# Morphological diversity and relationships among the IPGRI maize (Zea mays L) landraces held in IITA

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# Abstract

Genetic variability estimates in maize (*Zea mays* L) landraces is an important information for trait improvement for food and nutrition security. Genetic diversity information in the Sub-Sahara African maize landraces is lacking. Agromorphological trait evaluation is a practical approach for genetic diversity estimation. Our objective was to assess genetic diversity among 60 IPGRI maize landraces held in IITA, Ibadan, against a check, «Obatanpa GH». Twenty-one quantitative traits and five qualitative traits were field-evaluated in Ghana in 2011 and 2012 wet seasons in a three-replicated randomized complete block design experiment. Large phenotypic variation was identified in all traits except cob colour, principal grain colour, and number of ears per plant. A moderate within population variation based on pooled Shannon Diversity Index was  $0.68 \pm 0.28$ . Between population variation was largest in earliness, anthesis-silking interval, and grain yield. Genetic similarity of  $0.11 \pm 0.00$  based on squared on the basis of maturity characteristics, anthesis-silking interval, plant and ear heights, and grain yield. The first two principal components explained 67.89 % of the total variance. Four genotypes, TZm-1125 and TZm-1117 (5.0 Mg ha<sup>-1</sup>), TZm-1119 (5.4 Mg ha<sup>-1</sup>), and TZm-1139 (6.2 Mg ha<sup>-1</sup>) competed with the check (5.8 Mg ha<sup>-1</sup>) in grain yield. The IPGRI genotypes represent a large genetic reserve awaiting exploitation for trait improvement.

Keywords: maize, IPGRI landraces, agromorphological diversity, cluster analysis, principal components analysis

#### Introduction

Maize, an important food crop for over 70% of the population of Sub-Saharan Africa (SSA) requires continuous improvement in productivity and quality to meet current and future demands for food, feed and industrial purposes. This region faces rapid growth in population (Barriere et al, 2010) and constant threat of the negative effects of climate change, particularly, heat and drought stress to crops (Cairns et al, 2011). Current maize productivity in SSA stands at less than 2.0 Mg ha<sup>-1</sup>, except for Ethiopia and South Africa where grain yield surpasses 3.0 Mg ha<sup>-1</sup> on farmer's field (Abate et al, 2015). Having inadequate grain yield, Africa imports 28% of her maize (IITA, 2012).

Genetic variation is indispensable for effective maize improvement and relies on collection, conservation, and evaluation information. Although SSA has made significant collection of local maize landraces held in the International Institute of Tropical Agriculture (IITA), Ibadan, information on the genetic architecture of the collection is limited, leading to underutilization and reliance on the well-studied productive exotic varieties for improvement. Fowler et al (2000) reported of at least 73% reliance on exotic maize in SSA for trait improvement owing to supposedly less productivity of tropical maize (Dowswell et al, 1996; CIMMYT, 1988). Generally believed to be introduced into Africa, the landraces of SSA constitute a well-adapted, heterogeneous, and dynamic set which exhibit stability to the local environment, resistance to pest and diseases, drought tolerance, and low requirement of agricultural input. Without information on genetic diversity in the landraces, selection of diverse parents to maximize genetic variation and exploit heterotic groups is likely to be difficult. An exception is the ongoing utilization of the drought tolerant maize using varieties adapted to African climate in the droughttolerance breeding program (AATF, 2015).

Among the phenotypic, biochemical, and molecular methods (Mohammadi and Prasanna, 2003; Pejic et al, 1998) employed in assessment of genetic diversity, the phenotypic method offers an inexpensive approach for developing countries where labour cost is considerably low. Although the morphological characters are limited by environmental influence, low heritability, and chances of biased estimates of genetic distances compared with biochemical and molecular distances, they reveal comparable patterns of genetic variation in a species, and are the first markers used by breeders to identify promising parents for establishment of breeding populations (Camussi et al, 1985).

Current interest in the genetic diversity in African

maize using phenotypic markers has led to the estimation of genetic distance of 0.30 (Beyene et al, 2006) in 62 highland Ethiopian maize based on 15 morphological traits, a lower value than the SSR-based and AFLP-based mean genetic distance of 0.49 and 0.57, respectively. Obeng-Antwi (2007) reported congruence between phenotypic Euclidean distances and AFLP-based Jaccard distances in two populations of maize, but no correlation between the two distance measures. Cluster analysis of 294 landraces originating from Malawi, Zambia, and Zimbabwe using 34 phenotypic traits partitioned the set into three nonoverlapping groups (Magorokosho, 2006). Asare et al (2016) assessed a morphology-based genetic diversity in maize of lowland, mid-altitude and highland regions of Africa and reported large variability among the accessions with genetic similarity values of 0.26  $\pm$ 0.18. Similarly, morphological evaluation of 15 maize landraces in Northern Tanzania (Nestory and Reuben, 2016), 43 and 98 local and improved cultivars in Central and Northern Benin (Salami et al, 2015) and 87 landraces of Southern Benin (Salami et al, 2017) revealed large differences among accessions.

Some elite African inbred lines and accessions have in the past contributed to maize improvement in exotic lines, as those genotypes were reported to demonstrate good yield potential, disease resistance, and overall favorable agronomic performance (Nelson and Goodman, 2008; Tallury and Goodman, 1999).

The International Plant Genetic Resources Institute (IPGRI) of the Food and Agricultural Organization (FAO) maize collection constitutes a subset of the entire IITA collection, of which nothing is known about their genetic background, relationships and their geographical origins. This research was designed to study the agromorphological diversity and relationships among the IPGRI landrace maize population.

