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DEPARTMENT OF CROP AND SOIL SCIENCE

GENETIC DIVERSITY STUDIES OF TROPICAL IPGRI MAIZE (*Zea mays* L.) LANDRACES HELD IN IITA BY AGROMORPHOLOGICAL AND SIMPLE SEQUENCE REPEATS MARKER EVALUATION

BY

PATRICK TWUMASI

NOVEMBER, 2016

GENETIC DIVERSITY STUDIES OF TROPICAL IPGRI MAIZE (*Zea mays* L.) LANDRACES HELD IN IITA BY AGROMORPHOLOGICAL AND SIMPLE SEQUENCE REPEATS MARKER EVALUATION

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AGRONOMY (PLANT BREEDING)

BY

PATRICK TWUMASI

(BEd. Agriculture)

NOVEMBER, 2016

DECLARATION

I, Twumasi Patrick, hereby declare that except for the references to other people‟s work, which have been duly cited, this thesis hereby submitted by me for MPhil Agronomy (Plant breeding) is my own independent work and has neither in whole, nor part, been presented for a degree in Ghana or elsewhere.

..... ……..
TWUMASI PATRICK DATE (PG4240210)

(Student)

We declare that we have supervised the above student to undertake the study submitted herein and verify that he has our permission to submit.

..... ……..
DR. (Mrs.) ANTONIA .Y. TETTEH DATE
(Supervisor)

……..... ……..
PROF. RICHARD AKROMAH DATE
(Co-Supervisor)

…….. …….
DR. ENOCK A. OSEKRE DATE
(Head of Department)

DEDICATION

I dedicate entirely this work to my one and only beloved wife, Gifty Osei Bonsu and

my children, Malvin Dompreh Twumasi and Keren Antwiwaa Twumasi for their financial support, words of encouragement and serving as sources of inspiration for a better me tomorrow.

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LIST OF ABBREVIATIONS

AD	Anthesis Date

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
ASI	Anthesis to Silking Interval
CC	Cob Colour
CD	Cob Diameter
CIMMYT	International Maize and Wheat Improvement Center
CRI	Crops Research Institute
CRIG	Cocoa Research Institute of Ghana
CTAB	Cetyl Trimethylammonium Bromide
CV	Coefficient of variation
DNA	Deoxyribonucleic Acid
ED	Ear Diameter
EHT	Ear Height
EL	Ear Length
ELL	Ear Leaf Length
ELW	Ear Leaf Width
EN	Number of Ears per Plant
EP	Ear Position
EWT	Ear Weight
FAOSTAT	Food and Agriculture Organization of the United Nations
GWT	Shelled Grain Weight

H_e	Expected Heterozygosity
HKWT	Hundred Kernel Weight
H_o	Observed Heterozygosity
IFPRI	International Food Policy Research Institute
IGC	International Grains Council
IITA	Institute of Tropical Agriculture
INRA	Institut National de la Recherche Agronomique
IPGRI	International Plant Genetic Resources Institute
KA	Kernel Arrangement on Ear
KL	Kernel Length
KT	Kernel Thickness
KTEX	Kernel Texture
KW	Kernel Width
MAX	Maximum
MIN	Minimum
MMT	Million Metric Tons
NKR	Number of Kernels per Row
NRE	Number of Rows per Ear
NTSYS	Numerical Taxonomy System Software
OPV	Open Pollinated Varieties
PCA	Principal Component Analysis
PGC	Principal Grain Colour

PIC	Polymorphic Information Content
PLHT	Plant height
RFLP	Restriction Fragment Length Polymorphism
Rmp	Revolution per minute
SAS	Statistical Analysis Software
SC	Silk colour
SD	Silking Date
SD	Stalk Diameter
SD	Standard Deviation
SG	Stay Green
SIMQUAL	Similarity in Qualitative Data
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TL	Tassel length
UPGMA	Unweighted Pair Group Method based on Arithmetic Averages
YLD	Grain yield

ABSTRACT

Genetic diversity among maize (*Zea mays* L.) landraces reveals genetic backgrounds with respect to alleles, polymorphisms and heterozygosities, as well as relationships among genotypes. There has always been the need in Africa to identify useful alleles for maize improvement in a wide genetic base yet little is done to search for diversity among existing maize landraces. The IPGRI landraces of the IITA maize collection has neither record of geographical origin nor information on genetic diversity. The research objective was to estimate the level of genetic diversity, determine relationships among the landraces, and reveal evolutionary processes that have contributed to the genetic status of the population. A total of 60 landraces and a check, „Obatanpa GH" were evaluated by agromorphological characterization on 5 qualitative and 24 quantitative traits. Except for cob colour which was least variable with 98.0 % white and 2.1 % red, a large variability was observed for silk and grain colour, kernel texture and kernel arrangement Kernel arrangement with fairly equal distribution of straight, regular, irregular and spiral types was the most variable. On quantitative evaluation, large variability was demonstrated for all traits except number of ears per plant. Earliness ranged from 39 to 74 days with a mean of 54.8 ± 6.2 days to 50 % anthesis while days to 50 % silking covered 44 to 78 days and mean of 57.6 ± 6.3 days. Six early-maturing genotypes identified were TZm-149, TZm-1148, TZm1150, TZm-1157, TZm-1153, and TZm-1152. Mean anthesis-silking interval revealed genotypes for drought tolerance having 1.2 to 1.4 days of anthesis-silking interval in TZm-1188, TZm-1183, and TZm-1106. Many individual plants of these accessions exhibited protogyny. Mean grain yield ranged from 2.16 ± 0.4 $Mgha^{-1}$ to 6.19±1.7 $Mgha^{-1}$ of which the best performers with yield exceeding 4.2 $Mgha^{-1}$ were TZm-1185, TZm-1142, TZm-1213, TZm-1129, TZm-1143, TZm-1215, TZm-1150,

TZm-1211, TZm-1152, TZm-1101, TZm-1123, TZm-1100, TZm-1138, TZm-1112, TZm-1212, TZm-1130, TZm-1190, TZm-1118, TZm-1106, TZm-1144, TZm-1122, TZm-1125, TZm-1117, TZm-1119 and TZm-1139. Low to moderate broad sense heritability estimates of 0.00 for stay green and ear weight to 0.68 and 0.69 for earliness were recorded. The medium to high heritability estimates signify traits are under control of minimal additive and some dominance gene effects for a slow pace in progress in breeding. Besides the strong positive correlation of yield components with grain yield, all other correlation coefficients with grain yield were weak and nonsignificant ($P \leq 0.05$). Genetic similarities ranged from 0.00 to 0.80 with a mean of 0.14±0.15 indicating extensive genetic diversity. The UPGMA cluster analysis grouped genotypes into two main heterogeneous clusters, cluster I having earlymaturing, short plants with high grain yield and low anthesis-silking intervals whereas cluster II was of tall plants with poor grain yield. The first two principal components explained 85.0 % of the total variance with large contributions from plant height, ear height, anthesis, silking, ear leaf length, grain weight, grain yield, ear position, hundred kernel weight, kernel length, and kernel width. SSR profiling of 64 IPGRI genotypes at 12 loci produced a rate of polymorphism of 85.7 %, a total of 1,826 alleles ranging from 108 to 216. The number of alleles per locus ranged 3 to 10 with mean of 5.64±2.15 indicating lots of variability. The mean observed heterozygosity of 0.36±0.18 was not significantly different from the expected heterozygosity of 0.69±0.08, an indication of substantial mutation rate and polymorphism maintained by balancing selection. The high heterozygosity is also suggestive of a historical admixture event. Genetic distance by means of DICE similarity coefficient was 0.49±0.14. UPGMA clustering grouped the accessions into six clusters from which hybridization could be exploited. The large variability, polymorphism, and heterozygosity identified by both agromorphological and molecular assessments affirm the existence of wide

genetic diversity in the IPGRI genotypes and their possible beneficial contributions if exploited in maize improvement programmes.

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays*) is a member of the family Poaceae, the group of crops known as the grasses, which includes wheat, barley, and rye. It is generally believed that maize originated from Central America, specifically Mexico where it was domesticated and spread rapidly around the globe through trade routes (Matsuoka *et al*. 2002; Smith, 1998). Maize introduction to Africa can be traced back to the 1500s by the Portuguese traders (Sinha, 2007). To date, maize is grown in sub-Saharan Africa as the most important economic crop, and is used as food, feed, and a raw material for many industrial products. Maize is used as food for over 1.2 billion people in sub-Saharan Africa and Latin America (FAO, 2011). Presently, maize makes up more than 50 % of the total caloric intake (Sinha, 2007; McCann, 2005) and 53 % of the protein intake of local diets (Bressani, 1991).

Global maize production is estimated to be 785 million metric tons (MMT) with Africa producing about 51 million metric tons (6.5 %). Because this quantity is not enough for the population, Africa imports 28 % deficit of maize from other countries (IITA, 2012). Maize yield in developing countries has been consistently lower than that in developed countries primarily due to factors such as drought, use of landraces and old varieties. In contrast, developed countries cultivate hybrids and improved varieties (Munsch, 2009).

Maize landraces in Africa are adapted to various environments, from cold to hot, humid to drought, and on various elevations (Taba and Twumasi-Afriyie, 2008).

Some landraces are still being used as local cultivars in West and Central Africa, although the vast majority of the production areas are planted with modern commercial varieties. These landraces, though are believed to possess alleles for many important economic traits (Brandolini, 1969), they have not been utilized as valuable germplasm for breeding modern maize cultivars. For instance, more than 20 cultivars released in Ghana are bred from the superior offspring from „Obatanpa GH" derived from Population 63 genotype developed by the Crops Research Institute (CRI), Kumasi, Ghana in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan; the International Maize and Wheat Improvement Center (CIMMYT), Mexico; and the Sasakawa Global 2000 (Badu-Apraku *et al.*, 2006). Other cultivars in Ghana were derived from International Institute of Tropical Agriculture (IITA) maize lines (Sallah, 1998).

There appears to be dearth of information on the genotypic composition of maize landraces in West and Central Africa, hence they have not been utilized in maize improvement programs. Landraces are important genetic resources which serve as sources of biotic and abiotic stress resistance, yield, and disease resistance genes, quality and many useful agronomic characteristics, and comprise high genetic variability and fitness to the natural and anthropological environments where they have originated (Brandolini, 1969). Consequently, landraces represent a unique and valuable material for improvement of modern varieties adapted to changing environments (Rao, 2004; Heslop-Harrison, 2002). In view of this, efforts must be made to collect and conserve landraces and wild relatives for utilization in future breeding programs.

To promote the efficient use of genetic variation in the collection, information on genetic diversity and relationships within and among cultivars, traditional populations and their wild relatives is essential (Sidkar *et al*., 2010).

Considering the need to conserve plant genetic resources, more than 800 tropical maize accessions have been collected and deposited at the IITA Genetic Resource Center in Ibadan, Nigeria, with the collaboration of local germplasm institutions in many countries in Africa and the International Plant Genetic Resources Institute (IPGRI), Rome, Italy. This large number presents challenges and demands for more efficient management and cost-effective conservation. Management of germplasm collections encompasses assessment of genetic diversity and construction of a core collection to represent the variation within the group.

Genetic diversity analysis reveals genetic backgrounds and relationships of germplasm, and provides strategies to establish, utilize, and manage crop germplasm (Roussel *et al*., 2004; Brown-Guedira *et al*., 2000). It also offers the basis for devising future strategies for crop improvement, cultivar development, conservation, and sustainable use of crop germplasm for long-term crop improvement and reduction of vulnerability in plants to diseases. Measurement of genetic diversity is useful for enhancing genetic variation in base populations.

Despite the many benefits of genetic diversity analyses, there have been few reports of detailed assessment of genetic diversity among the African maize germplasm compared to the collections of other regions. For example, temperate maize genotypes such as the U.S. Corn Belt germplasm (Smith *et al*., 1997; Hallauer *et al*., 1988; Goodman and Stuber, 1983), North America (Smith, 1986; Goodman and Stuber, 1983; Kahler *et al*.,

1983), European maize genotypes (Hartings *et al*., 2008; Messmer *et al*., 1993;1992), France maize genotypes (Dubreuil *et al*., 1996) and Japanese maize inbred lines (Enoki *et al*., 2002) are fully classified into heterotic groups. Similarly, thousands of tropical maize germplasm at CIMMYT are listed to be evaluated (Warburton *et al*., 2005; 2002; Xia *et al*., 2005, 2004; Reif *et al*., 2003a,
2003b).

These efforts have led to the assignment of lines into heterotic groups for hybrid maize development, as well as identification of desirable traits for future breeding programs.

Records available on genetic diversity in African maize include assessment of few germplasm from Ethiopia (Legesse *et al*., 2007; Beyene *et al*., 2006), Ghana (ObengAntwi, 2007), Zimbabwe, Zambia and Malawi (Magorokosho, 2006), and six other countries in West Africa (Sanou *et al*., 1997). There is therefore the urgent need to study the genetic diversity in the African maize collection.

Since the 1970‟s, African maize has undergone changes arising from hybridization with genotypes of plant introductions from the U.S.A. and CIMMYT, Mexico with the aim of producing improved cultivars (Morris *et al*., 1999). An example is „Obatanpa GH‟, an open pollinated variety (OPV) and a quality protein maize (QPM) developed by the Crops Research Institute (CRI), Kumasi, Ghana in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan; the International Maize and Wheat Improvement Center (CIMMYT), Mexico; and the Sasakawa Global 2000 (SG 2000) (Badu-Apraku *et al.* 2006). As these practices are carried out, gene flow and genetic

erosion are inevitable. Some elite African inbred lines and accessions have also contributed to maize improvement in exotic lines, as they are reported to demonstrate good yield potential, disease resistance, and overall favorable agronomic performance (Mwololo *et al*, 2012). Among these are few TZi accessions of International Institute of Tropical Agriculture, Ibadan, together with Institut National de la Recherche Agronomique (INRA), Cameroon (Nelson and Goodman, 2008).

These point to the fact that there is useful distribution of genes in the African maize germplasm awaiting to be utilized to transform maize improvement in Africa. Assessment of the extent and distribution of genetic variation within plant populations has the capacity to increase the understanding of the historical processes underlying the genetic diversity. It can reveal both novel genes waiting to be exploited, as well as identify heterotic groups. This information finds uses in breeding for trait improvement and for management of the large number of germplasm in repositories.

Little is known about the genetic backgrounds and relationships including the geographical origins of the accessions collected by IPGRI and held by the Genetic Resource Center of IITA. In response to the lack of information on the geographical distribution of the IPGRI accessions, this research project was designed to reveal its potential exploitation in breeding programs.

In order to reveal the genetic backgrounds and relationships including variability within and among the IPGRI accessions, it is required that a combination of approaches such as morphological trait evaluation and molecular genotyping be applied to identify genes, reveal the richness of allelic polymorphisms, partition the population into heterotic groups, and identify a set of genotypes which maximize their diversity.

Information regarding genetic diversity of breeding materials especially landraces is indispensable for maize improvement. Genetic diversity of maize has usually been assessed based on morphological data characterization using descriptors (Goodman and Bird, 1977), and pedigree analysis through estimation of coancestry coefficients (Malécot, 1948).

Collecting and analyzing data by this technique is inexpensive in developing countries where labour cost is considerably low. Morphological evaluation is relatively simple and does not require sophisticated technology. Despite the simplicity, these descriptors alone present several limitations such as high demand of time and labour intensiveness.

Again, morphological characters are often influenced by environment, hence are limited in their reliability. In contrast, molecular markers such as SSRs (Warburton *et al*., 2002), AFLP (Beyene *et al*., 2006), RFLPs (Dubreuil *et al*., 1999) and SNPs (Yu *et al*., 2011) have proven to be powerful in discriminating among accessions. They are immune to environmental effects and have high heritability. Among these, SSRs have been widely used for the study of diversity including population structure and demographic history of domesticated species because of their high level of allelic diversity over RFLPs, AFLPs, or SNPs loci (McGregor *et al*., 2000; Powell *et al*., 1996). They are highly polymorphic, reliable (Smith *et al*., 1997), easy to generate, have low cost, are highly repeatable (Warburton *et al*., 2002), and are suitable for largescale investigations as needed for the characterization of genetic resources (Powell *et al*., 1996). Molecular markers are therefore superior to morphological and biochemical markers because they are more efficient and sensitive in detection of

distinct differences arising from mutations among genotypes at DNA level (Melchinger *et al*., 1991). They are however expensive and demand sophisticated equipment.

In a morphological study involving twenty-two traits Ruiz de Galaretta and Alvarez (2001) evaluated 100 landraces of maize from Northern Spain and came up with seven groups having promising breeding values. Beyene *et al*. (2005) researched into 62 traditional Ethiopian highland maize using morphological traits and molecular profiling by encompassing AFLPs and SSRs and concluded that variability existed among the selected genotypes. Hartings *et al*. (2008) reported a large genetic heterogeneity among 54 maize landraces originating from Italy on the basis of morphological and AFLP analyses and revealed four major clusters relating to their geographical origin.

Rebourg *et al*. (2001) examined genetic variation among 130 European traditional maize populations and split them into six groups on the basis of morphological and molecular analyses. Analysis of 294 landraces originating from Malawi, Zambia, and Zimbabwe using 34 phenotypic traits partitioned the set into three non-overlapping groups by cluster analysis (Magorokosho, 2006). Obeng-Antwi (2007) performed genetic diversity study on 92 maize landraces from Ghana and observed a large variability among accessions within groups (96 %) rather than among groups using AFLPs and agromorphological traits. Studies by the various researchers confirm the effectiveness of the combined use of morphological evaluation and molecular genotyping.

Therefore for a comprehensive study of the IPGRI genotypes held in IITA with little passport data the combined techniques must be applied to reveal their useful

characteristics in terms of allele diversity, unique genotypes worth incorporating in breeding programs, relationships among the genotypes, as well as their evolutionary history.

The main goal of this study was to estimate the level of genetic diversity and relationships among the tropical IPGRI maize landraces in the IITA germplasm repository.

The specific objectives are:

(1) To determine genetic variation in the IPRGI population by means of agromorphological traits evaluation
(2) To investigate the heritability, genotypic and phenotypic correlations among the IPGRI maize genotypes
(3) To estimate genetic diversity of the IPGRI genotypes using SSR profiling
(4) To assemble the IPGRI population into groups on the basis of genetic distance
(5) To determine the allele diversity and heterozygosity among the genotypes

The research is driven by the hypothesis given below:

That, the IPGRI maize landraces in IITA repository with little passport data are genetically diverse and contain alleles t hat can be exploited for maize improvement especially in Sub-Saharan Africa.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The role of maize in the world‟s agricultural economy

Maize (*Zea mays* L.), also known in some countries as corn is reckoned with rice and wheat as the three most produced and consumed cereal crop in the world. Over the past ten years, production of maize has surpassed that of wheat and rice. Data from United Nations Food and Agriculture Organization (FAO) covering the past five years of global cultivation of grains reveal that from 2007 to 2011 average maize production by top producing countries (United State, China, Brazil, and Argentina) was 719 million metric tons (MMT), while that for rice and wheat was 651 MMT and 571 MMT, respectively. Within this period, a yearly increase in maize production at a rate of 3.0 % was recorded, parallel to 3.5 % for wheat, and 2.2 % for rice (FAOSTAT, 2012).

Maize has worldwide significance as human food, animal feed and fodder, as well as a source of raw material for a large number of industrial products such as corn starch, starch-based products, in fermentation and distilling industries, and in recent times, as an alternative source of biofuel (Naylor *et al*., 2007). Owing to its numerous uses, demand for maize has escalated over the years. The IFPRI (2003) reported estimated global increase in demand for cereals from 1997 to 2020 at 2.1 billion mt of which maize makes up 852 MMT (45 %), 760 MMT for wheat (30 %), and 503 MMT for rice (32 %).

2.2 Maize production and consumption in Africa

Maize is believed to have been first introduced into Africa by the Portuguese traders in the 16th century. It has since become one of the continent‟s staple food crops, with more than 900 million Africans depending on it as their main food source where it supplies over 50 % of calories (Sinha, 2007; McCann, 2005) and about 63 % of the protein

intake in diets (Bressani, 1991). Of the 140 million hectares of maize grown globally, approximately 22 million hectares (15.7 %) are in sub-Saharan Africa (Pingali, 2001). Of these, 1.7 million ha are grown in the highlands, while 8.0 million ha are grown in the midaltitude areas and 12.3 million ha in the tropical lowlands (M‟mboyi *et al*., 2010). The major maize growing countries in Africa in 2015 were South Africa, (8 MMT), Nigeria (7 MMT), Ethiopia and Egypt (6 MMT each), and Tanzania (5.5 MMT), wheras Ghana produced 1.8 MMT.

Table 2.1 shows comparative statistics on global maize production of some selected regions in 2000 and 2015. Maize production by top forty-one countries in Africa in 2015 amounts to a total of 61 MMT, making about 6.29 % of global production (Index Mundi, 2015). This figure represents an increase in production by 38 % over 15 year period compared to 63 % growth in world maize production over the same period. Scientists forecast that the current climate variability will negatively affect maize production in Africa resulting in reduced growth rate and excess of demand over supply (Cairns *et al*., 2012). In Sub-Saharan Africa, demand for maize is expected to exceed 52 MMT by 2020 (Pingali and Pandey, 2000) and about 100 MMT would be required by 2050 (CIMMYT and IITA, 2010). Already Africa imports approximately 28 % of required maize (IITA, 2016), making the need for maize research, especially improvement in yield, disease resistance, and resilience to aboitic factors such as high heat index and drought of prime importance.

Globally, about 460 MMT (65 %), of total world maize production is used for feed purposes while about 15 % is used for food, and the remaining mainly destined for various types of industrial uses (Abbassian, 2006).

Table 2.1 Global production of maize in metric tons over 15-year period

	Production year (MMT)		
Region	2000	2015	Percentage change (%)
World	592,479,279	967,861,000	63
U.S.A	335,021,237	345,486,000	3
European Union-27	6,350,733	57,751,000	809
Africa	44,286,828	60,945,000	38

Source: FAOSTAT (2013) and Index Mundi (2015).

The new found uses of maize, essentially as a source of biofuel and population growth account for the estimates of trade (Table 2.2). Maize production, consumption and trade statistics, as well as forecast in 2013 to date reveal increasing demand over supply. Growth in production is estimated to be 3.7 % with a production rate of 1,025 as against 3.2 % growth in consumption with a consumption rate of 1,031 indicating a deficit by 2020.

Table 2.2 Global maize production, trade, consumption and forecast from 2013/ 2014 to 2019/ 2020 in million metric tons

All maize	2013/ 2014	2014/ 2015	2015/ 2016	2016/ 2017	2017/ 2018	2018/ 2019	2019/ 2020
Production	983	980	954	976	993	1,008	1,025
Trade	120	113	115	118	122	125	130
Consumption	939	961	965	982	999	1,015	1,031
Stocks	176	194	183	177	171	164	158

Source: IGC, International Grains Council, December 2014 Statistical update

While countries such as United States, China and Europe are net exporters of maize, Africa has over the years been a net importer. Maize imports in Africa accounted for 4.57 MMT in 1995 to 1997 at a cost of US$ 1.14 billion, 10.64 MMT in 2005 at US$ 2.25 billion, and in 2010, 13.9 MMT at a cost of US$ 3.04 billion. Conversely, countries

which are net exporters of maize accrue huge revenue from this activity. According to FARA (2009) in 2010, exports of 81 MMT of maize from the U.S.A. attracted US$16 billion, 21 MMT from Europe attracted US$5 billion, while 1.5
MMT exported from Africa attracted US$432 million (FAOSTAT, 2013).

2.3 Origin of maize

Maize is a cereal belonging to the grass family, Poaceae (formerly known as Gramineae) (USDA, 2005). Maize has a chromosome number 2n=2x=20. The exact origin of maize is unknown, however, it is believed to be native to South America, specifically, Central Mexico, where archaeological evidence of tiny ears of corn deposited in ancient village sites and in tombs of early Americans over 7000 years ago are discovered. In this region maize was domesticated from wild grass.

Additionally, phylogenetic analysis and microsatellite genotyping demonstrate that the Balsas River Valley in the highlands of southern Mexico is the region of a single maize domestication event which occurred over 9000 years ago. Prior to these findings, maize was believed to have been the product of multiple independent domestications from the wild relative, teosinte (Galinat, 1988; Kato, 1984). In this region, the oldest surviving maize types and those with high level of diversity are found. From here, maize spread to the Mexican lowlands and then was transported to other parts of the world through trade routes (Matsuoka *et al*., 2002).

It is generally agreed that teosinte (*Z. mexicana*) is an ancestor of maize, although opinions vary as to whether maize is a domesticated version of teosinte, (Galinat, 1988). From a taxonomic perspective, the genus *Zea* is divided into two sections (Iltis and Doebley,1980) namely, section *Zea*, encompassing the single class *Z. mays* L., and

section *Luxuriantes*, which includes the four classes *Z. Luxurians* (Durieu) R. M. Bird, *Z. nicaraguensis* Iltis and Benz, *Z. diploperennis* Iltis, J. F. Doebley and R. Guzman, and *Z. perennis* (Hitchock.) Reeves and Mangelsdorf. Section Z. *mays*, is subdivided into four subspecies, encompassing, the cultivated maize, *Z. mays* ssp. mays, and three wild taxa, namely, *Z. mays* ssp. *mexicana* (Schrader) (including; races Chalco, Central Plateau and Nobogame) Iltis (Central Mexico), *Z. mays* ssp.
Parviglumis var. *parviglumis* (race Balsas) Iltis and J. F. Doebley (Southern and Western Mexico), *Z. mays* ssp. *Huehuetenangensis* (race Huehuetenango) (Iltis and J. F. Doebley) J. F. Doebley (Eastern Guatemala) and the *Z. mays* species *Z. luxurians* (race Guatemala). All the wild species and subspecies of Z. *mays* are collectively known by the common name, „teosinte" (Kato, 1984; Beadle, 1939). The teosintes are ancient wild grasses found in Mexico and Guatemala having several differentiated forms giving rise to various races, species and plant growth habits (Doebley, 1990).

2.3.1 Cytological evidence

The wide array of the teosintes and their behaviour in hybridization with modern maize confounds evidence of teosintes as the progenitor of maize. The Mexican agronomist, José Segura performed successful hybridization between the Mexican annual teosinte (2n=2x=20) and maize (Harshberger, 1896). In contrast, maizeteosinte (*Z. luxurians*) hybrids exhibit two or more unpaired chromosomes during metaphase, hence are sterile while maize-teosinte (*Z. mays spp. Mexicana*) exhibit complete chromosomal pairing and full fertility (Beadle, 1932).

Emerson and Beadle (1932) demonstrated similarity in frequencies of crossing-over between maize-teosinte chromosomes and two variety maize chromosomes, which

provided a convincing evidence that maize and Mexican annual teosinte, *Z. mays spp. mexicana* were members of the same species (Beadle, 1972). In addition, the chromosomes of the two species were similar in arm lengths, centromere positions, and sizes and positions (interstitial positions) of knobs while knobs are only evident in telomeric positions in *Zea luxurians* (Kato, 1976; Longley, 1941) led to a conclusion that teosinte was ancestral to maize.

2.3.2 Isozyme evidence

Further evidence for the teosinte hypothesis is provided from isozyme analysis of 56 populations of teosinte, representing the entire geographic range of the wild taxa of *Zea*, and 99 populations of maize from Mexico and Guatemala (Doebley *et al*., 1987, 1984; Smith *et al*., 1985, 1984). In their studies encompassing 13 enzyme systems encoded by 21 loci, principal components analysis revealed that populations of maize and *parviglumis* could not be differentiated by their isozyme composition. A cluster analysis of the data also demonstrated that subspecies *parviglumis* was much similar to maize compared to the other teosintes, while maize and subspecies *mexicana* were distinct. Further support from allele frequencies revealed identical allele frequencies of *Z. mays* ssp. *parviglumis* or Balsas teosinte and that of maize, but distinct from that of *Z. luxurians*, *Z. diploperennis*, and *Z. perennis*
(Doebley, 2004).

2.3.3 Molecular evidence

The classical work of Matsuoka *et al*. (2002) employed microsatellite markers to investigate whether maize was the product of a single or multiple domestications from teosinte using phylogenetic analyses. Their findings confirmed that based on the

microsatellite data, modern maize is a product of a single domestication event from Balsas teosinte.

The microsatellite data go a bit further and imply that the populations of Balsas teosinte in the central portion of its distribution (where the states of Guerrero, Michoacan, and Mexico meet) are ancestral to maize. Second, Matsuoka *et al*. (2002) used microsatellites to date the time of the maize-teosinte divergence. The molecular dating indicates that maize and Balsas teosinte diverged about 9000 years ago, a date that agrees well with archaeological evidence (Piperno and Flannery, 2001).

2.4 Maize accessions and landraces

Accession is the general name given to types of a crop including wild relatives, progenitors (unimproved ancestral lines), landraces (local or traditional varieties), varieties (distinctly different lines), and cultivars (formally improved for a particular trait), and highly improved professionally-bred open pollinated varieties (OPV).

The concept of landrace is complex (Zeven, 1998) and is saddled with many inconsistencies resulting in an indefinable nature such that an all-embracing definition cannot be given. Brown (1978) and Rieger *et al*. (1991) described landraces as geographically or ecologically distinctive populations which are conspicuously diverse in their genetic composition both between and within populations. They differ from their wild relatives as they are regularly cultivated, but are not subjected to selection as the cultivars are and so demonstrate genetic heterogeneity.

Landraces have certain genetic integrity (de Carvalho *et al*., 2013). They are recognizable morphologically; farmers have names for them and differ in adaptation to

soil type, time of seeding, date of maturity, height, nutritive value, uses and other properties. Most importantly, they are genetically diverse (Harlan, 1975). Hawkes (1983) also supported this argument as he referred to landraces as „highly diverse populations and mixtures of genotypes".

Louette *et al*. (1997) described a landrace as farmers" variety which has not been improved by a formal breeding programme. Teshome *et al*. (1997) defined landraces as variable plant populations adapted to local agro-climatic conditions which are named, selected and maintained by traditional farmers to meet their social, economic, cultural and ecological needs. They further stated that in the absence of farmer"s manipulations, landraces may not exist in the ecological dynamics that are known today. Thus landraces and farmers are interdependent, in need of each other for their survival. Landraces are known to carry reservoir of genes which remain to be utilized in breeding programmes.

2.5 Biology of maize

2.5.1 Morphology of maize plant

Maize is an annual plant which often grows to about 2.5 m tall and matures in 100120 days, though some modern genotypes may reach maturity within 90 days.

Temperate cultivars are frequently shorter than tropical and subtropical cultivars (Gene Technology Regulator, 2008). It has an erect dominant stalk of 2.5 to 5 cm diameter with about 10 to 20 or more nodes having occasional tillers at the lower nodes. Beginning from the midsection of the plant, from each node grows a single leaf with total number of leaves varying from 12 to 30 in number in distichous arrangement. Leaf development ceases shortly before tasseling. Number of leaves in maize depends on

genotype and environment, specifically day length and temperature. Cold climate genotypes have fewer leaves than genotypes adapted to warm environments (Gene Technology Regulator, 2008).

Maize possesses two root systems which are fibrous and shallow. These include the embryonic and postembryonic roots (Feldman, 1994; Abbe and Stein, 1954). Hochholdinger (2009) gave a detailed review of the root system in maize. Essentially, the embryonic root system which consists of a primary root at the basal pole of the embryo bears many lateral root systems. Also present is the seminal roots which emerges from the scutellar node. The postembryonic root system includes the shootborne roots formed at the lower nodes, as well as lateral roots which originate from the pericycle of all roots. The aerial brace roots at the lower nodes provide support to the plant while the other roots provide water and nutrients to the plant. Figure 2.1 shows the morphological features of a mature maize plant. The stalk which terminates in an apical meristem bears the staminate flower and the pistillate flower.

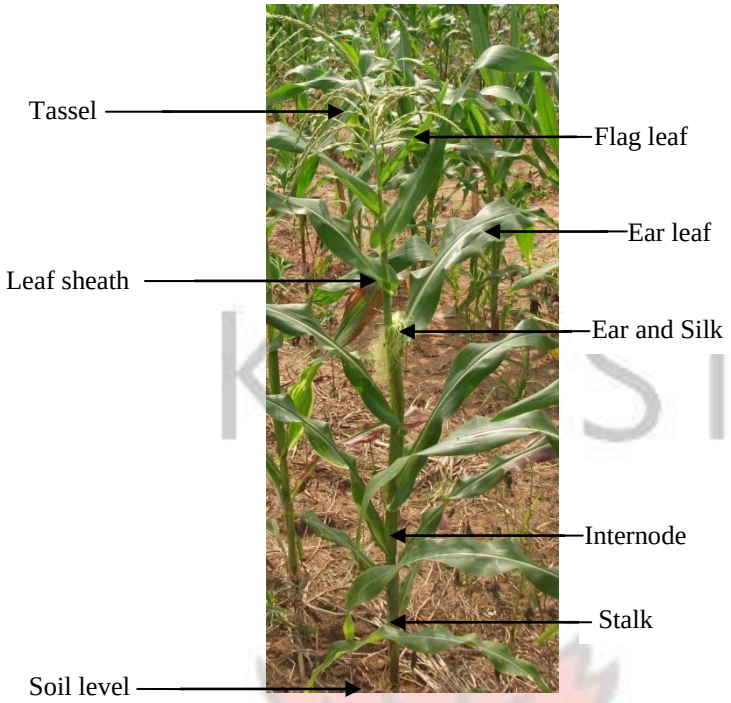


Figure 2.1 Morphological features of a mature maize plant

2.5.2 Maize inflorescence

Maize is a monoecious protandrous plant bearing male flowers in the tassel and female flowers on the lateral ear shoots of the same plant. The ears of the female"s inflorescence arise from axillary bud apices. The ear is covered with anumber of leaves called husks, which protect the grains from birds and insects. The thick axis of the ear, the cob, bears an even number of rows ranging from 4 to 30 ovaries each of which contains a single ovule.

The apical meristem of the stalk develop s into the tassel, the staminate structure. The tassel is a prominently branched structure at the top of the plant consisting of a central spike and a variable number of lateral branches (up to approximately 40) bearing flowers. The peduncle of the tassel grows vigorously, pushing the tassel out of the plant. The stamens consist of the anther which produces pollen grain and the filament which

holds the anther in position. Pollen is produced in the male spikelets of the tassel. Each spikelet consists of a pair of flowers (florets) enclosed in two large glumes (Cope and Gray, 2009).

2.5.3 Pistillate flower

Pistillate flowers of maize consist of a group of pistils each having a stigma, style (silk) and ovary containing the ovule. The silk which emerges from the base of the ear is stigmatic for most of its length. Ear shoot initiation occurs about 10 days after tassel initiation at 6-8 nodes below the tassel and infrequently at lower nodes (Bonnett, 1954).

2.5.4 Fertilization and embryogenesis

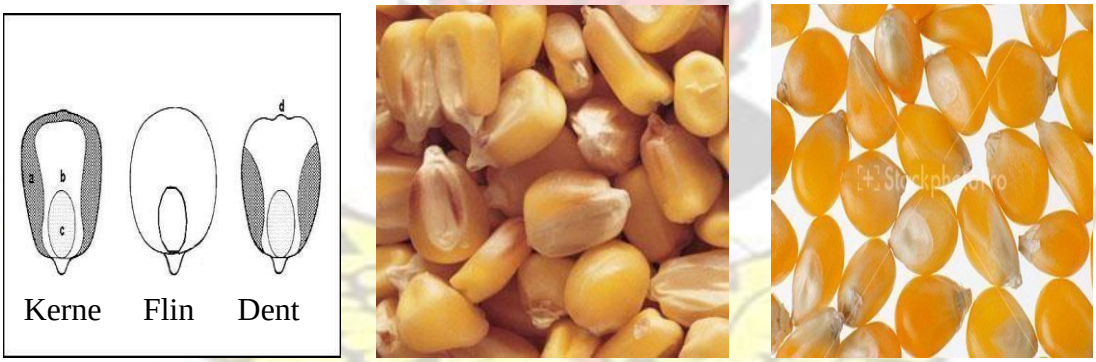
The monoecious nature of the plant facilitates both selfing and cross pollination. Reproduction in maize is initiated when pollen shed from a tassel fertilizes ovules located in the ear. Each tassel on a mature maize plant can produce up to 10 million pollen grains enclosed in anthers, which open few days, usually 3 - 5 days before the silks (stigmas) emerge. During anthesis, the anthers break open at the tips resulting in pollen shed which lasts for 5-8 days with a peak around the third day (IITA, 2013). Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe *et al*., 1988).

A single ear can produce up to 1,000 ovules, with each eventually producing a viable seed. Due to the protandry in maize, silks receive pollen as soon as they emerge. However, under certain conditions, when growth is vigorous and unstressed, pistillates mature before the staminate (protogyny). Nevertheless fertilization and yield may not

be adversely affected if anthesis occurs up to 5 days after silk emergence. The silks are for catching pollen. Pollen grains germinate on the moist and sticky hairs within few minutes after reaching the silks. The pollen tubes grow down the silks in 12-28 hrs to fertilize the ovules forming kernels or seeds (IITA,

2012).

