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Seed Quality Assurance in Maize Breeding Programs: Tests to Explain Variations in Maize Inbreds and Populations

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Abstract
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ABSTRACT

Maize (*Zea mays* L.) breeders are interested in evaluating the seed quality of their inbred lines, as seed quality has a strong relationship to field emergence. There is little information, however, on the influence of the seed quality of the inbred on field emergence of the hybrid. The objectives of this research were to (i) determine whether seed quality tests and a seed quality index of the inbred parents and F_2 seed are correlated with field emergence of F_1 hybrids, and (ii) determine how many tests are necessary to calculate this index. Standard germination (SG), saturated cold (SC), and soak (Soak) tests, and the inbred quality index (IQI) were calculated on inbred parents and their corresponding F_2 progeny, and field emergence was measured on associated F_1 hybrids produced in Clinton, IL in 2002 and 2003. The tests and index of the parental inbreds and F_2 progeny correlated poorly with early field emergence of the F_1 hybrids. All tests were required to calculate the seed quality index. By averaging several seed quality tests into a single index, the poor seed quality performance of inbreds and F_2 populations in some tests can be masked by other tests. The seed quality index might be useful when ranking inbreds based on seed quality but not as a selection tool.

For centuries people have selected maize germplasm to increase crop productivity. Today, breeders select for numerous desirable agronomic traits, such as high yield, disease resistance and grain chemical composition. In many cases, however, the seed quality characteristics of the selected materials are not evaluated (Burris, 2000). Seed quality is a critical factor affecting the early performance and growth of agricultural crops; therefore, the improvement of yield or nutritional traits should not be achieved at the expense of seed quality.

The Germplasm Enhancement of Maize (GEM) project, a cooperative breeding project between the public and private breeding sectors led by the USDA-ARS (Pollak, 2002), is introgressing exotic germplasm (used as the female parent in the original breeding cross) into commercial breeding materials to increase yield and improve grain quality. Seed scientists believe these exotic lines should be tested for seed quality before using in breeding programs (Munamava et al., 2004). Developing and testing inbred lines in hybrid combinations represent a large and expensive component of GEM and any breeding program. If a seed quality index can be used effectively to eliminate exotic breeding lines that may contribute to poor seed quality in the hybrid, the expense of developing these undesirable lines is eliminated.

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Seed quality is defined as the viability and vigor attributes of a seed that make possible the emergence of normal seedlings in a wide range of field environments. There are a number of tests used to assess seed quality for inbred and hybrid selection, including the SC test, the accelerated aging (AA) test (Association of Official Seed Analysts, 1983), and the Soak test (Martin et al., 1988). The fast green (FG) test evaluates another aspect of seed quality: pericarp integrity (Koehler, 1957). Some of these seed quality tests correlate well to seedling emergence under average or good field conditions (DeVries et al., 2007; Martin et al., 1988) but not under poor field conditions (Woltz and TeKrony, 2001). The results from these tests are used independently or are combined into a seed quality index to classify inbreds and hybrids according to seed quality. Navratil and Burris (1980) published equations to predict field emergence of maize inbred lines using the SG test, the cold test, root and shoot growth, and days to 50% emergence based on growing degree units (GDU). The laboratory tests that best predicted field emergence were different for various inbreds. While the equation including GDU to 50% emergence and SG test accurately predicted field emergence for most inbreds, the equation including the cold test was the best predictor for others. TeKrony et al. (1989a, 1989b) designed a vigor index based on the results of the SG test, the cold test, the AA test, and seedling growth rate. Field emergence was positively correlated with the vigor index. Low- and medium-vigor seed lots had lower emergence (TeKrony et al., 1989a) and reduced seedling growth rate (TeKrony et al., 1989b) compared to high vigor seed lots.

