KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

GENETIC DIVERSITY AMONG AFRICAN LOWLAND MAIZE ACCESSIONS ASSESSED BY MORPHOLOGICAL TRAITS AND SIMPLE SEQUENCE REPEAT (SSR) MARKERS

BY

KINGSLEY BAFFOE ADADE

NOVEMBR, 2016

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A THESIS SUBMITTED TO DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, KNUST, GHANA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF

MASTER OF PHILOSOPHY

IN

BIOTECHNOLOGY

BY

KINGSLEY BAFFOE ADADE

(BSc Agriculture)

NOVEMBER, 2016

DECLARATION

I declare that, I have wholly undertaken the study reported herein under the supervision of Dr. (Mrs.) Antonia Y. Tetteh and that except portions where references have been duly cited, this report is the outcome of my research and has neither in whole or part been submitted for any other degree at any other University.

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HEAD OF DEPARTMENT

DEDICATION

With the greatest of respect, this thesis is dedicated to my wife, Adwoa Aduako Owusu and children Jeffery Adom Baffoe-Adade and Antonia Aduako Baffoe-Adade.

ACKNOWLEDGEMENT

Bless the Lord oh my soul, great things He has done.

I express my sincere gratitude and appreciation to my supervisor, Dr. (Mrs.) Antonia Y. Tetteh for her guidance, advice and assistance during this research. Thank you for the confidence you had in me when I approached you as a graduate student and believing that I was capable of accomplishing this great task to this very completion. Gratitude and appreciation are hereby extended to the staff of the Department of Biochemistry and Biotechnology for helping me in diverse ways.

To my parents, Mr Kingsley Baffoe Adade and Madam Comfort Aduako Aboagye who enlivened me with great love to always aim high and trust God that my efforts will not be in vain, all I say is thank you and I am proud and eternally grateful for having you as my parents. I am greatly indebted to my brothers, Bismark Baffoe Adade, Darlington Twum Baffoe Adade and my mother-in-law, Madam Augustina Opoku for the constant love, prayers, encouragement and support they gave me during the difficult times of this research. My heart also goes out to my wonderful wife, Adwoa Aduako Owusu for her love, devotion and inspiration throughout these years of this research.

I am also grateful to a friend and a brother, Mr. Ebenezer Ofori of the Forestry Research Institute of Ghana, Kumasi for his assistance. To my fellow graduate students Nancy Coffie, Stephen Asare, Patrick Twumasi and Shadrack Obeng Asamoah, I say thank you and God bless you. Special appreciation goes to Dr. Francis K. Oppong of Ghana COCOBOD for his encouragement.

I also thank the IITA, Ibadan, Nigeria and Crop Research Institute of CSIR, Fumesua, Ghana, for providing the maize accessions used in the research. To the staff of the

Anwomaso KNUST Agricultural Experiment Station, especially Mr. Fetus Acheampong, I say God bless you all for your assistance. Final appreciation goes to the Kirkhouse team, Mr. Eric Brenya and Mr. Bernard Armooh for their support and assistance during the research.

Finally my thanks go to everyone who helped in diverse ways to make this study a success.

ABSTRACT

Information on genetic diversity among landraces can lead to identification of new alleles for maize improvement. Owing to growing concerns of climate change, reduced arable lands, population increase and increased maize usage in Africa, there is the need to explore our landraces which are believed to harbour rich reserves of alleles for crop improvement towards ensuring food security. The study sought to identify variability and estimate the genetic diversity in the African lowland maize population by means of agrophenomorphological and SSR markers. Sixty-four accessions originating from eight countries in Africa, spanning latitude 8.85° S in Tanzania to 12.9° N in Chad and longitude 39.3°E to 10.7°W at elevation range of 50 to 700 m.a.s.l. were evaluated for quantitative and qualitative traits. Forty-seven accessions evaluated on 31 traits constituted morphological study. The analysis of variance revealed significant differences ($P<0.001$) among accessions for all measured traits except cob colour, kernel thickness and ear number. The predominant qualitative traits of the accessions were pale yellow silk colour, regular kernel arrangement, mixed grain colours and flint grains borne on white cobs. Among the quantitative traits, the most variable quantitative trait was plant height and the least was ear position. With regards to earliness days to 50% anthesis ranged from 44 to 61 days and days to 50% silking from 50 days to 69 days, Anthesis-silking interval ranged from 3 days to 8 days. Grain yield varied from 2.1 to 6.3 $Mgha^{-1}$ with a mean of 4.0 $Mgha^{-1}$. Accessions which combined earliness of 47-50 days and high yield of 3.8-5.87 $Mgha^{-1}$ were TZm-1505, TZm-49, TZm-295, TZm-1427, TZm-1503, TZm386, TZm-343 and TZm-1522. The population was characterised by low to moderate broad-sense heritability of traits from 5% in kernel thickness to 44% in plant height. Earliness and grain yield exhibited low heritability

estimates of 16% and 27% respectively while a moderate heritability of 35% was revealed in anthesis-silking interval. Strong positive genetic correlation between grain yield and ear leaf width of 0.66 and ear leaf length of 0.57 were remarkable indicating correlated response to selection for grain yield. The genetic distance ranged from 0.00 to 0.88 with a mean of 0.28±0.19. Cluster analysis grouped the genotypes into three clusters. Grain yield in cluster I was controlled by the large values of ear characteristics whereas in cluster II, grain yield was driven by ear leaf length and ear leaf width. Cluster III was good for earliness. The first three PCA explained 79.10% of the total variance and the major contributors were plant height, ear leaf length, ear leaf width and grain yield.

Using SSR profiling, 64 accessions were evaluated across the 10 chromosomes of maize using sixteen primer sets. A total of 2,216 alleles ranging from 114 to 228 were detected across the genotypes with a mean of 170.46. Across the loci, number of alleles ranged from 2 to 10 with an average of 5.46. Observed heterozygosity ranged from 0.00 to 0.86 with a mean of 0.46 ± 0.30 while expected heterozygosity ranged from 0.26 to 0.78 with a mean of 0.65±0.14. The analysis revealed that on the basis of chi-square goodness-of-fit test, the observed heterozygosities and the expected heterozygosities were not significantly different.

Pair-wise genetic dissimilarity coefficient ranged from 0.30 to 1.00 with an average of 0.70±1.0.

A UPGMA clustering produced two main clusters irrespective of the place of origin. The unique accessions identified would be useful in maize improvement with regards to earliness, drought tolerance and grain yield. The higher heterozygosity and alleles

identified confirmed that the African landraces are a rich source of unique alleles yet untapped.

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CHAPTER ONE

INTRODUCTION

Maize (*Zea mays* L.) is a new world cereal belonging to the family Poaceae, which includes important staple crops such as wheat, rice, sorghum, barley, oats, and millet. Maize is believed to have originated from Central America, specifically Mexico (Gibson and Benson, 2002; McCann, 2001). Due to its adaptability and productivity maize spread rapidly around the globe along trade routes in the 15th and 16th centuries (Salvador, 1997) to other parts of the world including Africa.

Maize farming plays an important role in agriculture and the economies of many countries where it serves as a versatile raw material for many industrial products in the developed world, including corn starch and starch-based products. Maize is a raw material in fermentation and distilleries, a source of ethanol biofuel, and a major feed for livestock and poultry. However, in West and Central Africa, maize is a major staple food crop, making up more than 50% of the total caloric intake of local diets (Sinha, 2007) and 53% of the protein intake of local diets (Bressani, 1991).

Owing to the wide uses of maize and maize-based products, demand for maize is increasing around the world. Since 2009/2010 global maize consumption has gradually increased from about 800 to 944 million metric tons (mmt) in 2013/2014 and is projected to increase at a rate of about 2% per year such that consumption would reach 1,020 mmt by 2018/2019 (IGC, 2015). In contrast, production has increased from just about 800 to 948 mmt with a forecast of 1,016 mmt at a rate of less than 2% within the same period exhibiting an overall demand exceeding supply (IGC, 2013). The trend of increase in demand of maize arises from population growth, urbanization, and rising dietary preferences for consumption of animal protein, especially in developing

countries. Overall global rise in maize is attributed to use of maize as the major feed for livestock and poultry and as a key source of ethanol biofuel (IGC, 2013).

In sub-Saharan Africa, demand is expected to more than double from 27 mmt/year in 1995 to about 52 mmt/year by 2020 (Pingali and Pandey, 2000). The reduction in hunger by half and improvement in socioeconomic status of people living below the poverty line by the year 2015 through increase in agricultural productivity is one of the most important goals of the United Nations Millennium Development Goals (Sanchez and Swaminathan, 2005).

The area planted to maize in West and Central Africa has increased over the decades from 3.2 million hectares in 1961 to 8.9 million hectares in 2005, resulting in a corresponding increase in production from 2.4 to 10.6 mmt (IITA, 2009; FAOSTAT, 2006). Throughout this period, maize productivity in developed countries has been consistently higher than that of developing countries, the disparity attributed to use of landraces and old breeding materials, while developed countries use hybrids and improved maize varieties (Munsch, 2009).

Many countries in West and Central Africa have embarked on research focused on increasing the productivity of maize (CIMMYT, 1994). Until recently, average maize yield of 1.2 t/ha was recorded for sub-Saharan Africa, which is just below a quarter of the global average of 5.5 t/ha, and about a sixth of the average yield in U.S (7.8 t/ha, FAOSTAT, 2006). However, in recent years, advancement in maize productivity has been achieved through conventional breeding raising the productivity from 1.2 t/ha to over 2.0 t/ha, though some researchers report productivity as high as 3-5 t/ha. In 2005, six countries in Africa achieved double the amount consumed in the country, while eight other countries imported only 5-35%, and 11 countries also imported 57-100% of

the maize consumed in the country that year (FAOSTAT, 2007). Despite these advancements, maize productivity in West and Central Africa still remains below the world average. Development of high yielding maize cultivars is expected to contribute to reduction in hunger in this region.

Breeding for improved cultivars is dependent on allelic richness in the germplasm pool. Many alleles arise through forces of evolution, chartered by variations in climatic conditions, farmers‟ selection systems, mutation, migration, gene flow and recombination which contribute to diversity in any germplasm. The maize gene pool in West and Central Africa is reported to exhibit a wide diversity on the basis of isozyme polymorphism such that it is considered to be a secondary area of genetic diversification for maize (Brandolini, 1969).

Generally, estimation of genetic diversity provides a basis for devising future strategies for crop improvement, conservation and sustainable use. Cultivation of the same genotype over many generations leads to genetic erosion confining the subsequent breeding program (Cebolla-Cornejo *et al*., 2007). Genetic diversity is desirable for long-term crop improvement and reduction in vulnerability of plants to biotic and abiotic stress factors. The key to successful crop improvement is a continued supply of genetic variability and beneficial alleles which are normally derived from the landraces and wild relatives of modern cultivars. The landraces have historically been the source of novel alleles for improved nutritional quality, resistance to pests and diseases, as well as tolerance to drought and extreme temperatures (Dwivedi *et al*., 2008).

There have been few reports of detailed assessment of genetic diversity in the maize germplasm in sub-Saharan Africa (Legesse *et al*., 2007; Obeng-Antwi, 2007; Beyene *et al*., 2006; Magorokosho, 2006; Sanou *et al*., 1997), seriously limiting inbred line

development, assignment of lines into heterotic groups, identification of testers for inbred lines, and identification of genes for maize improvement. Conversely, genetic diversity estimates among maize germplasm in North America (Bretting *et al*., 1990; Smith, 1986; Goodman and Stuber, 1983; Kahler *et al*., 1986), in the International Maize and Wheat Improvement Centre, CIMMYT (Warburton *et al*., 2005; 2002) Europe (Hartings *et al*., 2008; Lucchin *et al*., 2003; Rebourg *et al*., 2001; Dubreuil *et al*., 1996; Messmer *et al*., 1993; 1992) and Japan (Enoki *et al*., 2002) have been evaluated.

Realizing the need for conservation of landraces and other wild relatives of maize in West Africa, the Plant Genetic Resources Research Institute of Ghana (PGRRI) organized a collection of about 400 landraces in 1991. A similar exercise in Burkina Faso led to a collection of 100 maize landraces by INERA (Institut d‟Etudes et de Recherches Agricole) from 1988 to 1994. In addition, the International Institute of Tropical Agriculture (IITA) has over 800 accessions of maize collected from over twenty-four countries in Africa. Assessing the genetic diversity in the collections should be useful for identifying sources of novel genes that can be incorporated into gene stocks of the national as well as the regional maize improvement programs.

To classify a collection as large as the African maize germplasm collection, very efficient marker protocols that directly evaluate genetic differences among accessions must be used (Melchinger, 1999). Important applications of morphological evaluation include the appropriate estimation of genetic relatedness among accessions (Smith and Smith, 1989), cultivar description (Smith and Smith, 1992), and the identification of quantitative trait loci (QTL) for important agronomic traits (Dudley, 1993). Revilla and Tracy (1995), Fountain and Hallauer (1996) and Obeng-Antwi (2007) employed morphological characterization to estimate genetic diversity in maize. Use of

morphological markers is time-consuming, labour-intensive and requires large populations of plants (Botha and Venter, 2000). Molecular markers are thus ideal for genetic diversity studies due to high heritability, accuracy and reproducibility contrary to morphological markers (Winter and Kahl, 1995).

Among the molecular markers, the microsatellite markers or simple sequence repeats (SSRs) offer greater reliability, reproducibility, discrimination, and are more costeffective and provide a high level of polymorphism in maize (Smith *et al*., 1997; Senior and Heun, 1993). The microsatellite markers since their development have been extensively used in various genetic diversity studies in maize. They have been used to establish the population structure and genetic diversity of elite maize germplasm in Europe and North America (Van Inghelandt *et al*., 2010). The genetic diversity within and among CIMMYT maize populations of tropical, sub-tropical and temperate germplasm was determined using SSR (Reif *et al*., 2004). Similarly, inbred lines in the U.S., India, Europe, Canada, South Africa, Thailand, Japan, CIMMYT and the International Institute of Tropical Agriculture (IITA) have been assessed using SSR (Ranatunga, 2009; Liu *et al*., 2003; Enoki *et al*., 2002; Gethi *et al*., 2002). Genetic diversity studies among African maize inbred lines present in the CIMMYT centres in Ethiopia and Zimbabwe have been determined using SSR markers (Legesse *et al.*, 2007). The genetic diversity and relationships among Ethiopian highland maize accessions were also determined using morphological and SSR markers (Beyene *et al*., 2006). Menkir *et al*. (2004) assessed the genetic relationships among tropical midaltitude inbred lines developed in Nigeria and Cameroun using amplification fragments length polymorphism (AFLP) and SSR markers.

Maize classifications based on elevation are grouped into three: highland, mid-altitude and lowland maize. Lowland maize varieties thrive best below 800 m.a.s.l. whereas

midaltitude and highland varieties perform best at 900 to 1,800 m.a.s.l. and >1800 m.a.s.l., respectively. There is little information on tropical African maize although they are reported to have a broad genetic base than the temperate maize (Betrán *et al*., 2003). It is estimated that, about 40 million hectares in the tropical lowland environments are under maize cultivation (Pingali, 2001).

Information on genetic diversity estimates of the collection would contribute to identification of useful genotypes for incorporation into breeding programs, for the organisation of the accessions into core subsets, and for management of maize collections in Genetic Resource Centers. In addition, genetic diversity estimates are employed in the assignment of inbred lines into heterotic groups, for identification of testers and is used to determine the requirement of maize introduction into a maize breeding program. Genetic diversity information also reveals the evolutionary history of populations. The history of maize in Africa has been a debatable subject among maize geneticists. The history of a crop population pre-determines the trend of improvement to undertake. In addition the history of a population defines its genetic stability and adaptability to different environments.

The main objective of the present research is to identify variability and estimate genetic diversity in the African lowland maize populations. The specific objectives are:

- To determine variability and estimate the genetic diversity among the maize populations by means of agro-morphological evaluation
- To determine genetic diversity by means of microsatellite profiling
- To identify genotypes with unique traits that can be incorporated into maize breeding programs

- To estimate the heterozygosity and its implications on the history of maize in Africa

Hypothesis

It is hypothesized that maize in Africa demonstrates variability generated from the forces of evolution including gene flow, mutation, genetic drift and natural selection and that the variability is maintained by these factors and several forms of natural selection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and history of maize

Maize belongs to the tribe *Maydeae* of the grass family Poaceae, tribe *Maydeae* comprising *Zea* and *Tripsacum*. The wild progenitor of cultivated maize is believed to be the teosintes. Evidence for the teosintes as the origin of cultivated maize was put forward by Doebley (2004), Matsuoka *et al.*, (2002a), Piperno and Flannery (2001), Watson and Dallwitz (1992) and Beadle (1932). Although maize and the teosintes are considered closest relatives in terms of their similar genomes and ease of crosshybridization, they exhibit extreme differences in their adult morphologies, such that taxonomists initially considered the teosintes to be more closely related to rice than to maize (Doebley, 2004). Both maize and teosintes have chromosome number of 2n=2x=20.

Teosinte is a common name for four perennial and annual species of the genus *Zea* (*luxurians, diploperennis, perennis and mays*), of which *Z. mays* ssp. *parviglumis*, is considered the direct ancestor of cultivated maize due to genetic similarities (Doebley,

2004; Matsuoka *et al.*, 2002b; Doebley, 1990a). The teosinte, *Z. mays* ssp. *parviglumis* is a small flowered species, native to southern and western Mexico which normally grows along streams and valleys (Doebley, 2004). Another species Z. *luxurians* is an annual teosinte from southeastern Guatemala (Watson and Dallwitz, 1992; Doebley, 1990b) having chromosomes that are cytologically distinct from those of maize (Beadle, 1932) considering that hybridization exhibits two or more unpaired chromosomes. Outside the genus *Zea*, the closest wild relative of maize is *Tripsacum* which includes gamma grass.

Evidence of teosinte as the progenitor of maize from cytological studies reveal all teosintes can be crossed to maize resulting in fertile hybrids except for the tetraploid Z. *perennis* (Wilkes 1967) and *Z. luxurians* whose hybrids are uncommon and exhibit partial sterility (Galinat 1983, Iltis 1983; Weatherwax 1935). *Zea mays* ssp. *mexicana* is known to have chromosomes that are cytologically similar to those of maize, and its hybrids with maize exhibit complete chromosomal pairing and full fertility. Doebley *et al*. (1984) showed that maize and *Z. mays ssp. parviglumis* are so similar in allozyme constitution that it would be difficult to apply allozymes to the study of introgression between them, however, the remaining teosinte taxa possessed
allozymes that are distinct from those of maize at several loci.

Within section *Zea*, subspecies *mexicana* and *parviglumis* are well differentiated for isozymes and ecologically with no overlap of their populations (Doebley *et al*., 1984) while subspecies *mays* and *parviglumis* show complete overlap in principal component analysis (Doebley, 1990a). Microsatellite studies reveal that the populations of *Z. mays* ssp. *parviglumis* in the central portion of its distribution
(where the states of Guerrero, Michoacan, and Mexico meet) are ancestral to maize (Matsuoka *et al*., 2002b). Molecular dating using microsatellite also indicates that

maize and *Z. mays* ssp. *parviglumis* diverged about 9,000 years ago, a date that agrees well with archaeological evidence (Piperno and Flannery, 2001). These molecular and archaeological evidences may support the school of thought that suggests Mexico as the centre of origin of maize.

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

Maize became the number one cereal crop in the world since 2001 (Asif *et al.*, 2006), when its global production, usage, and affordability 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 revealed that from 2007 to 2011 maize production increased from 789 to 883 mmt whereas that of rice and wheat increased from 656 to 722 mmt and 612 to 704 mmt, respectively. Returns from maize production over the same period amounted to about US $45 to $57 billion, while that of wheat and rice were $75 to $86 billion and $170 to $187 billion, respectively (FAOSTAT, 2012) making maize a relatively cheaper food crop. Table 2.1 shows global production statistics of maize, wheat and rice between 2007 and 2011.

Maize provides livelihood to over 70% of smallholder farmers in developing countries. Over 1.2 billion people in sub-Saharan Africa (SSA) depend on maize for both nourishment and income (Edmeades, 2013). Increase in population, dwindling arable lands, and the recent climate anomalies forebode shortage of maize in SSA where researchers allude to be most affected by the climate variability (Cairns *et al.*, 2013). It is therefore important to embark on interventions to improve maize productivity in SSA. One of the major interventions is to exploit the natural processes of evolution which enhance adaptation in natural populations and have the capacity to generate new alleles derived from the forces of evolution including mutation, recombination and gene

flow which enhance formation of new alleles on one hand, and genetic drift and natural selection which reduce variation in populations.

Table 2.1: Top cereal production statistics

Year	Maize		Wheat		Rice	
	Production (mt)	**Production value ($1000)**	**Production (mt)**	**Production value ($1000)**	**Production (mt)**	**Production value ($1000)**
2007	789,927,060	45,264,784	612,601,092	75,618,089	656,969,953	170,426,928
2008	829,104,646	49,686,167	683,153,270	82,004,246	688,527,157	178,745,135
2009	820,539,197	52,858,764	686,794,645	84,291,395	685,093,795	177,661,011
2010	850,445,143	54,617,721	653,355,358	81,554,403	701,127,975	181,912,414
2011	883,460,240	57,429,806	704,080,283	86,272,777	722,760,295	187,881,865
Total	**4,173,476,286**	**259,857,242**	**3,339,984,648**	**409,740,910**	**3,454,479,175**	**896,627,353**

2.3 Maize production and consumption in Africa

Of the top 25 maize producing countries, nine are in Africa, with a total production area of 17.4 million hectares which translates to 12.5% of the global area under maize cultivation (James, 2003). Prior to the 1980s maize productivity in Sub-Saharan Africa was below 1 t/ha. However, after the 1980s hybridization intervention, introduction of important traits including early maturity and disease resistance, as well as expansion in land area cultivated to maize led to a mean annual growth in productivity of 4.1%. In recent years, Africa‟s proportion of maize production is about 6.5% of the world‟s maize production and is led by South Africa which produces over 13 mmt/year followed by Nigeria with almost 8 mmt/year, then Egypt,

Ethiopia and Tanzania whose individual production is nearly 6 mmt/year (IGC, 2013).

By and large, the major maize producing countries in East Africa such as Kenya, Uganda and Mozambique each produce nearly 3 mmt/year, about double that of the key maize producers in West Africa including Ghana, Cameroon, Burkina Faso, Benin and Togo who produce not more than 1 mmt/year. Typically, many countries in Africa produce less than 1 mmt/year (USDA, 2014).

Africa imports about 28% of her required maize (IITA, 2009). The predominant maize kernels produced is white and dent of which about 95% is used for food, 4% for feed and limited quantity for manufacture of industrial products. The total area of maize production worldwide grew from 158 million hectares to 175 million hectares in 2007 to 2013 equivalent to 10.8% and is projected to reach 177 million hectares in 2018, an additional growth of 1.1%. In Africa, despite increase in maize production from 27 million hectares to 35 million hectares (29.6%) over the same period accounted for only 7% of the total world production area (FAOSTAT, 2015). In 2012 to 2014, maize production in Africa was about 70 mmt (6.9%) of the total world production of 1.017 billion mt.

For the period 1982 to 1993, Africa achieved a phenomenal growth in maize production by moving her production from 48 mmt/year to 65 mmt/year equivalent to 35% increase. In contrast, average total production of 70 mmt (FAOSTAT, 2015) in 2012 to 2014 was equivalent to 7.69% increase from the 1993 figure. Clearly, these statistics reveal that growth in maize production in Africa is dwindling. In the past, the large growth rate in maize was achieved through introduction of high-yielding, drought-tolerant, early and extra-early maturing varieties coupled with the combined activities of a collaborative network of scientists in the region (IITA, 2009).

The rise in maize production from 2012 to 2014 is forecast to be followed by a drop to 969 mmt in 2015/2016 and slowly rise to 993 mmt in 2016/2017. In the same period

global maize consumption rose from 785 mmt in 2008 to 940 mmt in 2014 while production increased from 800 to 1,017 mmt in the same period. Projections of consumption from 2014 to 2016/2017 are given as 971 mmt to 991 mmt (2.06%) compared to a growth in production of 2.47%, representing a marginal difference (IGC, 2014). The current trend in population growth climate anomalies, new found uses of maize for industrial products, and urbanization consumption of maize is likely to exceed production in the near future.

Maize consumption in sub-Saharan Africa is about 10% of total world production and since 2008, consumption has been in excess of production by over 10 mmt. In subSaharan Africa, consumption increased by 15% from 49,033 mmt to 57,167 mmt in 2008 to 2013. With the global consumption increase of 2.3% from 2013/2014 to 2017/2018, maize consumption in sub-Saharan Africa is projected to rise above 58,482 mmt. This increase in consumption requires a corresponding increase in production.

Africa spends close to US$2.0 billion annually on net imports of maize. The average annual value of maize imports rose from US$1.14 billion in 1995-97 to US$2.25 billion in 2005-07. In 2008, the total value of maize importation for Africa was reported to be US$3.1 billion. During the same period receipts from maize exports dropped from US$350 million to US$264 million (FARA, 2009).

Some countries in Africa such as South Africa, Ethiopia, Zimbabwe and Ghana had import values of US$27 million, US$14 million, US$16.2 million and US$21.5 million, respectively in 2011 (FAOSTAT, 2011). Much more money is expected to be spent on maize imports if these countries do not meet their deficit in terms of demand.

2.4 Introduction of maize to Africa

Maize is one of the crops believed to have been introduced to Africa from Mexico in the 16th century by the Portuguese but the time of introduction is still uncertain (Miracle, 1965). It is purported that the major entry point by the Portuguese was through the West Coast, while a minor introduction was through the Mediterranean route by the Arabs (Miracle, 1965; Portères, 1955). The only archaeological evidence of maize in West Africa dates back to pre-Columbian era of AD 1100 relating to a pottery found in Nigeria with an imprint resembling the ear of maize, which is contrary to the generally accepted introduction by the Portuguese (Miracle, 1965; Goodwin, 1953). Maize has since become a major staple food crop, making up more than 50% of the total caloric intake of local diets (Sinha, 2007; McCann, 2001).

