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Approaches to improve the determination of eligibility for plant variety protection: I evaluation of morphological characteristics

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Abstract

The demonstration of distinctness through comparisons of morphological characteristics is an essential requirement in order to obtain Plant Variety Protection (PVP) and registration. Desires for increased international harmonization and the increasing size of reference collections place increased emphasis on improving the efficiency of the process. Morphological characteristics are notoriously affected by environment and many may be correlated in their expression. We developed an approach using inbred lines of maize (*Zea mays* L.) to evaluate characteristics according to their performance for 9 parameters encompassing 3 categories of Variability, Power and Genotype by Environment interaction. These data provide a basis for selecting a reduced core set of characteristics with the goal of retaining discriminational ability while decreasing the time and resources required to obtain and to compare morphologies.

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 Vegetales (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, 1991). Characteristics that are used to test for distinctness, uniformity, and stability (DUS) under the currently applied UPOV scheme are morphological features. Amongst other criteria, 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". Detailed descriptions of characteristics and how they should be recorded for individual species of crop varieties are provided in the "Guidelines for the conduct of tests for distinctness, uniformity and stability for maize" (UPOV, 1999, 2009).

Important goals of UPOV include to achieve greater international harmonization (van Wijk, 2003) and to facilitate the process of DUS testing while maintaining standards required for PVP certification. Harmonization is required to simultaneously obtain protection in several countries (UPOV, 2000). Harmonization of methodologies also enables flexibility in determining who conducts growing tests, evaluates the data, and authors the test report.

It is well known that the stability or reproducibility of expression of morphological characteristics is reduced by interactions of the environment and especially when those characteristics are under complex genetic control (Comstock and Moll, 1963; Camussi, 1979; Camussi et al, 1983; Patterson and Weatherup 1984; Staub et al, 1996; Lombard et al, 2000; Bredemeijer et al, 2002; UPOV, 2003, 2007, 2008; Smykal et al, 2008). UPOV has considered the plasticity of morphological characteristics in determining which characteristics are most suitable for use in providing for greater harmonization or to facilitate the more efficient comparison of varieties. Characteristics are classified by UPOV (2002a) into one of three groups: 1) Qualitative Characteristics, "those that are expressed in discontinuous states (eg, sex of plant)". UPOV (2002a) states that "As a rule, the(se) characteristics are not influenced by the environment"; 2) Quantitative Characteristics and 3) Pseudo-Qualitative Characteristics. Species specific subsets of characteristics are then designated, taking their group classification into account, with the objective of identifying those that are the most appropriate as the basis upon which to provide descriptions that can i) facilitate international harmonization of databases (asterisked characteristics) (UPOV, 1999, 2008) or ii) allocate varieties into groups of most phenotypically similar varieties (UPOV, 1999, 2002a, 2008). Grouping characteristics are designated with the objective that "…even where recorded at different locations, (they) can be used to select varieties of common knowledge that can be excluded from the growing trial…or (information from them can be used) to organize the growing trial; so similar varieties are grouped

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together" (UPOV, 2002a).

These assignations of characteristics according to their use in facilitating international harmonization or to group like varieties are based upon information from plant breeders and other experts. However, since these characteristics were originally chosen (during the 1960s) for these purposes considerable additional research has been conducted into the genetic basis of inheritance for many of these characteristics. 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 be referred to as "quantitative" (Sourdille et al, 1991; Austin et al, 2001; Bredemeijer et al, 2002; Mickelson et al, 2002; Enoki et al, 2006; Li et al, 2007). For example, Coe et al (1988) noted that "some 20 loci affect the qualitative, quantitative, and distributional array of anthocyanin pigments". Mickelson et al (2002) found leaf angle in maize to be associated with nine Quantitative Trait Loci (QTL) on six chromosomes and concluded that "some differing QTL in other genetic backgrounds would be anticipated." Austin et al (2001) found plant height in maize to be associated with 34 QTL involving all 10 chromosomes. Ma et al (2007) describe 13 QTL on seven chromosomes being associated with kernel row number in maize while Upadyayula et al (2006) report total tassel length in maize to be associated with five QTL on five chromosomes.

A greater appreciation of the complex nature of genetic control for many morphological characteristics also raises greater awareness of the potential for gene x environmental interactions to affect the ultimate expression of these characteristics. This realization prompts a need to reevaluate the utility of the morphological characteristics that are currently used to describe maize inbred lines. The need to identify a set of characteristics that can provide a more efficient and reliable means of characterizing inbred lines of maize is highly desirable for evaluating distinctness and also for grouping similar inbred lines. It will also not be practically possible to achieve a globally harmonized system, optimally with descriptions that are made in different countries or regions being directly comparable, unless chosen characteristics are highly reliable and repeatable across environments. Finally, given the already huge size of many reference collections and the annual rate at which they increase, demands for more efficient and effective systems for evaluating eligibility for PVP certification only gain increasing importance.

Use of data from replicated trials is a prerequisite to examine the robustness, reliability, and discriminatory capabilities of morphological characteristics, both individually and in various combinations. The US Plant Variety Protection Office (USPVPO) requires data for characteristics to be generated by applicants from statistical analyses from replicated field trials. Consequently, we have access to morphological data recorded from replicated field plot trials, at least for publicly available inbred lines and for other inbred lines that we have direct access to, i.e., those developed by Pioneer Hi-Bred.

The goal of this study is to contribute toward improvements in the efficiency and precision of the current DUS process. As an initial step toward achieving these objectives we report upon the robustness and discriminatory abilities of morphological characteristics that are currently used by UPOV and individual PVP authorities, to evaluate distinctiveness of maize inbred lines. We designate several criteria which collectively can be grouped under three main paradigms: "Power", "Genotype x Environment" interaction (signal to noise ratio) and "Precision or Variability". We then evaluate and rank each morphological characteristic according to these criteria. These results then provide the basis for selecting a smaller, yet potentially equally effective and more cost-effective set of morphological characteristics for the determination of distinctiveness in maize. These selected candidate characteristics and their evaluation are reported upon in a subsequent paper.

Materials and Methods

Morphological data

We utilized data for morphological characteristics obtained from maize inbred lines that applicants for Plant Variety Protection are required to provide to the US PVP Office. The US PVP Office uses crop specific characteristics and guidelines that the Office established in 1971 to facilitate comparisons of new varieties with varieties of common knowledge. Applicants are requested to provide data for 53 morphological characteristics through their completion of "Exhibit C" (http://www.ams.usda.gov/science/pvpo/Forms/forms.htm). Additional data are also requested by the U.S. PVP Office, including insect and disease resistance, information for a further 6 agronomic traits, and there is an option to provide molecular marker data. Most morphological characteristics are identical to those requested by UPOV, a feature which facilitated the US joining UPOV in 1981. In contrast, however, the US PVP Office requires the recording of measurable characteristics in terms of their mean and standard deviation. This procedure differs from that described by UPOV where an alternative process of translating continuous data to discrete "notes" described by the expression of representative, "check" or "example varieties'. Also, the US PVP Office requests color characteristics to be recorded according to a Munsell color code whereas UPOV records color using a discrete 1-9 scale.

Inbred lines

We examined the morphologies of 365 inbred lines that had data obtained from 2 or more years (maximum of 8) of field trials. Seventy-two % of the inbreds had granted PVP certificates, 7% were pub-

licly available inbred lines used as checks, and the remainder (20.8%) had not yet been submitted to the U.S. PVPO. Each inbred was assigned a Comparative Relative Maturity (CRM) value (Eckert et al, 1987; Olson and Sander, 1988; Lauer, 1998). We allocated inbred lines into one of four maturity zones (MZ) according to the number of heat units that are required for the inbred to reach flowering and maturity; MZ1 = inbreds with maturity 70-90 Comparative Relative Maturity (CRM) (which corresponds to the maturity region of northern North America); MZ2 = 91-100 CRM, which corresponds to the maturity region of the northern U.S. Corn Belt; MZ3 = 101-115 CRM, which corresponds to the maturity region occupying the central U.S. Corn Belt; and MZ4 = 116-126 CRM, which corresponds to the maturity region of southern United States, northern Mexico and more tropical longer season environments. The inbreds were primarily comprised of a) Iowa Stiff Stalk Synthetic (BSSS) background (135 or 37%), b) Non-Stiff Stalk, Iodent background (140 or 38%). The remainder included lines related to Oh43, Mo17 and flint lines related to F2.

Preliminary analyses (not shown) suggested that there were influences of maturity on a number of characteristics. Consequently, we placed additional focus on the largest subset of 210 inbreds that are adapted to the central Corn Belt maturity zone (MZ3, 101-115 CRM). Of these inbreds, 70% had granted PVP certificates, 9% were publicly available checks, and 21% had not been submitted for PVP examination. This MZ3 subset of inbreds was primarily comprised of 92 lines (44%) related to BSSS and 97 lines (46%) non-stiff stalk Iodent lines.

Morphological Data

We obtained data describing 66 morphological characteristics (Table 1) during the period 1998-2005 from multi-location field trial plots. Two or three locations were planted each year in the U.S. located near Ankeny, Johnston, and Dallas Center, IA. Experiments were planted in late April or early May of each year using a randomized experimental design nested by flowering date. Plots were planted at approximately 69,000 to 79,000 plants per ha. Most characteristics classified as discrete were collected at the plot level and assessed to give a single determination from the observation of 28 to 32 plants per inbred line. Quantitative traits were recorded from five plants per plot.

Additional details of protocols for recording morphological characteristics can be found at: http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateC&navID=PlantVarietyProtectionOffice&rightNav1=PlantVarietyProtectionOffice&topNav=&leftNav=ScienceandLaboratories&page=PlantVarietyProtectionOffice&resultType=&acct=plntvarprtctn. We categorized these characteristics according to whether the data were classified as quantitative or discrete (qualitative) classes of expression. We included color in the discrete category. Data for quantitative traits are the mean from five plants per trial site. Characteristics which are purely qualitative were recorded according to the protocols described in Table 1.