The IQI (Hoegemeyer and Gutormson, 2000) also combines several seed quality tests into an index that can be used to rank inbreds according to seed quality. The following five seed tests are used in the calculation of the IQI: SC test, AA test, Soak

Abbreviations: FG, fast green test; GEM, Germplasm Enhancement of Maize; GDU, growing degree units; IQI, inbred quality index; PCA, principal component analysis; SC, saturated cold test; SG, standard germination test; Soak, soak test.

test, electrical conductivity (EC) of the steep water after 6- and 24-h imbibition periods, and FG test. The IQI is considered among maize breeders as a valuable tool to characterize seed quality of inbreds and their progeny (Munamava et al., 2004). However, there is no information on the correlation of this index with field emergence. Moreover, if a minimum of 2 replications of 100 seeds for each test are conducted as suggested by the authors of the index, 1000 seeds (approximately 340 g) per genotype are required to compute the IQI. Maize breeders seldom produce large amounts of inbred or hybrid seed during the early stages of inbred line development. The maize ears are hand-pollinated and bagged to assure the genetic purity of the seed. The number of seeds produced under these controlled pollination conditions are limited. A maximum of 300 to 500 seeds may be available for genotype selection and seed testing. This low number of seeds could restrict the use of IQI for evaluating seed quality early in a maize breeding program. Therefore, determining if the IQI could be calculated using fewer tests would benefit many breeding programs.

We collected a massive data set from germplasm that included inbreds, F_1 hybrids, and F_2 progeny. Parts of this data set were used to calculate genetic parameters of general combining ability and specific combining ability for seed quality (Goggi et al., 2007). We used the full data set from one location (Clinton, IL) in the present study to (i) determine whether seed quality tests and a seed quality index of the inbred parents and F_2 seeds are correlated with field emergence of F_1 hybrids, and (ii) determine how many tests are necessary to calculate this index.

MATERIALS AND METHODS

Seed Production

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. All the lines derived from a commercial inbred by commercial inbred breeding cross, so they have a narrow genetic base. However, the progeny lines were still segregating for seed quality (Goggi et al., 2007). The 50 lines planted in 2002 were derived by self-pollinating for 3 generations in a BC_1 (backcrossed once to the recurrent parent) selected for elevated protein content. The seed for this IQI study was obtained from a routine field evaluation of the 50 high protein lines with 40 of their respective test crosses. Self-pollinated inbred and F_1 seed (F_2 seed) from these materials were used for seed quality evaluations conducted in 2002. Seed from self-pollinating the three commercial tester lines, three additional commercial inbred lines, and two commercial hybrids produced at Clinton, IL in 2002 were also included.

Protein content of the self-pollinated seed was analyzed by near infrared reflectance to select the materials for the next growing season. Based on the 2002 protein content data of the inbreds from the preliminary screening trial, three female lines were selected. Two sister lines per female line were selected to make a total of six female lines for the 2003 experiments. Four male lines were selected, each with two sister lines. These are called sister lines because they are all derived from the same BC_1 S_2 family. A total of eight male parent lines were selected for the experiments of 2003. These 14 inbred lines and their corresponding 48 hybrid crosses between the male and female lines were self-pollinated in a nursery near Clinton, IL. Inbred and F_2 seed from these materials was used in this study. We chose to study the seed quality of the F_2 because all seed composition measurements were conducted in this generation, and because the seed produced by self-pollination of the F_1 is F_2. The entries in the 2002 and 2003 experiments were thus similar, but not identical.

The seed for these seed quality experiments was produced at Clinton, IL during the 2002 and 2003 growing seasons. The selected inbreds and hybrids were planted in a randomized complete block design with two replications in the 2002 and 2003 nurseries. Normal seed production practices of cultivation, insect and soil fertility management were used in maize breeding nurseries.

Seeds were harvested at physiological maturity as determined by black layer formation in the central row of seeds in each ear (approximately at a seed moisture concentration of 300 g kg^{-1} fresh weight). Husks were removed and ears were dried with ambient forced air to preserve maximum seed quality. At 130 g kg^{-1} fresh weight moisture content, ears were shelled using a laboratory size sheller (model LS91, Custom Seed Equipment, Altoona, IA). Small and shriveled seeds were removed during shelling. Seeds were stored for 30 to 90 d at 10°C and 50% relative humidity until laboratory analyses were performed. Seeds from all ears harvested from each replication were bulked due to limited seed supply (approximately 30 ears). Field replications were maintained as replications throughout the experiments, with no further subsampling.

