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# Approaches to improve the determination of eligibility for plant variety protection: II. Identification and Evaluation of a Core Set of Morphological Characteristics

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## Abstract

We compared the discriminational abilities of two sets of morphological characteristics among 210 inbred lines of maize (*Zea mays* L). One set of 62 characteristics comprised those required by UPOV and individual PVP authorities. A second core set of 28 characteristics was selected based upon an iterative process where characteristics were examined for their contribution according to three categories (Variabiity, Power, and Genotype by Environment [GxE] interaction) partitioned among nine parameters. An iterative peeling process involving cycles of 1) multivariate analysis to reveal contributions of characteristics to providing discrimination and 2) removal of characteristics to reveal underlying contributions of remaining characteristics was used to select a core set of 28 characteristics. The core set provided slightly less discrimination among inbred lines that were closely similar but was able to discriminate among inbred lines with use of less resources by removing characteristics that were duplicative, had little power of discrimination, or were particularly affected by GxE interactions.

**Keywords**: distinctness, maize, morphology, plant variety protection, UPOV

## Introduction

A new plant variety is eligible to be granted a Plant Variety Protection certificate under the auspices of the Union Internationale pour la Protection des Obtentions Vegetale (UPOV) provided that variety is uniform, stable, and can be shown to be distinct from all previously known varieties of common knowledge in that species. (UPOV, 2002a) requires that these characteristics "be sufficiently consistent and repeatable in a particular environment", "exhibit sufficient variation between varieties to be able to establish distinctness", and "be capable of precise definition and recognition".

It is well known that the expression of morphological characteristics is affected by environmental factors and by the complexity of their genetic control (Comstock and Moll, 1963; Camussi et al, 1983, 1985; Patterson and Weatherup, 1984; Staub et al, 1996; Lombard et al, 2000; Bredemeijer et al, 2002; UPOV 2003, 2007, 2008; Wurtenberger, 2006; Smykal et al, 2008). Nonetheless, UPOV has selected characteristics according to their general utility in characterizing maize inbred lines and hybrids and, more specifically, with regard to their use in facilitating international harmonization or to group like varieties. However, since these characteristics were originally chosen (during the 1960s) for these purposes considerable additional research has recently been conducted into the genetic basis of inheritance for many of these characteristics, including in maize. These studies reveal that the genetic basis of many morphological characteristics, including those once considered to be under fairly simple genetic control, can be more complex, and could therefore more appropriately referred to as "quantitative" (Sourdille et al, 1991; Austin et al, 2001; Bredemeijer et al, 2002; Micklelson et al, 2002; Enoki et al, 2006; Li et al, 2007).

In a previous paper (Law et al, 2011) we reported an evaluation of the robustness and discriminational abilities of morphological characteristics that are currently used by UPOV and individual PVP authorities, to evaluate distinctiveness of maize inbred lines. We evaluated these characteristics according to several criteria which were collectively grouped according to three main paradigms: "Power", Genotype x Environment interaction (signal to noise ratio) and "Precision or Variability" (Law et al, 2011).

The goal of the present study, therefore, is to utilize the evaluation results of the previous report (Law et al, 2011) as a primary basis for selecting a smaller, yet equally effective and thus more cost-effective core set of morphological characteristics for the determination of distinctness among inbred lines of maize and also for grouping similar inbred lines. We evaluate progress toward achieving this goal by comparing the discriminational abilities of the smaller set of characteristics with that provided by a larger set of 62 characteristics.

## Materials and Methods

Inbred lines used to provide data for this study have been described previously as have methods of

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analyses, and rankings of characteristics according to three main paradigms: "Power", Genotype x Environment interaction (signal to noise ratio) and "Precision or Variablity". The rankings and associations among characteristics and parameters have been provided by Law et al (2011).

***Examination of the relative contributions of characteristics and determining which characteristics should comprise the core set***

Our initial derivation of the methodological approach to select a smaller, yet equally useful core set of morphological characteristics was based upon preliminary investigations using information from, and further analyses of a 365 inbred set (Law et al, 2011). However, for the subsequent and final round of analyses presented here we used the 210 maturity zone 3 (MZ3) set of inbreds that covered a more discrete range of maturity. We made this decision because: 1) the subset of 210 inbreds was still large in number, 2) the set included the maturity range of inbred lines that are used to produce hybrids that are widely used in the most productive and corn-intensive agricultural region of the U.S., the central U.S. Corn Belt, and 3) by removing some of the discriminational power of maturity characteristics that was inherent when the more complete range of maturities was utilized (in the 365 inbred set) we were then able to focus the subject-matter of the analysis more equitably on all of the characteristics. Of the 66 characteristics reported upon previously (Law et al. 2011), 4 (KPEREAR, KPERROW, PVP_BARGLUME. NOTILLERPERPLT) were not reported upon consistently and were therefore eliminated from further analysis leaving 62 characteristics.

We developed and conducted an iterative process that comprised several cycles. Within each stage, or cycle of the process, information ranking each characteristic and results showing associations among parameters and their assignation into the paradigms of 1) "Power", 2) Genotype x Environment interaction (signal to noise ratio) and 3) "Precision or Variability" (Law et al, 2011) was used as a basis upon which to prioritize, primarily through elimination, the eventual selection of characteristics that would be retained for membership in the core set. Each individual step of the iterative process comprised: 1) multivariate analysis to facilitate examination of the relative contributions of morphological characteristics to be discriminative among 210 U.S. inbred lines, 2) removing or "peeling" away data for specific sets of characteristics, and then 3) re-examining the relative contributions of the remaining characteristics to showing discrimination among inbred lines.

The technical processes comprising the evidential based peeling strategy were as follows: A baseline Principal Component Analysis (PCA) was conducted, initially with all characteristics, referred to as P0, Subsequent peeling cycles were conducted (named P1, P2, P3 etc). Characteristics that were revealed as only weakly contributing to showing associations or discriminating among inbred lines from observation of the PCA plot were removed, or peeled, A second PCA was then performed after these characteristics were removed, or peeled from the analysis (labeled P1). The results of the P1 PCA therefore began to reveal a subsequent level or layer of inter-characteristic relationships beneath; a level of information which had been hidden, for example, when it was possible only to view the PCA at stage P0. Additional characteristics that were observed to be only weakly contributing to the discrimination among inbred lines based upon their placement in the PCA plot were removed and the PCA repeated (P2). Each peeling cycle of running a PCA analysis and removing characteristics was repeated until the increase in the % of total variation that was explained cumulatively by the Eigen values at each successive peeling cycle was minimal. During the later stages of peeling, we also temporarily removed data for some of the highly effective characteristics in order to be better comprehend the relative contribution of the remaining weaker characteristics in the absence of the confounding effects of the more highly effective characteristics. All characteristics that had been assigned as highly effective in prior stages of the peeling process were retained as members of the final selected core set of characteristics. In circumstances where dense clusters of characteristics were revealed on the PCA plot we also relied upon input from those among the coauthors who have years of first-hand practical experience in recording these characteristics to retain those which they regard as most robust and discriminative. At the end of the peeling process we had established a core set of 28 characteristics (Table 1) as potentially the most discriminative and reliable from among the whole set of 62 characteristics.

