (GCA) and specific combining ability (SCA). During summer 2002 and 2003, related sets of inbred lines were grown in replicated nurseries near Clinton, IL, and Ames, IA. Seed from each inbred was produced by self pollination. Some of the inbred lines grown in 2002 and all grown in 2003 were high-protein white lines that also had been selected for germination cold tolerance and high post-accelerated aging field emergence. In 2002, the mean percentage of standard germination test, saturated cold test, accelerated aging test, soak test, and fast green test for the group of selected high-protein white inbreds were significantly ($P \leq 0.05$) higher than the corresponding average values of the yellow inbred checks. There was genetic variability for seed quality in these sets of high protein white inbreds even after a very intense selection process for improved seed quality traits. GCA effects for seed quality were more important than the SCA effects, indicating that the additive effect of the inbreds was more important than the dominant effect to the final seed quality of the hybrids. Selecting inbreds for high seed quality early in the breeding program is beneficial and important for improving germination and field performance.

KEY WORDS: Maize; Seed quality; Seed composition; Inbred selection.

INTRODUCTION

Maize yield has improved significantly during the past six to eight decades (Duvick *et al*., 2004). These increases can be attributed to genetic progress and changes in cultivation conditions (Duvick and Cassman, 1999). The authors attributed some of the yield increase to relative changes in seed composition (i.e., increase in starch content at the expense of the protein level), which can have profound effect on seed quality (Munamava *et al.*, 2004).

Seed quality comprises the viability and vigor characteristics of a seed that allows it to grow and develop into a normal seedling under a wide range of field conditions (Bewley and Black, 1994). Seed quality is influenced by seed genetics, composition, and the environment where seeds are grown. Maize seed size and reserve deposition were greatly influenced by the availability of assimilates during grain filling (Borrás *et al.*, 2004; Gambín *et al.*, 2006). Protein content decreased and starch content increased

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TABLE 1 - *Entry number and pedigree description of the inbred lines evaluated in 2002 and 2003.*

Entries	Pedigree inbred	Entries	Pedigree inbred	Entries	Pedigree inbred
2002				2003	
2p01	HiPrM)-001-1-1	2p30	HiPrF)-016-1-5	3p51	HiPrF)-040-4-1-1-B
2p02	HiPrM)-002-4-1	2p31	HiPrF)-016-1-6	3p52	HiPrF)-040-4-1-2-B
2p03	HiPrM)-005-1-1	2p32	HiPrF)-040-2-1	3p53	HiPrF)-057-1-1-1-B
2p04	HiPrM)-005-1-2	2p33	HiPrF)-040-4-1	3p54	HiPrF)-057-1-1-2-B
2p05	HiPrM)-012-1-1	2p34	HiPrF)-047-4-1	3p55	HiPrF)-093-1-1-1-B
2p06	HiPrM)-012-1-2	2p35	HiPrF)-047-4-2	3p56	HiPrF)-093-1-1-2-B
2p07	HiPrM)-012-1-3	2p36	HiPrF)-047-4-3	3p57	HiPrM)-005-1-1-1-B
2p08	HiPrM)-018-3-1	2p37	HiPrF)-047-4-4	3p58	HiPrM)-005-1-1-2-B
2p09	HiPrM)-018-4-1	2p38	HiPrF)-047-4-5	3p59	HiPrM)-012-1-1-1-B
2p10	HiPrM)-032-3-1	2p39	HiPrF)-057-1-1	3p60	HiPrM)-012-1-1-2-B
2p11	HiPrM)-032-3-2	2p40	HiPrF)-061-1-1	3p61	HiPrM)-018-4-1-1-B
2p12	HiPrM)-032-3-3	2p41	HiPrF)-080-3-1	3p62	HiPrM)-018-4-1-2-B
2p13	HiPrM)-032-3-4	2p42	HiPrF)-080-3-2	3p63	HiPrM)-057-2-1-1-B
2p14	HiPrM)-032-3-5	2p43	HiPrF)-080-3-3	3p64	HiPrM)-057-2-1-2-B
2p15	HiPrM)-032-3-6	2p44	HiPrF)-082-4-1	3p65	IN456 (check)
2p16	HiPrM)-032-3-7	2p45	HiPrF)-082-4-2	3p66	IN725 (check)
2p17	HiPrM)-045-1-1	2p46	HiPrF)-082-4-3	3p67	IN447 (check)
2p18	HiPrM)-045-1-2	2p47	HiPrF)-093-1-1	3p68	IN436 (check)
2p19	HiPrM)-048-2-1	2p48	HiPrF)-093-1-2		
2p20	HiPrM)-048-3-1	2p49	HiPrF)-093-1-3		
2p21	HiPrM)-048-3-2	2p50	HiPrF)-093-1-4		
2p22	HiPrM)-057-2-1	2p51	IN354 (check)*		
2p23	HiPrM)-057-2-2	2p52	IN494 (check)		
2p24	HiPrF)-008-4-1	2p53	IN329 (check)		
2p25	HiPrF)-008-4-2	2p54	IN388 (check)		
2p26	HiPrF)-016-1-1	2p55	IN510 (check)		
2p27	HiPrF)-016-1-2	2p56	IN511 (check)		
2p28	HiPrF)-016-1-3	2p57	IN403 (check)		
2p29	HiPrF)-016-1-4				