#### Materials and Methods

#### Plant material

Sixty tropical maize accessions sampled from the maize collection of the IITA Genetic Resources Center, Ibadan, were studied (Table 1). The accessions were collected by the International Plant Genetic Re-

2

sources Institute, Italy (currently Biodiversity International). Records on their geographical origin are not available. An open-pollinated genotype, «Obatanpa GH», developed by the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research, Ghana, in collaboration with IITA, CIMMYT, and Sasakawa Global 2000, served as check.

# Field preparation

The field study was conducted in the wet seasons of March to July 2011 and April to August 2012 in the Kwame Nkrumah University of Science and Technology Agricultural Research Station, Anwomaso, Ghana. This station is located at latitude 6°41'28.4"N and longitude 1°30'58.8"W at an elevation of 277 masl and mean annual rainfall and temperature of 1,500 mm and 20°C, respectively. The accessions were planted on single row 6 m  $\times$  0.6 m plots with 15 plants, 0.5 m spacing between hills and 1.0 m alley between rows in a randomized complete block design with three replicates making a density of 42,000 plants ha-1. Rows were thinned to one plant per hill. Irrigation was performed as and when needed. Plants were fertilized at knee height by side dressing at 5.0 g per hill with 16-16-16 N-P2O5-K2O equivalent to 120:60:40 kg ha<sup>-1</sup> and top dressing with 50 kg ha<sup>-1</sup> sulphate of ammonia at ear emergence. Pre-emergence weeds were controlled with Round-up ready at a rate of 4 I ha-1. Post-emergence weeds were cleared by hand hoeing. Pests were controlled with Conpyrifos (48 %; 1.0 -1.5 | ha<sup>-1</sup>) and Cymethoate Super (1.0 - 1.5 | ha<sup>-1</sup>). Morpho-phenological trait evaluation

At physiological maturity, maize plants were evaluated using the IBPGR (1991) maize descriptor list. Five qualitative traits, namely, silk color (pale yellow = 1; red = 2), cob colour (0 = red; 5 = white), kernel arrangement (1 = regular; 2 = irregular; 3 = straight; and 4 = spiral), kernel texture (1 = flint; 3 = mixed; 5 = dent), and principal grain colour (0 = white; 1 = other colors) were measured as frequency on a plot basis. Twenty-one quantitative traits, namely, anthesis date (AD, number of days to 50% pollen shed), silking date (SD, number of days to 50% silking of at least 1 cm long), anthesis-silking interval (ASI, calculated as SD-AD), and tassel length (TL, length in cm of tassel

Table 1 - The IPGRI African maize landraces evaluated in 2011 and 2012 rainy s	season in Ghana.
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Entry	Accession Name						
1	TZm-1097	16	TZm-1119	31	TZm-1139	46	TZm-1182
2	TZm-1099	17	TZm-1120	32	TZm-1141	47	TZm-1183
3	TZm-1100	18	TZm-1121	33	TZm-1142	48	TZm-1184
4	TZm-1101	19	TZm-1122	34	TZm-1143	49	TZm-1185
5	TZm-1103	20	TZm-1123	35	TZm-1144	50	TZm-1187
6	TZm-1105	21	TZm-1125	36	TZm-1145	51	TZm-1188
7	TZm-1106	22	TZm-1126	37	TZm-1147	52	TZm-1190
8	TZm-1108	23	TZm-1128	38	TZm-1148	53	TZm-1193
9	TZm-1109	24	TZm-1129	39	TZm-1149	54	TZm-1194
10	TZm-1110	25	TZm-1130	40	TZm-1150	55	TZm-1195
11	TZm-1111	26	TZm-1131	41	TZm-1151	56	TZm-1211
12	TZm-1112	27	TZm-1132	42	TZm-1152	57	TZm-1212
13	TZm-1114	28	TZm-1136	43	TZm-1153	58	TZm-1213
14	TZm-1117	29	TZm-1137	44	TZm-1156	59	TZm-1214
15	TZm-1118	30	TZm-1138	45	TZm-1180	60	TZm-1215