***Evaluating the relative effectiveness of the core set of 28 characteristics compared to results obtained using the initial set of 62 characteristics***

The comparative degree of effectiveness for the 28 characteristics set to provide discrimination among inbreds, both quantitatively and qualitatively, to that provided by the 62 characteristic set of data was examined by 1) comparing associations among inbreds based upon comparisons of morphologies for the 62 characteristics with that provided by comparisons of morphologies of the 28 core set of characteristics and 2) comparing the degree of agreement between rank positions of inbred lines according to their placements based upon the initial 62 characteristic set.

Degree of concurrence among rank positions was assessed by using GenStat to form a similarity matrix generated from the 62 characteristics by 210 inbreds. Euclidean metric was used as the computational method appropriate to quantitative and continuous data from these characteristics. The set of each off-diagonal individual inbred by inbred similarity values

Table 1 - Listing of characteristics and designation as either members of the core set or, alternatively, the level of peeling at which they were removed from membership in the core set and the reason for their removal.

| Trait | Level of Peeling where Trait Removed | Reason for Trait Removal |
|---|---|---|
| %ROUND | P0 | High Chi-squared testing annual contribution to SSGxE and high Sergen GxE F-Ratio |
| BARGLUME | P1 | High CV%, high Sigma squared, high GxE Chi-squared, low inbred F (Power) |
| BRTANTHO | P5 | ~Core set~ |
| COBDIAMETR | P1 | Reduced ROE 76%, high Chi-squared |
| D10-90%P | P1 | High CV%, high Sigma squared, high Chi-squared, low "power" |
| DE-50%P | P4 | Weak when assessed within maturity set of traits |
| DE-50%S | P4 | Weak when assessed within maturity set of traits |
| EARDIAMETR | P5 | ~Core set~ |
| EARHT | P5 | ~Core set~ |
| EARINTLNG | P5 | ~Core set~ |
| EARLENGTH | P5 | ~Core set~ |
| EARROWALGN | P0 | High Sergen GxE F-Ratio |
| EARROWNUM | P5 | ~Core set~ |
| EARROWREG | P2 | High Sigma squared, low Min F, very low inbred F, high Chi-squared GxE, reduced % environments with significant Inbred differentiation |
| EARTAPER | P1 | High GxE F, |
| EARWEIGHT | P5 | ~Core set~ |
| EMERGGDU | P5 | ~Core set~ |
| GDU10-90%P | P1 | High CV%, high Sigma squared, low "power" |
| GDUE-50%P | P5 | ~Core set~ |
| GDUE-50%S | P4 | Weak when assessed within maturity set of traits |
| HUSKELENGTH | P5 | ~Core set~ |
| HUSKLENGTH | P5 | ~Core set~ |
| HUSKTIGHT | P4 | High GxE F, CV% |
| KLENGTH | P5 | ~Core set~ |
| KTHICKNESS | P5 | ~Core set~ |
| KTYPE | P5 | ~Core set~ |
| KWIDTH | P5 | ~Core set~ |
| KWT/100K | P5 | ~Core set~ |
| LFANGLE | P2 | Reduced ROE, low Min F, reduced % environments with significant Inbred differentiation |
| LFATTITUDE | P5 | ~Core set~ |
| LFLENGTH | P2 | Greatly reduced ROE, high Chi-squared GxE |
| LFNUMATE | P4 | Low "power" (Min F) |
| LFNUMBER | P4 | Low "power" (Min F) |
| LFWIDTH | P5 | ~Core set~ |
| NOEARS/STALK | P0 | Nil inbred discrimination |
| PLTHT | P5 | ~Core set~ |
| POLLSC | P4 | High Chi-squared GxE, reduced ROE |
| SCORALECOL | P4 | High GxE F, CV%, Sigma-squared |
| SCORANTHERCOL | P3 | Very high CV% and high Sigma-squared, low GxE |
| SCORCOBCOL | P2 | ~Core set~ |
| SCORDRYHSKCOL | P0 | High Sergen GxE F-Ratio |
| SCORENDOCOL | P3 | ~Core set~ |
| SCORFRSHSKCOL | P0 | Weakly significant inbred differences, high Sigma-squared |
| SCORGLUMECOL | P2 | High CV%, high Sigma-squared |
| SCORLEAFCOL | P0 | Nil inbred discrimination |
| SCORSILKCOL | P3 | Very high CV% and high Sigma-squared, low GxE |
| SHANKLNGTH | P5 | ~Core set~ |
| SHANKPOS | P3 | High CV%, high Sigma-squared, high Chi-squared GxE |
| SHED10%GDU | P4 | Weak when assessed within maturity set of traits |
| SHED50%GDU | P5 | ~Core set~ |
| SHED90%GDU | P4 | Weak when assessed within maturity set of traits |
| SHEPUB | P1 | High CV% and Chi-squared GxE |
| SILK50%GDU | P5 | ~Core set~ |
| STALKDIAM | P3 | Reduced ROE, high CV% |
| T#1RYBRANC | P3 | High CV%, high Chi-square GxE |
| T#2RYBRANC | P1 | Very high CV%, high Chi-squared |
| TASSELATTITUDE | P5 | ~Core set~ |
| TAXISFLDEN | P4 | Low "power" (Min F) |
| TBRANANGLE | P4 | High CV%, high Sigma-squared, Low Chi-squared GxE |
| TCENSPKLNG | P5 | ~Core set~ |
| TLENGTH | P5 | ~Core set~ |
| TPEDLENGTH | P5 | ~Core set~ |

was extracted into a single string and converted from similarities to % dissimilarity where 0% is identical. These steps were then repeated using data from the 28 characteristics core set x 210 inbreds. The pairs of % dissimilarities (some 22,000) were ranked for the 62 characteristics x 210 inbred and compared with the corresponding rankings for the 28 characteristics x 210 inbred data set.

We performed contrasting validation tests taking into account how inbred lines were assigned according to PVP status or germplasm constitution.

One grouping of inbreds was on the basis of "inbred protection status". Inbreds with granted PVP (145 inbreds), non-PVP material (52) and pub-

lic checks (13) were identified. A second means of grouping the 210 inbreds irrespective of "protection status" was on the basis of the pedigree lineage: 97 inbreds were classified as Stiff-Stalks (SS) whereas 107 were classified as Non-Stiff-Stalk (NSS), (6 were unclassified). A third classification was based on more detailed pedigree backgrounds thereby allowing the SS inbreds to be further classified as sub-SS types; namely SS1, SS2, SS3 or SS4 types (with 40, 26, 23, 3 inbreds respectively) and with a corresponding splitting of NSS into NSS1 (49 inbreds) and NSS2 (49 inbreds). Other inbreds unrelated to either SS or NSS were allocated as miscellaneous or not categorized.