* IN is a code for the yellow commercial inbred checks used in the experiments.

under a limited supply of assimilates, while oil content remained unchanged (BORRÁS *et al.*, 2002). There is wide genetic variation in maize oil content (DUNLAP *et al.*, 1995a,b). These changes in seed composition strongly influenced seed quality (MUNAMAVA *et al.*, 2004), and lines with high protein content had good seed quality, regardless of their oil content. It is important that breeders, especially those breeding for grain quality, evaluate the impact of selection early in their breeding programs to avoid seed quality problems in advanced lines that could result in poor germination and field emergence. To our knowledge, however, there are no previous studies analyzing the breeding improvement parameters of seed quality traits.

Hypothetically, if we select high protein content maize inbred lines for improved seed quality early in the breeding program, we should be able to improve the overall seed quality and reduce the phenotypic variability for this trait. To test this assumption, we conducted a set of experiments with three objectives 1) to evaluate the seed quality attributes of a group of maize inbreds selected for high protein content; 2) to assess whether early selection improves the seed quality and decreases the phenotypic variability of seed vigor in a group of inbreds; and 3) to calculate the breeding parameters of general (GCA) and specific combining ability (SCA) to provide guidelines for developing breeding lines with good seed quality.

MATERIALS AND METHODS

Seed production

The inbred lines used in this study are listed in Table 1. During summer 2002, seed from inbred lines and their corresponding testcross hybrids (made by crossing the lines to one or more of three commercial tester lines) were produced by self-pollination in Clinton, IL. The 50 lines planted in 2002 were derived by self-pollinating for three generations in a BC_1 (backcrossed once to the recurrent parent) selected for elevated protein content. These lines also had been selected for cold tolerance and high post-accelerated aging (AA) germination percentage during the early cycles of breeding (S_0 to S_1, and S_2 to S_3, respectively). Cold tolerance selection was performed by subjecting imbibed seeds to 5°C for 19 days and selecting those seeds that germinated. Additionally, all the female inbreds were selected after a vigor screening with the AA test. Before planting, seeds were aged at 43°C for 72 hours (Association of Official Seed Analysts, Aosa, 1983) and screened for survival in the field. Lines with 67% field emergence or above were selected (selection intensity was 0.80). Protein data of the self-pollinated seed of the inbreds grown in 2002, and the yield trial data of their hybrids were used to select the female and male lines used in 2003. Three female lines were selected, with two "sister" lines per selection, making six total lines used as a female parent. These are called "sister" lines because they are all derived from the same BC_1 S_2 family. Four male lines were selected, each with two sister lines, making eight total lines used as a male parent. The fourteen selected inbred lines were self-pollinated in fields near Clinton, IL and Ames, IA in an RCBD with two replications to produce the seed for this study. The entries in 2002 and 2003 experiments were thus related but were not identical. Seven commercial yellow inbred checks, which included the recurrent parents, were included in 2002. The two original recurrent parents and two parents of the check hybrid were included in 2003 as checks; checks were not selected for seed quality. In 2003 the 14 lines were crossed using a factorial to produce 48 crosses used in the seed quality analyses. Normal seed production practices of cultivation and insect and soil fertility management were used in both locations. Precipitation and maximum and minimum monthly temperatures for 2002 and 2003 are given in Table 2.

Ears of all entries were harvested at a seed moisture content of approximately 300 g kg^{-1}, which was approximately physiological maturity as determined by black layer formation. Husks were removed and ears were dried with ambient forced air. At 130 g kg^{-1} moisture content, ears were shelled using a laboratory size sheller (model LS91, Custom Seed Equipment, Altoona, IA). Seeds were stored 30 to 90 days in a cold room at 10°C and 50%

TABLE 2 - *Precipitation and maximum and minimum temperatures for Ames, Iowa and Clinton, Illinois for years 2002 and 2003.*

Location/year	Parameter	Month						
		May	June	July	August	September	October	Total
2002								
Ames, IA	Precip. (mm)[a]	112.3	71.1	133.6	122.4	32.0	38.1	471.4
	Temp. max. (°C)[b]	20.9	28.3	29.0	26.0	24.1	11.4	
	Temp. min. (°C)[c]	7.6	16.8	17.7	15.1	10.8	0.9	
	GDD_{10}[d]	172	351	412	338	252		1525
Clinton, IL	Precip. (mm)	159.8	97.3	101.3	243.3	45.0	80.5	609.9
	Temp. max. (°C)	21.1	29.4	32.2	29.4	27.8	16.7	
	Temp. min. (°C)	8.3	17.2	20.0	18.3	14.4	6.1	
	GDD_{10}	197	384	464	422	332		1799
2003								
Ames, IA	Precip. (mm)	95.3	60.1	89.0	21.8	87.4	20.6	374.2
	Temp. Max. (°C)	20.4	25.6	28.0	28.8	22.9	18.7	
	Temp. Min. (°C)	8.8	14.0	17.0	16.9	8.9	4.4	
	GDD_{10}	164	281	369	377	211		1402
Clinton, IL	Precip. (mm)	115.3	78.2	189.3	132.1	57.7	43.3	641.7
	Temp. Max. (°C)	21.7	26.1	29.4	29.4	23.9	18.9	
	Temp. Min. (°C)	10.6	13.9	17.8	17.8	11.1	6.1	
	GDD_{10}	214	302	412	412	253		1593

[a] Precip. (mm) - precipitation in millimeters.