Seed Quality Determination

The SG test was used to determine seed viability. The SC, AA, and Soak tests were used as vigor tests. The FG test was used to determine pericarp integrity (Koehler, 1957). The IQI was used for each line and was calculated as

$$\text{IQI} = \{\text{SC} + \text{AA} + \text{FG} + \text{Soak} + [200 - (\text{EC6h} + \text{EC24h})]\}/60$$

where SC = % germinated, AA = % germinated, FG = % non-damaged, Soak = % germinated, EC6h = electrical conductivity at 6 h μS cm^{-1} g^{-1} seed, and EC24h = electrical conductivity at 24 h μS cm^{-1} g^{-1} seed, and 60 = constant used to standardize IQI. The IQI values range from 1 (worst) to 10 (best) (Hoegemeyer and Gutormson, 2000).

Standard Germination Test

One hundred seeds per field replication 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 d. Seedlings were classified as normal, abnormal, or dead (Association of Official Seed Analysts, 2006).

Saturated Cold Test

A plastic grid rack of 60 by 40 cm was placed in a 61 by 41 by 5 cm tray. A single germination paper towel of 60 by 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 sifted through a 70-mm sieve was sprinkled over the paper towels to form a thin layer. The trays holding this media were chilled for 24 h at 10°C before planting. One hundred seeds per field replication 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 d, and then moved to 25°C for 3 d of continuous light. Seedlings were evaluated following the procedures of the Association of Official Seed Analysts (1992).

Accelerated Aging Test

One hundred seeds per field replication were placed on top of the screen inside AA boxes (Hoffman Manufacturing Co., Albany, OR), each 10 by 10 by 4 cm, and 40 mL of tap water were 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. Seedlings were evaluated after 7 d according to the procedures of the Association of Official Seed Analysts (1983).

Soak Test

One hundred seeds per field replication were soaked in 200 mL of deionized water for 48 h (Cerwick et al., 1995), and then planted in accordance with a SG test (Association of Official Seed Analysts, 2006).

Electrical Conductivity

Electrical conductivity was measured using the inoLab pH/Cond Level1 (Weilheim, Germany). Two replicates each of 100 seeds were weighed and soaked in 200 mL deionized water at 20°C. Electrical conductivity of the water was measured after 6 h and 24 h, according to the seed quality index methodology (Hoegemeyer and Gutormson, 2000). Conductivity was recorded in microsiemens cm^{-1} $gram^{-1}$ of seed ($\mu S\ cm^{-1}\ g^{-1}$ seed) (Association of Official Seed Analysts, 1983).

Fast Green Test

Fifty seeds were submerged in 0.1% FG solution for 15 to 30 s, rinsed under running tap water, and air-dried. Staining patterns were used to classify seed damage, with more stain indicating more pericarp damage (Koehler, 1957).

Field Emergence

To compare seed quality estimates in the laboratory and their relationship to field performance, seedling emergence of the F_1 in the field was also recorded. Hybrids were planted in a yield trial at the Clinton, IL location in a completely random block design with two replications. Field emergence was counted at the V2 growth stage of the maize planted in 2002 and 2003.

Statistical Analysis

F_1 Hybrid Field Emergence Predictions Using the Parental and F_2 Seed Quality

Pearson's correlation coefficients were computed between field emergence data of the F_1 and IQI, SG test, SC test, and Soak test of the inbreds and F_2 population to determine which test and generation correlated better with field emergence. To calculate the correlation between the test results from a parent (inbred) and the field emergence of the corresponding F_1, all F_1 hybrids with the same parent were averaged. Thus, the field emergence of each group of F_1 that shared a female or male parent were averaged and used as the field emergence value of the F_1 hybrid. To calculate the correlation coefficients of the F_2 progeny and field emergence of the F_1, the values of the F_2 test were correlated directly with the field emergence of the F_1.