The off-diagonal terms of the computed dissimilarity matrices were transferred into a vectors; one for inbred pair-wise dissimilarities computed from the full 62 characteristics and the other corresponding to the 28 characteristic dissimilarities.

Using either the full 62 and 28 characteristic sets, summary statistics and key percentile points were computed and histograms plotted for the set of pair-wise dissimilarities.

For each of the PVP, non-PVP, and check sets of inbreds, a Mantel Test (Manly, 1991) was used to compare the dissimilarity matrices between the entire set of 62 characteristics and the core set of 28. An initial correlation between the 62 and 28 characteristic sets was computed. The formal Mantel test then utilized large-scale permutations (10,000 in this case) of rows and columns to assess the proportion of such permuted data having an equal or higher association than that previously observed as the baseline association.

## Results

### *The peeling process and selection of the 28 characteristic core set*

The results of the P0 cycle of multivariate analyses for the MZ3 set of 210 inbreds are presented in Figure 1 as an explanatory aid to show how these data were interpreted in order to determine the characteristics which would be eliminated (or peeled) prior to the subsequent round of PCA and peeling. Percentage total variation accounted for by the first 2 axes was 54.4%; with 3 axes % total variation was 69.5%. Interpretation of the MZ3 PCA axes 1-3. The array of characteristics along each of the three axes of the PCA could be interpreted as follows: Characteristics with a moderate to high CV%, limited influence or interaction with environment low GxE, and

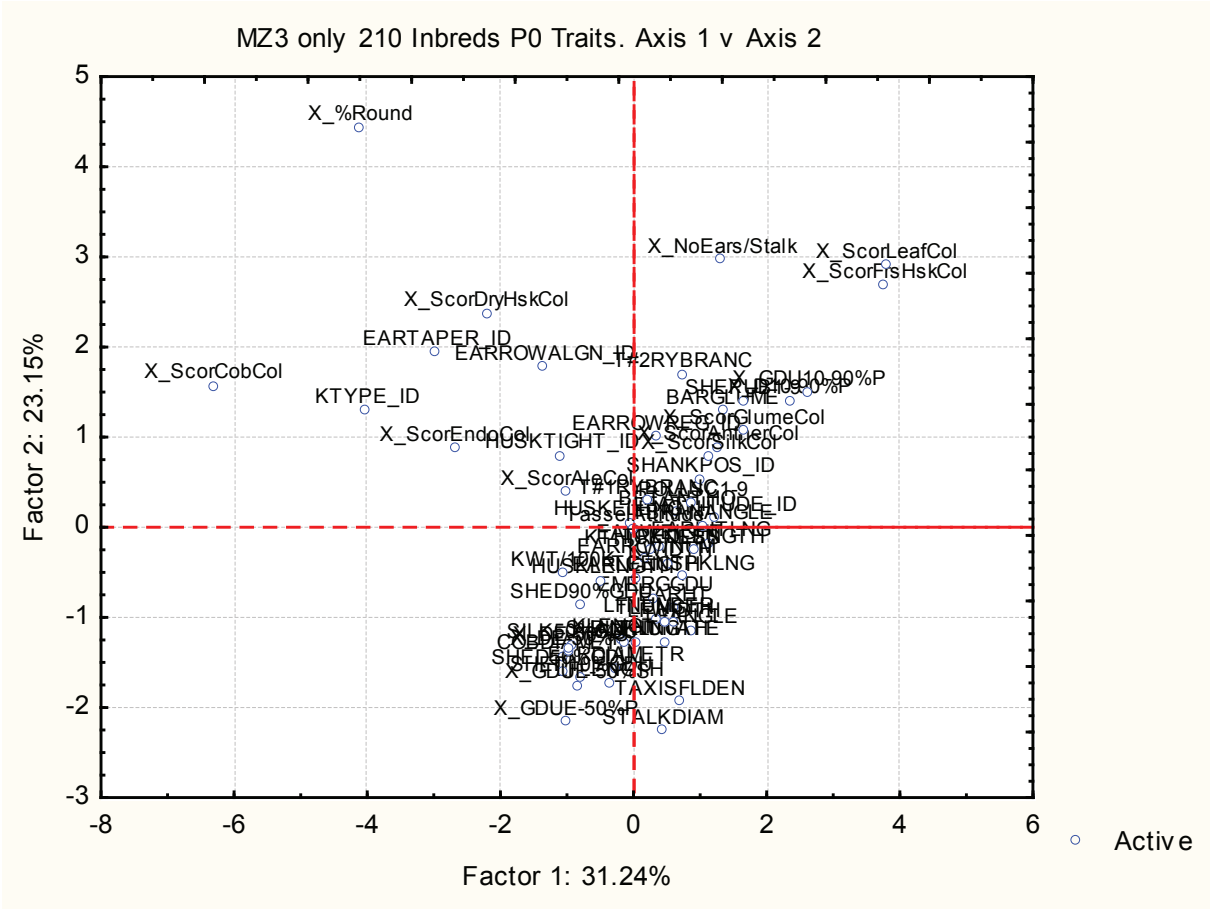


Figure 1 - Associations among characteristics revealed by principal components analysis using data from the 210 MZ3 inbreds. Factors 1 and 2 accounted for 31.2% and 23.2% of total variation, respectively.

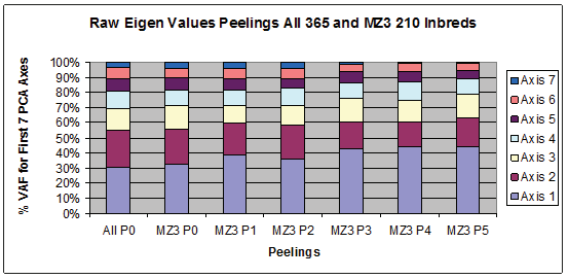


Figure 2 - Percent variation accounted for by the first 7 axes following multivariate analyses of characteristics prior to peeling (P0) for all 365 inbreds and for the 210 MZ3 set of inbreds and then subsequently after each of 5 peelings for the 210 MZ3 inbred set.

high power are arrayed along the negative part of axis 1 (e.g. traits SCORCOBCOL or KTYPE; characteristics with excessive "variability" in the terms of their high Sigma$^2$ and large CV% are on the positive part of axis 1.