[b] Temp. max. (°C) - average maximum temperature in °C.

[c] Temp. Min. (°C) - average minimum temperature in °C.

[d] GDD_{10} - growing degree-days in degree Celsius = [(minimum temperature + maximum temperature) × 2^{-1}] - 10°C accumulated per days.
If maximum temperature is >30°C, then maximum temperature = 30°C
If minimum temperature is <10°C, then minimum temperature = 10°C.

TABLE 3 - *Significance table for standard germination, saturated cold, accelerated aging (AA), fast green, electrical conductivity (EC), and soak tests for inbred lines harvested at Ames, IA and Clinton, IL in 2002 and 2003.*

	Standard germination	Saturated cold	AA	Fast green	EC	Soak test
2002						
GEN	***	**	NS	***	***	***
LOC	NS	**	NS	***	NS	NS
GEN*LOC	NS	**	NS	***	***	NS
2003						
GEN	NS	***	***	*	***	***
LOC	NS	***	*	NS	NS	NS
GEN*LOC	NS	***	NS	NS	***	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
NS, non significant at the 0.05 level of probability.

RH until laboratory analyses were performed. Seeds from 15 to 18 plants per entry were bulked within location due to limited seed supply.

Seed quality determination

The standard germination test (AOSA, 2004) was used to determine seed viability. Five additional seed quality tests were conducted: the saturated cold test, the AA test, the soak test, electrical conductivity of the steep water after 6- and 24-hour imbibition, and fast green (FG) test. All tests were replicated twice.

Standard germination test: One hundred seeds were germinated on moistened crepe cellulose paper and incubated at constant 25°C, with alternating light cycles of 4 h for a total of 12 h of light, for 7 days. Seedlings were classified as normal, abnormal, or dead (AOSA, 2004).

Saturated cold test: A plastic grid rack of 60 × 40 cm was placed in a 61 × 41 × 5-cm tray. A single germination paper towel of 60 × 30 cm was wrapped over the plastic grid rack serving as a wick, and two additional paper towels were placed on top. One liter of tap water was poured on the paper towels and allowed to soak through into the tray. Excess water was sufficient to keep the paper towels and soil saturated throughout the test. Sandy loam soil from a field was sifted through a 70-mm sieve and sprinkled over the paper towels to form a thin layer. The trays with media were chilled for 24 h at 10°C before planting. One hundred seeds were placed embryo side down on top of the soil (MARTIN *et al.*, 1988). The seed trays were placed in a dark cold room at 10°C for 8 days, and then moved to 25°C for 3 days of continuous light. Seedlings were evaluated following Association of Official Seed Analysts (AOSA) Rules for Testing Seeds (AOSA, 2004).

Accelerated Aging (AA) test: One hundred seeds were placed on top of the screen inside AA boxes (Hoffman Manufacturing Co., Albany, OR), each 10 × 10 × 4 cm, and 40 ml of tap water was added. Boxes were covered and placed in an AA chamber (Nuaire, Plymouth, MN) at 42°C for 96 h. Seeds were planted in moist crepe cellulose paper and placed in constant 25°C growth chambers with alternating light cycles of 4 h for a total of 12 h of light. Seedlings were evaluated after 7 days according to AOSA rules (AOSA, 2004).

Soak test: One hundred seeds were soaked in 200 ml of deionized water for 48 h (CERWICK *et al.*, 1995), and then they were planted in accordance to a standard germination test (AOSA, 2004).

Electrical conductivity (EC): Electrical conductivity was measured using the inoLab pH/Cond Level1 (Weilheim, Germany). Two replicates each of 100 seeds were soaked in 200 ml of deionized water at 20°C. Electrical conductivity of the water was measured after 6- and 24-h. Conductivity was recorded in microsiemens cm^{-1} $gram^{-1}$ of seed (μS cm^{-1} g^{-1} seed) (AOSA, 1983).

FG test: Fifty seeds were submerged in fast green solution for 15 to 30 s, rinsed under running tap water, and air-dried. Staining patterns were used to classify seed damage (KOEHLER, 1957), with more stain indicating more pericarp damage.

Statistical analysis

Our narrow-based breeding population was the equivalent of a BC_1: 25% contribution of one line for the female and 75% contribution of one line as the recurrent parent for the male. Sister lines were S_4 sisters from the same S_3 plant. Therefore, "sister" lines are treated as separate and independent lines.

Laboratory tests were conducted in a completely random design, and repeated two times. Data were analyzed as a two-way factorial with location and genotype as main factors. Analysis of variance was determined by general linear model procedures in Statistical Analysis System (SAS INSTITUTE, 1990). Because genotype × location effects were highly significant for some seed quality measurements, mean separation was conducted within location.