			Landrace			Check				
No.	Trait	Overall Mean $\pm$ SD <sup>1</sup>	Mean $\pm$ SD	Min <sup>2</sup>	Max <sup>3</sup>	$Mean \pm SD$	Min	Max	CV4 (%)	Mean square
1	AD (days)	54.8 ± 6.2	54.8 ± 6.2	39.0	74.0	48.8 ± 3.0	45.0	53.0	11.4	2,081.1***
2	SD (days)	$57.6 \pm 6.3$	$57.5 \pm 6.3$	44.0	78.0	$52.5 \pm 3.0$	48.0	56.0	11.0	1,500.8***
3	ASI (days)	$2.8 \pm 1.5$	$2.8 \pm 1.5$	-2.0	9.0	3.7 ± 1.1	3.0	6.0	53.7	47.3***
4	TL (cm)	$45.0 \pm 8.3$	45.1 ± 8.3	11.0	73.0	47.8 ± 7.1	29.5	65.0	18.4	425.3*
5	ELL (cm)	81.5 ± 13.8	81.6 ± 13.8	7.3	117.0	79.7 ± 15.5	45.0	105.5	17.0	204.6ns
6	ELW(cm)	7.4 ± 1.5	7.4 ± 1.5	2.0	12.5	8.3 ± 1.6	4.8	11.5	20.8	48.5***
7	PH (cm)	191.5 ± 47.0	191.9 ± 47.1	43.0	325.0	171.3 ± 35.3	75.0	249.0	24.5	25,021.0***
8	EH (cm)	97.2 ± 35.4	97.6 ± 35.5	7.0	325.0	77.7 ± 24.6	27.0	132.0	36.5	23,334.7***
9	StD (mm)	$19.9 \pm 3.6$	$20.0 \pm 3.6$	7.0	35.0	17.9 ± 3.2	11.0	25.4	18.0	242.7**
10	SG (%)	69.0 ± 221.0	68.9 ± 22.5	7.4	100.0	$75.2 \pm 3.5$	71.0	82.0	32.3	2,349.1*
11	EL (cm)	$15.04 \pm 2.9$	$15.0 \pm 2.9$	4.5	26.2	17.6 ± 2.2	9.0	21.0	19.7	330.8***
12	ED (mm)	$37.2 \pm 6.6$	$37.7 \pm 6.4$	15.0	55.5	$46.0 \pm 4.4$	38.9	55.1	17.7	3,341.4***
13	CD (mm)	$25.7 \pm 4.6$	$25.6 \pm 4.5$	10.0	43.0	$28.6 \pm 3.8$	23.9	42.1	18.0	424.3***
14	EN	$1.0 \pm 0.2$	$1.0 \pm 0.2$	1.0	3.0	$1.1 \pm 0.2$	1.0	2.0	15.6	0.1ns
15	NRE	$13.9 \pm 2.0$	$13.9 \pm 2.0$	6.0	22.0	13.4 ± 2.0	10.0	18.0	14.2	12.6ns
16	NKR	$28.6 \pm 7.2$	$28.9 \pm 7.0$	5.0	50.0	$28.9 \pm 8.3$	14.0	40.0	25.1	0.3ns
17	HK (g)	52.2 ± 13.6	53.5 ± 12.9	9.8	79.6	83.6 ± 14.3	49.2	117.2	26.1	44,535.7***
18	KL (mm)	8.5 ± 1.2	8.5 ± 1.2	5.0	12.5	$10.3 \pm 1.4$	8.0	12.8	14.2	147.8***
19	KW (mm)	$8.4 \pm 0.9$	$8.4 \pm 0.9$	3.5	11.9	$10.0 \pm 0.9$	8.8	12.4	10.9	117.9***
20	KT (mm)	$4.6 \pm 0.9$	$4.6 \pm 0.9$	3.0	9.0	$5.4 \pm 0.5$	4.2	6.9	18.7	29.8***
21	YLD (Mg ha-1)	3.8 ± 1.7	$4.0 \pm 1.6$	0.5	8.1	$5.8 \pm 2.3$	2.9	8.9	44.7	169.5***

Table 2 - Means, maximum, minimum, and mean squares of 21 agro-morphological traits on 60 IPGRI maize accessions evaluated in Ghana in 2011 and 2012.

<sup>1</sup>Standard deviation; <sup>2</sup>Minimum; <sup>3</sup>Maximum; <sup>4</sup>Coefficient of variation; AD = days to 50 % anthesis; SD = days to 50 % silking; ASI = anthesis-silking interval; TL = tassel length; ELL = ear leaf length; ELW = ear leaf width; PH = plant height; EH = ear height; StD = stalk diameter; SG = stay-green; EL = ear length; ED = ear diameter; CD = cob diameter; EN = ear number per plant; NRE = number of rows per ear; NKR = number of kernels per row; HKWT = hundred kernel weight; KL = kernel length; KW = kernel width; KT = kernel thickness; YLD = grain yield; \*\*P<0.01; \*\*\*P<0.001.

from flag leaf to tassel tip) were determined on ten competitive plants per plot. Other traits were ear leaf length and ear leaf width (ELL and ELW, length and width in cm, respectively, of leaf which subtends the uppermost ear), plant height and ear height (PH and EH, length of stem in cm from soil level to flag leaf and uppermost ear insertion point, respectively), and stalk diameter (StD, diameter of stem in mm at the second internode). Stay-green (SG, percentage green leaf area at physiological maturity), ear length (EL, length in mm), ear and cob diameter (ED and CD, diameter of ear and cob in mm of uppermost ear), number of rows per ear (NRE, number of kernel rows around the cob at 5 cm from the shank of uppermost ear), and number of kernels per row (NKR, average number of kernels in two rows on opposite sides of cob) were also evaluated. After harvest, traits assessed on plot basis included number of ears per plant (EN, calculated as number of ears with at least one fully developed grain divided by number of plants), kernel length (KL, length of kernel in mm from hilum to base), kernel width (KW, width of kernel in mm), kernel thickness (KT, thickness of kernel in mm), and one-hundred kernel weight (HK, mass of 100 kernels in g adjusted to 15% moisture content). Grain yield (YLD, shelled grain weight per plot adjusted to 125 g kg-1 moisture converted to Mg ha-1) was added.

#### Data analyses

Frequencies of plants per plot of the five qualitative scoring categories were calculated. For the quantitative traits, means, standard deviations, minimum and maximum values, as well as coefficient of variation (CV) were calculated. Using the entry means (X) and standard deviation ( $\sigma$ ), the accession scores were divided into six phenotypic classes (x) of equal width of 1.0 $\sigma$ , with class 1 values as  $x_i < X-3\sigma$  to class 6 values as  $x_i > X+3\sigma$ . The frequency of genotypes in the ith class ( $P_i$ ) or simply, the probability of finding the ith class, was used to calculate the standardized Shannon Diversity Index,  $H^1 = -\sum_i P_i(\ln P_i) / \ln(n)$  to assess the within-population variation (Shannon, 1948).  $P_i$  was computed as n/N, where  $n_i$  is the number of individuals of the i<sup>th</sup> class, and N is the total number of individuals; n is the number of classes. Analysis of variance (ANOVA) of the randomized complete block model assuming independent and heterogeneous error variance of environments was performed to test for differences in means:  $Y_{ijklm} = \mu + g_i + e_j + ge_{ij} + b_{k(j)} + r_{(lkh)} + \varepsilon_{iklm}$ .

