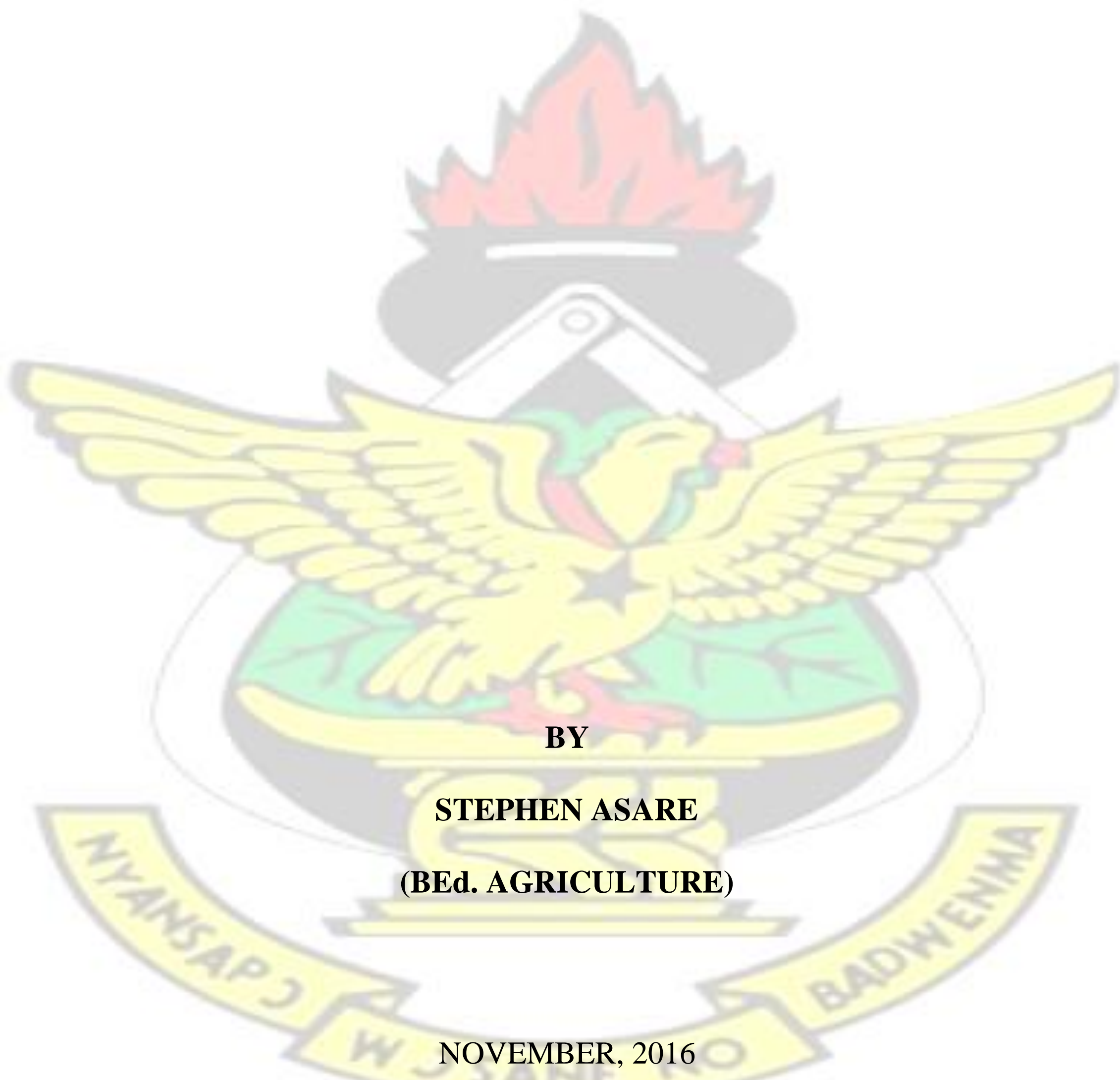
GENETIC ANALYSIS OF SIMPLE SEQUENCE REPEAT MARKERS AND MORPHOLOGICAL TRAITS VARIATION IN LOWLAND, MID-ALTITUDE AND HIGHLAND AFRICAN MAIZE (*Zea mays* L.) ACCESSIONS



BY

STEPHEN ASARE

(BEd. AGRICULTURE)

NOVEMBER, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

DEPARTMENT OF CROP AND SOIL SCIENCES

SCHOOL OF GRADUATE STUDIES

KNUST

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NYANSAPɔ WɔSANE NO BADWENMA

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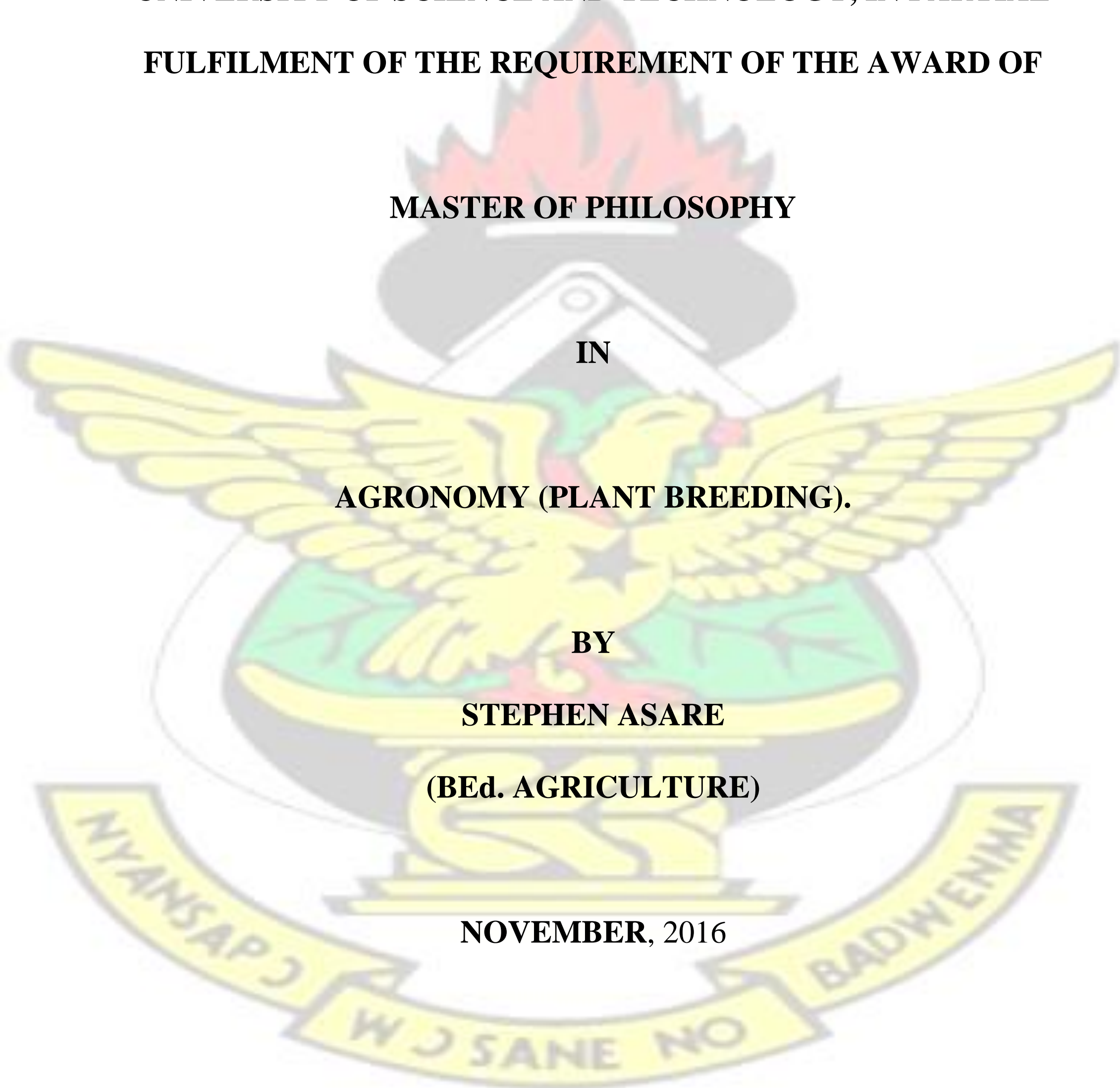

A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES OF THE FACULTY OF AGRICULTURE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT OF THE AWARD OF

MASTER OF PHILOSOPHY

IN

AGRONOMY (PLANT BREEDING).

BY

STEPHEN ASARE

(BEd. AGRICULTURE)

NOVEMBER, 2016

DECLARATION

I hereby declare that except for references cited in relation to other works, which have been duly acknowledged, this work is the outcome of my own original research and that this thesis has neither in whole nor part been submitted for any other degree at any other University.

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

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(Head of Department)

DEDICATION

I dedicate this work to Dr. (Mrs.) Antonia Tetteh for her unflinching support, my family and life partner.

KNUST

ACKNOWLEDGEMENT

What shall I render to my God; for all His mercy‟s store, I‟ll take the gifts He has bestowed, and humbly ask for more. My vows I will to His great name; before His

people pay, and all I have and all I am, upon His alter lay. Thank you Almighty God for doing such a wonderful thing in my life.

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I highly acknowledge the special assistance from the International Institute of Tropical Agriculture (IITA), Nigeria for the supply of maize seeds from their germplasm collections pool, Tropical Zea mays (TZm), which constituted the plant materials for this research work.

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ABSTRACT

Genetic diversity is a valuable resource for improvement in crop productivity and trait performance. Maize (*Zea mays* L.) is an important food security crop in Africa constrained with low yield typically below world average. The forces of population

escalation, diminishing arable land, and climate anomalies with their attendant low yield and crop loss arising from drought and emerging diseases indicate worsening food insecurity in Africa. However, genetic improvement in a wide genetic base is a relevant and logical strategy requiring availability of large reserve of alleles bearing these traits. This strategy necessitates studies into the well-adapted traditional African maize germplasm to identify, quantify, and explore the basis of variation in order to reveal the historical processes that have created and driven the level of variation for efficient exploitation. The main objective of this research was to investigate genetic diversity of maize originating from twelve countries, covering a wide geographical region in three mega environments in Africa, namely, lowland, mid-altitude and highland accessions. The accessions were tested under non-stressed environments in Ghana by evaluation of 29 agro-morphological traits on 35 genotypes, and 16 simple sequence repeat markers on 57 accessions held in IITA Genetic Resource Center. Accessions showed wide variations in silk color, kernel arrangement, principal grain color, and kernel texture except cob color. Significantly different mean squares and large coefficient of variation indicated substantial variation among the genotypes for all traits except anthesis-silking interval. Variability was highest in mid-altitude followed by lowland, and was least in the highland accessions. Being the most important traits, earliness and grain yield varied from 49 to 66 days to anthesis and 1.7 to 6.2 Mgha^{-1}, respectively. Anthesis-silking interval varied from 2 to 6 days. The study identified a single early-maturing genotype TZm-1376, with strikingly short ASI of 2 days which also possessed high yield of 5.6 Mgha^{-1} in consonance to the improved check „‟Obatanpa GH‟‟‟ with yield of 6.3 Mgha^{-1}. Unusual combination of early-maturing yet high-yielding accessions were identified in ten genotypes TZm-4, TZm-41, TZm-270, TZm-1521, TZm-275, TZm-14, TZm-33 TZm-37, TZm-1367, and

TZm-1376. Medium - maturing but high yielding genotypes which can be incorporated into breeding programs for improvement included TZm-1434, TZm-1356, TZm-1358 and TZm242. The broad sense heritability estimates were low for all traits in the accessions except earliness traits in the lowland genotypes. Significant ($p \leq 0.01$) positive genotypic correlations of grain yield with hundred kernel weight, kernel length, tassel length, and ear leaf width indicated that selection for these traits will lead to simultaneous increase in grain yield. Morphological genetic similarity measures ranged from 0.00 to 0.80 with overall mean of 0.26 indicating wide genetic variability among accessions. The African landraces were 23 %, 29 %, and 38 % similar in the mid-altitude, lowland and highland accessions which agreed with the level of variability revealed by the descriptive statistical measures. A total of 70 alleles ranging from 2-10 with a mean of 5.38 alleles per locus for the 14 SSR loci were identified. The total number of alleles generated across the loci was 1,908 over 57 accessions. Polymorphism Information Content ranged from 0.18 to 0.81 having an average of 0.64 and 93 % polymorphism rate. The high average expected heterozygosity of 0.64 indicates abundance of heterozygosity probably arising from historic admixture of two or more divergent populations. Molecular analysis revealed average dissimilarity coefficients of 0.70 for mid-altitude, 0.69 for lowland and 0.65 for highland accessions with an average of 0.70 ranging from 0.00 to 1.00.

These were consistent with the low similarity values produced by the morphological analysis. The UPGMA produced four and three main clusters for agro-morphological and SSR analysis, respectively, confirmed by the principal components biplots. Potential good clusters for exploiting heterosis in maize breeding programs were identified. The study has revealed wide genetic diversity in the accessions to permit their utilization as sources of alleles for improvement in performance and productivity of maize in Africa.

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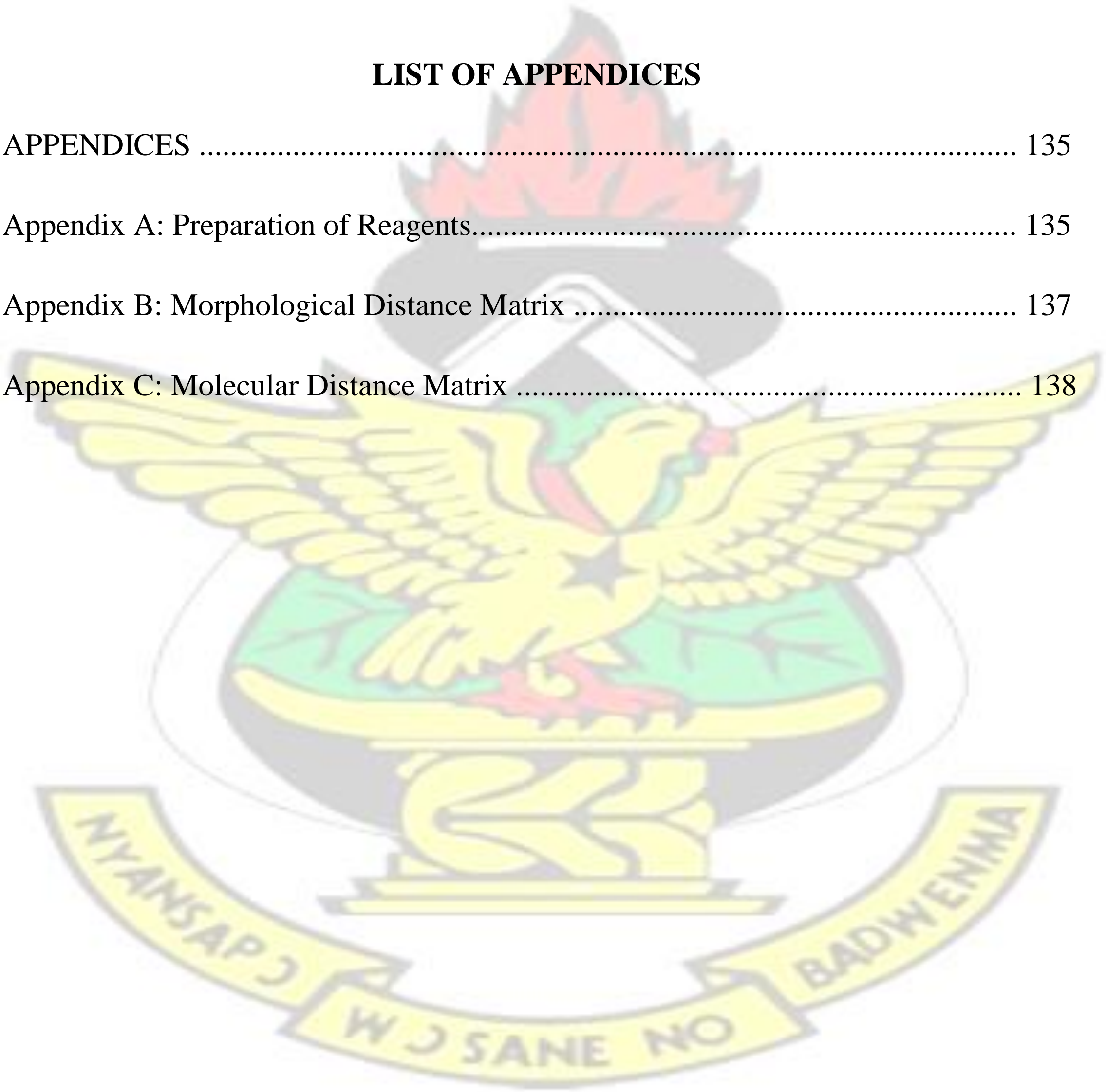

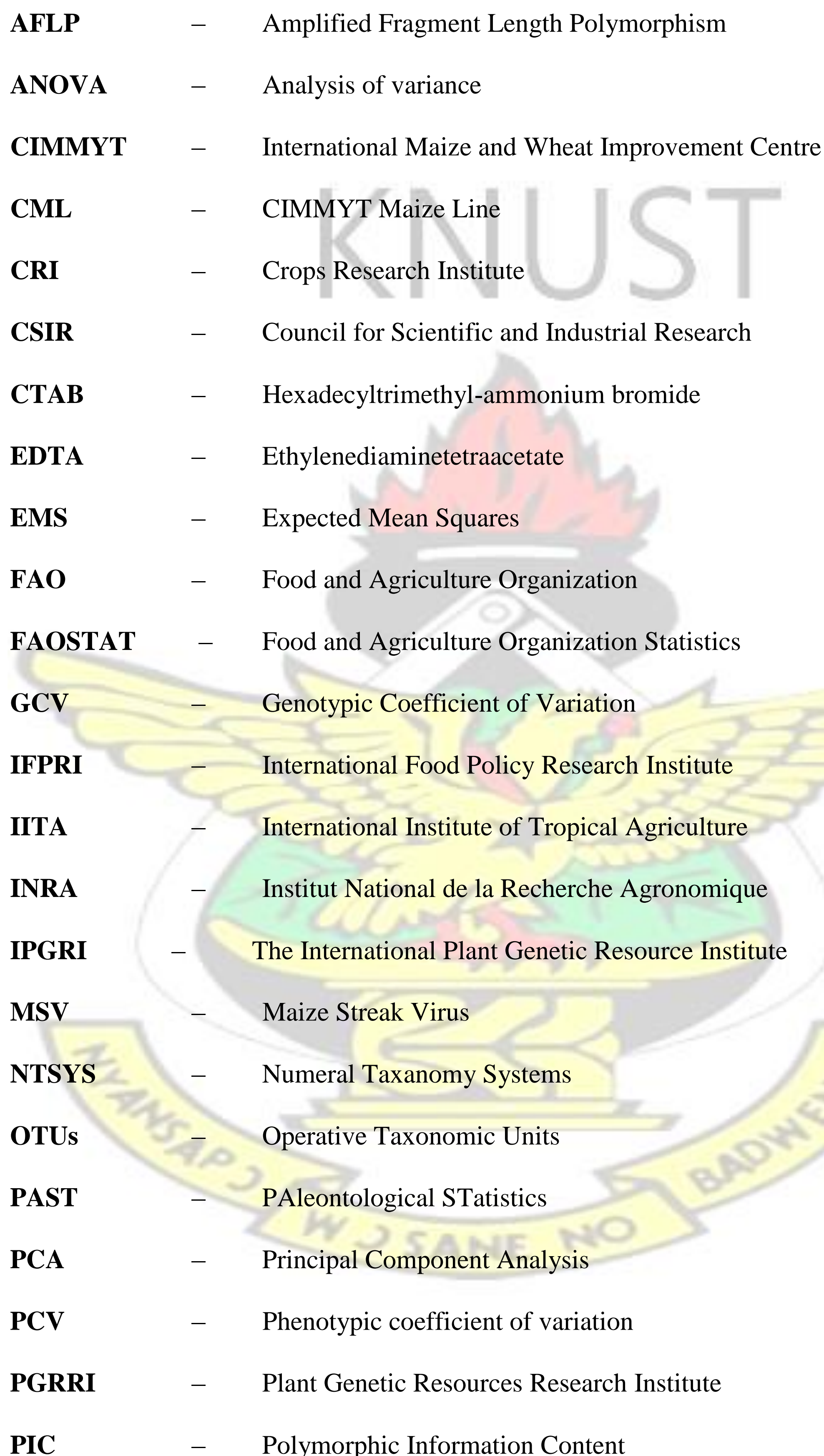

LIST OF ABBREVIATIONS

AFLP	–	Amplified Fragment Length Polymorphism
ANOVA	–	Analysis of variance
CIMMYT	–	International Maize and Wheat Improvement Centre
CML	–	CIMMYT Maize Line
CRI	–	Crops Research Institute
CSIR	–	Council for Scientific and Industrial Research
CTAB	–	Hexadecyltrimethyl-ammonium bromide
EDTA	–	Ethylenediaminetetraacetate
EMS	–	Expected Mean Squares
FAO	–	Food and Agriculture Organization
FAOSTAT	–	Food and Agriculture Organization Statistics
GCV	–	Genotypic Coefficient of Variation
IFPRI	–	International Food Policy Research Institute
IITA	–	International Institute of Tropical Agriculture
INRA	–	Institut National de la Recherche Agronomique
IPGRI	–	The International Plant Genetic Resource Institute
MSV	–	Maize Streak Virus
NTSYS	–	Numeral Taxanomy Systems
OTUs	–	Operative Taxonomic Units
PAST	–	PAleontological STatistics
PCA	–	Principal Component Analysis
PCV	–	Phenotypic coefficient of variation
PGRRI	–	Plant Genetic Resources Research Institute
PIC	–	Polymorphic Information Content

PROC GLM – Procedure General Linear Model

QPM – Quality Protein Maize

QTL – Quantitative Trait Loci

RAPDs – Random Amplified Polymorphic DNA

RFLPs – Restriction Fragment Length Polymorphisms

RNase – Ribonuclease

SAHN – Sequential Agglomerative Hierarchical Nesting

SAS – Statistical Analysis System

SCARs – Sequence characterized amplified regions

SIMINT – Similarity for interval data

SIMQUAL – Similarity for qualitative data

SNPs – Single Nucleotide Polymorphism

SSR – Simple Sequence Repeat

STS – Sequence Tagged Sites

TE buffer – Tris EDTA buffer

TZm – Tropical *Zea mays*

UPGMA – Unweighted Paired Group Method using Arithmetic averages

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CHAPTER ONE

INTRODUCTION

Maize (*Zea mays* L.) is a cereal belonging to the family Poaceae. It is believed to have originated from Central America, specifically Mexico (Gibson and Benson, 2002). It was first introduced to Africa by the Portuguese traders in the 16th century and has since become one of the continent"s staple food crops making up more than 50% of the total caloric intake of local diets (Sinha, 2007). The diversified uses for food, feed, and as a major industrial raw material for many products, including adhesives, textile, paint, xylose, ethanol, biofuel and a binder for pharmaceuticals make maize a very important crop in the global economy.

The demand for maize around the world is increasing. In 2010/11 world maize consumption was forecast to rise to 830 million metric tons (mmt), representing 2% increase in the previous year"s forecast while production in the same period was 823 mmt. Over the years, demand for maize has increased, without commensurate increase in supply. While the International Food Policy Research Institute (IFPRI, 2003) projected a demand of 852 mmts by 2020, the actual consumption of maize was 868 mmt in 2011/2012 , with a new projection of over 1,000 mmt by 2018/2019 (International Grain Council, 2013). With this tendency of consumption exceeding production, as well as actual consumption surpassing projections, the need to target improvement in maize productivity has become more important than ever. In subSaharan Africa, demand is expected to double from 27 mmt in 1995 to about 52 mmt by 2020 (Pingali and Pandey, 2000). As demand for maize increases around the world, there has been a commensurate increase in the acreage planted, as well as tremendous efforts by many countries to increase productivity of maize (CIMMYT,

1994). Prior to 2001, average maize yield of 0.9 to 1.2 t/ha was recorded for subSaharan Africa which is just below a quarter of the global average of 5.5 t/ha, and about a sixth of the average yield of 7.8 t/ha in the U.S.A. (FAOSTAT, 2006).

In recent years, impressive advancements in maize productivity have been achieved through conventional breeding in West and Central Africa raising the productivity from 1.2 t/ha to 3-5 t/ha. To support this, the Food and Agricultural Organization (FAO) reported that in 2005 six countries in Africa produced twice the amount consumed, while eight other countries imported 5-35% and 11 countries also imported 57-100% of the maize consumed in their respective countries (FAO, 2007). Nevertheless, maize yield in West/Central Africa still remains below the world average.

The key constraints to crop production globally are limited land and water resources, expanding population and abiotic and biotic stresses. In sub-Saharan Africa, the disparity in maize productivity is exacerbated by nutrient poor soils, drought, disease, and use of unimproved seed. The great demand for maize of both quality and quantity requires more rapid genetic improvement. Efforts have to be made to increase maize yield in Africa.

Genetic improvement of any crop begins with an evaluation of the genetic diversity present in the germplasm. Genetic diversity estimates provide valuable information for classification of germplasm for guidance in performing crosses in crop improvement programmes. It also provides the basis for devising strategies for conservation and sustainability. In the current climate variability phenomena, the need for developing genotypes having less vulnerability to drought, pest and disease resistance traits combined with high yield in a wide genetic base is relevant. Genetic

diversity information is useful for identification of useful genes among germplasm, for inbred line development, for assignment of inbred lines into heterotic groups, and for identification of testers.

To date only few reports of detailed assessment of genetic diversity among the accessions adapted to sub-Saharan Africa are documented. Genetic diversity studies on Ethiopian maize genotypes were reported by Beyene *et al*. (2006) and Legesse *et al*. (2007). In Ghana, Obeng-Antwi (2007) reported genetic diversity estimates of 90 landraces. Oppong *et al*. (2014) worked on genetic characterization of Ghanaian maize landraces using microsatellite markers. Some 294 Zimbabwe, Zambia and Malawi maize genotypes were evaluated for genetic diversity estimates (Magorokosho, 2006). Sanou *et al*. (1997) determined the diversity in some West African maize genotypes by means of isozyme diversity.

In contrast, genetic diversity among maize germplasm in North America (James *et al*., 2002; Bretting *et al*., 1990; Smith 1986; Goodman and Stuber, 1983 and Kahler *et al*., 1983.), in CIMMYT (Warburton *et al*., 2005; 2002; Xia *et al*., 2005; Carvalho *et al*., 2002), in Europe (Hartings *et al*., 2008; Okumus, 2007), and Asia (Enoki *et al*., 2002; Yuan *et al*., 2000) have been evaluated. As a result of this lack of information, maize breeding efforts in sub-Saharan Africa is seriously limited. To date, old breeding materials, and few newly developed inbred lines have been culled from CIMMYT lines, producing maize of narrow genetic base. While landraces are known to possess many useful alleles for crop improvement, the utilization of African landraces in breeding programs have not been exploited. For example, the popular maize genotype, „Obatanpa GH‟ which is in the pedigree of many of the maize cultivars produced in

Ghana was bred from CIMMYT Population 63 maize of Mexican origin (Badu-Apraku, 2006).

„Obatanpa GH" (Reg. no. CV-1, PI 641711), a tropically adapted, intermediate maturing, open-pollinated cultivar was developed by the Crops Research Institute (CRI), Kumasi, Ghana in collaboration with the International Institute of Tropical Agriculture (IITA), the International Maize and Wheat Improvement Center (CIMMYT), and the Sasakawa Global 2000. „Obatanpa GH" is a white dent and flint endosperm Quality Protein Maize (QPM) with elevated levels of lysine and tryptophan and was first released by CRI, Ghana in 1992 as „Obatanpa" to help improve the protein nutritional status and the health of a large population of lowincome groups in sub- Saharan Africa who depend on maize as a major component of their dietary protein intake (Sallah, 1998).

The Plant Genetic Resources Research Institute (PGRRI) in Ghana has in store some 400 maize accessions collected in 1991. The International Institute of Tropical Agriculture, Nigeria, also has over 800 maize accessions collected from many agroecological zones in Africa (www.iita.org, verified March 04, 2015). Evaluation of the genetic diversity estimates within the large African maize germplasm has the potential to reveal useful alleles for future maize breeding programs.

The markers commonly used in genetic diversity studies include morphological trait evaluation, isozyme and molecular markers. Accurate estimation of genetic diversity requires the use of very efficient marker protocols that detect fine genetic differences between the accessions. Maize breeders in India, as in most developing countries, have differentiated accessions mainly on the basis of major morphological characters such as plant height, anthocyanin pigmentation of various plant parts, tassel type, tassel

branching, number of days to flowering, ear characteristics, cob colour, grain colour and grain type (Virk and Witcombe, 1997). Although morphological descriptions are important for ascertaining the agronomic utility of germplasm, such descriptions are not very reliable because of complex genotype×environment interactions that require assessment in multiple environments (Enoki, 2002; Smith and Smith, 1989), are time-consuming, labour-intensive and require large populations (Botha and Venter, 2000). Isozyme analysis is relatively simple and less costly compared with molecular marker analysis; however inadequate genomic coverage, relatively low levels of polymorphism, developmental regulation and pleiotropic effects impose major constraints in effectively using these markers in genotype differentiation and analysis of genetic diversity (Dubreuil *et al*., 1996;
Smith and Smith, 1986).

Molecular markers have proved to be more powerful tools in genetic diversity and mapping studies. The available molecular markers include Random Amplified Polymorphic DNA (RAPDs), Restriction Fragment Length Polymorphism (RFLPs), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Sequence Tagged Sites (STS) and Single Nucleotide Polymorphisms (SNPs)

Among the different types of PCR- based DNA markers available for diverse applications in maize breeding, SSR markers (microsatellites) are often preferred because they are less costly, simple to prepare, offer greater reliability and reproducibility and more effective than the other markers (Smith *et al*., 1997). The SSR markers are robust, codominant, hypervariable, abundant, and uniformly dispersed in plant genomes. Senior and Heun (1993) reported that SSR loci provide a high level of polymorphism in maize. Pejic *et al*. (1998) and Smith *et al*. (1997)

reported of good correlation between SSR and RFLP diversity and pedigree-based measurements. Moreover, the efficiency of SSRs can be increased by running multiplexed reactions under automated electrophoresis conditions (Mitchell *et al*., 1997).

Genetic diversity study among the African maize inbred lines present in the CIMMYT Centers in Ethiopia and Zimbabwe has been determined using SSR markers (Legesse *et al*., 2007). Menkir *et al*. (2004) assessed the genetic relationships among tropical mid-altitude inbred lines developed in Nigeria and Cameroon, using AFLP and SSR markers. Beyenne *et al*. (2006) evaluated genetic diversity of traditional Ethiopian highland maize accessions by SSR markers. Reif *et al*. (2003) determined the genetic distance and heterosis in tropical maize populations by means of SSR markers. The genetic diversity estimates among maize accessions originating from Zambia, Zimbabwe, and Malawi were determined by
SSRs (Magorokosho, 2006).

Reif *et al*., (2004) determined genetic diversity within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by means of SSR markers. Warburton *et al*. (2008) estimated genetic diversity in CIMMYT nontemperate maize germplasm, including landraces, open pollinated varieties, and inbred lines using SSRs. Equivalent information on genetic diversity among West and Central African maize accessions is not available, hence they have not formed part of maize breeding programs in Africa.

The main objective of this research is to estimate genetic diversity and groupings among fifty-seven maize accessions originating from three ecological zones in

Africa, *viz,* lowland, mid-altitude and highland regions.

The specific objectives include:

1. Assessment of genetic diversity by means of morphological trait markers
2. Assessment of genetic diversity by means of SSR profiling
3. Determination of groupings within the maize collection for the purpose of hybrid breeding

Hypothesis of current research is based on two themes delineated as:

1. Over a long period of time, forces of evolution including mutation, recombination, selection, migration, and genetic drift have introduced allelic variation in the African maize germplasm pool
2. That the allelic variation can be estimated from marker polymorphisms

Justification

The information on genetic diversity study among the maize germplasm in Africa will be useful for identification of useful genotypes for enhancing the performance of commercial cultivars in future breeding programs, for application in organizing and managing the germplasm in Genetic Resource Centers, for widening the genetic base of the gene pool, and for identification of heterotic groups for hybrid breeding. Effective plant breeding and crop improvement programs for food security depend on the availability of genetic diversity information.

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize as an ancient crop

2.1.1 The role of maize in the world's agricultural economy

Maize (*Zea mays* L.) is one of the oldest crops cultivated around the globe and on similar scale as wheat and rice. Over the past ten years, production of maize has surpassed that of wheat and rice (FAOSTAT, 2012). 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 maize production by top producing countries was 719 million metric tons (mmt), while that for rice was 651 mmt, and that for wheat was 571 mmt (FAOSTAT, 2012). The data also reveal that the dollar value covering the past five years of global maize production for the same period was about US $48 to 54 billion, while that of wheat and rice were $66 to 77 billion and $161 to 180 billion, respectively (FAOSTAT, 2012).

The United States, China, and Brazil are the world's leading producers of maize (FAOSTAT, 2012) generating over 50 mmt of maize annually. Together, the U.S. and China produce approximately 60 % of the world maize crop (Smith *et al*., 2004). Maize accounts for 15-20 % of the total daily caloric intake in the diets of the people of many developing countries in Latin America, and over 50 % of same in Africa (Sinha, 2007; McCann, 2005; Dowswell *et al*., 1996). Approximately 68 % of the land devoted to maize is located in the developing world, however only 46 % of maize production occurs there, indicating the need for improving yields in developing countries where it is a major source of direct human consumption for many of the poor (Pingali and Pandey, 2000).

Empirical values suggest that, global demand for maize will increase above the demand for wheat and rice (M"mboyi *et al*., 2010; Pingali, 2001). For maize, a 50 %

increase in demand from its 1995 value of 558 mmt to 837 mmt by 2020 is expected (Martinez *et al*., 2011). The numerous uses of maize together with its new found uses as a source of biofuel have led to increase in demand around the world. Demand for maize in 2007/2008 was estimated to be 797 mmts while supply for same period was 779 mmts (International Grain Council, 2013), representing a deficit of 18 mmts. Current demand for maize is estimated to be 846 mmts in 2013/14 while production is 719 mmt, indicating a deficit of 127 mmt. The projection for maize demand in 2018/2019 is estimated to be 1,008 mmt, exceeding projected supply of
998 mmt (I.G.C., 2013).