The characteristics arrayed along the positive portion of axis 2 have a propensity to interact in a significant way with environments and can therefore be referred to as having detrimentally high "GxE" values (specifically expressed as large GxE F-Ratio (GXEF) [parameter 7 in Law et al, 2011] and % Inbreds with Significant GxE Interaction with Probability p<0.01 (SIGGXEP1) [parameter 8 in Law et al, 2011], (%ROUND is example of such a trait. The negative part of axis 2 could not be readily interpreted as there are many tightly bunched attributes involved in the observed relationship; however one feature is clear that all traits with reduced Range of Expression were included. Characteristics arrayed along axis 3 were associated with "Power" (effective discrimination between inbreds as shown by for example parameters MINF and Inbred_F).

Six characteristics were selected on the basis of observing results from the initial PCA and from results from a prior review of analytical data for each characteristic (Law et al, 2011) to be removed from candidature in the core set. These six characteristics were therefore the initial characteristics to be peeled from the next round (P1) of PCA. These characteristics and the rationale for their excision (in parentheses) were: 1) EARROWALGN (High Sergen GxE F-Ratio) (Law et al, 2011), 2) %ROUND (High Chi-squared testing annual contribution to SSGxE and High Sergen GxE F-Ratio), 3) NOEARS/STALK and SCORLEAFCOL (nil inbred discrimination), 4) SCORDRYKSKCOL (High

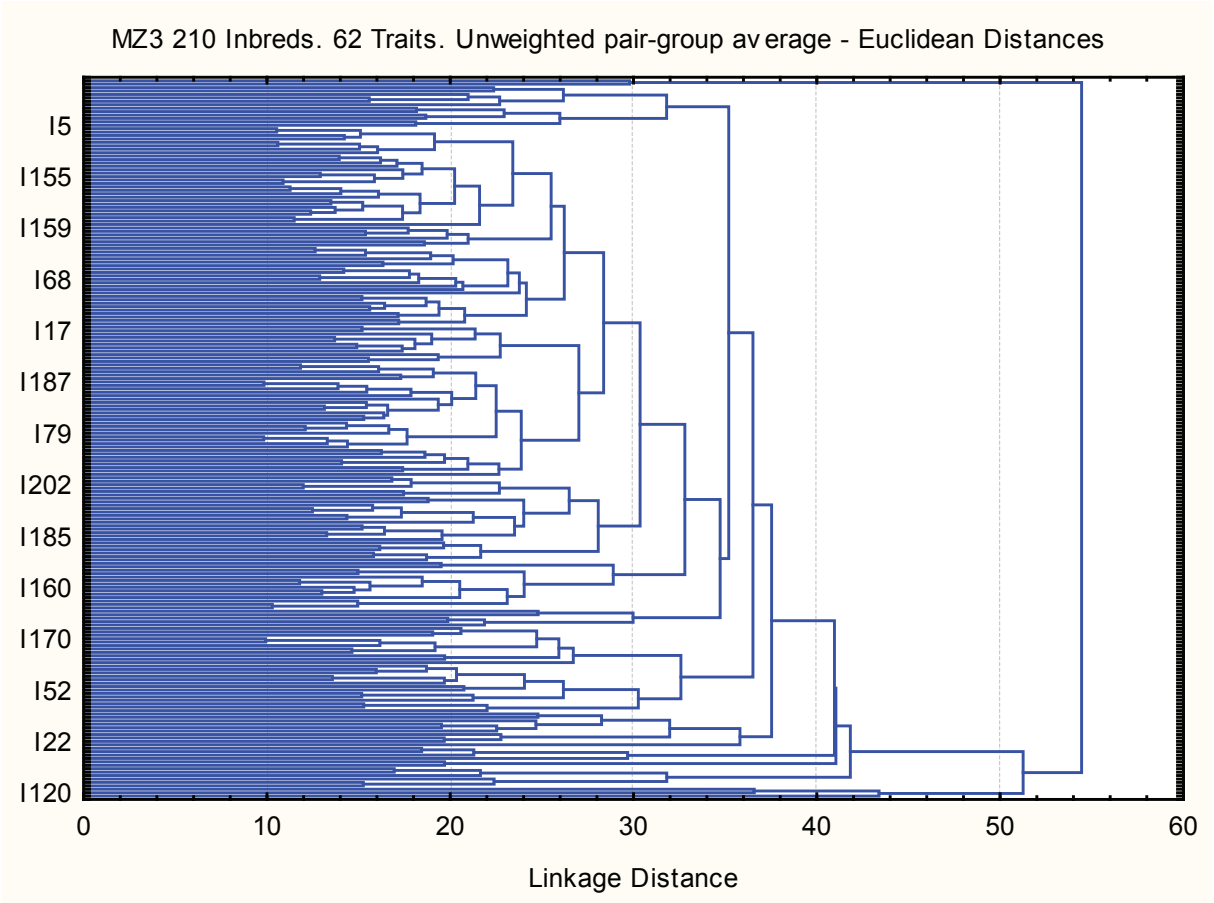


Figure 3 - Associations among 210 MZ3 inbred lines utilizing data from 62 characteristics following cluster analysis (unweighted pair-group average).