Sites in which to conduct the annual field trials (two or three locations) are selected from a total of eight locations in central Iowa. Each location has different soil types, and slightly off-set planting dates to spread work load. There were no replicated plots within a single location during an individual season. We partitioned inbreds as ”genotypes” and years as “environments”. We established by reviewing the results of cluster analysis and Principal Coordinates Analysis (Jambu,1991) (not shown) according to the criterion of less variation within a “site-family” cluster than between “site-families” that the eight sites could be clustered into five “site families”; thus “site families” were treated as replicates.

We obtained data for all characteristics in each of eight years (1998-2005) with the following exceptions: Kernel Type (KTYPE), Leaf Attitude (LFATTITUDE) (characteristics only recorded in 2002-2005), Tassel Attitude (TASSELATTITUDE) (only recorded in the period 2002-2005), Tassel Secondary Branch Number (T#2RYBRANC) (data available for 1999-2005), Number of Kernels per Row (KPERROW) (characteristic recorded 2004-2005), and Number of Kernels per Ear (KPEREAR) (recorded only in years 2004 and 2005).

We determined the criteria and statistical analytical procedures that we would use to evaluate characteristics as follows: Firstly, we considered hypothetically the attributes or features that would define an ideal characteristic. We considered that an ideal characteristic would be 1) highly repeatable, 2) highly reproducible, 3) highly discriminative or powerful and 4) independently informative. We defined repeatability as the degree of agreement in data observation taken by a single observer on one occasion with that by the same observer on another occasion, but the same day. Reproducibility could be partitioned into local and environmental. We considered local reproducibly as the degree of agreement in data observations taken by a single observer on one occasion with that by a different observer on the same or another occasion (low variability and high precision). We considered environmental reproducibility, (results over different environments locations and years), as genotype by environment interaction (GxE). We defined power as the ability to distinguish different inbred lines. High levels of agreement across years or environments would indicate a potentially powerful trait well able to distinguish inbreds. Alternatively, inconsistent evaluations might occur as a result of inherent variation, which could be expressed in terms of high characteristic CV%, or high noise (high variability or low precision), or due to structured variability such as genotype by environment interaction (GxE). We then considered, collectively, what additional features a

Table 1 - List of characteristics used in the current analysis of morphology listed according to their classification as quantitative or discrete (qualitative).

Name	Description	Units of measurement or scoring
Quantitative Characteristics		
%ROUND	% of kernels not passing through a 13/64 inch slot screen	%
COBDIAMETR	Cob diameter	mm
D10-90%P	Days from 10% pollen shed to 90% shed	Days
DE-50%P	Days from emergence to 50% of pollen shed	Days
DE-50%S	Days from emergence to 50% of plants in plot with silk extrusion	Days
EARDIAMETR	Ear diameter	mm
EARHT	Ear height	cm
EARINTLNG	Ear internode length	cm
EARLENGTH	Length of ear	cm
EARROWNUM	Number of rows of kernels	Number
EARWEIGHT	Ear weight	g
EMERGGDU	GDU to seedling emergence	Growing Degree Units (GDU)
GDU10-90%P	GDU from 10% to 90% pollen shed	GDU
GDUE-50%P	GDU from emergence to 50% of pollen shed	GDU
GDUE-50%S	GDU from emergence to 50% of the plants with silk extrusion	GDU
HUSKELENGTH	Husk extension length beyond ear	cm
HUSKLENGTH	Husk length	cm
KLENGTH	Kernel length	mm
KPEREAR	Number of kernels per ear	Number
KPERROW	Number of kernels per row	Number
KTHICKNESS	Kernel thickness	mm
KWIDTH	Kernel width	mm
KWT/100K	Weight per 100 Kernels (unsized sample)	g
LFANGLE	Leaf angle	degrees
LFLENGTH	Leaf length	cm
LFNUMATE	Number of leaves above top ear	Number
LFNUMBER	Nodes above ground	Number
LFWIDTH	Leaf width	cm
NOEARS/STALK	Number of ears per stalk	Number
PLHT	Plant height	cm
SHANKLNGTH	Shank Length	cm
SHED10%GDU	GDU to 10% pollen shed	GDU
SHED50%GDU	GDU to 50% pollen shed	GDU
SHED90%GDU	GDU to 90% pollen shed	GDU
SILK50%GDU	GDU to 50% of plants in plot with silk extrusion	GDU
STALKDIAM	Stalk diameter	cm
T#1RYBRANC	Number of primary tassel branches	Number
T#2RYBRANC	Number of secondary tassel branches	Number
TAXISFLDEN	Tassel axis floret density	Number florets per 4 cm of middle of central spike
TBRANANGLE	Tassel branch angle	degrees
TCENSPKLNG	Tassel central spike length	cm
TILLERPERPLT	Number of tillers per plant	Number
TLENGTH	Tassel length	cm
TPEDLENGTH	Tassel peduncle length	cm
Discrete (qualitative) characteristics		
BARGLUME	Tassel Bar glume (glume band) anthocyanin color development	1-5 scale. 1 = green/yellow, 2 = pink, 3 = red, 4 = dark red, 5 = purple
BRTANTHO	Brace root anthocyanin	1-4 scale. 1 = absent, 2 = faint, 3 = moderate, 4 = dark
EARROWALGN	Kernel row alignment	1-3 scale. 1 = Straight, 2 = Slightly curved, 3 = Spiral
EARROWREG	Regularity of kernel rows	Indistinct (1) Distinct (2)
EARTAPER	Ear Taper	1-3 scale. 1 = Slight, 2 = Average, 3 = Extreme
HUSKTIGHT	Husk tightness	1-9 scale. 1 =Very loose, 9 = Very tight
KTYPE	Kernel type	Flint to dent with intermediate “flint-dent” or “dent-flint” types
LFATTITUDE	Leaf Attitude base to tip	1-5 scale. 1 = erect, 5 = tip drooping relative to leaf base.
POLLSC	Pollen score	1-9 scale. 1 = no or few branches with low spikelet density, 9 = many branches and high spikelet density
PVP_BARGLUME	Tassel Bar glume (glume bands) color	Absent (1) Present (2)
SCORALEOL	Aleurone color	Color
SCORANTHERCOL	Anther color	Color
SCORCOBCOL	Cob color	Color
SCORDRYHSKCOL	Dry husk color	Color
SCORENDOCOL	Kernel endosperm color	Color
SCORFRSHSKCOL	Fresh husk color	Color
SCORGLUMECOL	Tassel glume color	Color
SCORLEAFCOL	Leaf color	Color
SCORSILKCOL	Silk color	Color
SHANKPOS	Position of ear at dry husk stage	1-3 scale. 1 = upright, 2 = horizontal, 3 = pendent
SHEPUB	Amount of leaf sheath pubescence	1-9 scale 1 = none, 9 = like “peach fuzz”
TASSELATTITUDE	Attitude of tassel branches from central axis of tassel to tip of branch	1-5 scale. 1 = upright, 5 = drooping

set of characteristics would optimally comprise. In this regard, the most informative and powerful set of characteristics would be comprised of: 1) individual characteristics that are independent and uncorrelated to ensure minimal duplication of effort in recording and processing data and 2) characteristics that are relatively inexpensive and practically easy to measure and to record.

We, therefore, established three categories: 1) Variability, 2) Power and 3) GxE by which to examine individual characteristics. Within these overall categories we then established nine specific parameters which we then used as the basis to measure the performance of each characteristic. These parameters are 1) Range of Expression (ROE), 2) Trait Coefficient of Variation (CV%), 3) Parameter Variance Components and Sigma^2 (S2), 4) Individual Environment Inbred Differentiation F-Ratio (MINF), 5) Individual Environment Inbred Differentiation Percentage Exhibiting Significant Inbred Differentiation (SIGINBRED ENVIRP1), 6) Inbred Discrimination F-Ratio (INBRED F), 7) GxE F-Ratio (GXEF), 8) % Inbreds with Significant GxE Interaction with Probability $p<0.01$ (SIGGXEP1) and 9) Chi-Squared Statistic for Testing Consistency of Contribution to SS GxE (CHIQ). We also examined associations among 1) characteristics and 2) among the nine parameters in regard to their contribution to Variability, Power, and GxE. We made these comparisons using both the 365 set of inbreds and the 210 subset of MZ3 inbreds. The nine parameters and methods for their measurement are described below:

Parameter 1: Range of Expression (ROE)

Characteristics that exhibit a wide range of expression across inbred lines would potentially be more informative and discriminative than characteristics that reveal relatively less diversity. Quantifying each characteristic for this attribute is therefore a useful source of information for determining relative utility of traits for determining distinctness. For both qualitative and quantitative characteristics, the range of expression is simply the difference between the maximum observed value over all inbreds, sites and seasons and the corresponding minimum value. Since characteristics are measured on different scales then such comparisons of ROE require a normalizing transformation. To establish an index for comparison purposes we utilized data from the largest and most genetically diverse set of 600 inbred lines that we had available in order to represent the widest observed range of expression that we have observed; for each characteristic the widest range of expression was indexed at 100%. Characteristics that retain the highest ROE index value in the sets of 365 inbreds and 210 MZ3 inbreds are preferable.

Parameter 2: Trait Coefficient of Variation (CV%)

For both qualitative and quantitative characteristics, mean and standard deviation are calculated and the standard deviation expressed as a percentage of the mean. The resulting CV% is dimensionless and can be used as a summary statistic for trait "precision". Characteristics with low CV% are preferable.

Parameter 3: Variance Components and Sigma^2 (S2)

GenStat software (Payne et al, 1996, 2006) was used with the same mixed model across each of the 66 characteristics to compute components of variance attributed to specific causal sources of inbred, site-families and year with corresponding interactions (results not shown). The component of particular interest here is the unattributable variation (sigma^2), effectively the experimental error. This is expressed as a % of the total observed variation to normalize comparisons across characteristics. Most desirable characteristics will have a low Sigma^2 as this feature represents unattributable "experimental noise".