In this model,  $Y_{ijklm}$  represents response from genotype *i*, in environment *j* in block *k* and replicate *l* on plot basis. The  $\mu$  is the overall mean,  $g_i$  is effect due to genotype *i*, *e* is the effect due to environment *j*,  $ge_{ij}$ is the effect of interaction between genotype *i* and environment *j* nested within environment *j*;  $r_{k0}$  is replication effect *k*, in environment *j*,  $b_{k0}$  is block effect *l*, nested within replication *k* and environment *j*, and  $\varepsilon_{jklm}$  is the error associated with the plot *m*, nested in replication *l*, block *k*, and environment *j*. The SAS 9.3 program (SAS Institute Inc, 2011) was employed for all statistical computations.

Pairwise genetic similarities based on squared correlation were computed. Mean genetic similarity for each accession was calculated as the average of betweenpopulation distances. Cluster analysis of the similarity matrix was based on hierarchical Unweighted Pair Group Method with Arithmetic Averages (UPGMA). Principal components analysis (PCA) comprised calculation of eigenvalues and their relative and cumulative percentages of the total variance, construction of

Table 3 - Maximum and average simila	ity of 60 IPGRI maize	populations and check ba	ased on squared correlation
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Accession	Max <sup>1</sup>	Mean	SD <sup>2</sup>	Accession	Max	Mean	SD	Accession	Max	Mean	SD
Obatanpa	0.67	0.19	0.19	TZm-1123	0.32	0.08	0.09	TZm-1150	0.63	0.19	0.17
TZm-1097	0.31	0.06	0.08	TZm-1125	0.33	0.07	0.07	TZm-1151	0.41	0.11	0.11
TZm-1099	0.41	0.09	0.10	TZm-1126	0.63	0.11	0.14	TZm-1152	0.78	0.15	0.17
TZm-1100	0.51	0.11	0.13	TZm-1128	0.75	0.13	0.17	TZm-1153	0.51	0.10	0.12
TZm-1101	0.63	0.12	0.15	TZm-1129	0.42	0.10	0.11	TZm-1156	0.16	0.05	0.05
TZm-1103	0.30	0.07	0.07	TZm-1130	0.46	0.08	0.09	TZm-1180	0.54	0.15	0.13
TZm-1105	0.59	0.13	0.14	TZm-1131	0.42	0.10	0.12	TZm-1182	0.48	0.13	0.13
TZm-1106	0.59	0.14	0.16	TZm-1132	0.75	0.15	0.18	TZm-1183	0.34	0.09	0.09
TZm-1108	0.63	0.13	0.16	TZm-1136	0.45	0.10	0.11	TZm-1184	0.31	0.05	0.08
TZm-1109	0.29	0.07	0.08	TZm-1137	0.37	0.08	0.09	TZm-1185	0.44	0.09	0.10
TZm-1110	0.46	0.09	0.10	TZm-1138	0.30	0.07	0.08	TZm-1187	0.41	0.09	0.11
TZm-1111	0.42	0.09	0.10	TZm-1139	0.57	0.14	0.14	TZm-1188	0.52	0.11	0.12
TZm-1112	0.44	0.10	0.11	TZm-1141	0.59	0.11	0.12	TZm-1190	0.45	0.11	0.13
TZm-1114	0.63	0.09	0.12	TZm-1142	0.52	0.12	0.13	TZm-1193	0.61	0.15	0.15
TZm-1117	0.46	0.09	0.11	TZm-1143	0.41	0.10	0.10	TZm-1194	0.38	0.11	0.09
TZm-1118	0.42	0.07	0.10	TZm-1144	0.55	0.11	0.13	TZm-1195	0.77	0.14	0.15
TZm-1119	0.25	0.06	0.06	TZm-1145	0.62	0.12	0.14	TZm-1211	0.45	0.09	0.11
TZm-1120	0.60	0.10	0.12	TZm-1147	0.55	0.12	0.13	TZm-1212	0.43	0.12	0.12
TZm-1121	0.77	0.14	0.16	TZm-1148	0.78	0.15	0.17	TZm-1213	0.45	0.11	0.11
TZm-1122	0.63	0.09	0.13	TZm-1149	0.47	0.12	0.12	TZm-1214	0.29	0.08	0.08
								TZm-1215	0.56	0.14	0.15

<sup>1</sup>Maximum; <sup>4</sup>Standard Deviation

biplots of the first two principal components to reveal the discriminatory power of the traits, as well as relationships among traits and accessions. Eigenvectors from the trait correlation matrix were extracted for construction of biplots. Test for significance of a PC was based on the sampling errors associated with the principal components of neighbouring eigenvalues as  $\Delta E > E_i (2 / N)^{1/2}$  where  $E_i$  is the i<sup>th</sup> eigenvalue,  $\Delta E$  is difference between neighbouring eigenvalues, and *N* is sample size (North et al, 1982). The NTSYSpc 2.2 software (Rohlf, 2009) was employed for cluster and principal component analyses. A by bootstrap analysis (Felsenstein, 1985) was conducted using the PAST software (Hammer et al, 2001).