Principal component analysis

Principal component analysis (PCA) is a statistical method that allows the reduction of a data set to its most relevant characteristics. In this situation, the original data set is linearly transformed into a smaller combination of uncorrelated variables (tests in our case), without losing the information from the original set of variables. In our case, the variable IQI is calculated by using the average of several tests. If two of these tests are highly correlated, only one of the tests is sufficient to calculate the average, thereby calculating the same information that is obtained when all the tests are used. Variance components analysis was used to determine the presence or absence of a systematic effect of replication. In the absence of a replication effect, data from the two replications could be pooled without generating aggregation effects. Correlations among all tests and the IQI, and PCA (Johnson and Wichern, 2002) were calculated using the open source statistical software R (R Foundation for Statistical Computing, 2004).

Variance component analysis (Searle et al., 1992) was used to detect whether data from the different replications could be pooled to increase the precision of the analyses. Pooling the replication data is appropriate only if there are no systematic effects between replications. In their absence, the observed variation originates only from random sampling variation among genotypes. The linear model used in the variance component analysis was:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where $i = 1,..., 50$ denotes the genotype; $j = 1, 2$ denotes the replication; y_{ij} denotes the value of a given test for genotype i and replication j; μ = the overall mean of a given test; and α_i denotes the genotype effect. The assumptions of the analysis are that α_i and ε_{ij} are independent, and normally distributed with Var $(\alpha_i) = \sigma^2_B$ (variation between genotypes) and Var $(\varepsilon_{ij}) = \sigma^2_W$ (variation within genotypes). The quantities Var (α_i) and Var (ε_{ij}) are called variance components.

If there are no systematic effects between replications, most of the total variability (defined as $\sigma^2_B + \sigma^2_W$) can be attributed to the genotype to genotype variation (thus, σ^2_W should be much smaller than σ^2_B). To estimate variance components, note first that $\sigma^2_W = E(MS_W)$, where E denotes the expectation function and MS_W denotes the mean squared error within genotypes. Thus, σ^2_W can be estimated by MS_W. Also, following standard calculations, we have that $E(MS_B) = 2\sigma^2_B + \sigma^2_W$, where MS_B denotes the mean squared error between genotypes, which implies that σ^2_B is estimated as $(MS_B - MS_W)/2$. This model was applied to the data from 2002 and

Table 1. Variance components and percentage variation attributed to each source of variability for various seed quality tests of hybrids at Clinton, IL, 2002 and 2003.

		Total variance		Between genotypes		Within genotypes	
Year	Test	Variance component	Variation	Variance component	Variation	Variance component	Variation
			%†		%		%
2002	Soak	5.11	100	3.29	64.47	1.81	35.53
	AA	31.31	100	26.19	83.66	5.12	16.34
	SC	20.34	100	16.30	80.15	4.04	19.85
	FG	72.00	100	64.00	88.89	8.00	11.11
	EC	0.58	100	0.17	29.23	0.41	70.77
	IQI	0.23	100	0.04	16.40	0.20	83.60
2003	Soak	4.37	100	2.72	62.25	1.65	37.75
	AA	12.72	100	9.62	75.62	3.10	24.38
	SC	15.08	100	11.66	77.35	3.42	22.65
	FG	265.95	100	250.13	94.05	15.82	5.95
	EC	0.40	100	0.09	23.37	0.30	76.63
	IQI	0.39	100	0.09	23.07	0.30	76.93

† Percentage variation attributed to each source of variability.