Parameter 4: Individual Environment Inbred Differentiation F-Ratio (MINF)

For each of the traits, inbreds each individual year were analyzed using SERGEN software using site-family factor as "replicates" as described previously. The inbred mean-squares value was then compared to the residual mean-square (the inbred by site-family interaction) to give the F-Ratio or the "power" of that trait. Examining each characteristic over all years allows the minimum inbred differentiation F-Ratio to be established (MINF). The minimum F-Ratio occurs when an environment is the least discriminating with respect to inbreds; possibly where there is excessive residual variation due to site-family replication or due to the set of inbreds exhibiting a compressed range of expression, possibly due to biotic or abiotic stress. This value differentiates characteristics according to their ability to perform effectively even in sub-optimal field trial conditions.

Parameter 5: Individual Environment Inbred Differentiation Percentage Exhibiting Significant Inbred Differentiation (SIGINBRED ENVIRP1)

Based on the same set of SERGEN analyses (see Law et al, 1997) an additional parameter was collected: F-Ratios were tested statistically at $p<0.01$ level for discrimination of inbred differences and the percentage of significant trials (years) was computed. Significance (at the agreed level of probability in this case $p<0.01$) is indicative that inbred differences exist and confirms that differences can be detected efficiently.

Parameter 6: Inbred Discrimination F-Ratio (INBRED F)

Traditionally, variety or genetic material interacting with environment is referred to as genotype by environment interactions (GxE). There is a wealth of published literature on this important subject; for example, see Yates and Cochran (1938), Allard and Bradshaw (1964), Crossa et al (1999), Yang (2002), van Eeuwijk et al (2005) and Holland (2007). Innovative theoretical approaches developed by Calinski et al (1987a, 1987b, 1989a, 1989b) have been incorpo-

Table 2 - Data for 62 traits and 9 parameters from analyses using 365 inbreds.

Master Order of Trait *	Range of Expression in the Selection of Data as % of Total Observable Range	Trait CV%	Analysis-model Un-attributable Error Sigma^2 as % of Total Variation	Inbred Discrimination Power Minimal F over Environments	Percentage of all Environments Where Inbred Discrimination is Significant with Probability $p<0.01$	Sergen Inbred Discrimination F-Ratio	Sergen GxE F-Ratio	Percentage of 365 Inbreds with Significant GxE Interaction with Probability $p<0.01$	Chi-Squared Statistic for Testing Consistency of Contribution to SS_{GxE}
BARGLUME	100.0	35.8	60.4	1.7	100.0	12.0	0.53	3.56	58.6
BRTANTHO	100.0	46.4	37.8	4.2	100.0	34.1	0.54	1.10	11.3
COBDIAMETR	100.0	10.0	15.5	4.5	100.0	108.5	1.34	13.70	32.9
EARDIAMETR	100.0	8.6	20.1	4.3	100.0	70.0	0.96	6.30	11.1
EARHT	100.0	21.5	25.0	2.6	100.0	47.0	0.60	3.29	7.9
EARINTLNG	100.0	15.2	41.6	1.8	100.0	21.2	0.48	1.64	20.2
EARLENGTH	100.0	14.6	19.5	4.4	100.0	81.1	0.70	4.11	12.3
EARROWALGN	100.0	23.3	33.4	1.3	87.5	20.0	2.10	32.05	17.7
EARROWNUM	100.0	11.4	31.4	3.6	100.0	69.5	0.60	2.74	26.0
EARROWREG	100.0	10.4	43.7	1.0	87.5	10.0	1.60	12.60	39.6
EARTAPER	100.0	22.5	27.8	4.5	100.0	26.5	2.69	36.99	11.1
EARWEIGHT	100.0	29.2	24.0	4.3	100.0	46.7	0.70	4.93	10.8
EMERGGDU	100.0	18.3	10.3	1.6	100.0	12.1	0.58	2.19	4.5
HUSKELENGTH	100.0	47.6	22.1	4.2	100.0	73.1	0.78	3.84	11.9
HUSKLENGTH	100.0	11.6	17.2	5.3	100.0	95.9	0.93	6.85	17.1
HUSKTIGHT	100.0	29.2	27.1	3.8	100.0	39.2	1.74	23.01	7.8
KLENGTH	95.7	9.9	23.0	4.3	100.0	59.3	0.86	6.85	15.9
KTHICKNESS	100.0	12.9	39.2	2.7	100.0	31.2	0.86	7.67	21.2
KTYPE	100.0	58.9	5.6	12.1	100.0	59.1	2.69	13.97	2.1
KWIDTH	100.0	7.7	34.1	2.7	100.0	33.4	0.75	4.11	10.4
KWT/100K	100.0	18.9	16.7	6.1	100.0	70.0	1.31	15.89	21.8
LFANGLE	98.6	29.3	11.5	2.2	100.0	25.6	0.53	1.64	15.1
LFATTITUDE	100.0	35.1	44.6	3.1	100.0	11.9	0.58	0.27	2.7
LFLENGTH	93.1	10.9	16.1	4.5	100.0	105.8	0.78	6.58	28.8
LFNUMATE	100.0	15.0	14.1	2.3	100.0	35.8	0.46	1.37	8.1
LFNUMBER	100.0	19.4	5.9	3.2	100.0	42.3	0.50	0.82	12.6
LFWIDTH	97.6	12.5	27.4	4.1	100.0	49.2	0.54	1.92	12.0
PLTHT	98.1	13.9	4.5	5.8	100.0	110.2	0.67	3.29	11.2
POLLSC1-9	100.0	32.4	43.6	1.7	100.0	21.3	0.68	3.84	14.5
SHANKLNGTH	82.3	31.7	11.3	2.9	100.0	47.2	0.74	5.21	6.5
SHANKPOS	100.0	51.8	41.1	2.6	100.0	16.2	0.70	3.01	18.8
SHED10%GDU	100.0	9.5	4.4	24.1	100.0	441.0	0.70	3.29	8.2
SHED50%GDU	100.0	9.4	4.2	24.5	100.0	448.0	0.77	4.66	6.4
SHED90%GDU	100.0	9.5	5.2	22.5	100.0	341.0	0.71	3.84	7.4
SHEPUB1-9	100.0	80.2	30.0	1.0	87.5	11.6	0.62	4.11	15.9
SILK50%GDU	100.0	9.9	4.7	20.5	100.0	372.8	0.80	5.48	2.8
STALKDIAM	89.8	23.6	13.9	2.5	100.0	26.8	0.56	2.19	18.4
T#1RYBRANC	100.0	56.2	7.9	3.3	100.0	79.1	0.55	4.11	23.1
T#2RYBRANC	100.0	117.4	10.9	3.2	100.0	29.6	0.45	1.92	17.7
TASSELATTITUDE	100.0	39.6	30.7	4.1	100.0	22.1	0.83	1.64	6.3
TAXISFLDEN	99.1	25.0	20.8	1.6	100.0	21.4	0.52	2.47	7.9
TBRANANGLE	100.0	43.2	40.8	2.8	100.0	30.1	0.54	1.64	3.1
TCENSPKLNG	100.0	17.3	29.8	3.0	100.0	48.5	0.53	1.37	8.2
TLENGTH	93.2	12.2	23.9	3.7	100.0	67.0	0.60	2.74	4.9
TPEDLENGTH	100.0	18.6	33.7	2.5	100.0	40.2	0.53	2.74	7.8
%ROUND	100.0	40.3	19.4	6.3	100.0	47.9	2.94	31.23	191.3
D10-90%P	100.0	43.2	55.0	1.0	25.0	4.3	0.41	0.55	24.8
DE-50%P	100.0	11.8	2.6	20.4	100.0	398.1	0.80	5.21	25.6
DE-50%S	100.0	12.0	3.1	17.7	100.0	344.6	0.76	4.66	20.0
GDU10-90%P	100.0	41.3	59.0	0.9	50.0	4.3	0.40	0.55	14.7
GDUE-50%P	100.0	10.3	3.9	21.6	100.0	444.7	0.75	4.66	8.2
GDUE-50%S	100.0	10.8	4.5	18.8	100.0	367.8	0.74	4.93	5.2
NOEARS/STALK	100.0	11.2	77.6	6.3	100.0	500.0	0.66	8.77	30.4
SCORALECOL	100.0	24.2	26.5	6.0	100.0	48.8	1.53	20.27	16.6
SCORANTHERCOL	100.0	73.5	46.5	2.8	100.0	25.3	0.51	2.19	8.8
SCORCOBCOL	100.0	42.5	9.1	14.2	100.0	191.0	2.82	34.25	7.6
SCORDRYHSKCOL	100.0	57.4	22.1	3.3	100.0	29.9	3.40	41.64	28.5
SCORENDOCOL	100.0	24.0	19.0	6.8	100.0	71.4	2.24	30.14	3.6
SCORFRSHSKCOL	100.0	16.7	80.6	1.0	50.0	4.3	0.50	0.00	4.8
SCORGLUMECOL	100.0	79.1	50.8	1.2	87.5	16.0	0.56	0.82	5.1
SCORLEAFCOL	100.0	17.9	93.4	0.8	0.0	2.5	0.37	1.64	43.7
SCORSILKCOL	100.0	73.2	43.7	2.3	100.0	28.7	0.55	3.84	12.4

*characteristics KPEREAR, KPERROW, TILLERPERPLT, PVP_BARGLUME were not included due to incomplete data

rated into a software package called SERGEN. SERGEN software (Calinski et al, 1992a, 1992b) allows a detailed genotype by environment analysis for each characteristic. The following four parameters are derived from analyses conducted using SERGEN software. The SERGEN analyses are briefly explained here together with a worked out example for a single characteristic in Supplementary Methods. As applied

here, SERGEN is univariate with a data model of the form: $y_{ij} = \mu + a_i + b_j + c_{ij} + e_{ij}$

y_{ij} is i^{th} variety at j^{th} environment averaged over replicates; μ overall mean; a_i is fixed effects of i^{th} variety; b_j is random effect of j^{th} environment; c_{ij} is random effect of interaction of i^{th} variety and the j^{th} environment; e_{ij} is experimental error.

Following the usual 'dot' notation to indicate summation, the varietal interaction deviations are modeled as $(y_{ij} - y_{i.} - y_{.j} + y_{..})$ and the environmental effects as $(\bar{y}_{.j} - \bar{y}_{..})$.