2.1.2 The origin of maize

Maize (*Zea mays* L.) with a chromosome number 2n=2x=20, is a member of the Poaceae (formerly known as Gramineae), the family of crops which includes the grasses such as wheat, rice, rye, oats, barley and sugarcane. The genus *Zea* comprises a group of annual and perennial grasses native to the Meso-American region, from Mexico to Nicaragua. In this region, many wild types of maize are found. Iltis and Doebley (1980) divided the genus *Zea* into two sections, namely, section *Zea* which comprises only *Z. mays* L., and section *Luxuriantes*, which includes *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 further partitioned into four subspecies, encompassing, the cultivated maize, *Z. mays* ssp. mays, and three wild taxa, namely,

Z. mays ssp. *mexicana* (Schrader) Iltis (Central Mexico), *Z. mays* ssp. *parviglumis* Iltis& J. F. Doebley (Southern and Western Mexico) and *Z. mays* ssp.

huehuetenangensis (Iltis& J. F. Doebley) J. F. Doebley (Eastern Guatemala). All the wild species and subspecies of *Z. mays* are collectively known by the common name,

„teosinte". The teosintes occur in two forms, the Mexican annual teosinte, which had earlier been named *Euchlaena mexicana* Schrader (Schrader, 1833) and a perennial form, *Euchlaena perennis* Hitchcock (Hitchcock, 1922). These types were so named outside *Zea* genus because of their wide morphological disparity from the domesticated maize with regard to the ear (Figure 2.1), providing no inkling to earlier researchers about the close relationship between them and domesticated maize.

Figure 2.1. Teosinte ear (left), modern maize ear (right), and their F1 hybrid (center). Source: Doebley (2001).

2.1.3. Cytological evidence

A successful experimental cross between the Mexican annual teosinte (2n=2x=20) and maize by the Mexican agronomist, José Segura implied that maize and teosinte were much more related than previously assumed (Harshberger, 1896). On the basis of demonstration of teosinte-maize hybrids with fully normal meiosis, fully fertile seeds, and identical linkage distances between genes in maize-maize crosses, Beadle and Emerson concluded that the Mexican annual teosinte was in the same species as maize (Emerson and Beadle, 1932; Beadle, 1932a, 1932b). In fact, other cytological studies also indicated that maize and the Mexican annual teosintes were indistinguishable. *Z. diploperennis, Z. perennis,* and *Z. luxurians* are clearly distinguished from maize by a

number of morphological, biochemical, and cytogenetic characteristics (Doebley *et al*., 1984; Doebley, 1983, Timothy *et al*.,
1979, Kato, 1976,).

Of the three subspecies of teosinte, *Z. mays ssp. huehuetenangensis* is clearly differentiated from maize by several independent lines of evidence (Doebley *et al*., 1984, Kato, 1976). *Zea mays ssp. mexicana* shows clear allozymic differences from maize, but *Z. mays ssp. parviglumis* is essentially indistinguishable from maize by all measures except morphology. Conversely, other maize-teosinte hybrids such as maize-*Z. luxurians* exhibit two or more unpaired chromosomes during metaphase and were partially sterile. Following these findings, the origin of maize became a subject of much contention among key maize researchers. Decades of research involving artificial hybridization of maize with *Tripsacum* (2n=2x=36), another family of grasses (Manglesdorf and Reeves, 1939; 1938) gave birth to „the tripartite hypothesis‟ which asserted that the progenitor of maize was a now-extinct wild maize from South America; that teosinte was a hybrid of a cross between maize and *Tripsacum*; that a major source of the diversity between modern varieties of maize had been an „infection of *Tripsacum* germplasm. Although this hypothesis was short lived, it gave way to a more reasonable proposal put forward by Beadle (1939) that the teosinte was the progenitor of maize, which had undergone four major gene changes to convert it to cultivated maize having solid cobs and naked kernels.

This became known as the „teosinte hypothesis.‟ As outlined by Beadle (Beadle, 1980; 1978; 1972; 1939), the teosinte hypothesis states that (*a*) teosinte provided a useful food source and ancient people cultivated it for this purpose, (*b*) during the cultivation of teosinte, mutations that improved teosinte‟s usefulness to humans arose and were

selected by ancient people, (*c*) as few as five major mutations would be sufficient to convert teosinte into a primitive form of maize, (*d*) different mutations controlled different traits. For example, one mutation would have converted the disarticulating ear-type of teosinte into the solid ear type of maize, and (*e*) over the course of time, humans selected additional major mutations plus many minor ones.

Another school of thought asserts that, the teosinte, known as *Z. mays* ssp. *parviglumis* shares a close genetic relationship with maize and available evidence indicates that it is the direct ancestor of maize (Matsuoka *et al*., 2002; Doebley, 1990a). This latter teosinte grows in the valleys of southwestern Mexico. In these regions, it grows commonly as a wild plant along streams and on hillsides, although it can also invade cultivated fields as a weed. It is most common in the Balsas River drainage of southwest Mexico and hence is also known as Balsas teosinte.

Other cytological evidence were provided by Longley (1941), who demonstrated that chromosome arm lengths, centromere positions, and the sizes and positions of knobs in Mexican annual teosintes are identical to those of maize. Longley (1941) noted that *Zea luxurians* has a primitive karyotype with knobs only in telomeric positions and different from maize and Mexican annual teosintes which possess additional knobs in interstitial positions, as was also observed by Kato (1976). Longley (1941) concluded that the Mexican annual teosintes might have been the ancestor of maize.
On the basis of morphology, the teosintes appear to be very similar to maize. *Zea mays* ssp. *mexicana* (Schrader) Iltis (Central Mexican annual teosinte) is a largeflowered, mostly weedy teosinte, with a broad distribution across the central highlands of Mexico, whereas *ssp. parviglumis* Iltis and Doebley is a smallflowered, mostly wild teosinte of southern and western Mexico. *Zea mays ssp. huehuetenangensis* (Iltis and

Doebley) Doebley is a narrowly distributed teosinte of the western highlands of Guatemala. *Zea diploperennis* Iltis, Doebley, and Guzman and *Z. perennis* (Hitchc.) Reeves and Mangelsdorf are respectively, diploid and tetraploid perennial teosintes with narrow distributions in Jalisco, Mexico. *Zea luxurians* (Durieu and Ascherson) Bird is an annual teosinte from southeastern Guatemala (Doebley, 1990).

2.1.4 Allozyme evidence

For the purpose of providing evidence for the teosinte hypothesis, isozyme analysis encompassing 13 enzyme systems encoded by 21 loci were tested on 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). A graph of the first two principal components revealed that populations of maize and subsp. *parviglumis* could not be differentiated by their isozyme composition. A cluster analysis of the data also demonstrated that subsp. *parviglumis* was most similar to maize compared to the other teosintes, while that of maize and subsp. *mexicana* were distinct. These analyses suggest that subsp. *parviglumis* and maize share a more recent common ancestor with one another than they do with the other teosintes. The fact that teosinte is wild and maize is fully domesticated leads to a conclusion that their common ancestor was also a teosinte.
With regard to allozyme distribution, *Z. mays ssp. mexicana*, which forms frequent hybrids with maize and has robust maize-like vegetative characteristics, is allozymically quite different from maize; however, *Z. mays ssp. parviglumis*, which rarely hybridizes with maize and appears more slender and grasslike, is essentially indistinguishable from maize (Doebley *et al*., 1984). For example, the isozyme locus *Glu1-7* is reasonably common in *Z. mays* but unknown in *Z. diploperennis, Z. perennis*, and *Z. luxurians*, with the exception of one plant of *Z. luxurians*. A more

convincing case of introgression was found with *Z. diploperennis*. One plant of this wild species possessed two allozymes (*Enpl*-8 and *Pgdl*-3.8) that are otherwise unknown in this species but are common in maize. Several additional allozymes (*Glu*1- 11, *Pgdl*-1.8, *Pgd*2-8, and *Pgm*2-7.2) occur in both maize and *Z. mays ssp. mexicana* in the central highlands of Mexico (Doebley, 1990a).

2.1.5 Molecular evidence

More recently, the phylogenetic analyses based on the microsatellite data strongly favor a single domestication event (Matsuoka *et al*., 2002) derived from *parviglumis* teosinte. The microsatellite data further demonstrates ancestral lineage of maize with the Balsas teosinte in Mexico. 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).

2.2 Introduction of maize into Africa

Knowledge of origin and introduction of crops presents to researchers vital information on diversity existing in genetic resources found in different regions of the world. Such information is of paramount importance for genetic improvement of crops for increase in food production. Following the domestication of maize from the wild grass teosinte in Central America about 9,000 years ago, the crop spread northwards and southwards and was particularly abundant in the Aztec and Inca empires in Central America at the time when the New World was discovered (Manglesdorf, 1974). In the 15th and 16th centuries, Europeans exported maize from the Americas to other parts of the world. The adaptability and productivity of maize facilitated its rapid spread around the world (McCann, 2005).

The origin of maize in Africa is a subject of much controversy. Two authors, Miracle (1965) and McCann (2005) provide an extensive account of the history of maize, in which both affirm that maize may have been brought into the continent in the Columbian era through opening of the New World market in North America, the slave trade, arrival and establishment of Portuguese settlements in Elmina, Gold Coast and in Mozambique, and the arrival of Christian missionaries. Maize was probably introduced to tropical Africa at more than one point and at different times.

History of maize in West Africa dates as far back as pre-Columbian era in A.D. 1100 (Goodwin, 1953) when a pottery work having an imprint of the ear of maize was found at Ife, Nigeria, which has since been the only archeological evidence. The earliest writings on maize in Africa however, were by the Portuguese who had been involved with slave trade. Notable among the writers was Valentim Fernandes in 1502, who referred to the crop in the West African coast as *milho zaburro*. Though this name was not immediately associated with maize, as historians thought the writer may have meant millet or sorghum, in the writings of the Italian historian Gian Battista Ramusio, this crop was referred to as *miglio zaburro* (Jeffreys, 1954). Though Fernandes recorded many instances when maize may have been transported to Africa, in his writings, he admitted that maize was already known to the African peoples by then, but may not have been a staple food.

In contrast to this hypothesis, Portuguese writers recorded that transportation of maize to Africa occurred through two trade routes, viz, „north to south‟ introduction across the Sahara by Arab traders, who carried mainly flint types from Spain through the Mediterranean to Egypt, and then the „Atlantic coastal‟ introduction through Santiago, Cape Verde Islands, by the Portuguese (Portères, 1955), who also brought in the „floury kernel‟ types from Brazil. The Portuguese transported maize to Western

Africa for the purpose of cultivation as cheap food for the huge slave market which had soared following the New World market in 1517 (Miracle, 1965). Few years after, two Portuguese authors indicated that, maize had become prolific in a vast area along the coast from the Gambia to São Tomè, as well as in the interior of West Africa from the Gambia river to Niger in 1795-97 (Mollien, 1820). It is purported that soon after the Portuguese discovered the Islands of Cape Verde, they established maize farms to support the slave trade. Whether the maize was brought with them or it was maize already available on the West African coast, it is not known. In the account of McCann, maize was never known to the African people in pre-Columbian times. He uses linguistic evidence to refute the existence of maize in Africa in pre-Columbian era.

Evidence from linguistics on Portuguese introduction to the interior of West Africa is portrayed in local dialects such as *mazza manputo* in Congo and Sudan, which means „grain of Portugal", *Masim porto* in northern Angola, *milho basil* in Portuguese Guinea, meaning Brazilian grain, and *aburo* of the Akans in Ghana, similar to *zaburro* of Brazilian maize (Miracle, 1965).

Regarding introduction of maize to East Africa, there are historical records of Portuguese introduction of maize to the Congo sometime after 1493; that it was known in Ethiopia by 1516; it was introduced into Mozambique in about 1570; that it was cultivated in Angola coast and near the mouth of the River Congo in the 7th century, particularly between 1617 and 1621; that by 1643, Portuguese settlers in Zanzibar and Pemba grew maize to supply the Portuguese garrison in Mombasa and was known in Kenya as early as 1848, where maize was only a garden crop. However, maize may not have been a staple food by

then. Uganda received maize between 1863 and 1900 (Miracle, 1965) after which it spread to all other parts of
East Africa.

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Spread of maize in southern Africa is attributed to white settlements in the 18th century where maize had become a staple food in Mozambique, and had spread to
Zambia (McCann, 2005).

Other countries in southern Africa, such as southern Malawi and Zimbabwe received maize at a later date in the nineteenth century (McCann, 2005; Weinmann, 1972). At the beginning of the 20th century, maize became a major food crop in Africa. In contrast to southern Africa where large-scale spread of maize is attributed to British white settlement and introduction of American white dent maize in the 19th century, West Africa has not seen recent introduction to maize except of introgression of Quality Protein Maize from Mexico.

2.3 West and Central Africa as secondary center of diversity for maize

Dowswell *et al*. (1996) and Manglesdorf (1974) stated that the current genetic diversity in maize is the result of long selection process practiced by the Native Americans in Central America before it was spread to other parts of the world.
Introduction of maize to Africa at various points in time and space, the long history of continuous cultivation of the varied types introduced by various groups, exposure to and adaptation in varied ecological regions, compounded with a myriad of biotic and abiotic stress factors besides farmers‟ selection over many generations have contributed to the availability of many landraces of maize. In Africa, several landraces therefore occur whose genetic diversity remains to be explored and may constitute a rich reserve of alleles for maize

breeding. Currently, only few attempts have been made to examine the genetic diversity in the landraces that has resulted from such selection.

Tanksley and McCouch (1997) stated that most maize diversity remains undescribed, poorly understood, and underutilized in modern plant improvement programs largely because of the difficulty in identifying useful genetic variants hidden in the background of low yielding local varieties or lines. Despite the under utilization of landraces in maize breeding programs in Africa due to presence of undesirable agronomic traits, they can serve as sources of genes for disease resistance, abiotic stress tolerance, and useful nutritional factors. Understanding genetic diversity is required for effective breeding programs (Reif *et al*., 2006) and for harnessing the desirable characteristics present in wild relatives and landraces.

2.4 Adaptability to different environments

Maize is a C4 plant, and hence, it is physiologically more efficient and has higher grain yield and wider adaptation over a wide range of environmental conditions. With regard to geographical locations, maize can be cultivated from latitude 58°N in countries such as Canada and the Russian Federation, where summer temperature ranges from 10°C - 35° to 40°S in Chile and Argentina (Shaw, 1988) having temperature of 15.4°C – 18°C and even beyond to the dry and high temperature zones in the tropics. Maize also grows well at below sea level in the Caspian Plain to an altitude as high as 3900 m.a.s.l. in the Andean mountains in South America (Farnham *et al*., 2003; Paliwal, 2000a).

2.4.1 Classification of maize into ecotypes

On the basis of geographical location of cultivation and latitude, maize is classified into two distinct types (Dowswell *et al*., 1996), viz., tropical and temperate maize.

Tropical maize grows in warmer climates between the equator and latitude 30° N and

30° S whereas temperate maize grows in cooler environments beyond 34° N and S. Subtropical maize grows between 30° and 34° both N and S. With regard to altitude of cultivation, tropical maize can be sub-classified into lowland (<900 m.a.s.l), midaltitude (900-1600 m.a.s.l) and highland (>1600 m.a.s.l) maize.

2.5 Maize accessions and landraces

Accession is the name given to types of crop including wild material, progenitors, landraces, varieties, and cultivars. The concept of landrace is complex (Zeven, 1998) and is fraught with many inconsistencies. Landrace encompasses the whole array of traditional varieties, including primitive cultivar, primitive variety, farmer‟s variety, and local variety (Villa *et al*., 2005). Variety and cultivar are terms which precisely refer to formally improved germplasm, hence their use for landrace is misleading. There are many varying definitions of landrace indicating lack of consensus among researchers. Stoskopf *et al*. (1993) defined landrace as genetically variable local cultivar with sufficient genetic identity to be morphologically identifiable. Obeng-Antwi (2007) declared his concept of landrace as a farmer‟s variety that has undergone no formal scientific improvement, and has become genetically differentiated over a period of time to be morphologically identifiable. In simple terms, a landrace is also defined as groups of genotypes that have evolved through natural selection or as genotypes resulting from artificial selection based on a grower‟s own criteria such that individuals in the group are morphologically identifiable. Many other authors present definitions of landrace based on their own understanding (Almekinders and Louwaars, 1999; FAO, 1998; Tudge, 1988; Fehr,
1987; Frankel and Bennet, 1970).

Landraces also exhibit genetic diversity, are locally adapted, and associated with traditional farming systems (Villa *et al*., 2005). Genetic diversity study among the maize landraces in Africa can be used for the purpose of identifying useful genotypes for enhancing the performance of commercial cultivars in future breeding programs, for application in organizing and managing the germplasm in Genetic Resource Centers, for widening the genetic base of the gene pool, and for identification of heterotic groups for hybrid breeding. Historically, landraces have been used as parent material for the development of improved varieties. Farmers adopting improved varieties also continue to maintain landraces due to their superior hardiness in marginal environments, lower fertilizer requirements, comparatively low seed cost and better resistance to rot in comparison to improved varieties.
(Bellon and Hellin, 2011; Keleman *et al*., 2009; and Almekinders *et al*., 1994).

The importance of landraces to crop improvement programs and their conservation has been affirmed many a time in the literature depicting how useful they are as genetic resources which carry abundance of potentially useful genetic variation
(Grenier *et al*., 2000; Hayward *et al*., 1993).

2.6 Biology of maize

2.6.1. Maize morphology

Maize plant is a tall (1-4 m), determinate annual grass having large, long, and alternating leaves in a distichous arrangement (Essau, 1977), borne singly at the nodes of a single and relatively thick erect stem (culm). The shoot terminates into the inflorescences, the male at the apex and female at the axil (Dhillon and Prasanna, 2001). Figure 2.2 shows the morphological features of a maize plant. Some cultivars may develop few elongated lateral branches or tillers. Many temperate cultivars are

shorter than tropical and subtropical types. Leaves are about one tenth as wide as their length. Mature maize plant can have approximately 30 leaves, with considerable variation in leaf number, size, and orientation depending on the variety, season, location and date of planting (Ritchie *et al*., 1992). Generally, tropical maize plants and late-maturing varieties develop more leaves than temperate and early-maturing genotypes cultivars. The internodes are short and thick at the base of the plant, but become long and thicker higher up the stem and then taper again. Maize roots demonstrate unique features that are typical of the cereals, but deviates from that of dicots, such as *Arabidopsis*. Hochholdinger (2009) gave a detailed account of maize root system. In the embryonic stage, primary and seminal roots develop. Seminal roots are adventitious roots that grow from the base of the stem during early seedling growth and take over the functions of the radicle. In the post embryonic stage, crown roots and lateral roots develop which develop from the lower nodes of stem below ground level. In the adult plant, brace roots develop on the shoot.

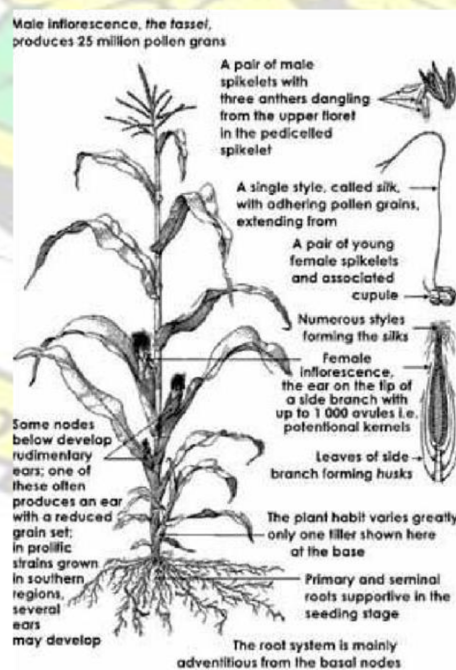

Figure 2.2 Morphological characteristics of the maize plant

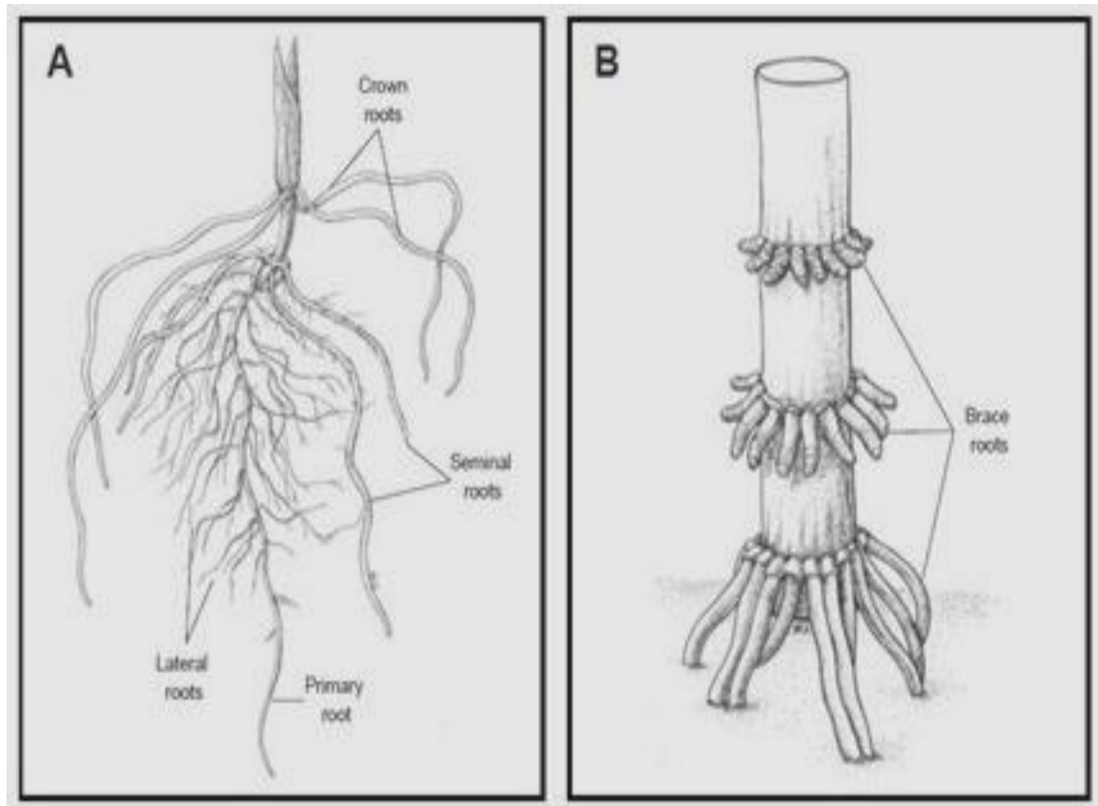

Figure 2.3 The root system of maize (A) Postembryonic lateral and crown roots Embryonic, primary and seminal roots and visible in 14-day-old wild type maize seedlings. (B)Aboveground shoot-borne brace roots of a 6-week-old plant (Hochholdinger, 2009).

2.6.2 Morphology of inflorescence and pollination

Maize is a monoecious plant having two types of inflorescence meristems, a terminal staminate flower or the tassel, and a lateral female inflorescence, the ear, which arises from the axillary meristem, five to six nodes below the tassel (Figure 2.2). Male and female inflorescences are identical at the rudimentary stages, but as they develop, some meristems are suppressed in the male or female to give rise to the morphologically disparate tassel and ear (McSteen *et al*., 2000; Cheng *et al*., 1983). Maize exhibits protandry in which the staminate flower matures earlier than the pistillate flower.

Anthesis in the male inflorescence begins a short distance from the tip of the central axis, and a little later at the tip of each rachis, followed by a gradual maturity which

proceeds downward along the axes of each rachis simultaneously. Pollen shed is intermittent and prolonged, begins two to three days prior to silk emergence and persists for five to eight days. A single panicle may produce up to 2×10^6 pollen grains per day (Jarosz *et al*., 2003), dispersed by wind to a distance of about 20 to 50 feet, thus favoring close to 97 % cross pollination. Pollen grain germinates immediately after settling on the silk. The pollen tube takes between 12 and 24 hours to reach and fertilize the ovule (Sleper and Poehlman, 2006). A very small, oneseeded dry, indehiscent fruit, the caryopsis, in which the actual seed coat is completely merged to the pericarp is formed.

The grain filling period in maize is approximately 8 weeks (Lee and Tollenaar, 2007) and goes through distinct stages of development named as, blister stage, milk stage, dough stage (dent stage in dent maize varieties) and physiological maturity spanning 55 – 65 days after silking (Farnham *et al*., 2003). The blister stage occurs 10 – 14 days after fertilization in which the husks, cob and shank develop rapidly and soon after, nutrients are accumulated in the developing kernels. The small blister-shaped kernels are filled with a clear fluid (Farrell and O"Keeffe, 2007).

The milk stage is characterized by the presence in the kernels of white fluid with high sugar content. During the dough stage, kernels are filled with a white paste and, starting at the tip of the kernel opposite the embryo, starch is deposited in the endosperm to give rise to distinct kernel textures, namely, floury (*Z. mays* var. *amylacea*), popcorn (*Z. mays* var. *everta*), dent (*Z mays* var. *indentata*) and flint (*Z mays* var. *indurata*) types. The other genotypes include sweet (*Z. mays* var. *saccharata* and *Z. mays* var. *rugosa*), waxy (*Z. mays* var. *ceratina*), amylomaize (*Z. mays* var. *tunicata*), and striped (*Z. mays* var. *japonica*) maize (Darrah *et al*., 2003; Paliwal,

2000c and Purseglove, 1972). At physiological maturity the endosperm reaches maximum dry matter with a moisture content of approximately 30-38 %, and formation of the hilum which seals off the kernel from the remainder of the plant (Paliwal, 2000e).

2.7 Maize types and their uses

Maize utilization is to some extent related to the grain types. Maize grain types are characterized by color and hardness. Eighty-five percent of global maize consists of yellow endosperm, 10-12 % white, and 5-8 % red, blue, purple, or black kernels (Morris, 1998). Yellow maize is used for feed and white for food. Flint maize is composed of hard starches, giving the kernel a rounded and vitreous surface. Dent maize is predominantly composed of soft starch that contracts on drying to give dented and opaque surface. A hard endosperm is present on the sides and base of the kernel. About 80 % global maize is dent and semi-dent, while 15 % is flint and semiflint texture. Dent maize is the most commonly grown maize for grain and silage.

Only 5 % is specialized grain like floury and waxy maize.

Maize of all grain types and colors are found in landraces in Sub-Saharan Africa, but the predominant ones are white dents, except where flints are preferred for their ease of pounding as in Malawi (M"boyi *et al*., 2010). Floury maize is commonly grown in the Andean region. Its endosperm is mainly composed of soft starch, making it easy to grind and process into foods. Waxy kernels contain starch similar to tapioca in which the starch is almost entirely amylopectin rather than the normal 70 % amylopectin and 30 % amylose. Waxy maize is preferred for food in some parts of East Asia and for some industrial uses. Pop maize kernels are characterized by a high

proportion of hard endosperm, much higher than in any other maize kernel. Pop maize is grown on a relatively small scale and is consumed worldwide as snack. Sweet maize is grown for green ears which are harvested at approximately 18 to 20 days after pollination when kernel moisture is about 70 %. The developing grain of sweet maize is higher in sugar content due to one or more recessive mutations blocking conversion of sugar to starch.

2.8 Genetic Diversity in Maize

According to Manglesdorf (1974), the phenotypic diversity present in maize today is the product of a long tradition of plant breeding practiced by Native Americans who played a major role in the development and adaptation of this crop to virtually every habitable environment in the Americas including deserts, tropical rainforest, and high mountains. The differentiation in African maize landraces is believed to arise from farmers‟ selection, the varied agroecological niches, and climatic stress factors (Sanou *et al*., 1997). Phenotypic and genetic diversity studies are aimed at identifying groups with similar genetic backgrounds for the purpose of conservation and utilization for trait improvement, for broadening the genetic base of modern maize, and for genotype identity in breeder‟s intellectual property rights (Franco *et al*., 2001).

Genetic diversity estimation among maize germplasm around the globe include that of North America (Tabanao and Bernado, 2005; James *et al*., 2002; Castiglioni *et al*., 1999; Dubreuil and Charcosset, 1998 ; Bretting *et al*., 1990; Kahler *et al*., 1986; Smith, 1986; Smith *et al*., 1985a,b; Goodman and Stuber, 1983) and European maize germplasm (Inghelandt *et al*., 2010; VazPatto *et al*., 2008; Okumus, 2007; Múdry and Ján Kraic, 2007; Le Clerc *et al*., 2006; Dubreuil and Charcosset, 1998; Ajmone Marsan

et al., 1998). Parallel studies have been carried out in China maize (Yao *et al*., 2008; Lu *et al*., 2002; Yuan *et al*., 2000), Japan (Enoki *et al*., 2002), among CIMMYT and non-temperate maize populations and inbred lines (Warburton *et al*., 2008; 2005; 2002, ,; Reif *et al*., 2004; 2003,; Xia *et al*., 2005; 2004;; Carvalho *et al*., 2002), Brazilian maize (Gimenes and Lopes 2000), and Argentina maize (Bracco *et al*., 2009).

Similar studies encompassing evaluation of landraces originating from different regions of Africa has not been conducted. Records available on genetic diversity in African maize include few maize accessions from Ethiopia (Legesse *et al*., 2007; Beyene *et al*., 2006;; Botha *et al*., 2000), Ghana (Oppong *et al*., 2014; Obeng-Antwi, 2007;), and some accessions from Zimbabwe, Zambia and Malawi (Magorokosho, 2006), and six other countries in West Africa (Sanou *et al*., 1997). Menkir *et al*. (2004) assessed the genetic relationships among tropical mid-altitude inbred lines developed in Nigeria and Cameroon. The result of lack of information on genetic diversity is that maize breeding efforts in sub-Saharan Africa is seriously limited such that progress in grain yield, abiotic and biotic stress tolerance and enhancement in grain quality is barely achieved while the usefulness of the collection remains unknown though size of collections steadily increases. To date, old breeding materials, and few newly developed genotypes have been bred from CIMMYT lines, producing improved maize of narrow genetic base.

While landraces are known to possess many useful alleles for crop improvement, African landraces have not been fully exploited. The IITA in Nigeria has in stock over 800 landraces whose genetic diversity information needs to be evaluated. It is important to characterize the diversity of the African maize landraces in order to

unearth their use and facilitate conservation. Furthermore, characterization is necessary for understanding of the purported historical introduction of maize into Africa, and for development of inbred lines for hybrid breeding.

2.8.1 Measures of Genetic Diversity

Genetic variation, relationships and association among crops (Mohammadi and Prasanna, 2003) have commonly been determined by pedigree information (Bernado, 1993; Messmer *et al*., 1993; Smith, 1988; Duvick, 1984), morphological and agronomic evaluations, molecular methods which include DNA-based techniques (Dudley *et al*., 1991); and isozyme analysis (Stuber *et al*., 1980; Stuber and Moll, 1972; Lamkey *et al*., 1978) and compositional data such as storage proteins and proximate composition (Imeri *et al*., 1987). The methods may be used individually or combined for better outcome as each has its individual strengths and limitations as well as combinations of the methods such as morphoagronomic, biochemical, and pedigree data.

2.8.2 Morphological and agronomic evaluation

The morphological and agronomic markers include traits expressed outwardly from the interaction of genes and environment. It includes characters such as plant height, stalk diameter, earliness, and grain yield. Morphoagronomic traits measurement is one of the foremost methods employed in crop characterization, estimation of genetic diversity, and germplasm management as they allowed quick and easy discrimination among phenotypes, and there abounds many statistical tools for analysis of the data. Their main limitation includes low level of polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992). To minimize the drawbacks, characters that are least influenced by environment are

commonly evaluated and this is complemented by subjection of the data to rigorous scrutiny by choice of appropriate experimental design and application of robust statistical analysis.

The maize descriptor list developed by the International Plant Genetic Resources Institute (IPGRI) is often employed for morphoagronomic characterization of crops. The descriptors evaluate in maize the most important discriminatory characters such as earliness, plant architectural traits, grain yield and yield components. Estimation of genetic diversity in maize on the basis of morphological descriptors were carried out on some European maize collections originating from Spain, Italy, Yugoslavia and Romania (Revilla *et al*., 2006; Gauthier *et al*., 2002; Ruiz de Galaretta and Alvarez, 2001; Gouesnard *et al*., 1997; Llaurado and Moreno-Gonzalez, 1993;Brandolini, 1970). Rebourg *et al*. (2001) carried out an assessment of 130 traditional European maize populations by means of molecular and morphological methods. Mikel (2008) evaluated the genetic diversity among 846 U.S. proprietary maize inbred lines registered from 1976 to 2005 by pedigree information, agronomic and morphological traits.

Other works on genetic diversity estimation by morphoagronomic evaluations are those of Harting *et al*. (2008) on Italian landraces and Le Clerc *et al*. (2006) on maize genotypes from France. Magorokosho (2006) carried out characterization, genetic diversity and assessment of relationships among maize from Malawi, Zimbabwe and Zambia and reported of wide morphoagronomical variability among the accessions for most traits. Ilarslan *et al*. (2002) found considerable genetic variation for morphological and agronomic traits in a collection of Turkish maize landraces. Little information is available on the phenotypic-based genetic diversity of maize landraces and varieties available in Africa. The genetic relationships among 62 Ethiopian

highland maize accessions were evaluated on the basis of 15 morphological traits (Beyene *et al*. 2005). Ninety maize landraces originating from Ghana were characterized by morphoagronomic traits and AFLP markers (ObengAntwi 2007). The large maize collection in IITA remains to be evaluated for genetic diversity.

2.8.3 Molecular markers

The advent of molecular markers has introduced higher efficiency assessment of genetic diversity and accurate prediction of genetic relationships among germplasm which is devoid of environmental influences. Molecular markers procedures overcome the drawbacks of agro-morphological markers which are time-consuming, labour-intensive and require large populations of plants (Enoki, 2002; Botha and Venter, 2000). The available molecular markers include the hybridization-based markers such as Restriction Fragment Length Polymorphisms (RFLPs) which make use of restriction enzymes (Botstein *et al*., 1980); polymerase chain reaction (PCR)based markers, including Random Amplified Polymorphic DNA (RAPDs) (Jacobsen and Hendren, 2007; Welsh and McClelland, 1990;), Sequence Tagged Sites (STSs), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al*., 1995) and microsatellites or Simple Sequence Repeats (SSRs) (Chin *et al*., 1996; Dib *et al*., 1996; Dietrich *et al*., 1996) and DNA sequence–based markers such as the single nucleotide polymorphism (SNPs) which are based on amplification by the polymerase chain reaction (PCR)

With the advent of molecular markers, estimation of the genetic variation within the subsets has been augmented, making way for classification to be refined (Warburton *et al*., 2008, 2005, 2002; Xia *et al*., 2005, 2004; Reif *et al*., 2004, 2003).

2.8.3.1 Microsatellite markers

In the early 1990s, microsatellite markers, which were first identified in humans were reported to be present in plant species as well, when the genome sequence of a number of plants were elucidated. Chin *et al*., (1996) discovered 69 out of 200 potential microsatellites in maize, many of which were AG/CT, CCT/GGA, and CCG/GGC repeats and were highly polymorphic, with 2-4 alleles. Senior *et al* (1996) developed 42 SSR markers from the maize sequence map of Coe *et al*.,

(1995). These primers were designated with the prefixes „*nc*" based on primer pairs designed by Senior and Heun (1993), while those designated with prefix „*phi*" were based on primer pairs developed by Chin *et al*. (1996), and the „*bnlg*" markers by

(Coe, 1996).