Normally more than 97 % of the seeds produced by a given plant result from cross pollination (Aldrich *et al*. 1975). Kernels emerge from the cobs arranged in one of four or five patterns, viz., spiral, straight, regular or irregular. The textures of kernels vary depending on the genotype and may be flint, dent, floury, pop, or waxy in white, yellow, red, blue, or purple colours (FAO, 2003).



A. Maize kernel texture B. Dent kernel

C. Flint kernel

Figure 2.2 Maize kernel textures. (A) The distribution of soft and hard starch in a single kernel; a= soft starch; b= hard starch, c= embryo, d= hull. (B) Indentations on dent kernel resulting from shrinkage of soft starch. (C) Flint kernel with slight or no indentations due to hardening of starch.

2.5.5 Growth stages of maize plant

Depending on variety and environmental conditions, maize typically matures between 90 to 120 days (Myrick, 1913). Days to maturity have led to the classification of maize

into three groups, viz. early-maturing (90-95 days), intermediate (105-110 days) and late-maturing (115-120 days) varieties (Myrick, 1913). Within its life span maize grows through various stages including the seedling stage, vegetative stage, reproductive stage then grain filling stage. Seedling stage normally begins one week after sowing when plants may have developed between 2-4 leaves. The vegetative stage then begins with the stem growing erect to its maximum height within 35–45 days after sowing. The reproductive stage involves tassel emergence, anthesis and silk development. The period between anthesis and silk emergence is known as anthesissilking interval which is a measure of protandry, and is related to tolerance to stresses which reduce photosynthesis at flowering.

The grain filling stage, which lasts over a period of about 8 weeks, involves three stages, namely the blister stage, milk stage and the dough stage (Lee and Tollenaar, 2007). Blister stage begins after fertilization when silks wilt and turn brown. Carbohydrates and nutrients rapidly accumulate in the developing kernels in the form of clear fluid. About 10 days after flowering, kernels shaped like small blisters appear. Milk stage begins 3 weeks after silking, when the kernels are filled with white, milky fluid. The fluid has high sugar content and kernels are most suitable for consumption as fresh maize. Following the milk stage, the sugar and water contents decrease while starch content increases.

The last stage is the actual grain-filling stage also known as the dough stage or physiological maturity stage. This stage occurs 55 to 65 days after silking, when lower leaves dry up and dry silks become brittle. The white paste in the kernel gradually solidifies to starch, starting from the top part of the kernels. Harvesting is normally done at this stage as the grains have physiologically matured.

2.6 Maize research in Ghana

2.6.1 Maize breeding in Ghana (varietal development)

Organized maize breeding in Ghana started in the 1930‟s (GGDP, 1986) with an objective of developing high and stable-yielding open-pollinated varieties for all six agro-ecological zones, namely, the Rain Forest, Deciduous Forest, Forest-Savanna Transition, Coastal Savanna, and Northern Savanna (Sudan Savanna and Guinea Savanna), Some open-pollinated varieties such as Mexican 17E in 1961, composite 2 in 1968, Golden Crystal, La Posta and Composite 4 developed in 1972 were released by the effort of some local Ghanaian breeders, especially M. K. Akposoe (Sallah, 1998).

In 1979 the Ghana-CIDA Grains Development Project supported the development of early-maturing varieties (Sallah, 1986). Quality Protein Maize (QPM) development programme was started in 1989 at the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) (Twumasi-Afriyie and Sallah, 1994; Twumasi-Afriyie *et al.,* 1994a, 1994b). In 1992 „Obatanpa GH‟ was released as an open-pollinated variety (OPV), with a yield potential of 4.6 tons/ha and has since been widely adopted in Ghana and other parts of Africa and beyond (Badu-Apraku *et al*., 2006; Twumasi-Afriyie *et al.,* 1994a, 1992,). „Obatanpa GH‟ is rich in lysine and tryptophan, the two amino acids that are known to play a key role in human and animal development.

Alongside the development of „Obatanpa GH‟, a QPM hybrid maize development programme was initiated in 1991. Three-way QPM hybrids, namely, GHllO-5

'Mamaba', GH132-28 'Dadaba', and GH2328-88 'CIDA-ba" developed in this programme were very productive, yielding between 6.3 and 7.3 t/ha on experimental fields, which represented an increase in grain yield of 19 to 38 % over „Obatanpa-GH" (Asiedu *et al*., 2001). These QPM hybrids were released for production in 1997. All three hybrids were medium-maturing genotypes (105-110 days) with moderate levels of resistance to maize streak virus.

Over the years, the CRI in collaboration with CIMMYT and IITA produced varieties having in addition drought-tolerance, *Striga* resistance, and were adapted to most agroecologies in Ghana. In 2010, new varieties released included high-yielding and earlymaturing OPVs such as CSIR-Omankwa, from the pedigree (TZE-W Pop STR QPM

C4), CSIR-Aburohema (EVDT-W99 STR QPM CO), and CSIR-Enii-Pibi

(GH110×Ent 75), an intermediate-maturing genotype.

2.7 Genetic diversity in maize

Genetic diversity refers to variation in nucleotides, genes, chromosomes, or whole genomes of organisms within or among populations (Frankharm *et al*., 2002). Genetic diversity in the African maize collection is critical as a resource to find new alleles that will improve grain yield to address food security and fight hunger and malnutrition in this region of the world which records highest incidence of poverty (Handley *et al*., 2009). Unimproved and wild relatives of cultivated genotypes constitute untapped genetic resource for yield increase and tolerance to biotic and abiotic stresses, as well as genes for improving kernel quality, such as protein, oil, and starch contents (Smith *et al*., 2005; Reif *et al*., 2005; Pollak, 2003).

Information on genetic diversity aids the organization of populations into core subsets for use by breeders and researchers (Warburton *et al*., 2002), for the conservation and management of germplasm, for identification of heterotic groups (Ajmone-Marsan, *et al*., 1998), and serves as a guide to the choice of testers for trials of hybrid combinations in breeding (Enoki *et al*., 2002). In addition, studies on the level and distribution of genetic variation within and among plant populations of a crop species reveal the historical and evolutionary processes governing the genetic diversity. Appreciable level of genetic variability in a population is required to achieve a successful long-term plant breeding program. Progress from selection is known to be directly related to the magnitude of genetic variance in the population (Tabanao and Bernado, 2005; Hallauer and Miranda, 1995; Helm *et al*., 1989).

Maize is perceived to be the most diverse crop plant known containing extensive diversity at both phenotypic and molecular levels (Buckler *et al*., 2006). The analysis of genetic relationship and germplasm diversity in crop species provide information about genetic diversity and serves as a platform for the development of new genotypes and breeding populations (Dwivedi *et al*., 2008; Mohammadi and Prasanna, 2003). Furthermore, in order to maintain long term genetic gain on desirable traits, and ensure a wide genetic base in breeding gene pools genetic diversity must be conserved and improved (Smith *et al*., 2005; Hallauer and Miranda, 1988). Regrettably, there have been few reports of detailed genetic diversity among the African maize genotypes compared to the U.S. Corn Belt germplasm (Goodman and Stuber, 1983), European genotypes (Dubreuil *et al*., 1996; Messmer *et al*., 1992), Japan maize inbred lines (Enoki *et al*., 2002), and CIMMYT tropical maize lines (Xia *et al*., 2005; Warburton *et al*., 2005, 2002).

Most of the West African maize populations and few inbred lines have been constituted from few local maize lines and introductions from the U.S. and CIMMYT maize germplasm, followed by recycling of elite inbred lines, a practice which leads to narrowing of genetic base. For example, the current few maize cultivars in Ghana were derived from the open-pollinated QPM variety „Obatanpa GH" as an inbred line (BaduApraku *et al*., 2006) for the development of QPM hybrids and synthetic varieties. The use of germplasm with narrow genetic base most often leads to vulnerability of the crop to environmental stresses. In the U.S., the pedigrees of most hybrids are derivatives of six to eight inbred lines (Gethi *et al*., 2002; Darrah and Zuber, 1986). In China, the parenthood of 91.6 % hybrids consists of about 20 elite inbred lines (Li *et al*., 2002).

Typical examples of the demerits of genetic uniformity are the Southern Corn Leaf Blight Epidemic caused by vulnerability of maize to the fungus, *Helminthosporium maydis* in U.S. hybrid maize bearing the male-sterile T-cytoplasm. In 1970 vast maize fields of the U.S. Corn Belt were destroyed by the disease. This incident created awareness of vulnerability of uniformity in genotypes to diseases and the importance of maintenance and exploitation of the available genetic diversity of maize to create a wide genetic base (Goodman and Brown, 1988). Similarly, the maize streak virus epidemic of West Africa in 1983 and 1984 in which many lives were lost due to famine was attributed to homogeneity in cultivated maize genotypes
(IITA, 1986).

Maize genetic diversity contained in germplasm banks and in breeding programs still remains unexploited (Carena and Wicks, 2006; Goodman, 2005; Warburton *et al*.,

2005, 2002; Reif *et al*., 2004; Labate *et al*., 2003) making studies on maize genetic diversity a very important springboard for effective maize breeding for food security in West Africa.

Although maize landraces represent an important source of genetic variability, they are under- exploited and their genetics is poorly understood (Molin *et al*., 2013). For instance, little is known about the genetic diversity as well as the structure and the influence of historical introductions in the International Plant Genetic Resources Institute (IPGRI) maize collections held in the Genetic Resource Center of IITA, though these genotypes may constitute a rich source of alleles for maize improvement.

IPGRI, the collector of maize used in the current study, is a global research organization that is mandated for plant development with a vision that agricultural biodiversity nourishes people and sustains the planet, has a primary focus of conservation of crop genetic resources in Genetic Resource Centers. IPGRI was known in 1974 as International Board for Plant Genetic Resources (IBPGR) until 1991. In 2006, IPGRI and the International Network for Improvement of Banana and Plantain (INIBAP), members of the Consultative Group for International Agricultural Research (CGIAR) became a single organization and subsequently changed their operating name to Biodiversity International. Biodiversity International delivers scientific evidence, management practices and policy options to the utilization and protection of agricultural biodiversity. In addition it works with partners in lowincome countries where agricultural biodiversity can contribute to improved nutrition, resilience, productivity and climate change adaptation (Wikipedia, 2015).

2.8 Estimation of genetic diversity

Four main approaches exist for assessing genetic diversity and relationships within and among various classes of germplasm including landraces, inbred lines, hybrids, synthetics and populations. The choice of a method is governed by availability of resources, time, labour, and extent of coverage of the genome. The methods include agro-morphological evaluation by means of descriptors, pedigree analysis through estimation of coancestry coefficients (Malecot, 1948), biochemical profiling encompassing isozyme or storage protein analysis, and finally, DNA-based molecular techniques (Mohammadi and Prasanna, 2003; Pejic *et al*., 1998). Each approach has its own strengths and drawbacks and hence their combined utilization is recommended to increase the resolving power of genetic diversity analyses (Singh *et al*., 1991).

2.8.1 Agromorphological trait evaluation

Agromorphological characterization of germplasm accessions is fundamental to progress in plant breeding (Lin, 1991). It employs both qualitative and quantitative descriptors such as colour, size, shape, growth habits and performance under different experimental conditions or treatments. Collecting and analyzing data by agromorphological traits is inexpensive and relatively simple with no requirement for sophisticated technology as with other means of genetic diversity estimation. However, the influence of environment and the low heritability of traits limit the applications of morphological evaluations. In addition, morphological evaluations are timeconsuming, labour-intensive, and require large plant population size (Botha and Venter, 2000).

Goodman and Bird (1977) reported of 14 clusters within the Latin American races and subraces of maize on evaluation of 20 ear characters from 219 accessions. On the

basis of twenty-two morphological traits, Ruiz de Galaretta and Alvarez (2001) evaluated 100 landraces of maize from Northern Spain and came up with seven different groups having promising breeding values. Hartings *et al*. (2008) reported a large genetic heterogeneity among 54 maize landraces originating from Italy on the basis of morphological and AFLP analysis and revealed four major clusters reflecting their geographical origin. Van Etten *et al*. (2008) evaluated 79 maize samples from Guatemala by means of ear characteristics, plant and ear height, stalk diameter, and grain yield and revealed 11 groups depicting divergence caused by isolationbydistance. Rebourg *et al*. (2001) examined genetic variation among 130 European traditional maize populations and split them into six groups on the basis of morphological and molecular analysis. Analysis of 294 landraces originating from Malawi, Zambia, and Zimbabwe using 34 phenotypic traits partitioned the set into three non-overlapping groups by cluster analysis (Magorokosho, 2006). Obeng-Antwi (2007) performed genetic diversity study on 92 maize landraces from Ghana and observed a large variability among accessions within groups (96%) rather than among groups.

2.8.2 Estimation of diversity by coancestry coefficients

Estimation of diversity by coancestry coefficients is based on pedigree records of genotypes. This approach does not demand use of sophisticated technology but depends on accurate pedigree records, and it cannot evaluate the effects of selection and gene drift (Messmer *et al*., 1993). As expected, variation in genotypes arises from both genetic and environmental factors, as well as interaction effects. More often, the magnitude of variability due to genotypic, and for that matter, heritability, as well as environmental components of diversity may be extracted by application of robust statistical analysis.

2.8.3 Isozyme and storage protein analysis

Biochemical profiling involves detection of isozymes by electrophoresis. Isozyme markers are codominant, inherited in a simple fashion, and are capable of detecting polymorphisms at the functional gene level, as such depict genetic base with high fidelity. The advantage of the use of isozymes is that it requires only small quantity of plant material for its detection. Nevertheless, only a few number of enzyme markers which do not cover the entire genome owing to their rare occurrence further limits the resolution of genetic diversity (Govindaraj *et al.*, 2015). Isozyme and storage protein analysis are limited in their ability to detect variation in nucleotide sequence that is silent with respect to the resulting amino acid sequence due to degeneracy of the genetic code.

2.8.4. Assessment of genetic diversity by molecular analysis

A molecular marker is a piece of DNA that indicates the site on a chromosome where differences in DNA sequences occur among members of the same species. It reveals polymorphisms at DNA level by detecting a particular gene or allele across a genome through probing. Molecular marker differentiates clearly the chromosomic traits by tagging the complementary pair of genes which it represents as well as flanking chromosomal coded or non-coded regions at the 5' and 3' end (Barcaccia *et al*., 2000).

DNA-based molecular techniques employ molecular markers such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR) or microsatellites, Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR) and Single Nucleotide Polymorphisms (SNPs) to identify polymorphisms among genotypes.

Molecular markers are superior to morphological, pedigree and biochemical data because they are more efficient and sensitive in detection of distinct differences arising from mutations among genotypes at the DNA level (Melchinger *et al*., 1991). Because they are abundant and cover the entire genome they are preferred markers for genetic diversity studies. Additionally, DNA-based molecular markers offer advantages such as, unresponsiveness to environmental effects, neutral to pleiotropy and epistatic effects, and opportunity to compare genotypes between generations and populations (Smith, 1988). Disadvantages of molecular markers include their high cost and requirement for sophisticated equipment. The SSRs (Warburton *et al*., 2002), AFLP (Beyene *et al*., 2006), RFLP (Dubreuil *et al*., 1999) and SNPs (Yu *et al*., 2011) offer more efficient method of estimating gene flow and classifying genotypes into groups, as well as for estimation of their genetic diversity.

Microsatellite (SSR) markers are short tandem repeats of 2 to 6 base pair repeat sequences which occur in varied copy numbers in the intergenic regions of the genome (Litt and Luty, 1989). SSRs are widely distributed throughout the genome of crop plants and are particularly useful for the study of population structure and demographic history of domesticated species because their high level of allelic diversity facilitates the detection of the structure of diversity more efficiently than
RFLP, AFLP, or SNP loci (McGregor *et al*., 2000; Powell *et al.*, 1996). Senior and Heun (1993) reported that SSR loci provide a high level of polymorphism in maize. Amplified SSR fragments from PCR may be separated on both polyacrylamide and high quality agarose gels (Senior *et al*., 1998).

Liu *et al.* (2003) analyzed the genetic diversity among 260 maize inbred lines of temperate tropical and subtropical origin using 94 SSR loci and identified 2,039 alleles.

Comparison of diversity in equivalent samples of inbreds and open-pollinated landraces revealed that maize inbreds capture about 80 % of the alleles in the landraces, suggesting that landraces can provide additional genetic diversity for maize breeding. Beyene *et al*. (2005) researched into 62 traditional Ethiopian highland maize in a comparative study, using morphological traits and molecular profiling by AFLPs and SSRs and concluded that variability existed among the selected genotypes. Menkir *et al.* (2005), conducted a genetic diversity study into 41 IITA *Striga*-resistant maize inbred lines by means of SSR analysis and revealed great deal of genetic variation estimated as similarity coefficients ranging from 0.21 to 0.92 with a mean of 0.48 ±0.003.

Magorokosho (2006) reported ample genetic diversity among 267 maize landraces collected from different agro-ecological zones in Zimbabwe, Zambia and Malawi and ancestral U.S.A. genotypes using SSRs. On the basis of DICE similarity coefficient, the genetic distance among these genotypes was high and ranged from 0.344 to 0.943 with a mean of 0.652. Moreover, it was evident that the genetic diversity introduced from the ancestral genotypes over 100 years ago had been preserved.

2.9 Measures of genetic diversity

Measures of genetic diversity quantify the variation and relationships within and among populations and/or individuals on the basis of some metric traits derived from agromorophological evaluation, or the typical binary data, fragment size, or allele frequencies of molecular markers. A range of genetic diversity measures are available and application of each for quantifying and characterizing variability is contingent on

the type of population (inbred lines, hybrids, landraces, etc), the status of the population in terms of Hardy-Weinberg equilibrium, an finally, the kind of data (Mohammadi and Prasanna, 2003).

The most common measures of genetic variation include rate of polymorphism, average number of alleles per locus, proportion of rare alleles, intra-population gene diversity for dominant loci, average polymorphic information content or expected heterozygosity for co-dominant loci, effective number of alleles, and genetic distance.

a) Rate of polymorphism (*Pj*)

Polymorphic loci are those having allele frequencies of less than or equal to 0.95 or 0.99 (Hartl and Clark, 1997). The limit of allele frequency, which is set at 0.95 or 0.99, is arbitrary, its objective being to help identify those genes in which allelic variation is common (Cavalli-Sforza and Bodmer, 1981). This measure of intrapopulation genetic diversity demonstrates that a gene is exhibiting variation. It is calculated from the number of polymorphic loci divided by total number of loci (both polymorphic and monomorphic) as presented in Equation (2.1).

$$P = \frac{n_p}{n_{total}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

where n_p = number of polymorphic loci; n_{total} = total number of loci, both monomorphic and polymorphic.

b) Average number of alleles per locus (A_P)

For codominant markers where alleles can be detected on gels, the average number of alleles per locus is calculated for polymorphic loci as total number of alleles in all loci divided by total number of polymorphic loci. It is given the formula

$$A_p = \frac{1}{k} \sum_{i=1}^{k} n_i \quad \text{..... (2.2)}$$

where, i = any locus; k = number of polymorphic loci; n_i = number of alleles detected per polymorphic locus.

c) Rare allele

Rare alleles are defined as those with frequencies of less than 0.005.

d) Average Polymorphic Information Content (PIC)

Polymorphic information content is a reflection of allele diversity and frequency among accessions. Average Polymorphic Information Content (PIC) (Botstein *et al*. 1980), also described as expected heterozygosity (Nei, 1987) at a locus is calculated for each SSR locus as:

$$PIC = 1 - \sum (P_i)^2 \quad \text{..... (2.3)}$$

where, P_i is the proportion of the population carrying the ith allele. PIC provides a guide to the markers that carry the most information for discriminating between genotypes.

e) Effective number of alleles (A_e)

It is the number of alleles that can be present in a population and measures the number

of equally frequent alleles that it would take to achieve a given level of gene diversity (Weir, 1990). Effective number of alleles makes it possible to compare populations where the number and distributions of alleles differ substantially. The formula is:

$$A_e = \frac{1}{\sum P_{i2}} = \frac{1}{1-h} \quad \text{..... (2.4)}$$

where P_i is the frequency of the i^{th} allele in a locus, and $h = 1-\sum P_i^2$ is the heterozygosity at a locus.

2.10 Determination of relationships among genotypes

2.10.1 Genetic distance

Genetic distance is the quantitative measure of genetic differences (similarity or dissimilarity) between individuals, populations, or species at the allelic level (Beaumont *et al*., 1998) arising from identity-by-state or identity-by-descent due to past mutation events from a common ancestor. In contrast, differences in populations arise from phenomenon such as founder effect or gene flow (Nei and Li, 1979; Kosman and Leonard, 2005). Genetic distance is an estimation of the evolutionary changes that have occurred since two populations diverged from being a single random ancestral mating population. Genetic distance ranges from 0 to 1. Low values of similarity coefficients indicate large genetic distances, while for dissimilarity estimates small values mean close genetic relationship.

Many distance measures are available but the choice depends on the kind of data, to be precise, interval data of morphological evaluations, allele frequency data of isozyme or DNA amplification products and presence or absence data. The most common distance

measure for morphological data is Euclidean distance or straight line measure. The Euclidean distance between two individuals is given by the square root of the sum of all squares of pairwise differences between two any individuals, A and B, having morphological measures (i) where i = 1,...., p represented by x_1, x_2, ..., x_p and y_1, y_2... and y_p. Equation 2.5 shows the mathematical expression for calculation of Euclidean distance.

$$d_{AB} = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + \ldots + (x_p - y_p)^2]^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots (2.5)$$

Other distance measures include correlation coefficient, Roger‟s distance (Roger), Cavalli-Sforza and Edward‟s (1967), and Nei (1972) distance. The correlation distance measure is a powerful estimation of genetic distance as data on metric traits having different units is standardized to allow for unbiased comparison among genotypes. For SSR molecu lar data analysis, in which repeat amplification products represent alleles, variation in allele frequencies may be estimated or bands may be scored as presence or absence to generate a binary data. A common distance measure which employs allele frequencies is Roger‟s distance, Cavalli-Sforza and Edward‟s (1967) arc and chord distances, and Nei‟s (1972) distance, *inter alia*.

Rogers‟ distance, d_{ij} is given by equation:

$$d_{ij} = \sqrt{\frac{1}{2l}\sum_k (x_{ki} - x_{kj})^2} \quad \ldots\ldots\ldots\ldots\ldots (2.6)$$

where l is the number of loci, x_{ki} and x_{kj} are frequencies of allele k for the entities i and j.

For binary data matrix, four measures of genetic distance are often used, namely, the Modified Roger"s distance GD_{MR} (Wright, 1978) (Equation 2.7), DICE, also known as Nei and Li"s (1979) coefficient (GD_{NL}), (Equation 2.8), Jaccard"s (1908) coefficient (GD_J) (Equation 2.9) and simple matching coefficient of Sokal and Michener (1958) (GD_{SM}) (Equation 2.9) which are hereby presented.

In these equations, X_{01} is the number of bands or allel es present in individual *j* only; X_{10} is the number of bands or alleles present in individual *i* only; X_{11} is the number of bands or alleles present in both individuals; X_{00} is the number of bands or alleles absent in both individuals; The simple matching and Modified Roger"s are examples of Euclidean distance measures.

$$GD_{MR} = \sqrt{\frac{X_{10} + X_{01}}{2l}} \quad \text{..... (2.7)}$$

$$GD_{NL} = 1 - \left(\frac{2X_{11}}{2X_{11} + X_{10} + X_{01}}\right) \text{ ..... (2.8)}$$

$$GD_J = 1 - \left(\frac{X_{11}}{X_{11} + X_{10} + X_{01}}\right) \text{..... (2.9)}$$

$$GD_{SM} = 1 - \left(\frac{X_{11} + X_{00}}{X_{11} + X_{10} + X_{01} + X_{00}}\right) \text{ ..... (2.10)}$$

A similarity measure between two individuals is essentially defined as the fraction of

observed bands in a banding pattern that are shared. It is therefore expected that in devising a similarity measure, equal weights would be assigned to shared presences (1s) and shared absences (0s). However, only the simple matching coefficient attaches importance to shared absences as shown in Equation 2.10.

Both DICE and Jaccard‟s coefficients disregard shared absences though DICE gives more weight to shared presences represented by the term $2X_{11}$. While the validity of these approaches is compromised, the suitability of a measure and correct interpretation of it remains unanswered. Despite these drawbacks, the DICE coefficient is widely preferred over the other measures. Kosman and Leonard (2005) argued that assessment of genetic similarity by the DICE, Jaccard‟s and simple matching coefficients in diploid organism with codominant markers may not be appropriate because there is no way of direct processing of fingerprint profiles as null alleles (absence of bands) is rarely observed in codominant markers, except for SSRs. They propose a new dissimilarity measure based on a transformation of a multialleleic banding pattern into homozygous and heterozygous states averaged over all loci. Contrary to this argument, multialleleic codominant banding patterns exhibit shared presence and absence (not arising from null alleles) making the measures valid.

Being landraces with no knowledge of ancestry of the genotypes, the DICE coefficient was a suitable dissimilarity measure to use to study the IPGRI accessions. Landry and Lapointe (1996) suggested the use of Jaccard or the DICE coefficients for genetic analysis involving molecular data after a comparative study of several coefficients with the use of RAPD.

2.11 Multivariate techniques for interpretation of genetic distance

Regardless of population size, a genetic distance among accessions is better visualized by application of various multivariate statistical techniques. These techniques group accessions into clusters on the basis of their genetic distance arising from multiple measurements on individual operative taxonomic units and analyses relationships among them. The most common multivariate techniques include cluster analysis, principal components analysis or principal coordinate analysis and multidimensional scaling, (Brown-Guedira *et al*., 2000; Thompson *et al*., 1998; Johns *et al*., 1997; Melchinger, 1993).

2.11.1 Cluster analysis

Cluster analysis (Hair *et al*., 1995) groups individuals on the basis of similarity in their characteristics such that members within clusters are homogeneous while members across clusters are heterogeneous. Two cluster methods are in common use, the hierarchical and nonhierarchical clustering based on (i) distance measurement by Johnson and Wichern (1992) and (ii) the more robust maximum likelihood estimation and Bayesian methods of Pritchard *et al*. (2000) developed to overcome the constraints of distance-based methods.

Mohammadi and Prasanna (2003) compared the most used hierarchical treeproducing cluster method to the less commonly-used non tree-generating nonhierarchical methods. The hierarchical method is agglomerative as it successively groups individuals and then merges them on the basis of their similarities. The single linkage, complete linkage and the Unweighted Pair Group with Arithmetic means (Panchen,

1992; Sneath and Sokal, 1973) are the most common cluster methods which convert distance measures into graphical forms.

2.11.2 Bootstrapping

Bootstrapping is a statistical method for obtaining confidence limits on phylogenies (Felsenstein, 1985). The statistical significance of cluster analysis may be performed by bootstrapping technique in which newer data sets are generated by resampling original data with replacement. The estimates of parameters of interest and their variances, as well as confidence intervals of the parameter estimates are obtained.

2.11.3 Principal components analysis

Principal components analysis (PCA) is a way of identifying patterns in data on the basis of similarities and differences (Lindsay, 2002). The technique was originated by Pearson (1901) and further developed to its present state by Hotelling (1933). PCA is a multivariate technique that uses ordination of multivariate data presented in a matrix form to reveal patterns or relationships inherent in a data on the basis of similarities or differences when projected in two-dimensional space. Classical account of PCA is provided by Johnson and Wichern (1992) and Joliffe (1986). On the two-dimensional space, variables that are similar appear close together while dissimilar variables are far apart. As such, only variables that capture essential data patterns are revealed.

PCA therefore reduces dimensionality of a multivariate data. In ordination, series of linear combinations of orthogonal variables known as PCs are created. Each PC explains successively some proportion of the total variance represented by an eigenvalue from the largest proportion, usually PC1, to the least contribution to the variance (PC_n) at which 100 % of the variance is explained. Hence, sum of eigenvalues

equals the total variance. Parameters associated with the PCs are given by the eigenvectors. Significance of PCA is determined from a scree plot at the point where the curve makes an elbow. Additionally, North *et al*. (1982) revealed that the sampling error associated with an eigenvalue is equivalent to the distance between two adjacent PCs in the formula (Equation 2.11) such that errors larger than the spacing between adjacent PCs indicate significance.

$$\text{Sampling error} = \lambda_i \sqrt{\frac{2}{n}} \quad \ldots\ldots\ldots\ldots\ldots (2.11)$$

where λ_i = a PC; $\Delta\lambda$ = change between neighbouring PCs; n= number of samples.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material

A set of 64 tropical maize landraces sampled from the 120 IPGRI (International Plant Genetic Resource Institute, Italy) maize germplasm collected from diverse locations in Africa and held in IITA (International Institute of Tropical Agriculture, Ibadan, Nigeria) was studied. The origin and passport data of these accessions are not available. In addition, an inbred line, „Obatanpa GH", served as check to represent the diversity available among current and historic lines used in breeding maize for subSaharan Africa. The accessions were evaluated in field trials in 2011 and 2012 wet seasons in Kumasi, Ghana, to determine phenotypic diversity and classify the landraces into groups for further evaluation. Additionally, a molecular evaluation encompassing simple sequence repeat marker profiling was performed on the landraces.

„Obatanpa GH" is an inbred line used for the development of Quality Protein Maize (QPM) hybrids and synthetic varieties in several maize breeding programs in Africa (Badu-Apraku *et al*. 2006). „Obatanpa GH" was developed by the Crops Research Institute (CRI), of the Council for Scientific and Industrial Research, Ghana. It was kindly supplied by the Institute, in Kumasi. 'Obatanpa GH" is a tropically adapted, intermediate-maturing, open-pollinated cultivar, whose endosperm is white, dent and flint. It was first released by CRI, Ghana, in 1992 in collaboration with the

International Institute of Tropical Agriculture (IITA), Ibadan, the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and the Sasakawa Global 2000.

Table 3.1 shows the list of IPGRI genotypes used in current study.

Table 3.1 The IPGRI African maize landraces used in current study

Entry	Accession Name	Entry	Accession Name	Entry	Accession Name	Entry	Accession Name	Entry	Accession Name

1	TZm-1097	15	TZm-1116	29	TZm-1132	43	TZm-1150	57	TZm-1193
2	TZm-1098	16	TZm-1117	30	TZm-1136	44	TZm-1151	58	TZm-1194
3	TZm-1099	17	TZm-1118	31	TZm-1137	45	TZm-1152	59	TZm-1195
4	TZm-1100	18	TZm-1119	32	TZm-1138	46	TZm-1153	60	TZm-1211
5	TZm-1101	19	TZm-1120	33	TZm-1139	47	TZm-1156	61	TZm-1212
6	TZm-1103	20	TZm-1121	34	TZm-1141	48	TZm-1180	62	TZm-1213
7	TZm-1105	21	TZm-1122	35	TZm-1142	49	TZm-1182	63	TZm-1214
8	TZm-1106	22	TZm-1123	36	TZm-1143	50	TZm-1183	64	TZm-1215
9	TZm-1108	23	TZm-1125	37	TZm-1144	51	TZm-1184	65	„Obatanpa GH“
10	TZm-1109	24	TZm-1126	38	TZm-1145	52	TZm-1185		
11	TZm-1110	25	TZm-1128	39	TZm-1146	53	TZm-1187		
12	TZm-1111	26	TZm-1129	40	TZm-1147	54	TZm-1188		
13	TZm-1112	27	TZm-1130	41	TZm-1148	55	TZm-1189		
14	TZm-1114	28	TZm-1131	42	TZm-1149	56	TZm-1190		

3.2 Location and conditions of experimental site

All accessions were grown in field trials at the Kwame Nkrumah University of Science and Technology Agricultural Experimental Station, Anwomaso, in Kumasi Metropolis in the Ashanti Region of Ghana, West Africa, from April to August 2011 and from March to July 2012, in the major rainy season. The geographical position of the station is latitude 6º 41‟ 28.4“‟ North and longitude 1º 30‟ 58.8“‟ West. The environmental conditions at this site are 1,500 mm rainfall, mean temperature of 25 º C, and a sandy loam soil texture with 1.8 % organic matter at pH 5.2.

Anwomaso forms part of the semi-deciduous agro-ecological zone and experiences an annual bimodal rainfall pattern with a high relative humidity. The major rainy season begins from middle of March and ends in July while the minor rainy season begins from September and ends in November. The month of August is fairly dry.

3.3 Land preparation, planting and experimental design

Ploughing and harrowing were carried out using tractor- mounted implements (disc plough and harrow) on a tractor. This was followed by weed control with Round Up

(Glyphosate at an application rate of 5 Lha^{-1}) and Gramoxone (Paraquat at a rate of 4.5 L/ha) two weeks after weed emergence. All entries were planted in an area of 2,160 m^2 in a randomized complete block design with three replications The experimental plots consisted of 6 m × 0.6 m row containing 15 plants per row with the planting distance of 0.90 m x 0.30 m, giving a planting density of 42,000 plants/ha. Plots were separated by an alley of 0.75 m and blocks were separated by 2 m. Recommended crop management techniques including regular irrigation as needed and application of fertilizer at a rate of 120:60:40 $kgha^{-1}$ of $N\text{-}P_2O_5\text{-}K_2O$ plus ammonium sulphate at a rate of 125 $kgha^{-1}$ at 21 days after planting, as well as at ear emergence. Post-emergence weeds were controlled by application of Atrazine (4.5 Lha^{-1}) and hand weeding with a hoe. Using Conpyrifos 48 % (1 L/ha) and Cymethoate Super (1.5 Lha^{-1}), the African maize stem borers (*Busseola fusca*) and common cutworms (*Agrotis segetum*) were controlled.

3.4 Data Collection

Two sets of data were collected, *viz*., agromorphological data, following the maize descriptor list developed by IPGRI, as well as SSR profiling data.

3.4.1 Morphological Data

At various stages of plant growth, morphological data on 5 qualitative traits consisting of silk colour, principal grain colour, kernel texture, cob colour, and kernel arrangement were collected (Table 3.2). Twenty-four quantitative traits covering plant architecture, ear and tassel related traits, and kernel characteristics, yield and yield component data were collected from 10 representative plants per plot following the maize descriptor list

of IPGRI and CIMMYT (1991). Table 3.2 gives a detailed description of the method of measurement of the various traits. Measurements were taken with meter rule, Vernier calliper, micrometre screw gauge, and weighing scale where relevant.

3.5 Statistical analyses of morphological data

3.5.1 Description of genetic diversity

For the qualitative evaluation, frequency of occurrence of genotypes in the various categories was determined. Means, standard deviations, minimum and maximum values, as well as coefficient of variation (CV) for the quantitative traits were calculated. Analysis of variance (ANOVA) was performed on each trait by means of PROC GLM to test for differences in means among the accessions. By considering accessions as random effects and replications and blocks within replications as fixed effects, analysis of variance was carried out and the variance components of genotypic, phenotypic, environmental effects were extracted from the expected mean squares (EMS) using SAS 9.3.1 (SAS Institute Inc. 2011). Table 3.3 shows the computations for determination of the variance components. Genotypic and phenotypic variance components were calculated from the linear functions of the mean squares represented by *M* and a subscript for the associated source of variation. Standard errors of the estimated variance components were computed using the method of Hallauer and Miranda (1981), and broad sense heritability estimate for each quantitative trait was also determined from a ratio of the variance components.

	Measurement Procedure	Abbreviation	Phenotypic data (units)	Trait	Definition
1	On a plot basis at anthesis date	AD	Anthesis date (days)	Quantitative	Number of days from planting to 50 % of the plants shedding pollen
2	On a plot basis at silking date	SD	Silking date (days)	Quantitative	Number of days from planting to 50 % of the plants having silks at least 1 cm long
3	On a plot basis at silking date	SC	Silk colour	Qualitative	Predominant colour of silk (Pale yellow = 1; red = 2)
4	On a plot basis at anthesis and silking date	ASI	Anthesis to silking interval (days)	Quantitative	Calculated as SD-AD
5	On ten plants taken at random within each row at blister stage	TL	Tassel length (cm)	Quantitative	Length of tassel from flag leaf level to tip
6	On ten plants taken at random within each row at blister stage	ELL	Ear leaf length (cm)	Quantitative	Length of the leaf which subtends the uppermost ear.
7	On ten plants taken at random within each row at blister stage	ELW	Ear leaf width (mm)	Quantitative	Width of leaf which subtends the uppermost ear.
8	On ten random plants at milk stage	PLHT	Plant height (cm)	Quantitative	Length of stem from soil level to the flag leaf insertion
9	On ten random plants at milk stage	EHT	Ear height (cm)	Quantitative	Length of stem from soil level to uppermost ear insertion node.
10	On ten random plants at milk stage	SD	Stalk diameter (mm)	Quantitative	Diameter of stem at the second internode.
11	On ten random plants at milk tage	SG	Stay green (%)	Quantitative	Estimation of green/dead leaf area: (1=10% dead leaf area to 10=100% dead leaf area
12	On ten random plantsat harvest (Physiological maturity)	KA	Kernel arrangement on ear (score)	Qualitative	The predominant arrangement of kernels on an ear 1=regular, 2=irregular, 3=straight, and 4=spiral)
13	On ten random plantsat harvest (Physiological maturity)	EL	Ear length (cm)	Quantitative	Length of ear located on the highest insertion point
14	On ten random plantsat harvest (Physiological maturity)	EP	Ear position	Quantitative	Calculated as EHT divided by PLHT
15	On ten random plantsat harvest (Physiological maturity)	ED	Ear diameter (mm)	Quantitative	Diameter of ear located on the highest insertion point

16	On ten random plantsat harvest (Physiological maturity)	CC	Cob colour (score)	Qualitative	Colour of cob after shelling (0=red; 5=white)

Table 3.2 Agro-morphol ı genetic diversity study.