In 1940 a Portuguese pilot described maize as a well-established crop in the Cape Verde islands. Dominique Juhé-Beaulaton a French scholar described the appearance of maize on West Africa‟s Gold Coast, specifically in Elmina beginning in the early 17th century.

On the basis of linguistics, historians suggest that the names given by local indigenes who interacted with the Portuguese traders reveal the history of maize introduction. For instance, as far back as in 1482, the Akans in Elmina, the permanent Portuguese settlement in Ghana called maize „*aburro*‟ purported to have been derived from the Portuguese words *milho zaburro*. The same word is used in Mozambique (McCann, 2001). In Malawi, speakers of Chichewa called it *chimanga* which literally means „from the coast‟ indicating a similar perception of its origin. *On the* Eastern African coast, the Kiswahili word for maize is *muhindi* which means the grain of India.

In the mid-sixteenth century, the local Kikongo around the Congo River and the Senegambia natives called maize *maza mamputo* and *tuba-nyo* which translates grain

of the white man (McCann, 2005). These natives gave an indication that maize was more likely to have been introduced by the slave traders than it being present in the pre-Columbian era, a belief which needs to be validated. Linguistic evidence suggests that the penetration of maize into interior Africa was from the coastal areas but the mode of introduction cannot be fully established (Miracle, 1965). Some evidence indicates that maize reached the northern Congo Basin after 1830 and in Uganda, as late as 1861 after its massive usage as food during the slave trade.

Maize, after its introduction has since undergone massive recombination, extensive selection by farmers and survival in extremes of environmental conditions such as severe drought in Africa.

The genetic diversity in maize in West Africa may therefore arise from admixture of different genotypes through different routes, exposure to a diversity of ecological niches and climatic stress factors combined with selection over many generations leading to differentiation of many landraces and cultivars across the region (Sanou *et al*., 1997). These landraces are distinguishable by various features such as their earliness, ear and kernel characteristics (Le Conte, 1976; Marchand, 1976; Robledo, 1976; Sarr, 1975), hence West Africa is considered as a secondary centre of genetic diversification for maize (Brandolini, 1969).

2.5 Maize research in Ghana

Maize cultivation in West Africa is largely done with landraces. Research into maize breeding in Ghana started in the mid-1950s (Agble, 1981) with the release of two improved early-maturing yellow varieties, „Nyankariwana I" and „Nyankariwana II" between 1954 and 1961 by J. McEwen (Sallah *et al.,* 1998). In 1972, M. K. Akposoe of Crops Research Institute of the Council for Scientific and Industrial Research

(CSIR), developed „Composite 4" and „La Posta CRI", both late-maturing varieties adapted to the Guinea Savanna zone, (Sallah *et al*., 1998; Agble, 1981). The period between 1983 and 1998 saw increase in breeding activities initiated by the Ghana Grains Development Project (GGDP), the Crops Research Institute (CRI) and the Savanna Agricultural Research Institute (SARI) both of the Council for Scientific and Industrial Research (CSIR) in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan, and the International Maize and Wheat Improvement Centre (CIMMYT).

Together, these institutes released 21 open-pollinated varieties in Ghana, these varieties possessed tolerance to lodging, improved husk cover and resistance to major diseases, including maize streak virus (MSV), *Striga* resistance, the maize stalk borer (*Eldana saccharina*), ear rot, and other key foliar diseases, such as leaf rust and downy mildew. Some of these genotypes are "Dobidi", „Aburotia", „Okomasa", „Dorke", and „Abeleehi" (Badu-Apraku and Menkir, 2006; Badu-Apraku *et al*., 2006; Morris *et al*., 1999; Sallah *et al*., 1993).

Presently, the major breeding objective is the production of drought-tolerant hybrids which combine high yield potential and earliness. New genotypes belonging to this class are „Etubi", „Enii-Pibi" as QPMs and „Opeaburoo", „Tintim" and „Aseda" as normal maize, both groups having an average yield potential of 7.0 t/ha (ObengAntwi *et al*., 2013).

An important breeding objective which was embraced in the 1990s was the development of Quality Protein Maize, a genotype whose lysine and tryptophan content are enhanced by introgression of *opaque*-2 gene (Bressani, 1991). This culminated in the release of „Obatanpa GH" which has since 1993 remained the dominant cultivar in Ghana and some parts of West Africa (Badu-Apraku *et al*., 2006).

The phenomenon of a trade-off between earliness and high yield such that increase in earliness leads to decrease in yield and vice versa is reported to be undesirable (Barrière *et al*., 2010) and breeders attempt to develop cultivars which combine earliness and yield with little success. Foreign varieties which possess both high yield and earliness have become a possibility through identification of quantitative trait loci (QTL) which manifest associations of high yield loci with reduction in number of days to silking (Barrière *et al*., 2010; Bouchez *et al*., 2002; Peter *et al*., 2002).

The maize germplasm in West Africa awaits studies to identify such favourable associations between earliness and high yield. Finally, efforts to enhance vitamin A content by the CRI of Ghana have led to the production of „Golden Jubilee", „Sotu baka" and „Abontem". Table 2.2 shows some improved maize varieties and their major characteristics.

Table 2.2: Maize varieties developed and released by the Crops Research Institute.

NAME OF VARIETY	DATE OF RELEASE	PEDIGREE/ LINE	MATURITY PEROID	NAME OF VARIETY	DATE OF RELEASE	PEDIGREE/ LINE	MATURITY PEROID
CSIR-Enii-Pibi	GH110xEnt75	2010	110	Okomasa	CIMMYT Pop 43SR	1988	120
CSIR-Omankwa	TZE-W Pop STR QPM C4	2010	90	Dobidi	CIMMYT Pop 43	1984	120
CSIR-Aburohema	EVDT-W99 STR QPM C0	2010	90	Safita 2	Pool 16	1984	90-95
CSIR-Abontem	TZEE-Y PopSTR QPM C0	2010	75-80	Kawandzie	CIMMYT Pop. 31	1984	90-95
CSIR-Golden Jubilee	Obatanpa/GH9866SR	2007	105-110	Aburotia	Tuxpeno P.B. C16	1983	105-110
CSIR-Aziga	Obatanpa/GH9866SR	2007	105-110	LaPosta	CIMMYT Pop 43	1972	120
CSIR-Akposoe	EV9990QPM	2007	105-110	Composite 4	Parental lines of central and South American origin	1972	120
CSIR-Etubi	GH110xEnt85	2007	105-110	Golden Crystal	-	1972	105-110
Mamaba	GH110xEnt5	1997	105-110	Composite 2		1968	120
Dadaba	GH132-28xP28	1997	105-110	Mexican 17	CIMMYT origin	1961	90-105
Cida-ba	GH2328x88	1997	105-110	Nyankariwana No. 1	-	1961	90-105
Dodzi	TZEEW SRBC3 (EV9990)	1997	80-85	GS I	-	1960	90-105
Obatanpa	CIMMYT Pop 63	1992	105-110	GS II	-	1960	90-105
Dorke SR	Pool 16	1992	90-95	GS III	-	1960	90-105
Abeleehi	CIMMYT Pop 8149SR	1990	105-110	C50	-	1942	90-105

Source: Crop Research Institute, Fumesua

2.6. Genetic diversity in maize

Genetic diversity refers to variation in nucleotides, genes, chromosomes, or whole genomes of organisms within and/or among populations. Genetic diversity in germplasm is the most valuable resource which contributes to progress in plant breeding. It is the custom of contemporary maize breeding programs around the world to utilize and recycle few elite genotypes constituting about 5% of the maize germplasm for production of new cultivars (Hoisington *et al*., 1999). In the United States, maize breeding is based on less than ten improved hybrids (Carvalho *et al*., 2004). This practice leads to genetic erosion and a narrow genetic base of the improved varieties leading to reduced fitness and increased susceptibility to biotic stress factors (Troyer, 1996; Hallauer, 1990; Smith, 1988; Troyer *et al*., 1988).

The Southern corn leaf blight epidemic in 1970 on hybrid maize caused by the fungus *Helminthosporium maydis* which led to a loss of more than 80% of U.S. corn fields was attributable to the narrow genetic base arising from the use of T cytoplasmic maize, the source of male sterility in hybrid seed production. The disease spread rapidly across U.S. and to other parts of the world including Japan, Africa, Philippines and Latin America in less than a year. Losses amounted to about US $1 billion. Similarly, the maize streak virus epidemic of 1983 in the West African Corn Belt was the result of widespread cultivation of uniform genotypes and drought conditions (Briddon *et al*., 1994; Pinner *et al*., 1988).

Assessment of genetic diversity estimates, their patterns, as well as relationships among genotypes or populations are important for breeding programs as they identify new alleles for crop improvement (Mohammadi and Prasanna, 2003) and reveal groups that can constitute new parental combinations for trait improvement (Pollak and Scott,

2005; Smith *et al*., 2005; Reif *et al*., 2004; Pollak, 2003). Landraces are known to represent a rich reserve of genetic variability and possess a wide array of genes not yet exploited for variety improvement in many traits.

Global maize accessions collected and documented at CIMMYT is about 27,451 including 300 wild species of *Zea* and *Tripsacum*. The IITA Genetic Resource Centre has in stock over 900 accessions of tropical *Zea mays* collected from various locations in Africa. Knowledge and exploitation of the genetic diversity among the accessions would obviate the risk of increasing uniformity in elite germplasm and ensure longterm selection gains (Messmer *et al*., 1993).

Genetic classification and estimation of diversity of maize germplasm was initiated over 60 years ago in the U.S. (Reif *et al*., 2004; Smith *et al*., 1997; Goodman and Brown, 1988; Hallauer *et al*., 1988; Kahler *et al*., 1986; Smith, 1986; Goodman and Stuber, 1983). Similarly, many countries in Europe (Messmer *et al*., 1993, 1992) embarked on same, including France (Dubreuil *et al*., 1996), Romania (Iuorå *et al*., 2001), Yugoslavia (Ignjatović-Micić *et al*., 2008), and Italy (Hartings *et al*., 2008). This was followed by Japan (Enoki *et al*., 2002) and Latin America (Warburton *et al*., 2005, 2002; Xia *et al*., 2005, 2004; Reif *et al*., 2003a, 2003b).

Nonetheless, in Africa, very little work on evaluation of genetic diversity in maize in Ethiopia (Legesse *et al*., 2007; Beyene *et al*., 2006), Ghana (Oppong *et al*., 2014; Obeng-Antwi, 2007), Zimbabwe, Zambia and Malawi (Magorokosho, 2006), and six other countries in West Africa (Sanou *et al*., 1997) exist. Genetic diversity estimation of the African maize germplasm from a wide geographical background has not been carried out. Moreover, there few reports on genetic diversity evaluation of the maize

landraces held in the IITA repository. There is also little information on the heterozygosity of the maize germplasm to inform the evolutionary history thereof.

2.7 Methods of estimation of genetic diversity

Historically, morphological evaluation was the main method employed for genetic diversity estimations (Obeng-Antwi *et al*., 2011; Farooq and Azam, 2002; Stadler, 1929). Demerits of morphological assessment include non-reliability of its markers as they are vulnerable to natural selection and their expression is partially under the influence of environmental factors (Hartings *et al*., 2008), low polymorphism, late expression and low heritability (Beyene *et al*., 2005).

Isozyme analysis for estimation of genetic variability is a better method for evaluation of genetic diversity and is widely used in many crops (Tanksley and Orton, 1983; Brown, 1979; Markert and Moller, 1959) for its simple, rapid, cheap and codominant expression which offer the ability to directly compare the magnitude and distribution of genetic diversity between different populations and species (Lu *et al*., 2002).

Isozyme assay has been used successfully to investigate genetic variability in maize populations in West Africa (Sanou *et al*., 1997), the U.S. (Kahler *et al*., 1986; Smith *et al*., 1985; Smith, 1984), China (Lu *et al*., 2002), Bolivia (Goodman and Stuber, 1983), India (Bhat and Chandel, 1998) and Mexico (Sanchez *et al*., 2000a; Doebley *et al*., 1985). A major demerit of protein and isozyme markers is their requirement of a different protocol for each isozyme system and the difficulty in automating the method hence it is limited in evaluation of genetic diversity (Farooq and Azam, 2002).

Molecular markers have been extensively used to improve plant breeding because they are relatively simple, easy to use, automatable, relatively faster to assay, are accurate and offer the ability to compare values from different populations. A major advantage

is that they are not influenced by the environment and differences can be seen at all stages (Farooq and Azam, 2002; Rafalski *et al*., 1996).

Molecular markers based on DNA polymorphisms detected by the polymerase chain reaction (PCR) offer many advantages such as high precision in detecting variation, as well as exploring genetic relationship among large populations, high heritability of the markers (100%), inexpensive and automatable compared to morphological and isozyme analysis (Winter and Kahl, 1995).

Of the most commonly used markers are the hybridization-based DNA markers of the restriction fragment length polymorphisms (RFLPs) (Botstein *et al*., 1980), and the PCR based DNA markers, including the randomly amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), sequence characterized amplified regions (SCARs) (Xu *et al*., 2001; Naqvi and Chattoo,1996) sequence tagged sites (STS) (Perry and Bousquet, 1998; Olson *et al*., 1989), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995), single nucleotide polymorphism (SNPs) (Geurra and Yu, 2005; Collins *et al*., 1998) and microsatellites or simple sequence repeats (SSRs) which offer higher polymorphism informative content measured as expected heterozygosity and average number of alleles while AFLPs showed the lowest level of polymorphism (Pejic *et al*., 1998).

2.8. SSRs for maize genetic diversity

Simple sequence repeats (SSRs), also known as microsatellites are short nucleotide sequences between 2-6 units in length, which may vary in number of tandem repeats, typically 3 to 100 times in an allele to give rise to polymorphisms within species (Tautz *et al.*, 1986). Simple sequence repeats make up about 14% of the genomes in eukaryotic species approximately three times more than in prokaryotes (Marcotte *et al*.,

1999). They arise from replication errors which create extra set of repeated sequences varying in length between genomes of species and creates genetic basis of variations. The variations in length of the DNA strand are traceable and allow for tracking genotype variations in breeding programs. The difference in DNA length is revealed by designing primers for the conserved sequences flanking the repeats which would be amplified by PCR then viewed on agarose or denaturing polyacrylamide gel electrophoresis.

Thus microsatellites have proved ideal for determining paternity, population genetic differences and relatedness as they are not only limited to the nuclear genome but are also present in chloroplast and mitochondrial genomes as repetition of guanine and cytosine (Soranzo *et al*., 1999). The first report of microsatellites in plants was by Condit and Hubbel (1991) suggesting their abundance in plant systems.

Microsatellites are ideal genetic markers owing to their abundance and uniform dispersal in genomes, hyper-variability, co-dominant nature, and amenability to automation (Hokanson *et al*., 1998; Powell *et al*., 1996; Röder *et al*., 1995; Morgante and Olivieri, 1993). For maize studies, the high polymorphic information content (PIC) provided by SSRs in comparison to AFLP, RFLP and other markers make them the marker of choice for genetic diversity evaluations (Le Clerc *et al*., 2005; Liu *et al*., 2003; Warburton *et al*., 2001; Senior *et al.*, 1998; Chin *et al.*, 1996; Gupta *et al*., 1994; Wang *et al.*, 1994; Helentjaris *et al*., 1988).

Liu *et al*. (2003) used microsatellites to estimate the diversity in 260 maize inbred lines. A total of 2,039 alleles were identified and a clustering analysis placed the inbred lines into five clusters corresponding to five major breeding groups. Reports of use of SSRs for assessment of genetic diversity in African maize accessions are few and cover small

geographical locations. Karanja *et al*. (2009) estimated genetic variation in 10 inbred lines from Kenya, CIMMYT and U.S.A. by means of
morphological traits and SSRs.

A cluster analysis separated the genotypes into four groups. Sixty-two Ethiopian highland maize accessions were evaluated for genetic similarities by means of morphological parameters and SSRs (Beyene *et al*., 2006). Legesse *et al*. (2007) evaluated thirty-five Ethiopian highland and 21 CIMMYT-Zimbabwe maize inbred lines on the basis of SSRs and partitioned the genotypes into five clusters with an average polymorphic information content of 0.58. Genetic variation among maize landraces from Zimbabwe, Zambia and Malawi were determined by morphological assessment and SSRs (Magorokosho, 2006).

In Ghana, 500 maize landrace populations were characterized by means of bulk SSR studies (Oppong *et al*., 2014). Evaluation of genetic diversity among landraces covering a large geographical region in Africa has become more important than ever as many maize breeding programs report of low and stagnant yields, challenges with emerging new diseases and stress conditions, and the need to develop nutritionallyenhanced genotypes. Additionally, estimation of the variation in African germplasm in terms of heterozygosity is essential to reveal the historical basis of variation in African maize germplasm and provides a guide to its utilization.

There is dearth of information on the genetic similarities among maize accessions originating from a wide geographical background in Africa. The findings from this study are expected to reveal useful germplasm and alleles that can be utilized in breeding programs for maize improvement with a concurrent widening of the genetic base.

2.9. Measures of genetic diversity

Measures of genetic diversity estimate the variation and relationships within and among populations and/or individuals on the basis of some metric traits. It facilitates reliable classification of accessions and identification of subsets of core accessions with possible utility for specific breeding purposes (Mohammadi and Prasanna, 2003). Typically, a combination of data sets from pedigree information (Bernardo, 1993; Messmer *et al*., 1993), passport data, morphological data (Bar-Hen *et al*., 1995; Smith and Smith, 1992), biochemical data obtained by analysis of isozymes (Lu *et al*., 2002; Hamrick and Godt, 1997) and storage proteins (Smith *et al*., 1987), as well as DNAbased marker data are used.

Accurate estimation of genetic diversity is reflected on the type of population, that is, inbred lines, pure lines and germplasm accession and whether they are in HardyWeinberg equilibrium. Because the sampling distribution may not be known, application of statistical theories to estimate the sampling variance of some genetic diversity measure is important (Weir, 1990; Brown and Weir, 1983) with the aim of reducing the sampling error. The most basic measures of genetic variation include (i) allelic richness, or the total number of distinct genotypes or the number of different alleles segregating in a population, and (ii) evenness which is the frequency of the genotypes or alleles (Frankel *et al*., 1995). Allele richness is affected by presence or absence of rare alleles and considers percentage of polymorphic loci which is subject to a large sampling error reducible by evaluating a large number of loci (Brown and Weir, 1983).

In qualitative terms, a marker is polymorphic if it has at least two alleles and its most frequent allele in the population has a frequency of at most 99% (Hartl and Clark, 2007). Measure of evenness, which is less affected by sampling error associated with

rare alleles, constitutes average observed heterozygosity, expected heterozygosity, and effective number of alleles (Mohammadi and Prasanna, 2003). In a quantitative sense, the degree of polymorphism is measured by two distinct parameters, heterozygosity and its variance (Nei and Rouchoudhury, 1974) and polymorphism information content (PIC) (Botstein *et al*., 1980).

2.10 Genetic distance

Genetic distance refers to the genetic divergence between species or between populations within a species. It is measured by a variety of parameters. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. In its simplest form, the genetic distance between two populations is the difference in frequencies of a trait. Genetic distance is a quantitative measure of genetic difference between individuals, population or species at the allelic level (Beaumont *et al*., 1998).

Many distance measures are available but the choice depends on the kind of data, to be precise, interval data obtained from morphological evaluations, allele frequency data from isozyme or DNA amplification products and presence or absence data. The most common distance measure for morphological data is Euclidean distance or straight line measure, estimated as similarity or dissimilarity. The Euclidean distance between two individuals is given by the square root of the sum of all squares of pairwise differences between two individuals, A and B, having morphological measures (i) where i = 1..., p represented by x_1, x_2,..., x_p and y_1, y_2... and y_p is shown in equation 2.1.

$$d_{AB} = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + ... (x_p - y_p)^2]^{\frac{1}{2}} \quad .... 2.1$$

Unlike Euclidean distance which is based on a metric character, Gower‟s genetic distance (Gower, 1971) coefficient estimates distance on both qualitative, where a match between two individuals is scored 0 and a mismatch is assigned 1, and quantitative traits which are calculated as the difference in trait value divided by the overall range of trait. Then follow a sum of the individual trait distance for each pair of individuals divided by the number of traits scored in both individuals. The distance measure based on correlation coefficient is a powerful estimation when considering multivariate variables. Distance measure based on correlation coefficient is able to increase accuracy scores when data is not normalised. Moreover, it has the power to detect associations between two or more variables than the classical methods such as Pearson measure of linear relationships.

For SSR molecular data analysis, in which repeat amplification products represent alleles, variation in calculated allele frequencies may be estimated or bands may be scored as presence or absence to generate a binary data. A common distance measure which employs allele frequencies is Roger‟s distance, Cavalli-Sforza and Edward‟s (1967) arc and chord distances and Nei‟s (1972) distance *inter alia*. Rogers‟ distance, RD_{ij} is given by Equation 2.2 (Mohammadi and Prasanna, 2003).

$$RD_{ij} = 1/2\ [\Sigma(X_{ai} - X_{aj})^2]^{1/2} \quad \ldots.2.2$$

Where X_{ai} and X_{aj} = frequency of the allele *a* for individual *i* and *j*, respectively.

When a binary data matrix is constructed from a molecular data, four measures of genetic distance are often used, namely, the Modified Roger‟s distance GD_{MR} (Wright, 1978), Nei and Li‟s (1979) coefficient, GD_{NL}, Jaccard‟s (1908) coefficient GD_J, and simple matching coefficient GD_{SM} of Sokal and Michener (1958), where *l* is the number of loci, X_{11} is the number of bands or alleles present in both individuals;

X_{00} is the number of bands or alleles absent in both individuals, X_{10} is the number of bands or alleles present in individual *i* only, and X_{01} is the number of bands or alleles present in individual *j* only. Simple matching and Modified Roger‟s are examples of Euclidean distance measures. The formulae for estimation of the genetic distances of a binary matrix data are presented in equations 2.3, 2.4, 2.5, and 2.6.

$$GD_{MR} = \sqrt{\frac{X_{10} + X_{01}}{2l}} \quad \ldots.2.3$$

$$GD_{NL} = 1 - \left(\frac{2X_{11}}{2X_{11} + X_{10} + X_{01}}\right) \quad \ldots.2.4$$

$$GD_{J} = 1 - \left(\frac{X_{11}}{X_{11} + X_{10} + X_{01}}\right) \quad \ldots.2.5$$

$$GD_{SM} = 1 - \left(\frac{X_{11} + X_{00}}{X_{11} + X_{10} + X_{01} + X_{00}}\right) \quad \ldots.2.6$$

Rogers's genetic distance is also less sensitive to the overestimation of distance produced by heterozygous loci and finite sample size than the Manhattan metric, Cavalli-Sforza and Edwards's distances, and the modified Nei's distance. The choice of a genetic distance measure for microsatellite marker is subject to many factors, especially models that make assumptions on the evolutionary forces, particularly mutation and drift that drive genetic change in the population under study. The stepwise mutations model assumes that mutations are cumulative, like a growing chain typical of microsatellites and tend to increase or decrease value of the allele one step at a time

rather than viewing every mutation event as resulting in a totally new allele of the infinite allele model. In effect, alleles resulting from stepwise mutations are related to the one before, it making it possible to trace evolutionary events in a population. In contrast, the infinite alleles model alludes that a mutation event changes an allele from a given state into a totally new allele unrelated to the previous allele which can then be subject to random selection or drift.

Microsatellite development is based on the stepwise mutations model, its distance measures incorporate small changes arising from mutation, while not ignoring drift also. For example, the Nei and Li (1979) genetic distance measure for co-dominant markers is a linear function of the co-ancestry (Melchinger, 1993), the Modified Rogers distance is widely preferred because of its statistical and genetic properties. Though the simple matching distance measure has Euclidean properties which facilitate use in analysis of molecular variance, its major demerit is undifferentiated scores for 0-0 and 1-1 matches (Mohammadi and Prasanna, 2003)

2.11. Multivariate techniques for interpretation of genetic distance

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

2.11.1 Cluster analysis

Cluster analysis (Hair *et al*., 1995) groups individuals on the basis of similarity in their characteristics such that accessions within clusters are homogeneous and among clusters are heterogeneous. Two cluster methods based on i) distance measurement by Johnson and Wichern (1992) and the more robust maximum likelihood estimation and Bayesian methods of Pritchard *et al*. (2000) developed to overcome the constraints of distance-based methods are commonly applied. Mohammadi and Prasanna (2003) compared the most used hierarchical tree-producing cluster method to the less commonly used nontree-generating non-hierarchical methods.

Hierarchical clustering is founded upon assessment of relatedness and distance among individuals such that objects that are nearby are more related than those that are far apart. Each cluster connotes the maximum distance which connects members so that different clusters have different maximum distances. Essentially, the hierarchical algorithm is agglomerative as it successively groups individuals and then merges them on the basis of their similarities in three distance categories, viz., minimum (single linkage), maximum (complete linkage), and the average distance, Unweighted Pair Group with Arithmetic Means, UPGMA (Sneath and Sokal, 1973; Panchen, 1992).

The second most common clustering method is the Ward‟s minimum variance method (Ward, 1963). Mohammadi and Prasanna (2003) gave an extensive review of seven methods of clustering namely, single linkage, complete linkage, UPGMA, Unweighted Pair Group method based on centroids (UPGMC), Median clustering, Ward‟s method, and Principal Component Analysis (PCA). Comparison of the clustering methods when applied to classification of barley germplasm collections

based on both qualitative and quantitative data on disease resistance (Peeters and Martinelli, 1989) and for the assessment of genetic diversity in dent and popcorn maize based on inter-simple sequence repeats (Kantety *et al*., 1995) demonstrated that UPGMA provided results that were consistent with known heterotic groups and pedigree information while PCA clearly separated the dent corn lines from the popcorn varieties.