A common dispersion matrix is assumed for all genotypes over environments. For other constraints see Pilarczyk and Kaminski (1995). Selected summary statistics from individual traits are collated from the expansive total output available from SERGEN. Using the SERGEN software GxE analyses were performed based on a maximum of 8 environments (years) on each of the 66 characteristics. As an example, we present one full GxE ANOVA table for a specific characteristic and the derivation of this and the following 3 statistical parameters in a Supplementary Methods Section. A univariate SERGEN analysis for each trait generated a single statistic (Inbred F) which identifies traits with high "inbred discrimination power". High values of Inbred F are desirable. Individual trait Inbred F-ratios, with the same degrees of freedom, can be ranked to identify the more "powerful" traits or conversely to flag traits that are potentially weak in terms of inbred distinction ability.

Parameter 7: GxE F-Ratio (GXEF)

Optimal characteristics are those that are not only powerful in terms of inbred differentiation (Inbred F-Ratios), but also robust across seasons. Characteristics that are robust to both inbreds and environments will have low or non-significant GxE F-ratios. In a similar manner and from the same univariate SERGEN ANOVA table to INBRED F (Parameter 6 above) we extracted the F-Ratio attributable corresponding to inbred GxE (GXEF). Traits with low GXEF are desirable as they indicate robust traits with limited influence due to different environments. GXEF, with similar degrees of freedom, can be used to compare and identify traits that are relatively robust with respect to environmental influences.

Parameter 8: % Inbreds with Significant GxE Interaction with Probability p<0.01 (SIGGXEP1)

GXEF is Parameter 7 above from the overall SERGEN ANOVA table but here, in SIGGXEP1, we assess the impact of environmental variation on individual inbreds under test. These analyses generate large tables of trait by inbred data which we distilled to a summary parameter: The percentage of inbreds which exhibit significant GxE with probability p<0.01. A desirable characteristic is one with a low percentage of inbred GxE which are significant with probability $p < 0.01$.

Parameter 9: Chi-Squared Statistic for Testing Consistency of Contribution to Sum of Squares for GxE (CHIQ)

The SERGEN software computes, for each individual trait, the total sum of squares attributable to GxE (SS_{GxE}) and partitions this to a percentage contribution of each environment (years). A computational restriction exists in that at least three environments (years) of data are required. If the contribution to SS_{GxE} for a characteristic is evenly distributed over each of the years then that characteristic is robust with respect to GxE with similar interactions in each environment. Most desirable characteristics are those with a low GxE impact in every year and not just low in years where total variation is low. The evenness of SS_{GxE} can be formally tested using Chi-squared analysis where the observed annual contribution to SS_{GxE} is used with the expectation of, for example, 12.5% per each of eight years. Characteristics with at least three years of data were similarly analysed with the appropriate adjustment of the expected % annual contribution.

Associations among characteristics and among parameters

Associations among the characteristics and among the nine parameters are likely to be complex and include some correlated structure. We, therefore, used multivariate analysis to show associations among both the characteristics and among the nine parameters. We utilized Principal Components Analysis or PCA, see for example Jambu (1991), which allows the original data (the trait by parameter matrix) to be transformed to a smaller number of uncorrelated or representative variables. We used the correlation matrix option based on experience gained from analyses of similar morphologically based description systems (see Weatherup, 1980 or Watson, 2000) and quantification of the moderate values of pair-wise correlations observed between traits.

Eigenvalues from the PCA analyses give the proportion of total variation that is accounted for in the PCA axes. Information was revealed about the set of selected statistical parameters in the form of a plot on "unit circle" of the high order PCA transformed axes where the area inside the unit circle was scaled to represent the region of the valid coordinates based on the PCA axes. The closer a plotted variable is positioned to the border of the circle, the better is its representation by the PCA axes in the plot. Results from the PCA analysis also allow "weights" to be assigned to the original parameters. The weighting of parameters will be an important consideration in a subsequent phase of selection of a set of characteristics that is optimized for effectiveness and efficiency in discriminating among maize inbred lines based upon their comparative morphologies. Comparisons among associations of characteristics for the 365 inbred and the 210 inbred sets were made using correlation analysis of eigenvector values for individual characteristics.

Table 3 - Data for the 9 parameters using 210 maturity zone 3 (MZ3) inbreds.

Master Order of Trait	Range of Expression in the Selection of Data as % of Total Observable Range	Trait CV%	Analysis-model Un-attributable Error Sigma^2 as % of Total Variation	Inbred Discrimination Power Minimal F over Environments	Percentage of all Environments Where Inbred Discrimination is Significant with Probability $p<0.01$	Sergen Inbred Discrimination F-Ratio	Sergen GxE F-Ratio	Percentage of 365 Inbreds with Significant GxE Interaction with Probability $p<0.01$	Chi-Squared Statistic for Testing Consistency of Contribution to SS_{GxE}
BARGLUME	100.0	35.0	63.1	1.8	100.0	9.3	0.54	4.29	60.4
BRTANTHO	100.0	46.0	35.1	4.3	100.0	33.7	0.63	0.95	13.9
COBDIAMETR	76.6	8.8	19.7	3.7	100.0	75.2	1.36	10.48	27.7
EARDIAMETR	79.0	7.2	27.0	3.3	100.0	39.4	1.08	5.71	17.5
EARHT	93.3	19.8	29.5	2.6	100.0	33.4	0.60	1.90	4.4
EARINTLNG	100.0	14.4	42.5	1.5	100.0	20.0	0.50	1.43	16.4
EARLENGTH	97.6	12.7	24.5	3.4	100.0	58.5	0.80	4.76	12.0
EARROWALGN	100.0	24.1	36.1	0.9	87.5	18.5	2.11	33.81	14.4
EARROWNUM	100.0	10.8	25.9	3.3	100.0	60.0	0.68	3.81	21.8
EARROWREG	100.0	9.2	50.0	1.0	87.5	6.0	1.55	11.43	25.5
EARTAPER	100.0	25.0	26.4	3.6	100.0	33.5	2.89	39.52	18.5
EARWEIGHT	100.0	26.0	29.8	3.5	100.0	29.8	0.74	3.33	8.2
EMERGGDU	100.0	18.5	9.7	1.5	100.0	12.1	0.66	2.86	9.0
HUSKELENGTH	100.0	46.5	22.2	4.4	100.0	73.0	0.83	4.29	10.2
HUSKLENGTH	96.1	10.0	21.0	4.4	100.0	71.5	1.02	6.19	15.7
HUSKTIGHT	100.0	26.8	30.8	3.2	100.0	30.0	1.77	20.95	4.7
KLENGTH	82.6	8.4	30.5	3.5	100.0	30.8	0.95	8.10	14.9
KTHICKNESS	92.6	12.6	40.2	2.1	100.0	28.0	0.94	8.57	19.5
KTYPE	100.0	55.8	5.1	12.7	100.0	50.5	3.45	20.48	0.8
KWIDTH	100.0	7.9	33.3	2.2	100.0	32.2	0.75	3.81	8.3
KWT/100K	91.4	18.1	19.7	5.9	100.0	50.6	1.13	12.86	17.5
LFANGLE	89.6	27.7	15.2	1.3	87.5	16.2	0.45	0.00	11.2
LFATTITUDE	100.0	35.6	49.2	3.0	100.0	10.8	0.62	0.48	0.3
LFLENGTH	78.7	8.9	20.8	3.6	100.0	63.6	0.89	5.24	21.3
LFNUMATE	93.5	13.9	16.0	2.0	100.0	24.6	0.49	1.90	6.4
LFNUMBER	97.8	18.2	9.8	2.2	100.0	22.4	0.50	0.95	13.0
LFWIDTH	90.2	10.9	33.5	2.7	100.0	31.7	0.58	2.38	9.8
PLTHT	93.9	11.5	8.4	3.3	100.0	56.2	0.64	2.86	13.2
POLLSC1-9	100.0	32.7	41.2	1.6	100.0	21.7	0.76	3.81	15.8
SHANKLNGTH	81.0	29.0	28.1	3.0	100.0	34.3	0.85	7.14	5.2
SHANKPOS_ID	100.0	53.0	39.9	2.2	100.0	13.3	0.69	1.90	20.2
SHED10%GDU	88.8	6.3	10.4	6.4	100.0	132.3	0.72	2.86	8.4
SHED50%GDU	89.0	6.2	9.4	7.2	100.0	134.0	0.83	4.29	8.2
SHED90%GDU	99.3	6.3	11.6	7.0	100.0	100.0	0.75	3.81	14.3
SHEPUB1-9	100.0	79.6	29.0	1.0	62.5	10.3	0.56	1.90	28.9
SILK50%GDU	92.7	6.8	9.8	6.1	100.0	119.3	0.86	5.71	5.7
STALKDIAM	76.6	22.4	14.4	1.8	100.0	18.7	0.53	0.48	8.4
T#1RYBRANC	100.0	56.8	20.9	3.3	100.0	81.3	0.60	4.76	23.4
T#2RYBRANC	100.0	122.4	26.3	2.7	100.0	33.9	0.64	3.81	43.1
TASSELATTITUDE	100.0	39.5	26.2	5.1	100.0	25.4	0.99	2.86	6.7
TAXISFLDEN	73.1	25.6	33.4	1.7	100.0	23.7	0.56	2.86	8.6
TBRANANGLE	98.5	44.2	40.3	2.6	100.0	31.8	0.58	1.43	3.1
TCENSPKLNG	100.0	15.9	30.9	2.5	100.0	44.7	0.54	1.43	3.6
TLENGTH	93.2	10.8	28.4	2.8	100.0	52.5	0.59	2.38	5.2
TPEDLENGTH	100.0	17.8	38.6	2.1	100.0	36.3	0.54	2.86	7.6
%ROUND	100.0	40.9	17.7	6.6	100.0	47.7	3.20	31.43	213.6
D10-90%P	100.0	42.6	52.7	1.0	50.0	4.3	0.45	0.48	28.1
DE-50%P	91.1	9.4	3.4	5.8	100.0	116.4	0.83	4.29	19.5
DE-50%S	90.0	9.6	4.1	5.0	100.0	107.0	0.84	6.19	21.0
GDU10-90%P	100.0	40.8	57.1	1.0	37.5	4.2	0.47	0.95	13.1
GDUE-50%P	79.4	6.7	8.4	5.9	100.0	133.3	0.80	5.24	10.8
GDUE-50%S	85.7	7.4	9.2	5.4	100.0	116.3	0.82	5.24	8.3
NOEARS/STALK	100.0	10.3	76.6	0.0	25.0	500.0	0.77	8.57	76.4
SCORALECOL	100.0	22.3	25.6	4.7	100.0	58.8	1.40	15.24	14.5
SCORANTHERCOL	100.0	74.2	48.2	2.7	100.0	21.7	0.54	2.86	10.9
SCORCOBCOL	100.0	39.7	9.0	23.3	100.0	179.0	3.05	36.67	10.3
SCORDRYHSKCOL	100.0	54.8	27.0	1.6	100.0	23.5	2.86	32.38	32.4
SCORENDOCOL	100.0	22.2	17.8	6.5	100.0	89.9	2.27	28.57	3.2
SCORFRSHSKCOL	100.0	54.8	79.3	0.9	0.0	4.0	0.51	0.00	4.8
SCORGLUMECOL	100.0	79.5	46.6	1.3	87.5	17.5	0.62	1.90	4.7
SCORLEAFCOL	100.0	17.1	93.2	0.8	0.0	2.2	0.42	0.95	45.7
SCORSILKCOL	100.0	71.9	42.8	2.2	100.0	28.5	0.57	3.81	10.6