Table 3.2 cont"d.

17	On ten random plants at harvest (Physiological maturity)	CD	Cob diameter (mm)	Quantitative	Diameter of cobs

	Measurement Procedure	**Abbreviation**	**Phenotypic data (units)**	**Trait**	**Definition**
18	On ten random plants at harvest (Physiological maturity)	NRE	Number of rows per ear	Quantitative	Number of kernel rows around the cob at a height of 5 cm from the shank of uppermost ear
19	On ten random plants at harvest (Physiological maturity)	NKR	Number of kernels per row	Quantitative	Average number of kernels in two rows on opposite sides of cob
20	On ten random plants at harvest (Physiological maturity)	HKWT	100-kernel weight (g)	Quantitative	Mass of 100 kernels adjusted to 15 % moisture content
21	On plot basis after harvest	EN	Number of ears per plant	Quantitative	Number of ears per plant calculated as number of ears (NE) with at least one fully developed grain divided by NP
22	On plot basis after harvest	KTEX	Kernel texture (score)	Qualitative	The texture of the kernel on the basis of starch distribution (1=flint and 5=dent)
23	On plot basis after harvest	PGC	Principal grain colour (score)	Qualitative	The predominant colour of the kernels (0=white, 1=other colours)
24	On plot basis after harvest	KL	Kernel length (mm)	Quantitative	Length of kernel from the hilum to the base
25	On plot basis after harvest	KW	Kernel width (mm)	Quantitative	Width of kernel
26	On plot basis after harvest	KT	Kernel thickness (mm)	Quantitative	Thickness of the kernel
27	On plot basis after harvest	EWT	Ear weight (kg)	Quantitative	Mass of ten randomly selected ears
28	On plot basis after harvest	GWT	Shelled grain weight (g)	Quantitative	Mass of shelled grains from the ten randomly selected ears
29	On plot basis after harvest	YLD	Grain yield	Quantitative	Shelled grain weight per plot adjusted to 125 g/kg moisture and converted to Mg ha^{-1}

Table 3.3 Analysis of variance for obtaining estimates of variance from mean squares (MS).

Source	Df	MS	Expected Mean Square
Year	y-1	M_y	$\sigma_{2e} + r\sigma_{2gy} + g\sigma_{2r(y)} + rg\sigma_{2y}$
Rep (year)	y(r-1)	M_{ry}	$\sigma_{2e} + g\sigma_{2r(y)}$
Genotype	g-1	M_g	$\sigma_{2e} + r\sigma_{2gy} + ry\sigma_{2g}$
Genotype×Year	(y-1)(g-1)	M_{gy}	$\sigma_{2e} + r\sigma_{2gy}$
Error	y(g-1)(r-1)	M_e	σ_{2e}

where g, y and r are number of genotypes, number of years, and replicates, respectively.

σ^2_e = environmental variance component σ^2_g = genotypic variance component σ^2_y = variance component associated with year σ^2_{gy}= variance component associated with g × y $\sigma^2_e = M_e$ = environmental variance component $\sigma^2_g = M_g = (M_g-M_{gy})/ry$ = genotypic variance component σ^2_y = $\{(M_y + M_e)-(M_{ry}+M_{gy})\}/rg$ variance component associated with year $\sigma^2_{gy}= (M_{gy} –M_e)/r$ = variance component associated with g×y $\sigma_{2r(y)}= (M_{ry} - M_e)/g$

The approximate variance, V, associated with each variance component, $\hat{\sigma}_i^2$, was expressed as a linear function of independent mean squares as shown in equation 3.1 (Snedecor, 1956),

$$V(\hat{\sigma}_{i2}) = f_2 \, df_{i2i} M_{i22} \quad \text{..... (3.1)}$$

where, V = variance; f = is the coefficient of the component of variance; df_i = is the degrees of freedom of the respective mean squares; $\square_i = \square 1$; M_i = are the mean squares used to determine the variance components.

Broad sense heritability (H^2), defined as the proportion of the total variance due to genetic effects was estimated as:

$$H^2 = \frac{\sigma_2}{\sigma^2 + g\sigma / r_e} \quad \text{..... (3.2) (Doolittle, 1987).}$$

$$\text{Thus } H \square \frac{\square_g^2}{\square_p^2}$$

where $\square_g^2$ is the genotypic variance, and $\square_p^2$ is the phenotypic variance component calculated as:

$$\sigma^2_P \square \sigma^2_g + \sigma / r_e^2 \quad \text{..... (3.3)}$$

The standard error of this heritability (H^2) was approximated with the equation of Hallauer and Miranda (1981) as:

$$SE\ H^2 = \square\square\square\frac{\square}{SE(\sigma\sigma)^2_{P\ g^2}}\square\square\square \quad \text{..... (3.4)}$$

where $SE(\sigma)_g^2$ is the square root of the variance of $(\sigma)_g^2$ and the denominator is the phenotypic variance (Knapp *et al.*, 1985; Knapp 1986). The genotypic and phenotypic coefficients of variation were estimated as:

$$GCV = 100(\sigma_G / \bar{X}) \quad \text{..... (3.5)}$$

$$PCV = 100(\sigma_P / \bar{X}) \quad \text{..... (3.6)}$$

where σ_G and σ_P are the genotypic and phenotypic standard deviations, respectively, and $\bar{X}$ is the population mean of the trait under consideration. Means for each trait were then standardized to avoid the influence of scale of measurements in different traits on the data.

3.6 Genotypic and phenotypic correlation and their standard error

Genotypic and phenotypic correlations were calculated between pairs of traits by considering maize accessions as random effects. Using the genotypic variance and covariance component estimates, the genotypic correlation between traits i and j was estimated as:

$$r_{Gij} = \frac{\sigma_{Gij}}{\sigma_{GiGj}} \quad \text{..... (3.7),}$$

$$r_{Pij} = \frac{\sigma_{Pij}}{\sigma_{PiPj}} \quad \text{..... (3.8)}$$

where r_{Gij} and r_{Pij} are the genotypic and phenotypic correlation coefficients between trait i and j respectively; σ_{Gij} and σ_{Pij} are the estimated genotypic and phenotypic covariance between traits i and j, respectively; σ_{Gi}, σ_{Gj}, σ_{Pi}, and σ_{Pj} are the genotypic and phenotypic standard deviations for traits i and j, respectively. All computations were implemented using PROC MIXED option of SAS which uses the Restricted Maximum

Likelihood Estimation method to generate variance and covariance components, as well as correlations and standard errors (Holland, 2006).

3.7 Assessment of relationships between genotypes

3.7.1 Data Standardization

The agro-morphological data was standardized to remove effect of differences of scale before using in multivariate analysis.

3.7.2 Euclidean distance measurement and cluster analysis

Owing to differences in scale of measurement, th e genetic distance made of Pearson correlation coefficient which standardizes the data to remove the undesirable effects of differences in scale was most appropriate. Squared correlation coefficient was considered as genetic distance (Edwards, 1976). From the diagonal pairwise distance matrix was calculated the minimum, maximum, and average genetic distance for the population.

3.7.3 Cluster analysis

The Unweighted Pair Group Method with Arithmetic Average (UPGMA) cluster analysis was performed on the correlation distance matrix to identify groups with similar morpho-agronomic and phenotypic characters. This method identifies groups that are homogeneous as possible and heterogeneous among groups (Franco *et al*., 2001) or associations between the descriptors. Dendrograms were generated from the cluster analysis. Adjustment between the distance matrix and the dendrogram was estimated by the cophenetic correlation coefficient (Sokal and Rolf, 1962). All calculations and analyses were performed using the appropriate options of NTSYS pc

2.21 (Rohlf, 2009).

3.7.4 Bootstrapping

Bootstrapping was performed on cluster genotypes using PAST software to generate bootstrap values at a resampling of 1000 times to obtain confidence limits for significance of clustering.

3.7.5 Principal components analysis

Principal component analysis (PCA) was performed on the correlation matrix in order to depict non-hierarchical relationships among the genotypes and determine the traits that are most effective in discriminating between accessions.

Through singular value decomposition, correlation coefficients, eigenvalues and eigenvectors, and relative and cumulative proportions of the total variance expressed by each trait were calculated using the EIGEN program. Two dimensional biplots were generated from the principal components to reveal relationships between traits.
All computations were carried out using the NTSYS software (Rohlf, 2009).

3.8 Genetic diversity in maize by means of SSR fingerprinting

Genomic DNA was extracted from maize leaf tissue using the CTAB procedure (Saghai-Maroof *et al*., 1984) of the Applied Biotechnology Center‟s Manual of Laboratory Protocols of CIMMYT with minor modifications by the Cocoa Research Institute of Ghana.

From the field grown plants were harvested about one centimeter square of young leaves from 15 plants of each accession under sterile conditions, bulked and placed on ice and transported to the laboratory for storage at -80 ^{o}C until ready for use. Each bulked sample was ground into powder in liquid nitrogen. To 0.1 g of the bulked sample was added 700 ul of 2 % CTAB buffer (Appendix A) incubated for 30 min at 65 ^{o}C in a sand bath with intermittent vortexing.

The mixture was centrifuged at 14000 r.p.m. for 15 min and the supernatant transferred into clean microfuge tubes. To the tube was added 400 ul of ice cold isopropanol and centrifuged at 14000 r.p.m. for 5 min to pellet nucleic acids. Pellets were washed twice with 500 ul of washing buffer and 400 ul of 80 % ethanol, airdried and resuspended in 300 ul of TE buffer and incubated with RNase 10 ug/ml for 30 min. To the mixture was added 11.2 ml of 2 M NaCl and the DNA pellet washed with 70% ethanol, resuspended in TE buffer and DNA stored at -20 ^{o}C until required for primer amplification. The quality of DNA was assessed by electrophoresis on 1 % agarose gel. Preparations of reagents are presented in Appendix A.

3.8.1 SSR primer selection

One hundred SSR primer sets selected from the maize genetic database (http://www.maizegdb.orf/ssr.php) were assayed for their preliminary discriminatory power on 64 accessions. Forty-eight primers amplified products. Primers which did not amplify as well as those which did not produce variety of bands were excluded. Finally, sixteen primers were selected to cover all ten chromosomes and to have at least one representation of each of the oligonucleotides di- (25 %), tri- (25 %), tetra- (25 %),

penta- (12.5 %), and hexa- (12.5 %) repeats. Table 3.4 shows the names of primers, their bin numbers, type of repeat, and forward and reverse primer sequences.

Table 3.4 Primer sets indicating the chromosomal number, repeat sequence, and annealing temperature

	Marker	Chrom. No.	Bin	Repeat	Repeat unit	Sequence (F/R)
1	phi001	1 1	1.03	Di	AG/CT	TGACGGACGTGGATC AGCAGGCAGCAGGTC
2	phi056	1 1	1.01	Tri	CCG/CGG	ACTTGCTTGCCTGCCG CGCACACCACTTCCCA
3	phi109642	2 2	2.03	Tetra	ACGG	CTCTCTTTCCTTCCGA GAGCGAGCGAGAGAC
4	nc133	2 2	2.05	Penta	GTGTC	AATCAAACACACACC GCAAGGGAATAAGGT
5	umc1399	3 3	3.07	Tetra	(CTAG)5	GCTCTATGTTATTCTT GGTCGGTCGGTACTCT

6	phi046	3 3	3.0 8	Tetra	ACGC	ATCTCGCGAACGTGTC TCGATCTTTCCCGGAA
7	phi076	4 4	4.1 1	Hexa	AGCGGG	TTCTTCCGCGGCTTCA GCATCAGGACCCGCA
8	phi085	5 5	5.0 7	Penta	AACGC	AGCAGAACGGCAAGG TTTGGCACACCACGAC
9	bnlg1371	6 6	6.0 1	Di	AG(22)	TTGCCGATAAGAACC ACGACCGGTGTGGTT
10	dupssr13	7 7	7.0 4	Di	(CA)12	TCGTTCGGTCCATGAA CAAATATCTCTCATCT
11	umc1066	7 7	7.0 1	Hexa	(GCCAGA)5	ATGGAGCACGTCATC AGCAGCAGCAACGTC
12	phi233376	8 8	8.0 9	Tri	CCG	CCGGCAGTCGATTAC CGAGACCAAGAGAAC
13	phi100175	8 8	8.0 4	Tetra	AAGC	TATCTGACGAATCCC GTACGTAACGGACGG

14	umc1279	99	9.00	Tri	(CCT)6	GATGAGCTTGACGAC CAATCCAATCCGTTG
15	bnlg1525	99	9.07	Di	AG(25)	AGGAATTGCGAGTCT CAACCCCCAAAATGA
16	umc1677	1010	10.05	Tri	(GGC)4	TGCAGCAAGTTTGGC CTCTTGATGAAGTTGA

T_m=Annealing temperature

3.8.2 Amplification and detection of bands

To amplify the DNA, a 10 ul reaction mix was prepared. The reaction mix consisted of 20 ng each of forward and reverse primer, 1 unit of Taq DNA polymerase, 200 µM of dNTP, 1× reaction buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 100 ug ml^{-1} of gelatin, with pH adjusted to 8.3), 30 ng of template DNA and topped up with deionized water. The reactions were amplified in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). The following thermal cycle was used; denaturation step of 1 min at 96 ^oC, followed by a touchdown procedure which encompassed denaturation at 96 ^oC for 1 min, annealing at 65 ^oC for 1 min and extension at 72 ^oC for 2 min. The annealing temperature was then reduced at each cycle by 0.5 ^oC until a final annealing temperature of 55^oC was reached. The last cycle was repeated 20 times and terminated at 72 ^oC for final extension.

The reaction was stored. After the reaction, 20 ul of the reaction mix was heated at 96 ^oC for 2 min and placed on ice. To each of the amplification products were added 10 ul loading dye (50 % deionized formamide, 40 % glycerol, 20 mM EDTA, 0.6 mg ml^{-1} of bromophenol blue) and 15 µl of the mix and 1 kb DNA ladder (Bioneer, South Korea) were loaded on 2 % agarose gels stained with 5 ul ethidium bromide. Electrophoresis was run at 120 V for 2 h after which the gels were photographed under UV light (Geldoc, BIO-RAD Laboratories, Inc.).

3.9 Statistical analysis of molecular data

3.9.1 Allele scoring and data analysis

Gel photographs were examined and bands were scored in binary form as presence (1) or absence (0) (Ghosh *et al*., 1997). By ensuring a maximum of two alleles per locus care was taken to prevent mis-scoring arising from faint and stuttering bands. Primers and/or accessions that showed 15 % or more missing data were eliminated (Warburton *et al*., 2002).

3.9.2 Estimation of genetic diversity within populations

3.9.2.1 Rate of polymorphism

The number of polymorphic loci was divided by total number of loci (both polymorphic and monomorphic) to give the rate of polymorphism, *P*, as shown in Equation 2.1.

3.9.2.2 Average number of alleles per locus (A_P) or allele diversity

The average number of alleles per locus was calculated as total number of alleles in all loci divided by total number of polymorphic loci (Equation 2.2)

3.9.2.3 Polymorphic information content or average expected heterozygosity

For each SSR locus the observed heterozygosity was obtained by dividing the number of heterozygous by the number of genotypes scored. The PIC or expected heterozygosity under Hardy-Weinberg equilibrium was calculated from the formula

PIC $= 1 - \sum (P_i)^2$ where P_i is the proportion of the population carrying the *i*th allele (Botstein *et al*., 1980). The average PIC over all loci was calculated as the average expected heterozygosity.

3.9.3 Estimation of genetic diversity among populations

3.9.3.1 Genetic distance and cluster analysis

Using the DICE coefficient, genetic distance between pairs of accessions was calculated from $GD_{NL} = 1 - \frac{2X_{11}}{2X_{11} + X_{10} + X_{01}}$

The dissimilarity matrix was subjected to UPGMA cluster analysis using the SAHN option of NTSYS to generate a tree diagram expressing the inter -relationships among the genotypes. All computations were carried out using the NTSYS version 2.21c (Rohlf, 2009). Statistical significance of the tree generated was ascertained by bootstrap analysis (Felsenstein, 1985) at a resampling frequency of 1000 times. The PAST software of Hammer *et al*. (2001) was used.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Morphological Description of Qualitative Traits

In this research sixty-one maize landraces belonging to the IPGRI collection held in IITA Genetic Resource Center, Ibadan, were evaluated for genetic variation by morphological and molecular diversity studies. The genotypes had no information on passport data pertaining to their origin and collection sites. The study was conducted in the major rainy season of 2011 and 2012 at the Kwame Nkrumah University of Science and Technology Agricultural Experimental Station, Anwomaso, Kumasi, Ghana. Genetic diversity estimates by morphological evaluations were obtained from 3,660 plants on 5 qualitative and 24 quantitative traits.

4.1.1 Qualitative trait description

Ample variability in all qualitative traits was observed except for cob colour and principal grain colour. Table 4.1 shows variation among the accessions based on the 5 qualitative traits over all accessions. Variation in silk colour was minor with pale yellow silks being the most common trait (86 %) with few red silks (14 %). Kernel arrangement was the most variable, characterized by four categories of arrangement in a fairly equal distribution. Kernel texture exhibited a wide variation with almost equal proportions of dent (44.32 %) and flint (55.68 %) kernels. Majority of the kernels were mixed colours (94 %) (yellow, red, purple and blue) in varied arrangements (50 % regular, 50 % mixed pattern) borne on white cobs (98 %).

The kernel texture and colour exhibited by the IPGRI genotypes were not typical of the West African collections which are usually dominated by white and dent kernels arranged in a predominantly regular pattern. However, the almost equal distribution of dent and flint is in consonance with the general knowledge that maize in Africa was

introduced by the Portuguese who brought in white and dent types, whereas the flints and yellows were introduced by the Arabs across the Mediterranean (Matsuoka *et al*., 2002). Figure 4.1 shows variation in kernel arrangement of the IPGRI genotypes.

Table 4.1 Qualitative trait measurement on 61 maize landraces of the IPGRI collection held in IITA grown in Ghana from April to August 2011 and 2012

No.	Trait	Description	Class	No. of plants	Percentage (%)
1	Silk colour	Pale yellow	1	3,140	85.79
		Red	2	520	14.21
2	Kernel arrangement	Regular	1	1,860	50.82
		Irregular	2	767	20.96
		Straight	3	361	9.86
		Spiral	4	672	18.36
3	Cob colour	Red	0	75	2.05
		White	5	3,585	97.95
4	Kernel texture	Flint	1	2,038	55.68
		Dent	5	1,622	44.32
5	Principal grain colour	White	0	237	6.48
		Other colours	1	3,423	93.52

Figure 4.1 Variability among the IPGRI tropical maize collection in IITA Genetic Resource Center, Ibadan. White, yellow, blue, red and purple kernels in regular and irregular arrangement.

4.2 Means, standard deviation, range and mean squares of quantitative traits of the IPGRI maize landraces

The mean square results were significantly different ($P \leq 0.01$) for all traits evaluated except number of ears per plant (EN) (Table 4.2). The most important sources of variation were earliness, yield and yield components, and plant height. The significantly different mean square of each trait is indicative of large phenotypic variability among the accessions.

The coefficient of variation provides an estimate of phenotypic variability. A large CV ranging from 10.93 % for kernel width to 53.72 % for anthesis-silking interval was observed (Table 4.2). Ear characteristics and earliness traits except ASI exhibited variability of less than 15 %. Plant characteristics demonstrated phenotypic variability between 17 % and 25 %, whereas yield and yield component traits showed phenotypic variability ranging from 25 % to 52 %. A similar trend in phenotypic variability was

reported for ear related traits (8 % to 14 %), plant architectural traits (6 % to 20 %) and kernel related traits (4 % to 28 %) among Eastern Serbian maize landraces (KneževićJaric *et al*., 2010). On yield and yield related traits, the current study recorded higher percentage of phenotypic variability than that reported by ObengAntwi *et al*. (2011) of

12 % to 39 % in two maize landraces in Ghana. The wide variability among the genotypes indicates substantial geneti c diversity and the possibility of enhancing the traits through selection.

Table 4.2 Means, standard deviation, range, standard error, coefficient of variation and mean squares of the 60 IPGRI maize landraces held in IITA collection

used in the study and check („Obatanpa GH“)

	Trait	Mean	Standard Deviation	Minimum	Maximum	Standard error	Coefficient of variation (%)
1	AD (days)	54.8	6.2	39.0	74.0	0.10	11.36
2	SD (days)	57.6	6.3	44.0	78.0	0.10	10.96
3	ASI (days)	2.8	1.5	-2.0	9.0	0.02	53.72
4	TL (cm)	45.0	8.3	11.0	70.0	0.14	18.41
5	ELL (cm)	81.5	13.8	24.0	117.0	0.23	16.98
6	ELW (cm)	7.4	1.5	2.0	12.5	0.03	20.81
7	PLHT	191.5	47.0	43.0	325.0	0.79	24.53
8	EHT	97.2	35.4	7.0	325.0	0.6	36.45
9	EP	0.5	0.1	0.0	1.3	0	21.31
10	StD (mm)	19.9	3.6	7.0	35.0	0.06	17.98
11	SG (%)	69	22.1	7.14	100	0.37	32.34

12	EL (cm)	15.04	2.96	4.5	26.2	0.06	19.72
13	ED (mm)	37.2	6.6	15.0	55.5	0.11	17.74
14	CD (mm)	25.7	4.6	10.0	43.0	0.08	18.02
15	EN	1.0	0.2	1.0	3.0	0.00	15.57
16	NRE	13.9	2.0	6.0	22.0	0.03	14.15
17	NKR	28.6	7.2	5.0	50.0	0.12	25.05
18	HKWT (g)	52.2	13.6	13.1	117.2	0.23	26.11
19	KL (mm)	8.5	1.2	5.0	12.8	0.02	14.19
20	KW (mm)	8.4	0.9	3.5	12.4	0.02	10.93

21	KT (mm)	4.6	0.9	3.0	9.0	0.01	18.71
22	EWT (kg)	0.1	0.1	0.01	1.2	0.00	51.92
23	GWT (kg)	0.7	0.3	0.1	1.6	0.01	44.73
24	YLD ($Mgha^{-1}$)	3.8	1.7	0.5	8.9	0.03	44.71

Acc = accession; AD = days to 50 % anthesis; SD = days to 50 % silking; ASI = anthesis to silking interval; TL = tassel length; ELL = ear leaf length; ELW = ear leaf width; PLHT = plant height; EHT = ear height; StD = stalk diameter; SG = stay green; EL = ear length; EP = ear position; ED = ear diameter; CD = cob diameter; NRE = number of rows per ear; NKR = number of kernels per row; HKWT = hundred kernel weight; EN = ear number per plant; KL = kernel length; KW = kernel width; KT = kernel thickness; EWT = ear weight; GWT = grain weight; YLD = grain yield; **P<0.01; ***P<0.001.

4.2.1 Earliness in the IPGRI landraces

Earliness was characterized by a wide variability supported by large mean squares and large coefficient of variation (Table 4.2). Number of days to anthesis was characterized by very early and extremely wide range of maturity spanning 39 days for TZm-1150 to a maximum of 74 days for TZm-1112 with an overall mean of 54.8 ± 6.2 days. Other reports of days to anthesis in maize are a minimum of 46 days and a maximum of 56 days with a mean of 51.3 days for Ghana landraces (Obeng-Antwi *et al*., 2011), 47 days to 74 days with a mean of 60.33 days for inbred lines originating from Nepal (Shrestha, 2013), 58 days to 81 days with a mean of 72 days for traditional Ethiopia highland maize (Beyene *et al*., 2006), 81 days to 94 days with a mean of 86 days for maize inbred lines originating from Bangladesh (Azad *et al*., 2012).

Similarly, number of days to silking demonstrated very early maturity ranging from 44 days for TZm-1148, TZm-1149, TZm-1150 and TZm-1215, to 78 days for TZm1112 and TZm-1132 with a mean of 57.6 ± 6.3 days (Table 4.2). Reports from other works in maize on number of days to silking are a minimum of 58 days to a maximum 80.5 days with a mean of 71.5 days for Ethiopian highland maize (Beyene *et al*., 2006). Maize inbred lines originating from Nepal exhibited a minimum of 50 days to maximum of 77 days to silking with a mean of 63.8 ± 7.1 days (Shrestha, 2013), while among Ghana maize landraces silking date occurred between 50 days to a maximum of 60 days with a mean of 55.3 days (Obeng-Antwi *et al*., 2011). Earliness in maize is desirable for breeding genotypes that are drought-tolerant as they can escape the short rainfall season typical of sub-Saharan Africa.

Compared to the check, „Obatanpa GH" whose mean number of days to anthesis was 48.8, six genotypes outperformed the check in earliness by exhibiting fewer mean number of days to anthesis and silking. The genotypes and their respective AD and SD values were TZm-1149 (44.0 and 45.8 days), TZm-1148 (45.5 and 49.3 days), TZm-1150 (45.8 and 48.2 days), TZm-1157 (47.6 and 50.0 days), TZm-1153 (47.6and 51.3 days) and TZm-1152 (48.2 and 51.8 days) (Table 4.3A). These extraearly genotypes may be good genetic resource for breeding for earliness.

A highly significant variation ($P<0.001$) in anthesis-to-silking interval (ASI) among all accessions was detected. This variation was supported by a large coefficient of variation (54 %) (Table 4.2). The ASI values, like the anthesis and silking days demonstrated a wide range of a minimum of -2 days for TZm-1106 to a maximum of 9 days for

TZm1132 and an overall mean of 2.8 ± 1.5 days. In all 19 individual plants showed protogyny in which nine plants of TZm-1106 and ten plants of TZm1183 had silk development earlier than anthesis by two days and one day, respectively. One hundred and forty individual plants had ASI of 0 days. ObengAntwi *et al*. (2012) on the other hand observed a large mean ASI of 5.9 ± 1.0 days with a much narrower ASI of a minimum of 3.8 days to a maximum of 8.3 days in maize landraces in Ghana.

On accession mean basis, mean ASI ranged from a least of 1.2 for TZm-1188 to 4.5 days for TZm-1128. In all, 53 accessions exhibited short ASI (1.2 to 3.5 days) than the check (3.7 days) (Table 4.3A). An ASI period of 2-4 days is considered ideal for drought tolerance (Dass *et al*., 2001). Although maize is predominantly protandrous, infrequently, under optimum environmental conditions in which plants are vigorous, some genotypes may demonstrate protogyny. It is worthy of note that the wild relatives, as well as hybrids between the progenitor teosintes and cultivated maize are by and large protogynous (Magoja and Pischedda, 1984). The occurrence of this ancestral trait in the landraces portrays that further studies are required to evaluate the maize collection for the origin of the maize.

4.2.2 Plant characteristics

Mean square results for all plant architectural traits were highly significant. Plant height (mean square of 3,429.7; P<0.001) and ear height (mean square of 2,311.6; P<0.001) were the most important traits in terms of variability. A large coefficient of variation 24.53 % and 36.45 % for plant height and ear height, respectively, demonstrates ample variability which can be harnessed for crop improvement (Table

4.2). Plant height ranged from a minimum of 43 cm for TZm-1148 to a maximum of 325 cm for TZm-1132 with a mean of 191.54 ± 47.0 cm (Table 4.2). On accession mean

basis the least plant height of 125.9 cm was in TZm-1149 to the tallest accession TZm1111 having mean height of 236.1 (Table 4.3A). The mean plant height of the check was 171.3 cm.

Similar maize plant heights of 96.5 cm to 171.1 cm with a mean of 135.60 ± 19.85cm (Ranatunga *et al*., 2009), 161.0 cm to 288.0 cm and a mean plant height of 217.8 ± 14.4cm among Ethiopian highland maize accessions (Beyene *et al*., 2006), and 110.0 cm to 215.0 cm with a mean of 166.0 ± 27.43 cm among Italian genotypes (Hartings *et al*., 2008) are reported. Fourteen plants of six genotypes had some individual plants heights exceeding 300 cm. These are TZm-1101, TZm-1111, TZm-1128, TZm-1132,

TZm-1183, and TZm -87. These genotypes demonstrated mean heights of 203 cm to 236 cm (Table 4.3A). Four genotypes were very short and exhibited mean heights lower than 150 cm. These are TZm-1149 (125.9 cm), TZm-1147(141.2 cm), and TZm-1148 (143.6 cm), and TZm-1150 (144.2 cm). Very tall maize plants with thin and long basal internodes are at high risk of lodging (Remison and Dele, 1978).

No.	Acc	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	EL
1	Oba	8.8 (45-53) 3.0	52.5 (48-56) 3.0	3.7 (3-6) 1.1	47.8 (29-65) 7.1	79.7 (45-105) 15.5	8.3 (4-11) 1.6	171.3 (75-249) 35.3	77.7 (27-132) 24.6	17.6 (9-21) 2.2
2	Tzm-1097	55.4 (50-62) 4.8	58.7 (55-64) 3.7	3.2 (1-5) 1.4	51.0 (40-70) 6.1	90.4 (70-105) 7.8	6.8 (4-9) 1.4	199.5 (137-294) 33.8	92.8 (50-151) 22.1	16.9 (11-22) 2.6
3	Tzm-1099	55.8 (51-61) 3.5	59.2 (56-68) 3.9	3.4 (1-7) 1.9	41.3 (21-55) 9.2	75.6 (40-104) 16.2	6.5 (3-9) 1.4	181.6 (99-260) 48.5	99.3 (47-153) 32.4	12.2 (8-16) 2.8
4	Tzm-1100	58.0 (54-65) 4.1	60.2 (56-67) 3.7	2.2 (1-4) 0.9	44.8 (24-65) 9.0	90.0 (67-111) 11.2	7.0 (4-11) 1.4	223.0 (163-295) 38.4	115.9 (65-160) 30.9	17.4 (13-26) 2.2
5	Tzm-1101	59.8 (49-67) 6.5	62.7 (52-70) 6.5	2.8 (1-4) 1.1	45.7 (27-60) 7.2	81.7 (60-108) 10.9	7.2 (5-11) 1.5	211.1 (139-307) 42.7	113.7 (51-170) 32.1	15.2 (12-19) 1.5
6	Tzm-1103	54.0 (49-58) 4.1	56.7 (51-64) 4.6	2.7 (0-6) 1.9	48.0 (30-61) 7.2	84.0 (62-105) 10.2	7.2 (4-10) 1.4	197.9 (110-258) 41.2	97.3 (42-146) 29.2	14.9 (10-19) 1.7
7	Tzm-1105	56.5 (52-61) 3.7	59.2 (55-62) 3.0	2.7 (1-4) 1.0	52.7 (40-65) 5.0	92.4 (62-108) 7.8	6.7 (5-9) 1.0	219.7 (157-275) 26.3	110.0 (75-142) 19.0	14.3 (8-20) 2.6
8	Tzm-1106	53.9 (48-60) 4.5	55.3 (50-64) 4.6	1.4 (-2-4) 2.0	40.1 (24-60) 8.5	69.4 (24-102) 21.2	6.8 (4-10) 1.6	159.5 (87-275) 57.7	73.4 (25-165) 41.3	15.0 (8-19) 2.5
9	Tzm-1108	55.0 (51-58) 3.1	57.0 (54-60) 1.9	2.0 (0-5) 1.7	48.8 (25-70) 8.7	86.6 (62-104) 8.7	7.3 (5-10) 1.2	199.8 (63-263) 38.6	97.3 (55-140) 21.5	13.6 (9-19) 2.2
10	Tzm-1109	49.6 (46-54) 3.2	52.9 (49-57) 3.1	3.4 (2-4) 0.7	43.1 (30-60) 7.1	76.4 (50-102) 11.6	6.9 (4-9) 1.3	174.6 (86-243) 37.5	79.0 (21-151) 31.3	15.6 (8-21) 3.4
11	Tzm-1110	54.0 (51-59) 3.3	56.5 (53-60) 3.0	2.5 (1-5) 1.2	49.9 (30-73) 8.6	87.0 (49-112) 13.3	7.4 (4-11) 1.5	199.6 (102-295) 43.2	104.1 (35-170) 33.1	15.6 (7-21) 2.6
12	Tzm-1111	59.6 (57-63) 2.5	63.0 (59-67) 3.1	3.4 (2-5) 1.0	47.2 (30-65) 9.2	92.3 (70-117) 10.0	8.5 (5-12) 1.4	236.1 (109-312) 46.4	134.1 (50-202) 36.8	15.4 (10-22) 2.7
13	Tzm-1112	54.7 (48-74) 8.3	58.2 (50-78) 8.6	3.5 (2-5) 1.0	45.6 (26-65) 10.0	79.2 (35-105) 18.7	8.3 (4-76) 9.2	172.5 (64-255) 55.2	86.3 (25-155) 33.5	16.1 (10-23) 2.7
14	Tzm-1114	50.6 (45-59) 4.5	52.8 (48-60) 3.8	2.2 (1-3) 0.9	44.4 (25-60) 7.9	78.6 (25-101) 13.3	7.0 (3-10) 1.2	174.5 (119-232) 29.5	82.3 (35-135) 20.5	14.6 (10-19) 2.3
15	Tzm-1117	59.2 (54-65) 3.6	62.3 (57-68) 3.3	3.2 (2-5) 1.1	44.4 (28-60) 7.6	83.3 (57-102) 13.2	6.8 (4-9) 1.0	196.0 (118-270) 47.7	109.3 (55-180) 34.8	16.4 (11-25) 3.4
16	Tzm-1118	53.8 (49-60) 4.6	55.2 (51-62) 4.0	1.4 (0-3) 1.1	44.8 (30-63) 5.7	82.7 (55-105) 10.3	7.4 (5-13) 1.2	199.1 (113-265) 30.1	105.6 (69-140) 18.1	15.0 (12-20) 2.2
17	Tzm-1119	55.7 (52-60) 3.3	58.6 (54-64) 3.9	2.8 (2-5) 1.1	47.9 (35-70) 7.1	87.6 (70-103) 7.4	7.5 (6-11) 1.1	214.4 (116-295) 46.5	110.4 (55-170) 32.4	16.2 (12-20) 2.2
18	Tzm-1120	58.6 (53-64) 4.6	60.5 (56-65) 3.7	1.8 (1-4) 1.1	46.4 (34-57) 6.7	87.9 (60-110) 12.0	7.6 (4-10) 1.6	193.7 (130-280) 46.4	94.3 (35-175) 41.8	16.0 (12-20) 2.5
19	Tzm-1121	59.8 (53-72) 5.8	63.1 (58-75) 5.4	3.4 (3-5) 0.8	47.1 (30-60) 7.6	84.1 (64-96) 7.9	7.7 (5-10) 1.2	201.9 (154-255) 27.3	106.2 (78-147) 17.7	15.7 (11-18) 2.1
20	Tzm-1122	55.5 (51-60) 3.9	58.2 (54-62) 3.3	2.7 (2-4) 0.8	46.0 (12-58) 7.0	82.0 (55-94) 7.1	7.9 (5-10) 1.0	197.6 (94-298) 38.0	103.4 (50-150) 27.4	15.8 (11-19) 1.8
21	Tzm-1123	59.6 (52-68) 6.5	61.7 (55-71) 6.2	2.0 (0-3) 1.1	44.4 (27-60) 8.6	82.5 (55-100) 9.0	7.7 (6-12) 1.1	191.7 (115-251) 32.2	91.6 (40-136) 27.3	16.2 (12-21) 1.9
22	Tzm-1125	56.6 (50-66) 5.3	58.5 (53-68) 4.9	1.9 (0-3) 1.0	47.4 (32-62) 6.8	88.1 (60-105) 10.1	7.6 (5-10) 1.2	201.3 (84-290) 49.8	106.7 (35-187) 30.5	15.6 (10-20) 2.4

Table 4.3A Means, minimum PGRI maize landraces held

in IITA collection and a check „Obatanpa GH“.