Moreover, Sokal (1986) and Rohlf and Wooten (1988) also confirmed that UPGMA gives the most accurate clustering method for classification in contrast to Lebeda and Jendrulek (1987) who report of identical performance of the six methods for classification based only on qualitative data. A major weakness of the UPGMA clustering method is its sensitivity to unequal evolutionary rates leading to faulty tree plots.

Relationship and classification of North American maize germplasm has been based on correlation, Karanja *et al*. (2009) used the Euclidean distance and the UPGMA in estimating genetic variation in inbred lines from Kenya, CIMMYT and U.S.A. by means of morphological traits and SSRs. Analysis of genetic diversity with SSRs among 42 CIMMYT maize inbred lines and 48 individuals from each of 7 populations were performed by simple matching coefficient distance measure on the binary matrix of the inbred lines and Nei‟s, Rogers‟ and Modified Rogers distance measures on the 53 SSR allele frequency matrix, respectively. On both sets of data, clustering was done by the UPGMA method (Warburton *et al*., 2002).

Investigation of genetic diversity and assignment into heterotic groups of 155 lowland tropical CIMMYT inbred lines were determined on 79 SSR allele frequencies by the Modified Rogers distance measure followed by UPGMA clustering (Xia *et al*., 2004).

Enoki *et al*. (2002) studied genetic diversity among 65 inbred lines comprising 51 Japanese highland maize and 14 lines introduced from U.S.A., Canada, and Europe by estimation of the Dice genetic distance measure on 60 SSR loci binary data followed by UPGMA clustering. Similarly, genetic diversity and relationship among 35 Ethiopian and 21 CIMMYT-Zimbabwe inbred lines was determined on 27 SSR binary matrix data through a Euclidean dissimilarity coefficient distance matrix and UPGMA clustering (Legesse *et al*., 2007). Beyene *et al*. (2006) used the Ward‟s minimum variance (Ward, 1963) method and Nei and Li‟s (1979) coefficient to assess genetic similarities on 20 SSR marker data among 62 traditional Ethiopian highland maize accessions. Magorokosho (2006) estimated the genetic distance between maize population originating from Zambia, Zimbabwe, and Malawi by means of Euclidean coefficient and Ward‟s modified location module. Oppong *et al*. (2014) evaluated over 500 Ghana maize populations with 20 SSRs, modified Rogers distance and clustering by UPGMA. There is scarcity of information on the use of the Dice coefficient for estimating genetic distance in the African maize germplasm.

2.11.2 Principal Component Analysis (PCA)

Multivariate data analysis was developed by Pearson (1901) for the social sciences. It was later developed again by Hotelling (1933) for the field of Educational Psychology. Other important recent authors of PCA analysis are Johnson and Wichern (2007) and Joliffe (2002). The application of PCA has been relevant in areas of agriculture, genetics (Menozzi *et al*., 1978; Cavalli-Sforza *et al*., 1994, 1993) biology, chemistry, ecology, food research, just to mention a few. The main idea of PCA is to reduce the dimensions of a data set with large numbers of variables while conserving the variance of the original data. PCA being linear, transforms the original data to new data sets of linear variables (PC) (Johnson and Wichern, 2007; Wilks, 2006; Jolliffe, 2002).

Generally the first PC has the maximum variance, followed by the second, third, etc. The PCA generates three important products, the eigenvalues, eigenvectors and scores, the dominant modes representing the most important characteristics from the original data. Generation of a scatter plot from two or more PCs in space reveals sets of similar individuals (Warburton and Crossa, 2000; Karp *et al*., 1997; Melchinger, 1993) and relationships between two or more variables (Mohammadi and Prasanna, 2003).

PCA has been one of the major tools used in genetic diversity studies. PCA was applied to maize genetic diversity assessment by Qi-Lun *et al*. (2008), Ho *et al*., (2005), Le Clerc *et al*. (2005), Xia *et al*., (2005, 2004), Reif *et al*. (2004, 2003) and Carvalho *et al*. (2002). Similarly maize researchers in Africa such as Obeng-Antwi *et al*., (2012, 2011) and Beyene *et al*. (2006) also used the PCA technique in their respective maize genetic diversity studies. The statistical power of PCA in genetic diversity studies is evident with the use of descriptor list in which it is common practice to evaluate large number of both morphological traits and molecular parameters. The method reduces the large variables into only few ones that carry majority of the variance.

In this study, a combination of the most reliable methods of clustering using UPGMA and PCA were used to assess the genetic variability in the African lowland genotypes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

A total of 64 lowland *Zea mays* accessions obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were evaluated. The genotypes originated from seven countries spanning latitude -8.85º S in Tanzania to 12.9º N in Chad and longitude 39.3ºE to 10.7ºW at elevation range of 50 to 700 m.a.s.l. The genotypes originated from Burkina Faso, Chad, Congo, Togo, Guinea, Benin and Tanzania. In addition a check variety, *'*Obatanpa GH" developed by the Crops Research Institute of the Council for Scientific and Industrial Research (CSIR), Fumesua, Ghana was included 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 accessions with regard to their designation, country of origin, collection sites and their precise locations (longitudes, latitudes and altitudes).

The study was divided into two sections, viz., morphological and molecular evaluation of genetic diversity. Forty-seven accessions of the morphological study were evaluated in two field trials from April to August 2011 and from March to July, 2012 at the Anwomaso Agricultural Experimental Station of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Table 3.1: Tropical Zea mays accessions of the IITA Genetic Resource Maize Collection employed in current study

No	Accession name	Collection site	Country	Altitude (m.s.a.l)	Longitude	Latitude
1	TZm 1503	-	Burkina Faso	340	1.67W	12.33N
2	TZm 1504	-	Burkina Faso	340	1.67W	12.33N
3	TZm 1505	-	Burkina Faso	340	1.67W	12.33N

4	TZm 1507	-	Burkina Faso	340	1.67W	12.33N
5	TZm 1176	-	Burkina Faso	< 750	1.67W	12.33N
6	TZm 1506	-	Burkina Faso	340	1.67W	12.33N
7	TZm 1508	-	Burkina Faso	340	1.67W	12.33N
8	TZm 1509	-	Burkina Faso	340	1.67W	12.33N
9	TZm 295	N‟djamena	Chad	310	14.78E	12.90N
10	TZm 310	Bongor	Chad	500	15.83E	9.87N
11	TZm 342	Lere pala	Chad	450	14.3E	9.58N
12	TZm 343	Lere pala	Chad	500	14.35E	9.58N
13	TZm 305	Tchad	Chad	300	14.5E	12.86N
14	TZm 335	Dik mogo	Chad	500	16.93E	10.45N
15	TZm 1427	Oyo Bokouele	Congo	315	16.3E	1.00S
16	TZm 386	Mbanra	Congo	335	15.98E	1.70S
17	TZm 398	Mbon	Congo	650	15.07E	2.08S
18	TZm 378	Oyo Abo	Congo	300	16.08E	1.12S
19	TZm 381	Oyo Kouda	Congo	310	15.37E	1.45S
20	TZm 394	Gounbome	Congo	350	15.85E	1.97S
21	TZm 399	Onzala	Congo	400	15.42E	1.62S
22	TZm 404	Etoumbi kelle	Congo	420	14.67E	0.13S
23	„Obatanpa GH“	-	CRI-Ghana	250	1.62W	6.67N
24	TZm 1522	Kodoro	Guinea	0 -1750	10.77W	10.97N
25	Tzm 1526	Kaaha Yaudhe	Guinea	0– 1750	10.77W	10.97N
26	TZm 1531	Kehali	Guinea	0 – 1750	10.77W	10.97N
27	TZm 1534	Difim Kaaha bodhe	Guinea	0 – 1750	10.77W	10.97N
28	TZm 1525	Samaya	Guinea	409	10.77W	10.97N
29	TZm 1543	Tormelin Binyo	Guinea	0 – 1750	10.77W	10.97N
30	TZm 1548	Mikaaha Bodhe	Guinea	0 – 1750	10.77W	10.97N
31	TZm 120	Save Alafia	Benin	250	3.22E	8.15N
32	TZm 121	Save Alafia	Benin	250	3.22E	8.15N
33	TZm 123	Onesse	Benin	250	2.43E	8.47N
34	TZm 124	Onesse	Benin	250	3.22E	8.47N
35	TZm 125	Onedeme Savalon	Benin	280	3.22E	8.02N
36	TZm 126	Onedeme Savalon	Benin	200	3.22E	7.95N

37	TZm 127	Onedeme Savalon	Benin	250	2.57E	8.15N
38	TZm 130	Agove Bante	Benin	290	3.22E	8.38N

Table 3 cont,,d

No	Accession name	Collection site	Country	Altitude (m.s.a.l)	longitude	latitude
39	TZm 132	Kyoota Cove	Benin	125	2.20E	7.22N
40	TZm 134	Zonguanado Wometo	Benin	90	3.22E	7.23N
41	TZm 137	Cove Bohicon	Benin	125	3.22E	7.23N
42	TZm 167	Sogbonou	Benin	110	1.82E	6.55N
43	TZm 143	Djidja	Benin	230	3.22E	7.35N
44	TZm 144	Kasseho	Benin	200	3.22E	7.42N
45	TZm 145	Kasseho	Benin	200	3.22E	7.42N
46	TZm 146	Soclogbo	Benin	225	3.22E	7.78N
47	TZm 147	Soclogbo	Benin	225	3.22E	7.78N
48	TZm 148	Agomy Adjahonme	Benin	175	3.22E	7.1N
49	TZm 149	Agomy Adjahonme	Benin	175	1.85E	7.1N
50	TZm 152	Adjahonme Agomy	Benin	290	3.22E	7.03N
51	TZm 155	Onedeme Lokossa	Benin	75	3.22E	6.72N
52	TZm 157	Aplahowe	Benin	195	3.22E	6.93N
53	TZm 183	Lobe Keton	Benin	175	3.22E	7.03N
54	TZm 185	Lolae Adja Onere	Benin	125	3.22E	6.98N
55	TZm 190	Lobe Onilahi	Benin	135	3.22E	7.08N
56	TZm 43	Iringa Morogoro	Tanzania	700	36.35E	7.57S
57	TZm 3	Korogwe	Tanzania	410	38.33E	4.93S
58	TZm 46	Kilimaewa Lokanga	Tanzania	130	39.07E	7.33S
59	TZm 49	Mitandango Tingi	Tanzania	50	39.3E	8.75S
60	TZm 1300	-	Togo	<986	1.22E	6.14N
61	TZm 1304	-	Togo	<986	1.22E	6.14N
62	TZm 1297	-	Togo	<986	1.22E	6.14N
63	TZm 1301	-	Togo	<986	1.22E	6.14N
64	TZm 1302	-	Togo	<986	1.22E	6.14N
65	TZm 1449	-	Togo	<500	1.22E	6.14N

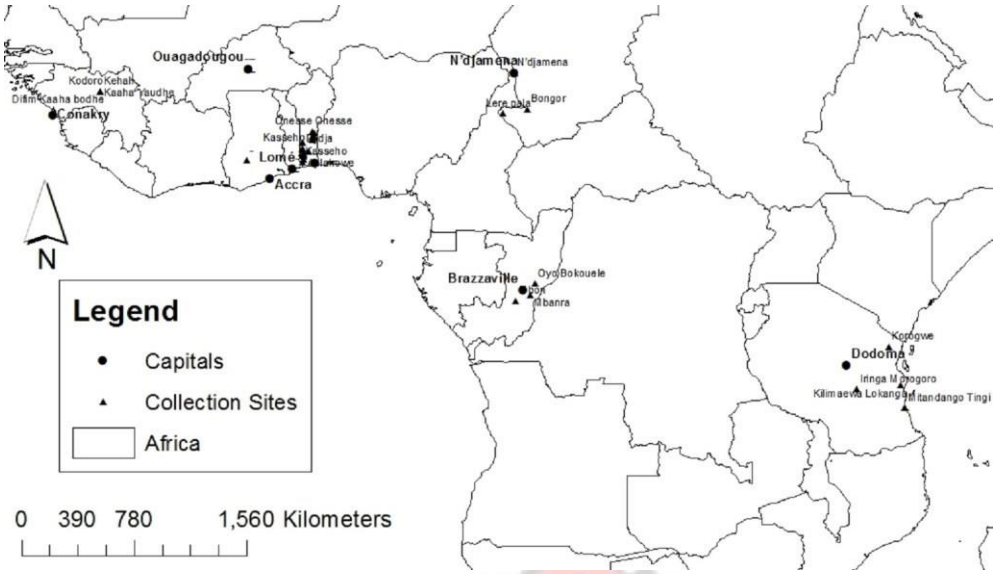

Figure 3.1: Schematic map of Africa depicting collection sites and capital cities.

3.2 Study site

The Anwomaso Experimental Station is located at latitude 6.69ºN, longitude 1.52ºW and an altitude of 277 m.a.s.l. The field has a slight inclination and conditions of sandy loam and well-drained soil at pH 5.2, 1.8% organic matter, average monthly temperature of 25 ºC, and a mean annual rainfall of 1500 mm. Two rainy seasons are recorded from March to July and from September to November.

3.3 Preparation, planting and experimental design

The field was prepared for planting by ploughing and harrowing, followed by weed control with Round-Up (Glyphosate, 360 g/L) applied at 5.0 Lha^{-1} for pre-emergence weeds. Plot dimension was 36 m × 9 m, each having 6 m × 0.6 m rows to which 15 plants of the same accession were planted. Distance between rows was 0.75 m and distance between blocks was 2 m. The planting density was 20,000 plants ha^{-1}. The experimental design was a randomized complete block with three replicates. The dimensioned 6 m x 0.6 m plots were containing 15 plants per row.

Maize was planted at a spacing of 60 cm x 30 cm with one maize seed per hill. Recommended agronomic practices such as irrigation and pest and disease control were followed. Fertilizer was applied at a rate of 120:60:40 kg ha^{-1} of NPK (nitrogen:phosphate (P_2O_5):potash (K_2O)) plus 125 $kgha^{-1}$ of sulphate of ammonia, equivalent to 50 g/plant at 21 days after planting and at ear emergence stage. Atrazine (4.5 L/ha) was applied to control post-emergence broad leaves and notorious grasses were controlled by hand weeding. Insect pests including maize stem borers (*Busseola fusca, Sesamia calamistis*) and cutworms (*Agrotis spp.*) were controlled with Conpyrifos 48% (1-1.5 L/ha) and Cymethoate Super (1-1.5 L/ha).

3.4 Morphological evaluation

3.4.1 Morphological data collection

For each plot, 31 morphological parameters of the IBPGRI and CIMMYT (1991) maize descriptor list consisting of 5 qualitative and 26 quantitative traits covering plant architecture, ear and tassel related traits, and kernel characteristics and yield were collected from 10 competitive plants per plot. Table 3.2 shows the list of traits, their definitions and method of measurement on the plants. Measurements were taken with meter rule, micrometre screw gauge, vernier calliper, and weighing scale as appropriate with each data involved.

Table 3.2: List of 31 morphological descriptors used in current study

	Measurement procedure	Abbreviation	Phenotypic data	Trait	Definition (units)
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 (%)	Qualitative	Estimation of green/dead leaf area: (1=10% dead leaf area to 10=100% dead leaf area

Table 3.2 cont"d

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

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 (Physiological 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 ten random plants at harvest (Physiological maturity)	NE	Number of ears harvested	Quantitative	Total number of ears harvested from 10 randomly selected experimental plants
22	On plot basis after harvest	NP	Number of harvested plants	Quantitative	Total number of plants from which ears were harvested from
23	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 number of harvested plants (NP)
24	On plot basis after harvest	KTEX	Kernel texture (score)	Qualitative	(1=flint and 5=dent),
25	On plot basis after harvest	PGC	Principal grain colour (score)	Qualitative	(0=white, 1=other colours),
26	On plot basis after harvest	KL	Kernel length (mm)	Quantitative	Length of kernel from the hilum to the base.
27	On plot basis after harvest	KW	Kernel width (mm)	Quantitative	Width of kernel
28	On plot basis after harvest	KT	Kernel thickness (mm)	Quantitative	Thickness of the kernel
29	On plot basis after harvest	EWT	Ear weight (kg)	Quantitative	Mass the ten randomly selected ears.
30	On plot basis after harvest	GWT	Shelled grain weight (GWT)	Quantitative	Mass of shelled grains from the ten randomly selected ears
31	On plot basis after harvest	YLD	Grain yield (YLD)	Quantitative	Shelled grain weight per plot adjusted to 125 g/kg moisture and converted to Mg ha^{-1}

3.4.2 Statistical analyses

Morphological variability in the qualitative data was evaluated by calculating frequencies and percentages of plants exhibiting each trait using PROC FREQ option

of SAS 9.3.1. On the quantitative data, means, standard deviation, minimum and maximum values, as well as coefficient of variation (CV) for the forty-seven accessions were calculated using PROC MEANS of SAS (Statistical SAS Institute, Cary, NC, 2011). Analysis of variance was performed on each trait by means of PROC GLM to test for significance of variation between accessions. Maize accessions were considered as random effects while replications and blocks within replications were considered as fixed effects for the purpose of extracting variance components. The form of ANOVA and generation of expected mean squares (EMS) involving genotypes and environments are presented in Table 3.3.

Table 3.3: Analysis of variance for obtaining estimates of variance components.

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

where

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 × y

The genotypic and phenotypic variance components were extracted from the linear functions of the mean squares represented by M and a subscript 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×y

$\sigma_{2r(y)} = (M_{ry} - M_e)/g$

Standard errors of the estimated variance components were computed using the method of Hallauer and Miranda (1981). Snedecor (1956) has shown 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

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

where *f* = is the coefficient of the component of variance f_i =

is the degrees of freedom of the respective mean squares

$\square_i = \square 1$ and

M_i = are the mean squares used to determine the component of variance

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

$$H_b^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2/r_e} \quad \ldots.3.2 \text{ (Doolittle, 1987)}$$

and $\sigma^2_P \square \sigma^2_g + \sigma^2/r_e$ is the phenotypic

variance component .

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

$$SE\,H^2_b = \square\square\square \frac{\square SE(\sigma^2_g)\square}{\sigma^2_P}\square\square\square \quad \ldots.3.3$$

where $SE(\sigma^2_g)$ is the square root of the variance of (σ^2_g) and the denominator is the phenotypic variance (Knapp 1986; Knapp *et al*., 1985).

The genotypic and phenotypic coefficients of variation were estimated as

$$GCV. . .\square = \frac{100(\square^G)}{\bar{X}} \quad \ldots.3.4$$

$$PCV. . . \square = 100(\square^{P}) \Big/ \bar{X} \quad \ldots.3.5$$

where $\square_G$ and $\square_P$ 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 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.

3.4.3 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 variance and covariance component estimates, the genotypic and phenotypic correlations between traits *i* and *j* were estimated as:

$$r_{Gij} \square \frac{\square_{Gij}}{\square\square_{GiGj}} \quad \ldots.3.6$$

$$r_{Pij} \square \frac{\square_{Pij}}{\square\square_{PiPj}} \quad \ldots.3.7$$

where r_{Gij} and r_{Pij} $\square_{Gij}$ $\square_{Gi}$ $\square_{Gj}$ $\square_{Pi}$ $\square_{Pj}$ are the estimated genotypic and phenotypic covariance between traits *i* and *j*, respectively, and $\square_{Gi}$, $\square_{Gj}$, $\square_{Pi}$, and $\square_{Pj}$ are the genotypic and phenotypic standard deviation for traits *i* and *j*, respectively. All computations were implemented using

PROC MIXED option 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).

3.5 Assessment of relationships between genotypes

3.5.1. Distance measurements and cluster analysis

The agro-morphological data was standardized before using in multivariate analysis by applying the YBAR and STD options in NTSYS-pc software version 2.2 (Rohlf, 2009). Relationships between genotypes were assessed by calculating correlation distance 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 a hierarchical method which groups by means of average distances, the Unweighted Pair Group Method with Arithmetic Average (UPGMA). 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. The statistical significance of the tree output was determined by means of bootstrapping (Felsenstein, 1985) using PAST software (Hammer *et al*., 2001).

3.5.2 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 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 between traits were investigated by means of graphing the principal components in 2D plots in NTSYS-pc 2.2 (Rohlf, 2009).

3.6 Molecular analyses

3.6.1 Genomic DNA Extraction

Leaf tissue was collected from a bulk of 15 plants of each of the 64 accessions at 3 weeks after planting. Samples were placed on ice cubes in an ice chest and transported to the laboratory. Genomic DNA was isolated from the bulked leaf samples by the CTAB (Saghai-Maroof *et al*., 1984) procedure according to the

Applied Biotechnology Center"s Manual of Laboratory Protocols (www.cimmyt.cgiar.org/ABC/Protocols/manualABC.html) with little modifications by the Kirkhouse Trust mobile laboratory from CRIG. The fresh harvested leaf samples were ground in 2.0 ml microtubes to fine powder in liquid nitrogen. Six hundred microliter of 2% CTAB and 0.1% (0.5µl) of β-mercaptoethanol were then added.

Samples were incubated in a sand bath at 65 ºC for 30 min with intermittent vortexing, then cooled at room temperature and equal volumes of chloroform:isoamyl alcohol (24:1) (Appendix A2) were added to reduce foaming during extraction.

Samples were mixed thoroughly by several inversions of tubes before centrifuging at 14000 r.p.m. for 15 min and the aqueous phase transferred into clean 1.5 ml tubes. Equal volumes of chloroform: isoamylalcohol (1:1 v/v) (Appendix A3) were added and tubes were inverted gently several times and centrifuged to separate the aqueous phase from the organic phase. The aqueous phases containing the DNA were transferred into

new clean 1.5 ml tubes. Nucleic acids were precipitated by adding two thirds volume of ice cold isopropanol to samples. They were kept overnight at -20

o
C to enhance precipitation. Samples were centrifuged at 14,000 r.p.m. for 5 min to pellet nucleic acids. Isopropanol solution was decanted and pellets were washed with washing buffer and centrifuged at 6,000 r.p.m for 4min. DNA pellets were washed in 80% ethanol, and then centrifuged at 6,000 r.p.m. for 4 min after decanting washing buffer. Ethanol was decanted and pellets were dried until the smell of ethanol was no longer detectable. DNA pellets were suspended in 100 µl TE buffer (Appendix A5) containing RNAse (10mg/ml) and centrifuged at high speed for 30 sec to remove all insoluble materials. The DNA isolates were then stored in -20^{o}C freezer for amplification with SSR primers.

3.6.2 SSR primer selection

The SSR markers used in this study were chosen from the maize GDB database (http://www.maizegdb.orf/ssr.php) in the University of Missouri, populated by Matsuoka *et al*., 2002a; Sharopova *et al*., 2002; Warburton *et al*., 2002; Senior *et al*., 1998; Chin *et al*., 1996) based on repeat units and bin location to provide a uniform coverage of the entire maize genome. A total of one hundred set of primers were assayed for their preliminary discriminatory power using samples from 20 accessions from which primers that failed to amplify were excluded from the study. Finally, sixteen primers were selected from this preliminary evaluation for amplification of the DNA templates to cover all ten chromosomes and to have at least one representation of each of the oligonucleotide as di- (25%), tri- (25%), tetra- (25%), penta- (12.5%), and hexa- (12.5%) repeats. Table 3.4 shows the primers used indicating their bin location and their average annealing temperature of the forward and reverse primers.

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

Marker	**Chromosome**	**Bin**	**Repeat**	**Repeat Unit**		**Primer Sequence (F/R)**	TM (used)
bnlg1597	1	1.09	Di	AG(34)	Forward	GATAATCTCGTCTCGCCAGG	
	1				Reverse	CATAAAAGGATGCCGACGAC	58
phi002	1	1.08	Tetra	AACG	Forward	CATGCAATCAATAACGATGGCGAGT	
	1				Reverse	TTAGCGTAACCCTTCTCCAGTCAGC	63
nc133	2	2.05	Penta	GTGTC	Forward	AATCAAACACACACCTTGCG	
	2				Reverse	GCAAGGGAATAAGGTGACGA	56
phi453121	3	3.00	Tri	ACC	Forward	ACCTTGCCTGTCCTTCTTTCT	
	3				Reverse	CAAGCAAGACTTTTGATCAGCC	58
umc1399	3	3.07	Tetra	(CTAG)5	Forward	GCTCTATGTTATTCTTCAATCGGGC	
	3				Reverse	GGTCGGTCGGTACTCTGCTCTA	63
phi072	4	4.01	Tetra	AAAC	Forward	ACCGTGCATGATTAATTTCTCCAGCCTT	
	4				Reverse	GACAGCGCGCAAATGGATTGAACT	63
nc130	5	5.00	Tri	*AGC*	Forward	GCACATGAAGATCCTGCTGA	
	5				Reverse	TGTGGATGACGGTGATGC	57
bnlg1237	5	5.06	Di	*AG(29*	Forward	TGGCGCGATTTTCTTCATAT	
	5				Reverse	AAAGAGCAACCTTCAACGGA	54
bnlg1695	5	5.07	Di	*AG(30*	Forward	ACCAAATCCTCATCTCGGAA	
	5				Reverse	CAATCTCCCCAAAATCTCGA	55
phi299852	6	6.07	Tri	*AGC*	Forward	GATGTGGGTGCTACGAGCC	
	6				Reverse	AGATCTCGGAGCTCGGCTA	60
umc1066	7	7.01	Hexa	*GCCAGA)5*	Forward	ATGGAGCACGTCATCTCAATGG	
	7				Reverse	AGCAGCAGCAACGTCTATGACACT	62
phi080	8	8.08	Penta	*AGGAG*	Forward	CACCCGATGCAACTTGCGTAGA	
	8				Reverse	TCGTCACGTTCCACGACATCAC	62
bnlg1525	9	9.07	Di	*AG(25)*	Forward	AGGAATTGCGAGTCTTCCAA	
	9				Reverse	CAACCCCCAAAATGAACAAA	54
phi022	9	9.03	Tetra	*GTGC*	Forward	TGCGCACCAGCGACTGACC	
	9				Reverse	GCGGGCGACGCTTCCAAAC	63
umc1367	10	10.03	Tri	(CGA)6	Forward	TGGACGATCTGCTTCTTCAGG	
	10				Reverse	GAAGGCTTCTTCCTCGAGTAGGTC	62
umc1196	10	10.07	Hexa	CACACG	Forward	CGTGCTACTACTGCTACAAAGCGA	
	10				Reverse	AGTCGTTCGTGTCTTCCGAAACT	62

3.7 Amplification and detection of SSR 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 µg ml^{-1} of gelatin, with pH adjusted to 8.3), 30 ng of template DNA and topped up with deionized water. The reactions were amplified in an Eppendorf Mastercycler from Germany by a process of denaturation step of 1 min at 96 ^oC, followed by a touchdown procedure which encompassed denaturation at 96 ^oC for 1 min., annealing at 65 ^oC for 1 min and extension at 72 ^oC for 2 min. The annealing temperature was then reduced at each cycle by 0.5 ^oC until a final annealing temperature of 55 ^oC was reached.