Selection efficiency for improved seed quality was evaluated using *t* test (TTEST procedure in SAS). Mean seed quality of the white high-protein inbreds was compared with the mean of the checks.

General (GCA) and specific combining ability (SCA) effects of the inbreds in 2003 was analyzed in SAS (SAS INSTITUTE, 1990) using Proc Mixed with estimates determined with Restricted Maximum Likelihood method and Satterthwaite confidence intervals about the variance components. Male, female and location were main effects. The experiment was analyzed as a factorial in a RCBD. Data was analyzed with genotype and their interactions as fixed effects, and genotype x location interactions as random effects. The model used was:

$Y_{iklp} = U_i + L_{ki} + R(L)_{lki} + M_{pi} + F_{pi} + (M\times F)_{pi} + (M\times L)_{kpi} + (F\times L)_{kpi} + (M\times F\times L)_{lkpi} + E_{lkpi}$

Where,

U_i = mean effect for trait $_i$

L_{ki} = effect of location $_k$ on trait $_i$

$R(L)_{lki}$ = effect of replication $_l$ in location $_k$ on trait $_i$

M_{pi} = effect of genotype of the male parent $_p$ on trait $_i$

F_{pi} = effect of genotype of the female parent $_p$ on trait $_i$

$(M\times F)_{pi}$ = effect of the genotypic interaction between parents on trait $_i$

$(M\times L)_{kpi}$ = effect of the interaction for genotype of male parent $_p$ on trait $_i$

$(F\times L)_{kpi}$ = effect of the interaction for genotype of female parent $_p$ on trait $_i$

$(M\times F\times L)_{lkpi}$ = effect of the interaction between genotype $_p$ and location $_k$ on trait $_i$

E_{lkpi} = error term

RESULTS AND DISCUSSION

Seed quality in 2002

The genotype × location (G × L) interactions for standard germination, AA, and soak tests were nonsignificant (Table 3). However, the G × L interactions for saturated cold, FG and EC were significant, indicating that the environment where the inbreds were grown influenced some aspects of seed quality. In July and August 2002, the average daytime and nighttime temperatures in Clinton averaged 3°C higher than in Ames (Table 2). In addition, the growing degree-days (GDD) in Clinton were 136°C higher than in Ames. Heat stress during grain filling is known to shorten seed filling (TeKrony and Hunter, 1995) and to cause reductions in kernel weight and starch, protein, and oil contents in the seed (Wilhelm *et al.*, 1999). Inbreds evaluated at Ames, however, did not produce adequate quantity of seed to conduct all tests. The average weight of 100 seeds in Clinton was significantly ($P \leq 0.05$) lower than in Ames (21.5 and 24.5 g, respectively) (data not shown). The G × L interaction for weight of 100 seeds was also significant (data not shown); thus differences in inbred responses to seed size were likely due to the differences in daytime and nighttime temperatures between these two locations. Heat stress, and especially warmer temperatures during the night, is associated with negative changes in seed quality (Keigley and Mullen, 1986). Precipitation, however, was normal at both locations.

Table 4 shows the mean for standard germination, soak, and accelerated aging test for both locations in 2002. The G × L interaction for these tests was not significant in 2002, thus results are presented for both locations combined. Standard germination test results ranged from 83.50 to 100%, from 79.00 to 99.00% in the soak test, and from 68.50 to

Table 4 - *Percentage standard germination, soak, and accelerated aging (AA) tests for inbred lines at both locations harvested in 2002.*

Entry	Standard germination	Soak test	AA
	%		
2p03	97.50 abc	93.50 abc	82.00
2p05	95.00 abcde	88.50 bcd	73.00
2p07	96.00 abcde	94.50 abc	84.00
2p08	96.00 abcd	97.50 ab	83.50
2p09	95.00 abcde	99.00 ab	88.50
2p10	99.00 abc	96.50 ab	88.50
2p11	94.50 abcde	94.50 abc	86.50
2p16	92.00 abcde	93.50 abc	97.00
2p17	95.50 abcd	93.50 abc	90.00
2p18	89.50 de	93.50 abc	86.50
2p20	97.50 abc	95.25 abc	91.75
2p22	92.00 abcde	91.00 abc	68.50
2p23	97.00 abcd	93.50 abc	90.50
2p24	96.50 abcde	97.25 ab	95.25
2p25	91.50 bcde	92.00 abc	86.00
2p26	98.85 abc	98.00 ab	96.50
2p27	97.00 abcd	94.50 abc	94.50
2p28	99.00 abc	98.00 ab	97.00
2p29	99.50 ab	96.50 ab	91.00
2p30	100.00 a	98.50 ab	89.00
2p31	98.00 abc	95.50 abc	94.00
2p33	96.00 abcde	96.00 abc	91.50
2p34	95.00 abcde	94.50 abcf	92.50
2p35	97.75 abc	98.75 ab	91.75
2p37	93.50 abcde	94.50 abc	92.00
2p38	97.00 abcd	96.00 abc	97.50
2p39	96.50 abcde	96.00 abc	85.00
2p40	98.00 abc	98.50 ab	88.00
2p41	98.00 abc	98.50 ab	94.00
2p42	99.50 ab	98.75 ab	93.00
2p43	98.50 abc	99.00 a	84.50
2p44	97.50 abc	97.00 ab	90.00
2p45	95.75 abcde	95.50 abc	89.00
2p46	97.50 abc	94.00 abc	89.00
2p47	96.00 abcde	90.00 abcd	89.00
2p48	97.25 abc	97.75 ab	94.25
2p49	95.50 abcde	96.50 ab	91.00
2p50	92.50 abcde	90.00 abcd	91.50
2p53	95.50 abcde	94.50 abc	92.50
2p54	89.00 e	83.00 de	83.00
2p55	91.00 cde	91.00 abc	83.00
2p56	92.00 abcde	86.50 cd	80.00
2p57	83.50 f	79.00 e	84.50