23	Tzm-1126	54.9 (49-62) 4.8	57.1 (51-65) 4.7	2.2 (0-3) 1.1	52.8 (35-70) 6.4	85.7 (55-117) 13.2	7.4 (6-10) 1.1	204.8 (130-285) 40.3	96.9 (45-154) 23.9	13.9 (10-18) 2.7

No.	Acc	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	EL
24	Tzm-1128	64.5 (59-72) 4.9	69.1 (63-76) 4.7	4.5 (3-6) 1.3	41.0 (20-59) 9.3	88.3 (61-112) 10.9	8.4 (6-12) 1.4	231.5 (110-315) 58.2	139.0 (49-280) 44.4	15.7 (11-22) 2.1
25	Tzm-1129	53.8 (50-60) 3.9	56.5 (53-62) 3.4	2.7 (2-3) 0.5	46.5 (30-65) 8.5	81.4 (61-104) 10.2	7.2 (4-11) 1.4	171.6 (80-262) 49.3	85.1 (20-152) 38.4	14.8 (9-19) 1.8
26	Tzm-1130	58.8 (54-65) 4.1	62.2 (56-68) 5.0	3.3 (2-6) 1.4	45.2 (30-61) 7.9	83.6 (60-110) 13.4	7.3 (4-10) 1.9	193.9 (115-290) 49.8	98.5 (40-186) 37.7	16.0 (11-23) 3.1
27	Tzm-1131	52.2 (47-58) 3.2	55.6 (51-60) 2.9	3.4 (2-5) 0.9	46.1 (30-60) 6.7	80.6 (35-100) 12.1	7.0 (3-9) 1.3	193.3 (115-265) 40.3	93.1 (30-145) 31.0	14.6 (11-19) 2.1
28	Tzm-1132	69.7 (65-73) 3.4	73.6 (68-78) 3.6	3.9 (1-9) 2.5	44.1 (30-60) 6.0	88.4 (69-107) 8.9	9.4 (7-12) 1.0	233.1 (153-325) 35.7	160.7 (70-325) 46.0	17.4 (11-23) 1.9
29	Tzm-1136	56.9 (53-60) 3.1	60.0 (57-65) 2.8	3.0 (0-5) 1.7	41.9 (28-55) 6.1	84.8 (59-102) 9.7	8.9 (7-12) 1.5	198.7 (143-280) 27.4	96.7 (55-164) 22.1	14.6 (8-21) 3.2
30	Tzm-1137	50.3 (46-54) 3.2	53.8 (51-58) 2.7	3.5 (2-5) 1.3	45.0 (16-70) 9.0	77.4 (46-96) 10.9	7.6 (5-10) 1.2	174.6 (70-230) 31.2	91.0 (31-134) 19.6	13.8 (8-21) 3.3
31	Tzm-1138	56.4 (51-61) 4.1	59.3 (54-65) 4.0	2.8 (2-4) 0.7	47.3 (27-62) 8.5	88.3 (65-105) 10.0	7.5 (4-10) 1.5	211.8 (123-270) 40.8	112.1 (35-155) 33.4	17.9 (11-23) 2.5
32	Tzm-1139	54.6 (46-68) 8.4	57.9 (51-74) 8.6	3.3 (1-6) 1.7	48.7 (30-62) 7.2	83.8 (55-105) 12.2	7.8 (6-11) 1.3	185.5 (121-270) 41.6	81.5 (40-122) 25.5	17.3 (12-24) 2.6
33	Tzm-1141	55.3 (51-58) 2.7	58.8 (54-62) 2.7	3.5 (2-5) 1.0	45.9 (30-65) 6.4	80.5 (50-100) 9.0	8.2 (5-10) 1.1	202.2 (101-287) 38.7	113.5 (55-171) 28.9	13.9 (6-20) 3.4
34	Tzm-1142	52.4 (50-58) 3.1	54.5 (52-58) 2.4	2.1 (0-4) 1.3	47.9 (29-63) 7.1	87.1 (58-105) 10.8	8.6 (5-75) 9.9	211.7 (118-270) 31.9	100.9 (60-160) 20.7	14.5 (11-18) 2.2
35	Tzm-1143	54.4 (50-60) 3.5	56.4 (52-62) 3.4	2.0 (1-3) 0.6	43.1 (28-60) 9.5	80.8 (49-100) 13.3	7.3 (5-11) 1.7	190.1 (140-270) 38.6	89.4 (50-160) 28.3	15.9 (11-44) 3.6
36	Tzm-1144	49.2 (46-58) 4.2	51.8 (49-60) 3.7	2.7 (1-4) 1.0	37.7 (21-60) 11.7	69.7 (30-101) 20.1	6.9 (2-10) 2.0	162.7 (60-225) 41.2	77.1 (37-120) 24.5	13.8 (8-19) 3.4
37	Tzm-1145	59.3 (54-66) 4.0	63.8 (58-74) 5.2	4.5 (3-8) 1.8	45.7 (30-57) 5.5	83.7 (69-101) 8.3	7.2 (6-11) 1.0	230.9 (150-300) 41.7	132.5 (80-193) 30.8	16.9 (7-25) 4.3
38	Tzm-1147	47.6 (44-50) 2.5	50.0 (46-52) 2.3	2.4 (1-5) 1.3	40.1 (25-60) 8.0	66.7 (26-100) 16.7	6.3 (3-10) 1.3	141.2 (81-226) 31.8	59.6 (15-11424.3)	12.2 (7-20) 3.3
39	Tzm-1148	45.5 (43-47) 1.3	49.3 (44-52) 2.7	3.8 (1-6) 1.8	37.9 (11-56) 10.5	65.4 (34-86) 14.5	6.3 (3-10) 1.6	143.6 (43-223) 47.1	62.6 (8-128) 29.7	11.8 (5-15) 2.8
40	Tzm-1149	44.0 (41-46) 1.8	45.8 (44-48) 1.7	1.8 (1-4) 1.1	38.1 (19-54) 7.5	69.3 (41-102) 14.3	6.9 (3-11) 1.7	125.9 (52-208) 40.9	55.0 (19-102) 25.3	12.6 (7-19) 3.2
41	Tzm-1150	45.8 (39-51) 4.0	48.2 (44-54) 3.4	2.3 (1-5) 1.5	40.7 (15-55) 8.1	67.1 (37-102) 17.2	6.1 (3-10) 1.9	144.2 (52-241) 46.9	62.3 (10-120) 31.4	12.8 (5-19) 3.3
42	Tzm-1151	50.2 (46-53) 2.2	53.7 (51-57) 1.9	3.4 (1-7) 1.8	45.2 (30-60) 7.4	79.2 (47-102) 12.8	7.6 (3-10) 1.6	173.9 (83-292) 47.4	78.1 (25-140) 32.5	13.9 (10-19) 2.1

43	Tzm-1152	48.2 (46-53) 2.6	51.8 (48-58) 4.2	3.7 (2-7) 1.8	42.5 (23-62) 9.6	73.9 (40-96) 16.6	6.5 (3-10) 1.9	156.1 (52-217) 43.4	69.2 (20-125) 31.8	13.1 (9-15) 1.8
44	Tzm-1153	47.6 (44-53) 2.9	51.3 (49-57) 2.7	3.7 (2-5) 1.1	43.8 (25-55) 7.1	73.1 (40-101) 12.7	7.4 (3-11) 1.5	149.8 (67-222) 32.9	67.3 (17-124) 24.2	12.2 (6-19) 2.6

Table 4.3A cont‟d

45	Tzm-1156	49.5 (47-55) 2.6	52.5 (51-56) 1.6	3.1 (1-5) 1.3	47.7 (18-65) 8.1	82.2 (50-105) 10.8	7.8 (5-10) 1.1	190.8 (114-260) 39.5	100.1 (50-160) 34.0	15.3 (12-19) 1.8
46	Tzm-1180	55.3 (51-61) 4.1	58.0 (54-65) 4.1	2.7 (1-4) 1.0	45.5 (21-60) 7.5	77.4 (30-98) 16.0	7.2 (2-10) 1.6	187.9 (55-270) 47.5	92.8 (30-150) 27.7	13.8 (11-20) 1.9

No.	Acc	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	EL
47	Tzm-1182	54.6 (50-60) 4.3	57.5 (53-63) 3.5	2.9 (0-5) 1.6	49.4 (35-60) 6.1	82.6 (58-96) 8.3	7.4 (3-9) 1.3	200.5 (115-275) 38.0	101.8 (34-145) 29.1	14.[1] (10-19) 1.8
48	Tzm-1183	56.7 (52-61) 3.6	58.0 (54-62) 3.0	1.3 (-1-3) 1.3	44.3 (25-60) 8.1	81.8 (37-102) 12.8	8.2 (4-11) 1.4	217.5 (109-310) 38.0	114.9 (49-170) 27.6	15.8 (11-26) 2.8
49	Tzm-1184	55.6 (49-62) 5.2	57.5 (52-65) 5.0	2.0 (1-3) 0.8	47.7 (35-60) 5.4	89.0 (63-100) 7.7	7.5 (5-10) 1.0	208.3 (94-280) 37.0	104.8 (61-152) 21.3	14.9 (9-20) 2.6
50	Tzm-1185	58.1 (54-61) 2.7	60.8 (58-64) 2.4	2.7 (2-4) 0.8	45.7 (30-65) 7.4	85.2 (62-103) 7.9	8.3 (6-11) 1.2	208.7 (138-295) 39.9	117.4 (59-180) 28.9	16.6 (12-23) 2.6
51	Tzm-1187	59.0 (53-65) 4.5	62.0 (57-68) 4.2	3.0 (2-4) 0.6	44.3 (30-60) 6.6	83.1 (60-103) 8.1	7.5 (5-10) 1.3	203.0 (89-315) 44.6	111.0 (12-180) 37.8	17.1 (13-20) 1.5
52	Tzm-1188	52.7 (47-59) 4.6	53.9 (49-60) 4.3	1.2 (0-2) 0.7	47.2 (35-60) 5.3	88.8 (60-110) 10.1	7.4 (5-11) 1.2	202.8 (141-268) 29.7	99.7 (58-155) 19.7	14.8 (11-20) 2.1
53	Tzm-1190	56.2 (49-63) 5.6	58.2 (51-65) 5.0	2.0 (0-4) 1.2	44.6 (28-57) 6.4	89.4 (71-104) 7.2	7.6 (5-10) 1.1	215.2 (125-274) 33.7	122.7 (58-185) 31.8	17.2 (8-23) 3.8
54	Tzm-1193	62.4(59-69) 3.5	66.3 (62-74) 3.8	3.8 (2-7) 1.8	46.1 (38-55) 5.5	87.2 (75-102) 9.3	8.2 (6-11) 1.4	211.9 (143-275) 43.7	118.1 (70-168) 29.6	15.9 (11-25) 3.9
55	Tzm-1194	54.9 (50-61) 4.4	57.2 (52-64) 4.1	2.3 (1-5) 1.4	44.5 (22-60) 7.5	79.3 (55-95) 9.8	7.4 (5-10) 1.2	186.6 (74-245) 45.9	96.7 (30-163) 30.7	13.7 (10-18) 2.1
56	Tzm-1195	51.6 (46-58) 4.5	54.4 (48-62) 4.7	2.8 (1-4) 1.1	43.8 (29-68) 8.7	80.7 (7-103) 16.0	7.0 (3-11) 1.3	174.7 (107-245) 35.6	85.2 (7-132) 27.6	13.9 (9-18) 2.2
57	Tzm-1211	54.5 (48-59) 4.1	57.5 (51-64) 4.2	3.0 (1-5) 1.2	43.3 (27-57) 8.7	83.2 (50-103) 12.3	7.7 (6-9) 0.8	204.2 (121-265) 31.6	98.3 (60-13417.6)	15.4 (13-20) 1.9
58	Tzm-1212	56.8 (53-62) 3.1	60.2 (56-64) 3.0	3.4 (2-5) 0.9	44.1 (28-60) 7.3	77.1 (50-100) 11.1	7.7 (6-10) 1.0	188.9 (130-273) 39.2	106.7 (70-170) 27.8	16.7 (10-19) 2.4
59	Tzm-1213	54.6 (50-61) 4.0	57.6 (52-67) 5.0	3.1 (1-6) 1.5	43.9 (30-60) 6.5	79.4 (63-95) 7.7	6.8 (4-10) 1.4	186.2 (112-252) 37.3	88.3 (9-155) 29.8	15.1 (12-19) 1.8
60	Tzm-1214	57.3 (54-62) 3.3	59.9 (57-64) 2.7	2.6 (1-4) 1.1	44.4 (30-55) 6.9	86.5 (60-99) 7.3	7.8 (5-11) 1.5	211.5 (120-280) 32.1	106.1 (70-164) 24.0	15.1 (11-22) 2.6
61	Tzm-1215	49.7 (43-54) 4.1	51.6 (44-59) 4.7	1.9 (0-5) 1.7	39.6 (21-56) 8.2	63.4 (29-95) 17.8	5.7 (3-10) 2.0	152.4 (80-245) 43.1	67.5 (15-120) 28.2	13.7 (9-24) 3.2

[1] AD= anthesis date; SD = silking date; ASI = anthesis to silk interval date TL = tassel length; ELL = ear leaf length; ELW = ear leaf width PLHT = plant height; EHT = ear height; StD = stalk diameter; EL = ear length

Table 4.3A cont"d

Mean square for ear number per plant (EN) was not significant, however, ample phenotypic variability (CV= 15.57 %) was recorded from plant to plant. Mean ear number was 1.0 ± 0.2 with a range of 1 to 3 (Table 4.2). In all, 76 plants (2 %) of TZm1097, TZm-1183, and TZm-1184 frequently produced 2 to 3 ears in both seasons while the check genotype, „Obatanpa GH" consistently produced one ear per plant. Azad *et al*. (2012) reported a minimum of 1.0 to a maximum of 1.3 ears per plant with a mean of 1.09 for maize inbred lines originating from Bangladesh. Prolificacy in maize is a desirable trait which has high correlation with yield (Stuber *et al*., 1966; Goodman, 1965; Robinson *et al*., 1951). Harris *et al*. (1976) demonstrated that prolific plants produced more grain than non prolific plants. The genotypes in current study which demonstrated extra ear number would be useful to breeders as good genetic material for yield improvement based on prolificacy.

On tassel length, a minimum of 11 cm observed in TZm-1148 and a maximum of 70 cm which occurred in seven accessions, namely, TZm-1126, TZm-1108, TZm-1110, TZm-1119, TZm-1100, TZm-1137, and TZm-1097 with an overall mean of 45±8.2 cm. Mean TL of accessions was high and ranged from 37.7±11.7 cm in TZm-1144 to 52.8±6.4 cm in TZm-1126 (Table 4.3A). The IPGRI accessions were similar to the traditional European maize inbred populations which reportedly exhibited tassel lengths ranging from 35.5 cm to 67.5 cm with a mean of 56.0 cm (Rebourg *et al*., 2001). Conversely, Hartings *et al*. (2008) reported mean tassel length of 20.2±3.42 cm and a range as low as 13.0 cm to a highest value of 28.0 cm for Italian landraces. Mean tassel length of 37.09 cm was recorded for inbred lines originating from
Rampur, Chitwan, and Nepal (Shrestha, 2013).

Tassel traits are important characters that influence yield either physiologically by competing for photosynthates or physically by a shading effect (Gue and Wasson, 1996). It is generally known that high yielding plants produce large tassels and vice versa. Therefore in hybrid breeding programmes an ideal male parent is supposed to have large tassels that can produce large amount of pollen, whereas an ideal female plant should have smaller tassels to permit partitioning of carbohydrates for increased ear size (Upadyayula *et al*., 2006). Indeed, the continuous yield enhancement in hybrid maize in the U.S. Corn belt following the green revolution era from 1930s to 2009 has been accompanied by continuous decline in tassel size (Hammer *et al*., 2009; Duvick *et al*., 2004; Duvick and Cassman, 1999). Further work is required to ascertain an equivalent mechanism in tassel size transformation among the landraces that may be incorporated into breeding programmes for hybrid development.

The ear leaf length, ear leaf width, and stalk diameter of the maize accessions showed highly significant ($P<0.001$) mean squares and CV of 16.98 %, 20.81 %, 17.98 %, respectively, such that these variabilities in ELL, ELW, and StD were deemed to be abundant. On plant to plant basis, the ELL range of 24 cm (TZm-1106) to 117 cm (TZm-1126 and TZm-1111) with an overall mean of 81±13.8 cm, ELW range of values and overall means of 2.0 cm (TZm-1144) to 12.5 cm (TZm-1118) and a mean of 7.4±1.5 cm, and StD of 7.0 mm (TZm-1215) to 35.0 mm (TZm-1136) with a mean of 19.9 ±3.6 (Table 4.2) were recorded.

Among the accessions, TZm-1215 had the shortest mean ELL, ELW, and StD (63.4 cm, 5.7 cm, 15.8 mm). The accession with longest mean ELL was TZm-1105 (92.4 cm), while TZm-1132 exhibited the widest ELW (9.4 cm). For stalk diameter, mean

values were lowest (15.8 mm in TZm-1215) and highest (27.8 mm) in TZm-1132. Beyene *et al*. (2006) reported similar values of mean ELL of 71.3 cm with a minimum of 51.8 cm and a maximum of 100.8 cm among Ethiopian highland maize. ObengAntwi *et al*. (2011) reported mean ELL of 78.1 ± 5.3 cm with a minimum of 60.0 cm and maximum of 90 cm in maize landraces in Ghana.

Other reports of ear leaf width are a mean of 9.8 cm in maize landraces in Eastern Serbia (Knežević-Jaric *et al*., 2010), and a mean of 7.7 ± 0.8 cm, ranging from 6.1 cm to 9.6 cm among maize landraces in Ghana (Obeng-Antwi *et al*., 2012, 2011). The unusually low values of ear leaf length and width in some of the plants may have resulted from environmental mishaps experienced on the rear side of the field during the first growing season.

The most important leaf on maize plant with regard to yield in grain, silage and biomass is the ear leaf, yet, there are hardly any reports on association between ear leaf dimensions and grain yield. Zheng and Lu (2013) reported on nine QTLs and seven QTLs in ear leaf length, ear leaf width and ear leaf area under low and high nitrogen conditions in maize, respectively.

The relationship between ELL and yield was evaluated by correlation analysis. A strong positive significant correlation with ear length ($r=0.83$; $P<0.001$) along with moderate positive significant correlation with number of kernels per row ($r=0.44$; $P=0.005$) were detected. Additionally, ELW showed moderate positive significant correlation with ear length ($r=0.59$; $P<0.001$). The analysis reveals that 19 % variation in number of kernels per row and 69 % variation in ear length are explained by ELL, whereas ELW explained

35 % variation in ear length. The positive correlations demonstrate that longer and wider ear leaf lead to increase in yield component traits probably due to greater accumulation of photosynthates that contribute to immediate supply of dry matter to the developing ear (Richards, 2000).

4.2.3 Ear characteristics

The traits, ear length, ear diameter, cob diameter, number of ears per plant, number of rows per ear, number of kernels per row, and ear weight constitute ear characteristics. Analysis of variance revealed highly significant differences ($P<0.001$) among the genotypes for all ear traits except number of ears per plant. In addition, high coefficient of variation of 14.15 to 51.92 % was detected for these traits (Table 4.2). Ear length varied from a minimum of 4.5 cm in TZm-1148, and TZm-1150 to a maximum of 26.2 cm in TZm-1100, and a mean of 15.04 ± 2.96 cm. In comparison to values of ear length reported by Hartings *et al*. (2008), Beyene *et al.* (2006), and Knežević-Jaric *et al*. (2010) who had mean values of 16.9 ± 2.87 cm with minimum of 12.0 cm and maximum of 24.0 cm, mean of 18.1± 2.2 cm with minimum of 14.5 cm and maximum of 22.7 cm, respectively, some genotypes in current study exhibited longer ears.

Moderate positive significant correlations were identified between EL and maturity dates, ear leaf dimensions, tassel size, and stalk diameter. Plants with long ears were also found to be late-maturing ($r=0.62$; $P<0.001$), tall ($r=0.76$; $P<0.001$), had long and broad ear leaves ($r\geq0.60$; $P<0.001$), long tassels ($r=0.66$; $P<0.001$), and large stalk diameter ($r=0.45$; $P=0.0$). Moreover, accessions with long ears also exhibited large number of kernels per row ($r=0.36$; $P>0.004$), and multiple ears ($r=0.32$; $P>0.01$), both of which accounted for their high grain yield.

Ear diameter ranged from 15 to 55.5 mm, cob diameter 10.0 to 43.0 mm, number of ears per plant 1 to 3, number of rows per ear 6 to 20, number of kernels per row of 5 to 50 and ear weight 0.01 to 1.2 kg. Large ear diameter was associated with large number of rows per ear, large hundred kernel weights, large kernel length and kernel width all of which accounted for their high grain yield. Moderate correlation coefficients of r = 0.43 to r = 0.55 at P<0.005 were obtained for the associations between ear diameter and these traits.

Invariably, plants with large number of rows per ear also had large ear diameter (r=0.43; P=0.0006) but low 100-kernel weight (r=-0.27; P=0.04), kernel width (r=0.42; P>0.0009), and ear weight (r=-0.35; P=0.0064). In plants with large number of kernels per row, their yield was contributed in part by their long ear length (r=0.36; P=0.004) and large kernel length (r=0.34; P=0.008).

Ear diameter had a range of 15 mm (TZm-1150) to 55.5 mm (TZm-1129). The mean ear diameter of 37.2 ± 6.6 mm is in consonance with 39 mm, 35 mm, and 40.1 mm of Hartings *et al.* (2008), Knežević-Jaric *et al*. (2010), and Beyene *et al.* (2006), respectively. Compared to the check, „Obatanpa GH", whose mean ear diameter was 46.0 mm, the following accessions exhibited similar values,TZm-1184 (39.5 mm), TZm-1138 (39.9 mm), TZm-1122 (41.0 mm), TZm-1111 (42.0 mm), TZm-1118 (42.0 mm), TZm-1195 (42.2 mm), and TZm-29 (44.3 mm) (Table 4.3B). As a yield component, ear diameter contributes to grain yield if most of its size is not contributed by the cob.

Concerning number of rows per ear, a mean of 13.9 ± 2.0 with minimum of 6 for TZm1156 to maximum of 22 for TZm-1142 were recorded. This mean was similar to the mean of 13.1± 2.42 and a range of 8 to 20 reported among Italian maize landraces (Hartings *et al*., 2008). Mean number of rows per ear of 13.5 ± 1.1 and range of 11.0 to 15.6 were observed by Obeng-Antwi *et al*. (2012) among Ghana maize landraces. Also reported was a mean of 13.6 for number of rows per ear in maize landraces in Eastern Serbia (Knežević-Jaric *et al*., 2010). On accession mean basis, 44 genotypes representing 72 % of the entire genotypes exhibited slightly higher number of rows per ear (13.5 to 15.6) than that of the check, „Obatanpa GH" (13.4) (Table 4.3B). This shows that genotypes of the present study may possess variety of alleles for number of rows per ear which may be exploited for yield improvement.

Regarding number of kernels per row, TZm-1190 had the minimum of 5 while a maximum of 50 was found in TZm-1139 and TZm-1128 with a mean of 45 ± 8.2 (Table 4.2). Beyene *et al*. (2006) on the other hand reported a mean of 27.42 ± 3.6 ranging from 18 to 36.6. A mean number of kernels per row of 25.72 ± 0.73 ranging from 19.80 to 34.47 were also reported by Azad *et al*. (2012) among inbred lines in Bangladesh. Those genotypes in current study which demonstrated unusually large would be good genetic resource for breeding for increased grain yield.

A Pearson correlation analysis revealed moderate positive significant associations of number of kernels per row with grain weight (r= 0.52; P<0.001) and grain yield (r= 0.54; P< 0.001). This interprets that a 27 % variation in grain weight and 29 % variation in grain yield are explained by number of kernels per row, suggesting that increase in number of kernels is associated with increase in yield.

4.2.4 Yield and yield components

Yield and yield components of the landraces demonstrated wide variability as indicated by the high coefficient of variation and highly significant (P<0.001) mean squares (Table 4.2). Grain yield ranged from 0.46 $Mgha^{-1}$ for Tzm-1136 to 8.14 $Mgha^{-1}$ for TZm-1139 with a mean of 3.8 ± 1.7 $Mgha^{-1}$ (Table 4.2). This highest yielding individual plant in accession TZm-1139 compared favorably with the highest yielding plant of the „Obatanpa" check of 8.7 $Mgha^{-1}$.

Mean grain yield for some maize landraces in Ghana is 2.7 $Mgha^{-1}$ with range of 1.3 to 4.5 $Mgha^{-1}$ (Obeng-Antwi *et al*., 2012). The average yield for Nigerian accessions was 3.0 $Mgha^{-1}$ with a range of 2.2 to 4.1 $Mgha^{-1}$ (Alika *et al*., 1993), while the yield of Ethiopian accessions averaged 2.6 $Mgha^{-1}$ with a range of 1.3 to 4.3 $Mgha^{-1}$ (Beyene *et al*., 2005).

On accession mean basis, yield ranged from 2.16 ± 0.4 $Mgha^{-1}$ for TZm-1132 to 6.19 ± 1.7 $Mgha^{-1}$ for TZm-1139 which outyielded „Obatanpa GH" (5.82±2.3 $Mgha^{-1}$). In Ghana the highest yielding OPV genotype is „Obatanpa GH", a white, intermediatematuring dent and flint open-pollinated QPM. This cultivar has been utilized for over thirty years for maize improvement in Ghana and some parts of Africa due to its superior qualities of early-maturing, high yield, and resistance to many of the diseases. Efforts to improve yield of maize in Ghana beyond that of „Obatanpa GH" has not been fruitful as there are indications of narrow genetic base of the breeding

materials and a possible attainment of a yield plateau as most of the improved genotypes share similar parentage with the plant introduction genotypes from CIMMYT.

Grain yield of all the accessions exceeded the average maize yield in Africa of 2.0Mgha^{-1}. Twenty-seven accessions demonstrated yield performance of 4.2 to 6.2 Mgha^{-1}, comparable to the highest maize productivity in Africa of 4.2 Mgha^{-1} achieved in South Africa. These high-yielding accessions included TZm-1185, TZm1142 TZm-1213, and TZm-1129 all having a yield of 4.2 Mgha^{-1}; TZm-1143 and TZm-1215 (4.3 Mgha^{-1}); TZm-1150, TZm-1211, TZm-1152, and TZm-1101 (4.4 Mgha^{-1}); and TZm-1123, TZm-1100, TZm-1138, and TZm-1112 (4.6 Mgha^{-1}). The other high-yielding accessions were TZm-1212, TZm-1130, TZm-1190, TZm-1118, and TZm-1183 (4.7 Mgha^{-1}); TZm-1106 and TZm-1144 (4.8 Mgha^{-1}); TZm-1122 (4.9 Mgha^{-1}); TZm-1125 and TZm-1117 (5.0 Mgha^{-1}); TZm-1119 (5.4 Mgha^{-1}); and finally, TZm-1139 (6.2Mgha^{-1}). These genotypes may be good candidates for incorporation into breeding programmes to increase genetic base while improving yield.

The relationship between yield and yield components was evaluated in a correlation analysis. Moderate to high positive significant correlations of yield with ear diameter (r=0.55; $P<0.001$), number of kernels per row (r= 0.54; $P<0.0001$), and kernel length (r= 0.73; $P<0.001$) were identified. Similarly, moderate positive significant correlation was observed in yield with kernel width (r=0.42; P= 0.0007). Hence, variation in grain yield was accounted for by 30 % variation in ear diameter, 29 % variation in number of kernels per row, and 53 % variation in kernel length, whereas kernel width explains

barely 18 % of the variation in yield. The positive correlations demonstrate that high values of yield components contribute to greater yield.

Hundred kernel weight (HKWT) ranged from a least value of 13.1 g for TZm-1120 to a maximum of 117.2 g for „Obatanpa GH" with a mean of 52.2 ± 13.6 g (Table 4.2). Obeng-Antwi *et al*. (2012) reported of relatively low values of HKWT of Ghana maize landraces ranging between 22.3 g to 35.4 g with a mean of 29.1 ± 2.7 g. Ranatunga *et al*. (2009), in a genetic diversity study of maize inbred lines from Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India reported HKWT values of 11.1 g to 36.8 g with a mean of 21.83 ± 0.8 g. The large HKWT of „Obatanpa" check was expected as it is an improved cultivar. On accession mean basis, HKWT ranged from 42.3± 6.6 g (TZm-1132) to 66.7±9.0 g. However, besides „Obatanpa GH", the second maximum HKWT was in TZm-1150 and TZm1211 which had 79.6 g. The African maize germplasm therefore possesses promising genotypes which may be exploited as sources of alleles for breeding for enhancement in grain yield.

As expected, a strong positive significant correlation between HKWT and kernel length (r=0.79; P<0.001) and kernel width (r=0.83; P<0.001) were identified in which 62 % and 68 % of the variation in HKWT is explained by kernel length and kernel width, respectively. The positive correlations demonstrated that longer and wider kernel dimensions contribute to kernel weight and hence yield in maize.

Values of kernel width, kernel length and kernel thickness were similar to those of other regions being 7.5 ± 0.9 mm in TZm-1180 to 9.3 ± 0.8 mm in TZm-1195 (10.0 ±

1.4 mm in „Obatanpa GH‟), 7.5 ± 1.1 mm in TZm-1137 and 7.5 ± 1.2 mm in TZm1193

TZm-1122 to 5.2 ± 1.0 mm in TZm -1110 (5.4 ± 0.5 mm in „Obatanpa GH‟), respectively (Table 4.3B). Reports of mean kernel widths, kernel length, and kernel thickness are 8.0 ± 1 mm, 9.0 ± 1.0 mm, and 4.0 ± 0.3 mm for Ghana maize accessions (Obeng-Antwi *et al*., 2012), kernel length of 11.1 mm (Knežević -Jaric *et al*., 2010) of Serbian maize and 9.3 mm in traditional European maize population (Rebourg *et al*. (2001).

On accession mean basis seven genotypes representing 11 % namely TZm-1138, TZm-1144, TZm-1111, TZm-1190, TZm-1137, TZm-1112 and TZm-1110 with means 5.0, 5.0, 5.0, 5.1, 5.1, 5.2 and 5.2 , respectively, ranked equally with „Obatanpa GH‟, the check (5.4) (Table 4.3B). Consequently these genotypes by equal competitiveness with the check, present good candidates in maize kernel breeding in improving yield.

to 10.2 ± 0.8 mm in TZm-1139 and 10.21.4 in „Obatanpa GH‟. Finally, 4.1±0.4 mm in

No.	ACC	EP	NRE	NKR	HKWT	EN	KL	KW	KT	YLD
1	Obatanpa	0.4 (0.3-0.7) 0.1	13.4 (10-18) 2.0	28.9 (14-40) 8.3	83.6 (49-117) 14.3	1.1 (1-2) 0.2	10.2 (8-13) 1.4	10.0 (9-12) 0.9	5.4 (4-7) 0.5	5.8 (3-9) 2.3
2	Tzm-1097	0.5 (0.3-0.7) 0.1	14.2 (12-20) 2.1	31.3 (20-44) 6.3	48.2 (37-56) 6.7	1.1 (1-2) 0.3	8.3 (7-10) 0.7	8.5 (6-10) 0.9	4.4 (4-7) 0.7	4.1 (2-5) 1.2
3	Tzm-1099	0.5 (0.4-0.7) 0.1	14.9 (12-20) 1.9	24.3 (17-35) 5.9	51.5 (28-63) 14.5	1.0 (1-1) 0.0	8.3 (7-11) 1.4	8.0 (7-10) 0.9	4.4 (4-8) 1.0	3.7 (1-6) 1.7
4	Tzm-1100	0.5 (0.3-0.7) 0.1	12.9 (10-16) 1.4	32.5 (23-41) 4.4	57.2 (35-75) 13.8	1.0 (1-1) 0.0	8.6 (70-11) 0.9	8.7 (7-10) 0.7	4.6 (4-6) 0.4	4.6 (3-7) 1.6
5	Tzm-1101	0.5 (0.3-0.7) 0.1	14.6 (10-18) 1.9	32.8 (24-40) 3.3	45.7 (26-54) 11.6	1.0 (1-2) 0.2	8.6 (7-11) 0.9	7.9 (7-10) 0.8	4.5 (4-7) 0.8	4.4 (3-5) 0.7
6	Tzm-1103	0.5 (0.3-0.6) 0.1	12.2 (9-16) 1.6	31.4 (19-42) 5.1	54.8 (49-66) 5.9	1.0 (1-2) 0.2	8.9 (7-12) 1.2	9.0 (7-11) 0.8	4.6 (4-6) 0.6	3.8 (3-5) 0.8
7	Tzm-1105	0.5 (0.4-0.6) 0.1	13.4 (8-18) 1.6	27.8 (15-43) 6.1	48.8 (31-66) 13.6	1.0 (1-1) 0.0	8.0 (7-10) 1.0	8.3 (7-11) 0.9	4.5 (4-6) 0.5	3.4 (1-5) 1.4
8	Tzm-1106	0.4 (0.3-0.6) 0.1	14.5 (12-18) 1.5	29.2 (16-45) 7.8	58.0 (45-66) 8.3	1.0 (1-2) 0.2	9.5 (8-12) 1.0	8.6 (7-10) 0.8	4.3 (3-5) 0.4	4.8 (3-6) 1.4
9	Tzm-1108	0.5 (0.3-1.3) 0.1	14.8 (12-18) 1.5	26.6 (19-39) 4.1	45.3 (35-54) 7.2	1.0 (1-1) 0.0	8.2 (7-10) 0.8	7.9 (6-10) 0.5	4.3 (4-5) 0.4	3.3 (1-5) 1.3
10	Tzm-1109	0.4 (0.2-0.6) 0.1	13.9 (10-20) 2.1	29.4 (14-42) 7.8	51.3 (34-61) 11.0	1.0 (1-2) 0.1	8.1 (6-11) 1.4	7.9 (7-10) 0.8	4.7 (4-9) 1.0	4.0 (1-6) 1.9
11	Tzm-1110	0.5 (0.3-0.9) 0.1	14.7 (12-18) 1.7	25.6 (8-43) 8.1	52.3 (34-73) 12.6	1.1 (1-2) 0.2	8.0 (5-10) 1.3	8.3 (7-10) 0.8	5.2 (4-8) 1.0	3.7 (1-7) 1.9
12	Tzm-1111	0.6 (0.4-0.7) 0.1	14.4 (10-18) 1.6	25.4 (8-40) 6.9	60.2 (39-77) 14.8	1.0 (1-2) 0.1	8.4 (7-11) 1.1	8.7 (5-11) 1.0	5.0 (4-8) 1.2	3.5 (2-5) 1.1
13	Tzm-1112	0.5 (0.3-0.8) 0.1	13.8 (8-20) 2.4	30.1 (10-42) 7.3	58.4 (54-66) 4.4	1.0 (1-2) 0.2	8.8 (7-12) 1.1	8.5 (7-10) 0.9	5.2 (4-9) 1.3	4.6 (2-7) 1.7
14	Tzm-1114	0.5 (0.3-0.7) 0.1	13.2 (10-18) 1.7	27.6 (14-46) 5.5	45.4 (36-59) 7.7	1.0 (1-1) 0.0	7.6 (6-10) 0.8	8.1 (7-9) 0.6	4.9 (4-7) 0.9	2.6 (2-4) 1.0
15	Tzm-1117	0.6 (0.4-0.9) 0.1	13.9 (10-16) 1.8	33.8 (18-46) 6.6	54.9 (40-66) 9.6	1.0 (1-1) 0.0	8.9 (8-11) 0.9	8.5 (8-11) 0.6	4.3 (4-6) 0.6	5.0 (3-7) 1.7
16	Tzm-1118	0.5 (0.4-1.0) 0.1	14.3 (12-18) 1.8	30.8 (20-41) 5.2	56.1 (44-61) 6.5	1.0 (1-1) 0.0	8.7 (7-11) 1.0	8.6 (6-10) 0.9	4.8 (4-6) 0.7	4.7 (3-5) 1.0
17	Tzm-1119	0.5 (0.3-0.9) 0.1	13.9 (8-18) 2.1	31.5 (20-43) 5.2	63.5 (54-73) 7.3	1.0 (1-1) 0.0	8.6 (7-12) 1.1	8.7 (7-11) 0.8	4.9 (4-8) 1.1	5.4 (3-6) 1.2

Table 4.3B cont‟d

Table 4.3B Means, ranges, a draces held in IITA collection

Table 4.3B cont‟d

and a check „Obatanpa GH‟.

