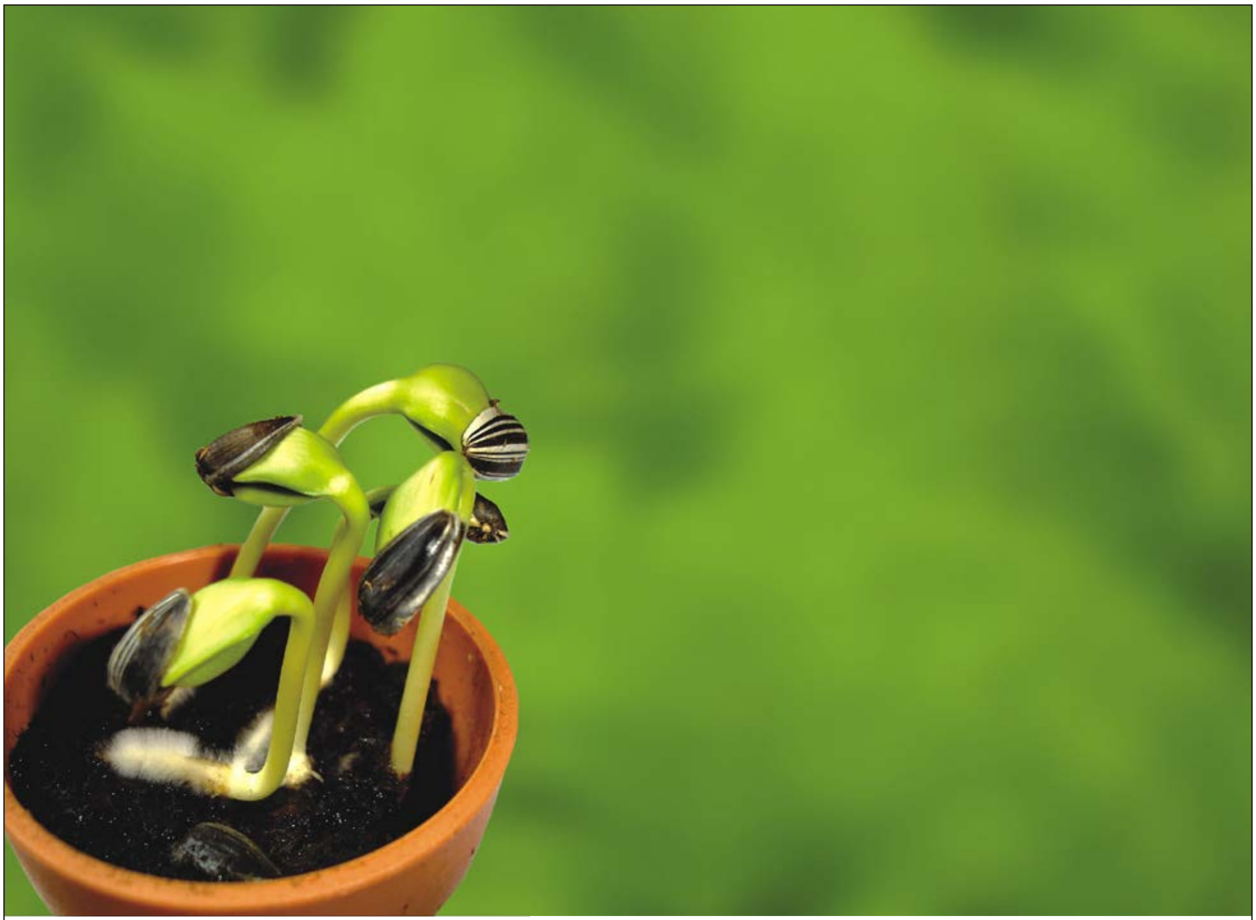

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Digital Seed Morpho-metric Characterization of Tropical Maize Inbred Lines for Cultivar Discrimination