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability. AA means are not significantly different ($P \leq 0.05$).

TABLE 5 - *Percentage saturated cold test, fast green, and average electrical conductivity (EC) for inbreds harvested at the Clinton location in 2002.*

Entry	Saturated cold test	Fast green	Avg EC
	%		µS cm^{-1} g^{-1} seed
2p03	88.50 ab	26.00 o	3.3 cdefghij
2p05	80.50 ab	41.00 jklmno	4.8 abcd
2p07	74.50 ab	59.00 cdefghij	3.8 cdefgh
2p08	86.00 ab	43.00 hijklmn	2.9 efghij
2p09	92.00 ab	50.00 ghijklm	1.9 ij
2p10	82.50 ab	32.00 no	3.2 cdefghij
2p11	77.50 ab	52.00 fghijklm	4.0 bcdefg
2p17	92.50 ab	59.00 cdefghij	2.2 ghij
2p18	82.00 ab	53.00 efghijkl	2.9 efghij
2p20	82.00 ab	59.00 cdefghij	3.4 cdefghij
2p22	68.00 ab	50.00 ghijklm	5.5 ab
2p23	71.00 ab	39.00 klmno	5.9 a
2p24	86.00 ab	35.00 no	2.8 efghij
2p25	82.50 ab	41.00 jklmno	2.7 efghij
2p26	95.50 a	78.00 ab	2.8 efghij
2p27	85.00 ab	78.00 ab	3.7 cdefghi
2p28	85.50 ab	62.00 bcdefgh	3.9 cdefgh
2p29	87.50 ab	72.00 bcd	2.7 efghij
2p30	87.00 ab	75.00 bcd	3.1 cdefghij
2p31	81.00 ab	78.00 ab	3.9 cdefgh
2p33	87.00 ab	57.00 cdefghij	2.1 hij
2p34	83.50 ab	51.00 ghijklm	2.6 efghij
2p35	90.00 ab	58.00 cdefghij	2.8 efghij
2p37	83.50 ab	71.00 bcde	2.7 efghij
2p38	88.00 ab	61.00 bcdefghi	2.9 efghij
2p39	85.50 ab	68.00 bcdefg	3.4 cdefghij
2p40	89.00 ab	79.00 ab	3.8 cdefgh
2p41	87.00 ab	79.00 ab	2.0 hij
2p42	88.50 ab	67.00 bcdefgh	2.4 fghij
2p43	92.00 ab	91.00 a	1.8 j
2p44	74.00 ab	56.00 defghijk	3.5 cdefghij
2p45	85.50 ab	63.00 bcdefgh	2.6 efghij
2p46	85.50 ab	64.00 bcdefgh	3.9 cdefgh
2p47	74.00 ab	78.00 ab	4.2 bcdef
2p48	75.00 ab	75.00 bcd	3.1 cdefghij
2p49	89.00 ab	70.00 bcdef	2.7 efghij
2p50	81.50 ab	64.00 bcdefgh	2.3 fghij
2p53	91.00 ab	49.00 hijklm	3.0 defghij
2p54	69.00 ab	52.00 fghijklm	4.4 bcde
2p55	80.50 ab	51.00 ghijklm	4.8 abc
2p56	69.00 ab	56.00 defghijk	4.2 bcdef
2p57	64.50 b	37.00 lmno	3.6 cdefghij

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 6 - *Percentage saturated cold test, fast green, and electrical conductivity (EC) for inbreds harvested at the Ames location in 2002.*

Entry	Saturated cold test	Fast green	Avg EC
	%		µS cm^{-1} g^{-1} seed
2p16	93.00 a	58.00 a	2.2 a
2p20	84.00 a	69.00 a	3.8 bc
2p23	94.00 a	81.00 a	2.5 bc
2p24	82.50 a	67.00 a	2.5 bc
2p26	94.00 a	92.00 a	5.5 c
2p28	95.50 a	77.00 a	2.7 bc
2p35	83.50 a	73.00 a	3.9 bc
2p42	93.50 a	87.00 a	2.8 bc
2p45	88.50 a	61.00 a	2.8 bc
2p48	95.00 a	83.00 a	4.1 bc