Table 2. Pearson's correlation coefficients between seed quality of inbreds and F_2 progeny and field emergence of the hybrid for replication 1 and 2 at Clinton, IL, in 2003.†

Field emergence	Pearson's correlation coefficients			
	SG	SC	Soak	IQI
Replication 1				
Female parent	−0.25	0.31	−0.38	0.02
P value‡	0.63	0.55	0.46	0.96
Male parent	0.43	0.04	0.18	−0.05
P value	0.29	0.92	0.67	0.91
F_2 population	0.17	0.10	0.11	−0.11
P value	0.23	0.48	0.44	0.46
Replication 2				
Female parent	0.34	−0.06	0.27	0.17
P value	0.50	0.92	0.60	0.75
Male parent	−0.49	0.29	0.25	0.22
P value	0.21	0.49	0.55	0.60
F_2 population	−0.01	-0.07	−0.22	−0.36
P value	0.97	0.64	0.13	0.01

† SG, standard germination test; SC, saturated cold test; Soak, soak test; IQI, inbred quality index. Two replications of 100 seeds were tested and results were used to calculate the IQI.

‡ If the probability value is significant ($P < 0.05$) the Pearson's correlation coefficient is different from 0.

2003, and the results are presented in Table 1. This table gives the estimated values for the variance components, as well as the percent of total variability they represent.

RESULTS AND DISCUSSION

The results from the variance component analysis indicated that the variation within the genotype for the EC test was much larger than variation between genotypes, which makes aggregation over replications impossible. These results also held true for the IQI due to the strong correlation between IQI and EC. Based on these findings, pooling the data from the two replications would erroneously increase the strength of correlation, and it would induce unknown aggregation effects for EC and IQI. Therefore, the statistical analysis was performed separately on each replication, of each year, at each location.

F_1 Hybrid Field Emergence Predictions Using the Parental and F_2 Seed Quality

To determine if field emergence of the F_1 hybrid is associated with the seed quality information from the parent lines or the F_2 progeny, Pearson's correlation coefficients were calculated between the seed quality test results of the parents and F_2, and the field emergence of the F_1 hybrid at Clinton, IL, in 2002 and 2003. Pearson's correlation coefficients were calculated for both years and replications. The results of all the correlations calculated were very similar and are presented in Table 2. The SG, SC, Soak, and IQI of the inbred and F_2 correlated poorly with early field emergence of the F_1 hybrid. The SC and Soak tests of the female parent correlated slightly better to field emergence than the male parent, indicating some maternal effect for seed quality. Burris (1977) also reported significant maternal effect for seed germination and vigor, as determined by the SG test and shoot and root dry weight. In our study, the *P* values indicated that the Pearson's correlation coefficients were not significant at $P < 0.05$.

Although in our experiments the SC and Soak tests showed similar results, Martin et al. (1988) reported that the SC test had a greater correlation with maize field emergence than the Soak test. However, as the field conditions deteriorate and become more stressful to the seed, the accuracy of the SC test and the AA test in predicting field emergence decreases (Woltz and TeKrony, 2001). In 2002, the hybrid trials of the F_1 were planted late due to unusually cold, wet conditions during the second half of April and first half of May. Temperatures remained cold throughout the month of May, immediately after planting. The maximum daily temperatures for the 10 d following planting were between 10 and 12°C and the minimum fluctuated between 3 and 5°C. In 2003, the hybrid trials of the F_1 were planted very early (April) with above-average rainfall (Illinois State Water Survey, 2006). These stressful growing conditions could have contributed to the failure of the seed vigor tests of the inbreds and the F_2 progeny to correlate well with early field emergence of the corresponding F_1 hybrids. Similar results were reported by other researchers (DeVries et al., 2007). Although results could be different under normal growing conditions, farmers routinely encounter stressful planting conditions. This lack of a strong correlation between tests and field emergence is a significant problem associated with seed quality of hybrid and inbreds. Our results also indicate that there is not a single test or a group of tests that will accurately predict the field emergence of the hybrid based on the seed vigor of the parental lines or the F_2. Thus, the use of only one or two tests is a good alternative to more complicated indexes, since the early emergence prediction capabilities of the index under stressful field environments are similar to those of the individual seed quality tests.