Additional 978 SSR markers, assigned prefixes „*umc*", were developed by Sharopova *et al*. (2002) to create dense genetic maps. The SSR markers, their primer sequences, map positions and polymorphisms are deposited at the Maize GDB at www.agron. missouri.edu. The SSRs offer several advantages over other markers as they are PCR generated, their polymorphisms can be detected in short periods of time by simple experiments, they are codominant markers and segregate in Mendelian fashion (Senior *et al*., 1998). Additionally, the SSRs are hypervariable, abundant, and uniformly dispersed in plant genomes, offer greater reliability and reproducibility and are more cost-effective over other markers (Smith *et al*., 1997). Pejic *et al*. (1998) and Smith *et al*. (1997) reported of good correlation between SSR and RFLP diversity and pedigree-based measurements. Moreover, the efficiency of SSRs can be increased by running

multiplexed reactions under automated electrophoresis conditions (Mitchell *et al*., 1997).

SSR markers were used to study genetic diversity in North American maize (Inghelandt *et al*., 2010; Ho *et al*., 2005; Xia *et al*., 2005, 2004), in China maize (Yao *et al*., 2008); and in tropical CIMMYT maize inbred lines (Reif *et al*., 2004, 2003; Warburton *et al*. 2008; 2002; VazPatto *et al*., 2008; Tabanao and Bernado, 2005).

Genetic diversity study among some African maize inbred lines present in the CIMMYT centers in Ethiopia and Zimbabwe have been determined using SSR markers (Legesse *et al*., 2007). Menkir *et al*. (2004) assessed the genetic relationships among tropical mid-altitude inbred lines developed in Nigeria and Cameroon, by means of AFLP and SSR markers. Beyenne *et al.,* (2006) evaluated genetic diversity of traditional Ethiopian highland maize accessions by SSR markers.
The genetic diversity estimates among maize accesions originating from Zambia, Zimbabwe, and Malawi were determined by SSRs (Magorokosho, 2006).

Conversely, the genetic variation within African maize accessions remains undescribed, poorly understood, and underutilized in modern crop improvement.

2.9 Genetic diversity evaluation

Genetic diversity assessment encompasses three levels of analyses depending on the nature of the data collected, and the genetic material under study. Typically, the data set may be , morphological , isozyme (e proteins electrophoresis), and DNA-based markers assessed on diverse materials such as germplasm accessions, inbred lines, or clones, populations, hybrids, and species. Since each of these data sets provide

different types of information, the choice of analytical method depends on the objective(s) of the experiment, the level of resolution required, the resources and technological infrastructure available, operational time and constraints (Karp *et al*., 1997).

The three levels of genetic diversity analyses are broadly divided into intrapopulation and interpopulation estimates as (i) quantification of genetic diversity, (ii) quantification of genetic relationships, and (iii) expression of the relationships in terms of classification and/or ordination (Cavalli-Sforza and Bodmer, 1981).

Many measures of genetic diversity are available in the literature including number of alleles per locus or allele richness, rate of polymorphism, observed and expected heterozygosity, effective population size (N_e). A gene is described as polymorphic if the frequency of one of its alleles is is less than or equal to 0.95 or 0.99 (CavalliSforza and Bodmer, 1981).

1. The rate of polymorphism refers to number of polymorphic loci (n_i) divided by the total number of loci (n_{total}), both polymorphic and monomorphic. It is expressed as

$$P = \frac{n_{pi}}{n_{total}} \quad .....(2.1)$$

(2) Average number of alleles per locus – this is the sum of all detected alleles in all loci, divided by the total number of loci and is expressed as

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

where k = the number of loci; n_i = the number of alleles detected per locus. This parameter is best applied with codominant markers.

(3) Effective number of alleles (A_e). This is the number of alleles that can be present in a population. It is represented as

$$A_e = \frac{1}{1-h} = \frac{1}{\sum P_i^2} \ldots\ldots (2.3)$$

where P_i= frequency of the *i*th allele in a locus; h= $1-\sum P_i^2$ = heterozygosity in a locus.

(4) Average expected heterozygosity (H_e). This is also known as Nei‟s genetic diversity index (*D*). It is the probability that, at a single locus, any two alleles, chosen at random from the population are different to each other. The value of H_e is an estimate of the overall extent of genetic variability in the population and it ranges from 0 to 1. It is calculated as an average of the sum of intralocus heterozygosity for both 2-allele and multi-allele loci. The value of H_e calculated from allele frequencies is that expected under Hardy-Weinberg equilibrium. It becomes necessary to compare the expected heterozygosity to the observed heterozygosity, H_o, in order to make inferences about the evolutionary history of the population (Lynch and Walsh, 1998).

(5) The effective population size (N_e) (Wright, 1931), is another important measurement which can indicate the amount of genetic variability of a set of individuals in a given situation. *N*e the size of an ideal population that has the same amount of drift in allele frequency, or the same rate of decrease in heterozygosity, as the actual population (Vencovsky and Crossa, 2003). It is a basic parameter that largely determines allelic retention, preservation, and conservation over generations and is particularly useful when studying genetic diversity of landraces.

2.9.1 Quantification of genetic relationships

Quantification of genetic relationships between operative taxonomic units (OTU) requires calculation of genetic distances. The basic measure of genetic diversity is genetic distance. Beaumont *et al*. (1998) defined genetic distance as any quantitative measure of genetic difference at either the sequence or allelic frequency level that is calculated between individuals, populations or species on the basis of what they do not have in common. It may be expressed as dissimilarity measure arising from divergence in space and time through evolutionary changes, such as mutation, migration and genetic drift that have occurred since two populations existed as a single random mating population. A small genetic distance indicates close

relationship while large genetic distance indicates distant relationship.

2.9.2 Determination of distance measures

2.9.2.1 Genetic similarity

There are many measures of genetic distance depending on the kind of data, *viz*., allozyme, molecular or phenological. Genetic distance measures include Euclidean distance, commonly used for quantitative agromorphological data. It is calculated as the root of the squared difference in traits between pairs of accessions *i* and *j*, and

Gower‟s distance for both quantitative and qualitative data (Gower‟s, 1971), and Bray-Curtis dissimilarity (Bray and Curtis, 1957). Many distance measures are available for gene frequency data, such as DNA bands or amplification products on SSR and RFLP gels. Few of these are the Cavalli-Sforza and Edwards (1967) arc and chord distances, Nei‟s distance (Nei, 1972), and Rogers (1972) distance. For nucleotide sequence data, the Jukes and Cantor (1969) distance is commonly used. In some instances where amplification products are converted to presence or absence binary data, the qualitative distance measures often used include Nei and Li‟s (1979) coefficient, Jaccard‟s (1908) coefficient, simple matching coefficient (Sokal and

Michener, 1958), and Modified Rogers" distance (Wright, 1978), both of which are Euclidean-type distance measures. The text of Mohammadi and Prasanna (2003) provides extensive review of other genetic distance measures.

For data sets which present special problems with differences in scale of measurement such as plant height in cm and kernel width in mm, standardization of the data to remove the effect of scale is a requirement. Some genetic distance measures, particularly correlation, incorporate standardization of data and are recommended for genetic distance on agromorphological and gene expression profile data. The Pearson correlation coefficient serves as a „correlation distance" and offers advantages over Euclidean-type measurements in being unit-independent, does not only reveal the strength of the association (the similarity measure), but exposes opposing gene effects. Similarity measures based on correlation coefficients range from -1 to 1 where 1 means the two traits or OTUs are identical, -1 means they are perfect opposites, and 0 means they are completely uncorrelated. Often, absolute or squared correlation is used as a distance measure to reveal the strength of the relationship rather than the sign.

2.9.3 Statistical analysis of distance measures

2.9.3.1 Bootstrapping

The genetic distance measures among OTUs are provided in a matrix form. The overall genetic distance in the population under test is calculated as average or estimate of the distance measures. To determine how close the value is to the expected, accuracy expressed as variance or confidence intervals of the sampling distribution is determined. The common situation of having a single data matrix and not replicates of it implies that the variance of the sampling distribution can be obtained by repeatedly sampling from within the same data matrix with replacement to permit computation

of variances. This is known a bootstrapping. Resampling over multiple times (usually >100) produces the probability of finding a genotype at a given position on a dendrogram.

2.9.4 Expressing relationships in a genetic diversity study

For expressing relationships in a genetic diversity study, classification methods that group entries into clusters according to plant characters are used. Clustering methods can be hierarchical, non-hierarchical or overlapping. In hierarchical methods such as the Ward method (Ward, 1963), entries are organized into a tree or hierarchy where entries or groups are fused one at a time to entries or groups with the most similar patterns for all characters. In nonhierarchical methods such as the Gaussian Mixed model or Normix model (Wolfe, 1970), initial groups must be defined a priori, and then the method improves the initial groups by an iterative process that results in a solution that corresponds to a maximum (global or local) of the likelihood function. With the Overlapping, individuals may belong to more than one group.

The three main methods from clustering include clustering by simple linkage (or nearest neighbor), by complete linkage (or farthest neighbor) and clustering by average linkage (or UPGMA). Single linkage is fast which enables the analyses of large data sets. It is also statistically consistent under many models of evolution. It however produces a chaining effect which leads to poor resolution of individual groups complicating the interpretation of results. Complete Linkage produces very clear groups by using minimum values but sometimes tends to underestimate similarity between recognized clusters. UPGMA is the simplest method for constructing trees with a high level of accuracy. It is consistent in grouping biological data with relationships computed from different data sets. The greatest disadvantage of UPGMA

is that it estimates the same evolutionary speed on all lineages, implying the rate of mutations is constant over time and for all lineages in the tree.

Warburton *et al*. (2002) constructed dendrograms of inbred lines from the similarity matrix by the UPGMA method (Rolf, 1997) to visualize the patterns of diversity in the lines.

Ninety four inbreds were clustered based on the matrix of genetic similarities using the Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) clustering algorithm (Senior *et al*., 1998). Legesse *et al*. (2007) performed cluster analysis to generate a dendrogram using UPGMA as implemented in the NCSS software (Hintze, 1998). Enoki *et al*. (2002) also used Average linkage (UPGMA) cluster analysis performed with the matrix the matrix of GS estimate using appropriate procedures of the program NTSYS-pc (Rolf, 1989).

2.10 Comparison of morphological to molecular analysis

Morphological traits were among the earliest markers used in germplasm management, but they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith., 1992).

On the other hand, DNA markers can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related genotypes. Because expression of morphological traits is influenced by environmental factors they are often regarded as unreliable as they do not give consistent trend in genetic relationships among genotypes, in addition to their inability to compare data among different populations. Smith *et al.* (1991) stated that morphological data did not

provide a good estimation of the genetic distance of studied maize inbreds. Ortiz *et al*. (2008) stated that morphological data provided the initial basis for taxonomic studies of maize and have remained a mainstay of maize racial taxonomy to current times. In the absence of morphological data on germplasm, the management of genetic resources in gene banks would be difficult

(Bioversity International, 2007‟ Sanchez *et al*., 2000).
Molecular markers are considered the best tools in genetic studies, due to the possibility to differentiate genotypes at the DNA level even when dealing with a narrow genetic base.

The added advantage of molecular markers is that they are under small impacts of environments Nagy *et al*. (2003) have compared RAPD, SSR and morphological markers and concluded that both marker systems only partially reflect genetic relationships among observed maize inbreds. Only combined analysis supported with morphological data provides a close association among groups formed on the cluster analysis and pedigree data.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Material

A set of 57 tropical *Zea mays* (TZm) accessions were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The accessions were collected from twelve tropical African countries including Tanzania, Benin, Zimbabwe, Zambia, Chad, Democratic Republic of Congo, Malawi, Equatorial Guinea, Kenya, Somalia, Togo and Burkina Faso. These regions represent the lowland (200-800 m.a.s.l.), mid-altitude (900-1600 m.a.s.l.) and the highland (over 1600 m.a.s.l) zones of Africa. In addition, five inbred lines comprising „Obatanpa GH", Tzi-8 and Tzi-9 and two CIMMYT (International Maize and Wheat Improvement Center) maize lines, namely, CML-157 and CML-258 were chosen to represent the diversity available among current and historic lines used in breeding maize for West and Central Africa.

Table 3.1 presents information on the origin of maize landraces in current study including their designation, country of origin, collection sites, their longitudes, latitudes and altitudes. Also shown is a schematic map of origin of the accessions (Figure 3.1). The genetic diversity study was divided into two parts, *viz.*, morphological, in which 36 accessions and a single check, „Obatanpa GH" were evaluated in field trials; and molecular evaluation of the entire 57 accessions and the five checks aforementioned.

3.2 Morphological evaluation of genetic diversity in maize

Accessions were evaluated in field trials in April to August 2011 and in March to July 2012

Table 3.1. Maize landraces representing the lowland, mid-altitude and highland accessions sampled from the maize germplasm collection in IITA

No.	Acc. name	Cultivar Name	Country	Mega-environment	Altitude (m.a.s.l.)	Longitude (degrees)	Latitude (degrees)
1	TZm-2	Mziava	Tanzania	Lowland	310	38.30	-6.02
2	TZm-4	Katumani	Tanzania	Mid-altitude	1000	37.57	-3.38
3	TZm-5	Katumani	Tanzania	Mid-altitude	1000	37.55	-3.33
4	TZm-6	Katumani	Tanzania	Mid-altitude	940	36.85	-5.35
5	TZm-7	Katumani	Tanzania	Mid-altitude	1300	34.17	-1.90
6	TZm-8	Katumani	Tanzania	Mid-altitude	1240	32.72	-2.62
7	TZm-11	Katumani	Tanzania	Mid-altitude	1180	32.05	-2.98
8	TZm-13	Katumani	Tanzania	Mid-altitude	1160	31.02	-3.30
9	TZm-14	Katumani	Tanzania	Mid-altitude	1160	30.10	-4.65
10	TZm-19	Katumani	Tanzania	Highland	1900	31.23	-7.55
11	TZm-20	Katumani	Tanzania	Highland	2100	31.77	-8.35
12	TZm-22	Katumani	Tanzania	Mid-altitude	1600	32.50	-8.48
13	TZm-23	Katumani	Tanzania	Mid-altitude	1480	32.92	-8.97
14	TZm-30	Katumani	Tanzania	Highland	1900	33.50	-8.92
15	TZm-32	Katumani	Tanzania	Mid-altitude	1170	34.05	-8.85
16	TZm-33	Walingombe	Tanzania	Mid-altitude	1480	34.58	-8.85
17	TZm-37	Walingombe	Tanzania	Midaltiude	1500	34.90	-8.73
18	TZm-38	Walingombe	Tanzania	Highland	1900	34.90	-8.73
19	TZm-41	Walingombe	Tanzania	Mid-altitude	1520	35.92	-7.73
20	TZm-42	Walingombe	Tanzania	Mid-altitude	1580	36.08	-7.70
21	TZm-57	Manicaland	Zimbabwe	Mid-altitude	1600	31.15	-18.42
22	TZm-63	Chimanimani clifton	Zimbabwe	Mid-altitude	1280	32.93	-19.73
23	TZm-77	Rusape inyanga	Zimbabwe	Highland	1800	32.45	-18.38
24	TZm-78	Shitowa hololeu	Zimbabwe	Mid-altitude	1340	32.73	-18.57
25	TZm-91	Shurugwi nashava	Zimbabwe	Mid-altitude	1060	20.22	-19.87
26	TZm-156	Gbogan	Republic of Benin	Lowland	50	3.22	6.77
27	TZm-242	Chintubulungu	Zambia	Mid-altitude	>900	31.50	-9.75
28	TZm-243	Kanjilimini	Zambia	Mid-altitude	>900	28.83	-9.75
29	TZm-251	Solweizi	Zambia	Mid-altitude	>900	26.00	-12.5
30	TZm-270	Makandakund a	Zambia	Mid-altitude	>900	23.00	-13.5
31	TZm-273	Chinyinji	Zambia	Mid-altitude	>900	22.75	-13.25
32	TZm-275	Mwinhunga	Zambia	Mid-altitude	>900	24.33	-12.25
33	TZm-301	Karal	Chad	Lowland	300	14.80	12.92
34	TZm-384	Oyo bokouele	Congo	Lowland	290	16.10	-1.07
35	TZm-385	Oyo bokouele	Congo	Lowland	315	16.30	-1.00
36	TZm-1084	Zunde ngabu	Malawi	Lowland	100	31.50	-16.47
37	TZm-1095	Balaka	Malawi	Lowland	100	34.97	-16.57
38	TZm-1355	Guinea local	Equatorial Guinea	Mid-altitude	600-1220	1.50	10.00
39	TZm-1356	Guinea local	Equatorial Guinea	Mid-altitude	600-1220	1.50	10.00

Table 3.1 cont‟d

No.	Designation	Cultivar Name	Country	Mega-environment	Altitude (m.a.s.l.)	Longitude (degree)	Latitude (degree)
40	TZm-1357	Guinea local	Equatorial Guinea	Mid-altitude	600-1220	1.50	10.00
41	TZm-1358	Guinea local	Equatorial Guinea	Mid-altitude	600-1220	1.50	10 .00
42	TZm-1359	Chebolosinik	Equatorial Guinea	Mid-altitude	600-1221	1.50	10
43	TZm-1360	Githigu	Kenya	Highland	>1600	NA[1]	NA
44	TZm-1367	Mahindi	Kenya	Highland	>1600	NA	NA
45	TZm-1369	Kinyanya	Kenya	Highland	>1600	NA	NA
46	TZm-1376	Kiragoli	Kenya	Highland	>1600	NA	NA
47	TZm-1380	Muthanu	Kenya	Mid-altitude	500-1200	NA	NA
48	TZm-1413	Magadishu to Baidoba	Somalia	Lowland	497	44.97	2.47
49	TZm-1424	Makambako Iringa	Tanzania	Highland	1900	34.90	-8.73
50	TZm-1430	NA	Burkina Faso	Lowland	340	NA	NA
51	TZm-1434	NA	Togo	Lowland	<500	NA	NA
52	TZm-1437	NA	Togo	Lowland	<500	NA	NA
53	TZm-1502	NA	Burkina Faso	Lowland	340	NA	NA
54	TZm-1514	Chintubulungu	Zambia	Mid-altitude	1235	31.50	-9.75
55	TZm-1516	Kanjilimini	Zambia	Mid-altitude	1235	28.83	-9.75
56	TZm-1521	Mali 2	Guinea	Mid-altitude	600-1500	NA	NA
57	TZm-1523	Tangue	Guinea	Lowland	188	NA	NA
58	TZi-8	NA	Cameroon	NA	NA	NA	NA
59	TZi-9	NA	Cameroon	NA	NA	NA	NA
60	„Obatanpa GH‟	NA	Ghana	Lowland	277	-1.67	6.67
61	CML-157	NA	Mexico	Lowland	NA	NA	NA
62	CML-258	NA	Mexico	Lowland	NA	NA	NA

[1]Information not available

to determine phenotypic diversity and classify the landraces into groups for further evaluation. The genotype „Obatanpa GH‟, being a major source of inbred line has been used for the development of Quality Protein Maize (QPM) hybrids and synthetic varieties in several maize breeding programs in Africa (Badu-Apraku, 2006). This was developed by the Crops Research Institute (CRI) of the Council for

Scientific and Industrial Research, Ghana. The genotype ʻObatanpa GH" is an improved tropically adapted, intermediate-maturing genotype with white, dent and flint kernel texture. It was released in 1992 by the CRI in collaboration with IITA,

CIMMYT, Mexico, and Sasakawa Global 2000.

KNUST

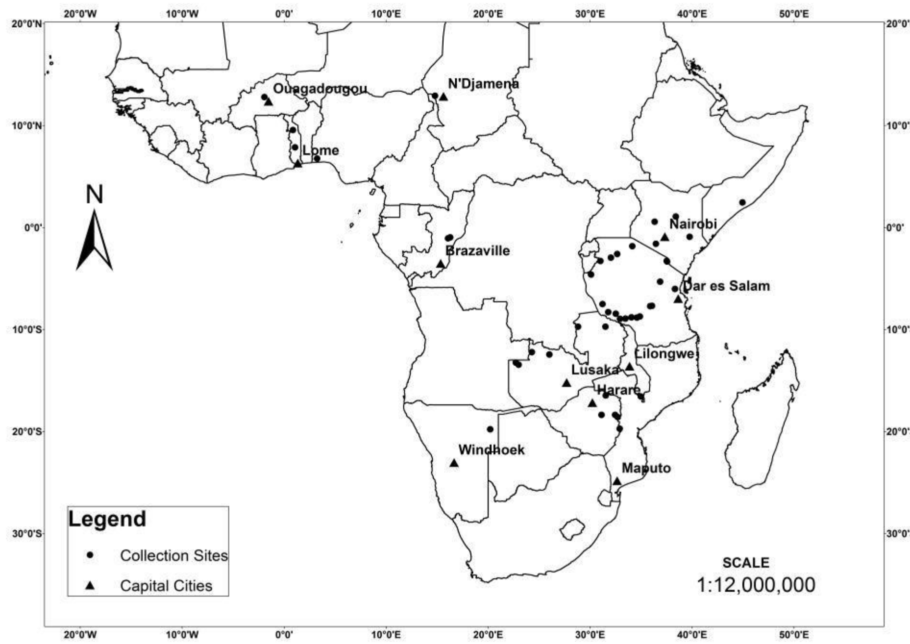

Figure 3.1 Schematic map of Africa depicting collection sites of maize in current study. Capital cities are indicated with shaded triangle and collection sites are shown as shaded bubble.

Genotypes TZi-8 and TZi-9 with accession numbers PI 506246 and PI 506247, respectively, were supplied by the North Central Regional Plant Introduction Station, Iowa State University, U.S.A. Both genotypes are inbred lines produced by the Institut National de la Recherche Agronomique (INRA), Cameroon, and IITA. TZi-8 is late-maturing with white and flint kernels and is resistant to both root lodging and maize streak virus (MSV). TZi-9 is medium-maturing and the kernels are white with dent and flint texture. Both inbred lines are used extensively in breeding programs. CML-157 is a lowland late-maturing inbred line from Mexico. It is a QPM genotype having

flint texture, white kernels and medium plant height. CML-258 is a lowland late-maturing normal maize inbred line from Mexico with a very short anthesissilking interval (ASI). It is a tall, low yielding plant, with dent and white kernels.

3.3 Location of experimental site

Field trials were carried out at the Anwomaso Agricultural Experimental Station of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. This site is located at longitude 1.61^{o} W and latitude 6.6^{o}N at an elevation of 277 m.a.s.l. The soil type is well-drained sandy loam with pH 5.2 and organic matter of 1.8 %. Mean annual rainfall in the station is 1500 mm and an average monthly temperature of 20 - 25 ºC. Anwomaso experiences an annual bimodal rainfall pattern with a high relative humidity. The rainy season is characterized by heavy rains from middle of March to July interspersed with a short dry spell in August followed by minor rains from September to November. The vegetation of the research site is semi-deciduous forest zone type with thick grass cover commonly dominated with Guinea grass (*Panicum maximum*) on a fairly flat topography.

3.4 Land preparation, planting and experimental design

Land preparation involved ploughing and harrowing, followed by pre-emergence weed control with Round-Up Ready (Glyphosate, 360 g/L) applied at 5.0 L/ha and Gramoxone (Paraquat) applied at 3.5 L/ha. All entries were planted in a randomized complete block design with three replications. An experimental plot consisted of 6 m × 0.6 m row planted to 15 hills per row. Plots were separated by 0.75 m and blocks were separated by 2 m alleys. Planting density was 4.2 plants/m^2. Recommended crop management techniques were applied. Irrigation was applied regularly as needed. Fertilizer equivalent to 120:60:40 kg ha^{-1} of N-P_2O_5-K_2O was applied at 21 days after

planting and sulphate of ammonia (125 kg/ha) at ear emergence. Postemergence weeds were controlled by application of Atrazine (4.5 L/ha) and hand weeding with a hoe. Maize stem borers (*Busseola fusca, Sesamia calamistis*) and cutworms (*Agrotis spp.*) were controlled using Conpyrifos 48 % EC (Chlorpyrifos ethyl) applied at 1.0 -1.5 L/ha and Cymethoate Super (1.0-1.5 L/ha) during the vegetative stage.

3.5 Morphological data collection

For each plot, 29 agromorphological parameters consisting of 5 qualitative and 24 quantitative traits covering plant architecture, ear and tassel-related traits, kernel characteristics, yield and yield component data were collected from 10 competitive plants per plot at random following the maize descriptor list developed by IBPGRI and CIMMYT (1991) (Table 3.2). Measurements were taken with meter rule, micrometre screw gauge, Vernier calliper, and electronic weighing scale as appropriate with each data.

3.6 Estimation of genetic diversity

Genetic diversity was estimated by three broad methods encompassing, (a) data description, (b) calculation of genetic distance among the accessions, and (c) determination of relationships among accessions.

3.6.1 Data description: statistical analyses of morphological data

Means, standard deviation, minimum and maximum values, as well as coefficient of variation (CV) for each population were calculated using SAS 9.3.1 (Statistical Analysis System, Cary, 2011). Analysis of variance (ANOVA) was performed on each trait by means of PROC GLM to test for significance of variation among accessions. Maize accessions were considered as random effects while replications

and blocks within replications were considered as fixed effects. The form of ANOVA and generation of expected mean squares (EMS) involving genotypes and environments are presented in Table 3.3.

Table 3.2. List of 29 morphological descriptors used in agromorphological evaluation of maize originating from three mega-environments and planted in 2011 and 2012 major rainy season in Ghana.

No.	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 stage	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 plants at 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 plants at harvest (Physiological maturity)	EL	Ear length (cm)	Quantitative	Length of ear located on the highest insertion point

14	On ten random plants at harvest (Physiological maturity)	EP	Ear position	Quantitative	Calculated as EHT divided by PLHT

Table 3.2 cont‟d.

No.	**Measurement procedure**	**Abbreviation**	**Phenotypic data (units)**	**Trait**	**Definition**
15	On ten random plants at harvest(Physiological maturity)	ED	Ear diameter (mm)	Quantitative	Diameter of ear located on the highest insertion point
16	On ten random plants at harvest (Phys. maturity)	CC	Cob colour (score)	Qualitative	Colour of cob after shelling (0=red; 5=white)
17	On ten random plants at harvest (Physiological maturity)	CD	Cob diameter (mm)	Quantitative	Diameter of cobs
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 color 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 $Mgha^{-1}$

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

Source	**df**	**MS**	**Expected Mean Square**
Year	y-1	M_y	$\sigma^2_e + r\sigma^2_{gy} + g\sigma^2_{r(y)} + rg\sigma^2_y$
Rep (year)	y(r-1)	M_{ry}	$\sigma^2_e + g\sigma^2_{r(y)}$
Genotype	g-1	M_g	$\sigma^2_e + r\sigma^2_{gy} + ry\sigma^2_g$
Gen*Year	(y-1)(g-1)	M_{gy}	$\sigma^2_e + r\sigma^2_{gy}$
Error	y(g-1)(r-1)	M_e	σ^2_e

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where df = degree of freedom; MS = mean square; *g* = number of genotypes (accessions); *y* = number of years; *r* = number of replicates; 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 $g \times y$. The genotypic and phenotypic variance components were extracted from the linear functions of the mean squares represented by „M" and subscript (s) which represents the associated source of variation.

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

Standard errors of the estimated variance components were computed using the method of Hallauer and Miranda (1981). Snedecor (1956) demonstrated that if the variance component was computed from a linear function of independent mean squares, the approximate variance, *V*, of a variance component $\hat{\sigma}_i^2$, is determined as

$$V(\hat{\sigma}_i^2) = \frac{2}{f^2} \sum \frac{M_i^2}{df_i + 2} \quad \text{..... (3.1)}$$

where f = is the coefficient of the component of variance; f_i = is the degree of freedom of the respective mean squares; □$_i$ = □1 and M_i = is the composite mean squares used to determine the variance component. Broad sense heritability (H^2), defined as the proportion of the total variance due to genetic effects was estimated as:

$$H^2 = \sigma_g^2/\sigma_P^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r) \quad \text{..... (3.2) (Doolittle, 1987)}$$

where σ_P^2 is the phenotypic variance component. The standard error (SE) of heritability (H^2) was approximated with the equation of Hallauer and Miranda (1981) as:

$$\text{SE } H^2 = \text{SE}(\sigma_g^2)/\sigma_P^2 \quad \text{….. (3.3)}$$

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

$$GCV = 100(\sigma_g/\bar{X}) \quad \text{….. (3.4)}$$

$$PCV = 100(\sigma_P/\bar{X}) \quad \text{….. (3.5)}$$

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. The means for each trait

were then standardized to avoid the influence of different scales of measurements in different traits on data interpretation and equalizing their effects in the final output of the cluster analysis (Anderberg, 1973) data and from this, a data matrix made of means of traits and accessions was constructed.

3.7 Genotypic and phenotypic correlation and their standard error

Genotypic and phenotypic correlations coefficients were calculated between traits by considering maize accessions as random effects. Using the genotypic and phenotypic variances and covariance component estimates, the genotypic and phenotypic correlations between traits *i* and *j* were estimated as:

$$r_{Gij} = \frac{\sigma_{Gij}}{\sigma_{Gi}\sigma_{Gj}} \quad \ldots.. (3.6)$$

$$r_{Pij} = \frac{\sigma_{Pij}}{\sigma_{Pi}\sigma_{Pj}} \quad \ldots.. (3.7)$$

where r_{Gij} and r_{Pij} are the estimated genotypic and phenotypic correlations between traits *i* and *j*, respectively; σ_{Gij} and σ_{Pij} are the estimated genotypic and phenotypic covariances between traits *i* and *j*, respectively, and σ_{Gi}, σ_{Gj}, σ_{Pi}, and σ_{Pj} are the genotypic and phenotypic standard deviation for traits *i* and *j*, respectively. All computations were implemented using PROC MIXED of SAS which uses the Restricted Maximum Likelihood Estimation (REML) method to generate variance and covariance components, as well as correlations and their standard errors (Holland, 2006).

3.8 Assessment of relationships among genotypes

3.8.1 Distance measurements and cluster analysis

The agro-morphological data was standardized before using in multivariate analysis. Relationships between genotypes were assessed by calculating distance as correlation coefficients to estimate the level of dissimilarity among all pairs of genotypes. To better view the distances among accessions, cluster analysis was carried out by means of the hierarchical method of Unweighted Pair Group Method with Arithmetic Average (UPGMA) which groups accessions on the basis of average distances. The Sequential Agglomerative Hierarchical Nesting (SAHN) in NTSYS was used. A dendrogram was generated from the cluster analysis. The adjustment between the distance matrix and the dendrogram was estimated by the cophenetic correlation coefficient (Sokal and Rolf, 1962). Correlations between the distance and dissimilarity matrices were performed using MXCOMP option. Assessment of the reliability of the nodes in the dendrogram and its statistical significance was determined by bootstrapping (Felsenstein, 1985) using PAST software (Hammer *et al*., 2001).

3.8.2 Principal components analysis

A principal component analysis (PCA) was performed on the accession-by-trait correlation matrix in order to depict non-hierarchical relationships among the genotypes and to determine the traits that are most effective in discriminating between accessions. Through singular value decomposition, the eigenvectors (principal component coefficients), correlation coefficients, and eigen values which explain relative proportions of the total variance, as well as cumulative proportions expressed by single traits were determined. Relationships among traits were investigated by means of graphing the principal components in biplots in NTSYS-pc

2.2 (Rohlf, 2009).

3.9 Genetic diversity in maize by means of SSR fingerprinting

From the field-grown plants at knee height 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 ^oC until ready for use. 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 with minor modifications by the Cocoa Research Institute of Ghana. Each bulked sample was ground into powder in liquid nitrogen. To 0.10 g of the bulked sample was added 700 ul of 2 % CTAB buffer (Appendix A1) incubated for 30 min at 65°C in sand bath with intermittent vortexing. The mixture was centrifuged at 14,000 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 14,000 r.p.m. for 5 min to pellet nucleic acids. Pellets were washed twice with 500 ul of washing buffer (Appendix A2) and 400 ul of 80 % ethanol, airdried and resuspended in 300 ul of TE buffer (Appendix A3) and incubated with 10 ug/ml RNase for 30 minutes. To the mixture was added 11.2 ml of 2 M NaCl (Appendix A4) 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 (Appendix A5).

3.9.1 SSR primer selection

One hundred SSR primer sets selected from the maize genetic database (http://www.maize gdb.orf/ssr.php) were assayed for their preliminary discriminatory

power on 16 accessions. Primers which did not amplify as well as those which did not produce variety of bands were excluded. Although many of the primers produced clear bands, only sixteen primers were selected due to cost constraints, to cover all ten chromosomes and to have at least one representation of each of the oligonucleotides as di- (25 %), tri- (25 %), tetra- (25 %), penta- (12.5 %), and hexa- (12.5 %) repeats from the preliminary evaluation for amplification of the DNA templates. Table 3.4 shows the names of primers, their chromosomal location, type of repeat, and forward and reverse primer sequences.

3.9.2 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. Each reaction was amplified in an Eppendorf Mastercycler (Germany), by a process of denaturation step of 1 min at 96°C, followed by a touchdown procedure which encompassed denaturation at 96 °C for 1 min., annealing at 65 °C for 1 min and extension at 72 °C for 2 min. The annealing temperature was then reduced after each cycle by 0.5 °C until a final annealing temperature of 55 °C was reached. The last cycle was repeated 20 times and terminated at 72 °C for 2 min. The reaction was finished with a continuous cycle at 4 °C. After the reaction, the reaction mix was heated at 96 °C 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 loaded along with 1 kb DNA ladder onto 2 % agarose gel (Bioneer, South Korea) containing 5 ul

ethidium bromide as the fluorescent dye. Electrophoresis was run at 120 V for 2 h after which the gels were photographed under UV light by means of UV transilluminator (Geldoc, BIO-RAD Laboratories, Inc.).

3.10 Statistical analysis of molecular data

3.10.1 Allele scoring and data analysis

Gel photographs were examined and bands were scored in binary form as presence (1) or absence (0). Care was taken to prevent mis-scoring arising from faint and stutter bands by ensuring a maximum of two alleles per locus to fulfil the diploid condition of maize. Lanes with no bands were also recorded a missing data. Primers and/or accessions that showed 15 % or more missing data were eliminated
(Warburton *et al*., 2002).