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE 7 - *A* t *test comparison between the mean percentage of all of the selected high-protein white inbreds and the checks, and of a subset of sister inbreds and their common ancestor for standard germination, saturated cold test, accelerated aging (AA), fast green (FG), electrical conductivity (EC), and soak tests for inbred lines harvested in 2002 and 2003.*

	Standard germination	Saturated cold	AA	FG	EC	Soak test
2002						
Mean of selected inbreds†	96.51	85.34	89.83	63.65	3.18	95.70
Mean of the checks‡	90.20	74.60	84.60	50.40	3.98	86.60
t value	4.21	3.06	2.53	3.38	-2.52	3.93
Probability > | *t*|	0.002	0.012	0.024	0.005	0.03	0.003
Mean of related inbreds††	97.03	86.34	91.63	69.00	3.05	96.41
Mean of common ancestor‡‡	95.50	91.00	92.50	49.00	3.00	94.50
t value	0.61	-3.35	-0.19	10.24	0.22	0.54
Probability > | *t*|	0.65	0.03	0.88	0.0001	0.85	0.68
2003						
Mean of selected inbreds§	87.90	87.49	89.88	71.49	5.41	93.14
Mean of the checksδ	95.16	90.13	92.99	71.29	4.54	97.22
t value	0.21	0.19	-0.13	0.42	1.49	-1.03
Probability > | *t*|	0.83	0.85	0.09	0.68	0.145	0.31
Mean of related inbreds§§	91.75	86.20	87.15	77.20	4.28	92.40
Mean of common ancestorδδ	98.75	91.25	96.75	69.50	2.97	97.25
t value	-3.71	-1.60	-4.03	1.00	4.11	-2.92
Probability > | *t*|	0.001	0.152	0.002	0.38	0.003	0.01

† Mean for each test includes inbreds 2p03 to 2p50.
‡ Mean for each test includes inbred checks 2p51 to 2p57.
†† Mean for each test includes sister inbreds 2p24 to 2p50.
‡‡ Mean for each test of the common parent 2p53.
§ Mean for each test includes inbreds 3p51 to 3p64.
d Mean for each test includes inbred checks 3p65 to 3p68.
§§ Mean for each test includes sister inbreds 3p51 to 3p64.
δδ Mean for each test of the common parent 3p65.

97.50% in the accelerated aging test (Table 4). The saturated cold test ranged from 64.50 to 95.50% in Clinton and from 82.50 to 95.50% in Ames (Tables 5 and 6). High-protein white inbreds (2p03 to 2p50) significantly performed better in all seed quality test than the yellow commercial lines (2p53 to 2p57) used as male checks in 2002, as determined by the *t* test (Table 7). Also, the mean EC of these high-protein white inbreds was significantly lower than the mean EC of the yellow checks, an indicator of superior seed quality. To evaluate the seed quality improvement of the progeny with respect to a common ancestor, closely related high-protein white female inbreds 2p26, 2p27, 2p28, 2p29, 2p30, 2p31, 2p40, 2p41, 2p42, 2p43, 2p48, and 2p49 were compared with inbred 2p53, a common parent of the breeding cross from which all these inbreds are derived. A *t* test comparison determined that the mean saturated cold test for this closely related subset of inbreds was significantly lower than that of their common yellow ancestor check (2p53) (Table 7). The yellow check 2p53 has excellent seed quality, but the seed quality of the white donor inbred is unknown. If the seed quality of the white donor was poor, it would likely lead to poor seed quality in the resulting progeny if selection for seed quality had not been practiced. Even if seed quality of the white donor inbred was good, a progeny line with poor seed quality could have segregated if selection pressure was not applied. These results indicate that selection for improved seed quality was very effective because the results of most tests are not significantly lower than 2p53, with the exception of saturated cold test.

Inbreds 2p28, 2p31, and 2p48 were among the high-protein white female inbreds with very high seed quality as determined by the standard germination test, the soak test, and the accelerated aging test (Table 4). These inbreds, however, had marginal germination in the fast green test and had high EC (Tables 5 and 6). These results indicate that although the overall seed quality of the group of inbreds was improved through early selection there is still genetic variation for seed quality.

The FG test showed that inbred lines 2p03, 2p10, and 2p24 had a highest percentage of pericarp damage at the Clinton location (Table 5). The EC values for these same inbreds were not different from the remaining inbreds, which indicates independence between these two tests. The FG test measures pericarp damage and the EC measures the

TABLE 8 - *Percentage standard germination, fast green, accelerated aging (AA), and soak tests for inbred lines at both locations harvested in 2003.*