The last cycle was repeated 20 times and terminated at 72 ^oC for 2 min. The reaction was finished with a continuous cycle at 4 ^oC. After the reaction, 20 µl of the reaction mix was heated at 96 ^oC for 2 min and placed on ice. To each of the amplification products were added 10 µl loading dye (50% deionized formamide, 40% glycerol, 20 mM EDTA, 0.6 mg ml^{-1} of bromophenol blue) and 15 µl of the mix and 100 bp DNA ladder (Bioneer, South Korea) were loaded on 2% agarose gels stained with 5 µl ethidium bromide. Electrophoresis was run at 120 V for 2 h after which the gels were photographed under UV light (Geldoc, BIO-RAD Laboratories, Inc.).

3.8. Statistical analysis of molecular data

3.8.1. 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 stuttering bands by ensuring a maximum of two alleles per locus as maize is a diploid plant. Lanes

with no bands were also recorded. Primers and/or accessions that showed 15% or more missing data were eliminated (Warburton and Crossa, 2002).

3.8.2 Estimation of genetic diversity within populations

3.8.2.1 Rate of polymorphism

Loci having allele frequencies of ≤0.95 were described as polymorphic. The rate of polymorphism was calculated as the number of polymorphic loci expressed as a percentage of total number of loci, both monomorphic and polymorphic.

3.8.2.2 Number of alleles per locus

Average number of alleles per locus (*A*) also known as allele diversity was calculated as

$$A = \frac{1}{K}\left(\sum_{i=1}^{k} A_i\right) \quad \ldots.3.8$$

where A_i is the number of alleles per locus, *i*, divided by number of loci, *K*.

3.8.2.3 Number of alleles per population

The number of alleles per population was calculated as

$$A_1 = \sum_{i=1}^{t} n_i \quad \ldots.3.9$$

where *t* is the number of accessions and *n* is the number of bands per accession

3.8.2.4 Heterozygosity

The locus by genotype binary data matrix was analysed for observed and expected heterozygosity for each locus. Observed heterozygosity, H_o, was obtained by direct counts of heterozygous bands divided by total number of genotype counts. Expected

heterozygosity, H_e, is calculated from allele frequencies under Hardy-Weinberg equilibrium as

$$H_e = 1 - \sum P_i^2 \quad \ldots.3.10$$

where P_i is the proportion of the population carrying the ith allele (Botstein *et al*., 1980). Average expected heterozygosity across loci was computed by summing expected heterozygosity across all loci and dividing by number of loci. The chi square goodness of fit test was employed to test equality of observed and expected heterozygosity.

3.8.3. Genetic distance as similarity coefficient

The qualitative binary data matrix, being binomial and not a normal distribution did not require standardization. Genetic distance was calculated based on Dice coefficient. 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. Dice coefficient was calculated using SIMQUAL subprogram in NTSYS (Rohlf, 2009) to generate a similarity matrix.

Genetic inter-relationships among the genotypes were determined by Sequential Agglomerative hierarchical clustering based on UPGMA analysis (Sneath and Sokal, 1973). Statistical significance of the tree generated 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.

3.8.4 Principal Components Analysis

Principal components analysis (PCA) was performed using the subroutine EIGEN. All computations were carried out in NTSYS-pc, Version 2.2 package (Rohlf, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSION

Studies on genetic diversity in tropical lowland maize accessions originating from Africa were carried out by means of morphological and molecular evaluations. Sixtyfive accessions from eight countries were sampled from the collections held in the International Institute of Tropical Agriculture (IITA) Genetic Resource Centre, Ibadan, Nigeria. Twenty-five accessions (38.5%) originated from the Republic of Benin, eight each (12.3%) from Burkina Faso and Congo, seven (10.8%) from Guinea, six each (9.2%) from Chad and Togo, four (6.2%) from Tanzania and one (1.5%) representation from Ghana served as a check. The trials were carried out from April to August 2011 and March to July 2012 at the Anwomaso Agricultural Experimental Station of the Kwame Nkrumah University of Science and Technology in Kumasi, Ghana.

4.1 Morphological description of qualitative traits

Morphological characterisation was carried out on 47 accessions only on qualitative and quantitative basis. A total of 2,820 plants were evaluated. The qualitative traits considered were silk colour, kernel arrangement, cob colour, kernel texture and principal grain colour. Table 4.1 shows distribution of qualitative traits among the accessions while Figure 4.1 shows the different kernel arrangements and colours. A large variability was observed in all qualitative traits except cob colour in which about 97% were white and 3% were red. The predominant qualitative description of the

lowland accessions was pale yellow silk colour, ears having regular kernel arrangement, mixed grain colours (yellow, purple, red) and flint grains borne on white cobs. The occurrence of both flint (47%) and dent (31%) kernel types confirm the historical introduction of maize through two routes into West Africa. Flint maize was introduced by the Arab traders through the Mediterranean and the dent white maize by the Portuguese along the coast (Miracle, 1965; Portères, 1955). Substantial occurrence of mixed kernels (22%) is indicative of the extent of hybridization between the dents and the flints over time and space (Trifunovic, 1978).

Table 4.1: Distribution of qualitative traits of the IBPGR descriptors among lowland maize accessions of Africa

No.	Trait	Description	Class	No. of plants	Total plants evaluated	Percentage (%)
1	Silk colour	Pale yellow Red	1 2	2,110 647	2,757	76.5 23.5
2	Kernel arrangement	Regular Irregular Straight Spiral	1 2 3 4	2,131 131 22 455	2,739	77.8 4.8 0.8 16.6
3	Cob colour	Red White	0 5	83 2,502	2,585	3.2 96.8
4	Kernel texture	Flint Mixed Dent	1 3 5	1,211 569 805	2,585	46.9 22.0 31.1
5	Principal grain colour	White Other colours	0 1	985 1,600	2,585	38.1 61.9

A B

Figure 4.1: Maize ear characteristics. A) Types of kernel arrangement. B) Types of kernel colours.

4.2 Range, mean, standard error, standard deviation and analysis of variance of pheno-morphological traits

The development of an effective breeding program and the efficiency of selection largely depend on the size of genetic variability existing in the population because it is prerequisite for finding the diversity of alleles and extent of association among the traits and genotypes. In the current study, substantial variability was detected in all traits except number of ears per plant (NE) and kernel thickness (KT) as revealed by the highly significant ($P<0.001$) mean squares and ample coefficient of variation (10.32 to 43.09%) (Table 4.2). This variability represents substantial genetic diversity that can be exploited for maize improvement.

4.2.1 Variation in earliness

Results of analysis of variance revealed highly significant ($P<0.001$) mean squares, moderate coefficient of variation for AD (12.63%), SD (11.33%), and high coefficient of variation for ASI (35.96%), all of which represent sufficient phenotypic variability that can be exploited for trait improvement via selection. A mean anthesis date of 53.81±6.79 days with a range of 33 days in TZm-1504 and TZm-1507 both from Burkina Faso to 69 days in TZm-125 and TZm-127 from Benin was observed. Number of days to silking also varied from a minimum of 37 days in TZm-1507 to a maximum of 76 days in TZm-125 with a mean of 59.77 ±6.77 days. The highly significant variation in phenological traits, AD and SD were characterized by a 26day interval between the anthesis of the earliest and the latest accessions and 39-day period between silking of the earliest and the latest accessions (Table 4.2).

Table 4.2: Mean, standard deviation, minimum, maximum, standard error, coefficient of variation and mean squares of agro-morphological traits evaluated in 47 lowland maize accessions evaluated in Ghana in 2011 and 2012

	Trait	Mean	SD[1]	Min.[2]	Max.[3]	SE[4]	CV[5] (%)	Mean Square (Accessions)
1	AD (days)	53.81	6.79	33.00	69.00	0.13	12.63	137.50***
2	SD (days)	59.77	6.77	37.00	76.00	0.13	11.33	133.35***
3	ASI (days)	5.96	2.14	2.00	14.00	0.04	35.96	10.45***
4	TL (cm)	49.14	7.25	24.50	77.00	0.14	14.75	89.38***
5	ELL (cm)	83.04	12.97	20.50	117.00	0.25	15.62	345.94***
6	ELW (cm)	8.72	1.51	3.90	12.80	0.03	17.32	4.61***
7	PLHT (cm)	194.12	44.72	43.00	330.00	0.85	23.04	3056.99***
8	EHT (cm)	100.20	32.58	17.00	218.00	0.62	32.52	1651.44***
9	StD (mm)	18.46	3.34	5.70	29.00	0.06	18.10	11.75***
10	EP	0.51	0.08	0.17	0.97	0.00	16.20	0.01***
11	SG (%)	67.73	18.88	20.00	100.00	0.36	27.88	777.70***
12	EL (cm)	19.37	4.63	3.00	31.00	0.09	23.89	35.90***
13	ED (mm)	40.61	4.19	23.68	58.90	0.08	10.32	39.51***
14	CD (mm)	25.15	3.15	15.15	42.10	0.06	12.52	24.75***
15	NRE	13.15	2.06	4.00	24.00	0.04	15.64	6.73***
16	NKR	27.23	5.94	6.00	45.00	0.12	21.83	52.97***
17	HKWT (g)	61.73	12.24	25.78	117.20	0.24	19.83	449.98***
18	EN	1.06	0.11	1.00	1.70	0.00	10.71	0.02ns
19	KL (mm)	9.97	1.08	6.41	13.76	0.02	10.79	2.34***
20	KW (mm)	8.77	1.01	4.28	12.35	0.02	11.48	2.28***
21	KT (mm)	4.65	0.72	3.00	8.81	0.01	15.46	0.31ns
22	EWT (kg)	0.08	0.04	0.01	0.27	0.00	43.09	0.002***
23	GWT (kg)	0.74	0.22	0.26	1.64	0.00	29.92	0.11***
24	YLD ($Mgha^{-1}$)	4.02	1.20	1.41	8.86	0.02	29.90	3.24***

1 2 3 4 5

Standard deviation; Minimum; Maximum; Standard error; Coefficient of variation; AD = days to 50% anthesis; SD = days to 50% silking; ASI = anthesissilking interval; TL = tassel length; ELL = ear leaf length; ELW = ear leaf width; PLHT = plant height; EHT = ear height; StD = stalk diameter; SG = stay green; EL = ear length; EP = ear position; ED = ear diameter; CD = cob diameter; NRE = number of rows per ear; NKR = number of kernels per row; HKWT = hundred kernel weight; EN = number of ears per plant; KL = kernel length; KW = kernel width; KT = kernel thickness; EWT = ear weight; GWT = grain weight; YLD = grain yield; *** P< 0.001; ** P< 0.01; * P< 0.05.

On accession mean basis, the two genotypes were the earliest, TZm-1507 of Burkina Faso (AD of 44 days, SD of 50 days) and TZm-144 of Benin (AD of 445 days and SD

of 52 days). In comparison to the mean of „Obatanpa GH" which is an intermediatematuring (AD 49 days, SD 55 days), the plant to plant data identified thirteen plants which flowered 2 to 12 days earlier and 15 plants which flowered at the same time with the check. Seven genotypes were earlier in their respective AD and SD values, namely, TZm-1504 (47 days, 54 days), TZm-146 (47 days, 54 days), TZm-1506 (47 days, 54 days), TZm-49 (48 days, 53 days), TZm-295 (48 days 55 days), TZm-1427 (48 days, 55 days), and TZm-137 (49 days, 58 days). Two genotypes the latest and exhibited the highest values of AD and SD, TZm-46 (61 days, 66 days), TZm-120 (62 days, 69 days) (Table 4.3).

Range of anthesis and silking dates of other maize genotypes were reported to be 49 to 54 days among Indian Quality Protein Maize hybrids (Atnafua and Nageshwar, 2014), 53 to 63 days among 17 Pakistan genotypes (Shamim *et al*., 2010), 52 to 76 days among sixty-two traditional Ethiopian highland maize (Beyene *et al*., 2005), 76 to 83 days in the „Nostrano di Storo" Italian maize landraces (Lucchin *et al*., 2003) and 79 to 91 days among 30 inbred lines in Bangladesh (Azad *et al*., 2012). The corresponding range of days to silking were 54 to 59 days (Atnafua and Nageshwar, 2014), 81 to 87 days (Lucchin *et al*., 2003), and 58 to 81 days among the Ethiopian highland maize (Beyene *et al*., 2005). The identification of very early-maturing genotypes in current study is in contrast to the general belief that landraces are frequently late-maturing while commercial varieties which have been improved for early maturity (Taba *et al*., 1998) are early. The accessions TZm-1507, TZm-144, TZm-1504, TZm-146, TZm-1506, TZm-49, TZm-295, TZm-1427 and TZm-137 would be of relevance to breeding programmes which target earliness as they can mature early and escape the drought season.

4.2.2 Anthesis-silking interval

A highly variable anthesis-silking interval (mean square 10.45, P<0.001, CV =35.96 days) ranging from 2 to 14 days with a mean of 5.96±2.14 days on plant to plant basis was recorded (Table 4.2). On accession mean basis, the genotypes exhibited long ASI values in excess of the two to three days earlier anthesis than silk emergence. In comparison to „Obatanpa GH‟ (mean ASI 4 days), two accessions demonstrated low mean ASI values, that is, 3 days in TZm-167, 4 days in TZm-155, and 4 days in TZm-130 (Table 4.3). The rest of the accessions had mean ASI exceeding 4 days considering maize pollen viability lasts 18 to 24 hours.

Delay in silk growth arising from reduced photosynthesis resulting from drought leads to increase in anthesis-silking interval (Bolaños and Edmeades, 1993; Westgate and Bassetti, 1990; Hall *et al*., 1981) and decrease in yield (Bolaños and Edmeades, 1993; Westgate and Boyer, 1986; Hall *et al*., 1981; Du Plessis and Dijkhuis 1967). In current study in which evaluations were carried out under nonstress conditions of adequate irrigation, the long and variable ASI periods exhibited by the landraces signify their genetic differentiation in sensitivity to abiotic stresses, mainly drought, low soil nitrogen, and low soil pH (Edmeades *et al*., 1999) such that genotypes demonstrating high ASI values beyond 7 days are expected to be drought sensitive whereas the low ASI genotypes may be tolerant to imposed drought stress.

Shrestha (2013) reported ASI values of between 6 to 9 days among sixty maize inbred lines in Nepal. TZm-167, TZm-155, and TZm-130 may be exploited for their short ASIs for efficient pollination during drought conditions.

4.2.3 Variation in plant characteristics

The mean square results revealed highly significant differences (P<0.001) in plant architectural traits. Combined with a moderate to high coefficient of variation (14.75% to 32.52%), a large reserve of phenotypic variability is made available for utilization in breeding programs. The range and mean values were for TL, 24.5 to 77cm, 49.14±7.25 cm, ELL 20.5 to 117, 83.04±12.97 cm; ELW 3.9 to 12.8 cm, 8.721.51 cm; PLHT 43 to 330 cm, 194.12±44.72 cm; EHT 17 to 218 cm, 100.2±32.58; and StD 5.7 to 29 cm, 18.46±3.34 cm. The minimum and maximum architectural traits were identified for TL in TZm-1300 and TZm-342; ELL in TZm310 and TZm-1300; ELW TZm-310 and TZm-3; PLHT in TZm-132 and TZm-1300; EHT in TZm-132 and TZm-1505 to TZm-343; and finally StD in TZm-1503 and TZm-46.

On accession mean basis, the range of plant traits were for TL 41.73±4.98 cm (TZm146) to 57.12±5.74 cm (TZm-127), for ELL 61.22±7.32 cm (TZm-144) to 97.34±7.20 cm (TZm-46), for ELW 6.92±1.10 mm (TZm-132) to 10.64±0.76 mm (Tzm-134), PLHT 135.34±28.68 cm (TZm-144) to 243.82±46.87 cm (TZm-342), EHT 62.47±15.42 cm (TZm-146) to 138.32±35.29 cm (TZm-342), and StD 16.47±3.61 mm (TZm-132) to 21.2±2.80 mm (TZm-342) (Table 4.3). Records of other maize genotypes indicate rather short and variable TL such as 13 to 28 cm and 44.7 cm to 51.9 cm among the Lombardy (Hartings *et al*., 2008) and „Nostrano di Storo" (Lucchin *et al*., 2003) maize landraces of Italy, respectively, and 58.3 cm to 73.3 cm among 59 races of maize in Mexico (Sanchez *et al*., 2000). Comparatively, the tassels of the lowland maize genotypes in current study were somewhat long. Long tassels affect grain yield as they reduce light availability to the lower canopy and utilize carbohydrate resources (Duncan *et al*., 1967). In addition to its relevance for seed production, variation in tassel morphology could be an indicator of genetic diversity in both wild

and cultivated maize. Plant height and stalk diameter determines the lodging resistance of the maize crop. Similarly other maize genotypes from Mexico, Ethiopia, Italy, India and Nepal recorded plant heights of 142.7 to 383.3 cm, 161 to 288 cm, 96.5 to 171.1, 110 to 215 cm and 95 to 211 cm, respectively (Shrestha, 2013; Ranatunga *et al*., 2009; Hartings *et al*., 2008; Beyene *et al*., 2005; Sanchez *et al*., 2000). The Italian landraces of Lucchin *et al*. (2003) exhibited a range of stalk diameters of 16.0 to 18.7 mm. Tall plants with slender stalks are often susceptible to lodging while stout plants are more resistant. In the current study there was no record of plant lodging despite occurrence of very tall plants of about 330 cm. Sanchez *et al*. (2000) reported a minimum of 64.5 cm to a maximum of 104.6 cm in ear leaf lengths with width varying from 6.2 cm to 11.5 cm among the 59 maize races in Mexico. Beyene *et al*. (2005) reported that among 62 highland maize genotypes in Ethiopia ear leaf length of 51.8 cm to 100.8 cm with a width ranging from 6.4 cm to 12.7 cm were recorded.

4.2.4 Ear and kernel characteristics and grain yield

Traits such as EL, ED, NRE, NKR, and EN constitute the ear characteristics which influence grain yield. Analysis of variance showed highly significant differences ($P<0.001$) in ear and kernel characteristics, as well as grain yield, except EN. Besides ED, CD, KL and KW which had low coefficient of variation (10.32 to 12.52%), phenotypic variability exceeded 15% and was considered large enough to be useful in trait improvement via selection.

The range of variability and overall mean in ear characteristics were EL 3 cm (TZm1304) to 31.0 cm (TZm-120, TZm-127, TZm-1505, TZm-342, TZm-386, TZm-43 and TZm-46) and 19.37±4.63 cm; ED 23.68 mm (TZm-146) to 58.90 mm (TZm-127) and 40.61±4.19 mm; CD 15.15 mm (TZm-132) to 42.1 mm („Obatanpa GH“)

with mean of 25.15±3.15 mm. Furthermore, NRE of 4 (TZm-49) to 24 (TZm-43) and mean

13.15±2.06; NKR of 6 (TZm-132) to 45 (TZm-1532) with mean 27.23±5.94, KL 6.41 mm (TZm-1505) to 13.76 mm (TZm-46) with mean of 9.97±1.08 mm; KW 4.28 mm (TZm-1503) to 12.35 mm („Obatanpa GH‟ and TZm-386) with mean of 8.77 ±1.01 mm, KT 3.0 mm (TZm-145) to 8.81mm (TZm-167) with mean of 4.65±0.72 mm, HKWT 25.78 g (TZm-1304) to 117.2 g (TZm-386) with mean of 61.73±12.24 g, EWT 0.01 kg (TZm-1507) to 0.27 kg („Obatanpa GH‟ and TZm-295) with mean of 0.08±0.04 kg and finally, grain YLD 1.41 $Mgha^{-1}$ (TZm-1507) to 8.86 $Mgha^{-1}$ („Obatanpa GH‟ and TZm-295), with mean of 4.02±1.2 $Mgha^{-1}$were identified. The minimum EN was 1 in several accessions to a maximum of 1.7 (TZm-49) with mean 1.06±0.11.

The accession means were EL of 14.68±2.76 cm (TZm-132) to 24.69±3.16 cm (TZm46), ED 31.14±3.43 (TZm-146) to 45.95±3.02 mm and 45.14±3.78 cm („Obatanpa

GH‟ and TZm-43), CD 20.72±1.60 mm (TZm-146) to 30.43± 2.08 cm (TZm-398), NRE 10.73±1.42 (TZm-146) to 15.69 ±1.60 (TZm-1304), NKR 20.11± 3.38 (TZm146) to 33.28±5.44 (TZm-1300), HKWT 40.27±8.79 g (TZm-1304) to 83.59±14.39 g, and 80.55±15.76 („Obatanpa GH‟ and TZm-386), KL 8.69±80.58 mm (TZm-146) to 11.71±1.34 mm (TZm-46), EWT 0.05± 0.01kg (TZm-146) to 0.15 ±0.1 and 0.14±0.04 kg („Obatanpa GH‟ and TZm-295) and grain YLD 2.11±0.14 $Mgha^{-1}$ (TZm-146) to 6.3±1.9 $Mgha^{-1}$ and 5.46±1.4 $Mgha^{-1}$ („Obatanpa GH‟ and TZm-386, respectively (Table 4.3). In comparison to the check which gave the highest grain yield, ten genotypes had high mean grain YLD exceeding 4.5 $Mgha^{-1}$. These were

TZm-167 (4.55 Mgha^{-1}) from Benin, TZm-1300 (4.57 Mgha^{-1}) from Togo, TZm-127 (4.59 Mgha^{-1}.) and TZm-134 (4.82 Mgha^{-1}) both from Benin, TZm-1503 (4.90 Mgha^{-1}) from Burkina Faso, and TZm-1534 (4.94 Mgha^{-1}) from Guinea. The other high yielding genotypes were TZm-1505 (4.99 Mgha^{-1}) from Burkina Faso, TZm-147 (5.08 Mgha^{-1}) from Benin, TZm-386 (5.46 Mgha^{-1}) from Congo and lastly, TZm-295 (5.87 Mgha^{-1}) from Chad.

Number of rows per ear ranged from 4 (TZm-49) to 24 (TZm-43). Number of kernels per row ranged from 6 (TZm-132) to 45 (TZm-1531) compared to 10.73 to 17.13 from Bangladesh maize (Azad *et al*., 2012), 7.0 to 13.9 for Ethiopian maize (Beyene *et al*., 2005), 9.0 to 16.3 among Indian varieties (Ranatunga *et al*., 2009), 12.0 to 14.6, 8.0 to 20 and 8.0 to 21.1 in European varieties (Hartings *et al*., 2008; Lucchin *et al*., 2003; Rebourg *et al*., 2001), number of kernels per row were reported to range from 19.8 to 34.47, 18 to 36.9 and 12.2 to 34.8 (Azad *et al*., 2012; Ranatunga *et al*., 2009; Beyene *et al*., 2005). On the basis of individual plants and accessions, the wide range observed in number of rows per ear and number of kernels per row is remarkable and indicative of the wide variation relative to yield in the accessions studied.

Results of the present study are in agreement with earlier reports of significant differences in all traits except number of ears per plant among Indian maize genotypes (Anshuman *et al*., 2013). In a similar study involving 300 maize accessions comprising open-pollinated varieties (OPVs) introduced into Zimbabwe, Zambia and Malawi from the U.S.A., historically important OPVs from Malawi, Zambia, and Zimbabwe, local landraces and improved varieties evaluated in Harare, kernel thickness demonstrated no variability (Magorokosho, 2006). Mean kernel thickness in these regions was 4.83 mm.

Similarly, other researchers reported ear lengths of 12 to 24 cm, 13.5 to 19.5 cm, 14.5 to 22.7 cm, 9.2 to 21.6 cm, 15.8 to 18.8 cm, 8.7 to 17.9 cm and 6.6 to 24.4 cm while ear diameter varied from 31 to 50 mm, 11.5 to 15.9 mm, 33 to 46 mm, 29 to 49 mm, 32.1 to 36.4 mm, 22 to 49.8 mm and 28 to 48 mm, respectively (Azad *et al*., 2012; Ranatunga *et al*., 2009; Hartings *et al*., 2008; Beyene *et al*., 2005; Lucchin *et al*., 2003; Rebourg *et al*., 2001; Sanchez *et al*., 2000) Ear length and diameter are a function of plant yield. Longer ears with large diameter would usually have higher number of kernels per ear or large grains.