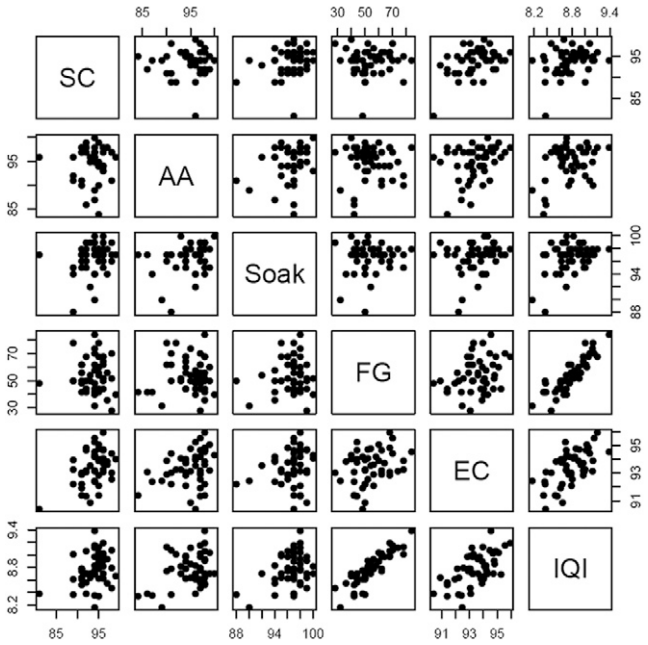

Fig. 1. Scatter plots between the saturated cold (SC), accelerated aging (AA), fast green (FG), soak (Soak), and electrical conductivity (EC) tests, and the inbred quality index (IQI) for data collected at Clinton, IL, Replication 1, 2003. Tests are listed in the diagonal squares. The dots inside the other squares represent the data. Data are in percentage for SC, AA, and FG tests. Data for EC are calculated as [200 – (EC6h + EC24h)] in μS cm^{-1} g^{-1} seed. Values for IQI range from 1 to 10.

Table 3. Correlation coefficients between seed quality tests and IQI of the F2 progeny seeds at Clinton, IL, in 2002 and 2003.†

	Correlation coefficients between tests				
	SC	**AA**	**Soak**	**FG**	**EC**
Replication 1, 2002					
SC		−0.03	0.33	−0.06	−0.06
AA			0.38	−0.12	−0.06
Soak				−0.02	−0.44
FG					−0.02
IQI	0.40	0.16	0.40	0.82	−0.16
Replication 2, 2002					
SC		0.51	0.53	0.31	−0.29
AA			0.09	−0.08	−0.09
Soak				0.08	−0.51
FG					−0.11
IQI	0.83	0.46	0.45	0.73	−0.33
Replication 1, 2003					
SC		0.03	0.24	−0.01	0.45
AA			0.32	0.029	0.36
Soak				0.095	0.38
FG					0.32
IQI	0.30	0.36	0.38	0.88	0.61
Replication 2, 2003					
SC		0.01	0.15	−0.07	0.09
AA			0.21	−0.16	0.20
Soak				0.23	0.29
FG					0.04
IQI	0.34	0.26	0.54	0.77	0.40

† SC, saturated cold test; AA, accelerated aging test; FG, fast green test; Soak, soak test; EC, electrical conductivity test.

Principal Component Analysis of Tests in IQI

The IQI represents an average of five tests. Statistically, if two or more of the tests are highly correlated, it would be possible to compute the index using only the uncorrelated components without missing much information. Initial analysis based on scatter plots between all possible combinations and tests showed similar patterns for years, locations, genotypes, and replications. Therefore, the data presented are only the results of the F_2 population from replication number 1 for seed produced at Clinton, IL, in 2003 (Fig. 1). When two tests are highly correlated, the scatter plot between data of both tests resembles a straight line. Scatter plots from our data set showed that there were no linear patterns between any two seed quality tests, which suggested a low linear correlation between the tests. To confirm the visual representation of the relationship among tests, correlation coefficients were calculated (Table 3). Based on the results of the scatter plots and the correlation coefficients, none of the tests could be omitted from the calculation of the IQI without losing information. Consequently, the information provided by the IQI cannot be obtained by using fewer tests.