#### Results

#### Variability in agromorphological traits

Ample variability in kernel texture and kernel arrangement, consisting of 55.68% flints and 44.32% dents distributed over 51% regular, 21% irregular, 18% spiral, and 10% straight kernel arrangement was identified. In contrast, color of silks (86% yellow), color of cobs (98% white), and color of kernels (94% mixed) were predominantly uniform and majority were not typical of the dent, white with regular kernel arrangement of the West African maize genotypes (Asare et al, 2016). Among the mixed colour, yellow was dominant, followed by purple and blue.

Pollen shed and silk emergence occurred on average at  $54.8 \pm 6.2$  days and  $57.6 \pm 6.3$  days, respectively, giving a protandry of  $2.8 \pm 1.5$  days (Table 2). Three accessions, TZm-1148, TZm-1149, and TZm-1150 were three to five days earlier than the check in both anthesis of  $45.5 \pm 1.3$  days,  $44.0 \pm 1.8$  days,  $45.8 \pm 4.0$  days, and silking of  $48.2 \pm 2.7$  days,  $49.3 \pm 1.7$  days, and  $45.8 \pm 3.4$  days, respectively (Supplementary Table 1). Protogynous genotypes were identified in TZm-1106 and TZm-1183 with silks emerging one to two days earlier than pollen shed. Though some accessions required longer days to anthesis, silking

was rapid giving anthesis-silking interval of less than 2 days in TZm-1188 ( $1.2 \pm 0.7$  days), TZm-1183 ( $1.3 \pm 1.3$  days), TZm-1118 ( $1.4 \pm 1.1$  days), TZm-1106 ( $1.4 \pm 2.0$  days), TZm-1120 ( $1.8 \pm 1.1$  days), TZm-1149 ( $1.8 \pm 1.1$  days), TZm-1125 ( $1.9 \pm 1.0$  days) and TZm-1215 ( $1.9 \pm 1.7$  days) (Supplementary Table 1). Average plant height was 191.5 ± 47.0 cm and ear height was 97.2 ± 35.4 cm (Table 2). The highest and lowest plant heights were recorded for TZm-1111 ( $236.1 \pm 46.4$  cm) and TZm-1149 ( $125.9 \pm 40.9$  cm), respectively, whereas for ear height it was in TZm-1132 ( $160.7 \pm 46.0$  cm) and TZm-1149 ( $55.0 \pm 25.3$  cm), respectively (Supplementary Table 1).

On the basis of highly significant mean squares, substantial differences in mean values, and large coefficients of variation, the phenotypic differences between the landraces and the check were deemed to be highest in anthesis and silking dates, anthesis-silking interval, plant and ear height, stay-green, hundred kernel weight, and grain yield (Table 2). Compared to the check, the landraces exhibited late maturity, short anthesis-silking interval, large values of plant height, ear height and stalk diameter, but lower scores for ear and kernel characteristics and grain yield (Table 2).

Mean number of rows per ear  $(13.9 \pm 2.0)$  and number of kernels per row  $(28.9 \pm 7.2)$  of the landraces were comparable to the check  $(13.4 \pm 2.0)$  and  $(28.9 \pm 8.3)$ , respectively. Mean one hundred kernel weight of landraces  $53.5 \pm 12.9$  g was considerably lower than the check  $(83.6 \pm 14.3 \text{ g})$ . With regard to prolificacy, all plants had at least one ear with 2.3% bearing two to three ears to give average of  $1.0 \pm$ 0.2 in a range of 1.0 to 1.3 ears per plant. Majority of the individual plants of TZm-1184, TZm-1120, TZm-1183 and TZm-1097 had 2 to 3 ears. The landrace populations, compared to the check, had low mean values of stay-green (69.0  $\pm$  22.1% vs. 75.2  $\pm$  3.5%), ear length (15.0  $\pm$  2.9 cm vs. 17.56  $\pm$  2.2 cm), ear diameter (37.2  $\pm$  6.6 mm vs. 46.0  $\pm$  4.41 mm), kernel length (8.5  $\pm$  1.2 mm vs. 10.3  $\pm$  1.4 mm) kernel width (8.4  $\pm$  0.91 mm vs. 10.0  $\pm$  0.86 mm) and kernel thickness (4.6  $\pm$  0.9 mm vs. 5.4  $\pm$  0.47 mm).

Mean grain yield of landraces varied from 0.5 Mg ha<sup>-1</sup> to 8.1 Mg ha<sup>-1</sup> with a mean of  $4.0 \pm 1.6$  Mg ha<sup>-1</sup>, and was lower than the check, which had range 2.9 Mg ha<sup>-1</sup> to 8.9 Mg ha<sup>-1</sup> and mean  $5.8 \pm 2.3$  Mg ha<sup>-1</sup> (Table 2). On accession mean basis, mean grain yield varied from the lowest value of  $2.2 \pm 0.4$  Mg ha<sup>-1</sup> in TZm-1132 to the highest value of  $6.2 \pm 1.7$  Mg ha<sup>-1</sup> in TZm-1139. Grain yield in the landraces were governed by large number of rows per ear exceeding 13.0 and number of kernels per row greater than 30, whereas in the check a major contributor to the high yield was one hundred kernel weight over 80.0 g. Other genotypes with grain yield of at least 5.0 Mg ha<sup>-1</sup> included TZm-1119 (5.38 Mg ha<sup>-1</sup>), TZm-1117 (5.0 Mg ha<sup>-1</sup>), and TZm-1125 (5.0 Mg ha<sup>-1</sup>) (Table 3).