Table 4.3 shows that the landraces were relatively taller, late-maturing, with longer ASI and lower yield than the improved check. However, because the improved varieties were poorly represented in current evaluation, there is need for further testing. The improved variety with the shortest ASI („Obatanpa GH") produced the highest yield. Cob diameter ranged from 15.15 mm (TZm-132) to 42.1 mm. Few authors have reported on the variations in cob diameter. Among the Italian landraces Hartings *et al*. (2008) and Lucchin *et al*. (2003) reported a range of 19 to 33 mm and 22.4 to 27.8 mm, respectively. Similarly, 16 to 27 mm was reported by Sanchez *et al*. (2000) among the Mexican race. In contrast to the other researchers, the significantly wide range observed in our accessions confirms the initial definition of the wide variability in the accessions.

Kernel length varied from a minimum of 6.41 mm (TZm-1505) to 13.76 mm (TZm46), kernel width from 4.28 mm (TZm-1503) to 12.35 mm („Obatanpa GH") and kernel thickness from 3 mm in TZm-135 to 8.81 mm in TZm-167. Kernel characteristics are of much importance as the kernels form the most important part of the crop in terms of maize economics. The kernel characteristics inform the breeder on selection of an accession for improvement. The large variability in grain yield and yield

components indicates that it is possible to enhance yield through hybridization and selection using the local landraces. This observation is in agreement with the belief that landraces constitute rich sources of alleles for trait improvement (Drinic *et al*., 2012; Acquaah, 2007; Stolton *et al*., 2006; Matsuoko *et al*., 2002).

Shamim *et al*. (2010), Miguel Angelo *et al*. (2008) and Saika and Sharma (2000) all reported similar results of variability in various traits of maize, including plant height, ear height, anthesis days, and grain yield. These results are expected as maize is considered as a widely diverse crop. There was also variability in each of the twentysix morphological traits with respect to plant earliness, plant architecture and yield and yield components across the 47 accessions. Table 4.3 shows the means and standard deviations of the various traits across the 47 accessions.

Table 4.3: Means and standard deviation of twenty-six morphological traits for forty-seven African lowland maize

ACC	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	StD	SG	EL	EP	ED
„Obatanpa GH“	48.83 (3.04)	52.41 (3.03)	3.59 (1.04)	48.14 (7.51)	82.38 (4.04)	8.31 (1.48)	176.37 (34.20)	79.50 (24.34)	18.21 (3.20)	74.65 (4.59)	22.00 (4.59)	0.44 (0.09)	45.95 (4.46)
TZm-120	61.67 (4.50)	68.83 (4.64)	7.17 (1.08)	50.35 (6.96)	90.22 (9.01)	8.28 (1.15)	195.78 (31.76)	100.88 (23.09)	17.22 (2.75)	88.33 (9.05)	18.84 (4.05)	0.51 (0.07)	41.53 (3.55)
TZm-121	58.31 (2.08)	64.64 (2.97)	6.34 (2.09)	49.42 (5.02)	91.36 (6.91)	9.17 (1.29)	195.69 (33.85)	102.47 (22.73)	18.70 (2.50)	68.98 (23.24)	19.01 (3.77)	0.52 (0.06)	39.79 (3.20)
TZm-123	58.32 (1.82)	64.95 (2.08)	6.63 (1.60)	52.19 (7.04)	90.07 (6.06)	9.23 (0.84)	217.86 (33.72)	118.37 (26.27)	19.43 (2.94)	83.05 (15.00)	20.32 (3.11)	0.54 (0.06)	39.26 (3.41)
TZm-124	54.83 (1.79)	59.67 (2.30)	4.83 (1.22)	50.08 (8.29)	78.62 (12.56)	9.29 (1.36)	188.98 (35.15	97.01 (24.97)	17.36 (3.47)	78.33 (13.55)	19.10 (3.22)	0.51 (0.08)	41.33 (4.14)
TZm-125	60.09 (6.02)	65.91 (6.81)	5.82 (1.42)	46.56 (5.96)	77.27 (7.85)	8.73 (1.20)	195.02 (36.54)	101.69 (22.96)	20.09 (3.16)	80.00 (14.27)	16.09 (3.71)	0.52 (0.06)	39.77 (2.57)
TZm-126	59.03 (2.86)	65.24 (4.72)	6.20 (2.21)	50.86 (6.16)	86.97 (9.29)	10.07 (0.87)	208.81 (33.19)	116.60 (22.95)	18.87 (2.58)	73.90 (15.65)	19.51 (4.37)	0.56 (0.05)	40.42 (3.55)
TZm-127	59.49 (5.28)	64.46 (5.43)	4.97 (1.53)	57.12 (5.74)	89.06 (8.22)	9.82 (1.55)	207.07 (41.31)	110.11 (29.64)	20.53 (2.58)	81.69 (6.99)	20.04 (4.30)	0.53 (0.06)	44.73 (4.04)
TZm-130	53.56 (2.15)	57.42 (2.76)	3.86 (0.69)	42.27 (5.23)	71.41 (13.23)	7.91 (1.06)	159.41 (36.07)	77.54 (20.96)	16.89 (3.12)	77.54 (18.83)	15.88 (3.77)	0.49 (0.09)	39.39 (4.23)
TZm-1300	58.76 (1.77)	64.90 (2.59)	6.14 (1.08)	47.19 (7.79)	91.99 (11.82)	9.72 (1.29)	219.27 (40.95)	120.57 (30.92)	20.29 (2.69)	83.10 (11.27)	19.30 (5.31)	0.55 (0.07)	43.20 (3.05)
TZm-1304	59.76 (1.23)	65.84 (2.08)	6.07 (1.29)	44.05 (5.50)	80.06 (11.05)	8.15 (1.38)	213.81 (42.87)	116.99 (30.68)	18.50 (3.54)	74.91 (12.89)	17.77 (6.41)	0.54 (0.07)	40.37 (3.02)
TZm-132	51.22 (2.55)	56.02 (0.82)	4.80 (1.87)	43.55 (6.40)	71.28 (12.48)	7.92 (1.10)	156.03 (51.38)	74.46 (30.33)	16.47 (3.61)	61.86 (10.74)	14.68 (2.74)	0.48 (0.11)	38.66 (3.33)
TZm-134	58.00 (2.25)	64.83 (2.43)	6.83 (0.38)	53.14 (6.39)	90.59 (8.53)	10.64 (0.76)	219.69 (34.62)	121.66 (26.25)	20.53 (2.16)	66.67 (18.10)	24.13 (3.08)	0.55 (0.06)	39.01 (3.23)
TZm-137	48.50 (5.41)	58.05 (4.30)	9.55 (1.29)	43.48 (5.99)	69.09 (8.51)	6.92 (0.94)	166.75 (31.61)	81.33 (17.51)	16.58 (2.64)	50.71 (15.00)	17.68 (3.50)	0.49 (0.07)	36.96 (4.01)
TZm-1427	48.33 (10.16)	54.50 (8.53)	6.17 (2.21)	56.31 (4.96)	93.47 (8.24)	9.79 (1.00)	218.58 (39.57)	112.40 (33.65)	20.22 (2.31)	61.67 (17.87)	23.23 (3.50)	0.51 (0.07)	41.81 (3.05)
TZm-143	53.00 (2.10)	59.00 (1.65)	6.00 (1.54)	48.28 (5.17)	79.03 (8.46)	8.03 (0.94)	181.08 (45.93)	90.84 (32.77)	16.75 (3.10)	78.33 (13.55)	19.64 (3.64)	0.49 (0.07)	39.23 (3.20)
TZm-144	43.50 (1.91)	51.17 (2.13)	7.67 (2.23)	42.20 (4.72)	61.22 (7.32)	6.96 (1.04)	135.34 (28.68)	63.12 (15.69)	16.53 (3.77)	73.33 (9.51)	16.33 (2.91)	0.47 (0.08)	38.56 (2.40)
TZm-145	53.97 (2.29)	58.55 (2.41)	4.59 (1.20)	47.84 (6.15)	80.02 (9.47)	7.17 (0.83)	166.39 (38.23)	82.03 (26.18)	17.21 (3.36)	62.76 (17.15)	17.30 (3.60)	0.49 (0.09)	39.23 (2.86)
TZm-146	46.83 (1.47)	54.00 (1.93)	7.17 (2.36)	41.73 (4.98)	65.02 (9.08)	7.18 (0.98)	139.05 (31.85)	62.47 (15.42)	17.09 (3.77)	80.00 (15.40)	17.03 (3.48)	0.45 (0.07)	31.14 (3.43)
TZm-147	57.00 (3.03)	64.50 (4.97)	7.50 (2.78)	55.34 (5.42)	90.58 (10.80)	10.01 (0.93)	216.24 (38.69)	120.39 (31.53)	20.34 (2.43)	70.00 (15.40)	22.00 (3.27)	0.55 (0.06)	43.68 (4.20)
TZm-148	54.33 (2.30)	58.50 (1.72)	4.17 (0.91)	49.38 (6.63)	82.09 (9.57)	8.44 (0.84)	196.81 (36.50)	101.84 (22.93)	17.73 (3.56)	68.33 (10.76)	17.20 (3.30)	0.52 (0.05)	37.35 (2.77)

Table 4.3 cont"d

TZm-149	57.96 (2.87)	66.30 (3.52)	8.33 (2.86)	46.00 (6.59)	80.86 (10.20)	7.49 (1.59)	183.49 (59.24)	96.89 (44.06)	17.15 (3.84)	55.79 (13.88)	17.82 (4.29)	0.51 (0.08)	40.01 (3.36)
TZm-1503	48.83 (8.91)	56.67 (8.00)	7.83 (2.36)	50.91 (5.71)	87.73 (15.80)	8.66 (1.63)	184.57 (35.64)	84.14 (31.14)	16.86 (5.16)	49.76 (14.61)	21.94 (4.76)	0.44 (0.10)	43.17 (3.48)
TZm-1504	46.67 (10.06)	54.00 (9.88)	7.33 (0.95)	46.67 (3.18)	74.31 (6.83)	8.70 (0.96)	214.51 (31.85)	120.95 (21.67)	16.94 (2.54)	66.67 (18.10)	14.83 (2.24)	0.56 (0.04)	40.29 (3.08)

ACC	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	StD	SG	EL	EP	ED
TZm-1505	47.33 (8.99)	54.00 (8.77)	6.67 (2.30)	50.92 (6.65)	78.70 (15.84)	8.07 (1.48)	169.68 (40.85)	75.07 (30.42)	17.63 (3.04)	60.00 (15.40)	20.38 (5.77)	0.43 (0.09)	41.55 (3.71)
TZm-1507	43.50 (9.70)	50.00 (9.98)	6.50 (2.00)	49.15 (9.56)	83.37 (13.49)	8.35 (1.55)	183.40 (37.84)	85.94 (29.00)	17.92 (2.85)	67.72 (20.88)	19.69 (4.60)	0.46 (0.09)	41.66 (3.40)
TZm-152	55.67 (2.77)	63.50 (3.89)	7.83 (1.79)	47.63 (6.00)	79.32 (11.93)	9.81 (1.00)	184.89 (34.68)	98.53 (20.64)	18.66 (2.87)	76.67 (17.14)	18.73 (3.48)	0.53 (0.06)	39.93 (3.45)
TZm-1522	50.11 (0.92)	55.89 (1.73)	5.79 (1.93)	48.67 (7.40)	86.86 (8.20)	8.30 (1.50)	186.71 (35.64)	87.57 (26.36)	17.60 (2.57)	60.00 (10.35)	15.84 (3.83)	0.46 (0.07)	40.66 (3.76)
TZm-1526	51.85 (1.98)	56.03 (2.02)	4.19 (1.80)	50.91 (5.32)	85.46 (11.98)	9.04 (1.43)	197.15 (38.71)	98.44 (28.19)	18.80 (2.44)	62.71 (18.55)	19.36 (4.60)	0.50 (0.09)	43.21 (3.49)
TZm-1531	54.83 (1.79)	61.00 (2.97)	6.17 (2.63)	48.03 (6.14)	89.35 (11.04)	8.96 (0.93)	203.96 (39.56)	106.50 (31.73)	19.68 (2.17)	55.00 (16.21)	22.31 (2.97)	0.51 (0.07)	44.12 (2.95)
TZm-1534	54.00 (1.55)	59.34 (1.73)	5.34 (0.48)	49.01 (6.38)	86.13 (9.64)	8.56 (1.29)	225.36 (49.46)	121.64 (41.08)	20.79 (3.14)	68.31 (18.02)	22.12 (4.93)	0.53 (0.08)	44.41 (4.15)
TZm-155	54.00 (1.43)	57.50 (1.72)	3.50 (0.50)	46.63 (7.03)	78.25 (8.00)	8.59 (0.96)	181.62 (42.16)	95.66 (29.49)	18.22 (3.19)	48.33 (9.05)	19.96 (3.27)	0.52 (0.07)	38.30 (3.12)
TZm-157	58.00 (3.03)	63.50 (3.33)	5.50 (2.16)	46.96 (7.03)	76.73 (9.92)	7.94 (1.35)	178.13 (34.06)	91.48 (24.59)	18.11 (3.47)	55.00 (11.27)	15.92 (2.85)	0.51 (0.08)	39.59 (3.37)
TZm-167	56.54 (2.38)	59.95 (2.44)	3.32 (0.47)	53.85 (6.43)	86.49 (10.49)	8.71 (1.14)	200.70 (31.70)	102.49 (23.08)	18.05 (2.04)	52.81 (19.53)	20.39 (2.59)	0.51 (0.08)	41.05 (2.95)
TZm-183	58.48 (8.90)	63.72 (8.00)	5.24 (1.17)	49.15 (6.29)	83.16 (12.53)	7.70 (1.34)	179.99 (38.35)	92.00 (25.77)	16.69 (3.30)	39.66 (11.69)	18.23 (4.05)	0.51 (0.07)	39.67 (2.41)
TZm-185	57.17 (2.88)	63.00 (4.12)	5.83 (2.13)	46.09 (4.34)	81.81 (8.54)	8.44 (0.89)	191.57 (31.65)	104.18 (21.07)	17.02 (2.55)	73.33 (15.03)	19.19 (3.14)	0.54 (0.06)	39.90 (2.40)
TZm-190	54.17 (2.43)	58.50 (2.08)	4.33 (1.39)	52.24 (4.98)	80.34 (9.43)	9.71 (0.86)	204.65 (35.17)	110.08 (24.39)	17.46 (2.31)	73.33 (21.52)	19.50 (3.28)	0.54 (0.06)	38.54 (2.73)
TZm-295	47.83 (8.36)	54.50 (8.04)	6.67 (0.48)	55.93 (6.67)	88.69 (9.85)	9.65 (1.45)	202.90 (35.43)	100.99 (29.81)	20.24 (2.77)	55.00 (16.21)	21.83 (4.27)	0.49 (0.09)	43.33 (3.34)
TZm-3	55.67 (4.23)	61.33 (4.02)	5.67 (1.90)	44.93 (7.36)	80.07 (14.56)	9.12 (1.85)	188.48 (46.12)	95.05 (34.42)	18.60 (3.76)	65.00 (9.66)	20.30 (4.66)	0.49 (0.09)	40.71 (3.23)
TZm-310	55.09 (1.84)	60.93 (1.53)	5.84 (0.90)	45.47 (6.71)	78.38 (15.23)	8.44 (1.79)	200.86 (43.03)	106.10 (32.60)	17.46 (2.88)	75.26 (12.55)	18.63 (4.96)	0.52 (0.09)	39.68 (3.83)
TZm-342	52.17 (4.56)	58.00 (3.99)	5.83 (2.36)	54.77 (6.62)	91.49 (10.41)	9.19 (1.38)	243.82 (46.87)	138.32 (35.29)	21.20 (2.80)	53.33 (16.12)	22.91 (5.33)	0.57 (0.09)	41.24 (2.25)

Table 4.3 cont‟d

TZm-343	49.66 (6.12)	56.52 (6.75)	6.86 (1.81)	51.07 (7.29)	89.24 (12.41)	9.02 (1.58)	215.53 (44.95)	114.45 (35.30)	19.56 (3.28)	58.97 (19.71)	20.50 (4.10)	0.52 (0.09)	41.06 (3.02)
TZm-386	49.10 (8.73)	56.55 (8.35)	7.45 (1.73)	52.02 (5.07)	88.74 (8.89)	9.55 (1.26)	219.99 (37.05)	116.52 (26.16)	18.97 (1.96)	72.07 (13.61)	21.95 (4.20)	0.53 (0.06)	41.00 (3.75)
TZm-398	57.45 (3.05)	63.62 (5.11)	6.17 (2.17)	45.94 (6.12)	87.94 (11.52)	8.45 (1.85)	204.34 (43.46)	98.61 (34.16)	19.16 (3.84)	68.97 (17.84)	17.97 (5.03)	0.47 (0.09)	43.69 (3.94)
TZm-43	57.07 (5.91)	62.48 (6.75)	5.41 (1.20)	52.16 (5.32)	88.66 (11.35)	9.72 (0.82)	219.34 (39.52)	116.20 (31.43)	20.16 (2.42)	89.66 (8.16)	21.22 (4.31)	0.52 (0.06)	45.14 (3.78)
TZm-46	61.17 (3.10)	66.33 (3.12)	5.17 (0.69)	52.58 (4.43)	97.34 (7.20)	9.37 (1.20)	210.26 (35.12)	110.03 (28.29)	21.00 (3.12)	76.67 (16.12)	24.69 (3.16)	0.52 (0.08)	41.35 (3.25)
TZm-49	47.58 (7.93)	52.92 (7.62)	5.35 (1.75)	51.15 (5.55)	80.53 (10.46)	8.05 (1.08)	167.87 (46.78)	83.44 (31.99)	18.03 (3.60)	66.15 (24.90)	16. (4.07)	0.49 (0.10)	36.53 (1.78)

ACC	CD	NRE	NKR	HKWT	NP	NE	EN	KL	KW	KT	EWT	GWT	YLD
„Obatanpa GH“	28.35 (3.78)	13.68 (1.81)	28.30 (8.84)	83.59 (14.39)	7.04 (3.87)	7.44 (4.25)	1.04 (0.08)	10.13 (1.46)	9.99 (0.9)	5.38 (0.5)	0.15 (0.10)	1.17 (0.3)	6.30 (1.9)
TZm-120	26.37 (3.51)	14.32 (2.09)	27.17 (6.37)	57.49 (8.68)	17.49 (8.90)	17.49 (8.90)	1.00 (0.00)	9.39 (0.87)	8.49 (1.04)	5.00 (1.25)	0.08 (0.03)	0.71 (0.2)	3.83 (1.09)
TZm-121	23.66 (2.44)	13.75 (1.83)	26.73 (5.00)	63.16 (9.51)	10.25 (1.72)	11.88 (1.24)	1.17 (0.13)	9.86 (0.85)	8.99 (0.54)	4.59 (0.6)	0.08 (0.01)	0.68 (0.1)	3.70 (0.52)
TZm-123	24.30 (2.15)	13.41 (1.93)	27.95 (4.23)	59.71 (2.96)	21.03 (7.95)	21.71 (8.67)	1.02 (0.03)	9.75 (0.99)	8.86 (0.65)	4.75 (0.69)	0.10 (0.01)	0.77 (0.11)	4.20 (0.62)
TZm-124	27.40 (2.60)	13.68 (2.26)	26.17 (4.94)	60.35 (6.04)	21.58 (4.11)	21.75 (4.10)	1.01 (0.02)	10.04 (0.84)	8.69 (0.75)	5.00 (0.63)	0.10 (0.03)	0.83 (0.25)	4.49 (1.38)
TZm-125	25.06 (2.49)	13.78 (2.12)	28.18 (6.43)	54.76 (8.57)	15.00 (10.93)	15.73 (11.63)	1.03 (0.06)	9.79 (0.87)	8.33 (0.87)	4.47 (0.54)	0.08 (0.05)	0.76 (0.16)	4.15 (0.87)
TZm-126	25.81 (2.47)	13.63 (1.87)	27.18 (5.86)	54.83 (5.15)	14.93 (5.65)	16.57 (7.27)	1.10 (0.12)	9.26 (0.91)	8.49 (1.14)	4.58 (0.65)	0.09 (0.02)	0.75 (0.16)	4.09 (0.87)
TZm-127	28.33 (3.47)	14.62 (1.99)	29.52 (3.94)	61.34 (2.57)	16.46 (9.21)	17.21 (9.64)	1.04 (0.04)	9.84 (1.03)	8.58 (0.88)	4.35 (0.48)	0.09 (0.03)	0.85 (0.08)	4.59 (0.43)
TZm-130	24.42 (2.80)	12.89 (1.94)	25.15 (5.88)	55.69 (10.02)	12.11 (4.69)	13.00 (5.19)	1.07 (0.10)	9.80 (0.87)	8.57 (0.69)	4.62 (0.76)	0.07 (0.01)	0.66 (0.08)	3.57 (0.44)
TZm-1300	26.45 (2.24)	13.78 (1.69)	33.28 (5.44)	55.55 (6.44)	11.36 (6.72)	12.36 (6.81)	1.10 (0.20)	9.61 (0.78)	9.00 (0.72)	4.63 (0.86)	0.09 (0.03)	0.84 (0.18)	4.57 (0.98)
TZm-1304	23.79 (2.47)	15.69 (1.60)	27.43 (3.90)	40.27 (8.79)	12.53 (7.32)	14.78 (8.87)	1.14 (0.15)	9.53 (0.59)	7.37 (0.6)	4.51 (0.61)	0.06 (0.02)	0.6 (0.05)	3.25 (0.29)
TZm-132	24.54 (2.20)	12.45 (2.32)	24.65 (6.33)	56.46 (5.38)	10.82 (2.19)	11.73 (2.28)	1.09 (0.09)	9.66 (0.80)	8.46 (0.81)	4.32 (0.56)	0.08 (0.03)	0.64 (0.18)	3.48 (0.97)
TZm-134	24.19 (1.98)	12.07 (1.91)	30.48 (5.30)	69.92 (9.20)	12.67 (2.71)	15.50 (3.01)	1.25 (0.24)	9.62 (0.64)	9.04 (0.88)	4.62 (0.98)	0.11 (0.02)	0.89 (0.13)	4.82 (0.69)
TZm-137	22.96 (2.33)	12.15 (1.68)	22.98 (5.37)	66.71 (6.18)	12.63 (5.84)	13.17 (6.00)	1.04 (0.06)	9.67 (0.71)	8.68 (1.01)	4.56 (0.81)	0.07 (0.02)	0.65 (0.1)	3.54 (0.53)
TZm-1427	25.29 (2.14)	11.64 (1.72)	28.32 (6.27)	78.33 (18.19)	9.88 (2.72)	10.72 (3.30)	1.09 (0.13)	10.50 (0.68)	9.82 (0.59)	4.57 (0.65)	0.09 (0.03)	0.7 (0.14)	3.82 (0.74)
TZm-143	23.90 (2.35)	12.68 (1.66)	25.33 (5.92)	56.64 (11.20)	19.50 (4.72)	20.83 (6.25)	1.06 (0.08)	9.86 (0.77)	8.48 (0.56)	4.44 (0.58)	0.08 (0.01)	0.68 (0.13)	3.66 (0.69)

Table 4.3 cont"d

TZm-144	23.26 (2.14)	11.88 (1.65)	22.24 (5.56)	57.44 (3.00)	13.75 (3.60)	14.08 (4.06)	1.02 (0.04)	9.63 (0.64)	8.38 (0.89)	4.87 (0.72)	0.06 (0.02)	0.53 (0.16)	2.87 (0.85)
TZm-145	23.31 (2.26)	13.54 (1.64)	23.21 (4.46)	54.32 (4.98)	17.25 (4.40)	18.14 (4.98)	1.05 (0.06)	9.28 (0.76)	7.64 (0.6)	4.27 (0.52)	0.07 (0.02)	0.61 (0.13)	3.30 (0.68)
TZm-146	20.72 (1.60)	10.73 (1.42)	20.11 (3.38)	53.57 (3.24)	13.57 (5.02)	15.00 (6.47)	1.09 (0.12)	8.69 (0.58)	8.33 (0.82)	4.57 (0.59)	0.05 (0.01)	0.39 (0.03)	2.11 (0.14)
TZm-147	28.15 (2.93)	12.96 (1.87)	31.50 (5.18)	69.81 (12.25)	20.29 (9.37)	20.29 (9.37)	1.00 (0.00)	10.12 (0.70)	9.26 (0.86)	4.64 (0.64)	0.12 (0.02)	0.94 (0.11)	5.08 (0.61)
TZm-148	22.01 (1.95)	13.05 (1.81)	25.38 (4.00)	51.95 (11.37)	16.00 (2.10)	18.17 (2.76)	1.14 (0.12)	9.36 (0.85)	8.11 (0.63)	4.62 (0.54)	0.08 (0.01)	0.64 (0.09)	3.44 (0.51)
TZm-149	25.50 (2.43)	13.37 (1.74)	25.82 (5.96)	59.26 (5.78)	15.10 (8.69)	17.14 (10.29)	1.10 (0.08)	9.40 (1.47)	8.5 (0.83)	4.86 (0.7)	0.08 (0.05)	0.78 (0.25)	4.23 (1.37)
TZm-1503	25.45 (2.52)	13.63 (1.85)	32.77 (4.81)	79.42 (3.49)	12.93 (6.60)	13.11 (6.80)	1.01 (0.02)	11.30 (0.99)	7.61 (1.95)	4.51 (0.47)	0.10 (0.04)	0.90 (0.26)	4.90 (1.4)
TZm-1504	23.98 (1.81)	13.71 (1.44)	23.91 (4.96)	58.51 (13.31)	19.52 (7.58)	20.21 (8.21)	1.03 (0.04)	9.90 (0.62)	8.25 (0.77)	4.59 (0.59)	0.08 (0.02)	0.67 (0.12)	3.61 (0.67)