Results

We present the data for 62 characteristics analyzed from: i) 365 inbreds collectively covering four-zones (Table 2) and ii) the subset of 210 inbreds categorized into maturity zone three (the central US Corn Belt) (Table 3).

Parameter 1: Range of Expression (ROE)

The majority (53 or 85%) of traits retained an ROE

of 100% when 365 inbreds were examined compared to the baseline 600 inbreds; the lowest index score for any characteristic (shank length) was 82.3% (Table 2). When inbreds were restricted by maturity to the MZ3 set of 210 then 34 (55%) characteristics retained an ROE index of 100% (Table 3). Among these were most of the ear characteristics and all of the color characteristics. The lowest ROE index scores for the 210 MZ3 inbred set were for tassel axis floret density (TAXISFLDEN) (73.1%), cob diameter COBDIAMETR) and stalk diameter (STALKDIAM) (76.6%), leaf length (LFLENGTH) (78.7%), ear diameter (EARDIAMETR) (79%), and GDU from emergence to 50% pollen shed (GDUE-50%P) (79.4%).

Parameter 2: Trait CV%

CV% values for characteristics generally ranged from 6% to 80% with number of secondary tassel branches (T#2RYBRANC) >100% for both the 365 and 210 MZ3 sets of inbreds (Tables 2 and 3). The median CV% over characteristics was 19.4%; 24 characteristics had CV% below 11% while 26 characteristics had CV% above 25%. CV% for the majority of characteristics when measured using the 210 MZ3 set were approx. 1-4% lower than when measured using the 365 inbred set. GDU from emergence to 50% pollen shed (GDUE-50%P) and a group of other characteristics also associated with maturity showed a reduction of at least 30% when measured using the 210 MZ3 subset compared to the 365 set of inbreds. However, there were also examples of the reverse trend. The most striking example was an increase in CV% for MZ3 inbreds for number of tillers per plant (TILLERPERPLT) which showed an increase of 30%. Data scores for this characteristic were singular and exceptional with data scores of 1 for nearly all inbreds with a very small number of non-one's.

Parameter 3: Variance Components and Sigma^2 (S2)

The Sigma^2 (unattributable variation) values are reported as the percentage of the total observed variation; a low value indicates characteristics with a high "signal to noise ratio" and thus, at least on the basis of this parameter, potentially very effective to distinguish among inbred lines. Characteristics with low Sigma^2 included those associated with maturity. The highest Sigma^2 was for leaf color (SCORLEAFCOL)

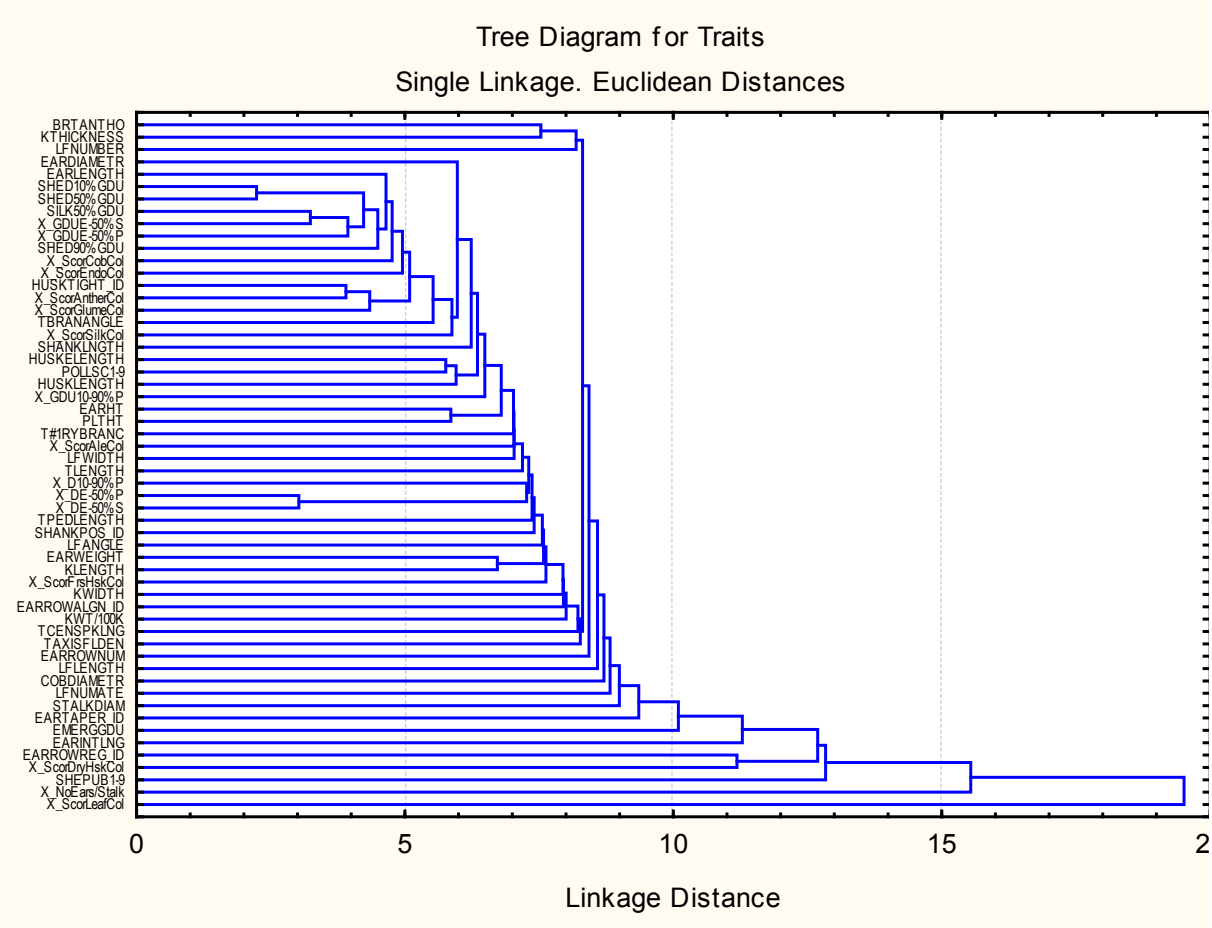

Figure 1 - Associations among characteristics with respect to their annual contribution to total SS_{GxE}. following removal of three outlier characteristics (BARGLUME, PVPBARGLUME, and %ROUND).