The within population variation assessed by SDI ranged from 0.12 (ear number) to 0.93 (grain yield) with a mean of 0.72  $\pm$  0.23. The variability was neither equally present in all populations for the same trait, nor for all traits in the same population. Mean SDI values were high for anthesis date (0.89  $\pm$  0.13), silking date (0.86  $\pm$  0.16), anthesis-silking interval (0.84  $\pm$  0.16), plant height (0.82  $\pm$  0.09), one hundred kernel weight (0.86  $\pm$  0.19), and grain yield (0.93  $\pm$  0.07). Among the populations, mean SDI values ranged from 0.38  $\pm$  0.16 in TZm-1148 to 0.84 $\pm$  0.18 in TZm-1106 (Supplementary Table 2).

### Genetic distance and cluster analyses

Genetic distance based on pairwise squared correlations revealed widespread and low similarities among accessions. Genetic similarities ranged from 0.00 to 0.78 with an average of  $0.11 \pm 0.13$ . Minimum distances of all accession pairs were 0.00. Fourteen



Figure 1- Dendrogram of UPGMA clustering of 60 IPGRI maize accessions and a check, "Obatanpa GH" evaluated on 21 quantitative traits based on similarity by squared correlation showing their bootstrap values.

percent of the pairwise distances were 0.00 making some accession pairs very distinct from others. The closest pairs were TZm-1148/TZm-1152 (0.78), TZm-1121/TZm-1195 (0.77), TZm-1128/TZm-1132 (0.75). The check was very similar to TZm-1101 (0.67). Based on average distances, the most distant accessions were TZm-1156 (0.05), TZm-1184 (0.05), TZm-1119 (0.06), TZm-1097 (0.06) (Table 3).

The UPGMA cluster analysis produced two clusters. Cluster I with 36 accessions had range and average distance of 0.00 to 0.78 and 0.11  $\pm$  0.12, respectively. The check variety grouped with cluster I (Figure 1). Members of cluster I were early maturing with mean days to anthesis and silking of 53.7  $\pm$  5.93 and 56.3  $\pm$  5.8 days, short mean plant height of 183.57  $\pm$ 48.2 cm, ear height 91.6  $\pm$  34.9, stalk diameter 19.4  $\pm$  3.6, possessed large mean kernel width 8.6  $\pm$  0.9 mm and mean kernel length 8.8  $\pm$  1.1, mean hundred kernel weight of 57.2  $\pm$  11.9 g and largest mean grain yield of  $4.2 \pm 1.7$  Mg ha<sup>-1</sup>. The 24 members of cluster II were characterized by intermediate to late maturing genotypes with mean days to anthesis and silking of 56.5  $\pm$  6.2 and 56.4  $\pm$  6.5, respectively, long mean anthesis-silking interval of 2.96 ± 1.5 days, tall plants 204.7  $\pm$  42.2, large values of ear height 106.8  $\pm$  34.5 cm, low hundred kernel weight 48.2 ± 12.3 g) and low mean grain yield of  $3.59 \pm 1.5$  Mg ha<sup>-1</sup>. Principal components analysis

Of the five principal components with eigenvalues greater than one, the first two were significant (P < 0.05) and explained 67.89% of the total variance (Table 4). The PC1 explained 42.2% of the variance and delineated important traits as anthesis and silking dates, tassel length, ear leaf length, ear leaf width, plant height, ear height, stalk diameter, ear length, and number of kernels per row. The PC2 explained 24.7% of the total variance and identified ear diameter, hundred kernel weight, kernel length, kernel width, and grain yield as major contributors to the variance. A plot of the first two PCs (Figure 2) depicted a high correlation in earliness and plant architectural traits with a range of values spanning 0.71 to 0.94 and were inversely associated with ear diameter, one hundred kernel weight, kernel width, kernel length and grain yield and yield components at -0.71 to -0.85 (Table 4).

The PC1 axis delineated the accessions high grain yield to include TZm-19, TZm-17, TZm-1125, TZm-1122, TZm-1212, TZm-1144, TZm-1106, and TZm-1183. PC2 axis distinguished the early maturing and short height genotypes such as TZm-1147, TZm-1148, TZm-1149, TZm-1150, TZm-1152, TZm-1144, TZm-1215, «Obatanpa GH», TZm-1132, TZm-1139, TZm-1149, and TZm-1121 were isolated from all other accessions. Both cluster analysis and the principal component biplots confirmed that accessions which developed late were not productive (Figure 2).

Table 4 - Principal components analysis of 60 IPGRI maize accessions «Obatanpa GH» (check) evaluated in Ghana in 2011 and 2012 using 21 agro-morphological traits.

Trait	PC1	PC2
AD	0.90	0.10
SD	0.89	0.13
ASI	0.21	0.20
TL	0.55	-0.09
ELL	0.88	-0.05
ELW	0.71	-0.08
PH	0.94	0.05
EH	0.93	0.12
StD	0.87	0.16
SG	0.17	0.28
EL	0.73	-0.43
ED	0.21	-0.71
CD	0.28	-0.36
NRE	0.30	0.13
NKR	0.53	-0.33
HK	-0.16	-0.91
EN	0.09	-0.29
KL	-0.12	-0.85
KW	-0.09	-0.80
KT	-0.05	-0.27
YLD	0.06	-0.82
Eigenvalues	6.86	4.16
Individual percentages	42.24	25.65
Cumulative percentages	42.24	67.89