Table 4.3 cont"d

ACC	CD	NRE	NKR	HKWT	NP	NE	EN	KL	KW	KT	EWT	GWT	YLD
TZm-1505	25.78 (2.07)	12.45 (1.79)	31.06 (5.04)	63.24 (9.33)	13.35 (6.30)	14.14 (6.94)	1.04 (0.06)	10.68 (1.55)	9.11 (0.86)	4.52 (0.58)	0.11 (0.05)	0.92 (0.33)	4.99 (1.79)
TZm-1507	25.31 (2.53)	12.61 (2.15)	24.88 (4.97)	57.55 (4.02)	14.79 (9.98)	15.14 (10.54)	1.01 (0.03)	10.96 (0.97)	9.32 (0.86)	4.58 (0.85)	0.07 (0.03)	0.58 (0.18)	3.13 (0.98)
TZm-152	26.78 (2.31)	13.23 (1.60)	23.82 (5.35)	63.11 (4.42)	16.71 (7.12)	17.79 (7.58)	1.06 (0.10)	9.35 (0.95)	8.33 (0.84)	4.68 (0.58)	0.09 (0.03)	0.68 (0.21)	3.69 (1.12)
TZm-1522	26.84 (2.58)	13.02 (1.14)	26.81 (4.56)	66.70 (12.46)	14.25 (10.09)	14.46 (10.38)	1.01 (0.01)	10.10 (0.66)	9.20 (0.5)	4.39 (0.58)	0.09 (0.05)	0.77 (0.16)	4.18 (0.87)
TZm-1526	27.86 (2.37)	13.66 (1.72)	27.72 (3.60)	57.70 (2.37)	17.26 (10.74)	17.68 (10.92)	1.02 (0.04)	9.39 (1.02)	8.54 (0.61)	5.00 (0.96)	0.08 (0.04)	0.78 (0.16)	4.21 (0.85)
TZm-1531	28.95 (2.47)	14.33 (1.67)	33.14 (4.90)	55.97 (2.80)	15.67 (7.43)	16.06 (7.65)	1.02 (0.04)	9.73 (0.66)	8.43 (0.46)	4.52 (0.66)	0.08 (0.02)	0.69 (0.07)	3.73 (0.39)
TZm-1534	28.75 (3.67)	15.06 (1.65)	27.75 (4.01)	61.25 (11.89)	13.00 (9.33)	15.08 (11.13)	1.09 (0.12)	9.66 (0.80)	8.68 (0.66)	4.94 (0.71)	0.09 (0.06)	0.91 (0.21)	4.94 (1.13)
TZm-155	24.45 (2.37)	12.60 (1.62)	23.25 (4.78)	60.94 (4.73)	18.67 (5.51)	19.17 (5.35)	1.03 (0.03)	9.45 (0.52)	8.81 (0.77)	4.71 (0.64)	0.08 (0.01)	0.6 (0.08)	3.23 (0.43)
TZm-157	24.01 (2.37)	13.09 (1.69)	26.76 (4.91)	61.83 (12.76)	14.55 (3.38)	16.10 (4.47)	1.10 (0.13)	9.99 (0.88)	8.73 (0.80)	4.63 (0.70)	0.09 (0.03)	0.72 (0.21)	3.92 (1.15)
TZm-167	23.70 (2.22)	11.98 (1.50)	28.48 (4.44)	61.52 (4.50)	15.86 (5.73)	16.21 (5.66)	1.03 (0.06)	10.57 (0.83)	8.96 (0.71)	4.45 (0.86)	0.1 (0.01)	0.84 (0.06)	4.55 (0.34)
TZm-183	24.65 (2.11)	13.05 (1.76)	25.93 (4.33)	53.03 (13.03)	8.89 (5.86)	9.84 (6.72)	1.16 (0.21)	9.99 (1.22)	8.98 (0.81)	4.89 (0.74)	0.06 (0.03)	0.70 (0.17)	3.79 (0.93)
TZm-185	22.47 (1.88)	13.72 (1.22)	24.10 (4.69)	66.80 (18.24)	18.50 (4.65)	19.00 (5.70)	1.02 (0.04)	10.18 (0.68)	8.42 (0.57)	4.42 (0.51)	0.09 (0.02)	0.77 (0.18)	4.20 (0.98)
TZm-190	23.81 (2.18)	13.13 (1.85)	25.58 (4.34)	62.11 (5.24)	24.17 (3.16)	26.33 (4.96)	1.09 (0.08)	9.87 (0.80)	8.64 (0.67)	4.85 (0.7)	0.08 (0.01)	0.72 (0.07)	3.93 (0.38)
TZm-295	26.82 (2.09)	12.77 (1.62)	32.17 (6.01)	77.73 (13.19)	14.17 (5.25)	14.33 (5.45)	1.01 (0.02)	10.66 (0.90)	9.25 (0.81)	4.69 (0.75)	0.14 (0.04)	1.08 (0.30)	5.87 (1.65)
TZm-3	24.58 (2.67)	11.79 (1.60)	27.08 (7.05)	60.94 (2.52)	13.30 (4.16)	13.30 (4.16)	1.00 (0.00)	10.53 (0.87)	8.93 (0.86)	4.49 (0.68)	0.09 (0.03)	0.73 (0.25)	3.94 (1.38)
TZm-310	24.53 (1.92)	12.52 (1.50)	28.80 (4.68)	59.29 (3.27)	8.48 (5.99)	8.85 (6.32)	1.03 (0.06)	9.81 (1.20)	9.05 (0.69)	4.54 (0.84)	0.07 (0.05)	0.79 (0.17)	4.28 (0.90)

Table 4.3 cont"d

TZm-342	25.17 (1.61)	12.45 (1.25)	29.85 (3.47)	63.28 (3.61)	12.50 (8.10)	13.83 (9.75)	1.07 (0.15)	9.82 (0.80)	9.87 (0.68)	4.91 (0.55)	0.07 (0.02)	0.63 (0.05)	3.40 (0.30)
TZm-343	24.18 (2.43)	14.74 (2.28)	29.42 (4.40)	65.25 (12.29)	12.12 (8.37)	12.52 (8.92)	1.02 (0.04)	10.49 (0.94)	8.06 (1.08)	4.38 (0.53)	0.07 (0.04)	0.78 (0.16)	4.24 (0.89)
TZm-386	24.28 (2.85)	12.02 (1.45)	30.22 (6.29)	80.55 (15.76)	7.20 (2.58)	8.43 (4.04)	1.12 (0.16)	11.42 (1.02)	10.22 (0.86)	4.84 (0.75)	0.10 (0.05)	1.01 (0.26)	5.46 (1.40)
TZm-398	30.43 (2.08)	15.29 (1.47)	30.91 (4.26)	50.14 (6.90)	13.75 (9.71)	14.11 (10.01)	1.02 (0.04)	9.41 (0.39)	8.85 (0.74)	4.54 (0.95)	0.06 (0.02)	0.57 (0.04)	3.08 (0.21)
TZm-43	27.17 (3.23)	14.17 (2.99)	26.34 (4.97)	63.20 (11.97)	10.95 (4.48)	11.47 (5.16)	1.03 (0.07)	11.10 (0.73)	9.79 (0.82)	4.57 (0.49)	0.08 (0.02)	0.70 (0.22)	3.78 (1.20)
TZm-46	24.02 (2.50)	12.81 (1.42)	27.40 (5.91)	74.52 (3.99)	10.76 (4.21)	11.28 (4.84)	1.03 (0.08)	11.71 (1.34)	9.83 (0.62)	4.99 (0.82)	0.08 (0.01)	0.68 (0.12)	3.69 (0.64)
TZm-49	22.35 (2.10)	10.87 (2.21)	27.23 (5.29)	56.20 (3.13)	8.21 (5.42)	8.52 (5.16)	1.10 (0.24)	9.48 (0.52)	8.13 (0.62)	4.68 (0.39)	0.05 (0.02)	0.70 (0.2)	3.81 (1.10)

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

4.3 Variation in genotypes by country of origin

The accessions were grouped by country and evaluated for their variability in earliness, anthesis-silking interval, plant height, ear leaf length and width, number of rows per ear, number of kernels per row, 100-kernel weight, and grain yield. Analysis of variance showed highly significant differences ($P<0.001$) among the genotypes of different countries. Besides the check, Chad (4.46 $Mgha^{-1}$) and Guinea (4.27 $Mgha^{-1}$) accessions showed the highest yields followed by genotypes of Burkina Faso (4.13 $Mgha^{-1}$) while the yields of and Congo (4.07 $Mgha^{-1}$), Benin (3.86 $Mgha^{-1}$), Togo (3.92 $Mgha^{-1}$), and Tanzania (3.80 $Mgha^{-1}$) were low. The Chad accessions were characterized by large HKWT (66.63 g), Large NKR, early-maturing, long ASI (6 days) but tall plants having long ELL and large ELW. The Guinea accessions however had low HKWT (60.37 g), large NRE (14.05), large NKR, moderate ASI (5 days) and medium PLHT (215.97 cm), ELL (86.96 cm) and ELW (8.72 cm). The Burkina Faso accessions were distinguished as the earliest (AD =47 days), longest ASI (7 days), as well as short plants with smallest ELL (80.99 cm) and ELW (8.45 cm) whereas Congo accessions were characterized by largest HKWT (68.85 g), tall plants (214.35 cm) and largest ear leaf characteristics (ELL 90.08 cm, ELW 9.27 cm). Despite the late-maturity (AD 59 days), tallest plants (PLHT 216.61 cm), large ear leaf dimensions (ELL 86.19 cm, ELW 8.95 cm), largest NRE (14.73) and NKR (30.38), the Togo accessions had relatively low grain yields (3.92 $Mgha^{-1}$) owing to their low HKWT (47.99 g).

Table 4.4: Means of grain yield, 100-kernel weight, anthesis-silking interval, number of ears per plant, Number of rows per ear, plant height and ear leaf characteristics for 47 maize accessions grouped into country of origin.

Country	N	YLD ($Mgha^{-1}$)	HKWT (g)	NRE	NKR	ASI	AD	PLHT	ELL	ELW
Ghana	50	6.30^{a}	83.59^{a}	13.68^{b}	28.30^{cd}	3.59^{e}	48.83^{e}	176.37^{e}	82.38c	8.31d
Chad	220	4.46^{b}	66.63^{bc}	13.06^{c}	30.12^{ab}	6.30^{bc}	51.15^{d}	215.97^{a}	87.04b	9.08a
Guinea	199	4.27_{bc}	60.37^{d}	14.05^{b}	28.90^{bc}	5.37^{d}	52.73^{c}	203.44^{b}	86.96b	8.72bc
Burkina Faso	221	4.13^{cd}	64.66^{c}	13.12^{c}	28.04^{cd}	7.08^{a}	46.58^{f}	188.10^{cd}	80.99c	8.45cd
Congo	155	4.07^{de}	68.85^{b}	13.07^{c}	29.86^{ab}	6.59^{b}	51.59^{cd}	214.35^{a}	90.08a	9.27a
Togo	99	3.92^{de}	47.99^{e}	14.73^{a}	30.38^{a}	6.12^{c}	59.25^{a}	216.61^{a}	86.19b	8.95ab
Benin	1416	3.86^{de}	59.74^{d}	13.02^{c}	25.89^{e}	5.92^{c}	55.34^{b}	186.09^{d}	80.45c	8.57cd
Tanzania	221	3.80^{e}	63.98^{c}	12.47^{d}	27.00^{de}	5.40^{d}	55.63^{b}	197.28^{bc}	86.85b	9.09a
	Mean square	52.80 ***	9254.34 ***	78.78 ***	1032.23 ***	126.66 ***	3474.11 ***	54776.95 ***	4489.52 ***	25.79 ***

Means in a column with different letters are significantly different at $P<0.05$;***$P<0.001$; YLD = yield; HKWT = hundred kernel weight; AD = days to 50% anthesis; ASI = anthesis-silking interval; NKR = number of kernels per row; NRE = number of rows per ear; HKWT = hundred kernel weight; PLHT = plant height.

4.4 Genotypic and phenotypic variance, genotypic and phenotypic coefficients of variation, and broad sense heritability estimates, of evaluations on lowland maize accessions

4.4.1 Genotypic and Phenotypic variance

Estimates of genotypic and phenotypic variances of the traits are presented in Table 4.5. Substantial genotypic and phenotypic variances were present in the maize populations for all traits other than ear weight and ear position, kernel traits and grain yield. All values of phenotypic variance were larger than that of genotypic variances. In general, a small and variable proportions (5 to 45%) of the phenotypic variance was attributable to the genotypic variance giving rise to low estimates of broad-sense heritabilities. Only 5% of the variance in KT accounted for genotypic variance whereas for ASI, ELL, ELW, PLHT, EHT, ED, CD, and HKWT between 30 to 45% accounted for genotypic variance with about 60% arising from environmental influence. In a similar manner, over 75% of the variance in AD, SD, STD, SG, NRE, NKR, KL, KW, and grain YLD was due to environment rather than genotype. Heritability estimates for the lowland accessions studied were generally low. The estimates ranged from 5% to 44%. Marginal heritability values of 5%, 10%, 11%, 16% and 18% were recorded for KT, EP, EWT, AD, SD and StD, respectively. Low estimates of 21%, 27%, 28% and 29% for SG, NRE, NKR, YLD, GWT, EL, KL and
KW, respectively were observed, while ELW, ELL, ASI, EHT, HKWT, ED and PLHT also had heritability estimates of 33%, 34%, 35%, 36%, 37%, 38% and 44% respectively.

Though these values are lower compared to heritabilities of ≥70% in Italian maize landraces (Hartings *et al*., 2008), and 0.27 to 0.82 for southern Africa landraces

(Magorokosho, 2006), they are not different from the range of expected heritability estimates for maize under non-stress growing conditions of less than 30% for grain yield and most of its components to between 50% and 70% for plant morphological and phenological traits reported by Hallauer and Miranda-Filho (1988). The low heritability estimates suggest that though it is possible to improve on the trait, slow progress would be made through many cycles of recurrent selection.

Estimates of phenotypic coefficient of variation for all traits were generally about four times higher than genotypic values. Highest values were recorded for PLHT (227%), EHT (240%), SG (182%), and HKWT (109%). The genotypic coefficient of variation for these traits were 55%, 58%, 42% and hundred kernel grain weight 26%, respectively (Table 4.5). Moderate to low genotypic and phenotypic coefficients of variation were recorded for earliness traits, tassel length and number of kernels per row.

Table 4.5: Genotypic and Phenotypic variance, phenotypic and genotypic coefficient of variation (CV) and broad-sense heritability estimates and respective standard errors for agromorphological traits of lowland African maize accessions grown in Ghana in 2011/2012

Trait	Phenotypic coefficient of variation (PCV%)	Phenotypic variance	Genotypic coefficient of variation (GCV%)	Genotypic variance	H^2 ± SE
AD	44.65	46.17	10.82	7.36	0.16 ± 0.12
SD	39.11	45.92	9.22	7.21	0.16 ± 0.12
ASI	26.12	3.99	6.21	1.40	0.35 ± 0.07
TL	26.29	51.35	6.17	14.08	0.27 ± 0.04
ELL	60.24	164.40	14.11	55.53	0.34 ± 0.05
ELW	7.63	2.28	1.72	0.74	0.33 ± 0.05
PLHT	227.93	1087.63	54.66	476.67	0.44 ± 0.06
EHT	240.01	677.27	57.59	241.89	0.36 ± 0.06
StD	9.53	8.30	2.28	1.46	0.18 ± 0.04
SG	182.83	348.61	42.36	74.01	0.21 ± 0.07
EL	19.28	23.86	4.53	6.68	0.28 ± 0.04
ED	0.21	17.08	0.06	5.94	0.35 ± 0.05
CD	14.12	9.96	3.35	3.81	0.38 ± 0.05

EP	14.25	0.01	3.30	0.00	0.10 ± 0.04
NRE	7.53	4.20	1.75	0.89	0.21 ± 0.04
NKR	28.85	33.76	6.79	6.95	0.21 ± 0.05
HKWT	109.22	146.89	25.66	55.03	0.37 ± 0.08
KL	3.49	1.14	0.80	0.32	0.28 ± 0.06
KW	3.87	1.01	0.91	0.29	0.29 ± 0.06
KT	1.07	0.51	0.22	0.03	0.05 ± 0.02
EWT	0.52	0.00	0.13	0.00	0.11 ± 0.10
GWT	2.38	0.04	0.54	0.01	0.27 ± 0.10
YLD	12.84	1.10	2.99	0.30	0.27 ± 0.10

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

For phenotypic coefficients of variation, AD (44.65%), SD (39.11%), TL (26.29%), and NKR (28.85%); and for genotypic coefficient of variation AD (10.82%), SD (9.22%), TL (6.17%), and NKR (6.79%) were recorded. The least variability of <10% was observed in ear and kernel traits (Table 4.5). The high estimates of PCV and GCV for these traits in the present study denote substantial variation which can be exploited for genetic improvement through selection, as well as strong influence of environmental factors on their expression. These results are in agreement with reports from Praveen Kumar *et al*. (2014), Abirami *et al*. (2005), Singh *et al*. (2003) who also reported high PCV and GCV values for grain yield per plant, ear height, hundred kernel weight, and plant height in maize.

4.4.2 Genotypic and phenotypic correlations

Table 4.6 shows the matrix of genotypic and phenotypic correlations among traits. There were mostly weak and non-significant correlations among the four sets of traits,

viz., plant architecture, yield and yield components, earliness, and kernel traits. The weak correlation indicates low genetic relationship between these traits under the optimum conditions at which the evaluation was carried out. Generally, genotypic correlations were larger than phenotypic correlations implying that association among these traits were greatly under genetic control. The low correlation values among traits were observed in southern African maize (Magorokosho, 2006) and in tropical and European maize and varieties originating from the Americas (Rebourg *et al*., 2001).

Besides these traits, approximately 50% of the correlation values were above 0.50, indicating a relatively high correlation among these traits for the current maize accessions in this study. Generally genotypic correlation (r_g) had relatively higher values than the phenotypic correlation values (r_p). Falconer (1989), reports that genetic correlation represents the heritable association between two traits while a phenotypic correlation is a combination of genotypic and environmental effects hence significant phenotypic correlation without a significant genetic correlation has no value (Atnafua and Nageshwar, 2014).

When the value of „r_p" is greater than „r_g", it shows that the apparent association of the traits was not largely due to genes but due to favourable environmental conditions present during the study (Ashraf *et al*., 2011). In the current study all the negative correlations among the traits were observed to be non-significant for both genotypic and phenotypic correlations. The strong positive and significant phenotypic correlation was between AD and SD (r_p = 0.96) while the most associated traits genotypically was observed between plant height and ear height (r_g = 1). Grain yield showed negative and non-significant correlation with earliness traits and ranged between -0.10 and -0.30. Generally, earliness is negatively correlated with grain yield, as a greater biomass is

required for the synthesis and accumulation of grain components (Atnafua and Nageshwar, 2014; Sallah *et al*., 1997b). Early-maturing varieties typically have low grain yield, while late-maturing varieties have higher yields (Sallah *et al*., 1997a). There is usually a trade-off between earliness and yield (Barriere *et al*., 2010) such that increase in yield leads to decrease in earliness and vice versa. Currently, as climate anomalies have increased globally, studies to combine earliness and high yield have dominated many maize breeding programs.

In the current study, eight very early–maturing accessions were found to have high yield. These were TZm-1505, TZm-49, TZm-295, TZm-1427, TZm-1503, TZm-386, TZm-343 and TZm-1522. Sallah *et al*. (1997a) reported relatively high yields of 3.8 to 5 $Mgha^{-1}$ for extra-early and early maturing improved varieties grown in Ghana. Regarding grain yield and architectural traits, there were positive and significant genotypic correlations. Results from the study also revealed correlation values of 0.50 and 0.35 between grain yield and plant height and ear height respectively.

An R^2 value of 0.25 and 0.12 indicate that 25% and 12% of grain yield variation is explained by plant height and ear height. Whereas many of the tall accessions were found to have grain yield of 3.1 and 5.9 $Mgha^{-1}$, some short accessions of height below 1.8 m recorded high yields of 3.7 to 5.0 $Mgha^{-1}$. These accessions include TZm-1505, TZm-49, TZm-157, TZm-183, TZm-143, TZm-149, TZm-1503, TZm1522 and TZm-124. Results also showed moderate positive significant correlation between grain yield and ear traits, ear length (0.61), ear diameter (0.69), cob diameter (0.53), kernel length (0.46), kernel weight (0.47) and number of kernels per row (0.60) with almost a perfect correlation with hundred kernel weight (0.97). The strong positive and significant

association between these traits is favourable in crop yield improvement through selection.

These results are in agreement with earlier studies by Atnafua and Nageshwar (2014), Bočanski, *et al.,* (2009), Singha and Prodhan (2000), Singh and Dash (2000), Tyagi *et al*., (1998), and Manivannan (1998) who reported strong positive and significant correlation of maize grain yield with yield component traits such as ear length, ear diameter, hundred kernel weight and kernel width.

Table 4.6: Phenotypic (lower diagonal) and genotypic (upper diagonal) correlation coefficients among traits evaluated for 47 African lowland maize accessions grown in Ghana in 2011 and 2012.

	AD	SD	ASI	TL	ELL	EW	PL	EHT	EL	EP	ED	CD	NR	NK	HK	KL	KW	YLD
AD		**0.90***	**-0.26**	**0.19**	**0.74***	**0.77***	**0.70***	**0.77***	**0.11**	**0.93***	**0.30**	**0.33**	**0.68***	**0.41**	**-0.45**	**-0.08**	**-0.03**	**-0.25**
SD	0.96*		**0.17**	**0.14**	**0.73***	**0.77***	**0.72***	**0.80***	**0.11**	**0.93***	**0.24**	**0.32**	**0.67***	**0.46**	**-0.38**	**-0.07**	**-0.07**	**-0.30**
ASI	-0.15	0.14*		**-0.12**	**-0.08**	**-0.05**	**0.0**	**0.01**	**-0.05**	**-0.05**	**-0.16**	**-0.03**	**-0.07**	**0.11**	**0.18**	**0.02**	**-0.08**	**-0.10**
TL	0.08	0.05	-0.11		**0.77***	**0.71***	**0.71***	**0.64***	**0.66***	**0.40***	**0.49***	**0.30***	**-0.02**	**0.65***	**0.64***	**0.50***	**0.52***	**0.75***
ELL	0.29*	0.22*	-0.09	0.71*		**0.70***	**0.86***	**0.78***	**0.69***	**0.46***	**0.63***	**0.43***	**0.34***	**0.76***	**0.45***	**0.41***	**0.44***	**0.52***
EW	0.16*	0.13	-0.11	0.64*	0.69*		**0.80***	**0.83***	**0.60***	**0.88***	**0.53***	**0.40***	**0.15**	**0.62***	**0.50***	**0.37***	**0.43***	**0.66***
PL	0.27*	0.20*	-0.09	0.59*	0.70*	0.69*		**1.0***	**0.61***	**0.94***	**0.56***	**0.39***	**0.41***	**0.73***	**0.27***	**0.30***	**0.45***	**0.50***
EHT	0.27*	0.25*	-0.07	0.49*	0.58*	0.63*	0.93*		**0.54***	**0.97***	**0.97***	**0.30***	**0.40***	**0.63***	**0.14**	**0.17**	**0.36***	**0.35***
EL	0.08	0.07	-0.03	0.60*	0.64*	0.57*	0.57*	0.46*		**0.24**	**0.49***	**0.28***	**0.04**	**0.58***	**0.70***	**0.53***	**0.62***	**0.61***
EP	0.29*	0.27*	-0.06	0.24*	0.30*	0.40*	0.58*	0.82*	0.18*		**0.14**	**-0.01**	**0.33***	**0.24**	**-0.18**	**-0.23**	**0.09**	**-0.18**
ED	0.12	0.09	-0.09	0.39*	0.49*	0.35*	0.42*	0.82*	0.45*	0.05		**0.87***	**0.66***	**0.69***	**0.42***	**0.40***	**0.41***	**0.69***
CD	0.13	0.12	-0.02	0.23*	0.34*	0.31*	0.28*	0.19*	0.29*	0.0	0.77*		**0.61***	**0.57***	**0.16**	**0.02**	**0.29***	**0.53***
NR	0.39	0.37	-0.07	0.06	0.27	0.13	0.31	0.26	-0.02	0.14	0.47	0.46		**0.36**	**-0.19**	**-0.09**	**-0.23**	**0.01**
NK	0.1	0.10	0.01	0.45	0.59*	0.47*	0.46*	0.33*	0.48*	0.08	0.56*	0.46*	0.19		**0.34***	**0.36***	**0.18***	**0.60***
HK	-0.19	-0.18	0.01	0.31*	0.26*	0.25*	0.16*	0.12	0.46*	0.04	0.25*	0.07	-0.23*	0.26*		**0.73***	**0.68***	**0.97***
KL	-0.17	-0.17	-0.02	0.37*	0.36*	0.24*	0.24*	0.15	0.44*	-0.01	0.40*	0.07	-0.08	0.36*	0.47*		**0.56***	**0.46***
KW	-0.07	-0.09	-0.08	0.29*	0.33*	0.29*	0.25*	0.18*	0.42*	0.03	0.24*	0.19*	-0.33*	0.23*	0.40*	0.39*		**0.47***
YLD	0.03	0.01	-0.05	0.37*	0.36*	0.33*	0.28*	0.25*	0.37*	0.17*	0.47*	0.30*	0.14*	0.59*	0.49*	0.36*	0.21*	

*P<0.05:

4.5 Genetic distance measurement and cluster analysis

Genetic dissimilarity among maize accessions was estimated by means of correlation distance coefficients generated from the data matrix of 47 accessions and 18 quantitative traits. The distance matrix, which represents an estimate of the genetic distance between pairs of accessions, is shown in Appendix B1. Dissimilarity for the entire data ranged from 0.00 to 0.88 with a mean of 0.28±0.19. A correlation distance coefficient of 0.28 represents a large dissimilarity among the accessions, hence a wide genetic diversity.