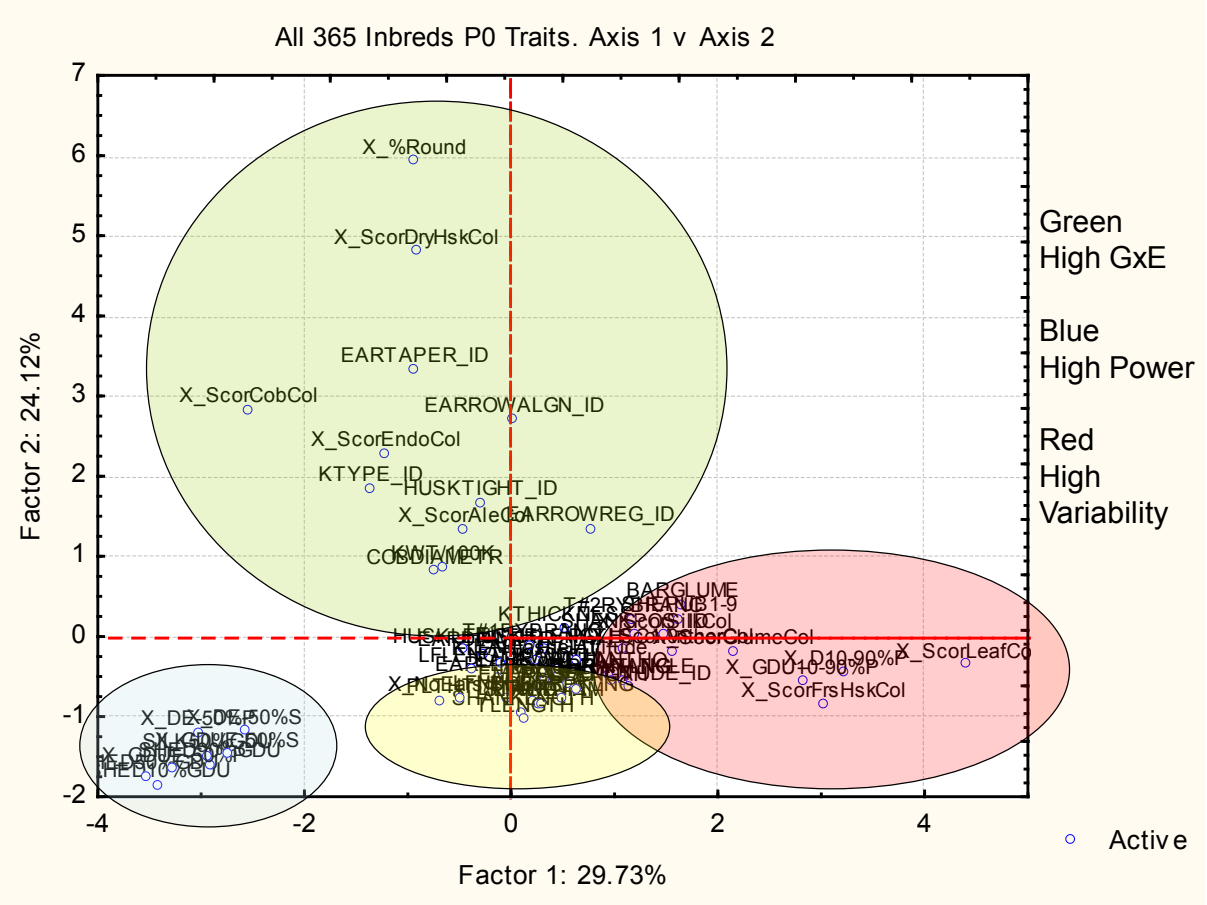

Figure 2 - Associations among characteristics and their assignations into classes (High GxE, designated in green), High Power (designated in blue) and High Variability (designated in red) using data from 365 inbreds shown by the first two factors expressing 29.7% and 24.1% of total variation, respectively.

(93.4%) and an additional 6 characteristics had Sigma² values over 50% (Table 2). For the MZ3 inbred set (Table 3) most characteristics (40) had increased Sigma² values of over 46%. There was a doubling of Sigma² for six of the group of eight maturity characteristics; albeit from low initial levels of Sigma², and also a substantial increase for number of primary tassel branches (T#1RYBRANC), number of secondary tassel branches (T#2RYBRANC) and shank length (SHANKLNGTH). In contrast, there were modest (5%) reductions in Sigma² for 22 characteristics compared to the 365 inbred set.

Parameter 4: Individual Environment Inbred Differentiation F-Ratio (MINF)

With the 365 inbred set (Table 2) each of the characteristics associated with the physiological process of "maturity", e.g., pollen shed and silk exertion, had large (<20) minimal F values. Exceptions were the pair of traits GDU from 10% to 90% pollen shed (GDU 10-90%P) and Number of days from 10% to 90% pollen shed (D10-90%P) which, apart from leaf color (SCORLEAFCOL), showed the lowest Minimal F of all characteristics. Cob color (SCORCOBCOL) and kernel type (KTYPE) also had high minimal F values indicative of, at least in respect of this parameter, potentially powerful PVP traits. In contrast, several characteristics had minimal F values below 2.0.

When MINF was measured using the MZ3 subset of inbreds (Table 3), 46 characteristics had lower values than based on the full set of inbreds; an average reduction of nearly 30%. The greatest reduction in MINF was for the set of maturity traits (each characteristic had a 70% smaller MINF compared to the 365 inbreds). Sixteen characteristics had increased Minimum F when measured using the MZ3 inbred subset. Characteristics cob color (SCORCOBCOL) and kernel type (KTYPE), not only retained a high MINF, they were the most powerful traits of all with respect to this parameter when measured using the 210 inbred subset. Characteristics with moderate levels of MINF and, which were also relatively unaffected by subsetting of inbreds, were (in ranked order): percentage of round kernels (%ROUND), tassel attitude (TASSELATTITUDE), husk extension length (HUSKELENGTH), brace root anthocyanin (BRTANTHO), number of primary tassel branches (T#1RYBRANC) and shank length (SHANKLENGTH) closely followed by weight of 100 kernels (KWT/100K) and ear height

(EARHT).

Parameter 5: Individual Environment Inbred Differentiation Percentage Exhibiting Significant Inbred Differentiation (SIGINBRED ENVIRP1)

Most characteristics contributed to significant inbred discrimination in each environment (each of eight years) (Table 2). In contrast, the characteristic leaf color (SCORLEAFCOL) had no statistically significant environments that provided inbred discrimination when assessed at the usual level of probability ($p<0.01$) or with a reduced stringency $p<0.05$. It was only with a weak stringency of $p<0.1$ and then for only a single environment (of a possible eight) that this characteristic achieved significance in terms of inbred differentiation.

Characteristics days from 10% to 90% pollen shed (D10-90%P) and tassel bar glume color (PVP_BARGLUME) exhibited significant inbred differentiation in 25% environments. Characteristics GDU from 10% to 90% pollen shed (GDU10-90%P), number of ears per stalk (NOEARS/STALK), and fresh husk color (SCORFRSHSKCOL) exhibited statistically significant inbred differences in 50% of the environments. Four traits expressed inbred differences at a statistically significant level ($p<0.01$) in seven out of 8 environments (years).

When the 210 MZ3 inbreds were used to measure this parameter (Table 3) all of the characteristics that were weak when measured using the 365 inbreds were also weak. In addition, characteristics leaf angle (LFANGLE), fresh husk color (SCORFRSHSKCOL) (no significant environments) and number of ears per stalk (NOEARS/STALK) (25% environments significant) showed large declines (in contrast to 100% environments when measured using the 365 inbreds).

Parameter 6: Inbred Discrimination F-Ratio (INBRED F)

Four characteristics exhibited particularly weak inbred discrimination "power" when measured using the 365 inbreds. These were: leaf color (SCORLEAFCOL), GDU from 10% to 90% pollen shed (GDU 10-90%P), number of days from 10% to 90% pollen shed (D10 -90%P), and fresh husk color (SCORFRSHSKCOL) (Table 2). Other characteristics had large inbred

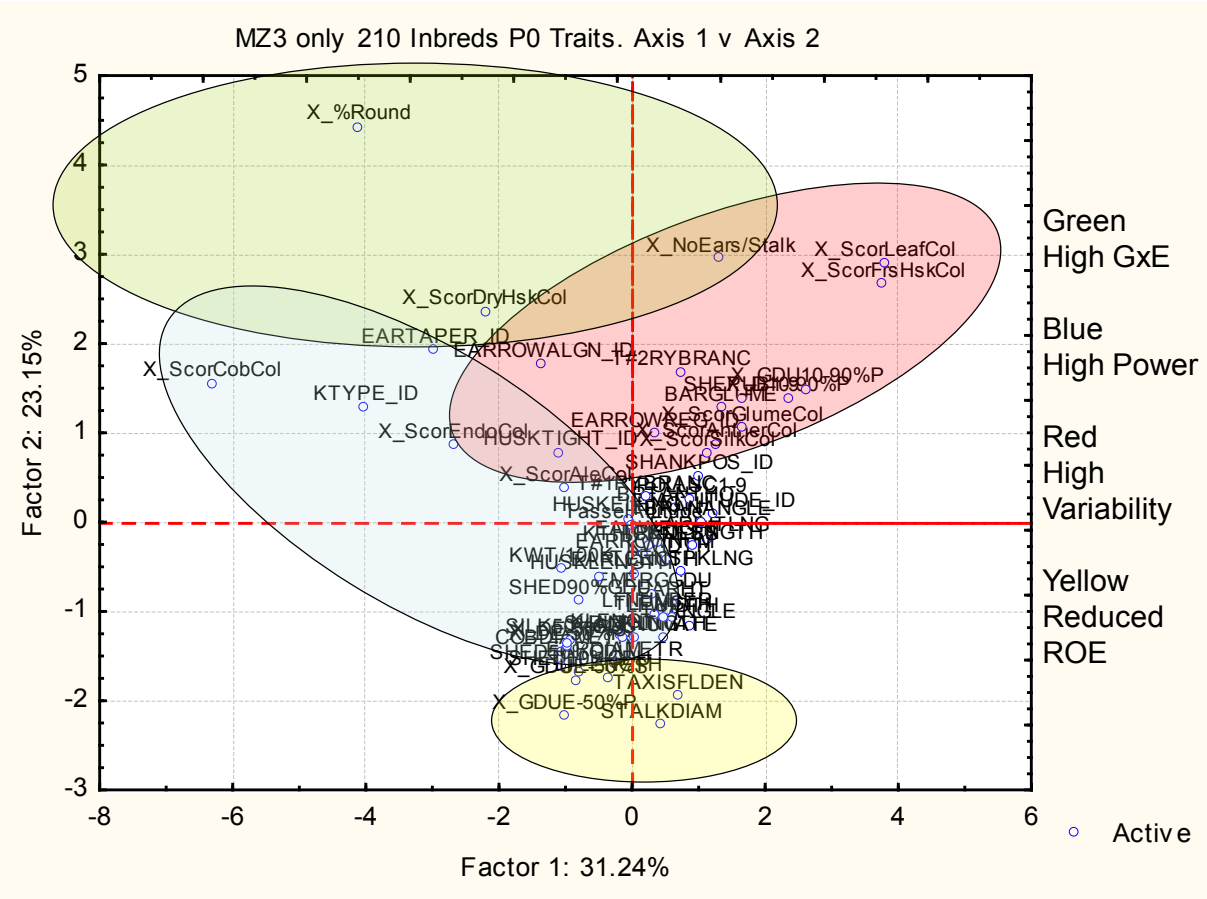

Figure 3 - Associations among characteristics and their assignations into classes (High GxE, designated in green), High Power (designated in blue), High Variability (designated in red) and Reduced ROE (designated in yellow) using data from 210 MZ3 inbreds shown by the first two factors expressing 31.2% and 23.2% of total variation, respectively.

F values. Each of the eight characteristics associated with maturity had F-Ratios of over 300, a value only exceed by number of ears per stalk (NOEARS/STALK) with a value of 500. Other characteristics with very effective inbred discrimination "power" included plant height (PLTHT), cob diameter (COBDIAMETR), leaf length (LFLENGTH), husk length (HUSKLENGTH), ear length (EARLENGTH), and number of primary tassel branches (T#1RYBRANC).