3.10.2 Estimation of genetic diversity within populations

The binary data matrix was first analyzed for rate of polymorphism (*P*) given by

$$P = \frac{n^{pi}}{n_{total}} \times 100\% ..... (3.8)$$

where n_{pi} is number of polymorphic loci and n_{total} is the total number of loci, both polymorphic and monomorphic; average number of alleles per locus (A) also known as allele diversity was calculated as sum of all detected alleles with frequency ≤0.95 divided by the total number of loci

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

Marker	Chr.[1]	Bin	Repeat	Repeat Unit	Primer Sequence (F/R)	T_M (°C)	Annealing temp. (°C)
bnlg1597	1	1.09	Di	(AG)34	GATAATCTCGTCTCGCCAGG(F) CATAAAAGGATGCCGACGAC(R)	59.40 57.30	58.0
phi002	1	1.08	Tetra	AACG	CATGCAATCAATAACGATGGCGAGT(F) TTAGCGTAACCCTTCTCCAGTCAGC(R)	61.30 64.60	63.0
nc133	2	2.05	Penta	GTGTC	AATCAAACACACACCTTGCG(F) GCAAGGGAATAAGGTGACGA(R)	55.30 57.30	56.0

phi101049	2	2.10	Tetra	AGAT	CCGGGAACTTGTTCATCG (F) CCACGTCCATGATCACACC (R)	56.00 58.80	57.0
phi046	3	3.08	Tetra	ACGC	ATCTCGCGAACGTGTGCAGATTCT(F) TCGATCTTTCCCGGAACTCTGAC(R)	62.70 62.40	63.0
phi073	3	3.05	Tri	AGC	GTGCGAGAGGCTTGACCAA(F) AAGGGTTGAGGGCGAGGAA(R)	58.80 58.80	59.0
bnlg1565	4	4.09	Di	AG(27)	TCGGAGACGAGGCTGAAC(F) CTGGAGACGTTTGGTGTCAA(R)	57.30 59.40	58.0
phi213984	4	4.01	Tri	ACC	GTGACCTAAACTTGGCAGACCC(F) CAAGAGGTACCTGCATGGC(R)	62.10 58.80	60.0
dupssr10	5	5.04	Di	(AC)22	AGAAAATGGTGAGGCAGG(F) TATGAAATCTGCATCTAGAAATTG(R)	53.70 54.20	54.0
bnlg1371	6	6.01	Di	AG(22)	TTGCCGATAAGAACCAAACA(F) ACGACCGGTGTGGTTACATT(R)	53.20 57.30	55.0
phi034	7	7.02	Tri	CCT	TAGCGACAGGATGGCCTCTTCT(F) GGGGAGCACGCCTTCGTTCT(R)	62.10 63.50	63.0
umc1161	8	8.06	Hexa	(GCTGGG)5	GGTACCGCTACTGCTTGTTACTGC(F) GCTCGCTGTTGGTAGCAAGTTTTA(R)	64.40 61.00	63.0
phi065	9	9.03	Penta	CACTT	AGGGACAAATACGTGGAGACACAG(F) CGATCTGCACAAAGTGGAGTAGTC(R)	62.70 62.70	63.0
umc1279	9	9.00	Tri	(CCT)6	GATGAGCTTGACGACGCCTG(F) CAATCCAATCCGTTGCAGGTC(R)	61.40 59.80	61.0
phi041	10	10.00	Tetra	AGCC	TTGGCTCCCAGCGCCGCAAA(F) GATCCAGAGCGATTTGACGGCA(R)	63.50 62.10	63.0
umc1196	10	10.07	Hexa	CACAGC	CGTGCTACTACTGCTACAAAGCGA(F) AGTCGTTCGTGTCTTCCGAAACT(R)	62.70 60.60	62.0

[1]Chromosome and is

expressed as

$$n = \frac{1}{k}\sum_{i=1}^{k} n_i \quad \text{..... (3.9)}$$

where k = the number of loci; n_i = the number of alleles detected per locus. The

Effective number of alleles (A_e) was calculated as

$$A_e = \frac{1}{1-h} = \frac{1}{\sum P_i^2} \quad \text{..... (3.10)}$$

where P_i= frequency of the ith allele in a locus; $h = 1 - \sum P_i^2$ = heterozygosity in a locus.

The Polymorphism Information Content (PIC) for each SSR locus was calculated from

the formula

$$\text{PIC} = 1 - \sum (P_i)^2 \quad \text{..... (3.11)}$$

where P_i is the proportion of the population carrying the ith allele (Smith et al., 1997;

Bostein *et al*., 1980). This calculation is equivalent to the gene diversity term of

Weir (1996). The expected heterozygosity (H_e) or Nei"s genetic diversity index (D) was also calculated as average of all PIC values.

3.10.3 Similarity Coefficient

Genetic distance calculations were performed on the accession by loci binary matrix data based on similarity index. Being qualitative and binomial and not normal distribution, standardization was not required. The similarity coefficients were estimated by the Dice coefficient (Nei and Li, 1979) by means of SIMQUAL option in NTSYS 2.21c (Rohlf, 2009). The Dice coefficient was computed as $2a/(2a + b + c)$, where a is the number of SSR bands shared by genotypes in each pairwise comparison; b and c are the numbers of SSR bands present in one genotype and not present in the other. Cluster analysis was performed on the similarity coefficients matrix using the Sequential Agglomerative Hierarchical cluster method based on UPGMA analysis (Sneath and Sokal, 1973) of the NTSYS package.

Statistical significance of the tree generated from cluster analysis was ascertained by bootstrap analysis (Felsenstein, 1985) using the PAST software (Hammer *et al.*, 2001). A cophenetic correlation (Sokal and Rohlf, 1962) was calculated to test the reliability and goodness-of-fit-between the similarity matrix obtained from the cluster and the original similarity matrix.

3.11 Principal Components Analysis

Principal components analysis (PCA) was performed using the subroutine EIGEN to produce eigenvalues and eigenvectors which reveal both total variance and the loci that are important to the variance. Biplots were generated to reveal associations among traits, accessions, and traits and accessions. All computations were carried out using the NTSYS-pc Version 2.2 package (Rohlf, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSION

In the current research maize accessions originating from three mega environments in Africa were evaluated for genetic variation by morphological and molecular diversity studies. The study was conducted in the major rainy season of 2011 and 2012 at the Agricultural Experimental Station Anwomaso, Kwame Nkrumah University of Science and Technology Kumasi, Ghana.

4.1. Morphological evaluation of maize from three mega environments Thirty-six maize accessions belonging to three mega environments and originating from 14 regions in Africa held at the IITA Genetic Resource Center were studied. The mega environments were low elevation (200-800 m.a.s.l.), mid elevation (9001600 m.a.s.l.), and high elevation (above 1600 m.a.s.l.). Five accessions (14 %) originated from the highland regions of Tanzania and Kenya, twenty accessions (55 %) from Equatorial Guinea, Guinea, Tanzania and Zambia represented the midaltitude collections, while eleven accessions (31 %) originated from the lowland regions of Chad, Tanzania, Congo, Guinea, Malawi, Somalia, and Togo. One cultivar from Ghana, „Obatanpa GH"" was used as a check. In all 2,160 plants were evaluated for a total of 29 phenotypic characters, made of 5 qualitative and 24 quantitative traits.

4.1.1 Qualitative description of maize accessions

Table 4.1 shows the distribution of qualitative traits among the 35 accessions studied in addition to the check. Large variabilities were identified among the plants of different accessions for all qualitative traits except cob color. Majority of the accessions had pale yellow silks with regular kernel

Table 4.1. Description of qualitative traits of lowland, mid-altitude, and highland African maize accessions held in IITA and evaluated in Ghana in April to August 2011 and 2012

No.	Trait	Description	Class	No. of plants	Percentage (%)
1	Silk color	Pale yellow Red	1 2	1231 929	57 43
2	Kernel arrangement	Regular Irregular Straight Spiral	1 2 3 4	1126 437 344 253	52 20 16 12
3	Cob color	Red White	0 5	162 1998	8 92
4	Kernel texture	Flint Dent	1 5	825 1335	38 62
5	Principal grain color	White Other colors	0 1	1097 1063	51 49

arrangement in addition to white and dent grains borne on white cobs. In general, the largest variability was detected in kernel arrangement in which each of the four categories was represented by substantial number of plants, although regular kernel arrangement was predominant (Table 4.1). In all, 1,126 (52 %) were regular, 437 (20 %) were irregular, 344 (16 %) were straight and 253 (12 %) had spiral arrangement. The predominant kernel texture was dent (62%). Variability in silk color, and principal grain color were also high, whereas cob color was the least variable with 1,998 cobs (92 %) being white and as few as 162 plants (8%) being red cobs.

The principal grain characteristics of the entire accessions in current study, which is white and dent kernels in regular arrangement on the cob confirm the historical fact that major introduction of maize into Africa was by the Portuguese who brought in the white and dent kernels while a minor quantity was introduced by the Arabs who carried the flint type (McCann, 2005; Miracle, 1965; Portères, 1955). Other grain colors were purple, yellow and red. Figure 4.1 shows phenotypic diversity among the

African maize genotypes from the three mega environments.

Figure 4.1 Phenotypic diversity among maize accessions collected from lowland, mid-altitude and highland regions of Africa. Ears show white, yellow, red, purple and blue kernels.

4.2. Variation in quantitative traits in the three mega environments

Means, standard deviations, range and mean squares of the 24 quantitative traits are presented in Table 4.2. Assessment of variability was based on significance of the mean squares from analysis of variance as well as magnitude of the coefficient of variation. Mean squares and coefficients of variation revealed large variability among all quantitative traits except anthesis-silking interval which increased in the order of 16 significant ($P<0.05$) mean squares in the highland population (MS=0.00 to 1,842, CV= 4.46 to 60.86 %), 21 significant ($P<0.05$) mean squares in the lowland population (MS= 0.00 to 1,397, CV= 10.54 to 57.15 %), and finally, 22 significant ($P<0.001$) mean squares in the mid-altitude populations (MS= 0.01 to 1,956, CV= 5.15 to 91.16 %) (Table 4.2). Besides anthesis-silking interval which was not variable in all three mega-environments, other traits which were also not variable were ear leaf width and grain weight in the lowland genotypes; tassel length, ear leaf length, ear position, stay green, stalk and cob diameter, and yield in the highland population; and only grain weight in the mid-altitude genotypes. Low values of coefficients of variation of less than 20 % were observed in ear number (4.46 - 15.92 %), anthesis date (5.62 - 10.69

%), silking date (5.91 - 10.54 %), tassel length (13.96 - 16.75 %), and kernel width (11.54 - 14.20 %) among accessions from all three mega environments, and were thus considered the least variable traits (Table 4.2). On the contrary, large coefficients of variation exceeding 25 % were observed in ear weight (57.15 - 91.16 %), ear height (32.27 - 35.85 %), hundred kernel weight (27.97 - 31.84 %), and yield (49.17 - 61.15 %). Grain yield was variable in only lowland and mid-altitude environments.

Beside the landraces, various classes of maize genotypes, including top-crosses and open pollinated varieties (Sampoux *et al*., 1989), hybrids (Ihsan *et al*., 2005) and inbred line populations (Sokolov and Guzhva, 1997) have also shown wide plant to plant variability in morphological and agronomic traits. Dijak *et al*., (1999) also reported significant amount of variability among long and short stature maize populations for ear and plant height. Similarly, Ullah (2004) and Shah *et al*. (2000) also reported significant amount of variability for ear height among different maize populations.

4.2.1. Earliness characteristics

A large variability in earliness, measured by mean squares and coefficients of variation was detected in all three mega environments accessions. Variability in both
AD and SD decreased in the order, lowland to mid-altitude to highland accessions. The mean squares and coefficient of variation for AD were 174.43 (P<0.001) and 10.69 % for lowland accessions, 114.98 (P<0.001), 9.86 % for mid-altitude accessions, and 15.73 (P<0.05) and 5.62 % for highland accessions. Similarly, mean squares and coefficient of variation for SD were 196.14 (P<0.001) and 10.54 %

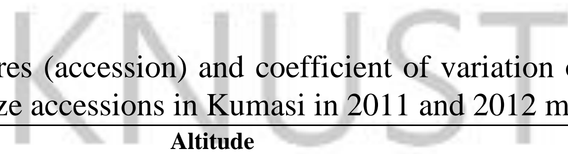
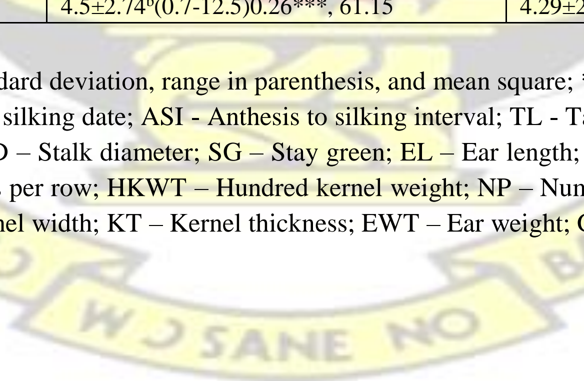


Table 4.2. Mean, standard deviation, range, mean squares (accession) and coefficient of variation of agro-morphological and phenotypic traits evaluated in 36 highland, mid-altitude, and lowland maize accessions in Kumasi in 2011 and 2012 major rainy season.

		Altitude			Overall Mean
No.	**Trait**	**High**	**Mid**	**Low**	
1	AD (days)	59.79±3.36(52-66)15.73* 5.62	58.52±5.77(43-73)114.98***, 9.86	58.21±6.23(45-71)174.43***, 10.69	**58.62±5.67**
2	SD (days)	63.27±3.74(55-71)32.52** 5.91	62.77±6.32(46-78)133.74***, 10.03	62.27±6.56(48-77)196.14***, 10.54	**62.68±6.10**
3	ASI (days)	3.48±1.58(2-9)3.61, 45.29	4.25±1.74(2-12)3.65, 40.91	4.05±1.46(2-8)3.48, 36.00	**4.08±1.66**
4	TL (cm)	46.62±6.51(30.5-63.5)6.22, 13.96	47.05±7.17(23-74)39.56***, 15.25	47.56±7.97(11.5-69)50.71***, 16.75	**47.14±7.34**
5	ELL (cm)	78.77±15.95(16-105)59.52, 20.25	78.18±17.49(21.6-113)314.71***, 22.38	81.12±15.71(26-112)316.51***, 19.35	**79.16±16.80**
6	ELW (cm)	8.52±1.59(4-12.6) 2.40* ,18.62	8.44±1.82(4-13.6)6.70***, 21.26	8.59±3.33(4-8.35)2.51, 38.75	**8.50±2.35**
7	PLHT(cm)	175.79±38.94(85-256) 1842**, 22.15	172.20±39.46(74-281)1956.11***, 22.92	173.28±37.67(75-272)1397.29***, 21.74	**173.03±38.85**
8	EHT (cm)	81.58±28.24(10-161) 1301***, 34.61	80.14±28.73(25-171)1265.78***, 35.85	83.89±27.07(22.5-180)1035.22***, 32.27	**81.49±28.20**
9	EP (cm)	0.46±0.10(0.1-0.8) 17.93, 22.26	0.46±0.09(0.2-1.0)79.05***, 19.18	0.48±0.09(0.2-0.9)79.63***, 18.92	**0.46±0.09**
10	StD (mm)	20.04±4.42(10-28)11.79*, 22.07	19.42±4.24(10-48.5)18.65***, 21.84	19.49±3.91(10-28)9.01**, 20.08	**19.52±4.17**
11	SG (%)	79.09±14.75(50-100) 206.90, 18.65	77.59±19.25(25-100)1179.35***, 24.81	82.71±13.45(50-100)307.67***, 16.26	**79.36±4.17**
12	EL (cm)	16.47±2.84(10.50-23) 0.02***, 17.26	16.12±3.5(7.5-28)0.01***, 21.69	16.51±3.42(7.50-26) 0.13***, 20.73	**16.29±3.40**
13	ED (mm)	40.64±8.42(23-56) 8.21***, 20.72	40.17±7.71(18.5-59.8)3.15***, 19.21	41.45±7.46(23-62.9)2.24*, 18.00	**40.62±7.76**
14	CD (mm)	25.06±5.19(2.7-35) 1.92, 20.70	26.66±6.39(12-50)13.49***, 23.98	27.13±5.71(11-49.1)20.15***, 21.04	**26.58±6.07**
15	EN	1.01±0.04(1-1.3)) 6.51***, 4.46	1.02±0.05(1-1.2)12.50***, 5.15	1.06±0.17(1-2)8.95***, 15.92	**1.03±0.10**
16	NRE	11.66±2.67(8-22) 103.21***, 22.94	12.61±2.27(8-20)69.33***, 17.99	13.46±2.65(8-22)65.97*, 19.71	**12.74±2.52**
17	NKR	32.69±6.33(16-51) 865.73***, 19.38	29.55±8.30(11-55)647.61***, 28.08	28.63±8.24(13-55)571.46***, 28.77	**29.70±8.13**
18	HKWT (kg)	72.74±20.34^{a}(42.-147.) 13.11*, 27.97	67.64±21.54^{b}(23.4-159.4)23.30***, 31.84	66.43±19.97^{c}(30.5-128.9)9.66***, 30.06	**67.98±20.99**
19	KL (mm)	9.79±1.73(6.5-12.8) 2.94**, 17.64	9.54±1.65(5.5-14.8)3.48***, 17.33	9.29±1.69(6.0-15.0)3.11***18.20	**9.49±1.68**
20	KW (mm)	9.91±1.1^{4b}(7.5-12.5) 1.33***, 11.54	9.62±1.24^{b}(5-13.0)1.55***, 12.94	9.19±1.31^{a}(4.9-13.2)0.73***, 14.20	**9.53±1.27**
21	KT (mm)	4.89±0.76^{c}(3.9-7.4) 0.00** , 15.56	5.12±0.86^{b}(5-13.0)0.06***, 16.75	5.16±0.78^{a}(3.5-8.4)0.00***, 15.08	**5.10±0.83**
22	EWT (kg)	0.12±0.07(0.03-0.3) 0.36**, 60.86	0.14±0.13(0.0-1.0)0.26***, 91.16	0.11±0.06(0.0-0.3) 0.133***, 57.15	**0.13±0.11**
23	GWT (kg)	0.91±0.51(0.35-2.1) 10.62**, 54.88	0.8±0.51(0.1-2.3)3.65, 61.15	0.79±0.39(0.1-1.8)3.49, 49.17	**0.83±0.47**
24	YLD (Mgha$^{-1)}$	4.92±2.70^{a}(1.89-11.6) 0.36, 54.88	4.5±2.74^{b}(0.7-12.5)0.26***, 61.15	4.29±2.11^{c}(0.7-9.5)0.13*, 49.17	**4.48±2.56**

Arrangement of data in the cell is in the order of mean, standard deviation, range in parenthesis, and mean square; *P<0.05; ** P<0.01, *** P<0.001 and coeffient of variation. AD – Number of days to anthesis; SD – Number of days to silking date; ASI - Anthesis to silking interval; TL - Tassel length; ELL – Ear Leaf length; ELW – Ear leaf width ; PLHT – Plant height up to flag leaf; 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; NP – Number of plants per plot; EN - Number of ears per plant; NE - Number of ears harvested; KL – Kernel length; KW – Kernel width; KT – Kernel thickness; EWT – Ear weight; GWT – Shelled grain weight; YLD - Grain yield. Means in a row with different letters are significantly differ at P≤0.05.



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for lowland, 133.74 (P<0.001) and 10.03 % for mid-altitude, and finally 32.52 (P<0.01) and 5.91 % for the highland accessions (Table 4.2). The highly significant mean squares combined with the fairly large coefficient of variation represent ample variation in earliness that can be exploited for breeding for early-maturing varieties.

The overall mean anthesis dates and mean silking dates for the three mega environment genotypes were 58.62±5.77 days and 62.68±6.10 days, respectively. In the lowland accessions, mean anthesis and silking dates were 58.21±6.23 and 62.27±6.56 days, respectively. Plants with least number of days to anthesis (43 days in TZm-8 from Tanzania) and longest number of days to anthesis (73 days in TZm251 from Zambia) were found in the mid-altitude genotypes. The same was true for silking dates of 48 days and 78 days, respectively. Earliness among the lowland genotypes was characterized by slightly higher values of AD and SD than the check variety, „Obatanpa GH", which was the earliest among the 36 genotypes with a mean AD of 48.8 days and mean SD of 52.5 days. The earliest genotype among the lowland accessions was TZm-2 with mean AD of 51.3 days and mean SD of 54.9 days (Table 4.3). Using a standard of flowering of 60 days after planting, only two additional genotypes in the lowland accessions, TZm-385 (AD of 53.7, SD of 57.7 days) and TZm-1523 (AD of 55.3, SD of 58.5 days) were important.

The pattern of variation in earliness among the mid-altitude accessions was not different. Mean anthesis and silking dates were 58.52±5.77 and 62.77±6.32 days, respectively. The earliest genotype was TZm-8 with AD of 49.3 days and SD of 53.2 days. Four other genotypes were classified as early. These are TZm-1521 (AD of 51.8 days, SD of 55.2 days), TZm-1514 (AD of 53.2 days, SD of 57.2 days), TZm-5 (AD of 55.8 days, SD of 60.2 days) and TZm-13 (AD of 56 days, SD of 59.5 days).

The highland accessions were considerably late. Their mean anthesis and silking dates were 59.79±3.36 and 63.27±3.74, respectively. Only one genotype in this class TZm-1376 (AD of 58.2 and SD of 60.5 days) was classified as early-maturing. Among all the genotypes studied, two genotypes exhibited extremely late maturity characteristics. These were TZm-1413 (AD of 66.8 days, SD of 71.3 days) and TZm42 (AD of 66.7 days, SD of 71.7 days) in the low and mid-altitude accessions, respectively.

Relatively different earliness characteristics are reported among different genotypes of diverse origins. Twenty maize genotypes of Ekiti State, Nigeria demonstrated mean anthesis date of 61.5±0.2 days and silking date of 62.7±0.7 days (Salami *et al*., 2007). Evaluation of 62 highland Ethiopian maize accessions revealed a minimum of 51.5 to a maximum of 76.0 days to anthesis with a mean of 65.1±3.2 days and silking range of 58.0 to 80.5 days with a mean of 71.5±3.0 days (Beyene *et al*., 2006). Azad *et al*. (2012) reported anthesis dates as late as 79.0 to 91.0 days with a mean of 83.0±0.5 days and delayed silking dates of 81.0 to 94.0 days with a mean of 85.9±0.55 days among Bangladeshi inbred lines. Likewise, Weiwei *et al*. (2012) reported AD of 84.3±1.7 and SD of 86.6±12.0 days for 498 maize accessions originating from wide geographical locations including Asia, Latin America and U.S.A.

This study has revealed that some African maize genotypes exhibit unusually short days to anthesis, majority of which were mid-altitude genotypes. Such genotypes represent rich sources of alleles for breeding for earliness since the short number of days is important for development of drought-resistant genotypes for marginal regions in tropical Africa (Tallury and Goodman 1999; Goodman 1985; Gerrish

1983).

Variability in anthesis-silking interval in all three mega environments was minimal as evidenced by the non significant mean squares, though a substantial coefficient of variation (36.0 to 45.29 %) was observed (Table 4.2). On plant basis, ASI ranged from 2 to 12 days with an overall mean of 4.08±1.6 days. Similar to the anthesis and silking dates, both the shortest and longest ASI occurred among plants of the midaltitude accessions. On accession mean basis, eight accessions exhibited mean ASI of less than 3.6 days. These are TZm-1523 (3.2 days), TZm-301 (3.3 days) of the low-altitude accessions; TZm-37 (3.2 days), TZm-1521 (3.3 days), TZm-13 and TZm-1516 (3.5 days each) of the mid-altitude genotypes; and TZm-1376 (2.3 days) and TZm-1367 (3.2 days) of the highland genotypes. The longest mean ASI of 6 days was observed in two genotypes, TZm-384, an early lowland genotype and TZm-1358, a late mid-altitude genotype (Table 4.3).

Climate change phenomenon with its consequential pressure on limited water resources in Africa has moved farmers to take decisions on earliness of their crops. Maize is particularly prone to drought conditions such that deficits of water for periods lasting one to two days during tasseling or pollination may bring about as much as 22 % loss in yield (Robins and Domingo, 1953). Depending on the geographical location and availability of irrigation resources, farmers may choose either early or vary late-maturing varieties. It is desirable that the ASI be short as it enhances tolerance to drought during flowering and ensures good grain filling (Bolanos and Edmeades, 1996; Edmeades *et al.,* 1993).

4.2.2. Plant architecture

In general, a large variability was detected in all plant architectural traits (TL, ELL, ELW, PLHT, EHT, and StD). The coefficients of variation in plant architectural traits

were largest in the highland accessions, followed by mid-altitude, and lowest in the lowland accessions indicating that plant to plant variation was least in the lowland accessions. Analysis of variance showed highly significant (P<0.001) differences in means of all plant architectural traits among the mid-altitude accessions but non significant TL and ELL in the highland accessions and non significant ELW in the lowland genotypes (Table 4.2). Nevertheless, coefficients of variation of at least 13.96 % represented enough variability among these traits.

The overall mean tassel length in the three mega environments was 47.14±7.3 cm with a range of 11.5 to cm for TZm-1523 of lowland Guinea to a maximum of 74 cm for TZm-242 of mid-altitude Zambia. Although mean tassel lengths for the mega environments were not different (46.6 to 47.6 cm), the range narrowed down with elevation spanning 11.5 - 69 cm for lowland, 23 - 74 cm for mid-altitude, and 30.5 - 63.5 cm for highland genotypes. The mean and standard deviations reveal that majority of the plants of the lowland accessions (47.56±7.97 cm) had longer tassel lengths exceeding the overall mean than that in the mid-altitude (47.05±7.17 cm) and highland accessions (46.62±6.5 cm). The long tassel lengths of the African genotypes were remarkable. Hartings *et al.* (2008) found short mean tassel length of 20.2±3.4 cm with a minimum and maximum value of 13.0 and 28.0 cm, respectively, among Italian maize accessions. Generally, the longer the tassel length the more enhanced its efficiency to shed pollen.

Ear leaf is one of the most important leaves in maize which relates to yield and plant morphology. Ear leaf characteristics mainly consist of ear leaf length (ELL), ear leaf width (ELW), and ear leaf area. Breeders strive to achieve ideal ear leaf characteristics encompassing large area by means of conventional breeding and marker assisted

selection (Zheng and Liu, 2013). Overall ear leaf length and ear leaf width were 79.16±16.80 cm and 8.50±2.35 cm, respectively. The smallest and largest ear leaf length of 16 cm and 113 cm, respectively, were recorded among the highland and mid-altitude genotypes, respectively.

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A correlation analysis revealed significant moderate positive correlation between ear leaf characteristics and all plant architectural traits (PLHT, EHT, EP, StD, and SG) (*r* = 0.41 to 0.61; P<0.01), and grain yield and some yield components (*r* =0.41 to 0.70; P<0.01). No significant correlation was detected between ear leaf characteristics and earliness. The contribution of ear leaf width to variation in yield and its components was more pronounced than that of ear leaf length in that ear leaf width correlated significantly with six yield-related traits involving EL (*r*=0.61), ED (*r*=0.47), HKWT (*r*=0.70), KL (*r*=0.55), KT (*r*=0.46), and grain YLD (*r*=0.61) whereas fewer significant correlations in ear leaf length with EL (*r*=0.67), HKWT (*r*=0.53), and KT (*r*=0.41) were observed. This finding suggests that breeding for large ELW is more important than longer ELL and would promote grain yield increase via improvement in 100-kernel weight, kernel length and kernel thickness. The R^2 values of 0.37, 0.22, 0.50, 0.30, 0.21 and 0.37 indicate that the ear leaf width explains 37 %, 50 %, 22 %, 30 %, 21 %, and 37 % of the variation in ear length, 100-kernel weight, kernel length, kernel thickness, and grain yield. Compared to the check which had mean ELL of 84.2 cm, eight accessions had much longer ELL of 86.1 cm in TZm-1359 and TZm-1516, 86.6 cm in TZm-41, 87.1 cm in TZm-384, 87.4 in TZm-251, 89.4 in TZm-1437, 91.3 in TZm-270 and the largest of 95.2 in TZm-1084. Similarly, mean ELW ranged from a minimum of 6.4 cm in TZm-42 to a maximum of 11.1 cm in TZm-270, the only accession exceeding the ELW of the check

(9.9 cm). Both accessions belonged to mid-altitude origin (Table 4.3). Hartings *et al*. (2008) reported mean ear leaf width of 9.53±1.4 cm for Italian maize landraces.

Plant height and ear height demonstrated large variabilities in all accessions as indicated by the large and significant mean squares (P<0.001) and large coefficient of variation (CV>20 %). Overall mean plant height and ear height were 173.0±38.9 cm and 81.49±28.20 cm, respectively (Table 4.2). The shortest and tallest plant heights were 74 cm for TZm-8 and 281 cm for TZm-41, both from mid-altitude Tanzania.

Typically, maize exhibit plant height of about 250 cm with heights up to 152.5 cm considered short. In current study, 668 plants, constituting 31 % of the total plant population exhibited heights in the range of 74 cm to 152.5 cm, hence short in height. On accession mean basis, plant heights ranged from 140 cm (TZm-5) to 208 cm (TZm-251) both of mid-altitude origin. Five genotypes were found to be very short in height, namely, TZm-5 (140 cm), TZm-42, (140.6 cm) TZm-2, (147.8 cm), TZm-20 (148.6 cm), and TZm-384 (149.1 cm) (Table 4.3). The check, „Obatanpa GH" and TZm-1084 had identical mean plant height of 174.1 cm. The genotypes of current study had plant heights similar to those of Ethiopia (161.0 to 288.0 cm with a mean of 217.8±14.4 cm) (Beyene *et al.*, 2006) and Italian landraces (110.0 to 215.0 cm with a mean of 166.0 ±27.4 (Hartings *et al.*, 2008). The heights of 131 European maize populations (82.4 to 206.7 cm with a mean of 164.2 cm) (Rebourg *et al.*, 2001) were not different from that of the African accessions considered in the current study. Similarly, Azad *et al*. (2012) reported mean plant height of 171.5±3.5 cm with a range of 114.2 to 219.8 among Bangladesh genotypes while Alan *et al*. (2013) also recorded among Turkey genotypes a mean of 214.61±5.1 cm and a range of 176.0 to 232.0 cm

plant heights. Mean plant heights reported for other maize genotypes include 213.2 cm (Salami *et al*., 2007) and 270.1±25.8 (Weiwei *et al*.,
2012).

Short plants resist lodging and drought and can be planted at higher densities. Tall plants usually yield better, however planting short plants at higher densities will compensate for the low yield (Voldeng and Blackman, 1975). These accessions would be beneficial in breeding for short plants which resist lodging.

Ear height ranged from a minimum of 10 cm for TZm-20 from highland Tanzania to a maximum of 180 cm for TZm-1084 from lowland Malawi with an overall mean of 81.5±28.2 (Table 4.2). On accession mean basis, least mean EHT of 55 cm in TZm42 and largest mean EHT of 113 cm in TZm-251 were observed (Table 4.3). As expected, the tall plants had larger EHT and short plants also had small EHT. The ear heights were similar to that of Ethiopian accessions which exhibited values ranging from 74.0 to 227.5 cm and mean of 125.9±26.3 (Beyene *et al*., 2006), Italian landraces with a range of 32.0 to 120.0 and mean of 77.01±22.42 (Hartings *et al*.,
2008), European inbred lines with a range of 20.9±129.3 and mean of 72.9 cm, (Rebourg *et al*., 2001), but taller than Bangladesh landraces which had EHT range and mean of 46.8 to 67.1 cm and 57.2±1.1 cm, respectively (Azad *et al*., 2012). Ear height is an important selection criterion for breeding for resistance to root and stock lodging.

Regarding stalk diameter, analysis of variance revealed significant differences ($P \leq 0.05$) among all three groups of accessions in addition to large coefficients of variation of at least 20 % (Table 4.2). These findings represent significant variability

that can be exploited in breeding for strong and tough stalks that can withstand lodging, beside their use as source of biomass. On plant to plant basis, the minimum and maximum stalk diameters were 10 mm inTZm-13, TZm-1360 and

TZm-1523 and 48.5 mm inTZm-273, respectively, with a mean of 19.52±4.17 mm. Accession mean stalk diameter was a least of 15.4 mm (TZm-5) to a largest of 23 mm (TZm-251). The mid-altitude genotype TZm-251 from Zambia was the tallest plant with a mean of 208.0 cm, had the largest ear height of 113.0 cm and the largest mean stalk diameter of 23.0 mm (Table 4.3). This confirms the usual morphology of the maize plant that the taller a plant is, the bigger the vegetative material (Hallauer and Miranda-Filho, 1988) hence more biomass. Such materials will be useful for silage preparation and for ethanol production.

Stay green is a desirable trait as it influences yield. Plants which remain green for longer periods have the capacity to accumulate more biomass. On plant basis, stay green varied from 25 % to 100 %. Significant mean squares (P<0.001) and sufficient coefficient of variation (16.24 to 24.81 %) indicated large variabilities among the lowland and mid-altitude genotypes whereas limited variability of CV of 18.65 % and a non significant mean square were observed among the highland genotypes

(Table 4.2). Mean stay green of the accessions varied from a minimum of 33.3 %

(TZm-42) to a maximum of 96.3 % (TZm-1084). About 95 % of the accessions stayed green till the milk stage. All plants of three genotypes, TZm-33, TZm-251 and

TZm-1084 remained fairly green till the physiological maturity stage.

4.2.3. Ear characteristics

Six traits, EL, ED, CD, EN, NRE and NKR constituted ear characteristics in current study. Analysis of variance revealed significant differences (P≤0.05) in all ear

characteristics among all accessions. Ample coefficients of variation ranging from 17.54 to 28.77 % were identified in all ear characteristics except EN, which had low values of 4.46 to 15.92 %. The range and overall mean of ear characteristics were, ear length of 7.5 to 28 cm with a mean of 16.29±3.4 cm, ED of 18.5 to 62.9 mm with a mean of 40.62±7.76 mm, CD of 2.7 to 50 mm with a mean of 26.58±6.07 mm, EN of 1 to 2 with a mean of 1.03±0.10, NRE of 8 to 22 with a mean of 12.74±2.52, and finally, NKR of 11 to 55 with a mean of 29.70±8.13. Compared to the overall mean, the lowland and highland accessions demonstrated longer and broader ears. Majority of the lowland accessions had large cob diameters with a mean of 27.13±5.71 mm compared to the overall mean of 26.58±6.07 mm. As regards grain yield, ears having large cob diameters are unfavorable. Nevertheless, the prolificacy of the lowland accessions (EN= 1-2) and largest mean number of rows per ear (13.46±2.65) (NRE) were expected to be compensatory (Table 4.2). In contrast, the highland accessions had the medium mean ear lengths (16.47±2.84 cm), average ear diameters (40.64±8.42 mm) and on average, one ear per plant (1.01±0.04). Productivity of the highland accessions was therefore expected to result from the medium ears and large number of kernels per row (32.69±6.33) (Table 4.2). The mid-altitude accessions had relatively shorter ears 16.12±3.5 cm, with smallest ear diameter (40.17±7.71), average cob diameter (26.66±6.39), one ear per plant, and fewer NRE (12.61±2.27) and NKR (29.55±8.3). Ear diameter is related to number of rows per ear while ear length is related to number of kernels per row.