Entry	Classification§	Standard germination	Fast green	AA	Soak test
		%			
3p51	Female	93.50 a	73.00 ab	87.25 a	93.50 ab
3p52	Female	95.75 a	65.50 ab	88.25 a	94.00 ab
3p53	Female	83.50 a	76.00 ab	78.50 ab	81.50 bc
3p54	Female	96.50 a	77.00 ab	93.00 a	97.50 a
3p55	Female	93.25 a	87.50 a	85.25 a	93.25 ab
3p56	Female	86.25 a	83.50 ab	89.25 a	91.75 ab
3p57	Male	80.00 a	39.50 b	95.50 a	98.00 a
3p58	Male	87.50 a	50.50 ab	84.25 a	97.75 a
3p59	Male	80.00 a	59.00 ab	93.00 a	91.75 ab
3p60	Male	74.25 a	66.50 ab	93.00 a	93.00 ab
3p61	Male	71.50 a	62.00 ab	92.00 a	89.00 ab
3p62	Male	80.25 a	73.50 ab	93.00 a	96.50 ab
3p63	Male	79.50 a	56.00 ab	68.50 bc	60.50 d
3p64	Male	73.50 a	61.33 ab	62.50 c	56.50 d
3p65	Check	98.00 a	69.50 ab	96.75 a	97.25 a
3p66	Check	82.00 a	65.00 ab	89.00 a	93.50 ab
3p67	Check	88.00 a	61.00 ab	84.75 ab	95.25 ab
3p68	Check	66.00 a	62.00 ab	67.00 bc	74.00 c

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.
§ Female, male or checks.

TABLE 9 - *Percentage saturated cold test and electrical conductivity for inbred lines produced in Clinton, Illlinois and Ames, Iowa in 2003.*

Entry	Classification§	Saturated cold test		Electrical conductivity	
		Ames	Clinton	Ames	Clinton
		%		μS cm^{-1} g^{-1} seed	
3p51	Female	88.50 ab	94.00 a	3.02 e	3.20 gh
3p52	Female	87.50 ab	92.50 a	4.19 cde	2.84 h
3p53	Female	—	91.50 ab	—	5.22 d
3p54	Female	—	96.50 a	—	4.26 ef
3p55	Female	87.00 ab	76.50 ab	5.36 cd	4.98 de
3p56	Female	77.50 ab	70.50 bc	4.95 cd	4.80 de
3p57	Male	92.00 ab	89.00 ab	3.81 de	3.73 fg
3p58	Male	90.50 ab	84.00 ab	5.72 c	3.93 fg
3p59	Male	88.00 ab	79.50 ab	7.30 b	4.82 de
3p60	Male	88.00 ab	93.50 a	5.37 cd	3.57 fgh
3p61	Male	—	74.00 ab	—	7.45 c
3p62	Male	95.00 a	86.50 ab	2.76 e	2.77 h
3p63	Male	—	56.00 c	—	10.23 b
3p64	Male	—	24.00 d	11.72 a	14.10 a
3p65	Check	94.50 a	88.00 ab	2.57 e	3.37 gh
3p66	Check	88.00 ab	89.50 ab	3.61 de	5.70 d
3p67	Check	87.00 ab	55.50 c	3.91 cde	5.37 d
3p68	Check	70.50 ab	—	7.25 b	—

Means within a column followed by the same letter are not significantly different at the 0.05 level of probability.
§ Female, male or checks

leakage occurring during the early stages of seed imbibition due to damage to the cell membrane (AOSA, 1983). Greater pericarp damage also increases the amount of seed leachates in the water and, therefore, EC increases. In these inbreds, however, this did not occur. It is conceivable that when selecting for a seed quality attribute, such as survival to AA, we inadvertently selected for other characteristics such as improved membrane structure and composition. Further research is needed to explain some of these changes.

Seed quality in 2003

In 2003, only the saturated cold test and electrical conductivity G × L interactions were significant (Table 3). These results allowed us to discuss genotypic differences for most seed quality parameters across both environments. In 2003, both locations suffered a drought; however, the water deficits in Ames were more severe than in Clinton. The total August 2003 rainfall in Ames was 21.8 mm, while GDD were similar at both locations. The historical average precipitation (1951 to 2004) for August is 97.8 mm. This drought coincided with seed maturation. BURRIS (1977) demonstrated that seed quality depends strongly on environmental conditions during seed development and maturation. Seed size in Clinton was significantly smaller than Ames (22 and 25 g, respectively) (data not shown). There was a significant G × L interaction for seed size, thus inbreds were affected differently by the drought. In general, seed quality of the 2003 inbreds as evaluated by the standard germination test was significantly ($P \leq 0.05$) lower than that of the 2002 inbreds (Tables 8 and 9). The standard germination percentage for most inbreds was in the 80s, which is considered below average. The standard germination test is conducted under ideal conditions (AOSA, 2004) and most commercial inbreds should germinate above the 90th percentile. The mean standard germination percentages of all the inbreds were 95.90 and 83.85% in 2002 and 2003, respectively. However, the saturated cold tests results in 2002 and 2003 were 84.31 and 82.50%; the accelerated aging test results were 87.20 and 91.40%; and the soak test were 91.20 and 95.20%, respectively. The selection progress for seed quality attributes from 2003 and 2004 seemed to improve the seed vigor of these selected lines.