INBRED F-values for the 210 MZ3 inbreds (Table 3) showed similar results (correlation of 0.77 between the 210 and 365 inbred sets). For the MZ3 inbreds there were 12 characteristics with increased INBRED F-Ratios (average increase of 10.9%). Eight maturity characteristics showed markedly weaker F-ratio values; at least 68% lower than for the 365 inbreds but from very high values (>300). Other characteristics: plant height (PLTHT), kernel length (KLENGTH), node number (LFNUMBER), and ear diameter (EARDIAMETR) also exhibited reduced inbred F-ratios for inbred differentiation when examined using data from the MZ3 inbred subset.

Parameter 7: GxE F-Ratio (GXEF)

Twelve characteristics had statistically significant GxE effects ($p < 0.01$) based on an analysis of the 365 inbreds (Table 2). With the exception of 3 quantitative characteristics 100 kernel weight (KWT/100K), cob diameter (COBDIAMETR) and percent round kernels (%ROUND), the remaining characteristics showing significant GxE effects were qualitative traits; 3 assessing "color"; aleurone color (SCORALECOL), endosperm color (SCORENDROCOL), cob color (SCORCOBCOL) and 5 "ID" traits; regularity of kernel rows (EARROWREG), husk tightness, (HUSKTIGHT), ear row alignment (EARROWALGN), ear taper (EARTAPER) and kernel type (KTYPE).

When measured using the 210 MZ3 inbred subset (Table 3), exactly the same 12 characteristics had significant GxE effects. Results for other characteristics were also very similar when measured using either 365 or 210 inbreds (correlation of 0.98).

Parameter 8: % Inbreds with Significant GxE Interaction with Probability p<0.01 (SIGGXEP1)

Twelve characteristics showed greater than 10% of all 365 inbreds with significant environmental interactions (Table 2). Eight characteristics showed greater than 20% of possible inbreds with significant interactions with environment. These characteristics, in increasing order of percentage observed inbred GxE interaction were: endosperm color (SCORENDROCOL), percent round kernels (%ROUND), ear row alignment (EARROWALGN), cob color (SCORCOBCOL), ear taper (EARTAPER), dry husk color (SCORDRYHSKCOL), and regularity of kernel rows (EARROWREG). The characteristic fresh husk color (SCORFRSHSKCOL) had nil observed inbreds with significant GxE and with a similarly low GxE F-ratio. Five characteristics had less than 1% of observed inbreds significant and a further 10 characteristics had between 1% and 2%.

Very similar results were found for most characteristics when the 210 MZ3 inbreds (Table 3) were measured (correlation of 0.98). The characteristic kernel type (KTYPE) was an exception showing an increase of 14% compared to results obtained using 365 inbreds. It should be noted, however, that this characteristic was only recorded for four years.

Parameter 9: Chi-Squared Statistic for Testing Consistency of Contribution to SS GxE (CHIQ)

Based on the 365 inbred set, 18 characteristics had significant Chi-squared ($p < 0.01$) for irregular contribution to SS_{GxE} (Table 2). These characteristics included three maturity characteristics; days from emergence to 50% pollen shed (DE-50%P), days from emergence to 50% silking (DE-50%S), days from 10% to 90% pollen shed (D10-90%P). Five other maturity characteristics were non-significant with GDU to 50% silking (SILK50%GDU) exhibiting the 3rd weakest Chi-squared value. Kernel type (KTYPE) was recorded in only four environments but was nonetheless very consistent with respect to annual contribution to the total SS_{GxE}.

For the MZ3 210 inbred set (Table 3) there were also 18 traits with significant Chi-squared ($p < 0.01$) for irregular contribution to SS_{GxE} including the same maturity physiological characteristics; number of days from emergence to 50% pollen shed (DE-50%P), number of days from emergence to 50% silking (DE-50%S), and number of days from 10% to 90% pollen shed (D10-90%P), but otherwise, not exactly the same characteristics; kernel type (KTYPE) was also non-significant.

Groupings of characteristics with respect to profile of annual contribution to total SS_{GxE}

Associations of characteristics with respect to their annual contribution to total SS_{GxE} were complex (not shown), although three characteristics; tassel bar glume anthocyanin color development (BARGLUME), tassel bar glume color (PVP_BARGLUME and percent round kernels (%ROUND) were clear outliers. The clustering was therefore repeated following removal of these characteristics (Figure 1).

There was a general lack of clustering or structure among characteristics with the exception of a set of 6 silk and pollen shed maturity traits that were associated (less than Euclidean Distance 5) indicating similar annual contributions by those characteristics to total SS_{GxE}.

Associations among characteristics and among parameters

Associations among characteristics when measured using the 365 and 210 inbred subset are shown in Figures 2 and 3, respectively. Characteristics are associated according to their contributions to 1) High GxE, 2) High Power, 3) High Variability, and 4) reduced ROE. The association of maturity characteristics that is formed in the lower left hand quadrant

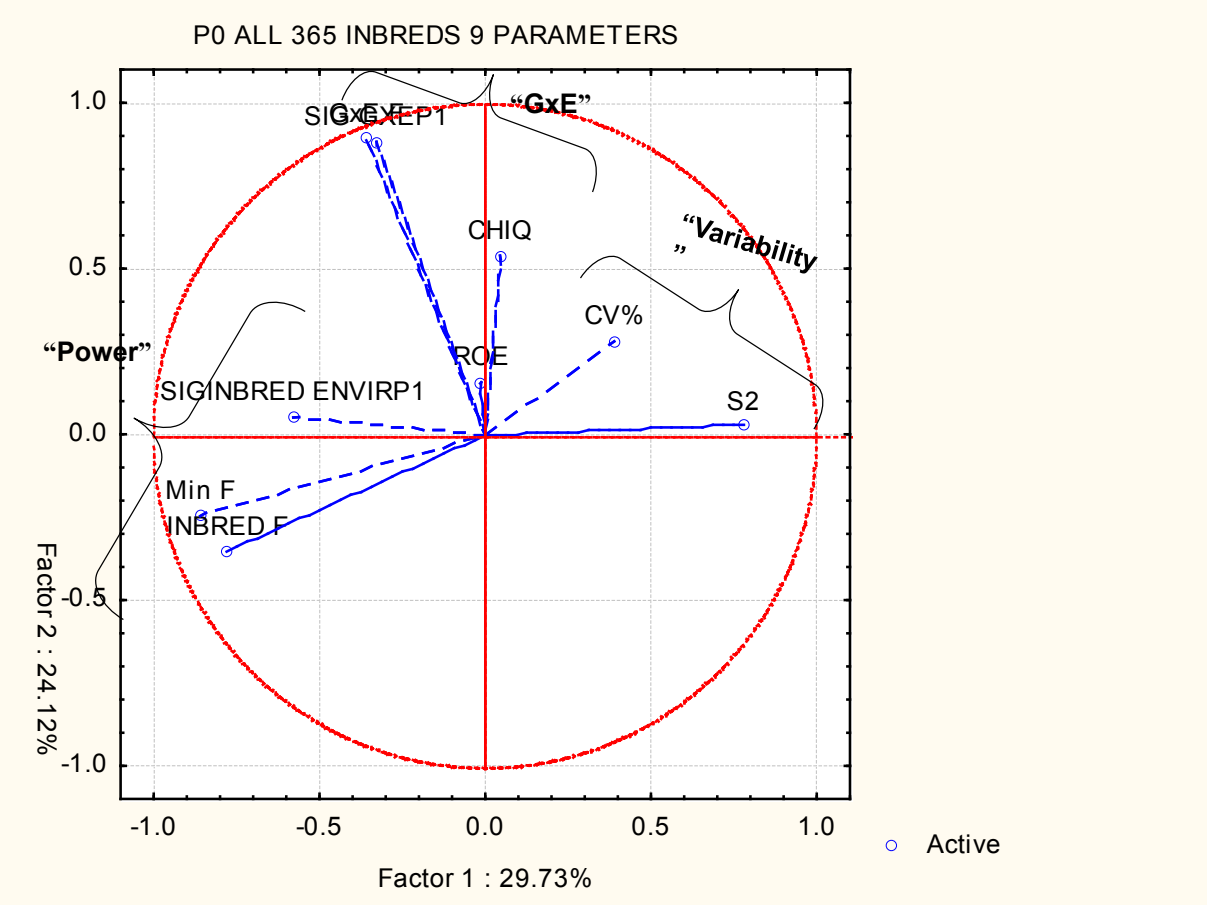

Figure 4 - Associations among each of the 9 parameters shown by the first two factors using multivariate analysis of data from 365 inbreds representing 29.7% and 24.1% of the variation, respectively.

for the analysis using the 365 inbreds (Figure 2) is not present for the MZ3 subset of 210 inbreds (Figure 3). Several of the same characteristics are spread out along the same axes regardless of whether the 365 inbreds or the 210 subset are the source of inquiry. These characteristics include: % round kernels (%ROUND), dry husk color (SCORDRYHUSKCOL), ear taper (EARTAPER), leaf color (SCORLEAFCOL), and fresh husk color (SCORFRSHSKCOL).

Associations among the nine parameters from measurements using 365 inbreds and from the 210 MZ3 inbred subset are shown in Figures 4 and 5, respectively where contributions of parameters vis-à-vis, the criteria of Power, GxE, and Variability are indicated. For the 365 inbred set (Figure 4) there was a correlation of 0.95 between GXE F ratio actual numbers of inbreds which express significant SIGGXEP, thereby indicative of parameter redundancy. A high correlation (0.9) was also evident among the 2 "power" statistics (INBRED F RATIO and INBRED MIN F) with the result that one parameter can be excluded without an overwhelming loss of information.

Results obtained using the MZ3 subset of 210 inbreds (Figure 5) showed similar associations among parameters; a high correlation (0.93) between GxE ratio and actual numbers of inbreds which express significant SIGGXEP1. The level of correlation between two power parameters (Inbred F Ratio and Inbred Min F) was reduced to 0.56 in contrast to the correlation value of 0.90 when measured using the wider maturity set of 365 inbreds.