The information in Fig. 1 and Table 3 also suggests that the FG test was very important for the overall value of the IQI, as indicated by the high correlation between FG and IQI. This high correlation is represented by the almost linear distribution of points in the scatter plot (Fig. 1) and the correlation coefficient values between FG and IQI ranging from 0.73 to 0.88 (Table 3). The FG is a test seldom used in the seed industry's quality assurance programs. It was originally developed to evaluate mechanical damage in maize. When pericarp damage is substantial, results from the test also correlated with low seed germination percentage (Koehler, 1957). But the test is very subjective and does not predict germination or vigor of a seed lot when the extent of the pericarp damage is small. In this study, the FG test results were strongly influenced by the seed analyst's personal interpretation of the staining patterns on the seed surface.

To further investigate the possibility of removing one or more seed tests from the calculation of IQI, the data were also analyzed using PCA. The primary goal is to construct linear combinations of the original variables (tests in our case) that account for as much of the original total variation as possible (for a detailed explanation of the method, see Chapter 8 of Johnson and Wichern, 2002). The Scree plot can be used as a visual tool to determine how many components are necessary to explain most of the original variation. Although the choice of the number of principal components that are sufficient to explain most of the variation is fairly subjective, it is customary to restrict this number to the factors that have variances above 1, when data have been scaled so that all variables have unit sample variance. Figure 2 illustrates the Scree plot for data of the F_2 population, replication 1 from seed harvested at Clinton, IL in 2003. Similar plots were graphed for the other replications and years (data not shown). Components 1 through 5 in the graph represent the linear combinations of the variables (tests). Based on the Scree plot, only the first principal component has variance greater than 1 and is therefore sufficient to explain most of the variability in the data. Close analysis of the loadings or coefficients of the tests entering the

Table 4. Loadings or coefficients of the individual variables (tests) in each principal component (or linear combination of the tests) and importance of the components expressed as proportion of the variance (and cumulative proportion of the component) for Replication 1 at Clinton, IL, in 2003.†

	Loadings of principal components or tests					Proportion of variance	Cumulative proportion of variance
	SC	AA	Soak	FG	EC		
Component 1	−0.450	−0.412	−0.441	−0.283	−0.594	0.369	0.369
Component 2	0.067	−0.495	−0.438	0.689	0.289	0.210	0.579
Component 3	0.723	−0.449	−0.117	−0.504	0.089	0.201	0.780
Component 4	−0.060	0.482	−0.733	−0.277	0.388	0.150	0.931
Component 5	0.516	0.390	−0.250	0.338	−0.636	0.069	1.000

† SC, saturated cold test; AA, accelerated aging test; FG, fast green test; Soak, soak test; EC, electrical conductivity test.

first principal component for the same location, replication, and year (Table 4) revealed that they range between -0.283 and -0.594. The values of the loadings also suggested that none of the component tests can be discarded without losing significant information. Perhaps an alternative methodology to calculate the IQI would be to conduct the seed quality tests using fewer seeds than recommended by the authors (Hoegemeyer and Gutormson, 2000). This approach would require further research to determine whether a smaller sample size would give the same information as the recommended amounts.

The IQI is a relatively new index for evaluating inbred lines (Hoegemeyer and Gutormson, 2000). In the past, lines were evaluated using tests that would closely resemble field conditions at planting (Martin et al., 1988) or evaluate seed tolerance to drying injury (Adegbuyi and Burris, 1988). When the number of seeds available is not a concern, the IQI could be an alternative for the ranking of inbreds or F_2 according to seed quality. There is a concern, however, that the group of inbreds and F_2 with IQI greater than nine [IQI values range from 1 (worst) to 10 (best)] do not necessarily rank high in all the individual seed quality tests. These findings are illustrated in Fig. 3. The graph represents the genotypes of the F_2 population from both locations and years with an IQI value greater than 9.25 (or approximately 25% of the F_2 population in this study). Also in Fig. 3 is the relative ranking in percentage of the same genotypes in other seed quality tests. Similar results were obtained for the inbreds (data not shown). These results indicate that poor seed performance of a genotype in a vigor test could be diluted or masked by the high score of other tests averaged into the IQI. Thus, an inbred or F_2 with a high IQI (in our case 9.25 or higher) does not necessarily have a high SC or AA percentage (i.e., 90% or higher). Because of the large sample size required to conduct the necessary tests for calculating the index and its low correlation to field emergence, the seed quality index might be useful when ranking finished inbreds based on seed quality (Munamava et al., 2004) but not as a selection tool. We doubt that decreasing the sample size would maintain the accuracy of the index, based on previous studies of sample size (Liu et al., 1999).