\*Boldfaced figures represent the highly correlated eigenvectors

#### Discussion

In the current study, we examined 60 landraces belonging to the IPGRI collection which have no available passport data. With no passport data, the likelihood of overlooking this group in characterization studies could be high. However, being landraces which have emerged from genetic, evolutionary, and anthropogenic events, they are likely to exhibit a large range of genetic variability, whose estimation is needed for sustainable exploitation. The large significant within and between population variability, and the low mean genetic similarity values underscored the existence of substantial genetic diversity within this collection. Our results concur with previous reports that the African maize landrace collection contains a large genetic variability in morphological traits. In this study, the large coefficients of variability for anthesissilking interval, ear height, and grain yield were comparable to Asare et al (2016) report of 36 to 45%, 32 to 36%, and 49 to 61%, respectively, though variation in stay green was higher in the IPGRI collection. Variability in ear height was about twofold larger than those of 77 Ghana accessions with 10.4% to 31.7% (Obeng-Antwi et al, 2012) and 19.65% and 20.43% (Salami et al, 2015, 2017), respectively, in local and improved varieties of 87 southern, 43 central and 98 Northern Benin landraces. Plant height and ear height were larger than those of European traditional populations with 164 to 166 cm and 73 to 77 cm, respectively (Hartings et al, 2008; Rebourg et al, 2001) but similar to the Benin landraces of 202 cm and 110 cm, respectively (Salami et al, 2017).

Such a large phenotypic diversity this much, reflects the population's heterogeneous geographical



Figure 2 - Principal components analysis of the IPGRI maize landraces evaluated in Ghana in 2011 and 2012 on 21 agro-morphological traits. (A) Accessions biplot (B) Traits biplot.

origin typical of many locations in Africa, a differential fitness to the environment, flexibility and survival in changing environmental conditions, as well as a wide range of farmer varietal preferences. The large diversity is consistent with the hypothesis of landraces harboring a reserve of rich genetic diversity for important economic traits. The data obtained will guide parental selection for maize improvement and broadening of the genetic base of breeding populations, and provide efficient conservation. The SDI values indicate a medium to large variation within the accessions, enough to select parents and make progress with yield enhancement. Earliness impacts droughttolerance by escape of the short rainfall season typical of Sub-Saharan Africa. An ASI period of 2-4 days is considered ideal for drought tolerance (Dass et al, 2001). Accessions TZm-1106, TZm-1183, and TZm-1188 with short mean anthesis-silking interval of 1.2 and occurrence of protogynous genotypes may be considered important for drought tolerance. The wide variability in mean grain yield of 2.16 to 6.18 Mg ha-1 is of particular importance as it depicts availability of both substantial variation and high grain yield genotypes for yield improvement. Previously, Obeng-Antwi et al (2012) had reported a mean grain yield of some 77 landraces collected from southern Ghana and four improved checks to be 2.7 Mg ha<sup>-1</sup> ranging from 1.3 to 4.5 Mg ha<sup>-1</sup>. Similarly, Alika et al (1993) estimated average yield for Nigerian maize landraces to be 3.0 Mg ha<sup>-1</sup> and ranged from 2.2 to 4.1 Mg ha<sup>-1</sup>, while the Ethiopian accessions averaged 2.6 Mg ha-1

with a range of 1.3 to 4.3 Mg  $ha^{-1}$  (Beyene et al, 2005).

The low mean genetic similarity value of 0.11  $\pm$ 0.002 indicate wide disparity among accessions and a divergence arising from allelic differentiation. Few studies have reported the diversity in morphology and genetic signatures among the African maize germplasm. The variability accumulates from demographic-driven selection, climate variability, seed flow, evolutionary history and environmental variability over SSA. Our goal is to explore the genetic variability in the African maize to better understand the context of maize differentiation and the conditions relevant to the divergence. We are not suggesting that phenotypic evaluation for genetic diversity is a better approach, but that, it provides a framework for assessing variability within a population having no available data on the geographical characteristics of the origin of the collection. We focus on phenotypic data because it is the first pragmatic approach to obtain information required by breeders for assessment of utility of any collection.

The early-maturing, short anthesis-silking interval, short plants and high yield genotypes of cluster I could be chosen for drought tolerance and offset the usual trade-off in earliness and grain yield (Barriere et al, 2010). The tall plants with wide stalk diameter genotypes of cluster II could equally be chosen exclusively for high biomass. Nevertheless, their long tassels and long ear leaves cannot be overlooked by reason of their low but highly significant correlation with grain yield (r = 0.27 to 0.32, P < 0.001). The

#### Twumasi et al

clusters represent uncorrelated groups which may be useful for future heterotic breeding as their trait performance may be governed by different sets of alleles.

The first statistically significant PCs interpret the response to variability due to earliness and small sized plants, whereas the second PC is interpreted as a response of late maturity to decreasing yield, a phenomenon that is less common in maize. The PCA results support a generally clear separation between plant architectural traits and yield and yield components and support a data reduction to 15 discriminatory traits, the remaining being noise. Our results suggest that different mechanisms affecting maturity, architectural, and grain yield traits control variation in the populations.

#### Conclusion

In conclusion, a large phenotypic variability in morphological traits, and the divergent genotypes exhibited by the IPGRI maize collection indicate a rich reserve of genetic diversity and alleles. Combined with the identification of two disparate clusters, sufficient information is present to include promising genotypes in maize improvement programmes via high grain yield and a combined early-maturing and short anthesis-silking interval for drought tolerance, a combination of traits most relevant to Sub-Saharan Africa maize productivity. The findings would also be useful in maize conservation management.