Additional comparisons of results from 365 inbreds across maturity zones with results from the 210 subset of maturity zone 3 inbreds

The contribution of the first axis to total variation was 29.7% and 31.2% (365 and 210 inbreds, respectively). Over 50% variation was contributed by the first two axes (for both inbred sets); over 75% variation was contributed by the first 4 axes, and over 90% by the first 6 axes (Supplementary Table 1). Correlations (in parentheses) for each PCA transformed axis, when comparing the 365 and MZ3 210 subset of inbreds were: PCA 1 (0.74), PCA 2 (0.66). PCA 3 (-0.53), PCA 4 (0.5), PCA 5 (0.85), PCA 6 (0.62), PCA 7 (0.54), PCA 8 (0.37) and PCA 9 (0.76). Correlations across the full set of PCA axes were statistically significant ($p < 0.01$) with the major contribution to these correlations from the large PCA coordinates.

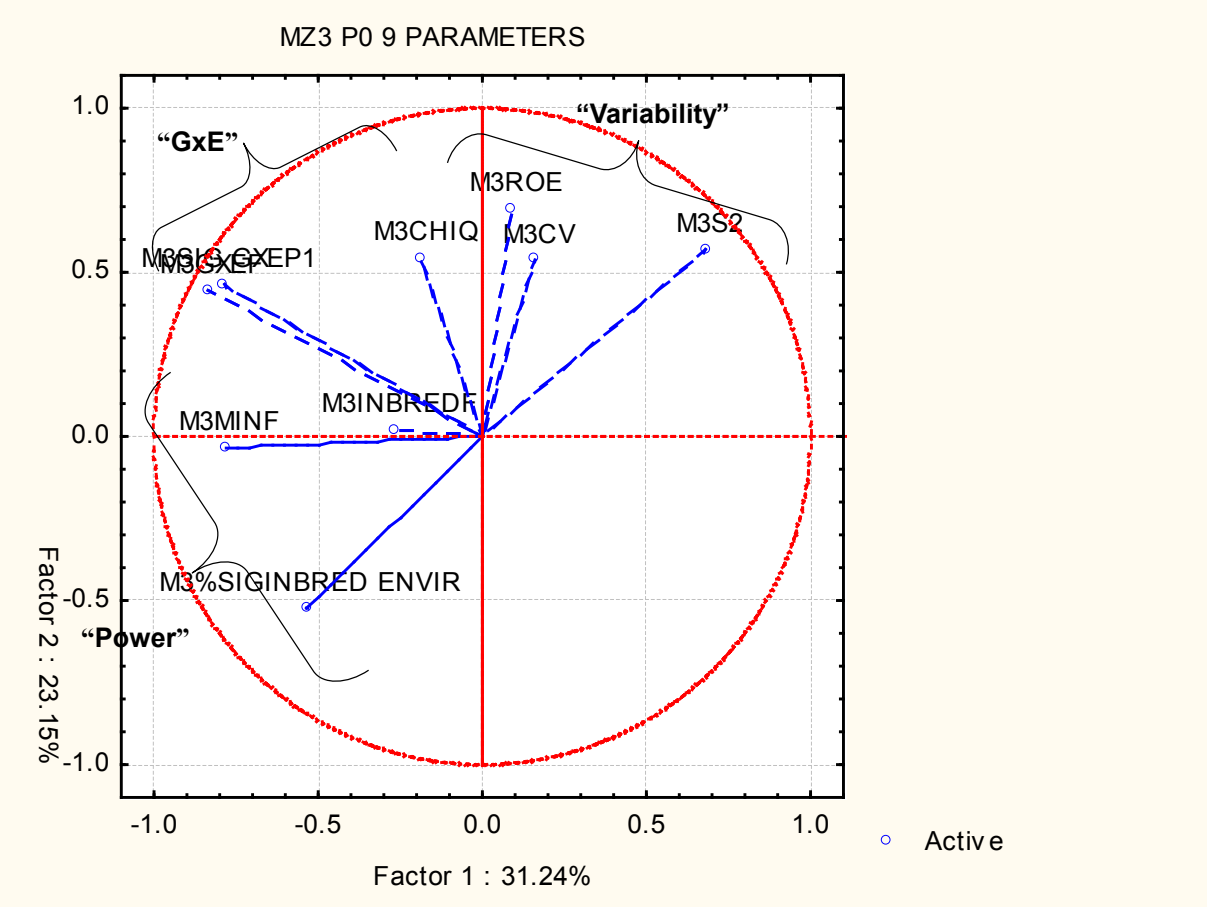

Figure 5 - Associations among each of the 9 parameters shown by the first two factors using multivariate analysis of data from 210 MZ3 inbreds representing 31.2% and 23.2% of the variation, respectively

Discussion

We used three criteria: 1) "Variability", 2) "Power" and 3) "GxE" to evaluate the relative abilities of each of 62 morphological characteristic to provide descriptions of maize inbred lines that are robust and discriminative. Within each of these criteria, several parameters were chosen to provide the statistical basis for evaluating the utility of individual morphological characteristic to provide robust discrimination among inbred lines.

The informative power of these parameters is evidenced by, for example, parameter 3 (Variance Components) and Sigma^2 (S2) where the relative size of the variance component and the robustness over reduced inbred data sets is a highly desirable attribute for effective and efficient PVP traits. Large Sigma^2 values point to characteristics where the useful discrimination power is being obscured or in some cases swamped by unattributable background variation. In other words the "signal to noise ratio" is low. For example, if Sigma^2, as percentage of the total observed variation, is 25%, then this result can be interpreted as showing that over a quarter of the total PVP description effort in recording this specific characteristic would be wasted. As another example, for Parameter 5: Individual Environment Inbred Differentiation Percentage Exhibiting Significant Inbred Differentiation (SIGINBRED ENVIRP1), when for any specific characteristic, the percentage of individual field trials where the F-Ratio for inbred differentiation achieved the target significance was low: this may indicate a potentially inefficient characteristic where the investment of resources fails to deliver comparable inbred differentiation. This may be for several reasons including high levels of unattributable background variation (high error mean square against which the inbred Mean-square is compared) and/or very low range of expression.

When results from the 365 inbred set, which included inbreds spanning from 70CRM to 126CRM were compared with the subset of 210 inbreds which spanned a more restricted range of maturities, (101CRM to 115CRM), representing the US Corn Belt, then major differences, as would be expected, were in regard to maturity characteristics. Selection of a set of inbred lines that represented a relatively small range of maturities would be expected to have the most effect on maturity and maturity related characteristics as the range of expression for

those characteristics will then have inevitably been reduced. Reductions in ROE were indeed found for the 210 subset of MZ3 inbreds for tassel axis floret density, cob diameter, stalk diameter, leaf length, ear diameter and GDU to 50% pollen shed. These results infer that these tassel, cob, stalk, leaf and ear characteristics are associated with maturity. In contrast, the majority of characteristics (85%) retained an ROE of 100% when examined using the 210 MZ3 subset of inbreds. The Parameter S2 which is a measure of signal to noise ratio also showed differential results for maturity characteristics when measured using the 365 or 210 MZ3 subset. Most (75%) of the maturity characteristics showed a doubling of S2 values when measured using the 210 MZ subset. Similar results were found for MINF where the greatest reductions (70%) were for maturity characteristics when the 210 MZ3 subset of inbreds was used as the data-source. Non-maturity characteristics were not much affected with regard to their parameter values when the results for the 365 and 210 sets of inbreds were compared.

These data show a wide range of values among the 62 characteristics for most of the nine parameters over which they were measured. For example, Trait CV% ranged from 7.7% to 80.2% (365 set of inbreds) and from 6.2% to 79.6% (210 MZ3 subset). Similarly, very wide ranges of parameter values were apparent for S2 and INBREDF. Other parameters also showed wide ranges of values: MINF (range 1.0-24.5) and SIGGXEP1 (range 0-41.6). Even the parameter SIG-INBRED ENVIRP1 which showed the least range of values (most at 100%), nonetheless provided useful information showing lower (more desirable) values for nine characteristics. These data, therefore, potentially provide a useful resource upon which to base the selection of subset of characteristics with the goal to select those which collectively can optimally satisfy the hypothetical attributes of ideal characteristics: 1) highly repeatable, 2) highly reproducible, 3) highly discriminative or powerful. Observation of the results from multivariate analyses, for both the individual characteristics and of the parameters within which they are associated, then provides information from which to make the final selection of characteristics upon the basis that they are independently and maximally informative.

That improvements in efficiency, robustness and discrimination are desirable is shown by relatively poor parameter values, in particular for characteristics that are required by UPOV to always be included in variety descriptions (the so-called asterisked characteristics) and also for those that are recommended by UPOV for grouping of maize inbred lines (UPOV, 2009). For example, the asterisked characteristic bar glume scored relatively poorly for five parameters (3,4,6,7,9). In contrast, the asterisked character Plant Height scored relatively well for all parameters, except for 7. Likewise, asterisked character, Number of Primary Tassel Branches, scored relatively well for all parameters although, in contrast, poorly for Parameter 2.

Given the interactions of morphological appearance with related physiological processes and the effects of environmental interaction, it is not surprising that results for many characteristics and for some parameters are not independent. For example, for Parameter 4 Individual Environment Inbred Differentiation F-Ratio (MINF), low values (e.g., below 2.0) may be indicative of low "power" generally (i.e., consistently low "signal to noise ratio") or interaction with environment (with a mix of acceptable seasonal F ratios for inbred discrimination, but also some weak or unacceptable F-ratios and weak inbred discrimination). The necessity to simultaneously consider values for numerous characteristics, each according to 9 parameters, in order to achieve the goal of selecting a subset of characteristics which can collectively provide for the more robust, reliable, and efficient discrimination of numerous inbred lines indicated the need to develop a data driven and iterative process specifically for this purpose. We report upon that selection and evaluation process in a subsequent paper.

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