Table 4.3B cont‟d

18	Tzm-1120	0.5 (0.2-0.7) 0.1	12.3 (8-16) 2.2	28.1 (18-42) 5.5	52.6 (13-80) 21.3	1.1 (1-2) 0.3	8.1 (6-11) 1.2	8.5 (6-10) 1.0	4.8 (4-8) 0.8	3.7 (1-6) 2.1

No.	ACC	EP	NRE	NKR	HKWT	EN	KL	KW	KT	YLD
19	Tzm-1121	0.5 (0.4-0.6) 0.1	14.3 (10-16) 2.0	29.9 (16-38) 5.5	44.2 (22-66) 15.1	1.0 (1-1) 0.0	8.0 (6-11) 1.0	7.6 (6-10) 1.0	4.6 (4-8) 1.0	3.6 (2-5) 1.2
20	Tzm-1122	0.5 (0.3-0.6) 0.1	13.8 (12-16) 1.4	31.0 (18-42) 5.2	61.0 (52-70) 6.6	1.1 (1-2) 0.2	9.3 (8-12) 0.8	8.5 (7-10) 0.6	4.1 (3-5) 0.4	4.9 (3-7) 1.6
21	Tzm-1123	0.5 (0.3-0.6) 0.1	13.0 (10-14) 1.4	33.7 (22-48) 5.2	55.9 (35-70) 13.7	1.1 (1-2) 0.2	8.6 (7-11) 1.1	8.5 (7-10) 0.9	4.8 (3-7) 1.3	4.6 (2-6) 1.4
22	Tzm-1125	0.5 (0.4-0.7) 0.1	13.8 (10-18) 1.8	30.4 (20-44) 5.9	58.9 (49-68) 6.7	1.0 (1-1) 0.0	9.6 (7-11) 1.0	8.7 (7-10) 0.7	4.4 (3-7) 0.7	5.0 (4-6) 0.9
23	Tzm-1126	0.5 (0.3-0.7) 0.1	13.9 (10-16) 1.2	28.6 (20-39) 5.3	47.2 (22-70) 17.4	1.0 (1-2) 0.1	8.3 (7-12) 1.4	8.2 (7-10) 0.9	4.4 (4-8) 0.7	3.7 (1-6) 2.0
24	Tzm-1128	0.6 (0.4-0.9) 0.1	14.7 (12-18) 1.3	33.7 (24-50) 5.9	43.3 (24-54) 11.2	1.0 (1-1) 0.0	8.4 (7-11) 1.1	8.0 (7-10) 0.7	4.4 (3-7) 0.9	3.6 (1-5) 1.4
25	Tzm-1129	0.5 (0.0-0.7) 0.1	15.6 (12-20) 1.9	27.7 (18-39) 5.1	53.1 (46-66) 7.3	1.0 (1-1) 0.0	8.8 (8-11) 0.9	8.9 (7-11) 0.9	4.2 (4-6) 0.4	4.2 (2-6) 1.7
26	Tzm-1130	0.5 (0.3-0.7) 0.1	14.2 (12-18) 1.8	27.8 (16-46) 8.7	49.5 (10-70) 22.3	1.0 (1-1) 0.0	7.9 (6-11) 1.5	8.4 (7-10) 1.0	4.8 (4-7) 0.6	4.7 (1-8) 2.6
27	Tzm-1131	0.5 (0.3-0.9) 0.1	13.7 (12-16) 1.3	30.6 (17-40) 5.2	45.3 (33-54) 9.1	1.1 (1-2) 0.2	8.2 (6-11) 1.1	8.1 (6-10) 0.7	4.6 (4-6) 0.5	3.8 (2-6) 1.6
28	Tzm-1132	0.7 (0.4-1.0) 0.2	14.4 (12-18) 1.2	29.6 (15-40) 6.2	42.3 (31-52) 6.6	1.0 (1-1) 0.0	7.9 (6-9) 0.8	8.1 (7-10) 0.6	4.3 (4-7) 0.6	2.2 (2-3) 0.4
29	Tzm-1136	0.5 (0.3-0.7) 0.1	15.4 (10-20) 2.7	25.9 (8-39) 8.6	46.4 (30-61) 10.0	1.0 (1-1) 0.0	7.8 (6-12) 1.3	8.2 (6-10) 0.9	4.5 (4-8) 0.7	3.1 (1-5) 1.3
30	Tzm-1137	0.5 (0.4-0.8) 0.1	12.6 (8-18) 2.6	24.3 (10-40) 9.5	51.7 (40-66) 8.1	1.0 (1-1) 0.0	7.5 (6-10) 1.1	8.9 (7-10) 0.8	5.1 (3-8) 1.3	2.9 (1-5) 1.5
31	Tzm-1138	0.5 (0.3-0.7) 0.1	14.7 (12-20) 2.0	32.3 (20-42) 6.6	53.4 (39-66) 9.6	1.0 (1-1) 0.0	8.7 (8-11) 0.9	8.2 (6-10) 0.7	5.0 (4-9) 0.9	4.6 (2-6) 1.5
32	Tzm-1139	0.4 (0.3-0.8) 0.1	13.7 (10-18) 2.0	36.6 (25-50) 6.0	66.7 (52-75) 9.0	1.1 (1-2) 0.2	10.2 (8-12) 0.8	9.0 (8-11) 0.6	4.4 (3-8) 0.8	6.2 (4-8) 1.7
33	Tzm-1141	0.6 (0.4-0.7) 0.1	12.7 (8-16) 2.0	28.3 (8-42) 8.5	48.0 (25-63) 14.5	1.0 (1-2) 0.1	8.0 (6-11) 1.2	8.5 (7-10) 0.9	4.3 (4-7) 0.6	3.2 (1-5) 1.6
34	Tzm-1142	0.5 (0.3-0.8) 0.1	15.3 (12-22) 1.7	29.4 (16-43) 5.2	47.2 (34-59) 10.1	1.0 (1-2) 0.2	8.2 (5-11) 1.2	7.9 (7-10) 0.7	4.4 (3-6) 0.5	4.2 (2-6) 1.4
35	Tzm-1143	0.5 (0.4-0.7) 0.1	14.0 (10-18) 1.5	32.2 (21-47) 8.4	53.9 (30-75) 15.5	1.0 (1-1) 0.0	8.6 (6-12) 1.2	8.7 (6-12) 1.2	4.5 (4-7) 0.6	4.3 (1-7) 1.8

Table 4.3B cont‟d

Table 4.3B cont‟d

36	Tzm-1144	0.5 (0.3-0.6) 0.1	13.8 (10-20) 2.5	28.3 (10-46) 10.2	55.7 (29-68) 14.6	1.0 (1-2) 0.2	8.6 (6-12) 1.5	8.3 (7-11) 0.9	5.0 (4-7) 1.2	4.8 (3-7) 1.4
37	Tzm-1145	0.6 (0.4-0.8) 0.1	15.3 (10-18) 2.0	32.2 (16-42) 7.4	47.2 (30-56) 10.0	1.0 (1-1) 0.0	8.4 (7-11) 1.1	7.9 (6-10) 0.8	4.3 (4-7) 0.7	3.2 (1-4) 1.1

No.	ACC	EP	NRE	NKR	HKWT	EN	KL	KW	KT	YLD
38	Tzm-1147	0.4 (0.2-0.7) 0.1	13.6 (10-16) 1.5	23.3 (13-37) 6.1	50.9 (36-61) 10.7	1.0 (1-1) 0.0	7.9 (7-10) 0.7	8.2 (8-10) 0.6	4.9 (4-9) 0.8	3.2 (2-5) 1.1
39	Tzm-1148	0.4 (0.1-1.0) 0.1	14.5 (12-18) 1.5	23.3 (10-36) 7.8	51.8 (46-59) 4.6	1.0 (1-1) 0.0	8.6 (8-10) 0.8	8.1 (7-10) 0.7	4.4 (3-8) 0.9	3.2 (1-5) 1.5
40	Tzm-1149	0.4 (0.3-0.9) 0.1	11.6 (10-16) 1.5	24.9 (14-43) 7.4	53.6 (37-66) 11.6	1.0 (1-1) 0.0	8.5 (6-11) 1.1	9.0 (6-11) 0.9	4.4 (4-8) 1.0	2.6 (1-4) 1.5
41	Tzm-1150	0.4 (0.1-0.7) 0.1	12.9 (8-18) 2.2	26.2 (12-47) 8.3	56.2 (38-80) 16.3	1.0 (1-2) 0.1	8.9 (6-11) 1.3	8.6 (8-10) 0.6	4.6 (4-8) 0.9	4.4 (2-7) 2.3
42	Tzm-1151	0.4 (0.3-0.7) 0.1	14.0 (10-18) 1.6	28.0 (17-45) 6.1	59.1 (43-77) 13.1	1.0 (1-2) 0.1	9.2 (7-11) 0.9	8.9 (7-11) 0.7	4.2 (4-8) 0.7	3.9 (1-7) 2.2
43	Tzm-1152	0.4 (0.2-0.6) 0.1	15.2 (12-18) 1.2	28.1 (15-44) 5.7	55.5 (37-66) 9.6	1.0 (1-1) 0.0	8.8 (7-11) 0.7	8.3 (7-10) 0.7	4.5 (4-8) 0.9	4.4 (2-6) 1.1
44	Tzm-1153	0.4 (0.3-0.9) 0.1	13.7 (6-16) 2.3	26.3 (9-43) 7.3	53.6 (40-63) 8.8	1.0 (1-1) 0.0	8.5 (5-12) 1.1	8.3 (5-11) 0.9	4.6 (4-7) 0.6	2.6 (2-5) 1.1
45	Tzm-1156	0.5 (0.3-0.8) 0.1	14.1 (12-16) 1.4	31.5 (20-43) 5.0	52.1 (42-59) 6.7	1.0 (1-1) 0.0	8.9 (8-11) 0.8	8.3 (7-10) 0.6	4.3 (3-7) 0.6	4.1 (2-6) 1.2
46	Tzm-1180	0.5 (0.4-0.8) 0.1	14.5 (10-20) 1.8	28.2 (18-38) 5.1	45.6 (37-52) 5.6	1.0 (1-1) 0.0	8.0 (6-10) 0.9	7.5 (6-9) 0.9	4.5 (3-7) 0.8	3.5 (2-5) 1.3
47	Tzm-1182	0.5 (0.3-0.6) 0.1	12.4 (8-16) 1.8	28.9 (20-41) 4.3	45.3 (30-56) 11.0	1.0 (1-2) 0.2	7.9 (7-11) 1.0	8.2 (8-10) 0.4	4.5 (4-7) 0.6	3.4 (2-5) 1.4
48	Tzm-1183	0.5 (0.3-0.9) 0.1	12.7 (10-18) 1.8	29.6 (18-43) 6.2	64.3 (50-75) 9.0	1.2 (1-2) 0.4	9.7 (7-12) 0.9	8.9 (7-10) 0.6	4.5 (4-9) 0.8	4.7 (3-7) 1.3
49	Tzm-1184	0.5 (0.4-0.7) 0.1	13.9 (12-16) 1.4	27.2 (17-36) 4.4	58.4 (41-73) 11.3	1.3 (1-3) 0.6	8.3 (6-10) 1.0	8.5 (4-11) 1.5	4.9 (4-8) 1.2	3.9 (2-6) 1.6
50	Tzm-1185	0.6 (0.4-1.0) 0.1	14.1 (10-18) 1.7	32.7 (18-44) 5.6	54.1 (40-73) 13.2	1.0 (1-1) 0.0	8.5 (6-12) 1.1	8.8 (7-11) 0.9	4.6 (3-8) 1.1	4.2 (2-6) 1.4
51	Tzm-1187	0.6 (0.1-1.2) 0.2	14.2 (9-16) 1.4	31.4 (19-42) 5.5	60.0 (42-73) 11.8	1.0 (1-1) 0.0	8.9 (8-12) 1.0	9.1 (6-12) 1.0	4.9 (4-7) 0.7	4.6 (4-6) 0.9
52	Tzm-1188	0.5 (0.3-0.6) 0.1	14.2 (12-18) 1.6	31.1 (20-47) 5.3	45.8 (34-63) 10. 2	1.1 (1-2) 0.2	7.9 (7-10) 0.9	7.8 (6-10) 0.7	4.3 (4-6) 0.6	3.8 (2-6) 1.3
53	Tzm-1190	0.6 (0.4-0.8) 0.1	13.3 (10-16) 1.5	30.9 (5-43) 9.0	66.6 (44-80) 11.6	1.0 (1-2) 0.1	8.9 (7-11) 1.0	8.9 (7-12) 1.0	5.1 (4-7) 0.7	4.7 (2-7) 1.6
54	Tzm-1193	0.6 (0.4-0.60.1)	14.0 (8-18) 2.5	26.0 (14-45) 9.0	47.8 (33-54) 8.2	1.0 (1-1) 0.0	7.5 (6-10) 1.2	8.2 (7-9) 0.6	4.9 (4-7) 1.2	3.1 (2-4) 0.6

Table 4.3B cont‟d

Table 4.3B cont‟d

	ACC	EP	NRE	NKR	HKWT	EN	KL	KW	KT	YLD
	Tzm-1211	0.5 (0.4-0.6) 0.1	13.2 (8.0-16) 1.6	28.7 (10-37) 5.1	59.6 (32-80) 20.2	1.0 (1-2) 0.1	9.0 (7-13) 1.6	8.3 (7-11) 0.9	4.6 (4-9) 0.8	4.4 (2-7) 2.3
	Tzm-1212	0.6 (0.5-0.7) 0.1	14.7 (12-18) 1.5	27.0 (15-38) 5.9	61.2 (53-66) 4.9	1.0 (1-1) 0.0	9.1 (8-12) 1.1	8.8 (8-10) 0.4	4.6 (4-5) 0.3	4.7 (3-6) 1.3
	Tzm-1213	0.5 (0.0-0.8) 0.1	14.3 (12-18) 1.8	30.2 (21-38) 3.7	53.0 (43-63) 7.5	1.0 (1-1) 0.0	8.8 (8-11) 0.8	8.2 (7-10) 1.0	4.6 (4-7) 0.8	4.2 (3-5) 0.9
	Tzm-1214	0.5 (0.3-0.6) 0.1	13.0 (10-18) 2.0	27.4 (17-46) 6.5	61.0 (45-73) 9.1	1.0 (1-1) 0.0	8.7 (5-11) 0.9	8.9 (7-11) 0.8	4.9 (4-7) 0.8	3.7 (1-6) 1.8
	Tzm-1215	0.4 (0.1-0.6) 0.1	13.5 (10-18) 1.6	26.6 (14-37) 6.4	56.5 (49-63) 5.8	1.0 (1-1) 0.0	8.8 (7-10) 0.8	8.2 (7-10) 0.7	4.6 (3-8) 0.9	4.3 (3-6) 1.0
55	Tzm-1194	0.5 (0.4-0.7) 0.1	13.5 (10-16) 1.4	25.5 (14-38) 6.2	60.6 (45-73) 9.2	1.1 (1-2) 0.2	9.2 (7-12) 1.1	8.7 (7-11) 0.9	4.7 (4-9) 0.8	3.9 (2-6) 1.4
56	Tzm-1195	0.5 (0.0-0.9) 0.1	13.2 (8-16) 1.9	26.2 (13-40) 6.3	65.6 (55-73) 7.7	1.0 (1-1) 0.0	8.9 (8-12) 1.0	9.3 (7-11) 0.8	4.7 (4-8) 0.8	3.8 (3-6) 1.1

o.

7

8

9

0

1

[2]EP= ear position; NRE = number of rows per kernel; NKR = number of kernels per row; HKWT = hundred kernel weight; EN = ear number per plant; KL = kernel length; KW = kernel width; KT = kernel thickness; YLD = yield

4.3 Heritability, phenotypic variance and genotypic variance of 24 quantitative traits on the 60 IPGRI genotypes and a check „Obatanpa GH"

Within the scope of environments and the population in current study, the phenotypic variance were substantially higher than genotypic variance leading to low and medium estimates of broad sense heritability in the range of 0.00 to 0.69 among the traits. Ample genotypic and phenotypic variances existed in the maize populations for all the traits considered, except stay green, ear position, ear number, and ear weight (Table 4.4). Of all the traits, plant height and ear height demonstrated highest values of phenotypic variance (1,541.07 and 848.01) and genotypic variance (464.28 and 324.26), respectively. Similar reports on genotypic variances of plant height and ear height in other maize populations are 348.70 and 228.50 (Sultan *et al*., 2014), and 155.34 and 12.24 (Rajesh *et al*., 2013). Other values of phenotypic variances of plant height in maize are 192.88 and 245.20 (Alan *et al*., 2013) and 14.70 for ear height (Rajesh *et al*. 2013).

Typically, heritability varies from trait however in current study, related traits demonstrated similar heritability estimates AD and SD (0.69 vrs. 0.68), PLHT and EHT (0.30 vrs. 0.38), NRE and NKR (0.10 and 0.10), GWT and YLD (0.18 vrs. 0.14). In contrast, ear leaf length and ear leaf width demonstrated wide variations in phenotypic and genotypic variances as well as heritabilities (0.18 and 0.05). There are hardly any reports on heritability of ELL and ELW. Heritability estimates of as high as 0.96 for ELL and ELW were obtained in a study on 239 recombinant inbred lines (RIL) under low and high nitrogen regimes (Zheng and Lui, 2013).

Trait	Phenotypic coefficient of variation (PCV) (%)	Phenotypic variance	Genotypic coefficient of variation (GCV) (%)	Genotypic variance	$H^2 \pm SE$
AD	30.19	28.42	8.50	19.64	0.69±0.05
SD	31.26	31.50	8.46	21.44	0.68±0.05
ASI	18.57	2.11	29.55	0.27	0.13±0.05
TL	18.85	56.05	8.91	6.93	0.12±0.03
ELL	46.98	166.48	9.37	30.67	0.18±0.05
ELW	5.00	4.71	10.60	0.22	0.05±0.02
PLHT	234.36	1,541.07	13.70	464.28	0.30±0.05
EHT	291.83	848.01	20.75	324.26	0.38±0.06
StD	11.13	31.23	9.51	2.07	0.07±0.02
SG	0.54	12.89	31.77	0.95	0.07±0.05
EL	0.40	20.72	23.00	2.60	0.13±0.03
EP	0.42	0.01	16.33	0.00	0.19±0.04
ED	22.88	31.48	9.09	4.70	0.15±0.05
CD	18.79	20.46	10.16	2.52	0.12±0.05
NRE	5.27	3.97	7.64	0.39	0.10±0.04
NKR	32.97	50.52	13.24	5.03	0.10±0.04
HKWT	91.90	104.85	14.56	39.45	0.38±0.08
EN	0.22	0.03	7.11	0.00	0.01±0.02
KL	3.56	1.21	7.91	0.23	0.19±0.05
KW	2.20	0.85	6.19	0.12	0.14±0.04
KT	2.34	0.75	8.86	0.02	0.03±0.03
EWT	0.22	0.09	21.75	0.00	0.00±0.00
GWT	2.51	0.05	19.80	0.01	0.18±0.08
YLD	12.52	1.34	18.57	0.19	0.14±0.08

Table 4.4 Estimates of phenotypic and genotypic variances, broad sense heritability estimates and standard errors of the 61 IPGRI maize landraces held in IITA collection used in the study

AD = days to 50 % anthesis; SD = days to 50 % silk ;ASI = anthesis to silk interval; TL = tassel length; ELL = ear leaf length; ELW = ear leaf width; PLHT = plant height; EHT = ear height; StD = stalk diameter; SG = Stay green; EL = ear length ; EP = ear position; ED = ear diameter; CD = cob diameter; NRE = number of rows per kernel; NKR = number of kernels per row; HKWT = hundred kernel weight; NP = number of plants harvested; NE = number of ears harvested per plot; EN = ear number per plant; KL = kernel length; KW = kernel width; KT = kernel thickness; EWT = ear weight; GWT = grain weight; YLD = yield

Similar estimates of heritability of 0.68 for days to anthesis and 0.50 for days to silking were reported for F1 hybrids and nine parental maize lines planted in the Northern Guinea and Sudan Savanna zones of Nigeria (Aminu and Izge, 2012). In contrast, relatively high estimates of 0.88 for days to silking (Anshuman *et al.,* 2013) and 0.89 for days to anthesis (Rajesh *et al*., 2013) are reported. The high heritability estimates for earliness traits indicate large genetic contribution to the phenotype in the environment in current study and better opportunity for selecting plant material for improvement.

On plant height, relatively high estimates of 0.41, 0.59, 0.61, 0.81, and 0.99 were observed by Atnafua and Rao (2013), Aminu and Izge (2012), Rajesh *et al*. (2013), and Anshuman *et al* (2013), respectively. Other heritability estimates of ear height include 0.39 (Atnafua and Rao, 2013), 0.54 (Magorokosho, 2006), 0.58 (Aminu and Izge, 2012), and very high values of 0.83 and 0.99 (Rajesh *et al*., 2013; Anshuman *et al*., 2013), respectively.

The low heritability estimates of HKWT in current study was supported by similar values of 0.42 (Atnafua and Rao, 2013), and 0.32 (Aminu and Izge, 2012). On the contrary, very high heritability estimates for HKWT of 0.96 and 0.99 were observed by Anshuman *et al* (2013) and Rajesh *et al*. (2013), respectively. Except for PLHT and EHT, the low heritability estimates of all traits indicate that environmental effect on the trait was substantially more than that of genotypic and it will be possible to select for improvement in the traits, albeit at a slow pace.

4.4 Phenotypic and genotypic correlation of agro-morphological quantitative traits

Phenotypic and genotypic correlations complement heritability estimates in achieving an effective indirect selection scheme compared to direct selection. Genotypic correlation coefficient is the heritable association between two variables (Falconer, 1989). However phenotypic correlation includes both genotypic and environmental effects. Hence significant phenotypic correlation without significant genotypic correlation has no value.

In the present study, genotypic correlation coefficients were generally higher than the corresponding phenotypic correlation values. Of the 136 genotypic correlation coefficients 68 were significant ($P<0.05$) and positive (0.01 to 1.00), whereas only 22 correlations were negative and non-significant and ranged from -0.01 to -0.34. Similarly, 84 phenotypic correlations were significant and positive (0.02 to 0.97, $P<0.05$) while 20 were negative (-0.02 to -0.60) and nonsignificant (Table 4.5). Other reports of higher genotypic correlations than phenotypic correlations in studies on maize phenological traits were observed by Duvick (2001), Mohammadi *et al*. (2003), and Aminu and Izge (2012).

Association between earliness, plant architectural traits, yield and yield components were characterized by moderate to large significant positive correlations (r=0.31 to 0.99; $P\leq0.05$) except for ear diameter, cob diameter, hundred kernel weight, kernel width, and yield. Early maturing genotypes exhibited low hundred kernel weight, kernel length, kernel width, and yield attributable to insufficient time for accumulation of photosynthates and confirming the trade-off between earliness and yield (Sultan *et al*.

2014; Aminu and Izge 2012). Strong and moderate positive significant genotypic correlation (r=0.99, P<0.05) and (r=0.42, P<0.05) were identified between days to anthesis and anthesis-silking interval and days to silking and anthesis-silking interval, respectively.

All plant architectural traits exhibited moderate to strong positive significant correlations with AD and SD (r= 0.31 to 0.94). Among all correlations of traits with ASI only ELW was important (r= 0.61). All ear and kernel characteristics and grain yield exhibited weak non significant correlations with earliness except EL and NKR (r=0.56 to 0.76). Other strong significant positive correlations were between grain yield and HKWT (r=0.92), KL (r=0.77), KW (r=0.71) and NKR (r=0.51).

Table 4.5 Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients among traits evaluated for 61 maize accessions

	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	EL	EP	ED	CD	NKR	HKWT	KL	KW	YLD
AD		**0.99***	**0.33**	**0.32***	**0.78***	**0.76***	**0.94***	**0.77***	**0.76***	**0.93***	**0.09**	**0.11**	**0.59***	**-0.30**	**-0.20**	**0.19**	**0.04**
SD	0.97*		**0.42***	**0.31***	**0.76***	**0.80***	**0.72***	**0.80***	**0.75***	**0.93***	**0.09**	**0.11**	**0.56***	**0.31**	**-0.21**	**-0.17**	**0.00**
ASI	0.06	0.32*		**0.02**	**0.10**	**0.61***	**0.00**	**0.01***	**0.19**	**-0.05**	**-0.01**	**0.07**	**0.03**	**-0.19**	**-0.18**	**0.07**	**-0.34**
TL	0.18*	0.15*	-0.07		**0.80***	**0.28**	**0.70***	**0.64***	**0.82***	**0.40**	**0.18**	**0.21**	**0.50***	**-0.08**	**-0.15**	**0.02**	**0.27**
ELL	0.40*	0.35*	-0.09	0.65*		**0.70***	**0.89***	**0.78***	**1.00***	**0.46***	**0.09**	**0.13**	**0.56***	**-0.10**	**-0.24**	**0.02**	**0.08**
ELW	0.28*	0.26*	0.00	0.32*	0.69*		**0.80***	**0.83***	**0.60***	**0.88***	**0.13**	**0.09**	**0.30**	**0.11**	**0.03**	**0.30**	**-0.19**
PLHT	0.23*	0.20*	-0.09	0.60*	0.70*	0.70*		**0.99***	**0.61***	**0.94***	**0.56***	**0.39**	**0.73***	**-0.27**	**0.30***	**0.45***	**0.50***
EHT	0.27*	0.25*	-0.07	0.50*	0.60*	0.63*	0.93*		**0.54***	**0.97***	**0.97***	**0.30***	**0.63***	**0.14***	**0.17***	**0.46***	**0.35***
EL	0.31*	0.29*	-0.01	0.51*	0.65*	0.54*	0.57*	0.46*		**0.24***	**-0.06**	**0.01**	**0.58***	**-0.30**	**-0.30**	**-0.01**	**0.00**
EP	0.30*	0.27*	-0.6	0.24*	0.30*	0.40*	0.58*	0.82*	0.18*		**0.14***	**-0.01**	**0.24***	**-0.18**	**-0.23**	**0.09***	**-0.18**
ED	0.07	0.06	-0.02	0.21*	0.26*	0.21*	0.42*	0.82*	0.17*	0.05		**0.82***	**0.02**	**0.60***	**0.52***	**0.66***	**0.55***
CD	0.09	0.08	-0.02	0.25*	0.26*	0.17*	0.28*	0.19*	0.16*	0.02	0.00		**-0.16**	**0.17**	**-0.07**	**0.25**	**0.03**

grown in Ghana in 2011 and 2012.

NKR	0.27*	0.25*	-0.04	0.23*	0.40*	0.21*	0.46*	0.33*	0.27*	0.08****	0.25*	0.08		**0.07**	**0.28**	**0.19**	**0.51***
HKWT	-0.20	-0.22	-0.11	0.03	0.04	0.12*	0.16****	0.12****	0.11	0.04****	0.49*	0.28*	0.02		**0.82***	**0.92***	**0.92***
KL	0.10	-0.12	-0.10	0.10	0.09	0.07	0.24****	-0.15	0.02	-0.01	0.51*	0.18*	0.37*	0.66*		**0.75***	**0.77***
KW	-0.15	-0.18	0.07*	0.01	0.05	0.11	0.25	0.18****	0.08	0.03*	0.32*	0.20*	0.07	0.73*	0.55*		**0.71***
YLD	0.00	-0.04	-0.16	0.16*	0.24*	0.17*	0.28*	0.25*	0.21*	0.17*	0.50*	0.28*	0.47*	0.54*	0.62*	0.35*	

*P<0.05).

The negative nonsignificant phenotypic and genotypic correlations of grain yield with earliness traits was not unexpected as late-maturing plants more often produce better yield than early-maturing genotypes. Such negative correlations between earliness and grain yield is widely reported in literature (Atnafua and Rao, 2013; Barriér *et al*., 2010; Sultan *et al*., 2014). Few studies using quantitative trait loci (QTL) have identified some associations of high yield loci with reduction in number of days to silking (Barrier *et al*., 2002). The negative correlation between ASI and grain yield indicates that short ASI genotypes ordinarily produced higher grain yield. This result was not unexpected as receptive silks would have viable pollen for kernel set. In contrast, Aminu and Izge (2012) observed a strong positive significant genotypic correlation (0.87, $P<0.01$) between anthesis-silking interval and grain yield.

Of the plant architectural traits only plant height and ear height showed important phenotypic and genotypic associations ($P<0.05$) with yield and yield component traits. The correlations of plant height with the yield component traits are listed as EL (r_p =0.57, r_g =0.61), ED (r_p =0.42, r_g =0.56), NKR (r_p =0.46, r_g =0.73), KL (r_p =0.24, r_g =0.30), KW (r_p =0.25, r_g =0.45), YLD (r_p =0.28, r_g =0.50), except HKWT (r_p =0.16, r_g =-0.27). The positive and significant associations indicate that yield of maize is associated with plant biomass.

The positive significant correlations between PLHT and the yield and yield components are reported in several studies in maize characterization (Atnafua and Rao, 2014; Alvi *et al*., 2003; Tyagi *et al*., 1998; Manivannan, 1998).

The general strong positive significant genotypic correlation between plant architectural traits and earliness (r_g= 0.72 to 0.94) was demonstrated in the current study. The phenotypic

correlations were rather weak but significant (r_p= 0.20 to 0.27). Results of the current study are in agreement with earlier report by Beyene *et al*. (2005) who observed significant and positive association between earliness and plant height. In contrast, Iqbal *et al*. (2009) reported negative significant genotypic association at level and negative nonsignificant phenotypic associations. Atnafua and Rao (2013) obtained negative nonsignificant associations between days to silking and plant height at both genotypic and phenotypic levels. The contrary view reports might be due to the different genetic make-up of the experimental material and differences in the environmental condition encountered in the two studies.

4.5 Genetic distance and cluster analysis of 60 IPGRI maize accessions held in IITA and a check, "Obatanpa GH"

Genetic similarity among 60 IPGRI genotypes held in IITA and a check, "Obantanpa GH" was estimated by means of correlation coefficient distance measure on 24 quantitative traits. The genetic distance matrix is shown in Appendix B1. A wide variation in correlation coefficient ranging from -0.087 to 0.89 with a mean of 0.27 ± 0.18 was recorded among the IPGRI maize landraces. A total of 1,590 pairwise associations out of 1,830, representing 86.9 % of the accession pairs had genetic distances below 0.50. These pairs are minimally related and are disparate in their traits expression. The squared correlation coefficient represents genetic distance. In all, average genetic distance based on squared correlation was 0.14 ± 0.15 in a range of 0.00 to 0.79. The study has revealed that the IPGRI landraces considered in current study are highly variable and contain substantial genetic diversity which can be beneficial to the African maize breeding programmes.

The UPGMA cluster analysis generated a dendrogram shown in (Figure 4.2). The genotypes grouped into two major clusters, cluster I and cluster II. Cluster I with 37

members was heterogeneous with two subclusters and had a range and average distance of 0.00 to 0.80, and 0.25 ± 0.18, respectively. The check variety grouped with cluster I.

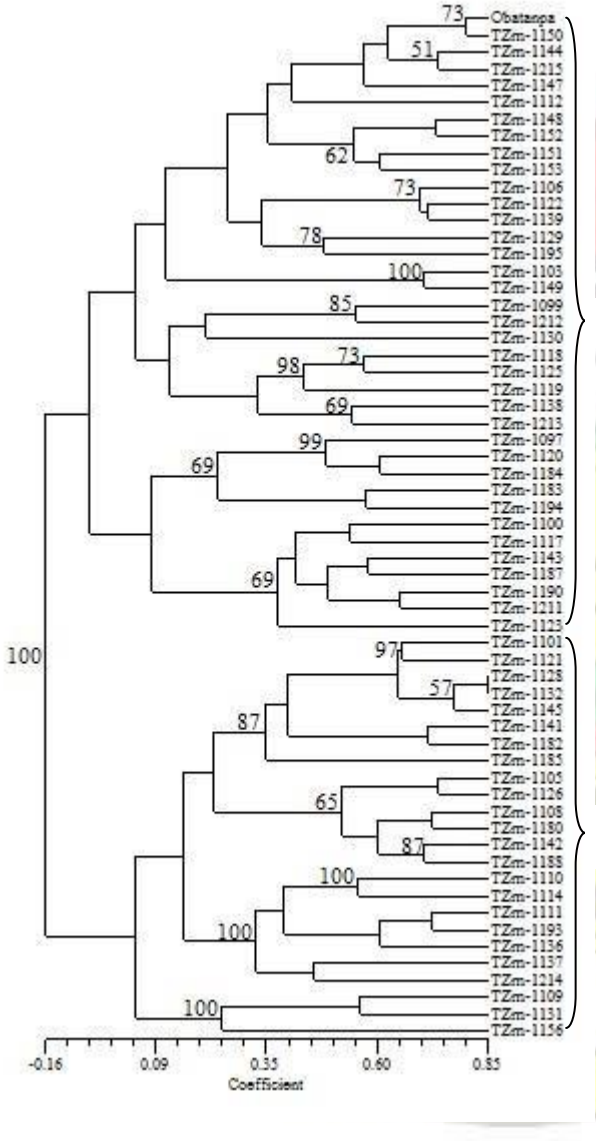


Figure 4.2 Dendrogram of 24 quantitative traits on 60 IPGRI maize accessions held in IITA and a check, "Obantanpa GH" based on correlation distance coefficient of dissimilarity index using UPGMA cluster method with corresponding bootstrap values.

Cluster II had a membership of 24 genotypes with two subclusters, and average genetic distance of 0.30 ± 0.20 in a range of 0.00 to 0.85. Table 4.6 shows the list of clusters and their membership.

Table 4.6 Clusters and subclusters of the 60 IPGRI and „Obatanpa" check accessions from the UPGMA cluster analysis

Cluster	Subcluster	Accessions	Average genetic distance	No acc

I	A	„Obatanpa GH“, TZm-1150, TZm-1144, TZm-1215, TZm1147, TZm -1112, TZm - 1148, TZm-1152, TZm-1151,TZm1153, TZm-1106, TZm-1122, TZm-1139, TZm-1129, TZm1195, TZm -1103, TZm - 1149, TZm-1099, TZm-1212, TZm1130, TZm -1118, TZm - 1125, TZm-1119, TZm-1138, TZm1213	0.29	25
I	B	TZm-1097, TZm-1120, TZm1184, TZm-1183, TZm-1194, TZm-1100, TZm-1117, TZm-1143, TZm-1187, TZm-1190, TZm-1211, TZm-1123	0.31	12

II	A	TZm-1101, TZm-1121, TZm1128, TZm-1132, TZm-1145, TZm-1141, TZm-1182, TZm1185, TZm-1105, TZm-1126, TZm-1108, TZm-1180, TZm1142, TZm-1188	0.37	14
II	B	TZm-1110, TZm-1114, TZm1111, TZm-1193, TZm-1136,	0.32	10
		TZm-1137, TZm-1214, TZm1109, TZm-1131, TZm-1156,		

Cluster I contained the earliest maturing genotypes with mean AD (53.52 days) and SD (56.17 days) values of about one day earlier than the overall mean of 54.68 and 57.46 days, respectively (Table 4.7). The mean anthesis-silking interval of cluster I genotypes (2.65 days) was lower than that of the overall mean of 2.79 days. Cluster IB genotypes demonstrated very short ASI values and protogyny including TZm1190. Other accessions with lower ASI values than the cluster mean were TZm-1120 (1.8 days), TZm-1123, and TZm-1100 (2.2 days).

Earliness in cluster I influenced all plant architectural traits as well as two yield related traits, the cob diameter and NRE by producing smaller plant sizes and fewer kernel rows per ear. Surprisingly, though the genotypes had smaller cobs, the ear diameters (38.5 mm) were larger than that of the overall mean (37.84 mm). Additionally, all other yield related traits outperformed that of the overall mean. Mean grain yield in cluster I (4.33 $Mgha^{-1}$) exceeded the overall mean (4.00 $Mgha^{-1}$) by 8.25%.

Trait	Overall Mean	SE	Cluster I Mean	SE	VAR	Cluster II Mean	SE	VAR
AD	54.68	0.10	53.52	0.13	-1.15	56.53	0.17	1.85
SD	57.46	0.11	56.17	0.13	-1.29	59.54	0.18	2.08
ASI	2.79	0.02	2.65	0.03	-0.14	3.01	0.04	0.22
TL	45.10	0.14	44.21	0.19	-0.89	46.56	0.21	1.46
ELL	81.56	0.23	79.66	0.33	-1.90	84.67	0.31	3.11
ELW	7.42	0.04	7.22	0.05	-0.20	7.75	0.07	0.34
PLHT	191.54	0.79	183.15	1.05	-8.38	205.72	1.16	14.18
EHT	97.24	0.60	90.53	0.75	-6.71	108.67	0.95	11.43
StD	20.06	0.10	19.46	0.13	-0.60	21.07	0.15	1.01
SG	69.00	0.39	66.35	0.49	-2.65	72.55	0.57	3.55
EL	15.04	0.06	15.02	0.08	-0.02	15.03	0.09	-0.01
EP	0.50	0.00	0.48	0.00	-0.01	0.52	0.00	0.02
ED	37.84	0.12	38.53	0.15	0.69	36.93	0.18	-0.91
CD	25.68	0.08	25.57	0.11	-0.10	25.79	0.13	0.11
NRE	13.86	0.04	13.71	0.05	-0.15	14.09	0.06	0.23
NKR	28.93	0.13	29.07	0.17	0.13	28.84	0.20	-0.09
HKWT	53.98	0.24	57.89	0.31	3.91	48.84	0.35	-5.14
EN	1.03	0.00	1.04	0.00	0.01	1.02	0.00	-0.01
KL	8.54	0.02	8.86	0.03	0.32	8.15	0.03	-0.40
KW	8.45	0.02	8.64	0.02	0.19	8.20	0.03	-0.26
KT	4.62	0.02	4.64	0.02	0.02	4.58	0.03	-0.04
EWT	0.12	0.01	0.14	0.01	0.02	0.09	0.00	-0.03
GWT	0.74	0.01	0.80	0.01	0.06	0.65	0.01	-0.08
YLD	4.00	0.03	4.33	0.04	0.33	3.55	0.04	-0.45

Cluster I genotypes departed from the usual trade-off between earliness and grain yield in maize (Sultan *et al*. 2014; Aminu and Izge 2012). The current research has demonstrated that the unutilized African genotypes carry unique alleles and combinations of alleles which may be exploited for both drought tolerances by escape and grain yield simultaneously. Further studies are required to ascertain the physiological mechanism which governs earliness and high yield in these genotypes.