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ABSTRACT

Cultivar discrimination of parental inbred lines is essential for genetic purity of F1 hybrid seed products. It is also essential for the purpose of maintenance of agronomic performance, variety registration, issuance of breeders' rights, proper labeling of seed products and protection of investments in plant breeding. Digital imaging analysis had been identified as a fast and reliable method for variety discrimination and was applied in this study. With the aid of a digital imaging software (Veho™), 10 seed morpho-metric traits were digitally measured and/or calculated from captured images of 17 inbred lines drawn from the *Striga*-resistant tropical maize population. The seed metric data were subjected to Pearson's correlation analysis, the Principal Component Analysis (PCA) and the Single Linkage Cluster Analysis (SLCA). All the parameters showed positive correlation except embryo angle, seed shape factor and circularity index. Seed area, length, perimeter and flatness index contributed the largest variability within the population (eigenvectors = 0.332, 0.328, 0.323 and 0.318), respectively, suggesting their potential usage for cultivar discrimination by digital imaging of maize seeds. Dendrogram constructed from the SLCA showed four major clusters of the inbred population. The clusters constitute a classification tool for future evaluation of genetic purity of test inbred and F1 hybrid samples within *Striga* resistant maize population. Results of this study demonstrated applicability of digital imaging for analysis of seed genetic quality and will ultimately improve the hybrid seed sector in Nigeria.

Key words: Tropical maize, seed geometry, digital imaging, clustering analysis, cultivar identification, genetic purity

INTRODUCTION

The hybrid maize seed industry in Nigeria is steadily growing through activities of public research institutions and a budding commercial seed sector (Daniel and Adetumbi, 2006). The International Institute Tropical Agriculture (IITA), a Consultative Group of International Agricultural Research (CGIAR) center is responsible for the development of over 100 maize inbred and hybrid populations adapted to various agro-ecologies of Nigeria and constituting up to 98% of parental lines used for commercial hybrid seed production in Nigeria (Manyong *et al.*, 2003). The proliferation of parental stock and commercial F1 hybrid seeds in Nigeria elicited the challenge of genetic purity of seed stocks. Since distinctness of parental inbred lines is essential for genetic purity of F1 hybrid seed products (Sujiprihati *et al.*, 2003; Daniel and Oloyede, 2010), reliable and fast cultivar identification is imperative for distinctness and genetic purity of commercial hybrid seeds in the Nigerian seed sector.

Laboratory method for measuring seed lot purity involved ocular observation with the purity board which at best is slow, have low reproducibility and possess a degree of subjectivity which is hard to quantify. Hence there is need for fast, reliable and economical methods for genetic purity of seed lots. Several workers had demonstrated the bio-systematic significance of seed morphology and its importance in genotype discrimination (Fawzi *et al.*, 2010; Fawzi, 2011). However, the use of digital imaging of seed metrics to quantitatively discriminate cultivars offer more accurate evaluation of morphological (Keefe and Draper, 1986; Salimpour *et al.*, 2007; Geetha *et al.*, 2011) and even colour parameters (Dell'Aquila, 2006; Grillo *et al.*, 2011) than traditional ocular visualization with purity boards. Varietal characterization through image analysis with computer vision techniques also has advantages of speed of seed testing and extended usefulness of captured images in databases (Sainis *et al.*, 2006). Captured images in database are useful resources for analyzing genetic purity of inbreds and F1 hybrid progenies.

The present study seeks to apply digital seed image analysis to discriminate inbred lines of the *Striga*-resistant maize population, which contains a number of important parental stocks currently used for producing many tropically adapted commercial hybrid maize in Nigeria. The aim of the study was to establish clusters of the population based on seed morpho-metric data.

MATERIALS AND METHODS

Seeds of 17 tropically adapted maize inbred lines were collected from International Institute of Tropical Agriculture (IITA), Ibadan, Table 1. All the accessions were drawn from the *Striga*-resistant population adapted to the lowland humid tropical agro-ecologies. The digital imaging experiments were carried out at the Department of Plant Breeding and Seed Technology, University of Agriculture, Abeokuta, Nigeria on the inbred seed lots produced in 2009.