Undoubtedly, the African accessions in current study had ear lengths similar to those reported by other authors. Beyene *et al*. (2006) found Ethiopian highland accessions to have mean ear length of 18.14±2.2 cm with a range of 14.5 to 22.7 cm. Hartings *et*

al. (2008), reported mean ear length of 16.92±2.9 cm with a range of 12.0 to 24.0 cm among Italian maize landraces. European inbred lines had a mean of 13.8 and a range of 8.7 to 17.9 (Rebourg *et al*., 2001). Bangladesh landraces demonstrated mean ear length of 16.3±0.26 and a range of 13.5 to 19.5 (Azad *et al*., 2012). Other authors report mean ear length of 23.12±0.39 with a range of 20.0 to 25.0 (Alan *et al*., 2013) and 16.83±1.22 (Weiwei *et al*., 2012). On the basis of the long ear lengths, the African accessions in current study express the potential to contribute to high grain yield. Typically, the maize cultivars in Ghana have been developed from few genotypes from CIMMYT and IITA (Obeng-Antwi, 2012; Badu-Apraku *et al.,* 2006, Sallah *et al.,* 1993 and GGDP, 1988) to incorporate disease resistance and yield increase. Identification of accessions with long ear lengths substantiates the concept of tropical landraces as sources of new alleles that are yet to be exploited for crop improvement (Tallury and Goodman, 1999; Goodman, 1985; Gerrish, 1983).

Other maize landraces possess ear diameters of 39.1±0.2 mm ranging from 33.0 to 46.0 mm (Beyene *et al*., 2006), 40.1±4.7 mm with a range of 31.0 to 50.0 mm (Hartings *et al*., 2008) and 38.0 mm ranging from 23.0 to 49.8 mm (Rebourg *et al*., 2001). Furthermore, Weiwei *et al*. (2012) report mean ear diameter of 47.3±0.31 among 498 genotypes from wide geographical regions of the world.

4.2.4. Yield and yield components

Analysis of variance showed significant differences (P<0.001) in grain yield and all yield components (number of kernels per row, number of rows per ear, ear number, hundred kernel weigth, kernel length, kernel thickness, and ear weigtht) of all genotypes from the three mega environments. Substantial phenotypic variability (CV of 11.54 to 91.16 %) was detected among the accessions. Overall mean grain yield

was 4.48±2.56 Mgha^{-1} with a range of 0.7 to 12.5 Mgha^{-1} on plant basis. Besides the check which had the highest grain yield of 6.3 Mgha^{-1}, mean grain yield of accessions ranged from 1.7 to 6.2 Mgha^{-1} (Table 4.3). Seventeen accessions had grain yield values above the overall mean. These are TZm-1437 (4.6 Mgha^{-1}), TZm1424 (4.7 Mgha^{-1}), TZm-1356 (4.8 Mgha^{-1}), TZm-275 (4.9 Mgha^{-1}), TZm-1521 (4.9 Mgha^{-1}), TZm-1358 (4.9 Mgha^{-1}), TZm-1434 (5 Mgha^{-1}), TZm-33 (5 Mgha^{-1}), TZm-14 (5.2 Mgha^{-1}), TZm-41 (5.2 Mgha^{-1}), TZm-242 (5.3 Mgha^{-1}), TZm-37 (5.4 Mgha^{1}), TZm-1360 (5.6 Mgha^{-1}), TZm-1376 (5.6 Mgha^{-1}), TZm-1367 (6 Mgha^{-1}), TZm-4 (6 Mgha^{-1}), and TZm-270 (6.2 Mgha^{-1}) (Table 4.3).

Collectively, the highland accessions produced the highest mean grain yield of 4.92±2.70 Mgha^{-1} followed by the mid-altitude 4.5±2.74 Mgha^{-1} and the least was in the lowland accessions (4.29 Mgha^{-1}) (Table 4.2). Of the seventeen high yielding genotypes, 11 were from mid-altitude origin while 4 and 2 were highland and lowland accessions, respectively. Traits which contributed primarily to grain yield in the highland accessions included HKWT (72.74±20.34 g), KL (9.79±1.73 mm), KW (9.91±1.14 mm), and GWT (0.91±0.51 kg) all of which were higher than the overall mean. Grain yield for mid-altitude accessions were derived from KL (9.54±1.65 mm), KW (9.62±1.24 mm), and KT (5.12±0.86 mm), whereas that in the lowland genotypes was contributed solely by high KT (5.16±0.78 mm). Salami *et al*. (2007) reported mean grain yield of 4.1 Mgha^{-1} among some Nigerian maize accessions. The diverse origins of the high yielding accessions along with the large phenotypic variability detected among the accessions are indicative of presence of multiple genes for high yield. Maize breeding programs in Africa would benefit from utilization of these genotypes.

Table 4.3. Means of 24 traits measured in 36 tropical maize accessions belonging to lowland, mid altitude, and highland regions of Africa. Accessions were evaluated in Ghana in 2011 and 2012.

	Acc	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	SD	SG	EL	EP	ED	CD	NRE	NKR	HKWT	EN	KL	KW	KT	EWT	GWT	YLD
LOW	Obatanpa	48.8	52.5	3.7	48.1	84.2	9.9	174.1	75.6	18.0	75.2	17.3	0.4	46.5	28.9	13.4	30.6	84.5	1.0	10.2	9.9	5.5	0.2	1.2	6.3
	TZm-1084	63.5	67.8	4.3	50.3	95.2	9.3	174.1	101.0	18.7	96.3	14.9	0.6	39.8	23.7	12.3	31.9	73.6	1.0	10.4	9.4	5.0	0.1	0.8	4.4
	TZm-1095	60.8	64.7	3.8	48.4	77.2	8.6	204.3	104.4	19.3	89.4	15.9	0.5	42.8	24.1	9.6	28.0	73.9	1.0	9.6	10.3	5.4	0.1	0.8	4.2
	TZm-1413	66.8	71.3	4.5	44.8	81.1	8.3	174.4	85.1	21.7	86.9	18.1	0.5	35.6	21.0	12.7	27.1	63.0	1.0	9.3	9.2	5.4	0.1	0.7	3.7
	TZm-2	51.3	54.9	3.6	42.9	70.3	7.5	147.8	66.2	17.4	85.2	15.7	0.5	36.6	25.4	13.8	30.3	48.0	1.3	8.4	8.1	4.7	0.1	0.7	3.7
	TZm-1434	61.5	65.7	4.2	49.6	78.0	8.4	177.5	92.8	19.1	88.5	17.4	0.5	38.5	29.4	16.9	30.3	63.9	1.0	8.7	7.8	5.2	0.1	0.9	5.0
	TZm-1437	60.5	64.5	4.0	46.6	89.4	9.3	183.3	93.5	20.7	77.2	17.0	0.5	43.4	26.8	13.3	31.6	72.9	1.1	9.9	9.6	4.8	0.2	0.9	4.6
	TZm-1523	55.3	58.5	3.2	47.6	78.2	8.1	171.3	75.7	19.2	77.2	16.9	0.4	44.0	29.4	14.2	27.6	64.3	1.0	8.9	9.3	5.1	0.1	0.8	4.4
	TZm-301	59.2	62.5	3.3	46.2	78.2	8.4	175.2	85.3	20.4	81.7	14.1	0.5	39.3	25.4	12.5	32.0	56.0	1.0	9.2	9.0	4.8	0.1	0.7	3.8
	TZm-384	58.8	64.8	6.0	53.4	87.1	8.3	175.0	78.7	19.6	79.3	16.5	0.4	44.5	29.5	15.1	22.4	63.8	1.1	9.0	9.1	5.3	0.1	0.7	3.8
	TZm-385	53.7	57.7	4.0	45.2	73.6	8.4	149.1	64.4	20.1	72.9	17.6	0.4	45.0	34.8	14.2	23.3	66.9	1.1	8.5	9.4	5.8	0.1	0.6	3.4
Mid	TZm-42	66.7	71.7	5.0	45.2	66.6	6.4	140.6	55.0	17.6	33.3	13.4	0.4	38.0	23.0	10.0	33.0	46.9	1.0	10.0	10.0	3.5	0.5	0.3	1.7
	TZm-37	56.5	59.7	3.2	47.9	70.4	8.7	165.7	78.9	19.7	88.3	16.9	0.5	41.0	25.6	12.4	34.4	66.2	1.0	10.8	9.5	4.8	0.1	1.0	5.4
	TZm-41	59.0	62.7	3.7	50.8	86.6	9.5	197.7	98.1	20.8	81.7	15.5	0.5	40.2	39.0	14.2	27.9	69.2	1.0	10.5	10.0	5.0	0.1	1.0	5.2
	TZm-33	56.3	61.8	5.5	45.2	81.6	8.0	176.7	80.4	18.9	92.0	15.3	0.5	42.5	26.4	11.3	30.9	72.2	1.1	9.3	10.1	5.4	0.1	0.9	5.0
	TZm-4	59.3	63.5	4.2	46.1	74.7	8.7	171.	79.9	19.8	84.2	15.9	0.5	45.8	28.5	15.6	31.5	65.9	1.0	9.9	9.2	4.9	0.1	1.1	6.0
	TZm-270	62.0	66.3	4.3	51.8	91.3	11.1	162.7	77.3	20.9	82.8	13.7	0.5	44.6	26.5	12.6	32.3	93.8	1.0	12.0	10.7	5.5	0.2	1.2	6.2
	TZm-251	66.2	71.2	5.0	46.5	87.4	9.0	208.0	113.0	23.0	94.4	16.8	0.5	40.9	31.1	14.3	21.3	80.1	1.0	8.2	9.1	6.3	0.1	0.7	3.9
	TZm-13	56.0	59.5	3.5	48.2	76.5	7.9	165.3	70.6	18.4	82.2	17.0	0.4	38.5	25.9	12.2	26.4	62.8	1.0	9.0	9.4	5.3	0.1	0.7	3.9
	TZm-1356	59.5	63.5	4.0	43.4	63.3	8.4	165.9	80.7	17.2	70.7	17.3	0.5	36.5	26.6	12.2	23.3	61.7	1.0	9.3	9.0	5.3	0.1	0.9	4.8
	TZm-1358	61.0	67.2	6.2	49.6	82.3	9.7	181.2	88.2	22.3	84.5	16.2	0.5	42.0	27.8	14.7	30.1	66.4	1.0	9.5	8.9	5.5	0.1	0.9	4.9
	TZm-1359	63.2	67.0	3.8	48.0	86.1	7.3	188.8	95.9	20.5	77.0	16.7	0.5	34.7	22.0	10.5	33.0	66.8	1.0	8.7	9.4	5.3	0.3	0.8	4.4
	TZm-14	58.3	63.2	4.8	42.7	73.2	7.9	170.8	87.9	18.9	78.8	15.0	0.5	38.6	20.9	12.2	27.8	68.3	1.0	10.0	10.4	5.0	0.1	1.0	5.2
	TZm-8	49.3	53.2	3.8	45.2	76.5	7.7	154.6	66.0	18.3	83.1	17.1	0.4	36.8	25.0	12.9	26.8	62.0	1.0	9.2	9.6	4.9	0.1	0.8	4.1
	TZm-5	55.8	60.2	4.3	43.1	74.4	7.5	140.0	60.2	15.4	53.0	15.5	0.4	32.5	21.6	14.6	28.0	48.3	1.0	7.8	7.2	5.1	0.1	0.4	2.1
	TZm-275	57.7	62.7	5.0	48.4	77.9	7.0	164.9	64.9	17.5	73.0	14.9	0.4	45.1	28.0	12.2	30.8	74.7	1.1	9.5	10.1	5.0	0.1	0.9	4.9

	TZm-1516	58.3	61.8	3.5	47.5	86.1	9.1	190.1	92.9	20.6	82.2	16.7	0.5	42.1	30.5	12.9	28.7	60.7	1.0	8.6	9.8	5.4	0.1	0.8	4.2
	TZm-1521	51.8	55.2	3.3	45.8	78.5	8.7	163.5	70.1	19.7	75.2	16.3	0.4	41.7	29.2	12.8	31.4	66.0	1.0	8.7	9.5	4.8	0.1	0.9	4.9
	TZm-1514	53.2	57.2	4.0	48.2	73.3	8.4	161.7	70.0	19.5	65.0	16.2	0.4	36.2	21.2	10.3	29.9	69.7	1.0	9.7	10.1	5.1	0.1	0.7	3.8
	TZm-273	57.7	61.8	4.2	46.6	81.7	8.7	174.6	75.9	20.0	87.0	16.8	0.4	44.4	29.4	12.6	29.5	70.7	1.0	10.4	10.0	5.5	0.1	0.7	3.5
	TZm-242	62.5	66.2	3.7	50.9	75.4	9.2	199.6	97.0	19.7	83.3	14.8	0.5	41.6	25.1	11.8	34.1	80.8	1.0	9.8	10.6	5.1	0.1	1.0	5.3
High	TZm-1360	59.8	63.5	3.7	48.4	80.3	8.3	175.5	77.0	19.8	76.2	16.7	0.4	37.9	23.8	11.7	35.8	67.9	1.0	9.4	9.6	4.7	0.1	1.0	5.6
	TZm-1367	58.2	61.3	3.2	46.4	77.5	8.9	192.8	102.7	21.0	73.0	16.8	0.5	40.8	24.7	11.9	34.3	71.6	1.0	10.3	9.6	4.8	0.1	1.1	6.0
	TZm-1376	58.2	60.5	2.3	46.1	78.2	8.6	189.6	86.0	20.6	77.1	16.1	0.5	40.9	25.2	11.3	36.9	72.5	1.0	10.6	10.4	4.5	0.1	1.0	5.6
	TZm-20	61.3	65.7	4.4	45.8	74.7	7.6	148.6	62.1	17.7	88.3	16.4	0.4	40.9	27.2	12.4	28.4	59.5	1.0	8.1	9.2	4.6	0.1	0.5	2.7
	TZm-1424	61.5	65.3	3.8	46.5	83.1	9.2	172.4	80.1	21.2	80.8	16.2	0.5	42.8	24.3	10.9	28.1	92.2	1.0	10.6	10.9	5.7	0.1	0.9	4.7

4.3 Genotypic Variance, Phenotypic Variance, Genotypic and Phenotypic Coefficient of Variation and Heritabilities

The genotypic and phenotypic variances and broad sense heritability estimates for the lowland, mid-altitude, and highland genotypes are presented in Table 4.4. The broad sense heritability estimates were generally low for all traits in the three groups of accessions except earliness traits in the lowland genotypes. Heritability estimates of lowland and mid-altitude genotypes were generally higher than those of highland origin. The low heritability estimates for the lowland genotypes for grain yield, ear weight, kernel thickness, kernel width, kernel length, ear number, 100-kernel weight, number of kernels per row, number of rows per ear, ear diameter and ear length were 15.3± 0.14 %, 8.73± 0.12 %, 10.77± 0.12 %, 39.99± 0.13 %, 24.57± 0.12 %, 0± 0 %, 14.91± 0.17 %, 9.09± 0.09 %, 37.41± 0.14 %, 16.43± 0.15 %, 15.44± 0.09 %, respectively. Heritability estimates for number of days to anthesis, number of days to silking, anthesis-silking interval, tassel length, ear leaf length, ear leaf width, plant height, ear height, stalk diameter and stay green were 84.37±0.07 %, 83.44±0.07 %, 10.80±0.13 %, 5.05± 0.08 %, 14.50± 0.12 %, 2.38± 0.02 %, 14.95± 0.1 %28.45± 0.1%, 5.52± 0.09%, and 0.00± 0%, respectively.

The broad sense heritability estimates of the mid-altitude genotypes for grain yield, ear weight, kernel thickness, kernel width, kernel length, ear number, 100-kernel weight, number of kernels per row, number of rows per ear, ear diameter and ear length were 8.38± 0.16%, 50.85± 0.12, 29.08± 0.09, 33.69±0.11, 38.46± 0.12, 14.07± 0.12, 6.54± 0.12, 0.00± 0, 36.03± 0.10 , 10.87± 0.17, 11.70± 0.14, 15.50± 0.07, and 14.45±0.08, respectively. Heritability estimates for number of days to anthesis, number of days to silking, anthesis-silking interval, tassel length, ear leaf length, ear leaf width, plant height, ear height, stalk diameter and stay green were

56.02± 0.13, 58.70± 0.11, 5.41±0.09, 7.54±0.05, 3.07± 0.09, 32.68± 0.09, 13.60±0.1, 24.39± 0.1, 11.76± 0.08, and 38.90±0.12, respectively.

The broad sense heritability estimates of the highland genotypes for grain yield, kernel thickness, kernel width, kernel length, ear number, 100-kernel weight, number of kernels per row, and ear length were 22.81± 0.35%, 27.18±0.24, 21.44± 0.26%, 36.94±0.24%, 1.05± 0.18%, 30.58± 0.27%, 25.70± 0.24%, and 16.83±0.14%, respectively. Heritability estimates for EWT, NRE, CD, ED, StD, ELL, TL, ASI and AD were 0.0%. Heritability estimates for number of days to silking, ear leaf width, plant height, ear height, and stay green were 48.74±0.23%, 5.57±0.16 %, 23.87±0.19%, 34.49±0.22 %, and 7.33±0.21%, respectively.

The low heritability estimates of the plant architectural traits, ear characteristics, kernel traits and grain yield shows that there is possibility of genetic improvement through recurrent selection albeit this could be achieved through many cycles. The low values also imply that the phenotypic variation observed arose substantially from environmental effects and very little variation from genetic effects. Because quantitative traits are usually influenced by environmental effects, the trend observed is indicative of presence of multiple alleles involved in trait expression and that the traits may be improved in different environments. In earliness traits (AD, SD, ASI) and ear characteristics (ED, CD, NRE) heritability estimates were highest in lowland accessions and decreased to least values in the highland genotypes, whereas the opposite trend was observed for kernel and yield traits in which heritabilities were highest in highland accessions but

Table 4.4. Estimates of genotypic and phenotypic variances and broad sense heritabilities for lowland, mid-altitude and highland accessions on 26 morphological traits evaluated in 36 maize accessions in 2011 and 2012 in Ghana.

Trait	Lowland			Mid-altitude			Highland		
	V_G	V_P	% H^2± SE	V_G	V_P	% H^2± SE	V_G	V_P	% H^2± SE
AD	28.08	33.28	84.37±0.07	15.15	27.04	56.02±0.13	0.00	8.20	0.00±0.00
SD	31.66	37.95	83.44±0.07	18.53	31.57	58.70±0.11	4.54	9.31	48.74±0.23
ASI	0.23	2.13	10.80±0.13	0.15	2.78	5.41±0.09	0.00	2.48	0.00±0.00
TL	2.83	56.04	5.05±0.08	3.34	44.34	7.54±0.05	0.00	36.29	0.00±0.00
ELL	29.27	201.84	14.50±0.12	7.32	238.61	3.07±0.09	0.00	162.60	0.00±0.00
ELW	0.25	10.43	2.38±0.02	0.90	2.76	32.68±0.09	0.12	2.21	5.57±0.16
PLHT	147.47	986.64	14.95±0.10	148.89	1094.54	13.60±0.10	227.50	952.94	23.87±0.19
EHT	156.81	551.11	28.45±0.10	139.16	570.66	24.39±0.10	172.16	499.11	34.49±0.22
StD	0.50	9.09	5.52±0.09	1.51	12.81	11.76±0.08	0.00	10.96	0.00±0.00
SG	0.00	178.37	0.00±0.00	147.48	379.12	38.90±0.12	10.14	138.45	7.33±0.21
EL	5.82	37.70	15.44±0.09	6.23	43.16	14.45±0.08	7.97	47.37	16.83±0.14
ED	6.47	39.40	16.43±0.15	4.10	35.09	11.70±0.14	0.00	36.71	0.000.00±
CD	11.44	31.87	35.90±0.14	4.40	40.50	10.87±0.17	0.00	25.37	0.00±0.00
EP	0.00	0.01	0.00±0.0	0.00	0.01	0.00±0.00	0.00	0.00	0.00±0.00
NRE	2.73	7.29	37.41±0.14	1.78	4.94	36.03±0.10	0.00	7.06	0.00±0.00
NKR	5.33	58.65	9.09±0.09	0.00	51.96	0.00±0.00	11.04	42.94	25.70±0.24
HKWT	39.53	265.19	14.91±0.17	23.41	358.11	6.54±0.12	94.29	308.34	30.58±0.27
EN	0.00	0.03	0.00±0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00±0.00
KL	0.36	1.46	24.57±0.12	0.68	1.77	38.46±0.12	0.85	2.31	36.94±0.24
KW	0.45	1.14	39.99±0.13	0.43	1.28	33.69±0.11	0.27	1.25	21.44±0.26
KT	0.05	0.50	10.77±0.12	0.20	0.70	29.08±0.09	0.15	0.55	27.18±0.24
EWT	0.00	0.00	0.00±0.00	0.01	0.02	0.50±0.12	0.00	0.00	0.00±0.00
GWT	0.01	0.07	15.30±0.14	0.01	0.11	8.38±0.16	0.03	0.12	22.81±0.35
YLD	0.33	2.15	15.30±0.14	0.27	3.28	8.38±0.16	0.82	3.61	22.81±0.35

Where V_G =Genotypic variance, V_P= Phenotypic variance, H^2 = Heritability broad sense; and SE= standard error.

AD – Number of days to anthesis; SD – Number of days to silking date; ASI - Anthesis to silking interval; TL - Tassel length; ELL – Ear Leaf length; ELW – Ear leaf width; PLHT – Plant height up to flag leaf; 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 - Number of ears per plant; KL – Kernel length; KW – Kernel width; KT – Kernel thickness; EWT – Ear weight; GWT – Shelled grain weight; YLD - Grain yield.

decreased to lowest values in the lowland genotypes (Table 4.4). Magorokosho (2006) obtained low heritability estimates for earliness, plant architectural traits, ear characteristics, yield and its component traits ranging from 0.27 for TL to 0.90 for

NKR. In contrast to these findings, Rebourg *et al*. (2001), Satyanarayana and Sai Kumar (1995) and Ojo *et al*. (2006) recorded relatively high heritability estimates of 0.58 to 0.91 for plant architectural traits, ear characteristics, and yield and yield components. The similar trend in heritability estimates of NRE and NKR in lowland (37.41 %, 9.09 %, respectively) and mid-altitude genotypes (36.03 %, 0.00 %, respectively) which was reversed in the highland genotypes (0.00 %, 25.70 %) indicates differential sensitivity of genotypes to environment. In current study, the results of heritability estimates of NRE and NKR in the evaluation of highland maize in lowland environment suggests that informativeness of a trait would depend on genotype and environment. This result has important implications for breeding with genotypes of plant introductions. Hallauer and Miranda-Filho (1988) estimated heritabilities as 30 % for grain yield and its components and 50 % and 70 % for plant architectural and earliness traits, respectively. The maize germplasm in the current study showed similar result with grain yield and its components with heritabilities ranging between 18 % and 36 % (Table 4.4).

Similar results of high heritability estimates of about 84 % for earliness was reported by Llaurado and Moreno-Gonzalez (1993) for Spanish maize accessions. Like maize accessions originating from Zimbabwe, Malawi and Zambia (Magorokosho, 2006) the accessions in this study which were selected from a much wider geographical area also had low heritability estimates for the plant architectural traits PLHT (0.44), ELL (0.22), ELW (0.43), and TL (0.22) (Table 4.4). Similar values were also reported by Geraldi *et al*. (1985).

Despite the low heritabilities for some of the traits, there is sufficient residual genetic variability to exploit for trait improvement.

4.4 Genotypic and phenotypic correlations of selected traits with earliness and grain yield

As the world faces challenges with climate anomalies, earliness and grain yield have become the most important traits for maize improvement. Correlation analysis which reveals associations among traits offer an efficient method for selection at early generations. Genotypic and phenotypic correlations for all possible pairwise combinations for 18 traits are presented in Table 4.5. In general, low values of correlation coefficients were observed. Genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients. The genotypic correlation coefficients ranged from -0.01 to 1.00 while the phenotypic correlation coefficient was between -0.02 to 0.95. Earliness had a moderate significant ($P<0.05$) positive genotypic correlation of r_g=0.48 to 0.54 and low significant ($P<0.05$) positive phenotypic correlations of r_p=0.27 to 0.30 exclusively with plant architectural traits. Association between earliness and grain yield and yield components was characterized by low negative non significant correlation coefficients. This finding was not expected as typically, earliness and yield demonstrate a trade-off such that early genotypes have low yield and late genotypes have higher yield.

The results further showed moderate to high positive significant ($P<0.01$) genotypic correlation of 0.42 to 1.00 and low to moderate positive significant ($P<0.01$) phenotypic correlations of 0.19 to 0.79, respectively, among plant architectural traits (TL, ELL, ELW, PLHT) and grain yield and yield components (EL, HKWT, KL, KW, and YLD). As expected under non-stress cultivation conditions, correlations between days to 50 % anthesis and days to 50 % silking were strong and positive ranging from 0.95 to 1.00 as was also reported by Magorokosho (2006) for genotypes in Southern

Africa and by Odongo *et al*. (1989) for Eastern Africa genotypes. On the contrary, correlation coefficients between flowering and anthesis silking interval were weak and not significant (r_p = 0.08 and 0.37; r_g =1.00). The 100-kernel weight, KL, KW and grain yield showed at least one significant positive correlation with TL, ELL, ELW, PLHT, and EHT in the range of r_g = 0.42 to 1.00. Moreover, grain yield had high significant positive correlation with HKWT and KW but weak positive non significant correlation with CD (r_g=0.04), NRE (r_g=0.20), NKR (r_g=0.07) and KW (r_g=0.72). Weak negative non significant correlation between EL (r_g=-0.17), ED (r_g=-0.37) with grain yield was also observed. Grain yield had weak positive significant phenotypic correlation with all traits except earliness traits which showed weak negative non significant phenotypic correlations. Moreover, all phenotypic correlations between kernel, ear and plant characteristics were positive and significant (Table 4.5).

The significant positive genotypic correlation of grain yield with HKWT, KL, TL, and ELW indicates strong dependence of grain yield on these traits and that selection for these yield-related traits leads to a simultaneous increase in grain yield. Grain yield is expected to benefit from simultaneous selection for these positively correlated traits. Ear diameter is related to number of rows per ear while ear length is related to number of kernels per row. In contrast, the negative correlation of grain yield with earliness shows that selection for earliness leads to reduced grain yield as was also reported by Magorokosho (2006). The moderate positive non significant correlations between plant height and yield (r_g=0.37 and r_p=0.47) indicate contribution of biomass of tall plants to yield (Edmeades *et al*., 1989). Salami *et al*.

(2007) recorded moderate positive significant correlation (r_g=0.65, P<0.01) between plant height and grain yield. Similar but stronger and significant correlations (r_g =

0.95 and r_p = 0.94, P<0.01) were reported by Bocanski *et al*. (2009). In contrast, Magorokosho (2006) recorded weak negative correlation between plant height and grain yield (r_g=-0.02 and r_p=0.07). Sumathi *et al*. (2005) found strong genetic correlation between grain yield and ear height, medium strong correlation between grain yield and number of kernels per row, low correlation between grain yield and plant height and negative correlation between grain yield and 100- kernel weight. Phenotypic correlations of yield with other variables were positive and significant except with anthesis date, silking date and anthesis silking date (Table 4.5).

Different physiological mechanisms are influenced by genetic and environmental factors that govern the variation in the sign of the correlation (Falconer, 1980). Selection for earliness leads to low grain yield due to shortened time for grain filling except for genotypes that mature early but have long grain filling period (Gasura *et al*., 2010). Generally, the late-maturing genotypes yielded more grain than the earlymaturing types (Table 4.3). The results of this study demonstrate the association of grain yield with the yield-related traits, HKWT and KL as well as with the plant architectural traits, TL and ELW.

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Table 4.5. Genotypic (upper diagonal) and Phenotypic (lower diagonal) correlation coefficients among 18 traits for 36 maize accessions evaluated in 2011 and 2012 in Ghana.

Trait	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	EP	EL	ED	CD	NRE	NKR	HKWT	KL	KW	YLD
AD		1.00***	1.00	0.40	0.52	0.13	0.54*	0.54**	0.52**	0.54*	-0.17	-0.22	-0.06	0.11	0.20	0.20	0.10	-0.20
SD	0.95**		1.00	0.41	0.51	0.08	0.48*	0.50**	0.50*	0.52**	-0.18	-0.23	-0.05	-0.01	0.13	0.14	0.07	-0.26
ASI	0.08	0.37***		0.57	0.37	-0.51	-0.17	0.04	0.36	0.42	-0.31	-0.34	-0.08	-1.00	-0.65	-0.53	-0.21	-1.04
TL	0.11	0.12	0.06		1.00 *	0.80*	0.31	0.37**	0.51	0.62*	0.91	0.67	0.05	-1.00	0.75**	0.61**	0.42	0.42**
ELL	0.11	0.12	0.07	0.49**		1.00**	0.54	0.57**	0.60	1.00**	0.45	0.92	0.18	-0.79	1.00	0.26	0.40	0.14
ELW	-0.02	-0.04	-0.06	0.32***	0.53***		0.56**	0.52**	0.56***	0.73**	0.62	0.56**	0.19	-0.34	1.00***	0.61***	0.35	0.92**
PLHT	0.18	0.14	-0.09	0.40***	0.47***	0.42**		1.00***	1.00***	0.59**	-0.04	0.41	-0.25	-0.65	0.60**	0.24	0.44**	0.37
EHT	0.32**	0.27*	-0.10	0.30***	0.50***	0.45**	0.88***		1.00**	0.52**	1.00**	0.22	-0.01	-0.35	0.52**	0.20	0.16	0.35
EP	0.36***	0.30**	-0.11	0.09	0.41***	0.35**	0.43***	0.79**		0.25	-0.36	0.04	0.26	0.07	0.57	0.19	-0.09	0.50
EL	0.15	0.16	0.07	0.48***	0.53***	0.55**	0.56***	0.49**	0.56*		0.44	0.55	-0.02	-1.00	0.84**	0.33	0.48**	-0.17
ED	-0.11	-0.07	0.12	0.22*	0.26**	0.38**	0.30***	0.79**	-0.04	0.42**		1.00	-0.07	-1.00	1.00	0.35	0.80	-0.37
CD	-0.16	-0.13	0.07	0.20*	0.11	0.24**	0.16	0.07	-0.07	0.16	0.46***		*0.66*	-1.00	0.31	-0.22	-0.05	0.04
NRE	0.002	-0.11	0.12	0.16	0.13	0.20*	0.05	0.06	0.01	0.18	0.28**	0.08***		-0.13	-0.10	-0.14	-0.54	0.20
NKR	0.00	-0.04	-0.14	0.29***	0.17*	0.20**	0.27**	0.19**	0.03	0.19**	0.12	-0.16	-1.00		-0.44	0.70	0.33	0.07
HKWT	0.05	0.05	0.02	0.29***	0.37***	0.42**	0.40***	0.34**	0.16	0.44**	0.45***	0.11	-0.43	0.16**		1.00***	1.00***	1.00***
KL	0.05	0.05	0.02	0.24**	0.25**	0.42**	0.18	0.18	0.13	0.26**	0.40***	0.04	-0.52	0.36**	0.43***		0.73***	0.91**
KW	0.08	0.07	-0.02	0.15	0.09	0.15	0.15	0.06	-0.04	0.18**	0.33***	0.01	-0.54	0.15	0.52***	0.56***		0.72
YLD	-0.14	-0.15	-0.07	0.22**	0.24**	0.42**	0.47***	0.41**	0.19*	0.40**	0.44***	0.18*	-0.35	0.50**	0.50***	0.45**	0.21*	

AD – Number of days to anthesis; SD – Number of days to silking date; ASI – Anthesis to silking interval; TL – Tassel length; ELL – Ear Leaf length; ELW – Ear leaf width ; PLHT – Plant height up to flag leaf; EHT – Ear height; EL – Ear length; EP – Ear position; ED – Ear diameter; CD – Cob diameter; NKR – Number of kernels per row; HKWT – Hundred kernel weight; KL – Kernel length; KW – Kernel width; KT – Kernel thickness; EWT – Ear weight ; GWT – Shelled grain weight; YLD – Grain yield; * P< 0.05; **P<0.01; ***P<0.001.

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4.5. Genetic distance and relationships among accessions and traits

Relationships among traits and accessions were investigated by similarity distance measures calculated as Pearson pairwise correlation coefficients for interval measures. Correlation distance measure was chosen as this coefficient is analogous to the robust Nei"s distance measure for gene frequency data. The distance matrix (Appendix B) generated was subjected to UPGMA cluster analysis. For the 36 accessions, 630 similarity coefficients were obtained. Unlike in correlation coefficient, the magnitude of the coefficient rather than the sign was important for estimating the strength of the similarity. The similarity measures were generally low and ranged from 0.00 to 0.80 with overall mean of 0.26, or 26 % similarity among accessions. Of the 630 pairwise similarity coefficients, nine accession pairs (TZm1084/TZm-384, TZm-1095/TZm-1514, TZm-1095/TZm-41, TZm-1360/TZm-1434,
TZm-1376/TZm-2, TZm-1437/TZm-1521, TZm-1523/TZm-2, TZm-2/TZm-20, and TZm-37/TZm-42) exhibited similarity coefficients of 0.00, 572 pairs (92 %) were below 0.5, while 49 were at least 0.50. The preponderance of very low similarity coefficients is indicative of a large genetic diversity among the accessions which were differentiated by both distance and elevation.

A genetic similarity of 0.0 interprets that the pairs do not share common alleles by reason of divergence possibly via adaptive natural selection (Camussi *et al*., 1985) in the past or isolation-by-distance. Relethford (2004) stated that the isolation-bygeographical distance model predicts that genetic similarity between populations will decrease exponentially as the geographic distance between them increases due to limitations on gene flow. Except for TZm-37/TZm-42 pair both of which originated from mid-altitude Tanzania, all other accession pairs having 0.0 similarity coefficient had diverse origins. For example, TZm-2 was from lowland Tanzania whereas TZm20 was from highland Tanzania, TZm-1523 from lowland Guinea, and TZm-1376 from

highland Kenya. As expected, accessions from different mega-environments were more dissimilar than those of similar origins, as was also corroborated by Hernandez (1985) and Beyene *et al*. (2006). The most similar accession pair was TZm-251/TZm-37 of mid-altitude Zambia and mid-altitude Tanzania, respectively.

A UPGMA cluster analysis of the distance matrix produced four main clusters (Figure 4.2). Clusters I and III were heterogeneous and differentiated into two subclusters each. Main cluster I contained 15 members which originated from the lowland and mid-altitude zones of Guinea, Tanzania, Congo, Zambia, Equatorial Guinea and Togo. The mean genetic distance was 0.30 or 30 % genetic similarity for both subclusters. The check variety from Ghana was identified in cluster I. Genotypes of cluster I included the earliest-maturing genotypes (3 to 4 days earlier than the overall mean) and small kernels (at least 0.35 mm shorter and narrower). Grain yield in this cluster was derived from large ear diameter exceeding the overall mean by 0.63 mm and high number of rows per ear of 1.3 rows more than the overall mean. It is worth noting that the small kernel sizes borne on large cob diameter in excess of 2.37 mm greater than the overall mean delimited the grain yield (Table 4.7).