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#### References

- AATF. African Agricultural Technology Foundation. 2015. Water Efficient Maize for Africa (WEMA) Project Collaboration, Intellectual Property and Licensing Background. www.aatf-africa.org/userfiles/Wema-Summary-Collaboration.pdf. verified March 20, 2017
- Abate T, Shiferaw B, Menkir A, Wegary D, Kebede Y, Tesfaye K, Kassie M, Bogale G, Tadesse B, and Keno T, 2015. Factors that transformed maize productivity in Ethiopia. Food Secur 7: 965-981
- Alika JE, Aken'Ova ME, Fatokun CA, 1993. Variation among maize (*Zea mays* L.) accessions of Bendel State, Nigeria. Multivariate analysis of agronomic data. Euphytica 66: 65-71
- Asare S, Tetteh AY, Twumasi P, Adade KB, and Akromah R. 2016. Genetic diversity in lowland, midaltitude and highland african maize landraces by morphological trait evaluation. Afr J Plant Sci 10: 246-257
- Beyene Y, Botha A, Myburg A, 2006. Genetic diversity in traditional Ethiopian highland maize accessions

assessed by AFLP markers and morphological traits. Biodiv Conserv 15: 2655-2671

- Cairns JE, Hellin J, Sonder K, Araus JL, MacRobert JF, Thierfelder C, Prasanna BM, 2013. Adapting maize production to climate change in Sub-Saharan Africa. Food Secur 5: 345–360
- Camussi A, Ottawano E, Calinski T, Kaczmarek Z, 1985. Genetic distances based on quantitative traits. Genetics 111: 945-962
- Canning D, Raja S, Yazbeck A, 2015. Africa's Demographic Transition Dividend or Disaster? Africa Development Forum, Washington, DC. World Bank and Agence Française de Développement. World Bank. https://openknowledge.worldbank. org/handle/10986/22036
- CIMMYT, 1988. Productivity of the tropical maize compared to temperate genotypes
- Cooper DH, Spillane C, Hodgkin T, 2001. CABI Technology and Engineering
- Dass S, Arora P, Kumari M, Dharma P, 2001. Morphological traits determining drought tolerance in maize. India J Agric Res 35: 190-193
- Dowswell CR, Paliwal RL, Cantrell RP, 1996. Maize in the Third World. Westview Press, Oxford
- Felsenstein J, 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791
- Fowler C, Smale M, Samy Gaiji S, 2000. Germplasm flows between developing countries and the CGIAR: An initial assessment. Global Forum on Agricultural Research, Rome, FAO and IPGRI
- Hammer Ø, Harper DAT, Ryan PD, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeont Electr 4: 9
- Hartings H, Berarado N, Mazzinelli GF, Valoti P, Verderio A, Motto M, 2008. Assessment of genetic diversity and relationships among maize (*Zea mays* L.) Italian landraces by morphological traits and AFLP profiling. Theor Appl Genet 117: 831-842
- IITA. International Institute of Tropical Agriculture, 2012. www.iita.org
- IBPGR, 1991. Descriptors for Maize. International Maize and Wheat Improvement Center, Mexico City - International Board for Plant Genetic Resources, Rome
- Magorokosho C, 2006. Genetic diversity and performance of maize varieties from Zimbabwe, Zambia and Malawi. PhD Thesis, Texas A&M University, USA
- Mohammadi SA, Prasanna BM, 2003. Analysis of genetic diversity in crop plant- salient statistical tool and considerations. Crop Sci 43: 1235-1248
- Nelson PT, Goodman MM, 2008. Evaluation of elite exotic maize inbreds for use in temperate breeding. Crop Sci 48: 85-92
- Nestory S, Reuben SOWM, 2016. Diversity of maize landraces collected at farm level in Northern Tanzania. Int J Agric Sci Res 5: 19-26

#### genetic diversity in maize

- North GR, Bell TL, Cahalan RF, Moeng FG, 1982. sampling error in estimation of empirical orthogonal functions. Monthly Weather Rev 110: 699-706
- Obeng-Antwi K, 2007. Genetic diversity in maize (*Zea mays* L.) landraces in Ghana. PhD Thesis. University of Reading, UK
- Obeng-Antwi K, Craufurd PQ, Menkir A, Ellis RH, Sallah PYK, 2012. Phenotypic diversity in maize landraces in Ghana. Int J Sci Adv Technol 2: 2221-8386
- Pejic I, Ajmone-Marson P, Morgante M, Kozumplick V, Castiglioni P, Tarmino G, Motto M, 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs and AFLPs. Theor Appl Genet 97: 1248-1255
- Rebourg C, Gouesnard B, and Charcosset A, 2001. Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation. Heredity 86: 574-587
- Rohlf FJ, 2009. NTSYSpc: Numerical Taxonomy System. Version 2.21c. Exeter Software: Setauket, New York

- Salami HA, Adjanohoun A, Padonou W, Yacoubou AM, Aly D, Yallou C, Sina H, Baba-Moussa L, 2015. Morphological diversity of corn's (*Zea mays* L.) local cultivar and improved varieties in central and north of Benin. Amer J Plant Sci 6: 2867-2877
- Salami HA, Sina H, Wallis NZ, Padonou W, Yallou C, Chabi-Sika K, Noumavo PA, Aly D, Adjanohoun A, Baba-Moussa L, 2017. Agro-morphological variability of *Zea mays* (L.) accessions collected in Southern Benin. J Plant Breed Crop Sci 9: 1-9
- SAS Institute Inc. 2011. Base SAS® 9.3 Procedures Guide: Statistical Procedures. Cary, NC: SAS Institute Inc.
- Shannon CE, 1948. A mathematical theory of communication. The Bell Syst Techn J 27: 379-423
- Tallury SP, Goodman MM, 1999. Experimental evaluation of the potential of tropical germplasm for temperate maize improvement. Theor Appl Genet 98: 54-61