Image analysis: Thirty seeds of each of the seventeen inbred lines were randomly sampled for digital imaging analysis in three replications. The imaging analysis was performed on individual seeds with the aid of a digital imaging software (Veho™, UK) installed on a USB digital microscope. The seeds were placed under the USB microscope lens in such a way that the embryo axis of the seeds was facing the lens of the camera under scanning light and magnification of x40. Images of each seed were captured and stored in a folder in the computer system. Measurements were taken by clicking on the captured images to transfer them to the measurement window of the software

Table 1: List of inbred genotypes in the IITA *Striga*-resistant population of tropical maize

Line No.	Line name	Line No.	Line name
1	TZSTR139	10	TZSTR184
2	TZSTR150	11	TZSTR185
3	5057	12	TZSTR186
4	TZSTR166	13	TZSTR187
5	TZSTR167	14	1368STR
6	TZSTR168	15	1393
7	TZSTR170	16	5057
8	TZSTR179	17	9540
9	TZSTR182		

where measurement icon menus were utilized to analyse the seed. Before actual measurements, the system was calibrated to millimeters under x40 magnification. The parameters measured on captured seeds were:

- Seed length-the distance between 2 points stretching from the base of the embryo axis to the tip of the endosperm of the maize seed
- Seed width-this is the length a line drawn across the widest section of the seed taken at right angle to the length of the seed
- Seed thickness-the dimension taken from one side across the surface of the endosperm area of the seed
- Seed area-product of seed length and width
- Seed perimeter-perimeter of a circle drawn around the seed touching all edges
- Embryo angle-value of the angle created by 2 lines joined at the base of the seeds and subtending to the tip of the seed on the 2 sides of the embryo axis

Seed shape parameters were derived from the measured parameters as follows:
Shape factor estimated as:

$$SF = (4\pi \times A)/P^2$$

where, A is area of the object, P is the perimeter of the object (Grillo *et al*., 2011):

- Flatness index-the flatness index was estimated as the ratio of seed length to seed width and thickness following Adewale *et al*. (2010) as:

$$FI = SL+SW/2\ ST$$

where, SL is seedling length, SW is seed width and ST is seed thickness.

- Eccentricity index-ratio of SL to SW according to Balkaya and Odabas (2002):

$$EI = SL/SW$$

- Circularity index-the square root of the ratio of actual area of seed object to the area of a circle with the same circumscribed shell. The estimation was done as:

$$CI = \sqrt{(A/AP)}$$

where, A is actual area of the object, AP is the area of a circle with a diameter equal to the circumscribed diameter or length of the object (Geetha *et al*., 2011).

Data analyses: The data was subjected to statistical analytical system (SAS™) procedures for Pearson correlation analysis, principal component analysis (PCA) and the single linkage cluster analysis (SLCA).

RESULTS

Mean, range and standard deviation values are reported in Table 2. Seed size based on seed length, thickness, perimeter and area were highest in inbred lines TZSTR139 and TZSTR186 and least in TZSTR166. Seed shape characteristics like flatness index and eccentricity index were also highest in lines TZSTR186 and TZSTR139. Inbred line 9540 with highest value of seed width and shape factor was least in circularity index. The range of embryo angle among the inbred lines did not show a regular trend with other seed metric values, except that line 1368STR with the highest embryo angle was least in circularity index.

Positive correlation exists among seed length and most other seed morpho-metric values except seed angle, seed shape and circularity index Table 3. Embryo angle was negatively correlated with other seed metric values except seed width and seed shape factor. Circularity index was negatively correlated to all other seed metric parameter other than flatness index.

Values of eigenvectors in the first two principal component axes showed that contribution of seed morpho-metric parameters to the variability in the population was highest in seed area with the vector loading of 0.332 (Table 4). Three other variables have eigenvector loadings above 0.3 in principal component 1, these are seed length, seed perimeter and flatness index with eigenvector values of 0.328, 0.323 and 0.318, respectively.

Figure 1 shows the dendrogram resulting SLCA to illustrate the relationship between the inbred lines on minimum distance scale. Table 5 shows a summary of major clusters from the dendrogram.