To better visualize the relationships among traits and accessions, cluster analysis was performed on the correlation matrix to generate a dendrogram based on Unweighted Pair Group of Arithmetic Mean (UPGMA) on 18 traits. Figure 4.1 shows the dendrogram generated from the correlation matrix. The dissimilarity coefficients ranged from -0.16 for „Obatanpa GH" and TZm-1522 to 0.85 for TZm-343 and TZm1504. Insertion of a reference line at -0.035 distance units classified the genotypes into three distinct clusters with sub-clusters irrespective of their geographical locations. A cophenetic coefficient of 0.71, demonstrated the high reliability and goodness-of-fit of the dendrogram to the correlation matrix of the data.

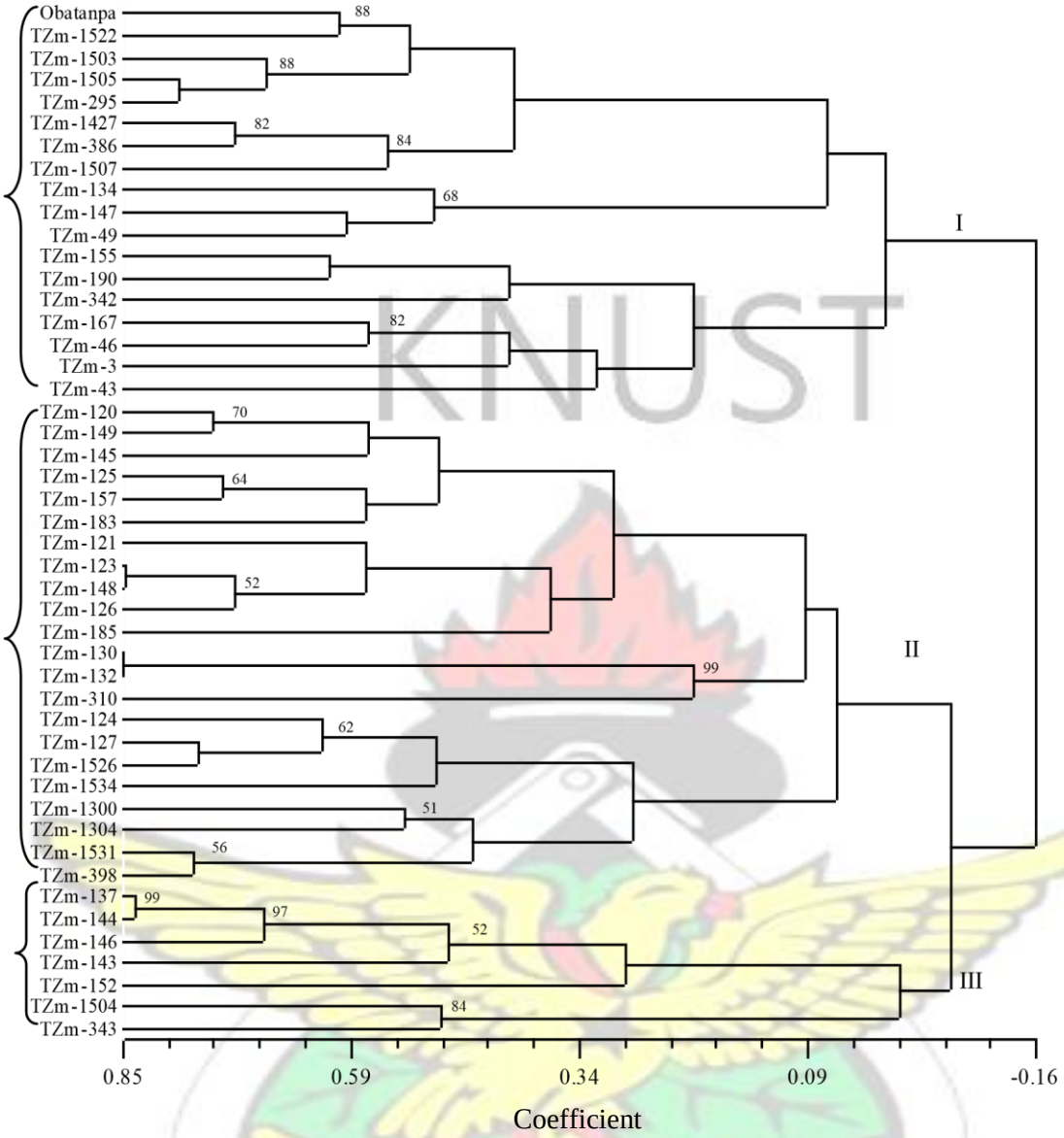

Figure 4.2: Dendrogram of 47 lowland Africa maize accessions on 18 agromorphological traits constructed from the correlation distance matrix using UPGMA cluster analysis.

Three clusters with diverse membership were generated, viz., cluster I (18 genotypes), cluster II (22 genotypes), and cluster III (7 genotypes) which were separated by an average genetic distance of 0.28. Although „Obatanpa GH" and TZm-343 occupied the extreme positions on the dendrogram, with a coefficient of 0.16, there were 15 other pairs of accessions which separated by a correlation coefficient of 0, including

„Obatanpa GH"/TZm-1531, „Obatanpa GH"/TZm-155, TZm-121/TZm-342, TZm125/TZm-3, TZm-130/TZm-150, TZm-130/152, TZm-130/TZm-46, TZm-132/TZm-

145; TZm-134/TZm-143; TZm-1427/TZm-144; TZm-146/TZm-155; TZm1505/TZm-46; TZm-148/TZm-43 and TZm-149/TZm-1531. On the contrary, the closest genotype pair related by the lowest dissimilarity genetic distance was TZm125/TZm-1427 (0.88). Five other genotypes were similarly closest in genetic distance such as TZm-130/TZm-132 (0.85) TZm-123/TZm-148 (0.84), TZm-120/TZm-386 (0.84), TZm-137/TZm-144 (0.83) and TZm-1505/TZm-126.

Cluster I was heterogeneous and subdivided into two subclusters, A and B. The genetic distance for cluster I ranged from 0.00 („Obatanpa GH"/TZm-155 and TZm1505/TZm-46) to 0.78 (TZm-1505/TZm-295) with an average of 0.30. Accessions in cluster I that were similar by at least 70% were TZm-1503/TZm-1505 (0.73), TZm1503/TZm-1505 (0.78), and TZm-1503/TZm-1505 (0.72). Subcluster IA was constituted from eleven members, „Obatanpa GH", TZm-1522, TZm-1503, TZm1505, and TZm-295, TZm-1427, TZm-386, TZm-1507, TZm-134, TZm-147, and TZm-49 with a genetic distance of 0.29. The accessions which were most similar to the check, „Obatanpa GH" by at least 60% were TZm-295 (0.68), TZm-1505 (0.66) and TZm-1522 (0.61).

Subcluster IB was formed from seven members, TZm-155, TZm-190, TZm-342, TZm-167, TZm-46, TZm-3, and TZm-43. Genetic distance among subcluster IB members ranged from 0.11 to 0.62 with an average of 0.33. Cluster I was derived from intermediate-maturing, least ASI genotypes having medium plant height and stem thickness, long ears with largest cob diameter which bore the longest, thickest and widest kernels and a correspondingly large 100-kernel weight. Nonetheless, because of

the few number of kernel rows per ear and the large cob diameter, the achievable grain yield was intermediate (4.36±1.4 $Mgha^{-1}$) (Table 4.7).

In a similar manner, cluster II was highly heterogeneous and grouped into two subclusters, viz., subcluster IIA populated by TZm-120, TZm-149, TZm-145, TZm125, TZm-157, TZm-183, TZm-121, TZm-123, TZm-148, TZm-126, TZm-185, TZm-130, TZm-132, and TZm-310 with range and mean genetic distance of 0.00 to 0.85, and 0.36, respectively. Accession pairs in cluster IIA that were substantially similar were TZm-120/TZm-149 (0.75). TZm-125/TZm-157 (0.74), TZm-123/TZm126 (0.76), TZm-123/TZm-148 (0.84) and TZm-130/TZm-132 (0.85). Subcluster IIB members were relatively less similar with a range of 0.01 (TZm-124/TZm-1304) to 0.77 (TZm-398/TZm-1531), and a mean of 0.41. Individual genotypes in cluster IIB were TZm-124, TZm-127, TZm-1526, TZm-1534, TZm-1300, TZm-1304, TZm1531, and TZm-398.

Genotypes of main cluster II were characterized by late-maturity, intermediate ASI, tall plants with long tassels, longest and widest ear leaves, high ear placement, intermediate ear length but widest ear diameter complemented by narrow cobs bearing large number of kernels per row with intermediate 100-kernel weights but the highest grain yield.

Main cluster III, the smallest of all was relatively homogeneous having seven genotypes TZm-137, TZm-144, TZm-146, TZm-143, TZm152, TZm-1504 and TZm343. The distance measure for this cluster ranged from 0.01 (TZm-146/TZm-1504) to 0.83 (TZm-137/TZm-144) with an overall cluster average of 0.33.

Table 4.7: Cluster means and standard deviations (SD) and their differences from the overall mean of 47 African lowland maize genotypes.

Traits	Overall mean and SD	**Cluster I mean and SD**	Variance	**Cluster II mean and SD**	Variance	**Cluster III mean and SD**	Variance
AD	53.77 (6.8)	**52.11 (7.7)**	-1.66	**54.50 (6.7)**	0.73	**49.12 (6.4)**	-4.65
SD	59.77 (6.8)	**57.86 (7.5)**	**-1.91**	**61.12 (7.6)**	1.35	**56.59 (6.4)**	-3.18
ASI	5.96(2.1)	**5.77 (2.2)**	**-0.19**	**6.62 (2.1)**	0.66	**7.47 (2.0)**	1.51
TL	49.14 (7.3)	**51.64 (7.0)**	2.5	**53.31 (6.0)**	4.17	**45.86 (6.3)**	-3.28
ELL	83.04 (13.0)	**86.43(12.3)**	3.39	**87.55 (10.9)**	4.51	**73.86 (12.9)**	-9.18
ELW	8.72 (1.5)	**9.12 (1.4)**	0.4	**9.64 (1.4)**	0.92	**8.10(1.5)**	-0.62
PLHT	194.1 (44.7)	**200.18(43.5)**	6.08	**202.82 (46.0)**	8.72	**176.65 (46.8)**	-17.45
EHT	100.2 (32.6)	**102.35 (33.8)**	2.15	**109.66 (34.4)**	9.46	**90.21 (32.0)**	-9.99
StD	18.46 (3.3)	**18.98 (3.2)**	0.52	**19.71 (3.0)**	1.25	**17.44 (3.3)**	-1.02
SG	67.73 (18.9)	**64.47 (18.9)**	-3.36	**67.67 (19.6)**	-0.06	**69.47 (18.6)**	1.74
EL	19.37 (4.6)	**26.41 (5.2)**	1.85	**23.58 (4.6)**	1.54	**22.81 (3.8)**	-1.72
EP	0.51 (0.1)	**0.50 (0.1)**	-0.01	**0.53 (0.1)**	0.02	**0.50 (0.1)**	-0.01
ED	40.61 (4.2)	**41.35 (4.0)**	0.74	**39.80 (4.4)**	-0.81	**38.15 (4.5)**	-2.46
CD	25.15 (3.2)	**25.30 (2.9)**	0.15	**24.9 (3.3)**	-0.25	**23.68(2.7)**	-1.47
NRE	13.15 (2.1)	**12.61 (2.0)**	-0.54	**11.99 (2.1)**	-1.16	**12.70 (2.1)**	-0.45
NKR	27.23 (5.9)	**28.42 (6.0)**	1.19	**29.82 (5.5)**	2.59	**23.90 (5.6)**	-3.33
HK	61.73 (12.2)	**68.15 (12.6)**	6.42	**65.64 (11.1)**	3.91	**60.00 (9.7)**	-1.73
EN	1.06 (0.1)	**1.05 (0.1)**	-0.01	**1.12 (0.2)**	0.06	**1.04 (0.1)**	-0.02
KL	9.97 (1.1)	**10.44 (1.1)**	0.47	**9.74 (0.7)**	-0.23	**9.64 (0.9)**	-0.33
KW	8.77 (1.0)	**9.20 (1.1)**	0.43	**8.83 (0.9)**	0.06	**8.36 (0.9)**	-0.41
KT	4.65 (0.7)	**4.69 (0.7)**	0.04	**4.64 (0.7)**	-0.01	**4.58 (0.6)**	-0.07
EWT	0.08 (0.0)	**0.09 (0.0)**	0.01	**0.09 (0.0)**	0.01	**0.07 (0.0)**	-0.01
GWT	0.74 (0.2)	**0.80 (0.3)**	0.06	**0.85 (0.1)**	0.11	**0.62 (0.2)**	-0.12
YLD	4.02 (1.2)	**4.36 (1.4)**	0.34	**4.59 (1.0)**	0.57	**3.38 (1.0)**	-0.64

AD = days to 50% anthesis; SD = days to50% silk ; ASI = anthesis-silking interval; TL = tassel length; EA = ear leaf length; EW = ear leaf width; PL = plant height; EHT = ear height; StD = stalk diameter; SG = stay green; EL = ear length; EP = ear position; ED = ear diameter; CD = cob diameter; NRE = number of rows per ear; NKR = number of kernels per row; HK = hundred kernel weight; NP = number of plants harvested; NE

= number of ears harvested per plot; EN = ear number per plant; KL = kernel length; KW = kernel width; KT = kernel thickness; EWT = Ear weight; GWT = grain weight; YLD = yield

4.5.1 Comparison of clusters with overall mean

Cluster I members were characterized by lower values of trait means in comparison to the overall mean in number of days to 50% anthesis and silking, number of rows per ear and ear number. All earliness traits demonstrated higher standard deviation than the overall standard deviation values. Additionally, cluster I demonstrated least values for anthesis-silking interval and stay green. In contrast, cluster I had higher than overall mean values for all plant architectural traits (PLHT, ELL, ELW, EHT, StD, and TL) except ear position and highest mean values for ear diameter, cob diameter, HKWT, KL, KW and KT (Table 4.7). Though cluster I had high values of yield components majority of the ear biomass was occupied by a large unusable cob diameter.

The standard deviations for these were either equal or higher than the overall individual standard deviations. It must be noted that, the average grain yield of cluster I genotypes was 4.36 $Mgha^{-1}$, a value slightly lower than the potential yield of 4.5 $Mgha^{-1}$ in sub-Saharan Africa (Prabhu *et al*., 2000). On the basis of the desirable phenomorphological characteristics exhibited by cluster I maize accessions, their incorporation into current maize breeding programs in Africa would be worthwhile but care must be taken to avoid genotypes with large cob diameter.

Cluster II genotypes demonstrated superior trait values in having the highest values for TL, ELL, ELW, PLHT, StD, NKR, EN, GWT and grain yield though they were late maturing. The late maturing characteristic permitted a better accumulation of biomass in terms of stalk diameter and plant height. Many of the genotypes had more than one ear which translated to high grain yield. The association of grain yield with prolificacy in maize was demonstrated by Lonnquist *et al*., 1967; Carena *et al*., 1998; Agrama, 1996 and Harris *et al*., 1976. The average grain yield of cluster II genotypes of 4.59

was in excess by 0.09 $Mgha^{-1}$ over the current potential yield of 4.5 $Mgha^{-1}$ in sub-Saharan Africa. The identification of such high performing genotypes as those of cluster II among the African maize accessions underscores the importance of embarking on genetic diversity studies. Indeed the findings confirm that the landraces are rich reservoirs of traits, alleles, and genotypes that can be useful to breeding programs.

Cluster III genotypes were the earliest but possessed the longest ASI (7.47 days). These genotypes exhibited the least plant architectural dimensions. PLHT (176.65 cm), TL (45.86 cm), StD (17.44 mm), ELW (8.10 cm), ELL (73.86 cm). Other traits such as the kernel dimensions, HKWT and hence grain yield were the least (Table 4.7). This trend was not unexpected as being early-maturing a short period was available for biomass accumulation. The earliness traits in cluster III genotypes would be of interest to breeding for drought tolerance by escape.

Comparatively, Shrestha (2013) in assessing 60 maize inbred lines from Nepal using morphological characterisation had six distinct clusters while this current study revealed three major clusters from the 47 genotypes. Likewise Azad *et al.* (2012) in assessing 30 maize inbred lines also reported of six cluster groups.

4.5.2 Principal Component Analysis

Principal component analysis identifies variables or set of variables that are highly correlated with each other and reveals traits that contribute most to the variance. Table 4.8 shows the principal components, eigenvalues and eigenvectors generated from the accession by trait correlation matrix. The first three principal components explained 79.1% of the total phenotypic variation in the accessions studied.

Table 4. 8: First three principal components, quantitative morphological traits in the 47 African lowland maize with eigenvalues, eigenvectors, relative and cumulative proportions of the total variation.

Traits	PC1	PC2	PC3
AD	0.399	**0.748**	0.020
SD	0.393	**0.743**	-0.024
ASI	-0.048	-0.063	-0.157
TL	**0.773**	-0.222	-0.212
ELL	**0.894**	0.036	-0.044
EW	**0.826**	0.062	-0.244
PLHT	**0.874**	0.259	-0.200
EHT	**0.796**	0.400	-0.309
EL	**0.762**	-0.316	-0.175
EP	0.474	**0.627**	-0.48
ED	**0.752**	-0.136	**0.573**
CD	**0.571**	-0.010	**0.685**
NRE	0.379	**0.537**	**0.606**
NKR	**0.762**	-0.126	0.266
HKWT	0.467	**-0.724**	-0.158
KL	0.464	**-0.591**	**-0.701**
KW	**0.503**	-0.491	-0.221
YLD	**0.624**	-0.392	0.228
Eigen values	7.292	3.488	1.894
Proportion	45.50%	21.80%	11.80%
Cumulative	45.50%	67.30%	79.10%

AD = days to 50% anthesis, SD = days to50% silk , AS = anthesis to silk interval, TL = tassel length, ELL = ear leaf length, EW = ear leaf width, PLHT = plant height, EH = ear height, 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, KL = kernel length, KW = kernel width, YLD = yield. Eigenvectors greater than 0.500 were considered important to the variance.

The first principal component (PC1) which explained 45.5% of the total variation was correlated with plant architecture (plant height, ear leaf length, ear leaf width, ear

height), ear traits (ear length, ear diameter, cob diameter, number of rows per ear), and yield components (kernel width and yield) as demonstrated by the eigenvectors.

The second principal component (PC2) explained 21.8% of the total variance and the traits that were most important were earliness (AD, SD), ear position, number of rows per ear, and yield components (HKWT, KL and KW). The third principal component (PC3) explained 11.8% of the total variation and the most important traits were ear characteristics including ear diameter, cob diameter, number of rows per ear, and kernel length (Table 4.8).

A plot of the first two principal components which accounted for 67% of the total variance revealed seven major correlation groups in which kernel characteristic (KL, KW, HKWT) and plant architecture (PLHT, EHT, ELW, ELL) were grouped. Ear position and number of rows per ear were grouped with earliness. The reproductive traits, ED, NKR, TL and EL were found in the same correlation group. Interestingly ASI was not grouped with any other trait (Figure 4.3). ASI, CD and YLD showed very little relationship with all the other groups.

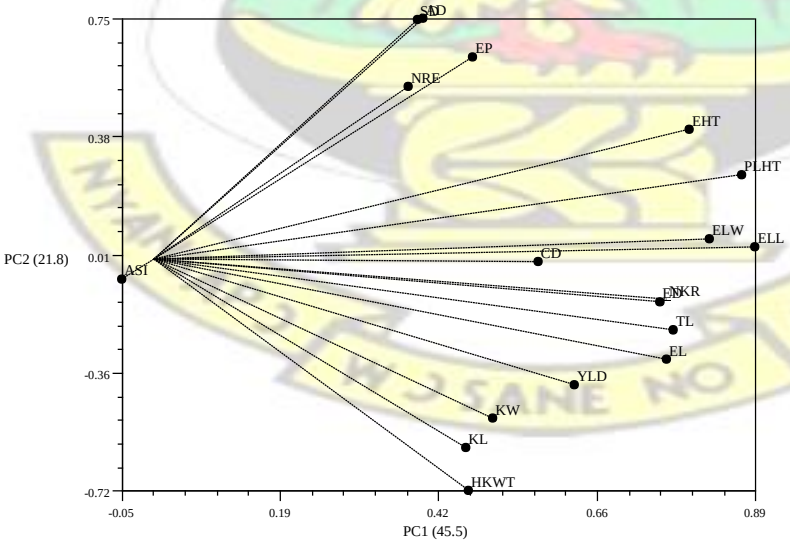

Figure 4.3: Association among the 18 pheno-morphological traits revealed by the first two principal components.

The topography of the PC biplot showed that all traits were correlated except ASI. An angle of approximately 180^{o} was formed between ASI and NRE, EP, AD and SD indicating no correlation between ASI and these traits. ASI is a drought-related trait in which small values signify early seed set in spite of drought conditions and high values mean delay in seed set and/or formation of open tips (when kernels at terminal end of the cob are unfilled) in response to water stress, leading to low values of NRE. The lack of association between ASI and NRE demonstrated signifies an inconsistent trend of some accessions having ears filled to the tip while others did not. This phenomenon would require verification in a drought stress environment.

Among the traits that were correlated, tight angles hence strong positive correlations were formed between four groups, viz., AD and SD, NRE and EP, NKR and AD, and finally ELW and ELL together with CD. On the basis of the length of the vectors, SD, AD, PLHT, ELL and HKWT contributed most to the variance.

Principal component one separated the accessions on the basis of plant architectural traits, ear traits and yield components incorporating accessions such as „Obatanpa GH", TZm-1522, TZm-1505, TZm-1503, TZm-386 and TZm-295. The second principal component separated accessions on the basis of earliness, ear position and number of rows per ear and yield components while the third principal component separated them on the basis of ear characteristics and kernel length. In all „Obatanpa GH", TZm-295, TZm-386, TZm-144, TZm-146 and TZm-1304 greatly contributed to the total variance (Figure 4.3). The origin of accessions did not influence the grouping.

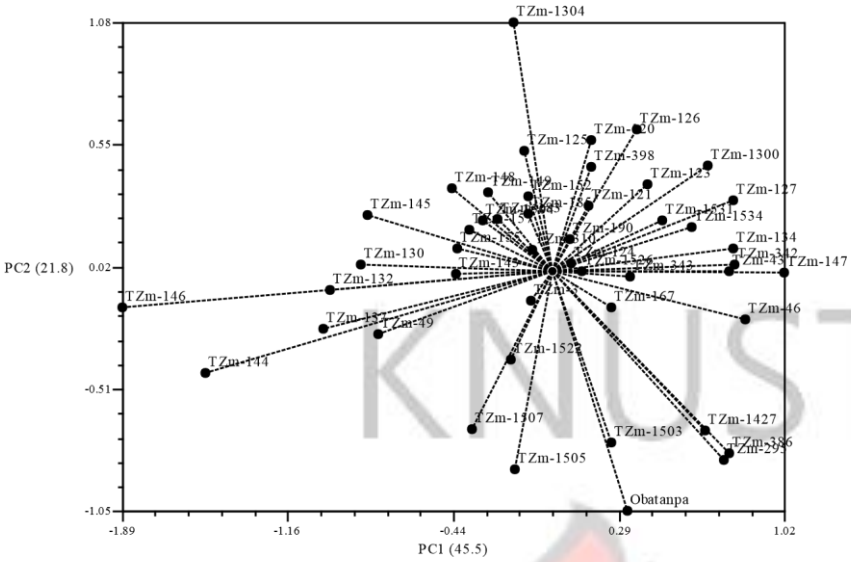

Figure 4.4: Association among the 47 African lowland maize accessions revealed by the first two principal components based on morphological traits.

These morphological results further strengthen the hypothesis of wide variation among maize landraces in Africa. In summary, the study reveals that the large variability in morphological parameters justifies its use for preliminary genetic diversity investigations. The three clusters revealed from the cluster analysis represent putative heterotic groups which breeders can make choices from for maize improvement. Hitherto, maize breeders in Africa relied on exotic materials for crop improvement but this study has revealed that the African lowland landraces constitute a rich source of alleles for genetic improvement of maize for many traits including earliness, plant height, drought tolerance, and grain yield. The study also revealed an unusual trend where genotypes in cluster 1 exhibited early maturity as well as high yield, contrary to the usual trade-off between earliness and yield (Barriere *et al.*, 2010). Plant lodging is normally associated with tall plants, however no occurrence of lodging was recorded despite the occurrence of very tall plants especially in cluster II.

The study further revealed genotypes with short ASI values which would be of great importance in developing drought tolerant cultivars (Clusters I and II) needed in the drought-prone regions of Africa.

4.6 Molecular genetic diversity in the African lowland maize

A total of 64 accessions were chosen from seven countries to represent diversity in lowland African maize. Twenty-five accessions (39.1%) were introduced from the Republic of Benin, eight each (12.5%) from, Congo and Burkina Faso, seven (10.9%) from Guinea, six each (9.4%) from Chad and Togo, four (6.3%) representation from Tanzania.

All 64 accessions produced good quality DNA that amplified well with 14 out of the 16 primers permitting good scoring on the gels. Bands were scored for presence (1) or absence (0) (Appendix B3). Figure 4.5 shows amplification of the SSR marker bnlg1525.