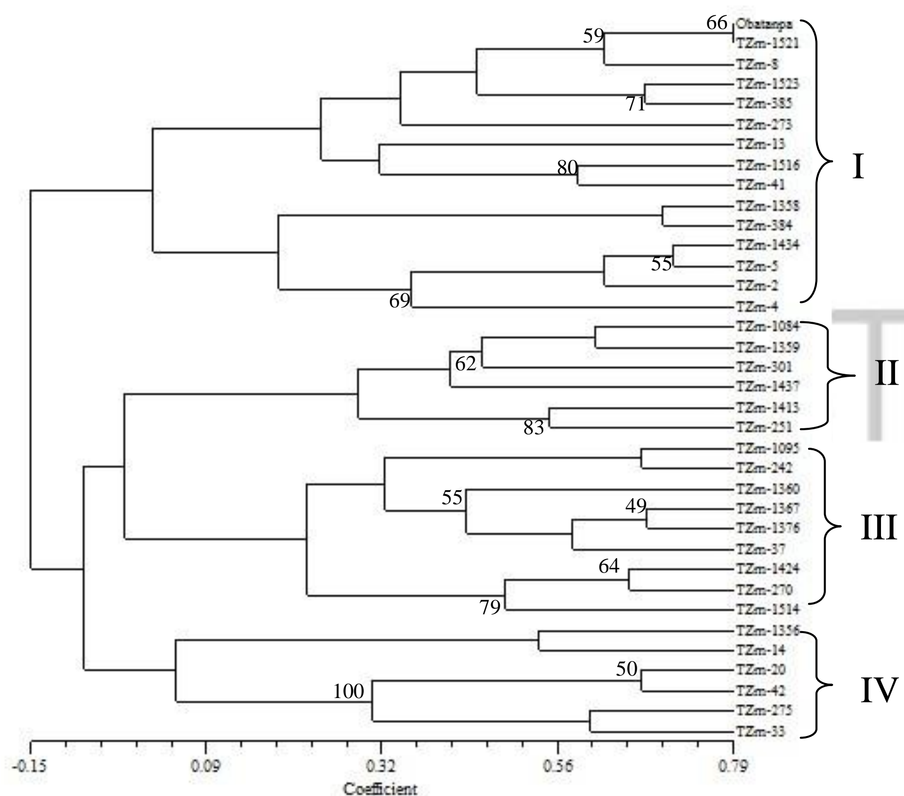


Figure 4.2. Dendrogram of 18 quantitative morphological traits on 36 maize accessions in the three mega environments of Africa based on correlation distance coefficient of similarity index using UPGMA cluster method with corresponding bootstrap values. A cophenetic coefficient of 0.70 indicated the high reliability and goodness-of-fit of the dendrogram with the values of the distance matrix of the data.

Table 4.6. Distribution of 36 maize accessions from three mega environment of Africa into clusters based on correlation coefficient distance measure.

Cluster no.	No. of Accessions	Accessions	Origin	Altitude
I	15	Obatanpa, TZm-1521, TZm-8, TZm-1523, TZm-385, TZm-273, TZm-13, TZm-1516, TZm-41, TZm-1358, TZm-384, TZm-1434, TZm-5, TZm-2, TZm-4	Ghana, Guinea, Tanzania, Congo Zambia, Equatorial Guinea, Togo,	Lowland, Mid-altitude,
II	6	TZm-1084, TZm-1359, TZm-301, TZm-1437, TZm-1413, TZm-251	Malawi, Equatorial Guinea, Chad, Togo, Somalia, Zambia	Lowland, Mid-altitude
III	9	TZm-1095, TZm-242, TZm-1360, TZm-1367, TZm-1376, TZm-37, TZm-1424, TZm-270, TZm-1514,	Malawi, Zambia, Kenya, Tanzania	Lowland, Mid-altitude, Highland
IV	6	TZm-1356, TZm -14, TZm -20, TZm-275, TZm-42, TZm-33	Equatorial Guinea, Tanzania, Zambia,	Mid-altitude, highland

Table 4.7. Overall means, cluster means, and standard deviation of the 36 highland, mid-altitude and lowland African accessions evaluated in Ghana by morphological trait measurement.

Trait	Overall Mean	Cluster I	Cluster II	Cluster III	Cluster IV
AD	58.61±5.67	55.86±5.39(-2.75)	63.22± 4.80(**4.61**)	59.19± 4.54(0.58)	59.97± 4.86(1.36)
SD	62.68±6.09	59.94± 5.92(-2.74)	67.39± 5.13(**4.17**)	62.74± 4.95(0.06)	64.76± 5.25(2.08)
ASI	4.08±1.66	4.08± 1.68(0.00)	4.17± 1.56(0.09)	3.56± 1.41(-0.52)	4.79± 1.75(**0.71**)
TL	47.14±7.34	47.31± 7.76(0.17)	47.06± 6.65(-0.08)	48.27± 7.48(**1.13**)	45.12± 6.25(-2.02)
ELL	79.16±16.79	79.22±16.19(0.06)	86.21± 12.70(**7.05**)	78.52± 18.35(-0.64)	72.9± 16.85(-6.26)
ELW	8.50± 2.35	8.55± 3.01(0.05)	8.59± 1.60(0.09)	8.99± 1.65(**0.49**)	7.54± 1.58(-0.96)
PLHT	173.03±38.85	168.90± 39.07(-4.13)	183.96± 33.8(**10.93**)	180.47± 40.95(7.44)	161.24± 34.77(-11.79)
EHT	81.49±28.20	77.02± 27.29(-4.47)	95.63± 25.47(**14.14**)	85.93± 27.90(4.44)	71.83± 26.96(-9.66)
StD	19.52±4.17	19.23± 4.41(-0.29)	20.83± 3.80(**1.31**)	20.17± 3.91(0.65)	17.96± 3.67(-1.56)
SG	79.36±4.17	79.41± 16.12(0.05)	85.6± 13.66(**6.24**)	79.56± 15.1(0.20)	72.69± 22.79(-6.67)
EP	0.46±0.09	0.44± 0.08(-0.02)	0.52± 0.08(**0.06**)	0.47± 0.09(0.01)	0.44± 0.09(-0.02)
EL	16.29±3.4	16.83± 3.23(**0.54**)	16.03± 3.25(-0.26)	16.26± 3.59(-0.03)	15.89± 3.43(-0.4)
ED	40.62±7.76	41.25± 7.87(**0.63**)	38.95± 8.25(-1.67)	40.94± 7.81(0.32)	40.26± 6.58(-0.36)
CD	26.58±6.07	28.95± 6.56(**2.37**)	25.00± 5.17(-1.58)	24.51± 5.09(-2.07)	25.35± 4.80(-1.23)
NRE	12.74±2.52	14.01± 2.30(**1.31**)	12.60± 2.14(-0.10)	11.39± 2.41(-1.31)	11.73± 1.93(-0.97)
NKR	29.70±8.13	28.3± 8.19(-1.40)	29.48± 8.94(-0.22)	32.64± 6.59(**2.94**)	29.03± 8.12(-0.67)
HKWT	67.98±20.99	64.21± 19.13(-3.77)	68.72± 23.50(0.74)	76.49± 22.03(**8.51**)	63.87± 17.02(-4.11)
EN	1.03±0.10	1.04± 0.14(0.01)	1.03± 0.09(0.00)	1.01± 0.03(-0.02)	1.04± 0.06(0.01)
KL	9.49±1.68	9.15± 1.72(-0.34)	9.28± 1.69(-0.21)	10.30± 1.54(**0.81**)	9.36± 1.37(-0.13)
KW	9.53±1.27	9.14± 1.34(-0.39)	9.27± 1.27(-0.26)	10.17± 1.04(**0.64**)	9.81± 0.93(0.28)
KT	5.10± 0.83	5.18± 0.79(0.08)	5.25± 1.01(**0.15**)	5.07± 0.72(-0.03)	4.81± 0.77(-0.29)
EWT	0.13± 0.11	0.11± 0.08(-0.02)	0.14± 0.13(0.01)	0.13± 0.11(**0.00**)	0.18± 0.16(0.05)
GWT	0.83± 0.47	0.81± 0.48(-0.02)	0.76± 0.42(-0.07)	0.96± 0.50(**0.13**)	0.75± 0.41(-0.08)
YLD	4.48± 2.56	4.44± 2.41(-0.11)	4.12± 2.29(-0.36)	5.2± 2.73(**0.72**)	4.06± 2.22(-0.42)

Cluster II consisted of 6 genotypes from of lowland and mid-altitude zones of Togo, Equatorial Guinea, Malawi, Chad, Somalia and Zambia in Africa (Table 4.6). The genetic distance of cluster II ranged from a minimum of 0.12 to a maximum of 0.61 with an average distance of 0.38. This cluster was characterized by late-maturing plants (4 to 5 days later than overall mean), maximum dimensions of plant architectural traits except ELW, enhanced HKWT and largest kernel thickness. The contribution of the yield components, ear length, ear diameter, HKWT and kernel thickness to grain yield was insubstantial and produced a cluster mean yield (4.12

Mgha^{-1}) making about 360 g below the overall mean grain yield (Table 4.7).

Cluster III comprised 9 accessions from all the mega-environments studied (lowland, mid-altitude and highland). The genotypes originated from Zambia, Malawi, Kenya, and Tanzania. Genetic distance in cluster III ranged from 0.1 to 0.68 with an average of 0.33. Two subclusters, IIIA and IIB could be identified having within average genetic distance of 0.43 and 0.54, respectively. The principal features of cluster III genotypes were medium-maturing with the shortest anthesis-silking interval, longest tassel length, and largest ELW, broad ears, and longest and widest kernels borne on narrow cobs. Furthermore, cluster III exhibited the largest number of kernels per row, highest HKWT and largest ear weight, all of which contributed to the largest mean grain yield of 5.2 Mgha^{-1}, approximately 720 g in excess of the overall average grain yield. The positive correlation between TL, ELW, and grain yield is favourable for early generation selection as breeders can select against low-yielding genotypes on the basis of short TL and small ELW, and vice versa. The positive correlation may be due to pleiotropy or linkage between genes controlling ELW, TL band grain yield.

Finally, cluster IV genotypes were characterized by late-maturity, longest ASI, shortest tassel and ear leaf lengths, smallest ELW, shortest dimension of plant architectural traits, least HKWT and EWT and smallest kernel thickness. Undoubtedly, cluster IV produced the lowest mean grain yield (4.2 Mgha^{-1}) of 420 g below the overall mean yield (Table 4.7). The 6 accessions of cluster IV originated from mid-altitude and highland Equatorial Guinea, Tanzania and Zambia. The fewer number of rows per ear may have been the consequence of the long anthesis-silking interval. Long periods between anthesis and silking make viable pollen unavailable for the late appearing silks (Hall *et al*., 1982), failure in ovary fertilization and eventual reduced kernel set (Hall *et al*., 1981). Anthesis-silking interval in most cases prolongs when

plants are exposed to water stress before anthesis since silking is unduly delayed (Bassetti and Westgate, 1993; Jacobs and Pearson, 1991; Herrero and Johnson, 1981).

Notwithstanding the non-stressed conditions ensured on the field, the long ASI may be due to genetic effects and not environmental. The average genetic distance in cluster IV was 0.23 with minimum and maximum distances of 0.04 to 0.54, respectively.

This phenomenon of late-maturing nonetheless short plants observed in cluster IV deviates from the norm and may have arisen from some form of environmental stress.

Generally, maize breeders the world over desire genotypes that are early-maturing, short in height, and possess high yield potential. Early-maturing would usually escape drought. High yielding varieties will lead to the production of higher food output thereby ensuring food security, raw materials for industries and contribute to increased grower returns for the numerous smallholder maize farmers in Africa.

The four clusters represent heterotic groups from which hybridization across promising genotypes is expected to enhance genetic gains. Clusters I and III are potentially good candidates for exploiting high grain yield and earliness in maize breeding programs in Africa.

4.6 Principal Components Analysis

The quantitative data was subjected to Principal Components Analysis which involved calculation of eigenvalues and eigenvectors to estimate the proportion of variance carried by each trait as well as presentation of the principal components on biplots to reveal associations among traits, among accessions, and traits and accessions. The first

four principal components which had eigenvalues greater than 1.0 explained 84.53 % of the total phenotypic variance (Table 4.8). In the first PC which accounted for 38.39 % of the total variance, the predominant traits were tassel length, ear leaf length, ear leaf width, plant height, ear position, ear length, ear diameter, hundred kernel weight, kernel length, kernel width and grain yield. The second principal component (PC2) explained 18.70 % of the total variance, with the earliness traits, AD, SD, and ASI predominating, whereas in the third (PC3) and fourth (PC4) principal components which accounted for 16.36% and 11.08 % of the total variance were dominated by the yield component, NKR and ASI, respectively. On the basis of the principal components traits that were most important (eigenvectors >0.76) in determination of genetic diversity in the maize accessions included ELW, PLHT, EL and HKWT.

A biplot of PC1 against PC2 (Figure 4.3) accounted for 57.09 % by increment and displayed four major correlation groupings, namely, group based on earliness, plant architectural traits, groups based on ear related traits, and lastly, groups based on kernel characteristics and grain yield. The earliness traits AD and SD were positively correlated but negatively correlated with grain yield and its components as shown by the respective acute angles between the vectors. Anthesis-silking interval recorded the maximum angle with yield and most of its component traits thereby explaining the negative correlation between these traits. Other traits which contributed minimally to the variance are NRE and ASI. In the plant architectural group, strong positive correlations were found among PLHT, ELL, EL, EHT and EP with principal contributions from PLHT and EL. In the accessions studied, tall plant also had long ears.

Table 4.8. Eigenvalues and eigenvectors, and cumulative percentage of variation explained by the first four principal components (PC) after assessing 18 morphological traits in 36 tropical maize accessions.

Variables	PC1	PC2	PC3	PC4
AD	0.39	**0.71**	0.40	0.23
SD	0.37	**0.75**	0.33	0.32
ASI	0.03	**0.52**	-0.21	**0.61**
TL	**0.66**	0.00	-0.20	0.21
ELL	**0.72**	0.17	-0.21	0.05
ELW	**0.79**	-0.21	-0.24	-0.12
PLHT	**0.78**	0.19	0.07	-0.34
EHT	**0.76**	0.38	0.11	-0.46
EL	**0.78**	0.18	-0.20	0.21
EP	**0.60**	0.47	0.15	**-0.51**
ED	**0.52**	-0.39	-0.42	0.37
CD	0.26	-0.09	**-0.78**	-0.01
NRE	-0.01	0.22	**-0.82**	-0.18
NKR	0.15	-0.39	**0.57**	-0.21
HKWT	**0.81**	-0.31	0.05	0.18
KL	**0.59**	-0.46	0.36	0.20
KW	**0.52**	-0.47	0.40	0.40
YLD	**0.63**	0.49	0.00	-0.30
Eigenvalues	6.10	2.97	2.60	1.76
Individual percentage	38.39	18.70	16.36	11.08
Cumulative Variance (%)	38.39	57.09	73.45	84.53

The third group was delineated by strong association between HKWT and ELW, their primary contributions to the variance, the secondary contribution of TL with CD playing a merely minor role. The major finding that genotypes with wider leaf width also had increased HKWT and grain yield would be valuable information for maize breeding programs as it can be used in early generation selection. Lastly, variance in the ear and kernel characteristics as well as grain yield grouping was dominated by KL, KW and ED in that order, with small effect from NKR (Figure 4.2A). Magorokosho (2006) in his study of genetic diversity and performance of maize varieties from Zimbabwe, Zambia and Malawi reported 17.6 % and 12.6% contributing to 30.2 % of the total variation for the first two principal components. Also, Hartings *et al*. (2008) in plotting PC1 (56.1) against PC2 (10.6) accumulatively accounted for 66.7% of the total variance in their study of genetic diversity among

54 Italian landraces based on morphological traits.

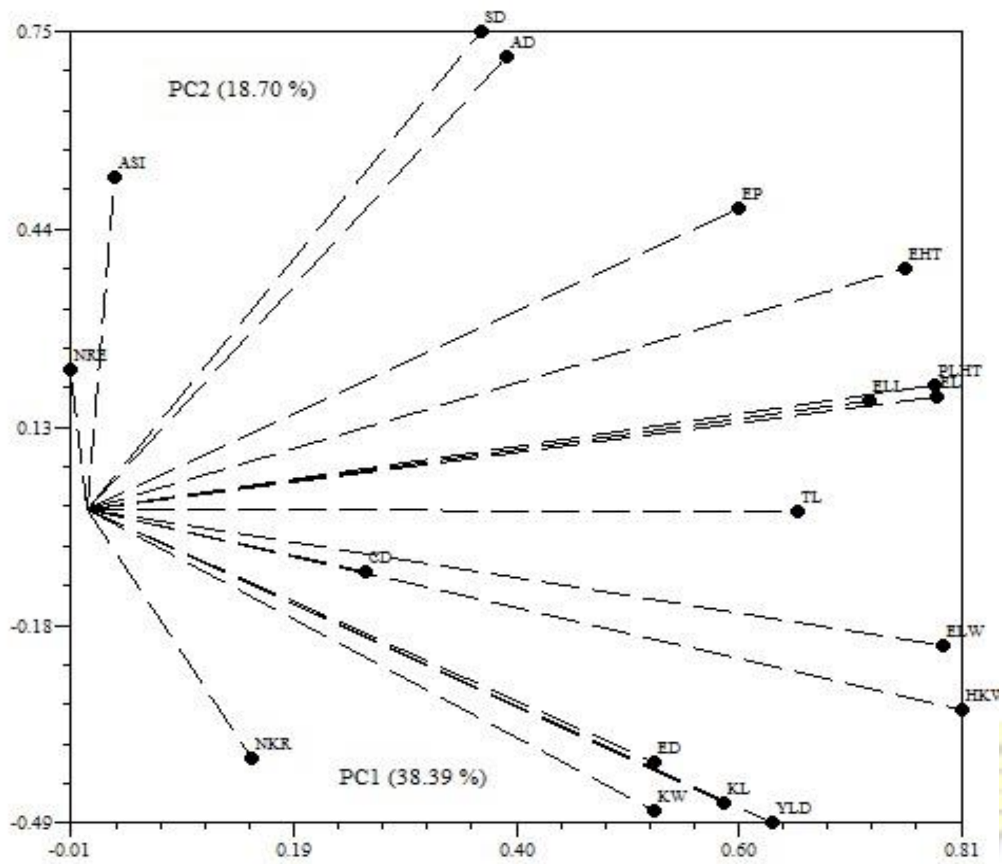
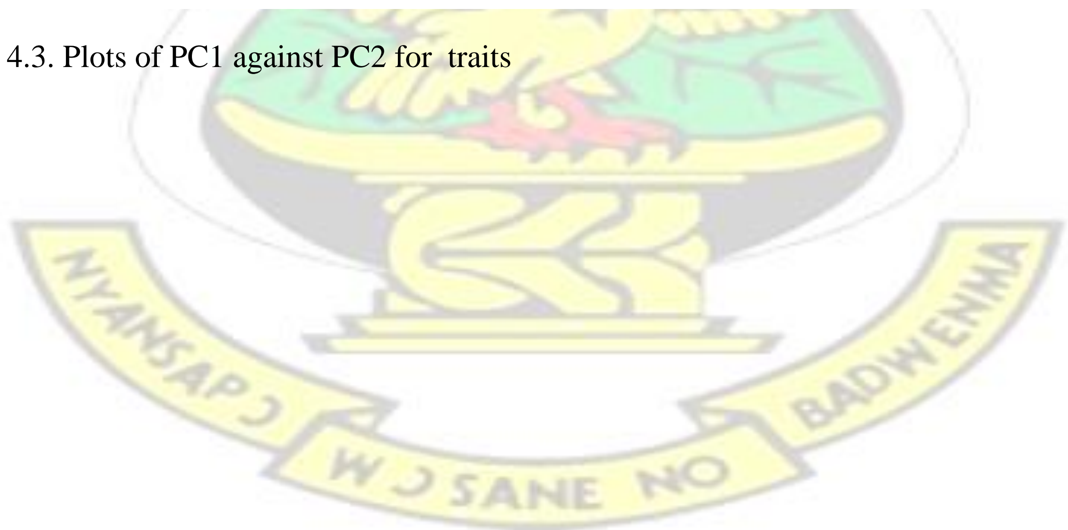


Figure 4.3. Plots of PC1 against PC2 for traits



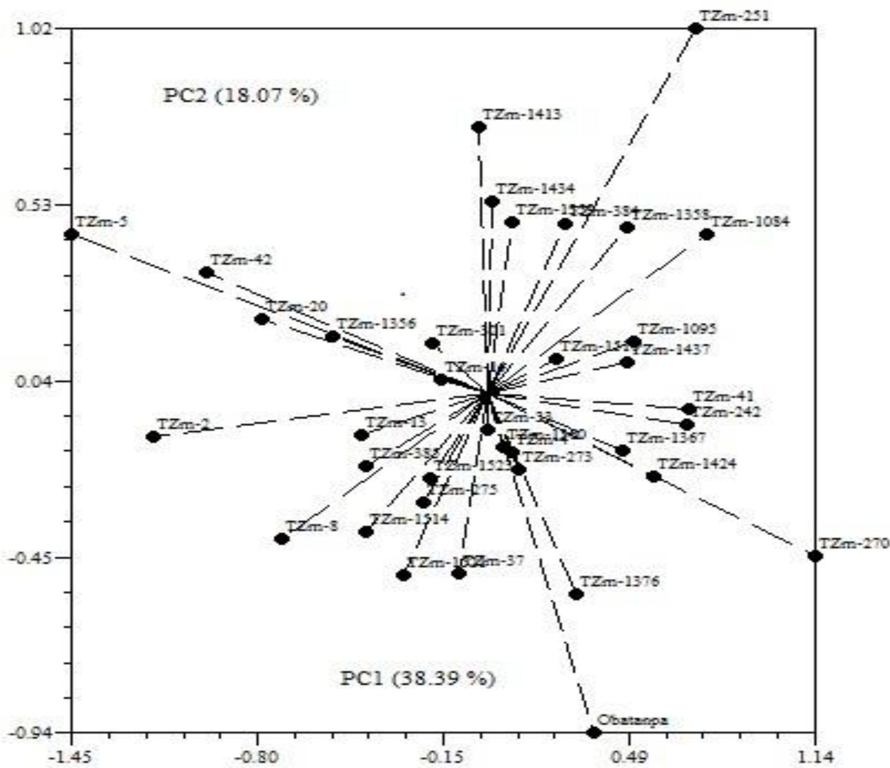


Figure 4.4 Plots of PC1 against PC2 for accessions including “Obatanpa”, the check.

The first PC separated the accessions on the basis of PLHT, large HKWT, EL, ELW and grain yield. Important members of this group are TZm-270, TZm-242, TZm1367, TZm-4, TZm-41 and TZm-37. The second PC partitioned the accessions according to earliness to incorporate members such as „Obatanpa GH‟, TZm-8, TZm-2, TZm-37, and TZm-1521. Accession TZm-270 and „Obatanpa GH‟ contributed most to the variance in PC1 and PC2 in being distantly related to the other members of their group. „Obatanpa GH‟ is a Quality Protein Maize, hence it was not surprising that it isolated from all other normal maize accessions. Similarly, TZm-2, TZm-5, TZm-251 were also isolated from the other accessions.TZm-270 stood out because it is a very high yielding genotype comparing favourably with Obatanpa, a high yielding inbred line from Ghana. A biplot of the first two PCs on the 36 accessions is shown in Figure 4.3.

4.7. Genetic diversity of maize by SSR profiling

The molecular genetic diversity study involved evaluation of 16 SSR primer loci on fifty-seven lowland, mid-altitude and highland maize accessions and five checks, "Obatanpa GH", TZi8, TZi9, CML157, and CML 258. All fifty-seven accessions together with the checks produced quality DNA which resulted in good amplification and genotyping of the data. Figure 4.4 shows typical gels in current study with amplification products.

KNUST

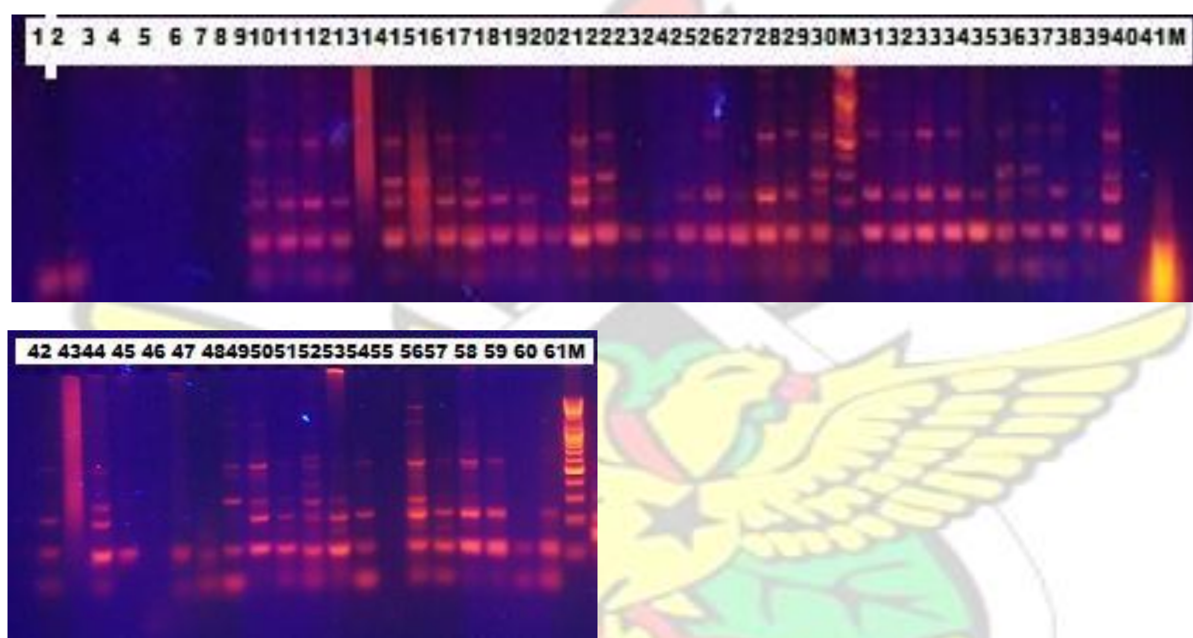


Figure 4.5. Agarose gel electrophoresis of 57 maize genotypes together with 5 checks using locus nc133 M = marker, 1= TZm-2, 2 = TZm-5, 3 = TZm-7, 4 = TZm11, 5 = TZm-14, 6 = TZm-20, 7 = TZm-23, 8 = TZm-4, 9 = TZm-6, 10 = TZm-8, 11 = TZm-13, 12 = TZm-19, 13 = TZm-22, 14 = TZm-30, 15 = TZm-32, 16 = TZm-37, 17 = TZm-41, 18 = TZm-57, 19 = TZm-33, 20 = TZm-38, 21 = TZm-42, 22 = TZm63, 23= TZm-77, 24= TZm-91, 25= TZm-242, 26= TZm-78, 27= TZm-156, 28= TZm-243, 29= TZm -251, 30 = TZm-273, 31 = TZi8, 32 = TZm-270, 33 = TZm-275, 34 = TZm-301, 35 = TZm-384, 36 = TZm-1084, 37 = TZi9, 38 = TZm-1355, 39 = TZm-385, 40 = TZm-1095 and 41= CML157, 42 = TZm-1357, 43 = TZm-1359, and 44 = TZm-1360, 45= 1356, 46= 1358, 47= 1367, 48= 1369, 49= 1380, 50= 1413, 51= 1376, 52= Obatanpa, 53= 1424, 54= 1430, 55= 1437, 56= 1514, 57=1434, 58=1502, 59= 1516, 60= 258, 61= 1521, 62= 1523.

Thirteen primers out of sixteen produced good amplification products expressed as clear and sharp bands free from stuttering. Primer phi002 failed to produce good

quality DNA. Each chromosome was represented by at least one SSR marker with chromosomes 4, 9, and 10 having 2 SSR markers each. Table 4.9 shows standard statistics of SSR loci, sequences, and number of alleles at each locus in addition to the polymorphism information content.

Besides locus phi046 which was monomorphic, and phi101049 which was discarded due large missing data, all other loci had at least 2 alleles. The range of polymorphism was a minimum of 2 alleles for locus umc1161 and umc1196 to 10 alleles for locus nc133. Majority of the SSR loci (80 %) had 3 or more alleles (Table
4.9). No rare allele was identified as all allele frequencies were higher than 0.005. The rate of polymorphism, *P*, was 93 %. For the 13 polymorphic loci, a total of 70 alleles were detected with an average of 5.38 alleles per locus. This value represents sufficient diversity within the African genotypes.

Effective number of alleles ranged from 1.22 to 5.26 with a mean of 3.25±1.27, while the average expected heterozygisity, H_e, for the entire population was
0.64±0.17. This value is also termed Nei"s genetic diversity, *D*. The disparity in the number of alleles and effective number of alleles is accounted for by differences in expected heterozygosity, which in this case is the PIC. Variation in heterozygosity is the corollary of differences in allele frequencies in a locus. In current study, two sets of loci, dupssr10/phi065 and bnlg1597/phi213984 have 5 and 4 alleles each but varying effective number of alleles as frequencies of 3.45, 3.23, 2.78, and 2.50, respectively. The highest allele frequency in dupssr10 was 0.41, whereas that in phi065 was 0.47. Similarly, highest allele frequency in bnlg1597 was 0.47 and that in phi213984 was 0.45, with all other

Table 4.9. Statistics of SSR loci, SSR marker sequence, annealing temperature, alleles per locus and polymorphic information contents of the 14 primers used in evaluation of 57 African maize accessions.

	Marker	**Chr.**[1]	**Repeat Unit**	**Primer Sequence (F/R)**	T_m (°C)	Alleles /locus	H_o	H_e	A_e
1	bnlg1597	1	(AG)34	GATAATCTCGTCTCGCCAGG(F) CATAAAAGGATGCCGACGAC(R)	58.0	4	0.33	0.64	2.78
2	phi002	1	AACG	CATGCAATCAATAACGATGGCGAGT(F) TTAGCGTAACCCTTCTCCAGTCAGC(R)	63.0	-	-	-	-
3	nc133	2	GTGTC	AATCAAACACACACCTTGCG(F) GCAAGGGAATAAGGTGACGA(R)	56.0	10	0.81	0.81	5.26
4	phi046	3	ACGC	ATCTCGCGAACGTGTGCAGATTCT(F) TCGATCTTTCCCGGAACTCTGAC(R)	63.0	1	-	1	1
5	phi073	3	AGC	GTGCGAGAGGCTTGACCAA(F) AAGGGTTGAGGGCGAGGAA(R)	59.0	6	0.59	0.76	4.17
6	bnlg1565	4	AG(27)	TCGGAGACGAGGCTGAAC(F) CTGGAGACGTTTGGTGTCAA(R)	58.0	9	0.29	0.70	3.33
7	phi213984	4	ACC	GTGACCTAAACTTGGCAGACCC(F) CAAGAGGTACCTGCATGGC(R)	60.0	4	0.20	0.60	2.50
8	dupssr10	5	(AC)22	AGAAAATGGTGAGGCAGG(F) TATGAAATCTGCATCTAGAAATTG(R)	54.0	5	0.41	0.71	3.45
9	bnlg1371	6	AG(22)	TTGCCGATAAGAACCAAACA(F) ACGACCGGTGTGGTTACATT(R)	55.0	3	0.56	0.53	2.13
10	phi034	7	CCT	TAGCGACAGGATGGCCTCTTCT(F) GGGGAGCACGCCTTCGTTCT(R)	63.0	8	0.79	0.81	5.26
11	umc1161	8	(GCTGGG)5	GGTACCGCTACTGCTTGTTACTGC(F) GCTCGCTGTTGGTAGCAAGTTTTA(R)	63.0	2	0.00	0.18	1.22
12	phi065	9	CACTT	AGGGACAAATACGTGGAGACACAG(F) CGATCTGCACAAAGTGGAGTAGTC(R)	63.0	5	0.17	0.69	3.23
13	umc1279	9	(CCT)6	GATGAGCTTGACGACGCCTG(F) CAATCCAATCCGTTGCAGGTC(R)	61.0	9	0.06	0.78	4.55
14	phi041	10	AGCC	TTGGCTCCCAGCGCCGCAAA(F) GATCCAGAGCGATTTGACGGCA(R)	63.0	3	0.30	0.60	2.50
15	umc1196	10	CACAGC	CGTGCTACTACTGCTACAAAGCGA(F) AGTCGTTCGTGTCTTCCGAAACT(R)	62.0	2	0.00	0.45	1.82
				Total		70		8.35	-
				Minimum		2	0.00	0.18	1.22
				Maximum		10	0.81	0.81	5.26
				Mean (H_e or D)		5.38	0.35	0.64	3.25
				SD		2.32	0.27	0.17	1.27
				$X_{2df1} = 2.66$					

[1]Chromosome

A_e =Effective No. of alleles, H_e=expected heterozygosity; Ho=observed heterozygosity

alleles occurring at varying frequencies. It was only loci umc1161 and umc1196 that had one allele dominating at frequency of 0.89 and 0.66, which reflected in the correspondingly least values of effective number of alleles of 1.22 and 1.82, respectively. Both loci were hexa repeats. In contrast, loci having the largest values of

A_e (>0.40), such as nc133, phi034, phi073 and umc1279 had fairly equal distribution of allele frequencies and were predominantly tri repeats.

The number of alleles per locus (5.38) obtained in current study is similar to those reported in other maize diversity studies. Warburton *et al*. (2002) in analyzing 57 CML with 85 SSR loci had an average of 4.9 alleles per locus. Lu and Bernardo (2001) evaluated 40 U.S. inbred lines with 83 SSR markers and identified overall average number of alleles to be 4.9 alleles. Senior *et al*. (1998) obtained 5.0 alleles from a study of 94 elite U.S. maize inbred lines with 70 markers. Pejic *et al*. (1998) examining 33 U.S. maize inbred lines with 27 SSR markers obtained an average of 6.8 alleles. Pinto *et al*. (2003) identified 4.16 alleles with 30 SSR loci. Xia *et al*. (2004) reported an average of 7.4 alleles with a range of 2 to18 alleles on evaluation of 155 tropical lowland inbred lines from CIMMYT recorded with 79 SSR markers. Finally, Magorokosho (2006) reported an average of 9.3 alleles with a range of 4 to 17 alleles in evaluation of 99 maize accessions from Zambia, Malawi and Zimbabwe with 23 SSR markers.

The number of alleles in current study is nonetheless higher than the findings of Bantte and Prasanna (2003) who obtained 3.25 alleles using 36 SSR loci in 23 QPM inbred lines from India. and Legesse *et al*. (2007) who reported a mean of 3.85 alleles on 56 CIMMYT inbred lines of highland and mid-altitude regions of Ethiopia and Zimbabwe African maize with 27 SSR loci. These investigators made use of agarose gel as is the case in this study. As heterogeneous as the landraces are with respect to their diverse geographical origin, their originality and, not having undergone any system of breeding, but open to hybridization by cross-pollination they are expected to be heterozygous having accrued mutations and recombinations over many generations to

give rise to expected substantial allelic richness. The relatively few number of alleles of 5.38 per locus identified may be attributed to the few markers (14) used to evaluate the 57 genotypes in current study. It is therefore expected that with increase in number of markers a higher number of alleles could be obtained.