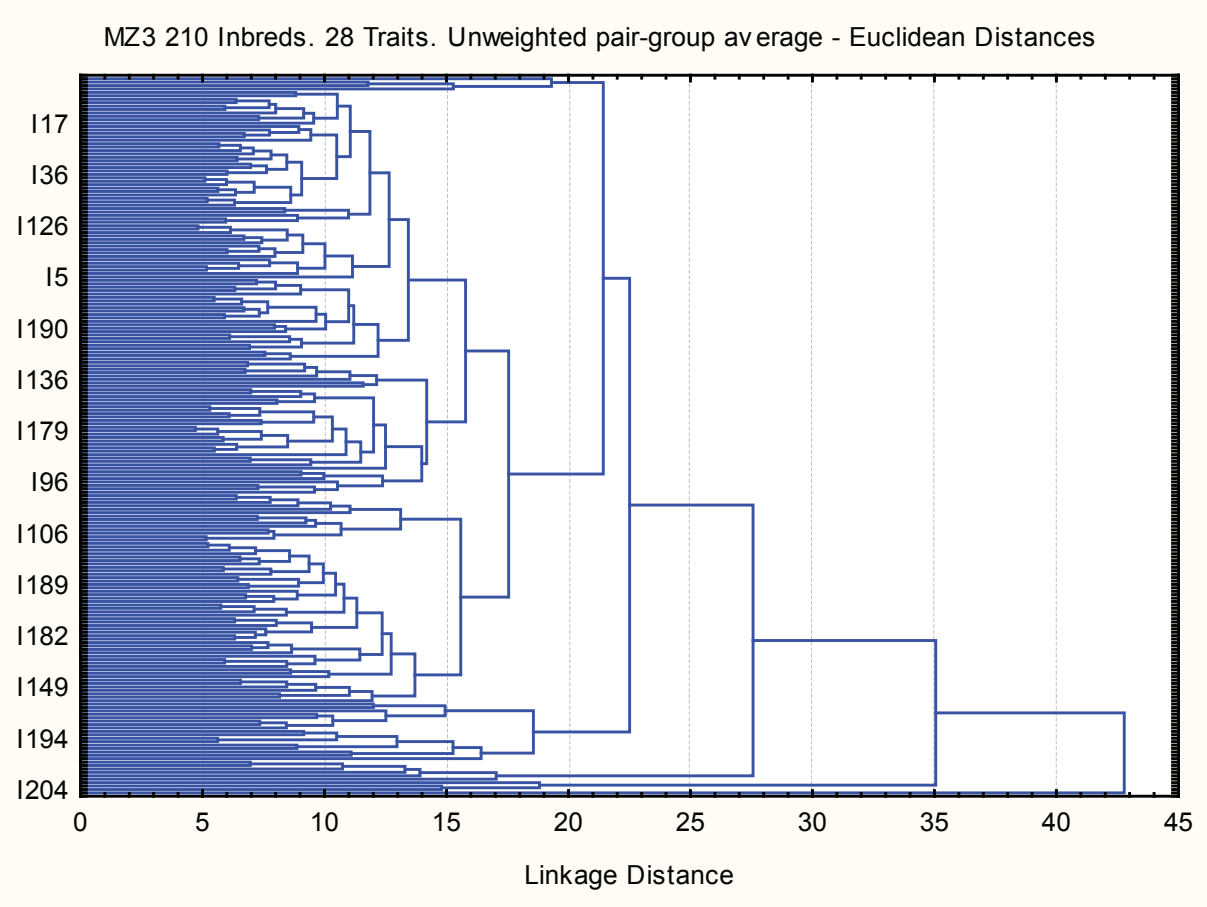


Figure 4 - Associations among 210 MZ3 inbred lines utilizing data from the 28 core set of characteristics following cluster analysis (unweighted pair-group average).

Sergen GxE F-Ratio) and 5) SCORFRSHSKCOL (weakly significance inbred differences, high sigma-squared).

Table 1 identifies the individual characteristics that were eventually designated as members of the 28 core set following the completion of the peeling process. For characteristics that failed to qualify as a member of the core set information is also provided in Table 1 to indicate at which stage of the peeling process they were eliminated and for what reason. Figure 2 presents the % variation that was expressed by the first 7 principal components during each stage of the peeling process P1 to P5 (and including at cycle P0 for the entire set of 365 inbreds). There was a general trend of a monotonic increase in the variation accounted for by the first PCA axis from about 30% to 45% but with reducing benefit at each successive peeling.

***Evaluation and validation of the Core Set of 28 characteristics. Comparison of pair-wise differences among inbred pairs calculated using 62 characteristics and the 28 core set of characteristics***

Associations of inbreds following multivariate analysis (unweighted pair-group average of Euclidean dissimilarities) calculated from the 62 characteristic dataset compared to that calculated from the 28 characteristics set are presented in Figures 3 and 4, respectively. When 62 characteristics were used the minimum linkage dissimilarity that pairs of inbreds were distinguishable was 10 whereas when 28 characteristics were used the minimum linkage dissimilarity of distinction was 5. Correlations for inbred pair-wise dissimilarities for inbreds from contrasting germplasm pedigree backgrounds were (correlation coefficient in parentheses): All Stiff Stalks (0.884), Stiff Stalk Group I (0.841), Stiff Stalk Group 2 (0.772), Stiff Stalk Group 3 (0.930), all Non-Stiff Stalks (0.853), Non-Stiff Stalk Group 1 (0.872), and Non-Stiff Stalk Group 2 (0.821).

***Degree of agreement between rank positions of inbred lines between use of 62 characteristics and 28 characteristics***

Inter-inbred dissimilarities when computed on a 0% - 100% scale using the core set of 28 characteristics resulted in an average dissimilarity shift over 22,000 pair-wise values of 0.097% compared to inter-inbred dissimilarities when computed using the 62 characteristics. Inter-inbred dissimilarities among the 210 inbreds when computed using the 62 characteristics showed that the smallest dissimilarity

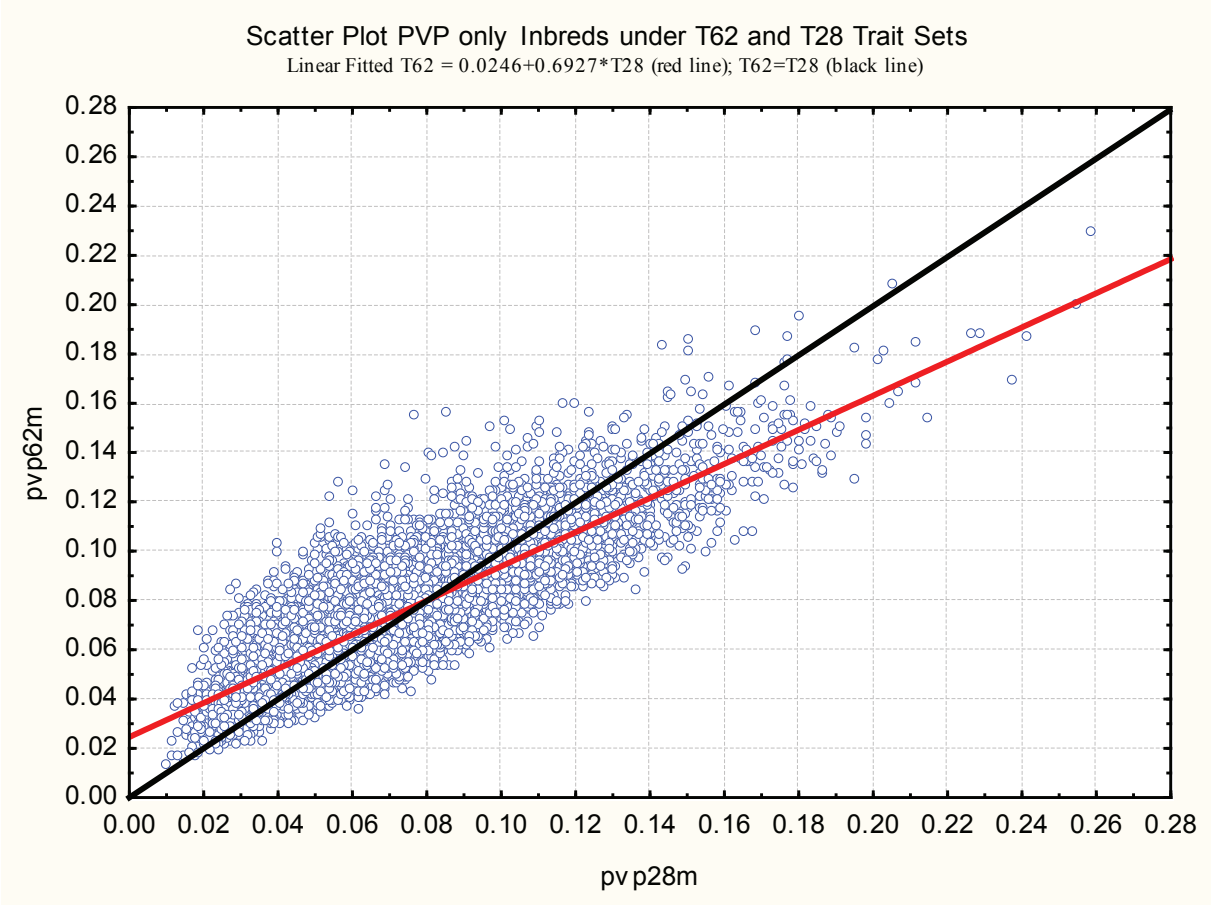


Figure 5 - Scatter plot of pair-wise distances between inbreds with data computed from 62 characteristics compared to the core set of 28 characteristics. Linear fitted line (red) compared to absolute equivalence (black line).