Table 2: Simple descriptive statistics of seed morpho-metric traits of tropical maize inbred population

	Mean	Minimum (genotype)	Maximum (genotype)	Standard error	Standard deviation
Seed length (mm)	2.99	2.51(TZSTR179)	3.47(TZSTR139)	0.04	0.30
Seed width (mm)	2.33	1.82 (TZSTR166)	2.83 (9540)	0.04	0.26
Seed thickness (mm)	1.35	0.89 (TZSTR166)	1.80 (TZSTR186)	0.03	0.21
Embryo angle (°)	68.41	57.52 (5057)	79.29 (1368STR)	0.77	5.53
Perimeter (mm)	9.95	7.80 (5057)	12.10 (TZSTR139)	0.13	0.96
Area (mm^2)	7.18	4.95 (TZSTR166)	9.41 (TZSTR186)	0.42	1.36
Shape factor	1.05	0.87 (TZSTR139)	1.16 (9540)	0.09	0.09
Flatness index	3.74	2.11 (TZSTR166)	5.38 (TZSTR186)	0.12	0.86
Eccentricity index	1.22	1.03 (1368STR)	1.38 (TZSTR139)	0.04	0.09
Circularity index	0.66	0.59 (9540)	0.73 (TZSTR166)	0.016	0.04

Table 3: Pearson Correlation coefficients of observed seed traits

	SL	SW	ST	EA	P	A	SF	EI	FI	CI
SL	1.00	0.71	0.76	-0.40	0.97	0.93	-0.16	0.28	0.89	-0.72
SW		1.00	0.58	0.16	0.71	0.92	0.45	-0.47	0.75	-0.97
ST			1.00	-0.43	0.72	0.73	-0.04	0.16	0.96	-0.59
EA				1.00	-0.39	-0.13	0.64	-0.70	-0.20	-0.13
P					1.00	0.91	-0.27	0.24	0.86	-0.68
A						1.00	0.15	-0.09	0.89	-0.90
SF							1.00	-0.80	0.004	-0.54
EI								1.00	0.09	0.40
FI									1.00	-0.75
CI										1.00

SL: Seed length, SW: Seed width, ST: Seed thickness, P: Perimeter of the seed, A: seed area, EA: The embryo angle, EI: Eccentricity index, SF: Shape factor, FI: Flatness index, CI: Circularity index

Table 4: Eigenvectors of seed morpho-metric parameter from the principal component analysis

Parameters	Prin1	Prin2
SL	0.328	-0.105
SW	0.287	0.270
ST	0.275	-0.043
EA	-0.090	0.434
P	0.323	-0.127
A	0.332	0.080
SF	-0.0002	0.506
EI	0.020	-0.497
FI	0.318	-0.007
CI	-0.283	-0.281

Table 5: Summary of cluster members from the SCLA of the 17 tropical inbred lines

Clusters	Linkage distance	Inbred lines
A	0.1-0.2	TZSTR168, TZSTR179
B	0.20-0.25	TZSTR139, TZSTR186, TZSTR150, TZSTR170, TZSTR182, TZSTR186
C	0.30-0.49	TZSTR139, TZSTR150, TZSTR168, TZSTR170, TZSTR179, TZSTR182, TZSTR184, TZSTR185, TZSTR186, TZSTR187, 1393
D	0.51 - 0.6	All the accessions