The average observed (0.35) and expected heterozygosity (0.64) under HardyWeinberg equilibrium values across the loci are presented in the Table 4.9. Application of the chi square goodness-of-fit test showed that there was no significant difference ($X^2 = 2.66$; $P<0.05$) between the observed and expected heterozygosity for the population. The expected heterozygosity was considered to be high.

The Polymorphic Information Content (PIC) of SSR loci ranged from 0.18 for umc1161 to 0.81 for phi034 and nc133 with the mean of 0.64±0.17. Over 70 % of the markers had a PIC value of 0.60 or more (Table 4.9) which represents high and significant informative and discriminatory power of the markers to detect disparities among maize accessions on the basis of their genetic relationships. The mean PIC of 0.64 is equivalent to mean expected heterozygosity for the entire population assuming Hardy-Weinberg equilibrium, a condition, generally expected among outcrossing species. This value is sufficient to indicate widespread genetic variation among the genotypes. The high heterozygosity in the maize population is suggestive of an isolate-breaking effect which may have occurred in the past to introduce new alleles to the maize population in Africa or due to a population admixture.
The mean PIC value (H_e) or gene diversity in the African landraces was higher (0.64) than those reported among various groups of inbred lines. Xia *et al*. (2004) obtained a range of PIC of 0.13 to 0.87 with an average of 0.60 in tropical lowland CIMMYT inbred lines. Smith *et al*. (1997) and Senior *et al*. (1998) found average PIC values of 0.62 and 0.59 in their SSR studies with 58 and 94 U.S maize inbred lines,

respectively. Legesse *et al*. (2007) reported average PIC value of 0.58 (ranging from 0.31- 0.71) among 56 highland and mid-altitude inbred lines of CIMMYT breeding programs in Ethiopia and Zimbabwe at 27 SSR loci. Matsuoka *et al*. (2002) stated expected heterozygosity of 0.60 among 101 US maize inbred lines at 46 SSR loci. Expected heterozygosity of 16 China maize inbred lines and one teosinte, *Z. mays* ssp.*mexicana*, was 0.05 to 0.83, with an average of 0.31 (Xu *et al*., 2013).

Higher values of mean heterozygosity were reported by Li *et al*. (2006) (0.66) in CIMMYT and Chinese inbred lines; Enoki *et al*. (2002) (0.69) for 65 inbred lines of the cold regions Japan at 60 loci; Pejic *et al*. (1998) (0.72) for 33 inbred lines of U.S. Corn Belt, Kostova *et al*. (2007) reported mean heterozygosity of 0.71 for 41 Bulgarian and 10 U.S. inbred lines. A high number of alleles and large gene diversity is ordinarily expected from large sample size and large proportion of di-nucleotide repeats employed (Liu *et al*., 2003). Di-nucleotide repeats are often more polymorphic and generate larger number of alleles than tri, tetra, penta and hexa repeat loci (Vigouroux *et al*., 2002) and consequently give high values of expected heterozygosity.

The four di-repeat SSR loci in current study were bnlg1597, bnlg1565, bnlg1371 and dupssr10. These gave mean allele number of 5.25 and expected heterozygosity of 0.67 in comparison with mean allele number of 9.3 and expected heterozygosity of 0.74 reported for the di-repeat loci in Enoki‟s work (Enoki *et al*., 2002) and 23.9 alleles and 0.839 gene diversity for the di-repeats of Liu *et al*. (2003). Surprisingly, the allelic content and gene diversity for the tri- and penta- repeats in current study were higher than those of the di-repeats. The tri, tetra, penta and hexa-repeats gave mean allele number of 6.75, 3.0, 7.5, and 2.0 and PIC values of 0.74, 0.60, 0.75, and 0.31, respectively. The relatively lower allelic mean value of di-repeats in current study might have been due to their difficulty in scoring arising from additional stutter bands

(Smith *et al*. (1997). It is expected that improvement in scoring could be achieved with the use of polyacrylamide gels which offer better resolution

Table 4.10. Polymorphic information score summary statistics by repeat class

Repeat class	Mean no. of alleles	Mean PIC value
2	5.25	0.67
3	6.75	0.74
4	3.0	0.60
5	7.5	0.75
6	2.0	0.31
3-6	4.8	0.60

4.8 Molecular genetic distance and relationships among accessions and traits

Pairwise genetic distances were determined using the DICE coefficient distance measure. Values ranged from 0.00, the most closely related pairs to 1.00, the most dissimilar genotypes.

Accession pairs that recorded distance of 0.00 included CML258/TZm-14, TZm-22, TZm-38 and TZm-8. The most dissimilar accessions were TZm-1516 with TZm-30, TZm-32, TZm-37, TZm-41, TZm-57, TZm-33, TZi8, TZm-275, TZm-1358 and TZm-1359. Also, CML258 was most dissimilar to TZm-6, TZm-4, TZm-33, TZm19, TZm-156, TZm-251, TZm-384, TZm-1084, CML157, TZm-1357, TZm-1380, TZm-1437, TZm-1514 and TZm-1502. Obatanpa was most dissimilar to TZm-1359 and TZm-1360 (1.00). The relationships among TZi9 and the other genotypes were closely related to TZm-4 (0.13) and dissimilar to TZm-1084 (0.71). TZi8 was similar to TZm-91 (0.49) and most dissimilar to TZm-4 (0.82) and TZm-33 (0.82). Also, CML157 was most similar to TZm-2 (0.39) but most dissimilar to TZm-13 (0.88). TZm-270 was in distant relationship with TZm-41(1.00) just as it occurred in the morphological principal components analysis but closely related to TZm-14 (0.44).

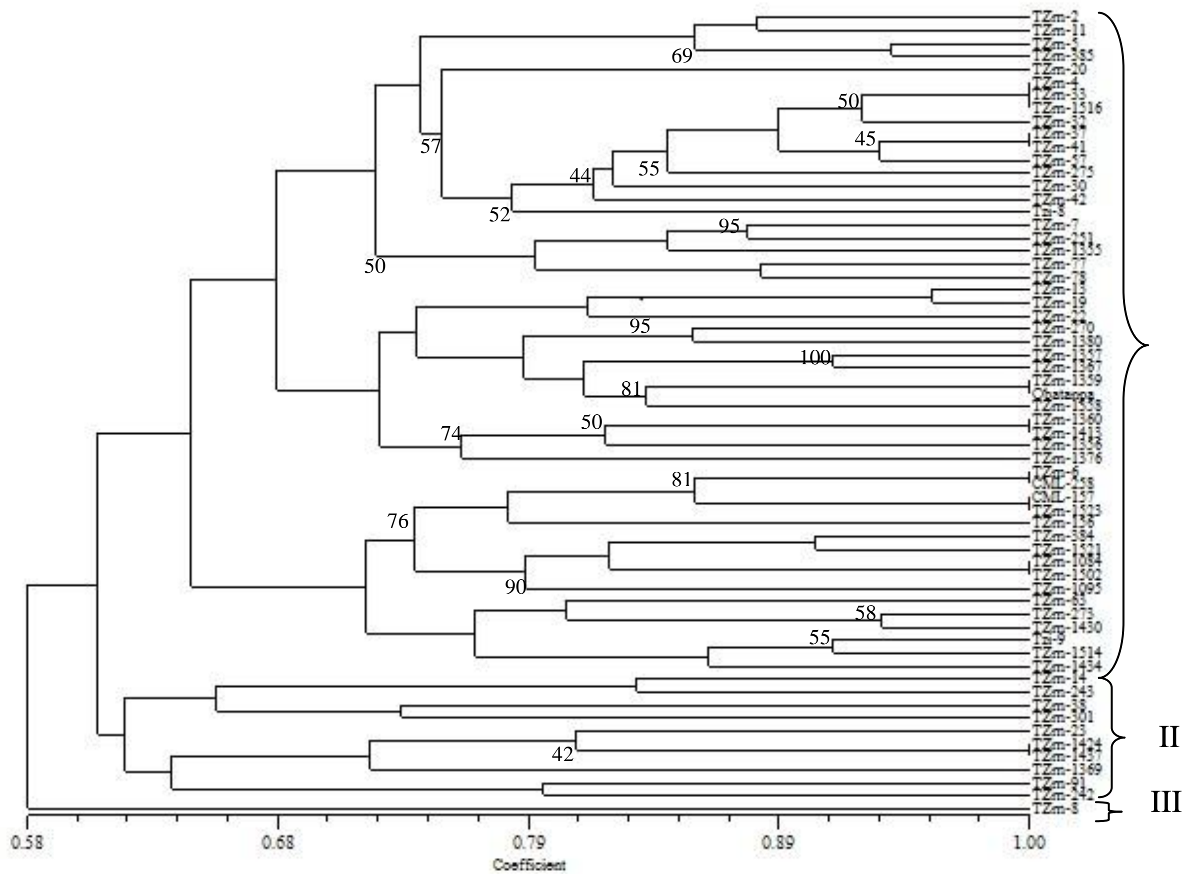
A UPGMA cluster analysis of the distance matrix revealed three main clusters

(Figure 4.5). Main cluster I was heterogeneous as it produced four subclusters. Members of subcluster IA were TZm-2, TZm-5, TZm-11, TZm-385, TZm-20, TZm42, TZm-30, TZm-275, TZm-57, TZm-41, TZm-37, TZm-32, TZm-1516, TZm-33, TZi8, and TZm-4. These mostly originated from the lowland, mid-altitude and highland zones of Congo, Tanzania and Zambia. In comparison to the morphological data, the principal features of cluster I genotypes were early- maturing with small kernel length and small kernel width borne on short ear length. Grain yield was derived from large ear diameter which was lower than the overall mean.

Subcluster IB was fairly homogeneous with a membership of 5 genotypes, namely TZm-7, TZm-251, TZm-1355, TZm-77 and TZm-78 which originated from midaltitude and highland regions of Equatorial Guinea, Tanzania, Zambia and Zimbabwe. Key characteristics of cluster IB genotypes include late-maturity plants (5 days later than overall mean), having maximum dimensions of plant architectural traits particularly, tall plants, long and narrow ears, enhanced HKWT and largest kernel thickness. Subcluster IC contained 14 members. The genotypes under this subcluster mostly originated from lowland, mid-altitude and highland zones of Tanzania, Zambia, Equatorial Guinea, Kenya, Somalia and Ghana. The check variety „Obatanpa‟ was identified with this subcluster. Members of subcluster 1C possessed long tassels, large ear leaf width with the highest number of kernels per row as well as high hundred kernel weight.

Subcluster ID had 16 members including TZm-6, CML-157, CML-258, TZm-1523, TZm-156, TZm-384, TZm-1521, TZm-1084, TZm-1502, TZm-1095, TZm-63, TZm273, TZm-1430, TZi9, TZm-1514 and TZm-1434. The genotypes originated from the lowland and mid-altitude regions of Tanzania, Zimbabwe, Zambia, Congo, Malawi, Burkina Faso, Togo, Guinea and Benin. Three out of the five checks were

identified with this cluster. The genotypes under this cluster were seen to have medium hundred kernel weight with a medium plant height. Main cluster II contained 10



genotypes. The genotypes originated from the lowland, mid-altitude and highland zones of Tanzania, Chad, Kenya, Togo, Zimbabwe and Zambia.

Finally, cluster III contained a single genotype, TZm-8 from the mid-altitude zone of Tanzania. This accession was early-maturing possessed small kernel length and width, having kernels borne on short ears.

I

Figure 4.6. Dendrogram of a UPGMA cluster analysis of 62 maize accessions based on SSR profiling showing bootstrap values.

The mid-altitude accessions were found in all the clusters, lowland accessions were in subclusters IA, IC, ID and main cluster II while highland genotypes were found in subclusters IA, IB, IC and main cluster II. From the dendrogram TZm-4, TZm-33 and TZm-1516 clustered together at a similarity of 0.99. The first two accession including TZm-4 and TZm-33 originated from Tanzania belonging to the midaltitude. These three tropical maize accessions were in close association with TZm-32.

Table 4.11. Distribution of 62 maize accessions from three mega environments in Africa into clusters based on Dice coefficient genetic distance measure.

Cluster	No. of Accessions	Accessions	Origin	Altitude
IA	16	TZm-42, TZm-30, TZm-275, TZm-57, TZm-41, TZm-37, TZm-32, TZm-1516, TZm-33, TZi8, TZm-4, TZm-20, TZm-385, TZm-5, TZm-11 and TZm-2	Tanzania, Zambia, Congo,	Lowland, Mid-altitude, Highland,
IB	5	TZm-78, TZm-77, TZm-1355, TZm-251, TZm-7	Tanzania, Zambia, Eq. Guinea, Zimbabwe,	Mid-altitude, Highland,
IC	14	TZm-1376, TZm-1356, TZm-1357, TZm-1413, TZm-1360, TZm-1358, TZm-1359, TZm-1367, TZm-22, TZm-1380, TZm-270, TZm-19, TZm-13 and Obatanpa.	Tanzania, Zambia, Eq. Guinea, Kenya, Somalia, Ghana	Mid-altitude, Highland, Lowland
ID	16	TZm-1434, TZm-1514, TZm-1430, TZm-273, TZm-63, TZm-1095, TZm-1502, TZm-1084, TZm-1521, TZm-384, TZm-156, TZm-1523, TZm-6, TZi9, CML-157 and CML-258	Tanzania, Zimbabwe, Zambia, Congo, Malawi, Burkina Faso, Togo, Guinea, Benin	Mid-altitude, lowland
II	10	TZm-38, TZm-301, TZm-14, TZm-243, TZm-23, TZm-1369 , TZm-1424, TZm-1437, TZm-91 and TZm-242	Tanzania, Chad, Kenya, Togo Zimbabwe, Zambia	Highland, Mid-altitude, Lowland
III	1	TZm-8	Tanzania,	Mid-altitude

4.9 Morphological and molecular genetic distances (GD) compared

Both the morphological and molecular genetic distance measures produced low similarity coefficients (Table 4.12). The similarity increased in the order of midaltitude, lowland and was highest in the highland elevations in each case. These indicate the presence of wider genetic variations in the African maize landraces with the mid-altitude accessions being the most diverse, followed by the lowland while the highland accessions were the least variable which was in consonance with the variability revealed by the statistical measures of mean squares and coefficient of variation.

Table 4.12. Genetic distances of African maize landraces revealed by similarity distance measure (morphology) and DICE (molecular) genetic distance coefficients, respectively.

Elevation/Diversity estimator		**Lowland**	**Mid-altitude**	**Highland**
Morphology (Similarity)	GD	0.29	0.23	0.38
Molecular (Dissimilarity)	GD	0.69	0.70	0.65
Molecular (Similarity =1- dissimilarity)	GD	1-0.69=0.31	1-0.70=0.30	1-0.65=0.35
Total		0.98	0.93	1.03

Breeding success in maize has historically relied on the naturally existing genetic diversity in the germplasm. Despite the consideration of Africa as the secondary center of diversity in maize, (Brandolini, 1969) assessment of genetic variability within her landraces has barely been carried out at both local and on a wide geographical range. Consequently, the use of the landraces has not been significant in breeding programs. The current climate anomalies with associated drought stress, disease epidemics, and poor grain yield requires active search for novel alleles from the highly adapted landraces genotypes and evaluation of their breeding potential.

In this study, the genetic diversity among lowland, mid-altitude and highland African maize accessions by means of morphological traits and SSR profiling was carried out.

Generally, a wide variability was observed among the different accessions. The level of variability followed the order mid-altitude, lowland, and highland accessions in a decreasing order. The key and highly variable traits which correlated with grain yield were earliness, ASI, TL, ELW, HKWT, NKR and KL.

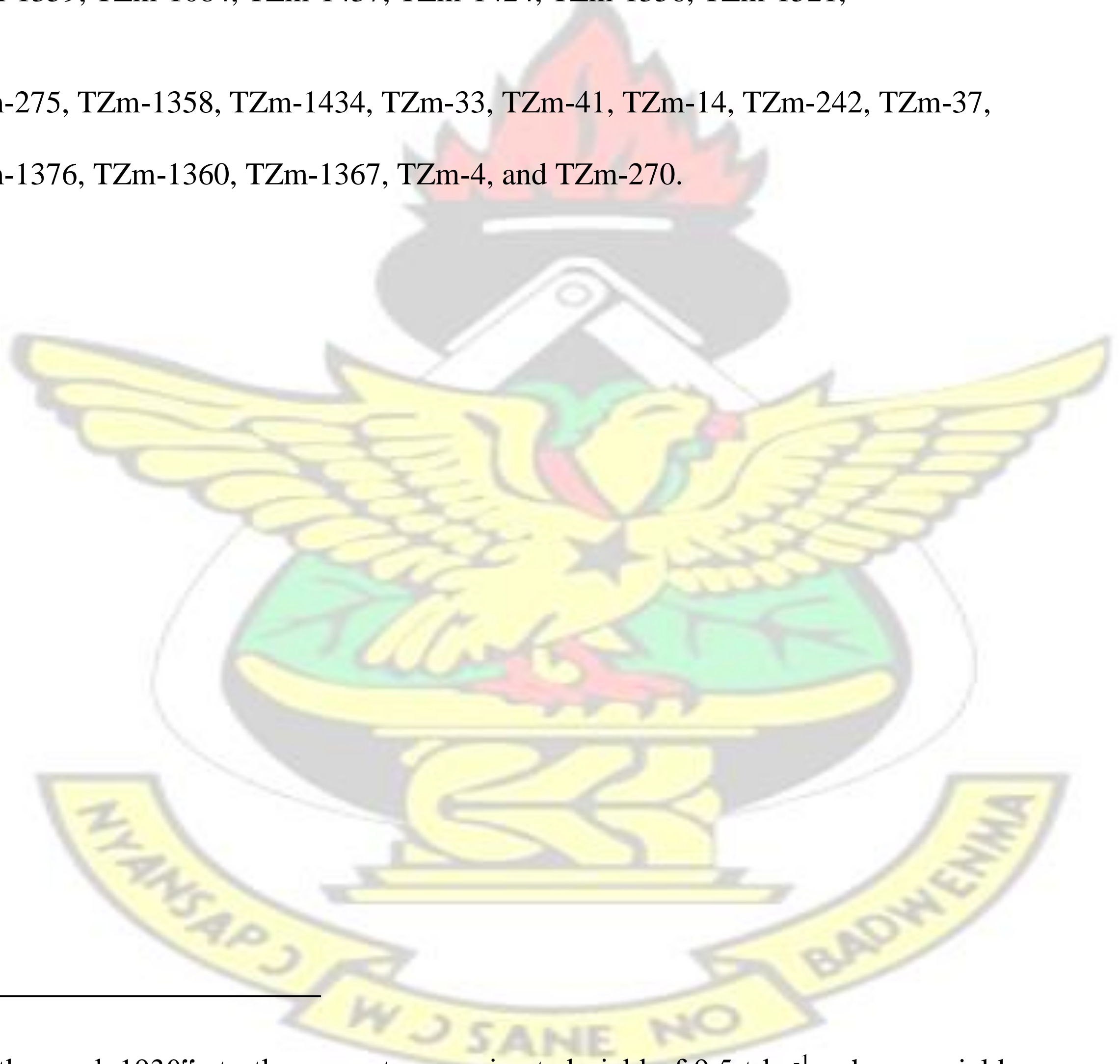
Accessions that emerged to be useful to breeding programs for earliness were the TZm-8, TZm-1521, TZm-13, TZm-33, TZm-37, TZm-5, TZm-275, TZm-1516, TZm-273, TZm-1514, TZm-14, TZm-270, TZm-4, and TZm-41 of mid-altitude
origin with the first five being the most early. In the lowland genotypes, accessions that were most early included „Obatanpa", TZm-2, TZm-1523, TZm-301, TZm-384, and TZm-385 with only TZm-1367 of highland origin. A unique combination of early-maturing and high-yielding trait was identified in TZm-4, TZm-41, TZm-270,
TZm-1521, TZm-275, TZm-14, TZm-33 TZm-37, TZm-1367, and TZm-1376. Majority of these were mid-altitude genotypes with only TZm-1367 and TZm-1376 from highland regions. Accession TZm-1376 in addition had extremely short ASI. These genotypes may be promising for breeding for drought tolerance through escape and avoidance.

Since its introduction into Africa from Mexico in the 16th century maize has adapted and established to the slightly hotter temperatures in various parts of Africa. Both morphological and SSR markers revealed large genetic diversity among the African maize collection, an indication of a wide assortment of alleles from ancestral genes plus those which have arisen or been lost through the forces of evolution, viz., migration (gene flow), intermating (recombination), and mutation.

Few studies on Ethiopian maize, southern African maize and Ghana maize have revealed the presence of large variability in the traditional populations (Oppong *et al*., 2014; Beyene *et al*. 2007; Magorokosho, 2006).

According Edgerton (2009) global Maize yield in 2009 averaged 4.9 t ha^{-1}, while yields in developing world still fall short at 3.1 t ha^{-1} (Pixley *et al*., 2009). Yields in the United States for instance have remarkably increased from an average of 1.6 t haTZm-1523, TZm-1359, TZm-1084, TZm-1437, TZm-1424, TZm-1356, TZm-1521,

TZm-275, TZm-1358, TZm-1434, TZm-33, TZm-41, TZm-14, TZm-242, TZm-37, TZm-1376, TZm-1360, TZm-1367, TZm-4, and TZm-270.



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[1] in the early1930"s to the current approximated yield of 9.5 t ha^{-1}, whereas yields currently obtainable in Ghana hover around 1.7 t ha^{-1} (MoFA, 2011; Edgerton, 2009). Genotypes having mean yield of 4.0 to 6.2 $Mgha^{-1}$which may be utilized into breeding for breeding for high grain yield included TZm-8, TZm-1516, TZm-1095,

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The primary goal of the research was to determine and compare genetic diversity of tropical maize accessions collected from three mega environments, namely lowland, mid-altitude and highland elevations belonging to twelve countries in Africa and held at the IITA Genetic Resource Center, Ibadan, Nigeria. Current breeding programs in Africa are confined to elite genotypes with neglect of the landraces, leading to genetic erosion. Though there have been plant introductions in the past from CIMMYT the current climate anomalies, population increase and biotic and abiotic stresses necessitate aggressive search for local genotypes which possess two fold properties, viz., useful genes and are highly adapted to the environment.

Being landraces and germplasm that have not been utilized in breeding programs it was a presumption that they would possess a large store of genetic diversity. However, no single study had evaluated maize germplasm collected from a large geographical area in Africa. The absence of a comprehensive database on morphological characteristics and molecular evaluation of African maize over a wide geographical region was necessary to quantify the genetic diversity and identify useful genotypes that could be beneficial to breeding programs in Africa and reveal the history of the African maize population.

It was therefore essential to carry out this work to determine weather the landraces are a rich source of genetic diversity,

To identify genotypes that have useful traits and unique alleles yet untapped

To narrow down the large number of accessions to only useful genotypes for incorporation into breeding programs

To determine if the extent of variation would reveal the historical basis of the diversity in the African maize germplasm.

Genetic diversity was determined by agro-morphological evaluation on five qualitative and twenty four quantitative traits. Although morphological evaluation provides information about variation in populations, they are influenced by environment, requires large plant population, have low heritability and low polymorphism hence, molecular profiling was also carried out to sharpen the estimation of genetic diversity. Simple Sequence Repeat unit (SSR loci) was chosen over other molecular marker methods because they detect polymorphism with high level of efficiency, are reliable, cost effective and the data easy to analyze and interpret.

Thirty-five accessions originating from the geographical area of longitude1.50, 38.30^0 E and latitude1.93 to 19.73^0 S, 6.77 to 10.0^0 N and an elevation of 100 m.a.s.l to 2100 m.a.s.l and a check, „Obatanpa GH" were used for the agro-morphological study while 57 accessions together with 5 checks constituted the molecular profiling.

In the morphological study, the large variabilities identified among the qualitative traits, especially kernel texture and color depicted a wide diversity. The relatively large proportion of dent kernels than flints supported the belief of major introduction of maize by the Portuguese through the West African route who principally introduced dent kernels and a minor introduction of flints through the Mediterranean route.

Regarding the quantitative traits, large variabilities were observed among the accessions as estimated by coefficient of variation and mean squares. The variability increased from highland to lowland accessions and was highest among the midaltitude accessions. The higher variability in the mid-altitude and lowland accessions was not surprising if the major entry of maize to the continent was through the West rather than

through the East as many of the lowland accessions originated from the Western African countries, such as Burkina Faso, Togo, Guinea, Benin, Chad as well as many of the mialtitude accessions coming from Equatorial Guinea. While this may seem to be true, it would not altogether be doubtful that the direction of variability from West and Central Africa then to the East provides a hint that probably, some maize genotypes may have been present in the West coast previously. Because all the highland accessions in current study were from a relatively narrower geographical area, particularly from the East, it was expected that variability among these accessions would be relatively lower compared to the much wider geographical areas spanning the Central and Western Africa where the lowland and mid-altitude accessions originated from.

All accessions were variable in all traits except anthesis-silking interval. The large variability in the traits may have originated from many generations of recombination, mutation, and gene flow. The diverse environmental conditions and adaptation in the three mega environments, such as soil properties, temperature, rainfall, and day length differences were expected to give rise to the wide variability in the germplasm and their attempt to adapt to the environments. Uniformity in ASI for all accessions regardless of origin is suggestive of genetic rather than environmental influence.

For the purpose of maize breeding in tropical Africa, the most important traits are earliness and grain yield. Three early-maturing accessions, TZm-8, TZm-2 and TZm-1521 were comparable to the check in anthesis-silking interval of 3.8 3.6, and 3.3 days respectively.

Accessions that had long ASI will be susceptible to drought. The study further identified a single early-maturing genotype TZm-1376, with strikingly short ASI of

2.3 days which also possessed high yield of 5.6 $Mgha^{-1}$ compared to the improved check with yield of 6.3 $Mgha^{-1}$. Indeed, this yield confirmed that short ASI promotes better fertilization and fruit set, hence better yield. The identification of earlymaturing yet high yielding accessions substantiates the fact that the landraces possess unique genes which have not yet been exploited. In modern maize breeding, there is usually a trade-off between earliness and yield. Current molecular biology tools seek to identify quantitative trait loci that link early-maturity to high yield, but with little success. The ten early-maturing and high-yielding accessions identified in current research including TZm-4, TZm-41, TZm-270, TZm-1521, TZm-275, TZm14, TZm-33 TZm-37, TZm-1367, and TZm-1376 would be valuable sources of genotypes to incorporate into breeding programs for improvement in earliness and grain yield. These genotypes may also be utilized for elucidation into the phenomenon of combined early-maturing and high grain yield.

As many as seventeen accessions (49 %) recorded grain yield above the average grain yield of 4.48 $Mgha^{-1}$ for the entire study. In terms of world average maize yield of 5.15 $Mgha^{-1}$, the important genotypes and their respective grain yields were TZm-14 (5.2 $Mgha^{-1}$), TZm-41 (5.2 $Mgha^{-1}$), TZm-242 (5.3 $Mgha^{-1}$), TZm-37 (5.4 $Mgha^{1}$), TZm-1360 (5.6 $Mgha^{-1}$), TZm-1376 (5.6 $Mgha^{-1}$), TZm-1367 (6 $Mgha^{-1}$), TZm-4 (6 $Mgha^{-1}$), and TZm-270 (6.2 $Mgha^{-1}$), all of which were mid-altitude and highland accessions.

The high heritability estimates for earliness traits (AD and SD) demonstrates faithful passing on of a these genes into their offsprings. The highest heritability in yield was found with the highland accessions. The Pearson correlation between plant

architectural traits and yield revealed that a better indicator of grain yield was the ear leaf width as this consistently produced high positive significant correlation with grain yield. Thus, in breeding practice, progress in development of high yield genotypes would benefit from selection on the basis of the ear leaf width.

On the whole, the morphological study gave average genetic distances measured as similarity coefficients of 0.29 for lowland, 0.23 for mid-altitude, and 0.38 for highland accessions. These values were coherent with the pattern of variability identified on the basis of the statistical measures of means, mean squares, and coefficient of variation that, variability was lowest in the highland followed by lowland, and highest in the mid-altitude accessions. Essentially, the African accessions were 23 %, 29 %, and 38 % similar in the mid-altitude, lowland and highland accessions with an average of 26 %. On the basis of morphological evaluation, the African accessions were only 26 % similar. This indicates that the genotypes are highly divergent there by providing an ample opportunity for improvement through selection.

Molecular profiling by SSR produced by the DICE dissimilarity coefficient was 0.69 for lowland accessions, 0.70 for mid-altitude, and 0.65 for highland accessions with a mean of 0.70. Dissimilarity ranges from 0 to 1.0, where values close to 1.0 demonstrates segregation among the groups. Similarity is equal to 1-dissimilarity. Reporting in similarity values, these values are equivalent to 0.30 for mid-altitude, 0.31 for lowland, and 0.35 for highland accessions, equivalent to 30 %, 31 % and 35 % similarity among the mid-altitude, lowland, and highland accessions, respectively. The high dissimilarity values of GD were congruent with the low similarity values of the morphological genetic distances. The implication here is that the morphological evaluations in current study were precise and accurate because the SSR profiling distance measures gave almost identical values with the morphological evaluations.

The high dissimilarity among genotypes of current study confirms that the African accessions are highly variable, and that this variability connoted the possibility of making progress in development of improved genotypes via selection. The consonance of morphological and molecular genetic distances indicates the existence of a very wide genetic divergence within the African maize landraces. This affirms that African landraces constitute a rich genetic resource which abounds with several useful alleles that can be incorporated into the maize breeding program in the face of the current challenge of climate change, food security issues and the dwindling arable land of the world. Genetic diversity estimation of maize is critical resource to locate and isolate useful alleles that will improve yield to fight global hunger and poverty particularly in sub-Saharan Africa.

The forces of evolution that drive increase in variation in populations include mutation, recombination, migration and gene flow. The high dissimilarity values show that there is segregation at many loci which may arise from historical hybridization among many divergent germplasm and so the historical fact that maize entry into Africa was by more than one route and at different times may be plausible or maize was already present on the continent when the Portuguese traders arrived. If this is true, then the question of maize probably already present in the African continent at the time of arrival of the Portuguese in West Coast of Africa would have to be explored further.

Heterozygosity is a parameter used to describe a population in terms of its history. It is synonymous to the Polymorphism Information Content. The high average expected heterozygosity of 0.64 obtained in this current study is an indication of the occurrence of a mixture of two or more populations over the years arising from either admixture or an isolate breaking effect. While Arab and Indian germplasm were transported from Mexico and are likely to be less heterogeneous, it is only the

admixture of divergent populations that can create a dissimilarity as high as that recorded in current study. One cannot also rule out the adaptations and transformations the maize crop has been exposed to that influenced the diversity in the population.

The UPGMA cluster analysis produced four and three groups each for the morphology and molecular analysis, respectively, confirmed by their principal components analysis. Clusters with desirable traits were identified constituting potential candidates for heterosis.

In conclusion, the study has (i) indicated that the diversity in African maize landraces is due to geographical locations, (ii) confirmed the existence of rich genetic diversity in the African maize germplasm, (iii) identified some unique traits which have not been reported, (iv) shown that all traits were variable except Anthesis
Silking Interval, (v) revealed a unique correlation between ear leaf width and grain yield (vi) the genetic diversity by agro-morphological evaluation gave a genetic similarity of 26 % (vii) the genetic diversity by SSR molecular profiling gave a genetic dissimilarity of 70 % indicated both morphological and molecular analysis confirm the existence of genetic diversity in African maize landraces.

The major limitations to this study were the few representations of the highland accessions in the morphological study and also, the use of few number of SSR loci in the molecular profiling.

5.2 Recommendations

- Any future research work should include more SSR primers proportional in number to the number of accessions studied
- Should use SNP markers

- An equivalent study which incorporate a larger and about equal number of accessions from each of the three mega environments would be necessary to confirm the genetic diversity estimates
- The use of polyacrylamide gel is also recommended since it gives a better resolution of SSR bands than agarose gel applied for current study.
- Appropriate precautions should be taken to ensure that equal numbers of accessions are used for both morphological and molecular analysis in order to ascertain the accuracy of the results obtained.
- The grain yield and ear leaf width association revealed by the Pearson correlation analysis must be subjected to regression analysis to ascertain any dependence of yield on ear leaf width.
- As a result of the high expected heterozygosity arising from this study, observed heterozygosity should be calculated in any future work and statistically compared to the expected in Fisher�someone's exact test to ascertain the fact that isolate breaking effect or admixture of two disparate populations has indeed occurred.
- In addition to agro-morphological evaluation, the compositional characteristics of the accessions must be carried to reveal variability in oil, protein and starch content for future breeding work
- The application of Mantel test to compare the performance of morphological genetic diversity estimates with that of molecular data is highly recommended.

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APPENDICES

APPENDIX A: PREPARATION OF REAGENTS

1. 2 % CTAB

Six hundred ml of 2% CTAB (Hexadecyltrimethyl-ammonium bromide) was prepared by transferring 0.5 g CTAB into an Erlenmeyer flask. To this was added

60 ml 1M TRIS (pH 7.5 at 65ºC), 84.0 ml of 5 M NaCl, and 60 ml 0.5 M EDTA (pH 8.0).
The volume was made up to 600 ml with 390.0 ml of deionized water. Just before use,
1.0 ml of 14 M β- mercaptoehanol was added.

2. Washing Buffer (500 ml)
Prepared by adding 380 ml of Absolute EtOH, 5 ml of 1 M NH_4OAc and 115 ml of dH_2O.

KNUST

3. TE buffer (1000 ml)
Mix in 200 ml beaker, 99 ml deionized or distilled water, 1 ml 1M Tris pH 8.0, 200 ul of 0.5 M EDTA. Tris Base (MW=121.10) 108.0 g, Boric acid (MW=61.83) 55.0 g, 0.5 M EDTA pH 8.0 40.0 ml.

4. 2 M NaCl
To make a 2 M aqueous solution of NaCl, dissolve 117 grams of NaCl in some distilled deionized water (the exact amount of water is unimportant; just add enough water to the flask so that the NaCl dissolves). Then add more water to the flask until it totals 1 liter.

5. 1% agarose gel.
1 g Agarose dissolved in 100 ml TBE

6. RNase (10 mg/ml)
Dissolve 100 mg of RNAse in 10 ml of 10 mM Tris - 7.5, 15 mM NaCl. Heat in boiling water for 15 min and allow to cool slowly to room temperature. Dispense into 1 ml aliquots and store at -20°C. Working stock may be stored at 4°C.

7. 1L of 5 M NaCl
Dissolve 292.5 g of sodium chloride (NaC1; M.W. 58.44) in 1L of deionized water.
Sterilize by autoclaving.

8. 0.5 M EDTA
Dissolve 186.12 g Na2EDTA•2H20 (MW=372.24) in approx. 750 ml of dH2O. Add NaOH pellets to bring pH to 8.0. After EDTA is in solution, bring to 1000 ml with dH2O. Autoclave.