(1-similarity) was 0.86% while the largest dissimilarity exceeded 28% (using a scale where 0% is zero dissimilarity and 100% equals totally dissimilar.

For pair-wise inbred dissimilarities, 9,953 (approximately 45%) expressed larger dissimilarities (greater discrimination) when computed using the 28 characteristics. Incidences of larger dissimilarities when based on the 28 core set of characteristics occurred more frequently for characteristics when dissimilarities computed using the 62 set were already relatively large. The average sacrifice in benefit in terms of reduced inter-inbred dissimilarity, by using data from the 28 core set of characteristics, was 1.13% of the maximal dissimilarity of 100%. Thus, the 28 characteristic set provided the basis for a slightly weaker power of discrimination but was advantageous in requiring less than half the resources that would otherwise be required to record and to analyse the initial full set of 62 characteristics.

The table of observed agreement for inclusion of the same inbred pairs over a range of pair-wise dissimilarities between the 62 inbred set and the core set of 28 characteristics for a range (10, 100, 500, 1,000, 2,000, 3,000, 4,000, 5,000, and 10,000) of pair-wise measures were (% inbreds in common computed from the 62 characteristic data with the 28 core set data in parentheses) were, in increasing order of inbred pairs and of inbred-pair dissimilarities: 10 (30%), 100 (36%), 500 (41.6%), 1,000 (47.2%), 2,000 (52.8%), 3,000 (57.9%), 4,000 (61.9%), 5,000 (65.6%) and 10,000 (nearly 50% of all possible inbred pairs) (78.8%).

***Do the small differences we have observed in the computation of pair-wise differences among inbreds for the reduced 28 set of characteristics 1) result in less discrimination (and thus potentially contribute to a less precise adjudication of distinctness for PVP purposes or 2) introduce bias that was not observed using data obtained from the 62 characteristic based test?***

To address these questions we plotted the 10,440 pair-wise dissimilarities from the 145 PVP only Inbreds comparing dissimilarities generated using the 62 and 28 characteristic sets (Figure 5). The linear fit equation was T62 = 0.0246 + 0.6927 · T28 (red line); the black line shows fitted line based on equality of T62 v T28. Observation of Figure 5 shows a small bias in favour of larger dissimilarity coefficients from the 62 characteristic set compared to the 28 characteristic core set. There was a positive shift of 0.0246

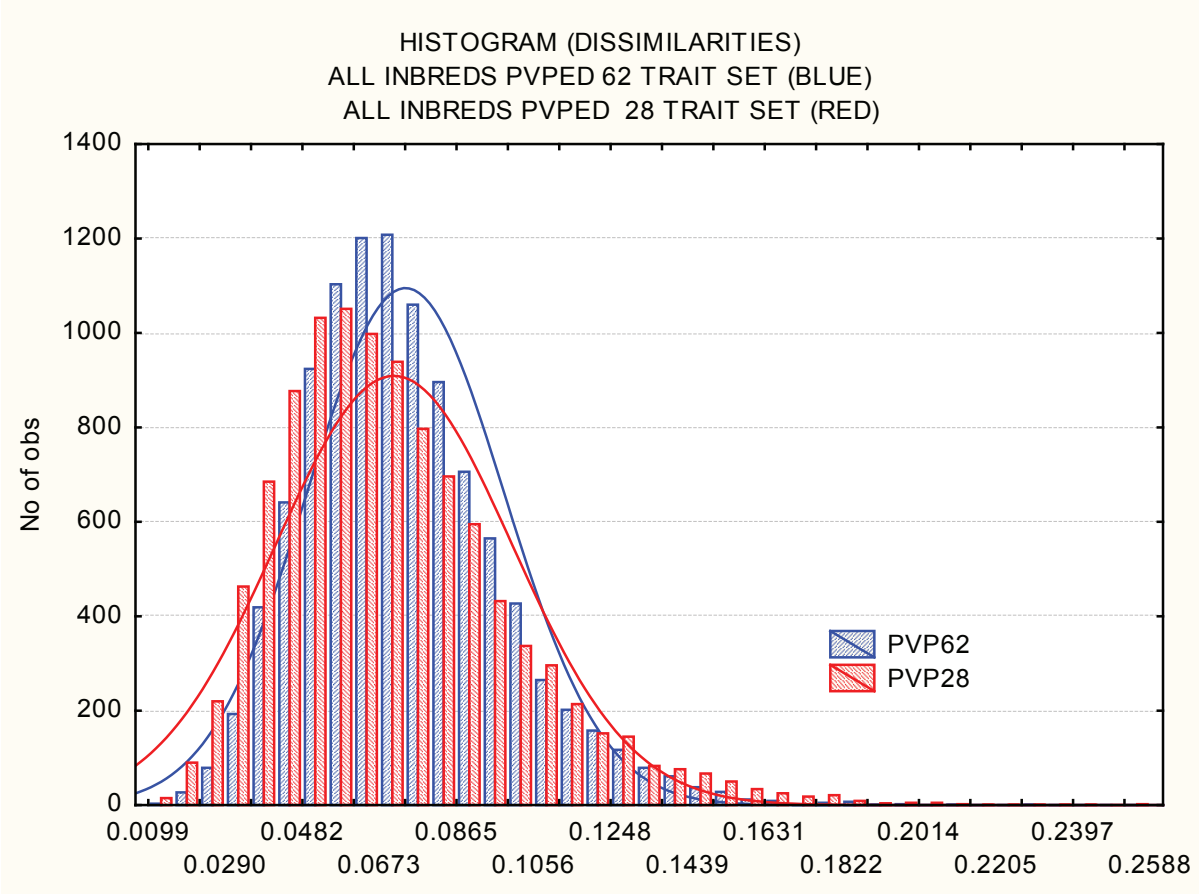


Figure 6 - Histogram showing frequency of occurrence for classes of pair-wise distances between inbreds computed from the initial set of 62 characteristics (blue) and the core set of 28 characteristics (red).

in dissimilarity calculated from the 62 characteristics set data when dissimilarities calculated from the 28 characteristic set would hypothetically reach zero. However, the pair-wise dissimilarity value of 0.08 is a fulcrum value (Figure 5). For the 28 characteristic set of data with dissimilarity values less than 0.08 there were many greater dissimilarities on the corresponding 62 characteristic (y-axis). Conversely, there were more dissimilarities greater than 0.08 when computed using the 28 characteristic set than using the 62 characteristic set.