Table 4.7 Cluster means and their standard errors for 24 characters in 60 IPGRI landraces held in IITA and “Obatanpa GH” (check).

[1]Variance = the difference in cluster means and overall mean

Cluster I plants were about 8 cm shorter in height and this confirms the general phenomenon that early-maturing plants are short as the precocity of the generative stage does not permit enough time for biomass accumulation during the vegetative stage. Short plant heights are of interest to breeding programmes for lodging resistance.

Among the thirty-seven cluster I genotypes, outstanding accessions with regard to earlymaturing and short plant height were the check (AD=48.8 days, PLHT =171.3 cm), TZm1149 (AD=44.0 days, PLHT =125.9 cm), TZm-1147 (AD=47.6 days, PLHT =141.2 cm), TZm-1148 (AD=45.5 days, PLHT =143.6 cm), TZm-1150 (AD=45.8 days, PLHT =144.2 cm), TZm-1153 (AD=47.6 days, PLHT =149.8 cm), and TZm-1152 (AD=48.2 days,
PLHT=156.1 cm).

On grain yield, TZm-1139 had the highest mean yield of 6.2 $Mgha^{-1}$ exceeding the yield of the improved check "Obatanpa GH" (5.8 $Mgha^{-1}$). Equally important highyielding genotypes were TZm-1119 (5.4 $Mgha^{-1}$), and TZm-1117 and TZm-1125 (5.0 $Mgha^{-1}$ each). The relatively high mean grain yield of cluster I genotypes was derived principally from the large ear diameter, number of kernels per row, kernel width, kernel thickness and long kernel lengths which nullified the effect of few number of rows per ear (Table 4.7).

Beyene *et al*. (2005) observed that early-maturing traditional Ethiopian highland genotypes had short heights. Unexpectedly, some members of this group of earlymaturing and short plants demonstrated higher grain yields than the mean grain yield of subSaharan Africa of 1.81 $Mgha^{-1}$, and exceeded that of Ghana (2.7 $Mgha^{-1}$, Obeng-Antwi *et al*., 2012), Nigeria (3.0 $Mgha^{-1}$, Alika *et al*., 1993), and Ethiopia (2.6 $Mgha^{-1}$, Beyene *et al*., 2005), as well as mean grain yield of Africa 1.7 $Mgha^{-1}$ (FARA, 2009). These represent a valuable set of genotypes which have the potential to transform maize breeding programmes in Africa for earliness against drought

conditions, as well as resistance to lodging, and better grain yield.

Some members of cluster I had the shortest ear length but the widest ear width giving them an appearance of thick short ears. Genotypes that demonstrated these traits include TZm-1099, TZm-1106, TZm-1122, TZm-1212, TZm-1118, TZm-1129, TZm-

1151 and TZm-1152.

Cluster II genotypes were late maturing with prolonged anthesis-silking intervals, demonstrated large values of plant architectural traits, such as tall plants with wide stalk diameter, in addition to larger cob diameter, extended stay green, and highest number of kernels per ear. All other yield related traits such as HKWT, GWT, number of ears per plant, ear length and grain size were smaller than that of the overall mean, leading to low grain yield. Cluster II genotypes were therefore to be chosen exclusively for high biomass. However, their long anthesis-silking intervals show drought sensitivity and poor grain yield arising from poor seed set of the asynchronous tassel and silk development.

The highest number of rows per ear of cluster II accessions cannot be overlooked since it is an important yield related trait that can be exploited for improved grain yield.

4.6 Principal components analysis of morphological traits

Principal components analysis revealed that first two principal components (PCs) with eigenvalues greater than 0.5 explained approximately 85.0 % of the total variance (Table 4.8). The PC1 accounted for 51.9 % of the variance with significant contributions from plant height and ear height followed by earliness, then yield and

yield related traits. Similarly, PC2 33.1 % of the variance which was explained by ear diameter, hundred kernel weight, kernel length, kernel width, grain weight and grain yield.

Table 4.8 Principal components analysis of 60 IPGRI maize accessions held in IITA and "Obatanpa GH" (check) based on 24 agro-morphological traits.

Trait	PC1	PC2
AD	**0.90**	0.04
SD	**0.90**	0.01
ASI	0.25	-0.17
TL	**0.53**	0.21
ELL	**0.86**	0.20
ELW	**0.69**	0.14
PLHT	**0.94**	0.11
EHT	**0.95**	0.02
StD	**0.81**	-0.14
SG	0.21	-0.26
EL	**0.77**	0.18
EP	**0.85**	-0.04
ED	0.10	**0.73**
CD	0.23	0.38

NRE	0.30	-0.06
NKR	0.42	0.44
HKWT	-0.27	**0.86**
EN	0.06	0.30
KL	-0.25	**0.82**
KW	-0.19	**0.72**
KT	-0.06	0.24
EWT	-0.10	0.11
GWT	-0.10	**0.89**
YLD	-0.09	**0.87**
Eigen values	7.50	4.78

Individual percentages	51.9	33.1
Cumulated percentages	51.9	85.0

AD = days to 50 % anthesis; SD = days to 50 % silk ;ASI = anthesis to silk interval; TL = tassel length; ELL = ear leaf length; ELW = ear leaf width; PLHT = plant height; EHT = ear height; StD = stalk diameter; SG = Stay green; EL = ear length ; EP = ear position; ED = ear diameter; CD = cob diameter; NRE = number of rows per kernel; NKR = number of kernels per row; HKWT = hundred kernel weight; NP = number of plants harvested; NE = number of ears harvested per plot; EN = ear number per plant; KL = kernel length; KW = kernel width; KT = kernel thickness; EWT = ear weight; GWT = grain weight; YLD = yield

Biplots of the PC1 and PC2 for the genotypes and traits are shown in Figure 4.3 and Figure 4.4, respectively. The topography of the accessions biplot showed a wide coverage of associations among genotypes indicating a wide variability and large genetic diversity among the accessions. Two large groups of genotypes were identified, while with few accessions TZm-1132, TZm-1139 and „Obatanpa GH" were separated from the rest. The two uncorrelated groups (180^{o} apart) may be considered for future heterotic groups in inbred line development, and their trait performance may be governed by different sets of alleles. Accessions with tight angles of < 90^{o} and angles exceeding 270^{o} are closely related. For example TZm-1097, TZm-1214, TZm-1185, TZm-1111, TZm-1138, TZm-1100, TZm-1125, TZm-1134, and TZm1112, were closely associated with each other than their association with TZm-1114, TZm-1121, TZm-1147, TZm-1149, and TZm-1132 (Figure 4.3).

Separation of „Obatanpa GH" from other genotypes was not unexpected as it is quality protein genotype. The relationship between TZm-1139 and „Obatanpa GH" was worth noting as it depicted a close association, hence similarity between them. By means of

the vector lengths and location on the biplot, genotypes which contributed most to the variance and were most divergent were „Obatanpa GH“, TZm-1139, TZm-1132, TZm-1149, and TZm-1147.

Hybridization across the two different heterotic groups could enhance maize improvement efforts in sub-Saharan Africa.

All traits contributed positively to the total variance as they skewed more to the right (Figure 4.4). Remarkable associations among traits were ear length, ear leaf length, and ear leaf width; hundred kernel weight and kernel length; stalk diameter and number of kernels per row and their association with anthesis-silking interval. Close associations

that were expected include earliness traits with plant architectural traits and grain yield with yield component traits. However yield and its related traits were uncorrelated with earliness and plant architecture traits in the discrimination o f the genotypes. Fifteen traits out of the twenty-four were most important to the association of the genotypes as they exhibited longest vectors. These traits included plant height, ear height, days to anthesis, days to silking, grain weight, grain yield, hundred kernel weight, ear leaf length, ear position, kernel length, stalk diameter, ear length, ear diameter, kernel width, and ear leaf width.

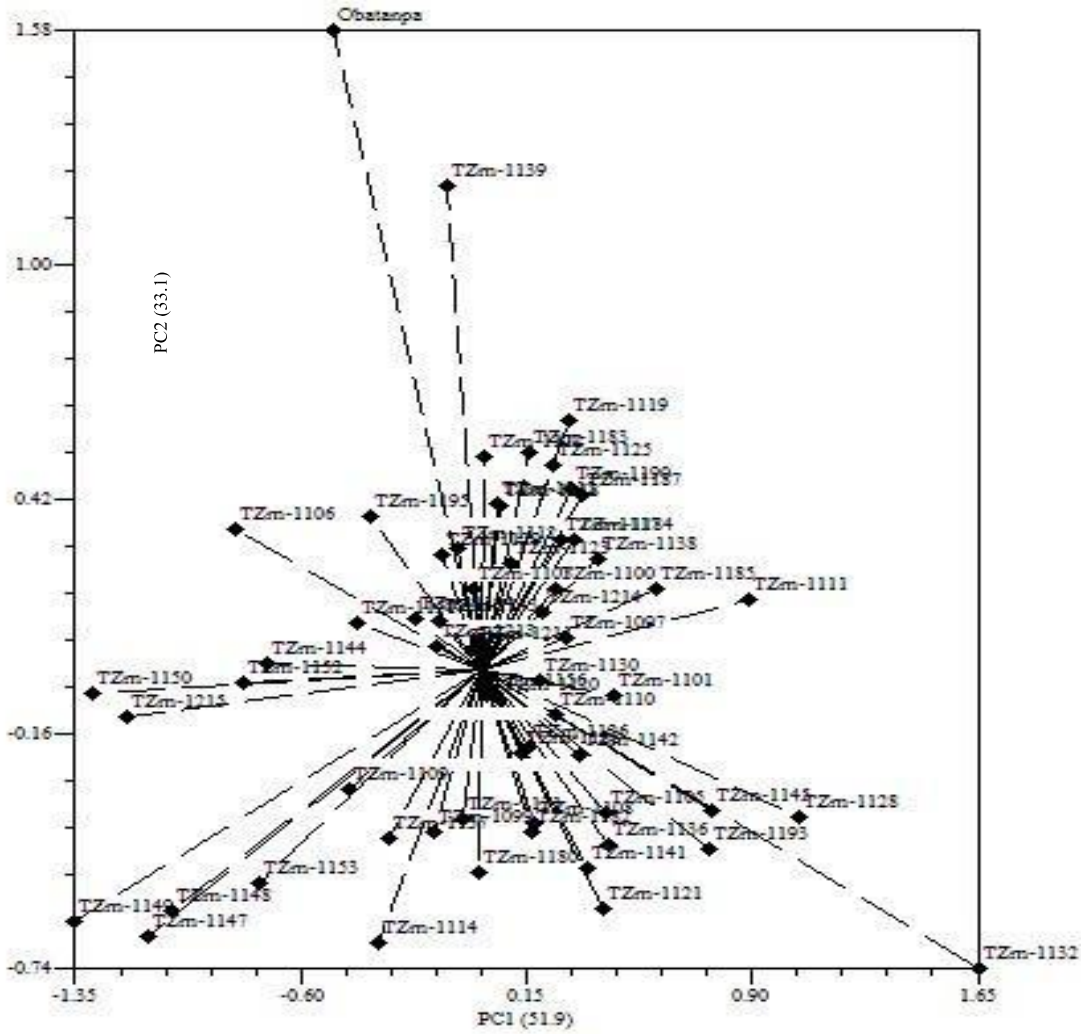


Figure 4.3 Principal components analysis biplot of 60 IPGRI maize accessions held in IITA including a check, “Obatanpa” based on 24 agromorphological traits

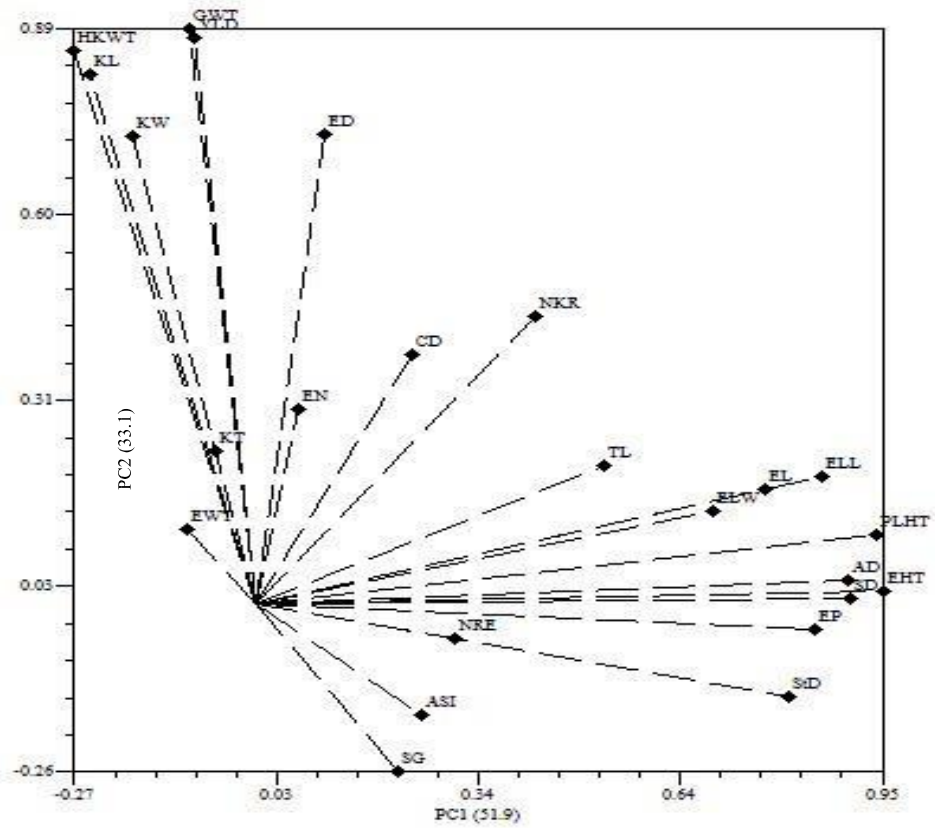


Figure 4.4 Principal component analysis biplot of 24 agro - morphological traits used on the accessions studied.

4.7 Molecular diversity in IPGRI maize accessions

A total of 64 accessions were considered in the molecular genotyping. The additional accessions were TZm-1098, TZm-1116, TZm-1146, and TZm-1189, were lost through damage during the morphological study. Sixteen SSR markers were used across 64 IPGRI genotypes. Figure 4.5 shows a gel picture of amplification products with phi076.

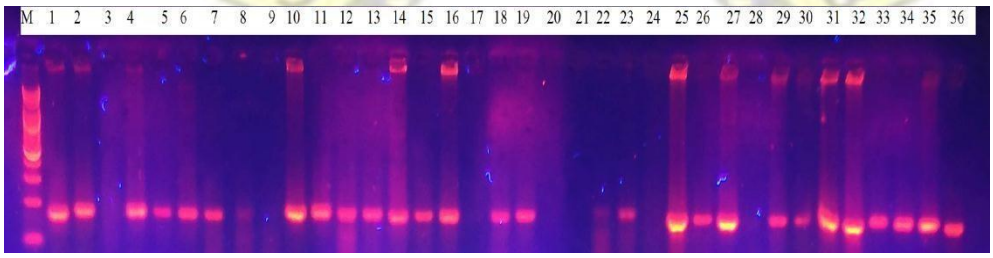


Figure 4.5 Amplification patterns of SSR loci in evaluation of genetic diversity in IPGRI maize. DNA samples were amplified with phi076 and run on 2 % agarose gel. Bands represent first 36 IPGRI maize

genotypes. M=Molecular ladder; 1=TZm-1097; 2=TZm-1099; 3=TZm-1100; 4=TZm-1101; 5=TZm1103; 6=TZm-1105; 7=TZm-1106; 8=TZm-1108; 9=TZm-1109; 10=TZm-1110; 11=TZm-1111; 12=TZm-1112; 13=TZm-1114; 14=TZm-1117; 15=TZm-1118; 16=TZm-1119; 17=TZm-1120; 18=TZm-1121; 19=TZm-1122; 20=TZm-1123; 21=TZm-1125; 22=TZm-1126; 23=TZm-1128; 24=TZm-1129; 25=TZm-1130; 26=TZm-1131; 27=TZm-1132; 28=TZm-1136; 29=TZm-1137; 30=TZm-1138; 31=TZm-1139; 32=TZm-1141; 33=TZm-1142; 34=TZm-1143; 35=TZm-1144; 36=TZm-1145 Lanes without bands show a common feature of SSRs exhibiting no amplification.

The scores representing presence or absence is presented in appendix D.

Of these, two were monomorphic, marker nc133, and phi046. Two other markers, bnlg1525 and bnlg1371 were of low discriminatory power and did not give good resolution of bands. The rate of polymorphism was 85.71 %. Table 4.9 shows the primary statistical data on the 12 polymorphic loci.

Table 4.9 Allele number, observed heterozygosity (H_o) and expected heterozygosity (H_e) of SSR polymorphic loci found in 64 accessions of IPGRI maize landraces held in IITA

	Locus	Bin	Repeat	Repeat Unit	No. of alleles per locus	No. of alleles across genotypes	H_o	H_e
1	phi001	1.03	Di	AG/CT	10	216	0.55	0.81
2	phi056	1.01	Tri	CCG/CGG	5	138	0.52	0.64
3	phi109642	2.03	Tetra	ACGG	9	184	0.08	0.79
4	umc1399	3.07	Tetra	(CTAG)5	5	168	0.28	0.73
5	phi076	4.11	Hexa	AGCGGG	4	162	0.05	0.57
6	phi085	5.07	Penta	AACGC	6	114	0.22	0.71
7	dupssr13	7.04	Di	(CA)12	3	118	0.37	0.59
8	umc1066	7.01	Hexa	(GCCAGA)5	6	188	0.36	0.69
9	phi233376	8.09	Tri	CCG	4	146	0.35	0.61
10	phi100175	8.04	Tetra	AAGC	6	120	0.47	0.76

11	umc1279	9.00	Tri	(CCT)6	5	164	0.66	0.73
12	umc1677	10.05	Tri	(GGC)4	3	108	0.41	0.61
				Total	66	1,826	-	-
				Min	3	108	0.05	0.57
				Max	10	216	0.66	0.81
				Mean	5.64	153.57	0.36	0.69
				SD	2.15	33.99	0.18	0.08
				X^2			2.37	
				Probability			0.15	

The twelve primers together produced 1,826 alleles across the 64 IPGRI maize landraces, and the allele number for SSR loci ranged from 3 for markers dupssr13 and umc1677 to 10 for marker phi001 (Table 4.9). The mean number of alleles per locus was 5.64 ± 2.15 with average observed heterozygosity 0.36±0.18 over a range of 0.05 to 0.66. The expected heterozygosity values ranged from 0.57 to 0.81 with a mean of 0.69 ± 0.08 (Table 4.9).

The mean expected heterozygosity of 0.69 exceeded 0.50 and is an indication of lots of variability within the IPGRI accessions. On the basis of chi square (X^2) value of 2.37 at 1 degree of freedom, the H_o was not significantly different ($P>0.05$) from the tabulated value of 3.84. The nonsignificant difference between the observed and expected heterozygosity, as well as the high value of H_e is indicative of three phenomena, viz., a high mutation rate, a historical admixture event involving previously isolated and differentiated populations, (Bertranpetit and Calafel, 2001; Cavalli-Sforza *et al*., 1994), and/or the high polymorphism of the SSR loci.

The SSR loci employed in fingerprinting studies are known to undergo high mutation rate to give rise to polymorphism. With regard to admixture, the discovery of an artefact

in Nigeria having the imprints of the ear of maize which dates back to 1100 AD provides support to the possible existence of maize in Africa in pre-Columbian times, though this has been a subject of debate among population geneticist for a long time. A possible admixture of the indigenous population with the introduced maize by the Portuguese may be the plausible explanation to the high heterozygosity.

To the best of my knowledge, this is the first study which has examined maize landraces collected from unidentified yet wide locations in Africa, typical of germplasm collections. The high heterozygosity would require further investigations encompassing larger population sizes to validate it. If an admixture occurred in the past, then a heterozygosity as high as this value has been maintained in favour of heterozygotes. Therefore the assumption that the origin of maize in Africa is solely from its introduction from Mexico leaves room for debate and this finding should generate interest in its validation.

This finding of the current research reveals the possibility of Africa as a secondary centre of diversity in maize, hence, a repository of alleles yet to be discovered for maize improvement in breeding programs. It is of prime importance therefore to embark on planned conservation of the African maize landraces which have adapted to the changing environment and must be conserved for both present and future utilization.

The mean expected heterozygosity value in the IPGRI accessions was higher than the reported 0.37 (Hartings *et al*., 2008), 0.46 (Obeng-Antwi *et al*., 2011) and 0.35 (Chittò *et al*., 2000) based on AFLP on Italian maize and, Ghana landraces and inbred lines,

respectively. The high expected heterozygosity value of current study is probably due to the high levels of allelic diversity of SSR primers than AFLP, RFLP or SNP loci

(Senior *et al*., 1998) and hence the high level of its polymorphism in maize (Senior and Heun, 1993).

The mean expected heterozygosity value was similar to that reported by Legesse *et al*. (2007) (0.58) on Ethiopian maize inbred lines, Senior *et al*. (1998) (0.59) on U.S. maize inbred lines, and 0.62 of Smith *et al*. (1997). High heterozygosity values are indicative of wide diversity in alleles which may be exploited for crop improvement in a wide genetic base.

All loci demonstrated expected heterozygosity values greater than 0.50, with over 83 % having PIC values greater than 0.60. Mean allele diversity was greatest in tetrarepeats (6.67), followed by di (6.50), hexa- (5.0), penta- (6.0), and the least being the tri- (0.45) repeats (Table 4.10). The three loci that were predominantly present across the genotypes were phi001 (216 alleles), umc1066 (188 alleles) and phi109642 (184 alleles), whereas loci umc1677 (108 alleles) occurred least among the genotypes (Table 4.9). The occurrence of 10 alleles per locus for phi001 was not unexpected as di-repeats typically show multiple alleles (Enoki *et al*., 2002; Senior *et al*., 1998; Smith *et al*., 1997) although they also produce additional stutter bands. In this work, stutter bands were carefully identified and excluded during band scoring.

Table 4.10 Mean PIC score summary statistics by repeat class

Repeat class	Mean number of alleles	Mean number of allelic loci across genotypes	Mean PIC value
Di	6.50	167.00	0.70

Tri	4.50	139.00	0.65
Tetra	6.67	157.33	0.76
Penta	6.00	144.00	0.71
Hexa	5.00	175.00	0.63

4.8 Genetic similarity estimates of molecular data

The genetic similarity estimates among the 64 IPGRI maize landraces was determined using the Dice (1945) coefficient distance measure. The pairwise distance measure ranged from 0.00 (TZm-1109/TZm-1211, TZm-1148/TZm-1211, TZm1116/TZm1211, TZm-1132/TZm-1211, TZm-1189/TZm-1211 and TZm-1130/ TZm-1187) to 1.00 (TZm-1150/TZm-1152) with an average of 0.49 ± 0.14 (Figure 4.5; Appendix C). Of the 2,016 pairwise distances, 997 pairs, equivalent to 49.45 %, had genetic distances below 0.5. These were deemed to be pairs that were minimally related. Of the remaining (50.5 %) pairs that demonstrated similarity coefficient beyond 0.5, as few as 25 pairs (1.24 %) had genetic similarities greater than or equal to 0.80. This pattern of low genetic similarities supports a wide genetic diversity and abundance of alleles as well as continued evolutionary mechanism to maintain it. Genotypes within a cluster have much genetic elements in common than intercluster genotypes. Therefore for breeding purposes the combined effects of genotypes from different clusters would be best.

The UPGMA cluster analysis revealed six clusters, three of which were highly heterogeneous, two were individually distinct genotypes and did not cluster with the rest of the accessions, while two other genotypes that were similar to the extent of forming a single cluster (Figure 4.5). Cluster I had 6 accessions representing 9.4 %, cluster II 17 genotypes (26.6 %), cluster III 37 accessions (57.8 %), cluster IV 1

accession (1.6 %), cluster V with 2 accessions (3.1 %) and finally cluster VI had one member (1.6 %) (Table 4.11). Cluster I membership included TZm-1097, TZm-1098, TZm-1099, TZm-1137, TZm-1213, and TZm-1194. This cluster showed mean genetic similarity of 0.53 ± 0.12 with no subclusters (Table 4.11).

Cluster II was made up of TZm-1100, TZm-1125, TZm-1119, TZm-1123, TZm-1121, TZm-1122, TZm-1180, TZm-1190, TZm-1182, TZm-1187, TZm-1184, TZm-1214, TZm-1185, TZm-1183, TZm-1188, TZm-1215 and TZm-1189. This cluster was heterogeneous with a mean genetic similarity of 0.59±0.13 and produced 2 subclusters (Table 4.11). The six accessions of subcluster IIA were fairly similar with average genetic distance of 0.60±0.09 and range of 0.39 (TZm-1100/TZm-1119) to 0.73 (TZm-1119/TZm-1123). Subcluster IIB consisted of 11 accessions with a mean genetic distance of 0.68±0.11. The most distantly related accession in this group was TZm-1184/TZm-1188 (0.40) while TZm-1184/TZm-1214 (0.92) was the most closely related.

Cluster III was the cluster with the largest number of accessions and the most heterogeneous with an average genetic distance of 0.54±0.13 and a range of 0.27 (TZm1138/TZm-1195) to 1.00 (TZm-1150/TZm-1152). It produced two large subclusters (Table 4.12). Subcluster IIIA had 25 accessions with a mean distance of 0.58±0.12. Subcluster IIIB consisted of 12 accessions with a mean genetic distance of 0.66 ± 0.12. The closest pair was between TZm-1142/TZm-1145 (0.91) while TZm-1141/Zm-1148 (0.43) was the least similar.

Cluster IV and cluster VI were each made of only one genotype TZm-1149 and TZm1211, respectively. On the other hand, Cluster V was made up of two accessions TZm-1118, and TZm-1138 with a high similarity coefficient of 0.73.

The current study was in agreement with Chittò *et al*. (2000) who obtained 6 cluster groupings with a mean genetic distance of 0.44 using AFLPs on 71 Italian maize inbred lines. Beyene *et al*. (2005) however, obtained 5 cluster groupings with a mean genetic similarity of 0.49 using 20 SSR markers on 62 traditio nal Ethiopian highland maize accessions. Magorokosho (2006) on the other hand identified 3 cluster groupings with mean genetic similarity of 0.652 on 99 African maize accessions from Zambia, Zimbabwe and Malawi using SSR markers. A genetic similarity of 0. 652 among American maize landraces was also obtained by Matsuoka *et al*. (2002). The majority of accession pairs with a relatively lower similarity values is an indication that the IPGRI collection is highly diverse.

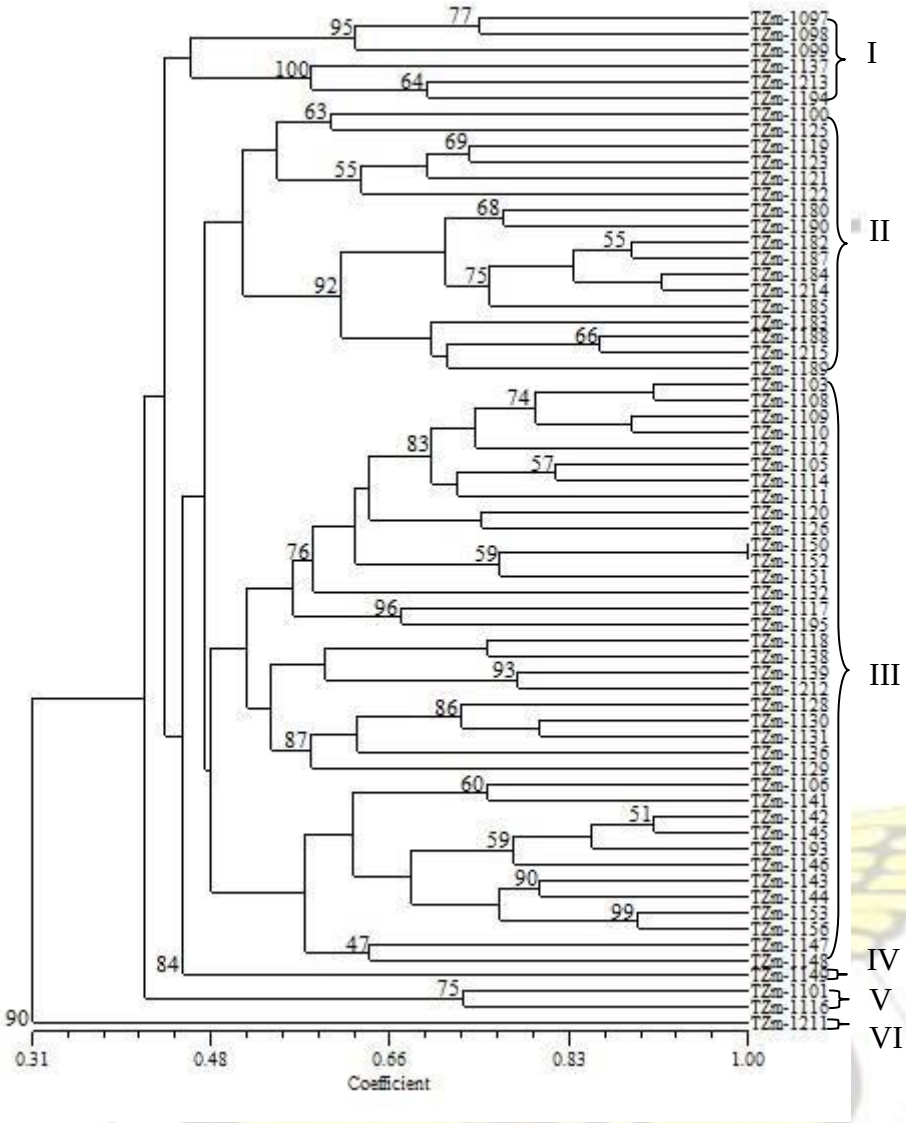


Figure 4.6 UPGMA dendrogram generated from Dice‟s similarity coefficients for 64 IPGRI maize landraces held in IITA with 12 SSR markers. Bootstrap values are shown at nodes of the tree plot.

Table 4.11 Clusters of the 64 IPGRI maize landraces held in IITA generated from SSR loci based on Dice coefficient genetic distance

Cluster	Subcluster	Accession	Number of accessions	Percentage

I		TZm-1097, TZm-1098, TZm-1099, TZm-1137, TZm-1213,TZm-1194	6	9.4
II	A	TZm-1100, TZm-1125, TZm-1119, TZm-1123,TZm-1121, TZm-1122	6	9.4
	B	TZm-1180, TZm-1190, TZm-1182, TZm-1187, TZm-1184, TZm-1214, TZm-1185, TZm-1183, TZm-1188, TZm-1215, TZm-1189	11	17.2
III	A	TZm-1103, TZm-1108, TZm-1109, TZm-1110, TZm-1112, TZm-1105, TZm-1114, TZm-1111, TZm-1120, TZm-1126, TZm-1150, TZm-1152, TZm-1151, TZm-1132, TZm-1117, TZm-1195, TZm-1118, TZm-1138, TZm-1139, TZm-1212, TZm-1128, TZm-1130, TZm-1131, TZm-1136, TZm-1129	25	39.1
	B	TZm-1106, TZm-1141, TZm-1142, TZm-1145, TZm-1193, TZm-1146, TZm-1143, TZm-1144, TZm-1153, TZm-1156, TZm-1147, TZm-1148	12	18.8
IV		TZm-1149	1	1.6
V		TZm-1101, TZm-1116	2	3.1
VI		TZm-1121	1	1.6

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The main goal of the study was to estimate the level of genetic diversity and relationships among 64 tropical IPGRI maize landraces held in the repository of the IITA using agromorphological traits and SSR markers. Motivation for the study was that, the IPGRI maize collection has hitherto not been evaluated to identify their utility in breeding programs, nor to inform conservation management, and to identify traits that are important for grain improvement. This goal has become more important than ever especially at a time when population escalation, dwindling arable land, climate anomalies and hostile climate projections are having a negative impact on the continent

of Africa. The negative impacts include reduced agricultural production despite efforts to breed for improved cultivars, primarily due to use of old breeding stocks and use of cultivars that have been bred from few exotic genotypes characterized by genetic uniformity, so they easily succumb to the current harsh environmental challenges. Unfortunately, in doing so, landraces which represent a reservoir of genes for trait improvement are relegated, resulting in genetic erosion.

Although comparative studies on foreign maize have been studied to a great extent, information on the African landraces is absent. Information on the historical events that have driven the genetic character of maize in Africa is required to explain the genetic characteristics and reveal their usefulness. There is dearth of information regarding the passport data of the IPGRI accessions. There has been no single study to evaluate the genetic diversity within and among the IPGRI maize accessions.

Traits that are most important in African maize include drought tolerance, high yield, and earliness. It has therefore become necessary to embark on aggressive search for genotypes that have adapted to the changing climate of Africa for sources of alleles for crop improvement, hence the current research. The objectives were 1) To ascertain that indeed the IPGRI landraces are reserves of genetic diversity; 2) To determine the historical events that have led to the evolution of the landraces; 3) To characterize the genotypes and identify those with desirable traits for exploitation in breeding programmes; 4) To assemble the IPGRI population into groups on the basis of genetic distance; and finally, 5) To investigate the heritability, genotypic and phenotypic correlations among the exhibited traits.

Sixty-four IPGRI accessions and a check were evaluated in 2011 and 2012 in Ghana to determine their phenomorphological characters based on 5 qualitative and 24

quantitative traits derived from the IPGRI/CIMMYT maize descriptor list. On the basis of frequencies of accessions within the 5 qualitative traits, considerable variation was identified among the landraces for most traits except cob colour and principal grain colour which were dominated by other colours especially yellow kernels borne on white cobs. Kernel texture exhibited a wide variability with almost equal proportions of dent (44.32 %) and flint (55.68 %) kernels. The fairly equal distribution of dent and flint is in consonance with the general knowledge that maize in Africa was introduced by the Portuguese who brought in white and dent types, whereas the flints and yellows were introduced by the Arabs across the Mediterranean. An overall description of the qualitative characteristics of the IPGRI accessions is yellow dent or flint kernels borne in regular and mixed arrangements on white cobs.

The large phenotypic variability, supported by both coefficient of variability and mean squares exhibited by all traits except number of ears per plant was an indication of ample genetic diversity arising from a variety of alleles accumulated with time. It is therefore possible to breed for improvement in these traits by selection.

On earliness, six genotypes outperformed the check „Obatanpa GH" by about 1 to 5 days for anthesis and 1 to 7 days for silking. These early-maturing genotypes which may be incorporated in breeding programs for drought escape include TZm-1149, TZm1148, TZm-1150, TZm-1147, TZm-1153, and TZm-1152. Among the six, TZm1150 and TZm-1152 exhibited high yield of 4.4 $Mgha^{-1}$. The combined earliness and high yield is an important trait that breeders strive to achieve as there is usually a trade-off between earliness and yield. The high heritability of earliness traits, indicative of large genetic effects, possibly additive gene effects demonstrates the high possibility of making progress in earliness by simple selection regimes. The identification of

earlymaturing yet high yielding and short plants substantiate the fact that the landraces possess unique genes which have not yet been exploited.

The two protogynous genotypes, TZm-1106 and TZm-1183 as well as twelve genotypes with mean ASI of 0 days (TZm-1103, TZm-1108, TZm-1118, TZm-1123, TZm-1125, TZm-1126, TZm-1136, TZm-1142, TZm-1182, TZm-1188, TZm-1190 and TZm-1215) would be good genotypes to consider for drought tolerance as their ASI values are very short. However, the low heritability of ASI indicates large environmental influence and a rather little genetic component. The implications for breeding for short ASI is that the trait may be controlled by dominance and epistatic gene effects, rather than additive and so progress in selection may be achieved rather slowly.

The occurrence of protogynous genotypes among the IPGRI collection suggests the presence of ancestral traits common with the teosintes. This finding is expected to engender active research into the origin and evolution of the IPGRI genotypes.