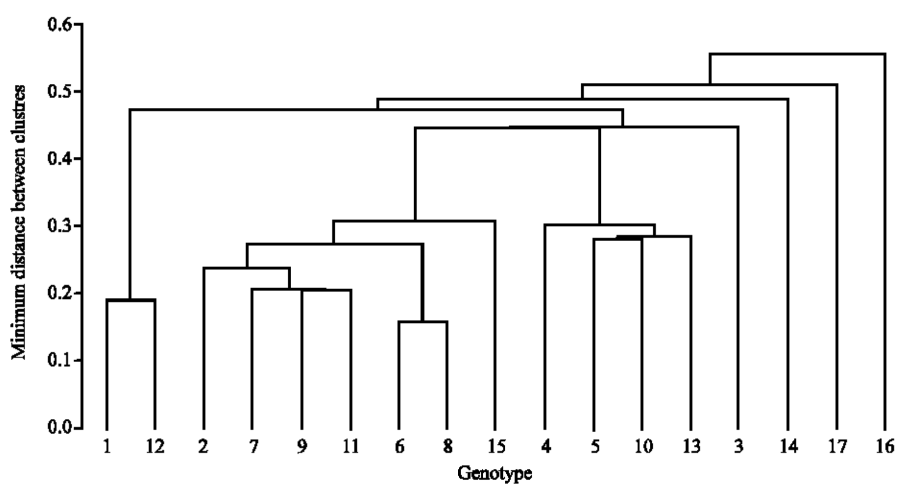

Fig. 1: Dendrogram resulting from single linkage cluster analysis (SLCA) of the inbred genotypes of *Striga*-resistant maize population

Based on minimum distance between clusters, four main clusters were identified, two inbred lines were scored in the cluster A at 0.1-0.2, six inbred lines were grouped between 0.2-0.25 in cluster B and twelve inbred lines were grouped in cluster C between 0.3-0.39.

DISCUSSION

The seed sector in emerging economies of the world is challenged with need to improve quality of seed products to meet global standards (Kizilaslan and Onurlubas, 2010; Daniel *et al.*, 2012). However, enhancing seed quality require investments in easily adaptable technologies, which makes seed digital imaging techniques suitable for evaluating genetic quality of tropical seed products. Seed quantitative descriptors generated by digital imaging analysis had been reported to better define cultivar identity in comparison to traditional sensorial descriptors which are categorical descriptors (El-Naggar, 2001; Shaheen, 2002; Dell'Aquila, 2006). Results of this study showed potential of various digital seed metric measurements on a tropical maize population for cultivar discrimination within the population.

In this study, correlation coefficients were generally high among most of the seed metric parameters which denote association between seed metric traits and their potential usefulness as discriminators in maize. Wyllie-Echeverria *et al.* (2003) also showed that seed size and shape metrics were important discriminators of *Zostera marina*. Though seed angle was key discriminatory quantitative variable in earlier digital imaging analysis works of Keefe and Draper (1986), it is negatively correlated variable with most seed size variables except seed width and shape variables except shape factor. This is understandable since embryo angle is the value of the angle created by 2 lines joined at the base of the seeds and subtending to the tip of the seed on the 2 sides of the embryo axis. Thus longer and narrower denote decreasing values of the angle. In essence, seed angle will be a useful quantitative seed descriptor in the opposite direction of most other descriptors reported in this work. All seed size parameters showed high correlation coefficients in both positive and negative directions, suggesting that they are potentially good quantitative taxonomic descriptions of maize seed morphological classification. Adewale *et al.* (2010) reported similar finding for Africa yam bean seeds. However, the PCA analysis showed seed area, length, perimeter and flatness index as contributing the largest variability in the first two principal components axes of the genotypes, suggesting they are the main seed metric variables to select for effective discrimination of the inbred cultivars and possibly their hybrid derivatives.

Cluster analysis is a method often used for genotype groupings in order to cluster entries that show similarities in one or more characters. For the purpose of cultivar discrimination, clustering analysis provides information on genetic alignment of parental population and genetic purity of subsequent generation that clusters with the parents (Eevera *et al.*, 2009). The dendrogram resulting showed how the inbred lines clustered based on seed morpho-metric parameters. This classification forms a basis for grouping of parental inbred lines based on digital seed morpho-metric measurements and constitutes background data for future evaluation of genetic purity of test inbred and F1 hybrid samples from crosses involving these parents.

CONCLUSION

These seed morpho-metric parameters evaluated in this study are useful discriminators of maize genotypes. Thus classifications obtained in this study can be used to discriminate seed lots and cultivars test and test the purity of seed samples.

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