TABLE 10 - *Significance table for standard germination, saturated cold, accelerated aging (AA), fast green, electrical conductivity (EC), and soak tests for crosses harvested in 2003.*

Source	df	Standard germination	Saturated cold	AA	Fast green	EC	Soak test
		P value					
2003							
LOC	1	0.001	0.001	0.001	0.03	0.001	0.001
Rep (LOC)	2	NS	0.001	NS	0.001	NS	NS
GEN	47						
Male	7	0.001	0.001	NS	0.002	0.001	0.001
Female	5	0.007	0.02	0.001	NS	0.001	0.001
Male*Female	35	0.02	NS	NS	NS	0.001	0.001
Male*LOC	7	NS	0.003	0.034	NS	0.001	0.001
Female *LOC	5	NS	NS	NS	NS	0.001	0.001
Male*Female *LOC	35	NS	NS	NS	NS	0.001	0.001
C.V. (%)		8.2	9.4	7.3	16.1	1.6	7.1

For the 2003 experiment, inbreds 3p51, 3p52, 3p53, 3p54, 3p55 and 3p56 were selected as high-protein white lines with the potential to be used as females to generate commercial hybrids. Inbreds 3p57, 3p58, 3p59, 3p60, 3p61, 3p62, 3p63, and 3p64 were selected as high-protein white inbreds to be used as male parents. Inbreds 3p65, 3p66, 3p67, and 3p68 were high-protein white checks. White inbred lines in 2002 and female lines in 2003 had higher standard germination in both years (96.20% in 2002 and 91.75% in 2003) (data not shown) than the checks (Table 7) and male parents (90.60% in 2002 and 79.90% in 2003) (data not shown). The saturated cold test results indicated that the female parent lines had better seed quality than the males. The mean saturated cold test percentage of the females was 83.93% at the Clinton location and 90.35% at Ames in 2002 and 87% at the Clinton location and 85% at Ames in 2003 (data not shown). Male mean saturated cold test was 74.80% in 2002 and 81.67% in 2003 (data not shown). Because seed quality of the hybrid largely depends on the female inbred parent, it is more important that the female parents have better seed quality than the male parents. However, male parent seed quality is still important for inbred and hybrid seed production.

Inbreds 3p61, 3p63, and 3p64 were among inbreds with the highest values for EC, indicating lower quality seed. The average EC of inbreds 3p63 and 3p64 was significantly higher (above 10 µS cm^{-1} g^{-1} seed) than the average for all inbreds (5.28 µS cm^{-1} g^{-1} seed) in Clinton. The EC of inbred 3p64 also was significantly higher (11.70 µS cm^{-1} g^{-1} seed) than the average of all inbred lines produced in Ames. The other tests also showed that these three inbreds, intended to be used as male parents, had poor seed quality.

GCA and SCA in 2003

In the GCA and SCA analysis, the replication within location effect was added to the model. Under this new model, the location effects were significant for all tests, including standard germination. This is probably due to the reduction in the error term used to test locations.

The GCA effects of male were significant for all tests, except for AA (Table 10). The female GCA effects were significant for all tests, except fast green. These results were not expected. It is generally believed that male seed quality attributes are not as important to the final seed quality of the cross. However, for this set of inbreds, the male seed quality attributes were significant to the final seed quality of the hybrid. Maybe this means that males can contribute to seed quality if early selection for seed quality on the males is used, as it was in these experiments. There was a strong maternal effect for most tests, which was expected. Previous research reported a strong maternal influence on seed quality (BURRIS, 1977). It was surprising that fast green was not significant. This test relates to the pericarp integrity, which is maternal tissue (BEWLEY and BLACK, 1994), thus a strong maternal effect was expected.

The additive genetic effects of females and males (GCA) are important for the seed quality

traits. If the seed quality of the inbreds is good, the resulting hybrid should have the superior characteristics of the parents. Thus, it is important to have good seed quality characteristics in the inbreds, to have good seed quality in the hybrid.

The SCA effects of female ¥ male interactions or nonadditive effects were significant for only half of the traits measured. The saturated cold test, AA and fast green were non-significant, while the standard germination, EC and soak tests were significant (Table 10). These results indicate that the combination of certain specific lines is very important for improving standard germination, EC and soak tests values in the hybrids, but not for the other seed quality traits.

Although these results only apply to this specific set of high protein white maize lines, it is important to evaluate other materials to see if these conclusions extend to typical yellow dented maize lines.

CONCLUSION

There were significant differences among genotypes for all tests but AA in 2002, and all tests but standard germination test in 2003 (Table 3). These results indicate that, even though the white inbreds had been selected for high protein content and good seed quality characteristics during the early cycles of breeding (S_0 to S_1), the maize seed still had genetic variability for seed quality. Consequently, additional improvement for seed quality can be made. For example, inbred 3p61 is a sister to inbred 3p62. Both these inbreds have opposite seed quality characteristics. Selecting for seed quality during inbred line development is beneficial, especially when working with wide breeding crosses such as these white × yellow seeded crosses. The results of selection are progeny inbred lines, many of which have good seed quality.

The GCA effects for seed quality are relatively more important than the SCA effects. The additive effects of the inbred in this study are more important to hybrid seed quality than the dominant effects. Early selection for good seed quality characteristics in this group of inbreds translated to good seed quality in the hybrids.

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