Twenty-seven accessions of the IPGRI genotypes demonstrated yield performance of 4.2 to 6.2 $Mgha^{-1}$ comparable to the highest maize productivity in Africa of 4.2 $Mgha^{1}$ achieved in South Africa. These accessions may be beneficial to breeding programs. They include; TZm-1185, TZm-1142, TZm-1213, and TZm-1129 all having a yield of 4.2 $Mgha^{-1}$, TZm-1143 and TZm-1215 (4.3 $Mgha^{-1}$), TZm-1150, TZm-1211, TZm- 1152, and TZm-1101 (4.4 $Mgha^{-1}$), and TZm-1123, TZm-1100, TZm-1138, and TZm1112 (4.6 $Mgha^{-1}$). The other high-yielding accessions were TZm-1212, TZm-1130, TZm-1190, TZm-1118, and TZm-1183 (4.7 $Mgha^{-1}$), TZm-1106 andTZm-1144 (4.8 $Mgha^{-1}$), TZm-1122 (4.9 $Mgha^{-1}$), TZm-1125 and TZm-1117 (5.0 $Mgha^{-1}$), TZm-1119

(5.4 $Mgha^{-1}$), and finally, TZm-1139 (6.2$Mgha^{-1}$).

The low heritability of yield indicates little genetic contribution but large environmental influence. The consequence for breeding for yield is that the trait may be controlled by dominance and epistatic gene effects, rather than additive and hence progress in selection may be achieved rather slowly.

Besides the strong correlation of grain yield with hundred kernel weight and kernel length, all other pairwise correlations with grain yield were weak and not significant.
Therefore, among the IPGRI genotypes, opportunity to improve traits by means of correlated response to selection may not be applicable. Selection at early developmental stages may not be possible. Notwithstanding this observation, some genotypes with combined earliness and high grain yield were identified.

The identification of a wide genetic distance estimated by a similarity coefficient of 0.27±0.18 means the collection is highly variable, represents a rich reserve of alleles and may be used to widen the genetic base of maize genepools in breeding programmes. In the absence of geographical information on origin of the accessions, grouping of the accessions into two main clusters on the basis of high yield and yield component traits, earliness and short plant height indicates two major mechanisms controlling maize performance within the IPGRI accessions, a combined earliness and yield on one hand, and tall late-maturing but low grain yield on the other hand. This splits the germplasm into two major classes, 37 accessions in cluster I rich in earliness and grain yield genes, and 24 accessions in cluster II for fodder production.

The PCA biplots confirmed the two major groupings however; the topography revealed four correlated groups which may be incorporated into two existing heterotic groups in breeding groups for hybrid breeding. Fifteen traits out of the twenty-four contributed most to the variance, and these traits may be relied on with high accuracy in future maize genetic diversity. The traits include anthesis and silking days, plant height, ear height, ear leaf length, ear position, stalk diameter, hundred kernel weight, kernel length, grain weight and grain yield as the most contributors to the variance (0.81 - 0.95) and to a relatively lesser extent, ear length, ear diameter and ear leaf width (0.69-0.77).

A polymorphism rate of 85 % and 1,826 alleles across the 64 genotypes with high mean number of alleles per locus of 5.64 ± 2.15 indicates richness of alleles and large diversity for the IPGRI genotypes preserved overtime. The high expected mean heterozygosity of 0.69 ± 0.08 in addition to the high mean allele number per locus is an indication of lots of variability within the IPGRI accessions. This could be the result of current evolutionary events such as mutation, as well as forces including balancing selection that have contributed in maintaining this level of heterozygosity from as it were, the time of maize introduction into Africa or the time of admixture of two major isolated populations. These two populations may be the documented maize introduction from Mexico and possibly, maize which may have existed in preColombian era when the Portuguese arrived on the West Coast in 1500. Further studies are required to substantiate the processes that have led to the high heterozygosity in the African maize landraces.

The wide genetic distance produced by Dice (1945) similarity coefficient of 0.49±0.14 together with the six cluster groupings of the accessions revealed by the UPGMA cluster algorithm implies that the accessions are divergent. The divergence

may probably have arisen from segregation after an initial hybridization event involving two or more differentiated populations. The genetic distance estimate therefore supports the level of heterozygosity in the IPGRI accessions.

The genetic similarity distances of 0.14 ± 0.15 and 0.49 ± 0.14 by both agromorphological and molecular pairwise associations, respectively, confirm variability for the IPGRI genotypes and prove the reliability of these two techniques in the evaluation of genetic diversity in maize.

Overall, the findings suggest (i) that indeed the IPGRI landraces held in IITA are reserves of large genetic diversity, (ii) the genotypes contain in them inherent and desirable alleles that have not been exploited and therefore can be used to improve maize breeding in Africa, (iii) putative heterotic groups on the basis of genetic distance, (iv) a unique correlation between earliness traits and grain yield, (v) the genotype TZm-1139 outperformed the check, „Obatanpa GH" in earliness, height and grain yield, (vi) had identified some accessions that show protogyny and can be used for breeding against drought.

The major limitations to this study were the lack of passport data regarding the IPGRI accessions and the use of few number of SSR loci in the molecular profiling.

5.2 Recommendations

- Any future study should involve more SSR primers proportional in number to the number of accessions.
- Further research work should be carried to ascertain the protogynous nature of some genotypes identified.

- More sensitive methods of gel electrophoresis such as polyacrylamide gels with silver staining should be used to give more highly resolved SSR bands.
- Further studies are required to ascertain the physiological mechanism which governs combined earliness and high yield in some identified genotypes.
- There is the need to study narrow sense heritability in future research of the IPGRI genotypes.

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APPENDICES APPENDIX A. PREPARATION OF REAGENTS

1. CTAB

2% CTAB (Hexadecyl trimethyl-ammonium bromide)
100 mM TrisHCl {pH = 8}
20 mM EDTA
1.4 M NaCl
0.1% (w/v) PVP (polyvinyl polypyrrolidine)
0.2% β-mercaptoethanol { added just before use}
0.1 mg/mL proteinase K {added just before use}

2. TE buffer (1000 ml)

1 M Tris pH 8.0 10 ml.
0.5 M EDTA pH 8.0 2 ml.
5 M NaCl 200 ml.
dH_2O complete volume to 1000 ml

3. Chloroform:isoamyl alcohol (24:1) Measure 960 ml/L Chloroform in beaker.

   Add 40 ml/L Isoamyl alcohol into the beaker.

4. Phenol/chloroform (1:1v/v)

   Weigh out 20g phenol in a glass beaker.

   Add 20ml chloroform cover with cling film and mix well over a period of a few hours until all the phenol has dissolved.

5. 5 M NaCl

   5 M sodium chloride
   Dissolve 292.2 g of sodium chloride (NaC1; M.W. 58.44) in 800 ml of H2O.
   Adjust the volume to 1 liter with H2O. Sterilize
   by autoclaving.

6. 10 mM Tris-HC1 (pH 8.0) /9
   Dissolve 121.1 g of Tris base in 800 ml of H_2O.
   Add 42 ml concentrated HCl.
7. 1.5 M $MgCl_2$

   Dissolve 20.33 g $MgCl_2$ (M. W. 203.30) in dH_2O to a final volume of 100 ml.

   Autoclave.

8. 1 M potassium chloride (KC1)
   Dissolve 7.45 g of potassium chloride (KC1; M.W. 74.55) in 80 mL of water and adjust volume to 100 ml with distilled H_2O.

9. Ethidium bromide solution (10 mg/mL)

Add 1 mg of ethidium bromide to 100 mL of water.
Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved.

10. 1% Agarose

Weigh 1g of agarose and add 200 ml of 10 X TBE and heat in a micro wave.

11. 2% agarose gel
Weigh 2 g of agarose and add 400 ml of the 10 X TBE and heat in a micro wave.

12. 80% ethanol (100 ml)

Measure and mix 80 ml of absolute ethanol, and 20 ml distilled water.

13. Washing buffer

Prepare 1 X Wash Buffer by mixing the 1-L 10 X concentrate with 9 L of deionized water.

14. 250µM dNTP,

dNTP mix (2.5 mM each of dCTP, dGTP, dATP, and dTTP)
To mix, place 250 µl of each nucleotide in a 10 ml tube and add 9000 µl of sterile ddH_2O to obtain a 2.5 mM concentration of each nucleotide.

15. 1 Liter10X TBE
Dissolve 108g Tris base, 55g Boric acid, and 40mls of 0.5M EDTA (pH 8.0) in 600ml ddH_2O. Adjust volume to 1 litre with deionized water and autoclave for 20 min.

16. 1 L 70 % ethanol,

700 ml of absolute ethanol

17. 1% agarose gel.

1 g Agarose dissolved in 100 ml TBE

18. . 0.5 M EDTA

Dissolve 186.12 g Na2EDTA.2H20 (MW=372.24) in approx. 750 ml of dH2O.

19. Add NaOH pellets to bring pH to 8.0. After EDTA is in solution, bring to 1000 ml with dH2O.

Autoclave

APPENDIX B. MORPHOLOGICAL DISTANCE MATRIX

	Obatanpa	TZm-1097	TZm-1099	TZm-1100	TZm-1101	TZm-1103	TZm-1105	TZm-1106	TZm-1108	TZm-1109	TZm-1110	TZm-1111	TZm-1112	TZm-1117	TZm-1118	TZm-1119	TZm-1120	TZm-1121	TZm-1122	TZm-1123	TZm-1125	TZm-1126	TZm-1128	TZm-1129	TZm-1130
Obatanpa																									
TZm-1097	0.07																								
TZm-1099	0.07	0.08																							
TZm-1100	0.07	0.00	0.12																						
TZm-1101	0.64	0.03	0.08	0.09																					
TZm-1103	0.09	0.08	0.52	0.24	0.08																				
TZm-1105	0.26	0.51	0.02	0.13	0.06	0.10																			

TZm-1106	0.28	0.21	0.01	0.02	0.01	0.04	0.49						

TZm-1108	0.24	0.21	0.02	0.08	0.05	0.12	0.28	0.08																	
TZm-1109	0.01	0.07	0.01	0.13	0.00	0.09	0.05	0.00	0.01																
TZm-1110	0.11	0.26	0.01	0.07	0.00	0.05	0.42	0.28	0.66	0.01															
TZm-1111	0.08	0.01	0.07	0.03	0.02	0.05	0.12	0.25	0.26	0.15	0.32														
TZm-1112	0.22	0.03	0.08	0.13	0.10	0.01	0.25	0.01	0.28	0.14	0.12	0.18													
TZm-1114	0.15	0.22	0.04	0.04	0.00	0.00	0.23	0.37	0.41	0.00	0.32	0.22	0.08												
TZm-1117	0.06	0.00	0.03	0.27	0.34	0.02	0.00	0.03	0.15	0.00	0.24	0.28	0.01												
TZm-1118	0.04	0.03	0.00	0.00	0.01	0.02	0.06	0.25	0.10	0.03	0.00	0.00	0.17	0.00											

TZm-1119	0.12	0.04	0.15	0.17	0.04	0.07	0.01	0.01	0.01	0.01	0.00	0.03	0.01	0.04	0.20										
TZm-1120	0.05	0.09	0.31	0.28	0.00	0.27	0.28	0.19	0.01	0.22	0.04	0.06	0.01	0.00	0.02	0.04									
TZm-1121	0.60	0.08	0.01	0.06	0.46	0.01	0.23	0.36	0.02	0.00	0.05	0.00	0.00	0.05	0.46	0.13	0.06								
TZm-1122	0.20	0.27	0.00	0.00	0.01	0.00	0.25	0.48	0.06	0.00	0.27	0.09	0.00	0.01	0.34	0.08	0.09	0.32							
TZm-1123	0.00	0.00	0.30	0.33	0.08	0.18	0.01	0.03	0.13	0.05	0.22	0.16	0.05	0.23	0.00	0.07	0.27	0.03	0.02						
TZm-1125	0.11	0.04	0.08	0.14	0.01	0.08	0.01	0.30	0.00	0.23	0.04	0.05	0.04	0.02	0.35	0.18	0.01	0.20	0.45	0.10					
TZm-1126	0.12	0.47	0.11	0.00	0.03	0.04	0.62	0.16	0.54	0.01	0.46	0.03	0.11	0.11	0.00	0.01	0.16	0.06	0.04	0.01	0.01				
TZm-1128	0.66	0.00	0.09	0.05	0.49	0.07	0.02	0.22	0.00	0.00	0.01	0.04	0.01	0.10	0.22	0.30	0.01	0.60	0.16	0.01	0.26	0.02			
TZm-1129	0.15	0.01	0.06	0.41	0.10	0.12	0.09	0.28	0.15	0.03	0.03	0.00	0.02	0.08	0.32	0.00	0.19	0.45	0.06	0.14	0.02	0.00	0.33		

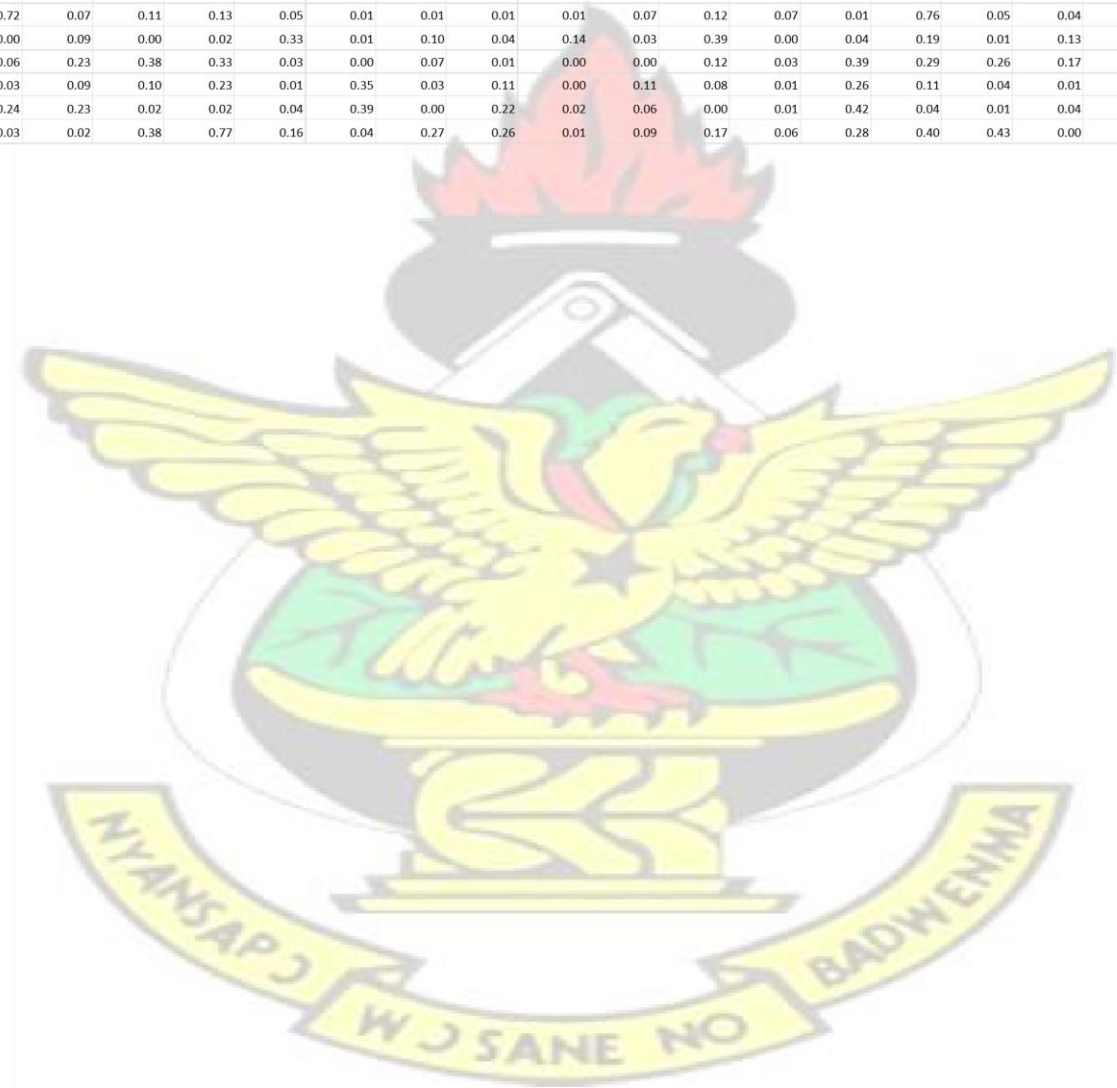
TZm-1130	0.21	0.12	0.13	0.00	0.11	0.16	0.06	0.10	0.06	0.04	0.08	0.05	0.01	0.03	0.03	0.00	0.05	0.13	0.15	0.00	0.21	0.01	0.13	0.00	

TZm-1131	0.23	0.41	0.03	0.03	0.23	0.02	0.22	0.35	0.00	0.24	0.03	0.08	0.01																						
TZm-1132	0.64	0.02	0.10	0.01	0.25	0.06	0.07	0.30	0.05	0.12	0.02	0.27	0.06																						
TZm-1136	0.14	0.00	0.05	0.28	0.00	0.36	0.01	0.07	0.29	0.01	0.18	0.37	0.00																						
TZm-1137	0.02	0.01	0.00	0.08	0.31	0.02	0.01	0.21	0.00	0.01	0.03	0.22	0.01																						
TZm-1138	0.25	0.22	0.00	0.01	0.34	0.08	0.08	0.01	0.39	0.10	0.11	0.00	0.20																						
TZm-1139	0.49	0.00	0.20	0.00	0.05	0.14	0.18	0.40	0.25	0.10	0.35	0.58	0.21																						
TZm-1141	0.21	0.00	0.02	0.03	0.01	0.07	0.13	0.66	0.00	0.05	0.02	0.13	0.01																						
TZm-1142	0.16	0.07	0.05	0.02	0.08	0.11	0.07	0.08	0.35	0.01	0.31	0.03	0.00																						
TZm-1143	0.03	0.00	0.23	0.18	0.01	0.10	0.07	0.17	0.14	0.01	0.16	0.35	0.01																						
TZm-1144	0.26	0.35	0.06	0.02	0.03	0.10	0.74	0.51	0.35	0.15	0.39	0.26	0.13																						
TZm-1145	0.62	0.04	0.28	0.00	0.51	0.13	0.10	0.23	0.08	0.04	0.03	0.06	0.14																						
TZm-1147	0.29	0.03	0.10	0.23	0.21	0.06	0.20	0.14	0.14	0.13	0.01	0.09	0.21																						
TZm-1148	0.20	0.12	0.32	0.38	0.09	0.21	0.35	0.24	0.05	0.26	0.08	0.02	0.04																						
TZm-1149	0.49	0.14	0.13	0.00	0.49	0.26	0.18	0.06	0.49	0.03	0.22	0.10	0.24																						
TZm-1150	0.65	0.11	0.00	0.00	0.19	0.03	0.31	0.49	0.43	0.04	0.32	0.40	0.14																						
TZm-1151	0.67	0.03	0.01	0.20	0.44	0.02	0.21	0.23	0.13	0.08	0.12	0.09	0.17																						
TZm-1152	0.23	0.02	0.15	0.23	0.03	0.16	0.27	0.37	0.03	0.36	0.08	0.18	0.03																						
TZm-1153	0.22	0.02	0.02	0.42	0.20	0.01	0.11	0.01	0.06	0.17	0.03	0.01	0.25																						

TZm-1156	0.00	0.04	0.14	0.00	0.01	0.04	0.01	0.05	0.00	0.12	0.00	0.17	0.10	0.01	0.06	0.00	0.07	0.08	0.01	0.02	0.00	0.04	0.00	0.09	0.22
TZm-1180	0.47	0.07	0.17	0.11	0.42	0.40	0.06	0.06	0.48	0.09	0.20	0.06	0.06	0.00	0.00	0.08	0.02	0.21	0.02	0.04	0.13	0.16	0.19	0.02	0.23
TZm-1182	0.20	0.31	0.17	0.09	0.03	0.25	0.56	0.72	0.04	0.02	0.14	0.04	0.00	0.00	0.19	0.00	0.42	0.33	0.30	0.02	0.09	0.34	0.10	0.34	0.06
TZm-1183	0.06	0.24	0.18	0.32	0.02	0.21	0.02	0.06	0.19	0.37	0.13	0.01	0.00	0.01	0.01	0.06	0.12	0.01	0.11	0.24	0.40	0.04	0.02	0.22	0.30

TZm-1184	0.00	0.11	0.07	0.00	0.08	0.02	0.22	0.11	0.34	0.11	0.54	0.33	0.11	0.15	0.09	0.14	0.26	0.02	0.12	0.00	0.02	0.21	0.11	0.02	0.00
TZm-1185	0.17	0.02	0.06	0.04	0.04	0.01	0.00	0.07	0.02	0.16	0.00	0.06	0.03	0.00	0.10	0.01	0.10	0.01	0.01	0.09	0.00	0.00	0.14	0.04	0.00
TZm-1187	0.01	0.13	0.01	0.19	0.00	0.06	0.10	0.09	0.39	0.16	0.25	0.07	0.00	0.31	0.00	0.00	0.01	0.01	0.00	0.16	0.01	0.39	0.01	0.05	0.00
TZm-1188	0.20	0.18	0.09	0.03	0.13	0.01	0.18	0.04	0.53	0.01	0.28	0.03	0.22	0.01	0.26	0.09	0.05	0.01	0.00	0.02	0.08	0.39	0.00	0.01	0.00
TZm-1190	0.00	0.09	0.10	0.63	0.00	0.17	0.01	0.00	0.18	0.24	0.04	0.01	0.02	0.06	0.00	0.15	0.15	0.01	0.01	0.14	0.18	0.04	0.00	0.40	0.14
TZm-1193	0.47	0.04	0.07	0.01	0.07	0.05	0.21	0.58	0.16	0.02	0.22	0.47	0.03	0.02	0.17	0.17	0.17	0.37	0.43	0.02	0.40	0.05	0.43	0.10	0.40
TZm-1194	0.19	0.21	0.00	0.01	0.21	0.07	0.02	0.07	0.14	0.30	0.02	0.00	0.02	0.02	0.03	0.00	0.02	0.04	0.00	0.00	0.14	0.07	0.11	0.03	0.20
TZm-1195	0.65	0.04	0.00	0.08	0.72	0.07	0.11	0.13	0.05	0.01	0.01	0.01	0.01	0.07	0.12	0.07	0.01	0.76	0.05	0.04	0.05	0.09	0.55	0.28	0.09
TZm-1211	0.00	0.04	0.06	0.21	0.00	0.09	0.00	0.02	0.33	0.01	0.10	0.04	0.14	0.03	0.39	0.00	0.04	0.19	0.01	0.13	0.00	0.08	0.07	0.58	0.05
TZm-1212	0.16	0.43	0.44	0.24	0.06	0.23	0.38	0.33	0.03	0.00	0.07	0.01	0.00	0.00	0.12	0.03	0.39	0.29	0.26	0.17	0.01	0.27	0.06	0.27	0.00
TZm-1213	0.03	0.04	0.10	0.12	0.03	0.09	0.10	0.23	0.01	0.35	0.03	0.11	0.00	0.11	0.08	0.01	0.26	0.11	0.04	0.01	0.00	0.03	0.04	0.36	0.02
TZm-1214	0.04	0.06	0.09	0.16	0.24	0.23	0.02	0.02	0.04	0.39	0.00	0.22	0.02	0.06	0.00	0.01	0.42	0.04	0.01	0.04	0.04	0.01	0.01	0.09	0.03
TZm-1215	0.34	0.16	0.08	0.02	0.03	0.02	0.38	0.77	0.16	0.04	0.27	0.26	0.01	0.09	0.17	0.06	0.28	0.40	0.43	0.00	0.15	0.21	0.27	0.22	0.05

Appendix B: Cont"d



	TZm-1131	TZm-1132	TZm-1136	TZm-1137	TZm-1138	TZm-1139	TZm-1141	TZm-1142	TZm-1143	TZm-1144	TZm-1145	TZm-1147	TZm-1148	TZm-1149	TZm-1150	TZm-1151	TZm-1152	TZm-1153	TZm-1156	TZm-1180	TZm-1182	TZm-1183	TZm-1184	TZm-1185	TZm-1187	TZm-1188	TZm-1190	TZm-1193	TZm-1194	TZm-1195	TZm-1196	TZm-1211	TZm-1212	TZm-1213	TZm-1214
TZm-1132	0.03																																	
TZm-1136	0.00	0.22																																
TZm-1137	0.02	0.05	0.05																															
TZm-1138	0.11	0.00	0.03	0.24																														
TZm-1139	0.01	0.64	0.34	0.14	0.00																													
TZm-1141	0.18	0.51	0.04	0.29	0.07	0.34																												
TZm-1142	0.11	0.03	0.26	0.07	0.30	0.06	0.01																											
TZm-1143	0.00	0.13	0.10	0.33	0.00	0.24	0.09	0.00																										
TZm-1144	0.10	0.21	0.05	0.02	0.03	0.24	0.24	0.12	0.05																									
TZm-1145	0.31	0.45	0.08	0.00	0.13	0.36	0.13	0.03	0.10	0.09																								
TZm-1147	0.01	0.20	0.00	0.01	0.28	0.05	0.10	0.09	0.00	0.28	0.06																							
TZm-1148	0.06	0.11	0.00	0.02	0.06	0.06	0.22	0.11	0.08	0.44	0.00	0.46																						
TZm-1149	0.08	0.12	0.14	0.09	0.59	0.17	0.00	0.21	0.11	0.12	0.37	0.20	0.03																					
TZm-1150	0.08	0.57	0.41	0.01	0.18	0.57	0.30	0.26	0.09	0.56	0.30	0.41	0.34	0.39																				
TZm-1151	0.08	0.40	0.03	0.03	0.22	0.37	0.19	0.08	0.00	0.19	0.23	0.37	0.49	0.38	0.54																			
TZm-1152	0.02	0.32	0.01	0.01	0.00	0.24	0.49	0.04	0.00	0.46	0.01	0.38	0.80	0.01	0.45	0.50																		
TZm-1153	0.00	0.03	0.00	0.15	0.23	0.05	0.00	0.03	0.08	0.05	0.00	0.33	0.50	0.19	0.16	0.61	0.35																	
TZm-1156	0.35	0.02	0.05	0.02	0.03	0.05	0.04	0.23	0.02	0.00	0.01	0.00	0.01	0.03	0.01	0.02	0.00	0.08																
TZm-1180	0.09	0.20	0.28	0.03	0.43	0.21	0.00	0.23	0.20	0.08	0.44	0.08	0.00	0.79	0.39	0.25	0.00	0.02	0.00															
TZm-1182	0.39	0.16	0.00	0.10	0.00	0.12	0.61	0.04	0.05	0.55	0.07	0.22	0.48	0.03	0.33	0.24	0.51	0.06	0.07	0.02														
TZm-1183	0.12	0.01	0.20	0.06	0.12	0.06	0.01	0.01	0.19	0.01	0.23	0.03	0.17	0.23	0.07	0.00	0.18	0.12	0.00	0.37	0.00													
TZm-1184	0.03	0.00	0.07	0.01	0.05	0.12	0.00	0.08	0.01	0.29	0.05	0.06	0.26	0.04	0.21	0.12	0.28	0.20	0.04	0.00	0.08	0.01												
TZm-1185	0.01	0.29	0.10	0.04	0.03	0.16	0.23	0.04	0.04	0.05	0.01	0.33	0.30	0.00	0.30	0.31	0.37	0.18	0.00	0.01	0.08	0.02	0.04											
TZm-1187	0.06	0.00	0.14	0.01	0.21	0.00	0.00	0.35	0.32	0.11	0.02	0.06	0.01	0.22	0.11	0.01	0.02	0.06	0.10	0.31	0.04	0.20	0.04	0.06										
TZm-1188	0.02	0.01	0.06	0.11	0.69	0.03	0.01	0.48	0.02	0.20	0.01	0.45	0.31	0.35	0.33	0.27	0.11	0.30	0.03	0.22	0.05	0.01	0.28	0.19	0.19									
TZm-1190	0.02	0.00	0.26	0.02	0.05	0.00	0.04	0.02	0.16	0.00	0.07	0.04	0.24	0.16	0.02	0.07	0.21	0.18	0.01	0.33	0.01	0.64	0.02	0.04	0.28	0.00								
TZm-1193	0.08	0.66	0.38	0.15	0.00	0.70	0.48	0.03	0.28	0.37	0.33	0.08	0.11	0.21	0.70	0.36	0.32	0.03	0.08	0.26	0.33	0.13	0.08	0.11	0.03	0.01	0.04							
TZm-1194	0.21	0.03	0.12	0.00	0.49	0.01	0.00	0.13	0.01	0.01	0.22	0.14	0.00	0.39	0.19	0.07	0.02	0.00	0.01	0.39	0.03	0.49	0.02	0.07	0.19	0.25	0.28	0.06						
TZm-1195	0.41	0.33	0.02	0.15	0.20	0.09	0.13	0.33	0.00	0.09	0.39	0.17	0.15	0.32	0.27	0.35	0.08	0.09	0.12	0.34	0.15	0.01	0.07	0.03	0.05	0.12	0.00	0.14	0.02	0.17				
TZm-1211	0.08	0.00	0.11	0.08	0.13	0.03	0.09	0.00	0.08	0.00	0.00	0.00	0.06	0.11	0.03	0.00	0.08	0.00	0.10	0.17	0.03	0.36	0.03	0.05	0.07	0.11	0.36	0.00	0.00	0.23	0.08			
TZm-1212	0.47	0.01	0.00	0.04	0.11	0.00	0.16	0.19	0.10	0.37	0.02	0.18	0.54	0.01	0.22	0.15	0.34	0.12	0.17	0.00	0.51	0.01	0.10	0.04	0.03	0.21	0.07	0.05	0.00	0.05	0.21	0.12		
TZm-1213	0.00	0.20	0.00	0.17	0.18	0.15	0.48	0.04	0.00	0.15	0.02	0.06	0.29	0.10	0.08	0.06	0.40	0.01	0.03	0.10	0.24	0.25	0.03	0.15	0.01	0.00	0.35	0.10	0.01	0.14	0.04	0.17	0.08	
TZm-1214	0.18	0.01	0.00	0.11	0.27	0.03	0.08	0.11	0.00	0.07	0.14	0.02	0.12	0.19	0.01	0.00	0.28	0.05	0.20	0.34	0.03	0.34	0.17	0.03	0.12	0.04	0.29	0.03	0.00	0.34	0.17	0.09	0.01	0.27
TZm-1215	0.22	0.39	0.21	0.07	0.02	0.36	0.56	0.29	0.04	0.63	0.14	0.26	0.41	0.06	0.68	0.25	0.51	0.03	0.05	0.06	0.57	0.01	0.15	0.20	0.09	0.16	0.00	0.53	0.28	0.06	0.21	0.03	0.43	0.33	0.04

APPENDIX C: MOLECULAR DISTANCE MATRIX

	TZm-1097	TZm-1098	TZm-1099	TZm-1100	TZm-1101	TZm-1103	TZm-1105	TZm-1106	TZm-1108	TZm-1109	TZm-1110	TZm-1111	TZm-1112	TZm-1114	TZm-1116	TZm-1117	TZm-1118	TZm-1119	TZm-1120	TZm-1211	TZm-1121	TZm-1122	TZm-1123	TZm-1125	TZm-1126

TZm-1097																								
TZm-1098	0.74																							
TZm-1099	0.67	0.58																						
TZm-1100	0.43	0.42	0.52																					
TZm-1101	0.48	0.25	0.53	0.52																				
TZm-1103	0.47	0.48	0.56	0.59	0.67																			
TZm-1105	0.42	0.53	0.38	0.64	0.52	0.69																		
TZm-1106	0.36	0.47	0.44	0.46	0.45	0.72	0.71																	
TZm-1108	0.43	0.38	0.40	0.56	0.44	0.91	0.80	0.77																

TZm-1109	0.31	0.27	0.34	0.45	0.53	0.72	0.69	0.72	0.87																

TZm-1110	0.48	0.36	0.43	0.57	0.53	0.74	0.82	0.70	0.86	0.89																					
TZm-1111	0.37	0.43	0.20	0.50	0.50	0.67	0.71	0.67	0.73	0.69	0.63																				
TZm-1112	0.38	0.38	0.39	0.59	0.40	0.78	0.69	0.73	0.70	0.74	0.73	0.62																			
TZm-1114	0.41	0.53	0.30	0.62	0.40	0.75	0.81	0.75	0.67	0.57	0.69	0.73	0.74																		
TZm-1116	0.46	0.24	0.55	0.63	0.73	0.74	0.59	0.57	0.67	0.55	0.64	0.58	0.64	0.60																	
TZm-1117	0.43	0.41	0.52	0.48	0.53	0.67	0.71	0.70	0.48	0.53	0.62	0.48	0.53	0.52	0.57																
TZm-1118	0.41	0.41	0.41	0.60	0.59	0.70	0.53	0.62	0.63	0.67	0.63	0.67	0.56	0.64	0.44	0.64															
TZm-1119	0.50	0.45	0.33	0.39	0.48	0.61	0.69	0.58	0.55	0.40	0.44	0.48	0.52	0.69	0.38	0.32	0.67														

TZm-1120	0.54	0.51	0.50	0.53	0.43	0.67	0.80	0.61	0.63	0.61	0.63	0.70	0.70	0.68	0.54	0.61	0.65	0.72													
TZm-1211	0.38	0.44	0.17	0.42	0.14	0.46	0.40	0.47	0.24	0.00	0.15	0.40	0.24	0.44	0.00	0.29	0.33	0.38	0.27												
TZm-1121	0.50	0.61	0.35	0.64	0.38	0.53	0.74	0.50	0.40	0.29	0.43	0.43	0.40	0.67	0.17	0.25	0.52	0.69	0.62	0.46											
TZm-1122	0.44	0.33	0.29	0.59	0.47	0.60	0.64	0.54	0.55	0.64	0.61	0.57	0.57	0.62	0.50	0.42	0.64	0.64	0.56	0.60	0.67										
TZm-1123	0.47	0.46	0.52	0.61	0.55	0.64	0.57	0.58	0.67	0.58	0.67	0.48	0.69	0.65	0.38	0.45	0.69	0.73	0.73	0.40	0.69	0.58									
TZm-1125	0.46	0.42	0.57	0.60	0.55	0.63	0.40	0.52	0.56	0.58	0.48	0.31	0.53	0.47	0.33	0.44	0.53	0.41	0.51	0.33	0.59	0.58	0.57								
TZm-1126	0.51	0.58	0.55	0.36	0.38	0.67	0.59	0.67	0.63	0.51	0.57	0.54	0.59	0.69	0.40	0.63	0.52	0.58	0.74	0.38	0.61	0.47	0.54	0.55							
TZm-1128	0.33	0.36	0.36	0.67	0.47	0.67	0.64	0.72	0.62	0.50	0.61	0.53	0.58	0.69	0.42	0.48	0.57	0.48	0.50	0.40	0.71	0.52	0.59	0.50	0.64						
TZm-1129	0.33	0.59	0.33	0.36	0.21	0.53	0.47	0.71	0.64	0.61	0.48	0.57	0.56	0.64	0.37	0.41	0.53	0.44	0.44	0.43	0.53	0.46	0.35	0.48	0.63						
TZm-1130	0.21	0.42	0.43	0.43	0.25	0.53	0.50	0.67	0.63	0.57	0.62	0.55	0.53	0.71	0.36	0.67	0.57	0.17	0.53	0.57	0.50	0.20	0.43	0.67	0.60						
TZm-1131	0.40	0.55	0.33	0.60	0.24	0.64	0.52	0.71	0.69	0.53	0.61	0.54	0.60	0.79	0.42	0.46	0.64	0.45	0.47	0.46	0.67	0.38	0.43	0.38	0.69						

TZm-1132	0.36	0.39	0.40	0.29	0.33	0.48	0.48	0.53	0.64	0.70	0.63	0.63	0.59	0.55	0.50	0.50	0.48	0.50	0.63	0.00	0.40	0.42	0.50	0.38	0.63
TZm-1136	0.37	0.56	0.51	0.55	0.30	0.42	0.35	0.51	0.47	0.48	0.59	0.42	0.51	0.63	0.38	0.52	0.55	0.27	0.41	0.29	0.42	0.36	0.41	0.50	0.54

TZm-1137	0.50	0.53	0.53	0.34	0.36	0.59	0.35	0.47	0.41	0.38	0.40	0.25	0.41	0.44	0.35	0.55	0.48	0.30	0.46	0.56	0.40	0.30	0.42	0.60	0.61