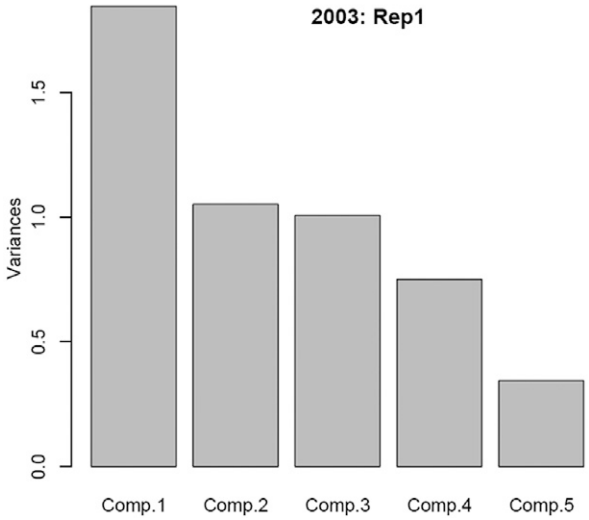

Fig. 2. Screen plot of the principal component analysis for the saturated cold (SC), accelerated aging (AA), fast green (FG), soak (Soak), and electrical conductivity (EC) tests performed on the data collected at Clinton, IL, Replication 1, 2003. Components 1, 2, 3, 4, and 5 are the linear combinations of the variables (or tests).

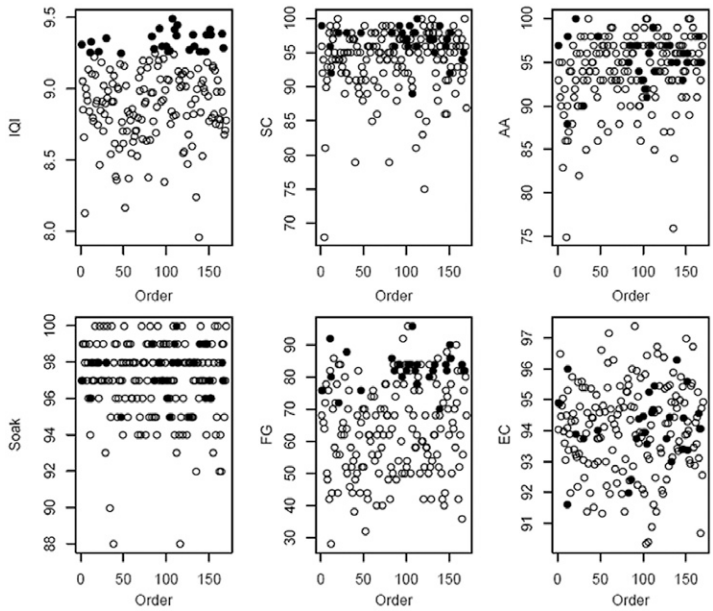

Fig. 3. Plots of ordered tests highlighting the genotypes with inbred quality index (IQI) values in the upper quartile (approximately equal to 9.25) in both years at Clinton, IL, compared with the distribution of the percentage values of the same subset of genotypes in the other seed quality tests that make up the IQI. Data are in percentage for the saturated cold (SC), accelerated aging (AA), and fast green (FG) tests. Data for electrical conductivity (EC) is calculated as [200 - (EC6h + EC24h)] in μS cm^{-1} g^{-1} seed. Values for IQI range from 1 to 10. A total of 160 data points were included in these graphs (closed circles are genotypes with an IQI³ 9.25). Open circles are all other entries.

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