These features are also shown by observing the histogram presented in Figure 6 which plots the frequency occurrence of the 10,440 pair-wise dissimilarity coefficients calculated for all pairs of 145 inbreds with granted PVPs based on the full set of 62 characteristics and also from using the set of 28 characteristics. There was a larger representation from the 62 characteristics based data in the central dissimilarity range 0.05 to 0.09 compared to representation from the 28 characteristic set. There were small additional representations in both tails of the distribution when dissimilarities were computed from the 28 characteristics set, more so for the low dissimilarity pairs. The distribution of all dissimilarities based on the 62 characteristic set was more leptokurtic than when based on the 28 characteristic set.

*Minimal dissimilarity values*

For the 62 characteristic set, the absolute minimal dissimilarity that was found for all pairs of PVP inbreds was (0.020) compared to 0.010 when calculated using the 28 characteristic set. However, it might be argued that the absolute minimum value could be prone to influence any extreme observation(s). Consequently, we also compared pair-wise dissimilarities using a more robust statistical approach; the pth percentile. We chose a range of values for p from 1% to 30% and the median (50th percentile). The percentile values are presented in Table 2 for p% = 1, 5, 10, 15, 20, 25, 30 for the main classifications of the inbreds (PVP, NON_PVP and ALL CHECKS); inbreds classified as NSS or SS (irrespective of PVP status) and the sub-classification within NSS and SS types. For each of the percentiles up to and including p = 50% (median) there was a reduced dissimilarity determination when based on the 28 characteristic data compared to when computed using the 62 characteristics with the level of “bias” reducing as p approaches 50%.

With the matrices of dissimilarities as input, the baseline association (product moment correlation)

Table 2 - Maximum, minimum, median and percentile of distributions (1%, 5%, 10%, 15%, 20%, 25%, and 30%) for dissimilarities among pairs of inbred lines based upon analyses of 62 or 28 core characteristics. Various groups of inbreds were analyzed: 1) All inbreds with PVP certificates granted; 2) all inbreds that do not yet have granted PVP certificates, 3) all inbreds used as checks; 4) all non-stiff stalk inbreds; 5) two subdivisions of non-stiff stalk inbreds, 6) All stiff-stalk inbreds, and 7) three subdivisions of stiff-stalk inbreds. N is number of pair-wise comparisons.

| 62 TRAITS | n | MAX | MIN | MEDIAN | p1 | p5 | p10 | p15 | p20 | p25 | p30 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ALL PVP | 10440 | 0.229 | 0.013 | 0.071 | 0.029 | 0.039 | 0.045 | 0.050 | 0.053 | 0.057 | 0.060 |
| ALL NON-PVP | 1326 | 0.260 | 0.033 | 0.095 | 0.041 | 0.054 | 0.062 | 0.067 | 0.072 | 0.076 | 0.081 |
| ALL CHECK | 78 | 0.397 | 0.062 | 0.189 | 0.077 | 0.093 | 0.104 | 0.115 | 0.125 | 0.133 | 0.146 |
| ALL NSS | 5671 | 0.231 | 0.020 | 0.079 | 0.032 | 0.045 | 0.051 | 0.056 | 0.060 | 0.063 | 0.066 |
| NSS1 | 1176 | 0.246 | 0.028 | 0.094 | 0.043 | 0.057 | 0.063 | 0.068 | 0.072 | 0.076 | 0.080 |
| NSS2 | 1176 | 0.264 | 0.032 | 0.100 | 0.044 | 0.056 | 0.065 | 0.072 | 0.077 | 0.080 | 0.085 |
| ALL SS | 4656 | 0.269 | 0.012 | 0.071 | 0.027 | 0.037 | 0.043 | 0.048 | 0.051 | 0.055 | 0.058 |
| SS1 | 780 | 0.257 | 0.026 | 0.105 | 0.040 | 0.051 | 0.063 | 0.069 | 0.075 | 0.080 | 0.085 |
| SS2 | 325 | 0.267 | 0.057 | 0.123 | 0.063 | 0.077 | 0.085 | 0.091 | 0.098 | 0.102 | 0.107 |
| SS3 | 253 | 0.329 | 0.044 | 0.117 | 0.051 | 0.058 | 0.065 | 0.071 | 0.078 | 0.086 | 0.092 |
| **28 TRAITS** | **n** | **MAX** | **MIN** | **MEDIAN** | **p1** | **p5** | **p10** | **p15** | **p20** | **p25** | **p30** |
| ALL PVP | 10440 | 0.259 | 0.010 | 0.066 | 0.023 | 0.032 | 0.038 | 0.043 | 0.046 | 0.050 | 0.053 |
| ALL NON-PVP | 1326 | 0.283 | 0.023 | 0.087 | 0.034 | 0.044 | 0.051 | 0.057 | 0.062 | 0.066 | 0.071 |
| ALL CHECK | 78 | 0.387 | 0.037 | 0.202 | 0.061 | 0.075 | 0.092 | 0.097 | 0.112 | 0.122 | 0.134 |
| ALL NSS | 5671 | 0.258 | 0.014 | 0.074 | 0.023 | 0.035 | 0.042 | 0.047 | 0.051 | 0.055 | 0.059 |
| NSS1 | 1176 | 0.295 | 0.023 | 0.092 | 0.033 | 0.047 | 0.055 | 0.061 | 0.066 | 0.071 | 0.074 |
| NSS2 | 1176 | 0.330 | 0.023 | 0.092 | 0.031 | 0.045 | 0.054 | 0.059 | 0.065 | 0.070 | 0.075 |
| ALL SS | 4656 | 0.286 | 0.011 | 0.070 | 0.022 | 0.032 | 0.039 | 0.043 | 0.047 | 0.051 | 0.055 |
| SS1 | 780 | 0.293 | 0.023 | 0.101 | 0.032 | 0.045 | 0.058 | 0.065 | 0.072 | 0.078 | 0.082 |
| SS2 | 325 | 0.340 | 0.039 | 0.125 | 0.053 | 0.067 | 0.077 | 0.084 | 0.089 | 0.093 | 0.100 |
| SS3 | 253 | 0.355 | 0.021 | 0.111 | 0.034 | 0.046 | 0.058 | 0.067 | 0.074 | 0.080 | 0.084 |

between matrices from calculated from the 62 characteristic set and the corresponding 28 characteristic set for 145 PVP inbreds was 0.8344. We used a Mantel test to compute the percentage of the 10000 associations from permuted data equaling or exceeding the baseline association for PVP as 0.00%.