TZm-1138	0.45	0.57	0.55	0.31	0.38	0.38	0.40	0.59	0.50	0.53	0.48	0.48	0.54	0.46	0.42	0.60	0.75	0.47	0.62	0.21	0.43	0.34	0.48	0.56	0.61
TZm-1139	0.42	0.48	0.47	0.27	0.30	0.59	0.41	0.67	0.57	0.56	0.50	0.44	0.55	0.52	0.33	0.55	0.53	0.50	0.53	0.38	0.30	0.31	0.48	0.50	0.61
TZm-1212	0.44	0.53	0.42	0.30	0.42	0.62	0.44	0.71	0.67	0.56	0.55	0.57	0.58	0.61	0.44	0.61	0.58	0.52	0.56	0.29	0.35	0.33	0.43	0.48	0.70
TZm-1213	0.47	0.48	0.55	0.22	0.42	0.59	0.40	0.44	0.40	0.41	0.35	0.38	0.34	0.43	0.50	0.62	0.55	0.50	0.46	0.14	0.38	0.37	0.42	0.48	0.48
TZm-1141	0.29	0.38	0.48	0.39	0.35	0.67	0.51	0.75	0.45	0.41	0.37	0.38	0.65	0.59	0.40	0.58	0.39	0.58	0.57	0.50	0.50	0.45	0.50	0.59	0.60
TZm-1142	0.48	0.45	0.46	0.19	0.33	0.38	0.46	0.71	0.40	0.35	0.48	0.43	0.43	0.59	0.32	0.52	0.38	0.67	0.50	0.56	0.50	0.44	0.55	0.44	0.63
TZm-1143	0.42	0.48	0.43	0.21	0.21	0.46	0.34	0.67	0.54	0.48	0.45	0.40	0.50	0.54	0.36	0.36	0.62	0.62	0.46	0.38	0.44	0.44	0.55	0.48	0.50
TZm-1144	0.47	0.53	0.48	0.17	0.32	0.55	0.48	0.63	0.61	0.55	0.63	0.38	0.56	0.64	0.33	0.42	0.64	0.59	0.50	0.44	0.42	0.50	0.54	0.43	0.67
TZm-1145	0.48	0.40	0.42	0.19	0.22	0.45	0.44	0.63	0.38	0.40	0.33	0.35	0.48	0.46	0.33	0.44	0.56	0.65	0.56	0.46	0.43	0.40	0.48	0.45	0.50
TZm-1146	0.46	0.42	0.47	0.08	0.30	0.34	0.28	0.53	0.38	0.27	0.43	0.17	0.36	0.54	0.40	0.38	0.30	0.58	0.40	0.40	0.47	0.29	0.44	0.46	0.53
TZm-1147	0.41	0.52	0.42	0.25	0.43	0.58	0.33	0.50	0.38	0.32	0.35	0.26	0.35	0.42	0.38	0.40	0.38	0.52	0.41	0.36	0.50	0.35	0.44	0.50	0.52
TZm-1148	0.48	0.54	0.50	0.09	0.14	0.38	0.38	0.50	0.42	0.44	0.38	0.42	0.43	0.38	0.44	0.50	0.48	0.43	0.59	0.00	0.24	0.30	0.42	0.22	0.52
TZm-1149	0.33	0.30	0.24	0.36	0.40	0.58	0.47	0.54	0.48	0.54	0.56	0.43	0.52	0.55	0.38	0.55	0.48	0.36	0.41	0.43	0.25	0.45	0.44	0.48	0.41
TZm-1150	0.33	0.33	0.11	0.21	0.38	0.53	0.64	0.46	0.56	0.59	0.67	0.59	0.50	0.64	0.43	0.40	0.35	0.55	0.69	0.15	0.53	0.53	0.48	0.33	0.58
TZm-1151	0.42	0.38	0.18	0.43	0.53	0.78	0.70	0.72	0.67	0.52	0.63	0.59	0.69	0.76	0.56	0.35	0.52	0.69	0.67	0.43	0.56	0.70	0.54	0.45	0.57
TZm-1152	0.16	0.25	0.08	0.30	0.24	0.46	0.67	0.63	0.50	0.64	0.63	0.76	0.73	0.78	0.56	0.55	0.33	0.42	0.59	0.17	0.43	0.50	0.46	0.22	0.41
TZm-1153	0.36	0.40	0.32	0.26	0.20	0.52	0.50	0.69	0.62	0.56	0.64	0.50	0.71	0.63	0.43	0.53	0.41	0.53	0.55	0.29	0.48	0.48	0.51	0.41	0.54
TZm-1156	0.30	0.40	0.26	0.19	0.20	0.43	0.44	0.73	0.53	0.48	0.54	0.50	0.63	0.64	0.42	0.45	0.31	0.52	0.51	0.27	0.43	0.37	0.42	0.31	0.54
TZm-1180	0.48	0.56	0.50	0.40	0.59	0.78	0.61	0.69	0.69	0.60	0.70	0.56	0.73	0.57	0.50	0.67	0.52	0.59	0.65	0.25	0.55	0.46	0.63	0.59	0.63
TZm-1182	0.50	0.42	0.52	0.50	0.50	0.45	0.54	0.44	0.40	0.43	0.59	0.32	0.57	0.62	0.36	0.57	0.35	0.58	0.59	0.32	0.53	0.56	0.62	0.57	0.55

TZm-1183	0.38	0.33	0.44	0.42	0.56	0.42	0.31	0.50	0.45	0.59	0.50	0.37	0.46	0.43	0.29	0.42	0.57	0.57	0.44	0.13	0.55	0.67	0.52	0.67	0.50
TZm-1184	0.36	0.23	0.43	0.42	0.71	0.53	0.31	0.44	0.64	0.62	0.74	0.40	0.58	0.45	0.50	0.55	0.50	0.57	0.52	0.12	0.38	0.53	0.61	0.53	0.50
TZm-1214	0.48	0.40	0.50	0.46	0.42	0.38	0.48	0.50	0.46	0.50	0.70	0.35	0.65	0.64	0.35	0.56	0.35	0.48	0.59	0.27	0.45	0.52	0.59	0.48	0.58
TZm-1185	0.47	0.52	0.30	0.37	0.35	0.67	0.53	0.64	0.52	0.53	0.61	0.43	0.63	0.69	0.36	0.50	0.50	0.50	0.46	0.42	0.50	0.70	0.48	0.50	0.61
TZm-1187	0.52	0.50	0.43	0.37	0.50	0.55	0.48	0.44	0.44	0.44	0.56	0.33	0.45	0.64	0.32	0.44	0.56	0.67	0.52	0.20	0.55	0.62	0.67	0.61	0.40
TZm-1188	0.44	0.57	0.54	0.45	0.47	0.57	0.54	0.37	0.30	0.38	0.45	0.13	0.48	0.52	0.29	0.45	0.42	0.43	0.39	0.25	0.67	0.50	0.48	0.67	0.46
TZm-1189	0.35	0.42	0.47	0.31	0.44	0.38	0.29	0.52	0.40	0.40	0.46	0.24	0.55	0.52	0.30	0.28	0.34	0.44	0.33	0.00	0.55	0.40	0.44	0.57	0.42

TZm-1190	0.43	0.53	0.49	0.38	0.33	0.48	0.53	0.42	0.40	0.50	0.53	0.44	0.63	0.57	0.34	0.50	0.34	0.44	0.59	0.22	0.52	0.46	0.51	0.59	0.52									
TZm-1193	0.41	0.59	0.33	0.22	0.25	0.48	0.50	0.67	0.45	0.43	0.52	0.50	0.38	0.72	0.31	0.52	0.42	0.53	0.50	0.50	0.57	0.27	0.40	0.29	0.57									
TZm-1194	0.29	0.37	0.45	0.30	0.42	0.58	0.38	0.57	0.63	0.61	0.47	0.43	0.52	0.48	0.44	0.60	0.48	0.43	0.50	0.15	0.33	0.32	0.46	0.48	0.64									
TZm-1215	0.29	0.42	0.35	0.35	0.35	0.52	0.45	0.40	0.38	0.43	0.38	0.35	0.44	0.45	0.20	0.48	0.40	0.48	0.46	0.36	0.47	0.50	0.44	0.48	0.50									
TZm-1195	0.23	0.32	0.19	0.40	0.44	0.70	0.53	0.54	0.67	0.55	0.67	0.43	0.64	0.58	0.50	0.67	0.40	0.45	0.54	0.18	0.40	0.35	0.52	0.35	0.54									

Appendix C Cont"d: Molecular Distance Matrix

	TZm-1128	TZm-1129	TZm-1130	TZm-1131	TZm-1132	TZm-1136	TZm-1137	TZm-1138	TZm-1139	TZm-1212	TZm-1213	TZm-1141	TZm-1142	TZm-1143	TZm-1144	TZm-1145	TZm-1146	TZm-1147	TZm-1148	TZm-1149	TZm-1150	TZm-1151	TZm-1152	TZm-1153	TZm-1156	TZm-1180	TZm-1182	TZm-1183	TZm-1184	TZm-1214	TZm-1185	TZm-1187	TZm-1188	TZm-1189	TZm-1190	TZm-1193	TZm-1194
TZm-1129	0.50																																				
TZm-1130	0.67	0.59																																			
TZm-1131	0.78	0.71	0.80																																		
TZm-1132	0.33	0.46	0.30	0.48																																	
TZm-1136	0.52	0.53	0.67	0.69	0.50																																
TZm-1137	0.46	0.50	0.71	0.52	0.29	0.54																															
TZm-1138	0.40	0.60	0.67	0.53	0.54	0.61	0.48																														
TZm-1139	0.33	0.48	0.50	0.50	0.62	0.49	0.56	0.56																													
TZm-1212	0.55	0.61	0.53	0.65	0.53	0.54	0.56	0.70	0.78																												
TZm-1213	0.18	0.35	0.25	0.36	0.38	0.44	0.60	0.53	0.58	0.65																											
TZm-1141	0.44	0.51	0.56	0.36	0.32	0.35	0.44	0.51	0.56	0.55	0.48																										
TZm-1142	0.40	0.50	0.53	0.35	0.45	0.36	0.55	0.57	0.59	0.50	0.52	0.65																									
TZm-1143	0.36	0.50	0.35	0.38	0.50	0.47	0.55	0.57	0.76	0.67	0.58	0.53	0.73																								
TZm-1144	0.40	0.57	0.44	0.50	0.40	0.52	0.59	0.67	0.59	0.58	0.55	0.56	0.81	0.80																							

TZm-1145	0.24	0.43	0.31	0.35	0.37	0.21	0.40	0.57	0.67	0.55	0.55	0.69	0.91	0.79	0.69																						
TZm-1146	0.36	0.40	0.44	0.29	0.38	0.39	0.58	0.43	0.50	0.46	0.48	0.44	0.87	0.76	0.73	0.77																					
TZm-1147	0.40	0.47	0.18	0.27	0.29	0.43	0.58	0.29	0.52	0.43	0.43	0.60	0.61	0.69	0.67	0.56	0.69																				
TZm-1148	0.13	0.48	0.20	0.30	0.43	0.40	0.50	0.63	0.48	0.45	0.61	0.43	0.56	0.61	0.74	0.58	0.52	0.64																			
TZm-1149	0.35	0.47	0.40	0.35	0.19	0.39	0.46	0.25	0.52	0.53	0.36	0.58	0.40	0.43	0.46	0.50	0.32	0.54	0.22																		
TZm-1150	0.29	0.36	0.38	0.33	0.55	0.32	0.29	0.33	0.48	0.48	0.35	0.38	0.48	0.42	0.46	0.42	0.42	0.53	0.47	0.60																	
TZm-1151	0.60	0.48	0.44	0.52	0.60	0.33	0.40	0.47	0.59	0.62	0.42	0.55	0.63	0.67	0.64	0.50	0.38	0.50	0.35	0.60	0.82																
TZm-1152	0.29	0.46	0.36	0.40	0.48	0.41	0.32	0.27	0.38	0.44	0.45	0.54	0.40	0.56	0.46	0.38	0.27	0.45	0.50	0.60	1.00	0.70															

TZm-1153	0.48	0.48	0.40	0.50	0.42	0.46	0.47	0.61	0.55	0.65	0.65	0.70	0.67	0.73	0.79	0.62	0.50	0.54	0.63	0.50	0.55	0.69	0.78														

TZm-1156	0.50	0.48	0.38	0.52	0.52	0.51	0.41	0.60	0.48	0.59	0.53	0.61	0.69	0.76	0.77	0.58	0.58	0.62	0.53	0.43	0.57	0.75	0.72	0.89													
TZm-1180	0.54	0.42	0.43	0.48	0.55	0.50	0.53	0.60	0.67	0.67	0.65	0.65	0.55	0.55	0.59	0.46	0.39	0.56	0.50	0.44	0.48	0.70	0.53	0.76	0.71												
TZm-1182	0.40	0.22	0.38	0.26	0.46	0.47	0.41	0.39	0.50	0.48	0.48	0.56	0.59	0.46	0.50	0.36	0.46	0.40	0.35	0.38	0.53	0.64	0.60	0.65	0.55	0.69											
TZm-1183	0.48	0.39	0.13	0.30	0.40	0.47	0.28	0.53	0.53	0.55	0.52	0.56	0.48	0.52	0.48	0.41	0.33	0.48	0.30	0.52	0.43	0.56	0.32	0.58	0.55	0.58	0.61										
TZm-1184	0.47	0.30	0.25	0.36	0.48	0.48	0.40	0.56	0.46	0.62	0.62	0.41	0.52	0.48	0.52	0.33	0.44	0.45	0.40	0.40	0.45	0.60	0.50	0.74	0.71	0.69	0.78	0.74									
TZm-1214	0.48	0.26	0.40	0.38	0.45	0.56	0.36	0.53	0.46	0.58	0.52	0.53	0.59	0.55	0.67	0.38	0.55	0.38	0.31	0.50	0.53	0.67	0.67	0.75	0.73	0.67	0.90	0.75	0.92								
TZm-1185	0.48	0.53	0.40	0.56	0.38	0.57	0.53	0.43	0.55	0.67	0.67	0.56	0.57	0.62	0.75	0.55	0.43	0.52	0.47	0.72	0.63	0.73	0.64	0.76	0.67	0.71	0.69	0.67	0.69	0.73							
TZm-1187	0.35	0.22	0.00	0.29	0.43	0.43	0.36	0.40	0.50	0.54	0.67	0.44	0.55	0.59	0.58	0.50	0.46	0.48	0.45	0.45	0.60	0.60	0.67	0.67	0.57	0.67	0.89	0.64	0.78	0.88	0.89						

TZm-1188	0.42	0.38	0.33	0.29	0.17	0.52	0.50	0.37	0.25	0.37	0.59	0.62	0.35	0.31	0.50	0.32	0.37	0.48	0.30	0.57	0.42	0.35	0.43	0.48	0.33	0.56	0.55	0.64	0.40	0.60	0.76	0.64				
TZm-1189	0.52	0.41	0.27	0.29	0.31	0.43	0.27	0.47	0.26	0.41	0.42	0.53	0.57	0.52	0.57	0.43	0.59	0.50	0.35	0.41	0.43	0.45	0.29	0.58	0.60	0.52	0.54	0.71	0.62	0.78	0.67	0.62	0.73			
TZm-1190	0.41	0.27	0.38	0.26	0.31	0.50	0.42	0.49	0.42	0.43	0.56	0.60	0.55	0.44	0.60	0.41	0.43	0.53	0.52	0.44	0.48	0.40	0.59	0.72	0.65	0.76	0.75	0.63	0.62	0.87	0.67	0.76	0.71	0.62		
TZm-1193	0.40	0.53	0.57	0.52	0.38	0.41	0.38	0.50	0.54	0.57	0.59	0.50	0.90	0.61	0.74	0.80	0.69	0.43	0.48	0.35	0.47	0.32	0.40	0.64	0.70	0.50	0.50	0.42	0.44	0.77	0.63	0.55	0.43	0.53	0.61	
TZm-1194	0.44	0.52	0.47	0.52	0.46	0.53	0.56	0.43	0.48	0.67	0.69	0.44	0.38	0.36	0.35	0.25	0.38	0.30	0.40	0.36	0.30	0.33	0.42	0.50	0.52	0.56	0.40	0.54	0.67	0.53	0.64	0.40	0.48	0.46	0.40	0.42
TZm-1215	0.40	0.30	0.38	0.18	0.24	0.40	0.40	0.29	0.32	0.37	0.52	0.60	0.38	0.36	0.43	0.35	0.41	0.52	0.42	0.48	0.38	0.33	0.44	0.43	0.38	0.43	0.44	0.74	0.52	0.63	0.60	0.57	0.86	0.69	0.67	0.52	0.59
TZm-1195	0.50	0.32	0.38	0.45	0.50	0.36	0.43	0.27	0.52	0.48	0.42	0.48	0.33	0.40	0.29	0.26	0.33	0.53	0.32	0.50	0.56	0.67	0.63	0.57	0.60	0.73	0.56	0.42	0.63	0.50	0.63	0.56	0.32	0.43	0.44	0.36	0.50

APPENDIX D: Binary score of SSR amplification product of 64 IPGRI maize landraces held in IITA.

	TZm-1097	TZm-1098	TZm-1099	TZm-1100	TZm-1101	TZm-1103	TZm-1105	TZm-1106	TZm-1108	TZm-1109	TZm-1110	TZm-1111	TZm-1112	TZm-1114	TZm-1116	TZm-1117	TZm-1118	TZm-1119	TZm-1120	TZm-1211	TZm-1121	TZm-1122	TZm-1123	TZm-1125	TZm-1126	TZm-1128	TZm-1129	TZm-1130	TZm-1131	TZm-1132	TZm-1136	TZm-1137	TZm-1138
phi001a	0	0	0	1	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	9	0	0	9	0	1	0	9
phi001b	0	0	0	1	0	0	0	0	9	9	9	9	9	9	9	9	1	0	0	0	0	0	0	0	0	9	0	0	9	0	0	0	9
phi001c	0	0	0	0	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	9	0	0	9	1	0	0	9
phi001d	0	0	0	0	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	9	0	0	9	1	1	0	9
phi001e	0	0	0	1	0	0	1	0	9	9	9	9	9	9	9	9	1	1	1	0	1	0	0	0	0	9	0	0	9	0	0	0	9
phi001f	0	0	0	0	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	9	0	0	9	0	1	0	9
phi001g	1	1	1	1	1	1	1	0	9	9	9	9	9	9	9	9	1	1	1	0	1	1	1	1	1	9	0	0	9	1	1	0	9
phi001h	0	0	0	0	0	0	1	1	9	9	9	9	9	9	9	9	0	1	1	0	1	1	0	0	1	9	1	0	9	1	0	0	9
phi001i	0	0	1	1	1	1	0	1	9	9	9	9	9	9	9	9	1	0	0	1	0	0	1	1	0	9	0	1	9	0	1	1	9

phi001j	1	1	0	0	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	9	0	0	9	0	0	0	9
phi056a	1	0	1	1	9	1	1	9	1	1	1	9	1	9	1	1	1	9	1	9	9	1	1	0	1	1	0	9	1	9	0	0	9
phi056b	1	1	1	1	9	1	1	9	1	1	1	9	1	9	1	1	1	9	1	9	9	1	1	1	0	0	1	9	0	9	1	1	9
phi056c	0	0	0	0	9	0	0	9	0	0	0	9	0	9	0	0	0	9	0	9	9	0	0	0	0	0	1	9	0	9	0	0	9
phi056d	0	1	0	0	9	0	0	9	0	0	0	9	0	9	0	0	0	9	0	9	9	0	0	0	0	0	1	9	0	9	0	0	9
phi056e	0	0	0	0	0	0	0	9	0	0	0	9	0	9	1	0	0	9	0	9	9	0	0	0	0	0	0	9	0	9	1	0	9
phi109642a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	9	0	0	9	0	9	0	0	0	1	0	0	0
phi109642b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	9	0	0	9	0	9	0	0	0	1	0	0	0
phi109642c	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	9	0	0	9	9	0	0	9	0	9	0	0	0	0	0	0	0

phi109642d	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	9	0	1	9	9	0	0	9	1	9	1	1	1	1	0	0	1

phi109642e	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	9	0	0	9	9	0	0	9	0	9	0	0	0	0	0	1	0
phi109642f	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	9	0	0	9	0	9	0	0	0	0	0	0	0
phi109642g	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	0	9	1	1	9	9	1	1	9	0	9	0	0	0	1	0	0	0
phi109642h	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	9	0	0	9	1	9	1	0	0	0	1	0	1
phi109642i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	9	0	0	9	0	9	0	0	0	0	0	0	0
umc1399a	0	0	0	1	9	9	9	1	1	1	1	1	1	1	1	0	1	0	0	0	0	1	0	0	0	1	1	9	1	9	1	0	1
umc1399b	1	1	1	0	9	9	9	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0	0	9	0	9	0	1	1
umc1399c	0	0	0	0	9	9	9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	9	0	9	0	0	1

umc1399d	0	0	0	0	9	9	9	1	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	0	0	9	0	9	0	0	1
umc1399e	0	0	0	0	9	9	9	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	9	0	9	0	0	1
phi076a	1	1	9	1	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	1	1	9	0	1	0	0	1	1	1	0	1	1	1
phi076b	1	0	9	0	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	0	0	9	1	0	1	0	0	0	0	1	0	0	0
phi076c	0	0	9	0	0	0	0	0	0	0	0	1	0	0	0	0	9	0	0	1	0	9	0	0	0	0	0	0	0	0	0	0	0
phi076d	0	1	9	1	0	1	1	1	1	0	0	1	0	1	0	0	9	1	1	1	1	9	1	0	1	1	1	1	1	0	0	0	0
phi085a	0	9	0	9	9	0	1	9	9	0	9	1	9	9	0	0	0	9	9	9	9	9	0	9	0	9	0	9	0	9	0	0	9
phi085b	0	9	0	9	9	0	1	9	9	1	9	1	9	9	0	0	0	9	9	9	9	9	0	9	0	9	0	9	0	9	0	0	9
phi085c	0	9	0	9	9	0	1	9	9	1	9	1	9	9	0	1	1	9	9	9	9	9	1	9	0	9	1	9	0	9	0	0	9

phi085d	0	9	0	9	9	1	0	9	9	1	9	1	9	9	0	1

9	9	9	9	9	0	9	1	9	1	9	1	9	1	1	9

phi085e	1	9	1	9	9	0	0	9	9	0	9	0	9	9	1	0	0		9	9	9	9	9	0	9	0	9	0	9	0	9	0	0	9
phi085f	0	9	0	9	9	0	1	9	9	1	9	0	9	9	0	0	0		9	9	9	9	9	0	9	0	9	0	9	0	9	0	0	9
dupssr13a	0	1	0	1	0	0	1	1	0	0	1	0	1	1	0	1	0		0	0	9	1	0	1	0	0	1	0	9	1	0	1	0	0
dupssr13b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	9	1	1	1	1	1	1	1	9	1	1	1	1	1
dupssr13c	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1		1	1	9	0	0	1	0	0	0	0	9	0	0	0	0	1
umc1066a	0	0	0	9	9	9	0	0	0	0	0	0	1	0	0	0	0		0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0
umc1066b	0	0	0	9	9	9	0	1	0	1	0	0	1	0	0	0	0		0	0	9	0	1	0	1	0	0	1	9	0	0	0	0	0
umc1066c	1	1	0	9	9	9	0	1	1	1	1	1	1	1	0	0	1		1	1	9	1	1	1	1	1	1	1	9	1	1	1	0	1
umc1066d	0	0	0	9	9	9	0	0	0	0	0	0	0	0	1	1		0	0	0	9	0	0	0	0	0	0	0	9	0	0	0	1	0
umc1066e	0	0	0	9	9	9	0	0	0	0	0	1	1	0	1	0	0		0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0
umc1066f	1	1	1	9	9	9	1	1	0	0	0	1	0	0	1	1	0		0	1	9	0	0	0	0	0	0	0	9	0	0	0	0	1
phi233376a	0	0	0	0	0	0	0	0	9	9	9	9	9	0	9	9	0		0	0	9	0	1	0	1	0	0	0	9	0	0	0	0	0
phi233376b	0	0	0	1	1	1	1	1	9	9	9	9	9	1	9	9	1		1	1	9	1	1	1	1	0	1	0	9	0	0	0	0	0
phi233376c	1	1	1	0	0	0	0	1	9	9	9	9	9	1	9	9	1		1	1	9	0	0	1	0	1	0	1	9	1	1	1	1	1
phi233376d	0	0	0	0	0	0	0	0	9	9	9	9	9	1	9	9	1		1	0	9	1	1	1	1	0	0	0	9	0	0	0	0	1
phi100175a	9	0	0	1	0	9	9	9	1	0	0	9	0	0	9	0	0		0	0	0	0	0	0	1	0	0	9	9	9	0	0	0	0
phi100175b	9	0	0	0	0	9	9	9	1	1	1	9	0	0	9	0	0		0	0	0	0	0	0	1	0	0	9	9	9	0	0	0	0

phi100175c	9	0	0	0	1	9	9	9	0	0	0	9	0	0	9	1	1	0	1	0	0	0	0	1	1	1	9	9	9	0	0	1	1

phi100175d	9	1	1	1	0	9	9	9	0	0	0	9	1	1	9	1	0	0	1	0	0	0	0	1	1	0	9	9	9	1	1	0	1
phi100175e	9	0	0	0	0	9	9	9	0	0	0	9	1	1	9	0	0	1	1	0	0	0	0	0	0	0	9	9	9	0	0	0	0
phi100175f	9	0	0	1	0	9	9	9	0	0	0	9	1	1	9	0	0	0	1	1	1	1	1	1	1	1	9	9	9	0	0	1	0
umc1279a	0	0	0	9	0	0	0	0	0	0	0	9	0	1	0	0	9	9	0	0	9	0	0	0	0	1	0	1	1	0	1	0	0
umc1279b	0	0	1	9	0	0	0	1	1	1	1	9	1	0	0	1	9	9	1	0	9	0	1	1	1	1	0	1	0	1	1	1	1
umc1279c	0	0	1	9	1	1	1	1	1	1	1	9	1	1	1	1	9	9	0	0	9	0	0	1	1	1	1	1	1	0	1	1	1
umc1279d	1	1	0	9	0	0	1	0	0	0	1	9	0	1	0	1	9	9	1	1	9	1	0	0	1	0	0	1	0	0	1	1	0
umc1279e	1	1	0	9	1	0	0	0	0	0	0	9	0	0	0	0	9	9	0	0	9	0	0	0	0	0	0	0	0	0	0	0	1

APPENDIX D: Binary score of SSR amplification product of 64 IPGRI maize lar

umc1677a	1	1	0	0	0	1	1	1	1	0	9	0	1	1	9	9	0	1	0	1	9	9	9	9	1	1	1	0	1	0	0	1	0		
umc1677b	0	1	0	0	0	1	1	1	1	1	9	1	1	1	9	9	1	0	1	0	9	9	9	9	1	0	1	1	1	1	1	1	1		
umc1677c	1	0	1	1	1	0	0	0	0	0	9	0	0	0	9	9	0	0	0	0	9	9	9	9	0	0	0	0	0	0	0	0	0		

Appendix D Cont"d

	TZm-1139	TZm-1212	TZm-1213	TZm-1141	TZm-1142	TZm-1143	TZm-1144	TZm-1145	TZm-1146	TZm-1147	TZm-1148	TZm-1149	TZm-1150	TZm-1151	TZm-1152	TZm-1153	TZm-1156	TZm-1180	TZm-1182	TZm-1183	TZm-1184	TZm-1214	TZm-1185	TZm-1187	TZm-1188	TZm-1189	TZm-1190	TZm-1193	TZm-1194	TZm-1215	TZm-1195
phi001a	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001b	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001c	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001d	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	1	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001e	0	0	0	0	0	0	0	1	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001f	0	1	1	0	0	0	0	0	0	0	0	1	1	9	1	1	1	0	0	1	1	1	1	1	1	1	1	9	1	1	0
phi001g	0	0	1	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1	1	1	1	1	1	1	1	1	1	9	1	1	1
phi001h	0	0	0	1	1	1	1	1	1	1	1	0	1	9	1	1	1	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001i	1	0	0	1	1	1	1	1	1	1	0	1	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi001j	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
phi056a	0	0	0	0	0	0	9	9	0	0	0	0	9	9	0	0	0	0	0	9	9	9	0	0	0	0	0	0	9	9	9
phi056b	1	1	1	1	0	1	9	9	0	1	1	1	9	9	1	1	0	1	1	9	9	9	1	1	1	0	1	0	9	9	9

phi056c	0	0	0	0	1	0	9	9	1	0	0	0	9	9	0	0	0	0	0	9	9	9	0	0	0	1	0	1	9	9	9
phi056d	0	0	0	0	1	1	9	9	1	1	0	0	9	9	0	0	1	0	0	9	9	9	0	0	0	1	0	1	9	9	9
phi056e		0	0	0	0	1	9	9	1	1	0	0	9	9	1	0	1	0	0	9	9	9	0	0	0	0	0	0	9	9	9
phi109642a	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	9	9	0	0	0	0	0	0	0	0
phi109642b	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	9	9	0	0	0	0	0	0	0	0
phi109642c	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	9	9	0	0	0	0	0	0	0	0
phi109642d	1	1	0	1	9	0	0	1	0	0	0	1	1	1	1	9	9	9	0	0	0	9	9	0	0	0	0	1	0	0	1
phi109642e	0	0	1	0	9	1	1	1	1	1	1	0	0	0	0	9	9	9	0	0	0	9	9	1	1	1	1	1	0	1	0
phi109642f	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	9	9	0	0	0	0	0	1	0	0

phi109642g	0	0	0	0	9	0	0	0	0	0	0	0	1	1	1	9	9	9	1	0	1	9	9	1	0	0	0	0	0	0	1

phi109642h	0	0	0	1	9	0	1	0	0	1	1	0	0	0	0	9	9	9	0	1	0	9	9	0	1	1	1	0	0	1	0
phi109642i	1	1	1	0	9	1	0	1	1	0	0	0	0	0	0	9	9	9	0	0	0	9	9	0	0	0	0	1	0	0	0
umc1399a	0	1	0	0	0	1	1	0	0	0	9	1	0	1	9	1	1	0	0	1	1	1	1	0	0	1	0	9	9	9	9
umc1399b	1	1	1	1	1	1	1	1	1	1	9	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	9
umc1399c	0	0	0	0	0	0	0	0	0	0	9	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	9
umc1399d	1	1	1	1	1	1	1	1	0	0	9	0	0	1	9	1	1	1	1	1	1	1	1	1	0	0	0	9	9	9	9
umc1399e	0	0	0	0	0	0	1	0	0	0	9	0	0	0	9	1	1	1	0	0	1	1	0	0	0	0	1	9	9	9	9
phi076a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0

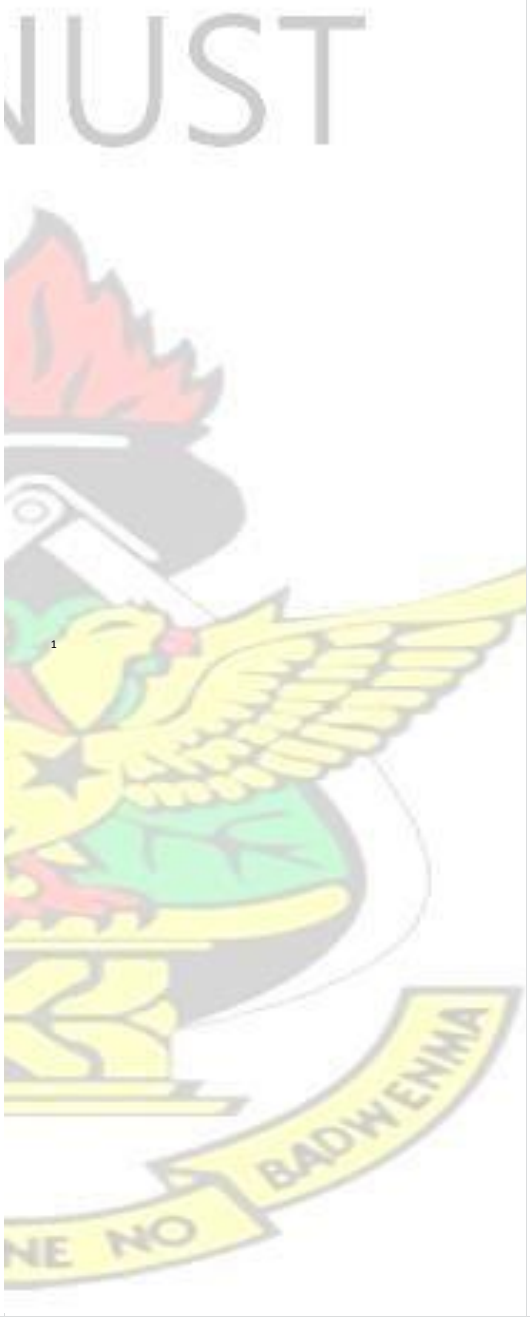
phi076b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1
phi076c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
phi076d	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	1	1	1	0
phi085a	0	0	0	0	9	9	9	9	0	0	9	0	9	1	1	0	0	0	9	0	0	9	0	9	9	0	0	0	0	1	0
phi085b	0	0	0	0	9	9	9	9	0	0	9	0	9	0	1	0	0	0	9	0	0	9	0	9	9	0	1	0	0	0	0
phi085c	0	0	0	0	9	9	9	9	0	0	9	1	9	0	1	0	0	0	9	0	0	9	0	9	9	0	0	0	0	0	0
phi085d	1	1	1	1	9	9	9	9	0	1	9	1	9	0	1	1	1	1	9	1	1	9	1	9	9	0	1	1	1	1	1
phi085e	0	0	0	0	9	9	9	9	1	0	9	0	9	0	0	0	0	0	9	0	0	9	0	9	9	1	0	0	0	0	0
phi085f	1	0	0	0	9	9	9	9	0	0	9	0	9	0	0	0	0	0	9	1	0	9	0	9	9	0	1	1	0	1	0

dupssr13a	0	0	0	9	9	9	9	0	9	9	0	9	9	0	1	1	1	1	1	0	9	1	1	1	1	1	1	1	0	9	1

dupssr13 b	1	1	1	9	9	9	9	0	9	9	0	9	9	1	0	0	0	1	0	1	9	0	0	0	1	0	0	0	1	9	0
dupssr13 c	0	0	0	9	9	9	9	1	9	9	1	9	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	9	0
umc1066 a	0	0	0	1	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0
umc1066 b	0	0	0	1	0	0	0	1	0	0	0	1	0	0	9	0	0	0	0	1	0	0	1	0	1	1	0	0	1	1	0
umc1066 c	1	1	0	0	1	1	1	1	1	1	1	0	1	1	9	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	0
umc1066 d	0	0	1	0	0	0	0	0	1	1	1	0	0	0	9	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1
umc1066 e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
umc1066 f	0	0	1	1	1	0	0	1	0	0	1	0	0	0	9	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0

phi233376 a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	9	0	9	0	9	9	9	1	0	0	1	0	0	0	0
phi233376 b	0	0	0	1	0	0	0	0	0	1	0	1	1	1	9	0	9	1	9	1	9	9	9	1	1	1	1	0	0	1	1
phi233376 c	1	1	1	0	1	1	1	1	1	0	1	0	0	0	9	0	9	0	9	0	9	9	9	1	0	0	0	1	1	0	0
phi233376 d	0	0	1	1	1	1	1	1	1	0	0	0	0	0	9	1	9	0	9	1	9	9	9	1	1	1	1	1	0	1	0
phi100175 a	0	0	9	0	0	9	9	9	9	9	9	9	9	0	0	0	0	9	0	9	9	0	0	9	9	9	0	9	9	9	9
phi100175 b	0	0	9	0	0	9	9	9	9	9	9	9	9	0	0	0	0	9	0	9	9	0	0	9	9	9	0	9	9	9	9
phi100175 c	0	1	9	0	0	9	9	9	9	9	9	9	9	0	0	0	0	9	0	9	9	0	0	9	9	9	0	9	9	9	9
phi100175 d	1	1	9	1	0	9	9	9	9	9	9	9	9	0	0	0	0	9	1	9	9	1	0	9	9	9	1	9	9	9	9
phi100175 e	0	0	9	1	0	9	9	9	9	9	9	9	9	1	1	1	1	9	1	9	9	1	0	9	9	9	1	9	9	9	9

phi100175f 0 0 9 1 1 9 9 9 9 9 9 9 9 9 1 1 1



1

9 1 9 9 1 1 9 9 9 1 9 9 9 9

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umc1279b	1	1	0	1	1	1	0	9	1	9	9	0	0	9	9	1	1	1	1	1	1	1	0	9	0	1	1	0	1	1	1
umc1279c	0	1	1	1	1	0	1	9	1	9	9	0	0	9	9	1	1	1	1	0	1	1	1	9	1	1	1	1	1	0	0
umc1279d	0	0	0	0	1	0	1	9	1	9	9	1	1	9	9	0	0	0	1	0	0	1	1	9	1	0	1	1	0	1	0
umc1279e	0	1	0	0	0	0	0	9	0	9	9	0	0	9	9	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0
umc1677a	1	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1	1	1	0	0	0	0	1	9	9	9	9	9	0	9	1
umc1677b	1	1	1	0	0	1	1	0	0	0	1	0	1	1	1	1	1	1	0	0	0	0	1	9	9	9	9	9	1	9	1
umc1677c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	9	9	9	9	9	0	9	0