9. washing buffers
Prepare 1X Washing Buffer by mixing the 1L 10X concentrate with 9 L of deionized water.

10. 1 L 70 % ethanol,
700 ml of absolute ethanol

11. 1 L 80% ethanol
800 ml of absolute ethanol

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12. 10X TBE Buffer, (Tris-Borate-EDTA) 108gm Tris base, 55gm Boric acid, 9.3gm Na4EDTA, Distilled H_2O to 1000ml, pH 8.3 (without adjustment)

13. Chloroform:isoamyl alcohol (24:1)
960 ml/L Chloroform

40 ml/L Isoamyl alcohol

14. 10 mM Tris-HC1 (pH 8.0)
    Dissolve 121.1 g of Tris base in 800 ml of H_2O.
    Add 42 ml concentrated HCl.

15. Ethidium bromide solution (10 mg/mL)
    Dissolve 100 mg of ethidium bromide in 10 ml sterile ddH2O. Wrap tube in aluminum foil and store at 4°C. Ethidium Bromide. Mutagen and cancersuspect agent. Wear rubber gloves when handling

16. 2% agarose gel
    Add 100 ml of x 10 buffer to 900 ml dH_2O to make x 10 TBE buffer.
Weigh 2 g of agarose gel and add 400 ml of the x 10 TBE and heat in a micro wave.

17. 250 µM dNTP,
    DNTP mix (2.5 mM each of dCTP, dGTP, dATP, and dTTP). Each set comes with 4 individual tubes containing dCTP, dGTP, dATP, and dTTP at 100 mM concentration. To mix, place 250 µl of each nucleotide in a 10 ml tube and add 9000 µl of sterile ddH2O to obtain a 2.5 mM concentration of each nucleotide.

18. 30 mM KCl,
    Dissolve 11.175 g of potassium chloride (KC1; M.W. 111.75) in 80 ml of H_2O and adjust volume to 100 ml with H_2O.
19. 100 mM Tris HCl {pH = 8} Add 300 µl of 1 M Tris-HCl pH 7.5, and 40 µl of 0.5 M EDTA-8.0 to 90 ml of ddH2O. Check pH by dropping a few µl onto a pH paper. If necessary, bring pH to 7.0. With HCl and make volume up to 100 ml.

20. 0.1 mg/mL proteinase K {added just before use}
    Dissolve 100 mg of proteinase K in ddH2O to a final volume of 10 ml. Dispense 200 µl aliquots into 0.5 ml tubes and store at -20°C.

21. 0.5% SDS.
    Dissolve 200 g lauryl dodecyl sulfate, sodium salt (MW=288.40) by adding it little by little to 800 ml dH20. After complete dissolution, adjust to final volume of 1000 ml.

A. Genetic Distances by Correlation based on agromorphological traits

B. Genetic Distance by Dice coefficient based on presence or absence of SSR alleles

APPENDIX B: MORPHOLOGICAL DISTANCE MATRIX

		Obatanpa	TZm-1084	TZm-1095	TZm-13	TZm-1356	TZm-1358	TZm-1359	TZm-1360	TZm-1367	TZm-1376	TZm-14	TZm-1413	TZm-1424	TZm-1434	TZm-1437	TZm-1514	TZm-1516	TZm-1521	TZm-1523	TZm-2	TZm-20	TZm-242	TZm-251	TZm-270	TZm-273	TZm-275	TZm-301	TZm-33	TZm-37	TZm-384	TZm-385	TZm-4	TZm-41	TZm-42	TZm-5	TZm-8
Obatanpa	lowland																																			
TZm-1084	lowland	-0.37																																		
TZm-1095	lowland	-0.21	0.31																																	
TZm-13	midaltitude	0.24	-0.26	-0.13																																
TZm-1356	midaltitude	-0.31	-0.15	0.10	-0.34																															
TZm-1358	midaltitude	-0.23	0.04	-0.28	0.00	0.03																														
TZm-1359	midaltitude	-0.62	0.61	0.47	-0.20	-0.10	-0.11																													
TZm-1360	highland	-0.01	0.06	-0.03	0.04	-0.37	-0.14	0.49																												
TZm-1367	highland	0.12	0.29	0.48	-0.49	0.16	-0.43	0.36	0.32																											
TZm-1376	highland	0.32	-0.09	0.27	-0.10	-0.23	-0.67	0.16	0.62	0.68																										
TZm-14	midaltitude	-0.20	0.06	0.27	-0.58	0.53	-0.18	0.17	-0.09	0.32	0.06																									
TZm-1413	lowland	-0.82	0.48	0.18	-0.32	0.26	0.36	0.55	0.05	-0.08	-0.27	0.31																								
TZm-1424	highland	0.21	0.09	0.38	0.02	-0.11	-0.34	-0.04	0.09	0.05	0.35	0.22	0.13																							
TZm-1434	lowland	-0.32	0.13	-0.37	-0.02	0.28	0.36	0.12	-0.15	-0.02	-0.45	-0.24	0.16	-0.77																						
TZm-1437	lowland	0.06	0.58	0.21	-0.48	-0.22	-0.20	0.22	-0.17	0.37	0.14	-0.01	0.14	0.09	0.00																					

TZm-1514	midaltitude	0.29	0.08	0.22	0.19	-0.21	-0.25	0.05	0.34	0.13	0.33	0.22	-0.26	0.42	-0.56	-0.27																					
TZm-1516	midaltitude	0.05	0.08	0.27	0.16	-0.14	-0.09	0.09	-0.26	0.11	-0.01	-0.40	-0.14	-0.27	0.13	0.44	-0.40																				
TZm-1521	midaltitude	0.79	-0.50	-0.39	0.19	-0.38	-0.16	-0.52	0.12	0.06	0.32	-0.40	-0.77	-0.08	-0.14	-0.01	0.02	0.29																			
TZm-1523	lowland	0.53	-0.50	-0.30	0.48	-0.24	-0.15	-0.56	-0.29	-0.18	-0.01	-0.54	-0.64	-0.27	0.22	-0.02	-0.40	0.44	0.64																		
TZm-2	lowland	0.07	-0.18	-0.65	-0.32	0.12	0.17	-0.25	-0.11	0.07	-0.17	-0.01	-0.25	-0.76	0.60	0.02	-0.33	0.03	0.37	0.28																	
TZm-20	highland	-0.43	-0.25	-0.34	0.09	0.02	0.18	-0.10	-0.19	-0.76	-0.52	-0.18	0.18	-0.19	0.13	-0.25	-0.28	-0.12	-0.21	0.03	0.08																
TZm-242	midaltitude	-0.04	0.03	0.67	0.11	-0.02	-0.41	0.41	0.51	0.50	0.62	0.08	-0.01	0.36	-0.30	-0.15	0.50	-0.04	-0.14	-0.24	-0.54	-0.32															
TZm-251	midaltitude	-0.46	0.23	0.34	-0.09	0.32	0.35	0.29	-0.49	-0.13	-0.53	0.01	0.55	-0.05	0.35	0.24	-0.56	0.44	-0.41	-0.06	-0.20	0.08	-0.16														
TZm-270	midaltitude	0.50	0.18	-0.09	0.19	-0.21	-0.19	-0.30	0.19	-0.03	0.25	-0.08	-0.20	0.65	-0.51	0.07	0.56	-0.38	0.14	-0.14	-0.42	-0.21	0.15	-0.49													
TZm-273	midaltitude	0.38	-0.37	-0.08	0.25	-0.38	-0.10	-0.58	-0.29	-0.41	0.10	-0.29	-0.32	0.40	-0.59	0.02	0.07	0.02	0.30	0.31	-0.24	0.18	-0.22	-0.22	0.29												
TZm-275	midaltitude	0.22	-0.53	-0.22	0.29	-0.21	-0.18	-0.25	0.07	-0.49	-0.08	0.03	-0.41	0.07	-0.32	-0.47	0.22	-0.42	0.14	0.19	-0.09	0.51	-0.07	-0.42	0.09	0.34											
TZm-301	lowland	-0.46	0.43	0.16	-0.39	-0.07	-0.03	0.48	0.21	0.49	0.25	-0.16	0.30	-0.45	0.39	0.45	-0.36	0.41	-0.14	-0.09	0.33	-0.12	0.12	0.11	-0.44	-0.33	-0.62										
TZm-33	midaltitude	0.15	-0.25	0.10	-0.27	-0.06	0.01	0.01	0.01	-0.13	-0.08	0.48	-0.16	0.15	-0.50	-0.11	0.28	-0.24	0.08	-0.27	-0.06	0.14	-0.12	-0.13	-0.07	0.16	0.60	-0.47									
TZm-37	midaltitude	0.38	-0.17	-0.10	-0.10	0.08	-0.48	-0.23	0.36	0.52	0.64	0.07	-0.51	-0.03	-0.09	-0.12	0.40	-0.35	0.27	0.07	0.24	-0.32	0.36	-0.80	0.36	-0.03	0.09	0.12	-0.17								
TZm-384	lowland	-0.06	-0.07	-0.22	0.56	-0.23	0.70	-0.23	-0.36	-0.72	-0.73	-0.41	0.08	-0.21	0.23	-0.29	-0.24	0.10	-0.09	0.29	-0.10	0.31	-0.43	0.36	-0.14	0.20	0.18	-0.32	-0.03	-0.61							
TZm-385	lowland	0.35	-0.53	-0.39	0.30	0.01	0.19	-0.76	-0.60	-0.52	-0.39	-0.37	-0.39	-0.05	0.03	-0.21	-0.39	0.23	0.51	0.68	0.21	0.29	-0.54	0.15	-0.08	0.52	0.20	-0.37	-0.03	-0.26	0.47						
TZm-4	midaltitude	0.31	-0.61	-0.56	-0.13	0.23	0.07	-0.60	-0.09	-0.04	0.01	0.01	-0.32	-0.36	0.32	-0.17	-0.44	-0.20	0.37	0.53	0.56	0.14	-0.33	-0.29	-0.13	0.05	0.25	-0.07	-0.07	0.38	-0.02	0.36					
TZm-41	midaltitude	0.22	-0.14	-0.04	0.48	0.15	-0.21	-0.21	-0.32	0.00	0.00	-0.41	-0.39	-0.27	0.20	-0.01	-0.15	0.59	0.27	0.47	0.09	-0.21	-0.13	0.15	-0.10	0.13	-0.22	0.04	-0.38	0.05	0.09	0.32	-0.07				

TZm-42	midaltitude	-0.64	-0.02	-0.05	-0.11	0.07	0.03	0.17	0.15	-0.38	-0.11	0.20	0.41	0.05	-0.20	-0.34	0.11	-0.47	-0.52	-0.47	-0.12	0.67	0.02	-0.21	-0.07	0.17	0.39	0.06	0.19	0.01	-0.02	-0.20	-0.09	-0.42			
TZm-5	midaltitude	-0.43	0.18	-0.61	-0.24	0.12	0.51	0.07	-0.16	-0.35	-0.60	-0.05	0.37	-0.62	0.71	0.07	-0.37	-0.13	-0.24	-0.13	0.65	0.45	-0.58	0.20	-0.35	-0.32	-0.14	0.27	-0.15	-0.25	0.23	-0.01	0.20	-0.15	0.21		
TZm-8	midaltitude	0.65	-0.32	-0.44	0.27	-0.22	-0.06	-0.47	-0.10	-0.11	0.03	0.09	-0.56	-0.03	-0.17	-0.21	0.35	-0.08	0.60	0.32	0.35	-0.32	-0.34	-0.39	0.17	0.23	0.21	-0.45	0.27	0.14	0.10	0.35	0.15	0.23	-0.37	-0.05	

APPENDIX C: MOLECULAR DISTANCE MATRIX

		TZm-2	TZm-5	TZm-7	TZm-11	TZm-14	TZm-20	TZm-23	TZm-4	TZm-6	TZm-8	TZm-13	TZm-19	TZm-22	TZm-30	TZm-32	TZm-37	TZm-41	TZm-57	TZm-33	TZm-38
TZm-2	lowland																				
TZm-5	Midaltitude	0.86																			
TZm-7	Midaltitude	0.81	0.75																		
TZm-11	Midaltitude	0.88	0.86	0.76																	
TZm-14	Midaltitude	0.61	0.57	0.65	0.69																
TZm-20	Highland	0.71	0.71	0.71	0.76	0.71															
TZm-23	Midaltitude	0.63	0.63	0.67	0.58	0.54	0.75														
TZm-4	Midaltitude	0.85	0.95	0.9	0.8	0.6	0.75	0.75													
TZm-6	Midaltitude	0.72	0.8	0.54	0.64	0.5	0.61	0.62	0.94												
TZm-8	Midaltitude	0.8	0.67	0.6	0.6	0.44	0.48	0.52	0.65	0.64											
TZm-13	Midaltitude	0.58	0.76	0.59	0.59	0.58	0.55	0.69	0.92	0.63	0.57										
TZm-19	Highland	0.56	0.63	0.54	0.61	0.57	0.54	0.62	0.65	0.58	0.55	0.96									
TZm-22	Midaltitude	0.6	0.56	0.57	0.5	0.6	0.5	0.57	0.71	0.53	0.67	0.88	0.75								
TZm-30	Highland	0.7	0.8	0.65	0.79	0.57	0.65	0.63	0.83	0.68	0.48	0.78	0.82	0.64							
TZm-32	Midaltitude	0.88	0.78	0.89	0.83	0.76	0.74	0.75	0.92	0.63	0.57	0.78	0.77	0.69	0.78						
TZm-37	Midaltitude	0.64	0.59	0.68	0.73	0.68	0.91	0.89	0.79	0.63	0.44	0.76	0.78	0.63	0.8	0.86					
TZm-41	Midaltitude	0.75	0.71	0.87	0.8	0.6	0.87	0.63	0.88	0.5	0.41	0.75	0.7	0.65	0.75	0.94	1				
TZm-57	Midaltitude	0.67	0.76	0.74	0.7	0.67	0.81	0.75	0.94	0.62	0.52	0.96	0.79	0.73	0.83	0.85	0.92	0.95			
TZm-33	Midaltitude	0.86	0.81	0.88	0.83	0.54	0.75	0.71	1	0.59	0.69	0.78	0.72	0.62	0.85	0.87	0.77	0.85	0.86		
TZm-38	Highland	0.64	0.54	0.5	0.64	0.74	0.68	0.67	0.45	0.46	0.58	0.44	0.67	0.57	0.53	0.53	0.67	0.61	0.5	0.58	
TZm-42	Midaltitude	0.7	0.69	0.73	0.77	0.44	0.63	0.75	0.81	0.53	0.62	0.69	0.75	0.62	0.78	0.87	0.81	0.78	0.74	0.88	0.53
TZm-63	Midaltitude	0.7	0.62	0.63	0.67	0.63	0.67	0.55	0.75	0.56	0.48	0.77	0.84	0.69	0.78	0.7	0.77	0.78	0.8	0.75	0.67
TZm-77	Highland	0.75	0.73	0.81	0.67	0.63	0.78	0.71	0.92	0.62	0.54	0.74	0.59	0.73	0.67	0.8	0.69	0.65	0.69	0.66	0.57
TZm-91	Midaltitude	0.68	0.7	0.61	0.58	0.54	0.74	0.67	0.71	0.61	0.5	0.63	0.58	0.67	0.71	0.5	0.67	0.65	0.78	0.69	0.63
TZm-242	Midaltitude	0.74	0.77	0.68	0.66	0.52	0.74	0.79	0.8	0.77	0.54	0.52	0.55	0.53	0.77	0.63	0.76	0.72	0.7	0.67	0.56
TZm-78	Midaltitude	0.82	0.69	0.87	0.65	0.6	0.74	0.71	0.88	0.64	0.6	0.7	0.57	0.63	0.68	0.81	0.7	0.7	0.69	0.72	0.5
TZm-156	Lowland	0.71	0.6	0.71	0.61	0.43	0.71	0.67	0.71	0.67	0.7	0.48	0.58	0.5	0.54	0.75	0.63	0.61	0.56	0.72	0.5
TZm-243	Midaltitude	0.68	0.6	0.79	0.68	0.83	0.75	0.71	0.8	0.67	0.55	0.63	0.54	0.56	0.57	0.69	0.6	0.6	0.66	0.55	0.59
TZm-251	Midaltitude	0.72	0.66	0.88	0.76	0.58	0.6	0.61	0.79	0.53	0.57	0.71	0.63	0.59	0.76	0.83	0.56	0.64	0.62	0.81	0.5
TZm-273	Midaltitude	0.8	0.58	0.7	0.72	0.63	0.75	0.77	0.89	0.68	0.68	0.54	0.63	0.5	0.64	0.7	0.73	0.71	0.63	0.71	0.74
Tzi-8		0.75	0.63	0.75	0.68	0.59	0.57	0.67	0.82	0.6	0.63	0.67	0.69	0.56	0.79	0.81	0.67	0.82	0.69	0.83	0.59
TZm-270	Midaltitude	0.71	0.66	0.79	0.68	0.44	0.61	0.57	0.82	0.57	0.67	0.81	0.72	0.78	0.75	0.85	0.7	1	0.75	0.86	0.59
TZm-275	Midaltitude	0.71	0.67	0.76	0.68	0.65	0.82	0.79	0.85	0.58	0.55	0.6	0.61	0.58	0.74	0.78	0.87	0.78	0.77	0.88	0.7
TZm-301	Lowland	0.61	0.57	0.68	0.71	0.63	0.71	0.75	0.7	0.58	0.58	0.63	0.74	0.63	0.79	0.65	0.73	0.65	0.69	0.79	0.73
TZm-384	Lowland	0.68	0.74	0.68	0.76	0.59	0.64	0.33	0.64	0.68	0.63	0.57	0.59	0.47	0.55	0.78	0.44	0.56	0.56	0.64	0.4
TZm-1084	Lowland	0.59	0.53	0.68	0.64	0.68	0.68	0.47	0.6	0.58	0.42	0.59	0.68	0.38	0.63	0.63	0.75	0.73	0.76	0.67	0.44
Tzi-9		0.53	0.53	0.4	0.5	0.33	0.6	0.38	0.13	0.47	0.65	0.44	0.71	0.43	0.59	0.54	0.59	0.55	0.48	0.59	0.71
TZm-1355	Midaltitude	0.73	0.67	0.82	0.78	0.84	0.73	0.6	0.91	0.54	0.46	0.74	0.67	0.62	0.71	0.75	0.71	0.75	0.73	0.79	0.68
TZm-385	Lowland	0.87	0.94	0.73	0.85	0.58	0.8	0.38	0.88	0.8	0.65	0.63	0.6	0.5	0.71	0.77	0.64	0.71	0.65	0.71	0.61
TZm-1095	Lowland	0.67	0.62	0.67	0.67	0.74	0.7	0.55	0.75	0.56	0.55	0.73	0.66	0.62	0.56	0.83	0.69	0.78	0.74	0.69	0.6
CML-157		0.39	0.61	0.48	0.41	0.43	0.57	0.71	0.8	0.62	0.43	0.89	0.77	0.81	0.68	0.56	0.65	0.64	0.84	0.59	0.47
TZm-1357	Highland	0.43	0.63	0.36	0.53	0.43	0.71	0.7	0.83	0.68	0.5	0.83	0.77	0.68	0.64	0.72	0.77	0.93	0.83	0.61	0.63
TZm-1359	Midaltitude	0.45	0.54	0.52	0.48	0.55	0.68	0.73	0.73	0.59	0.54	0.81	0.81	0.74	0.59	0.72	0.75	0.86	0.83	0.65	0.67
TZm-1360	Highland	0.67	0.78	0.71	0.75	0.57	0.67	0.67	0.79	0.62	0.65	0.78	0.77	0.69	0.75	0.75	0.58	0.69	0.68	0.8	0.57
TZm-1356	Midaltitude	0.48	0.56	0.64	0.52	0.64	0.68	0.78	0.79	0.59	0.48	0.79	0.59	0.67	0.56	0.71	0.63	0.71	0.69	0.62	0.56
TZm-1358	Midaltitude	0.61	0.7	0.68	0.65	0.61	0.74	0.71	0.82	0.6	0.59	0.7	0.67	0.7	0.64	0.75	0.7	0.82	0.76	0.76	0.75
TZm-1367	Highland	0.78	0.64	0.71	0.67	0.75	0.62	0.6	0.8	0.52	0.75	0.71	0.76	0.8	0.62	0.83	0.65	0.86	0.68	0.77	0.68
TZm-1369	Highland	0.58	0.57	0.59	0.52	0.62	0.63	0.76	0.62	0.48	0.58	0.69	0.65	0.71	0.63	0.56	0.62	0.45	0.63	0.63	0.52
TZm-1380	Midaltitude	0.62	0.71	0.63	0.68	0.46	0.58	0.59	0.65	0.59	0.79	0.67	0.63	0.67	0.53	0.67	0.5	0.67	0.65	0.7	0.67
TZm-1413	Lowland	0.6	0.67	0.8	0.67	0.73	0.6	0.73	0.73	0.53	0.6	0.91	0.73	0.67	0.73	0.91	0.67	0.6	0.67	0.73	0.62
TZm-1376	Highland	0.68	0.76	0.63	0.68	0.52	0.74	0.53	0.73	0.74	0.63	0.73	0.52	0.67	0.47	0.59	0.5	0.67	0.61	0.53	0.54
Obatanpa	lowland	0.53	0.63	0.58	0.53	0.37	0.68	0.67	0.6	0.53	0.63	0.8	0.79	0.69	0.68	0.67	0.75	0.8	0.72	0.8	0.69
TZm-1424	Highland	0.59	0.65	0.76	0.5	0.53	0.71	0.85	0.69	0.59	0.71	0.63	0.53	0.5	0.5	0.63	0.59	0.47	0.6	0.65	0.56
TZm-1430	Lowland	0.71	0.67	0.67	0.74	0.63	0.75	0.55	0.75	0.58	0.54	0.8	0.67	0.67	0.68	0.73	0.71	0.87	0.81	0.75	0.64
TZm-1437	Lowland	0.5	0.41	0.6	0.45	0.53	0.55	0.77	0.67	0.55	0.55	0.53	0.5	0.53	0.3	0.44	0.47	0.44	0.42	0.44	0.71
TZm-1514	Midaltitude	0.55	0.55	0.5	0.5	0.45	0.55	0.5	0.67	0.65	0.65	0.74	0.8	0.55	0.52	0.58	0.6	0.67	0.74	0.68	0.64
TZm-1434	Lowland	0.62	0.71	0.67	0.56	0.43	0.57	0.59	0.76	0.71	0.62	0.73	0.9	0.52	0.74	0.73	0.67	0.75	0.8	0.8	0.47
TZm-1502	Lowland	0.59	0.65	0.75	0.53	0.6	0.65	0.62	0.77	0.6	0.59	0.64	0.6	0.45	0.56	0.79	0.59	0.53	0.7	0.74	0.33
TZm-1516	Midaltitude	0.75	0.83	0.83	0.83	0.58	0.75	0.75	1	0.67	0.5	1	1	0.75	1	1	1	1	1	1	0.7
CML-258		0.2	0.4	0.4	0.4	0	0.4	0.4	0.4	1	0	0.4	1	0	0.4	0.4	0.4	0.4	0.4	0.4	0
TZm-1521	Midaltitude	0.77	0.85	0.85	0.75	0.46	0.69	0.69	0.85	0.85	0.77	0.73	0.69	0.54	0.73	0.73	0.69	0.63	0.81	0.81	0.45
TZm-1523	Lowland	0.45	0.55	0.55	0.55	0.36	0.36	0.45	0.64	0.82	0.55	0.64	0.73	0.38	0.64	0.64	0.5	0.45	0.57	0.64	0.33
		0.663115	0.669167	0.678305	0.650172	0.565789	0.668571	0.646909	0.757963	0.619623	0.571154	0.707059	0.6962	0.602245	0.663542	0.719787	0.675217	0.699778	0.692955	0.704419	0.572381

		TZm-42	TZm-63	TZm-77	TZm-91	TZm-242	TZm-78	TZm-156	TZm-243	TZm-251	TZm-273	Tzi-8	TZm-270	TZm-275	TZm-301	TZm-384	TZm-1084	Tzi-9	TZm-1355	TZm-385	TZm-1095
TZm-63	Midaltitude	0.71																			
TZm-77	Highland	0.6	0.63																		
TZm-91	Midaltitude	0.57	0.71	0.74																	
TZm-242	Midaltitude	0.61	0.7	0.73	0.79																
TZm-78	Midaltitude	0.6	0.69	0.89	0.62	0.78															
TZm-156	Lowland	0.71	0.57	0.66	0.51	0.65	0.74														
TZm-243	Midaltitude	0.47	0.66	0.69	0.58	0.73	0.74	0.55													
TZm-251	Midaltitude	0.72	0.69	0.73	0.53	0.57	0.84	0.73	0.69												
TZm-273	Midaltitude	0.67	0.79	0.48	0.52	0.72	0.74	0.76	0.81	0.73											
Tzi-8		0.69	0.72	0.52	0.48	0.67	0.66	0.58	0.77	0.69	0.77										
TZm-270	Midaltitude	0.81	0.78	0.72	0.61	0.64	0.77	0.7	0.57	0.8	0.7	0.74									
TZm-275	Midaltitude	0.74	0.71	0.6	0.64	0.65	0.67	0.72	0.61	0.7	0.79	0.69	0.75								
TZm-301	Lowland	0.71	0.77	0.53	0.64	0.61	0.58	0.67	0.66	0.66	0.73	0.66	0.66	0.82							
TZm-384	Lowland	0.61	0.57	0.68	0.48	0.5	0.6	0.76	0.64	0.79	0.58	0.5	0.64	0.57	0.48						
TZm-1084	Lowland	0.52	0.81	0.5	0.45	0.48	0.64	0.64	0.68	0.79	0.79	0.64	0.59	0.76	0.64	0.76					
Tzi-9		0.61	0.57	0.48	0.57	0.44	0.52	0.7	0.35	0.71	0.71	0.45	0.65	0.7	0.57	0.67	0.71				
TZm-1355	Midaltitude	0.63	0.83	0.7	0.67	0.6	0.78	0.48	0.78	0.88	0.79	0.63	0.7	0.73	0.7	0.6	0.76	0.52			
TZm-385	Lowland	0.57	0.7	0.6	0.6	0.67	0.75	0.7	0.65	0.7	0.8	0.55	0.75	0.78	0.65	0.83	0.86	0.75	0.7		
TZm-1095	Lowland	0.61	0.79	0.63	0.54	0.48	0.69	0.63	0.72	0.69	0.71	0.66	0.72	0.71	0.66	0.75	0.9	0.57	0.77	0.78	
CML-157		0.64	0.82	0.68	0.77	0.76	0.62	0.54	0.57	0.61	0.53	0.48	0.74	0.54	0.65	0.46	0.5	0.45	0.67	0.45	0.64
TZm-1357	Highland	0.72	0.78	0.61	0.64	0.65	0.59	0.64	0.55	0.55	0.68	0.55	0.77	0.64	0.55	0.54	0.62	0.79	0.67	0.71	0.78
TZm-1359	Midaltitude	0.65	0.81	0.65	0.67	0.64	0.6	0.67	0.67	0.52	0.64	0.63	0.78	0.67	0.74	0.56	0.69	0.64	0.56	0.64	0.81
TZm-1360	Highland	0.71	0.71	0.71	0.63	0.67	0.75	0.63	0.69	0.77	0.62	0.66	0.79	0.63	0.76	0.67	0.42	0.56	0.75	0.85	0.68
TZm-1356	Midaltitude	0.55	0.59	0.69	0.58	0.54	0.67	0.48	0.77	0.67	0.6	0.61	0.67	0.64	0.58	0.53	0.63	0.55	0.79	0.59	0.72
TZm-1358	Midaltitude	0.69	0.69	0.62	0.7	0.65	0.58	0.61	0.67	0.6	0.72	0.61	0.79	0.83	0.76	0.64	0.58	0.71	0.71	0.88	0.69
TZm-1367	Highland	0.76	0.8	0.64	0.54	0.54	0.61	0.58	0.68	0.6	0.7	0.71	0.79	0.69	0.68	0.67	0.5	0.5	0.82	0.64	0.84
TZm-1369	Highland	0.57	0.57	0.75	0.72	0.56	0.71	0.56	0.52	0.68	0.46	0.41	0.53	0.6	0.59	0.52	0.5	0.65	0.7	0.5	0.51
TZm-1380	Midaltitude	0.74	0.57	0.45	0.46	0.56	0.5	0.71	0.67	0.71	0.81	0.67	0.86	0.59	0.71	0.67	0.53	0.75	0.6	0.73	0.61
TZm-1413	Lowland	0.67	0.61	0.67	0.4	0.56	0.87	0.47	0.83	0.92	0.83	0.73	0.73	0.67	0.72	0.5	0.57	0.53	0.83	0.67	0.67
TZm-1376	Highland	0.39	0.56	0.73	0.58	0.63	0.67	0.53	0.67	0.44	0.5	0.48	0.57	0.45	0.54	0.67	0.44	0.33	0.67	0.73	0.72
Obatanpa	lowland	0.72	0.67	0.47	0.58	0.68	0.63	0.53	0.55	0.5	0.69	0.74	0.84	0.64	0.68	0.33	0.39	0.6	0.56	0.6	0.5
TZm-1424	Highland	0.6	0.45	0.75	0.55	0.7	0.8	0.7	0.75	0.71	0.67	0.53	0.53	0.6	0.65	0.53	0.45	0.53	0.67	0.5	0.5
TZm-1430	Lowland	0.59	0.81	0.58	0.63	0.59	0.67	0.58	0.7	0.81	0.94	0.75	0.83	0.78	0.67	0.64	0.89	0.67	0.86	0.87	0.78
TZm-1437	Lowland	0.47	0.63	0.63	0.45	0.65	0.7	0.65	0.75	0.61	0.7	0.43	0.57	0.52	0.61	0.53	0.53	0.5	0.79	0.53	0.58
TZm-1514	Midaltitude	0.68	0.84	0.38	0.45	0.43	0.5	0.7	0.57	0.7	0.85	0.65	0.74	0.7	0.61	0.68	0.84	0.92	0.74	0.83	0.79
TZm-1434	Lowland	0.85	0.85	0.47	0.52	0.63	0.57	0.76	0.58	0.67	0.81	0.79	0.71	0.75	0.67	0.73	0.87	0.83	0.6	0.75	0.7
TZm-1502	Lowland	0.74	0.61	0.65	0.45	0.48	0.7	0.8	0.65	0.88	0.75	0.6	0.6	0.74	0.5	0.83	1	0.75	0.8	0.67	0.74
TZm-1516	Midaltitude	0.93	0.93	0.58	0.75	0.73	0.67	0.5	0.67	0.75	0.83	1	1	1	0.87	0.53	0.73	0.58	0.8	0.75	0.73
CML-258		0.6	0.6	0.4	0.2	0.4	0.4	1	0.4	1	1	0.4	0.4	0.4	0.4	1	1	0.8	0.4	1	0.6
TZm-1521	Midaltitude	0.88	0.75	0.77	0.69	0.69	0.69	0.85	0.69	0.77	0.75	0.69	0.69	0.81	0.69	0.91	0.82	0.63	0.73	0.75	0.75
TZm-1523	Lowland	0.71	0.64	0.45	0.36	0.43	0.36	0.91	0.57	0.88	0.75	0.64	0.64	0.71	0.64	0.86	0.79	0.64	0.57	0.64	0.64
		0.659024	0.6995	0.620769	0.567105	0.604324	0.655	0.657714	0.65	0.711818	0.725	0.621935	0.701	0.678276	0.641786	0.652222	0.673846	0.63	0.698333	0.698261	0.680909

		CML-157	TZm-1357	TZm-1359	TZm-1360	TZm-1356	TZm-1358	TZm-1367	TZm-1369	TZm-1380	TZm-1413	TZm-1376	Obatanpa	TZm-1424	TZm-1430	TZm-1437	TZm-1514	TZm-1434	TZm-1502	TZm-1516	CML-258	TZm-1521
TZm-1357	Highland	0.84																				
TZm-1359	Midaltitude	0.81	0.89																			
TZm-1360	Highland	0.73	0.67	0.81																		
TZm-1356	Midaltitude	0.7	0.77	0.74	0.72																	
TZm-1358	Midaltitude	0.69	0.79	0.8	0.76	0.8																
TZm-1367	Highland	0.69	0.92	0.8	0.77	0.7	0.77															
TZm-1369	Highland	0.64	0.56	0.54	0.71	0.69	0.53	0.59														
TZm-1380	Midaltitude	0.71	0.85	0.72	0.71	0.67	0.81	0.72	0.35													
TZm-1413	Lowland	0.67	0.67	0.5	1	0.92	0.75	0.89	0.6	0.8												
TZm-1376	Highland	0.8	0.69	0.7	0.92	0.75	0.69	0.73	0.47	0.68	0.61											
Obatanpa	lowland	0.9	0.85	1	1	0.75	0.88	0.77	0.4	0.79	0.72	0.73										
TZm-1424	Highland	0.7	0.56	0.55	0.69	0.71	0.57	0.55	0.76	0.76	0.87	0.67	0.67									
TZm-1430	Lowland	0.71	0.85	0.73	0.59	0.71	0.76	0.79	0.58	0.8	0.67	0.61	0.61	0.6								
TZm-1437	Lowland	0.71	0.65	0.65	0.69	0.75	0.65	0.69	0.63	0.75	0.75	0.75	0.63	1	0.63							
TZm-1514	Midaltitude	0.71	0.88	0.86	0.56	0.7	0.75	0.77	0.47	0.88	0.6	0.53	0.63	0.5	0.89	0.7						
TZm-1434	Lowland	0.71	0.85	0.87	0.65	0.52	0.67	0.72	0.5	0.67	0.53	0.42	0.63	0.53	0.7	0.5	0.89					
TZm-1502	Lowland	0.62	0.78	0.57	0.56	0.71	0.6	0.71	0.65	0.65	0.73	0.4	0.4	0.76	0.7	0.67	0.87	0.8				
TZm-1516	Midaltitude	0.83	0.89	1	0.88	0.75	1	1	0.47	0.75	0.73	0.53	0.8	0.5	0.87	0.42	0.73	0.8	0.6			
CML-258		1	1	1	0.4	0.4	0.4	0	0.2	1	0.4	0.4	0.4	0.6	0.6	1	1	1	1	0.4		
TZm-1521	Midaltitude	0.83	0.8	0.71	0.54	0.62	0.69	0.64	0.5	0.77	0.64	0.55	0.45	0.69	0.63	0.63	0.82	0.81	0.88	0.73	0.8	
TZm-1523	Lowland	1	0.8	1	0.38	0.5	0.63	0.56	0.36	0.73	0.5	0.43	0.36	0.55	0.64	0.63	0.91	0.91	0.91	0.64	1	0.91
		0.761905	0.786	0.765789	0.696111	0.685294	0.696875	0.675333	0.495714	0.771538	0.645833	0.547273	0.558	0.636667	0.7075	0.65	0.87	0.864	0.8475	0.59	0.9	0.91

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