Results from the Mantel permutation test confirmed that the two matrices of the 145 PVPed inbred dissimilarities based on the full 62 traits and the reduced 28 traits were highly similar in structure and composition with quantification, based on the 10000 permutations, that the observed baseline association of 0.8344 is significant and not a "random" aberrant artifact but a real assessment. In similar manner the non-PVP inbreds (52 in number) baseline 0.8421 association is highly significant and hence the respective non-PVP matrices from T62 and T28 are highly similar.

By stringing 10,440 dissimilarities calculated from the 62 characteristics and from the 28 characteristics a paired t-test was computed with a t-value for the 145 PVP inbreds of 18.1355 which is highly significant $p < 0.001$.

Results of Mantel tests and pair-wise t-tests for this and other designated classes of inbreds for comparison among the 28 and 62 characteristics are presented in Table 3. The right-hand column shows probability with PVP, Non-PVP, ALL NSS, NSS2 and SS4 significant $p<0.01$ – i.e., the T62 and T28 are significantly similar when inbreds are classified by protection status and biological "type". Hence we can infer that the similarity matrices computed from the inbreds based on the full 62 characteristics and reduced 28 characteristic set are strongly related.

## Discussion

We used information from a set of summary parameters (Law et al, 2011) whereby results for each parameter allowed the potential strengths or weaknesses of each characteristic in respect of its ability to efficiently discriminate among inbred lines of maize to be quantified. Each of these statistical parameters were categorized into one of three main target groups: "power", "variability" and "GxE". At each stage of the peeling process, PCA was used to define new transformed axes that could individually be interpreted in terms of "power", "variability" and "GxE". Where characteristics resided on these axes contributed to decisions to retain them in the analysis and so potentially to establish them as members of the core set, or alternatively, to eliminate them from membership of the core set. This basis of decision making was refined by also examining associations among the individual parameters. Added to this information on individual characteristics was experience from years of collecting morphological data for the purpose of DUS testing that related to ease of recording and other practical aspects. We also removed characteristics if they were duplicative, and therefore redundant in their contributions to showing associations among inbreds.

We conducted an iterative process of examining associations among characteristics in regard to their contribution to showing discrimination among inbred lines and either nominating characteristics to be members of the core set because they contributed positively and relatively independently to discrimina-

Table 3 - Product moment correlations and results of t test analyses for comparisons among pairs of inbred lines according to PVP status or germplasm pedigree type (see text and Table 2 for definitions of acronyms).

| TRAIT SETS T62 v T28 | n | PRODUCT MOMENT CORRELATION | PERCENTAGE EXCEEDING THE BASELINE ASSOCIATION | T-VALUE PAIRED SAMPLE 2 TAIL TEST | DF | PROBABILITY PAIRED SAMPLE 2 TAIL T TEST |
|---|---|---|---|---|---|---|
| ALL PVP | 145 | 0.834 | 0.00 | 18.136 | 10439 | 0.000 |
| ALL NON-PVP | 52 | 0.842 | 0.00 | 10.649 | 1325 | 0.000 |
| PUBLIC CHECKS | 13 | 0.900 | 0.00 | -0.569 | 77 | 0.571 |
| ALL NSS including NSS_MISC | 107 | 0.853 | 0.00 | 18.970 | 5670 | 0.000 |
| ALL SS Including SS_MISC | 97 | 0.884 | 0.00 | -0.914 | 4655 | 0.361 |
| NSS1 | 49 | 0.872 | 0.00 | 0.498 | 1175 | 0.619 |
| NSS2 | 49 | 0.821 | 0.00 | 9.064 | 1175 | 0.000 |
| SS1 | 40 | 0.841 | 0.00 | -0.497 | 779 | 0.620 |
| SS2 | 26 | 0.772 | 0.00 | 1.083 | 324 | 0.280 |
| SS3 | 23 | 0.930 | 0.00 | 1.085 | 252 | 0.279 |
| SS4 | 3 | 0.941 | ~ | 12.135 | 2 | 0.007 |

tion among inbreds and were relatively easy or alternatively to eliminate them because they were redundant or failed to contribute positively to discrimination among inbreds. In this fashion, we selected a core set of 28 characteristics which we hypothesized could be used to efficiently discriminate among inbred lines of maize. We then evaluated that hypothesis by comparing discrimination power, pair-wise inbred associations, and associations among all inbred lines using data from the full set of 62 characteristics compared to using data from the 28 core set of characteristics.

We used several approaches to evaluate whether selection of this core set of 28 characteristics gave results that were comparable with those using a larger set of 62 characteristics. Among these approaches were those previously used to monitor and to validate changes in DUS testing systems including field trial designs (Weatherup, 1974, 1980, 1994a, 1994b; Law et al, 1999).

Overall associations among inbreds based upon comparisons of the 62 and 28 characteristic subset were examined using t-tests and Mantel tests. Results from T-tests showed that the overall PVP INBRED 10440 elements in the respective T62 and T28 sets were not significantly different nor were they for the inbreds that have as yet not been adjudicated for their PVP status. The results from the Mantel tests also showed the PVP INBRED 10440 elements in the respective T62 and T28 sets were not significantly different, nor were the non-PVP'd inbreds, and nor were any of the pedigree-based subsets. However, when detailed pair-wise associations of inbreds were examined at both extremes of the scale, highly-similar and highly-dissimilar, then agreements in rankings were generally relatively low. For example, for most-similar the agreement in ranking ranged from 30-36% (10-100 most similar inbred pairs).

We acknowledge that there was a marginal reduction of about 1% in discrimination power among inbred lines that have very similar morphologies. Nonetheless, Information from the subset of 28 characteristics provided a viable basis for showing morphologically based distinctness between each inbred line. We preferentially eliminated characteristics that were, duplicative or highly correlated or which, on balance, added noise or error to the data. We submit that it is important to evaluate characteristics that are duplicative or highly correlated, and to thus to prune the set of characteristics with those features in mind. Overuse, or overfitting of data obtained from highly correlated characteristics, will tend to manifest itself in amplifying the similarity, even further, of inbred lines that are already similar rather than to provide a less-biased assessment of distinctness. So long as morphological characteristics remain the basis for tests of distinctness then we advocate that a set of characteristics that is selected based upon individual and collective capabilities to discriminate represents a scientifically justified approach to identifying a core set of characteristics. Such a set of characteristics can contribute less bias and deliver cost–benefits to all those involved in the generation and comparison of morphological data for the purposes of testing eligibility for PVP certification. We offer this approach to evaluating morphological characteristics to researchers who are seeking more efficient means to utilize this class of data to identify and to characterize inbred lines or varieties in other crop species.

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