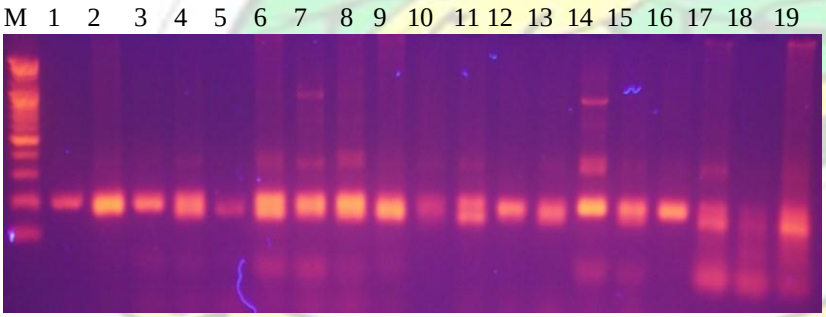

Figure 4. 5: SSR marker profile of 19 African lowland maize produced by primer bnlg1525.

M=molecular marker (100 bp); 1=TZm-335; 2=TZm-1503; 3=TZm-1507; 4=TZm-343; 5=TZm-127; 6=TZm-157; 7=TZm-1505; 8=TZm-149; 9=TZm-132; 10=TZm-310; 11=TZm-124; 12=TZm-144; 13=TZm-143; 14=TZm-399; 15=TZm-394; 16=TZm-185; 17=TZm-1522; 18=TZm-295; 19=TZm-134

Fourteen out of the sixteen primers amplified. Loci nc130 of chromosome 5 and phi 299852 of chromosome 6 failed to amplify. All chromosomes except chromosome 6 were represented by at least one primer locus. Thirteen out of the fourteen amplified loci representing 92.86% rate of polymorphism were detected while one locus, umc1399 was monomorphic. A total of 2,216 alleles were detected from the thirteen loci across 64 genotypes with an average number of 170.46 ± 33.76 alleles per SSR marker, in a range of 114 alleles for locus umc1066 to 228 alleles for locus bnlg1525. The number of alleles ranged from 2 (phi 072) to 10 (phi022) across the loci. A total of 71 distinct alleles were detected in the 13 polymorphic loci with an average of 5.46 ± 1.85 alleles per locus. These values signify that abundant genetic diversity resides within the lowland African genotypes. All primer loci except phi072 had four or more alleles. Table 4.9 shows summary of the standard statistical parameters of the molecular data.

Table 4. 9: Statistics of 13 SSR polymorphic loci, number of alleles per locus, number of alleles across accessions, and expected heterozygosity in 64 lowland African maize accessions.

SSR locus	Bin location	Repeat unit	No. of alleles per locus	No. of alleles across accessions	[1] H_o	[2] H_e	[3] Prob.
phi002	1.08	Tetra-	5	150	0.13	0.49	0.78
bnlg1597	1.09	Di-	7	206	0.61	0.76	0.98
nc133	2.05	Penta-	5	138	0.00	0.76	0.46
phi453121	3.00	Tri-	4	138	0.74	0.67	0.99
phi072	4.01	Tetra-	2	144	0.16	0.26	0.97
bnlg1237	5.06	Di-	6	174	0.74	0.76	0.80
bnlg1695	5.07	Di-	6	158	0.59	0.78	0.95
umc1066	7.01	Hexa-	6	114	0.02	0.64	0.46
phi080	8.08	Penta-	5	210	0.45	0.69	0.71
phi022	9.03	Tetra-	10	188	0.84	0.71	0.99
bnlg1525	9.07	Di-	6	228	0.67	0.70	0.99
umc1367	10.03	Tri-	4	196	0.86	0.56	0.69
umc1196	10.7	Hexa	5	172	0.54	0.70	0.99
Mean		Total	71	2,216	-	-	-
		Minimum	2	114	0.00	0.26	
		Maximum	10	228	0.86	0.78	
		Mean	5.46	170.46	0.47	0.65	
		SD	1.85	33.76	0.30	0.14	
		χ^2			2.13		
		Prob			0.17		

[1] [2] Observed heterozygosity; Expected heterozygosity or polymorphic information content; [3]Probability of a χ^2 at df = 1

No rare allele was identified as all allele frequencies were higher than 0.005. The observed and expected heterozygote frequencies were compared by the chi square goodness-of-fit. The chi square value of 2.13 at 1 degree of freedom showed that H_o was not significantly different (P>0.05) from the tabulated value of 3.84. In general, observed heterozygosity showed lower values than expected heterozygosity values except in three loci, phi453121, phi022, and umc1367, where the observed values were

higher (Table 4.9). The observed and expected heterozygosity ranged from 0.00 (nc133) to 0.86 (umc1367) and 0.26 (phi072) to 0.78 (bnlg1695), with mean values of 0.46 ± 0.30 and 0.65±0.14, respectively. The analysis revealed that the observed heterozygosities did not significantly differ (P >0.05) from the expected heterozygosities at all loci, hence the population was in Hardy-Weinberg equilibrium at each of the loci considered. In all, 76.92% of the loci (bnlg1597, nc133, phi453121, bnlg1237, bnlg1695, umc1066, phi080, phi022, bnlg1525 and umc1196) had expected heterozygosities of 0.60 or more (Table 4.9), an indication of abundant genetic variability, rich informativeness and large discriminatory power of those markers (Hartings *et al.,* 2008). Future studies in maize genotypes may rely on these loci for both distinguishing and grouping of genotypes in addition to determining genetic diversity estimates with certainty. This value is sufficient to indicate widespread genetic variation among the genotypes. The high heterozygosity in the maize population is suggestive of a historic admixture of populations of independent origins or an isolate-breaking effect in the past which may have introduced new alleles to the maize population in Africa.

Mateu *et al*. (1997) confirmed that admixture leads to high heterozygosity, an event, which causes the resultant population to have equal or higher heterozygosity than the individual populations. Moreover, old populations tend to have larger heterozygosity as they have preserved genetic variation, and balancing selection in favour of heterozygotes. It is expected that high values of average heterozygosity correlates with high levels of genetic variation. Additionally, high mutation rates at microsatellite loci can also give rise to high heterozygosity (Vigouroux *et al.*, 2002)

It must be noted that the total number of alleles in any genetic diversity study is usually proportional to the sample size and number of markers used. Hence the average number

of alleles per locus obtained in this study is relatively higher compared to those reported in other maize studies. Warburton *et al*. (2002) and Lu and Bernardo (2001) both reported 4.9 alleles per SSR locus for 40 U.S. inbred maize amplified with 83 SSR markers and 57 CML lines with 85 SSR markers, respectively. Senior *et al*. (1998) also reported an average of 5 alleles per locus in a study of 94 elite U.S. maize inbred lines with 70 SSR markers while 6.3 and 6.8 alleles per locus were reported by Reif *et al.* (2003) and Pejic *et al*. (1998) in examining 366 maize genotypes with 85 SSR and 33 U.S. maize lines with 27 SSR markers, respectively. Similarly, Ranatunga *et al*. (2009) and Beyene *et al*. (2006) reported average number of alleles of 4.9 and 6.0 among 62 traditional Ethiopian highland maize evaluated with 20 SSR markers and 45 maize genotypes examined with 22 primers,

respectively.

Despite the fewer number of SSRs in current study, the alleles identified per locus was high compared to the 2.3 reported by Kanagarasu *et al.* (2013) in evaluation of 27 inbred lines with 10 SSR primers and 3.1 by Kumar *et al.* (2012) in accessing 91 maize genotypes with 40 SSR loci. The high number of alleles recorded per locus is indicative of the presence of broad genetic base of the genotypes.

Majority of the alleles were produced by the di- and tetra- repeats while the tri-, penta- and hexa- produced fewer alleles. The di-repeats contributed 766 alleles representing 37.5%, the tetra produced 482 representing 23.6% while the tri-, penta- and hexa-repeats each contributed 334 (16.3%), 348 (17%) and 114 (5.6%), respectively (Table 4.10). The higher number of alleles contributed by the di- repeats was not unexpected as this phenomenon was demonstrated in another maize study (Pejic *et al*., 1998). Di-repeats have a higher probability for slippage to create variation in the genome. Consequently, the di-repeats in this study produced relatively higher number of alleles

than all the other repeat motifs with an average of 5.75 alleles per locus and 153.2 alleles across the entire population.

Table 4. 10: Information score summary by repeat class of SSR loci

Repeat class	Average number of alleles per loci	Average numb(alleles across genotypes	Average H_e
Di	5.75	153.2	0.75
Tri	4	167	0.62
Tetra	5.7	160	0.49
Penta	5	174	0.73
Hexa	6	114	0.64

Eleven out of the twelve (91.6%) of the SSR loci had PIC exceeding 0.3, indicating the good discriminatory power of the markers identified.

The mean H_e was similar to that reported for Japanese inbred maize of 0.69 (Enoki *et al*., 2002), 0.62 for the North American inbreds belonging to the Iowa Stiff Stalk and B73 (Smith *et al*., 1997), 0.60 for CIMMYT inbred lines (Xia *et al*., 2004), 0.59 of U.S. maize germplasm collection (Senior *et al*., 1998) and 0.58 of Ethiopian inbreds (Legesse *et al*., 2007).

On the contrary, the mean H_e value in current study was higher (0.45) than that of India and Mexico inbred lines (Kanagarasu *et al*., 2013) 0.54 (Choukan *et al*., 2006) and 0.51 (Aguiar *et al*., 2008) of Iranian maize inbred lines. The di-repeat SSR loci gave the highest mean H_e of 0.75 while the tri-, tetra-, penta- and hexa- SSR average PIC values ranged from 0.49 to 0.73 (Table 4.9). The highest mean PIC value of the di-repeat SSR loci is consistent with the results of Enoki *et al*. (2002), Smith *et al*.

(1997) and Senior *et al*. (1998). The combined high average PIC and the high number of alleles recorded indicate the presence of many rare alleles (Reif *et al*., 2004; Xia *et al*., 2004) in these African lowland landraces evaluated in this study.

A high heterozygosity of 0.65 obtained in the current research was not expected as it is generally believed that the African genotypes were predominantly introduced from a single source, that is, Mexico by the Portuguese (McCann, 2005, 2001; Miracle, 1965) and to a minor extent the Arabian route. A heterozygosity as high as 0.65 suggests high accumulation of mutations, a historic admixture involving previously differentiated populations or from the highly polymorphic SSR loci. The high level of heterozygosity is indicative of a wide allelic diversity modulated by other evolutionary forces such as mutation, recombination and gene flow which increase variability among genotypes or genetic drift and speciation which decreases variability. The plausible explanation to this finding is that the current Africa maize germplasm may have been formed from an already existing maize germplasm on the continent with Portuguese maize introduction. Further investigations into the genetics of the African maize germplasm are required to explain this level of heterozygosity. The artefacts of maize ears found in Nigeria which dated back to the pre-Columbian era may support the existence of maize in Africa prior to the Portuguese introduction. The high levels of heterozygosity also confirm the allelic richness of the landraces which are of importance in breeding programs.

4.7 Genetic similarity and cluster analysis based on SSR profiling

Genetic distance among the accessions was estimated by means of DICE dissimilarity coefficient. Genetic distances among the lowland population were characterized by a wide range of coefficients from 0.30 (Tzm-1505/TZm-145) to 1.00 and an overall average of 0.70±0.10. Ten accession pairs (TZm-121/TZm-1525, TZm-123/1507,

TZm-145/ TZm-190, TZm-157/1505, TZm-154/TZm-1503, TZm-154/TZm-1507, TZm-183/TZm-125, TZm-183/TZm-306, TZm-183/TZm-1304, TZm-183/TZm-1504) with genetic distance of 1.00 were unrelated. Additionally, the dissimilarity coefficients (96.58%) were predominantly high and exceeded 0.50 except for 69 out of the 2016 coefficients indicating that the accessions were highly variable. The pairwise genetic distance matrix is shown in Appendix B2.

To better visualize the relationships among SSR markers and accessions, a UPGMA cluster analysis was performed on the distance matrix to generate a dendrogram (Figure 4.5). Two distinct clusters independent of geographical origins were formed from the 64 genotypes. Cluster I was a large heterogeneous group made up of 57 genotypes, while main cluster II was uniformly constituted from only 7 genotypes. Cluster I formed 3 subclusters, viz., cluster IA (20 members, TZm-335 to TZm-43), cluster IB (26 members, TZm-1503 to TZm-149), cluster IC (11 members, TZm-132 to TZm-125). The 7 genotypes of cluster II were TZm-399 to TZm-147.

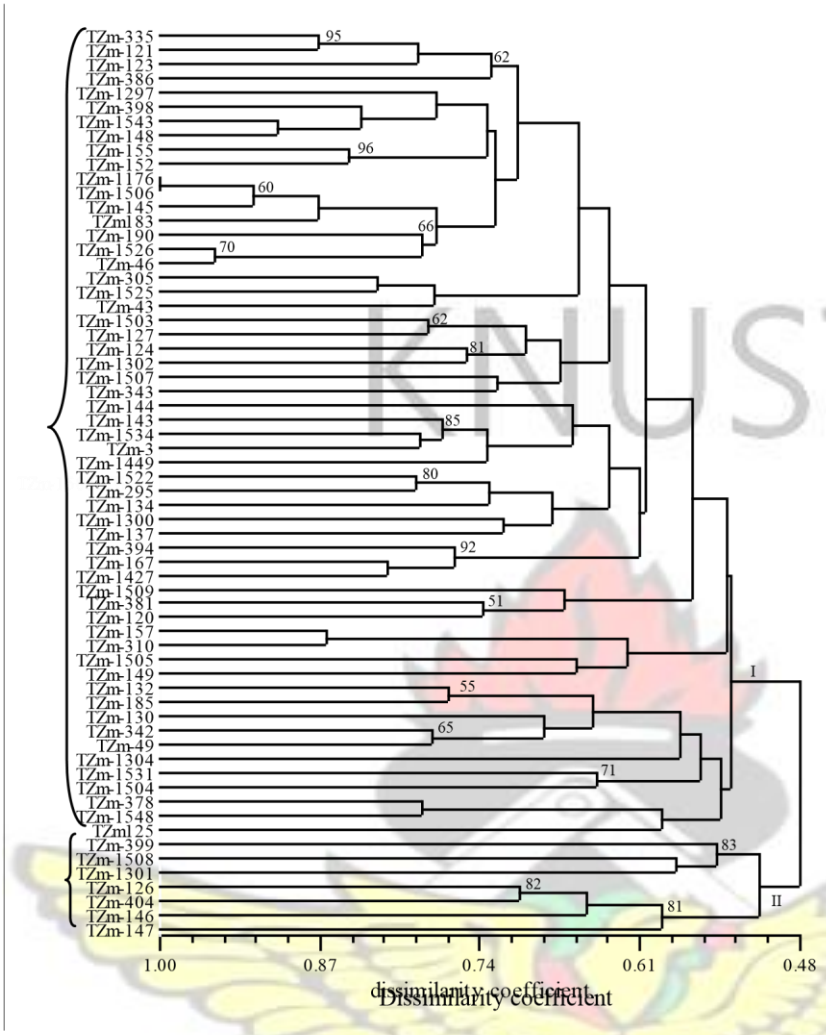

Figure 4.6 Dendrogram showing the relationship among sixty-four African lowland maize landraces based on 13 SSR primer pairs

The clusters belong to different groups and can form the basis for development of inbred lines for hybrid breeding for desirable traits.

The most similar accession pairs having the least dissimilarity coefficient of between 0.3-0.5 were, 35 in number. Although all were lowland genotypes, climatic conditions, farmers‟ selection and various environmental stresses in these wide geographical origins may have contributed to the observed dissimilarities.

The cophenetic coefficient of 0.80 demonstrates a good approximation of the dendrogram to the similarity matrix. The bootstrap figures demonstrated the percentage accuracy of location of genotypes on the dendrogram.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Genetic diversity in maize is of great value and plays a major role in breeding programs. Maize germplasm collections deposited in resource centres serve as a source of diversity and must be well characterised for effective exploitation in future works. The objective of this study was to determine the genetic diversity among the lowland African maize accessions. Following the growing concerns with respect to climate change and population increase with reduced arable lands there is the need to explore our landraces which have been found to be a store of rich traits and alleles which are very essential in developing genotypes with the potential to withstand the current severe climatic conditions.

Determining variations in existing landraces would lead to preserving traits and alleles of relevance from genetic erosion. However, there have not been any thorough investigations into the diversity of the lowland landraces in Africa across a wide geographical area. The absence of a widespread database on morphological and molecular characteristics of these lowland maize accessions in Africa was indispensable to quantify the genetic diversity and identify useful genotypes that could be beneficial to breeding programs in Africa.

With the assumption that landraces harbour huge potential for plant development, the study was set to determine the following:

a. Are landraces truly a rich source of genetic diversity?

b. Do the lowland genotypes actually harbour useful traits and unique alleles yet untapped?
c. Narrow down the large number of accessions in genetic resource centres to only useful genotypes for incorporation into breeding programs
d. Does the extent of variation reveal the historical basis of the diversity in the African lowland maize germplasm?

The research encompassed both morphological and molecular evaluation of the sampled genotypes followed by application of robust statistical techniques to substantiate the findings. Although morphological evaluations have been used in various maize studies, they are known to be influenced by environmental factors, require large plant population sizes, known to produce low polymorphism hence provide unreliable results. Thus molecular assessment by means of SSR profiling was carried out to strengthen the variability among the genotypes.

Simple Sequence Repeat markers were chosen over other molecular marker methods because they detect polymorphism with high level of efficiency, are reliable, cost effective and the data easy to analyse and interpret.

A total of 46 accessions originating from seven countries spanning latitude -8.85° S in Tanzania to 12.9° N in Chad and longitude 39.3°E to 10.7°W at elevation range of 50 to 700 m.a.s.l. and a check „Obatanpa GH" were employed in the morphological study while 64 accessions were used for the molecular evaluation. The morphological study revealed a large variability in qualitative traits including silk colour, principal grain colour, kernel arrangement and kernel texture. Cob colour was predominantly similar among the accessions. With regard to kernel texture, flint and dent types dominated the accessions confirming the generally accepted view of the two routes of maize

introduction to Africa; the Mediterranean and Portuguese routes. The combined analysis of variance revealed a wide variation in quantitative traits. The existence of large variability in all the traits signifies that it is possible to enhance the traits through selection especially for TZm-1505, TZm-49, TZm-295, TZm-1427, TZm1503, TZm-386, TZm-343 and TZm-1522. The early-maturing, yet high yielding accessions identified, demonstrates the fact that the landraces possess unique genes which have not yet been exploited. In modern maize breeding, there is usually a tradeoff between earliness and yield. However, recent molecular biology studies seek to identify traits that would link early-maturity to high yield, but with little success hence the need to exploit the landraces.

The importance of genotypes that exhibit short anthesis-silking intervals to the development of drought tolerant cultivars cannot be overemphasized. The three accessions, TZm-167, TZm-155, and TZm-130 of Benin having the shortest ASI would be important to maize breeding for drought tolerance. Such genotypes are of great potentials for developing high yielding-drought tolerant varieties needed in Africa. With increasing climatic pressure on plant survival and food security, the need for early maturing genotypes capable of escaping the stress cannot be disregarded. Owing to the current climate anomalies, studies globally have prioritised combining earliness and high yield traits in many maize breeding programs. The current study has revealed eight very early-maturing accessions which also possessed high yields.

Similarly, TZm-1505, TZm-49, TZm-157, TZm-183, TZm-143, TZm-149, TZm1503, TZm-1522 and TZm-124 with plant height less than 1.8 m exhibited high grain yield potentials (3.7 - 5.0 $Mgha^{-1}$). The high yielding accessions of grain yield in excess of

4.00 Mgha^{-1} (TZm-167, TZm-1300, TZm-127, TZm-134, TZm-1503, TZm1534, TZm-1505, TZm-147, TZm-386 and TZm-295) represent completely new

genotypes that could be incorporated into breeding programs.

The accessions also showed variability in yield and yield component traits across the lowland regions. Accessions from Benin recorded the shortest plant height (185.9 cm) although they were higher than the check from Ghana (171.8 cm) while the accessions from Togo recorded the tallest (216.61 cm). Accessions from Tanzania had the shortest anthesis-silking interval (5 days) across the countries while the longest was observed in accessions from Burkina Faso (7 days). It must be noted that the accessions from Tanzania, however, showed higher anthesis-silking interval compared to the check (4 days). The least number of kernels per rows was observed in accessions from Benin (25.78) while the highest was observed in Togo genotypes (30.32) which were also higher than what was observed from the check (26.63).

Similarly, the least number of rows per ear were observed in the genotypes from Tanzania (12.4) while the highest was from the genotypes from Togo (14.8). In spite of the observed variability in the yield components, there was no significant difference in yield across the countries compared to the check. The large variability in the yield and yield components indicates that it is possible to enhance yield through hybridization and selection using the local landraces which constitute a rich source of alleles.

The low to medium broad-sense heritability estimates indicate large environmental influence to moderate genetic effects on the traits considered. Heritabilities in excess of 0.30 suggest minor additive genetic effects and possibly some dominance effects too. It is possible to improve these traits via selection but not without difficulty.

Because heritability estimates are a function of genotype and the environment further work is needed to validate the estimates in different locations. Because the most important trait of maize is grain yield and breeders strive to shorten the cycles in each generation, correlated response to selection plays a significant role in the success of breeding.

The study revealed substantial amounts of genotypic and phenotypic variance present in the maize populations for all traits except ear position, kernel traits and yield and yield components. Phenotypic coefficient of variation for all traits were generally about four times higher than genotypic values with PLHT (227%), EHT (240%), SG (182%), and HKWT (109%) recording highest values. The high estimates indicate a substantial variation in these traits which can be exploited for genetic improvement. This further confirms the assertion that landraces are good materials to exploit for future genetic improvement for crop species.

In this study, traits which correlated with grain yield, the ear leaf width and tassel length in a positive and significant fashion, would be important to consider for selection for increased gain in yield.

Cluster analysis revealed three clusters, from which further studies can be tailored to crop improvement development. A correlation distance coefficient of 0.28 (28%) represents a large dissimilarity among the accessions hence a wide genetic diversity thus providing researchers and plant breeders with a great opportunity for crop improvement through selection.

Assessing both qualitative and quantitative morphological traits of existing landraces may be useful in maintaining their genetic diversity and preserving them from genetic erosion.

Molecular profiling by SSR produced by the DICE dissimilarity coefficient was 0.70. The high dissimilarity values are in agreement with the variations also revealed by the morphological evaluation. The inference here is that the morphological evaluations although generally not precise provides basic information on diversity studies which can further be evaluated by molecular markers.

The high values of dissimilarity are suggestive that most of these accessions may have originated and evolved from different ancestors confirming that the African accessions are highly variable, and that this variability indicates the possibility of making progress in development of improved genotypes via selection. It is therefore imperative that germplasm in genetic resource centres be fully evaluated before usage in breeding programs to avoid narrowing the genetic base of improved genotypes.

The current study confirms that the landraces harbour rich genetic resources with several useful alleles which can be incorporated into the maize breeding program in the face of the current challenge of climate change, food security and the declining arable land of the world. In the fight against global hunger and poverty particularly in sub-Saharan Africa, knowledge on genetic diversity of maize, an important strategic crop is critical in the quest for its improvement focusing on grain yield.

The forces of evolution that drive increase in variation in populations include mutation, recombination, migration and gene flow.

The molecular profiling of the accessions demonstrated high polymorphism in all the primer loci used in the current study. Number of alleles per locus ranged from 2-10 with phi022 being the most polymorphic primer locus. An average PIC of 0.65 was similar to those of Japan, North America, CIMMYT and Ethiopian genotypes but higher than those of Mexico and India. The African lowland maize possesses a higher

number of alleles and must be harnessed for maize improvement programs especially in contemporary times where the impact on food security has been pronounced.

The two groups revealed by the cluster analysis represent heterotic groups from which inbred lines may be produced for hybrid development. All primer loci were found to contribute to the total variance hence they are reliable and may be used for genotyping a larger number of maize accessions. Although they were all lowland accessions, some accessions were not related to the rest of the groupings and such accessions may have been introduced to Africa from disparate origins as purported by the two schools of thought that maize was introduced to Africa from two major trade routes, the Portuguese and Mediterranean introduction though there was evidence of preColombia maize. Nevertheless, the large number of clusters may have resulted from by divergence over time and space.

In comparison, morphological assessment grouped the four most promising genotypes in two different heterotic groups (TZm-1505, TZm-1503 and TZm-1522 in cluster I and TZm-49 in cluster II) while the molecular assessment placed them into four subclusters under cluster I. In summary, molecular markers proved to be a more reliable tool in identifying variation among the maize accessions than the phenomorphological traits.

Finally, results from both morphological and molecular studies revealed large variability and the presence of rich alleles in the African maize genotypes and must therefore be harnessed to produce inbred lines for developing hybrids most suitable for the sub-region. Secondly, the general notion that landraces harbour rich traits, alleles and genotypes has been largely confirmed by these studies.

In conclusion, the study has (i) indicated that geographical locations have not played any major role in the diversity in African lowland maize landraces , (ii) confirmed the existence of rich genetic diversity in the African lowland maize germplasm, (iii) shown that all traits were variable except number of ears per plant (NE) and kernel thickness (KT), (iv) revealed a no correlation between ASI and NRE, AD and SD (v) the genetic diversity by agro-morphological evaluation gave a genetic similarity of 28% (vi) 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 limitation to this study was the use of few number of SSR primers in the molecular profiling as the number of SSR are expected to be proportional to the sample size.

5.2 Recommendations

- Any genetic studies using morphological traits must be further validated with a molecular marker.
- Future research work on the maize collection should include more SSR primers proportional in number to the number of accessions studied to increase accuracy.
- Similar studies to cover larger number of lowland accessions over a wide geographical scope be conducted to confirm or otherwise, the genetic diversity estimates reported in this study.
- Determination of the inheritance of earliness and anthesis-silking intervals, ear leaf width and tassel lengths

- Studies to ascertain the mechanism of drought tolerance among the earlymaturing and short anthesis-silking interval genotypes be undertaken.

- The use of polyacrylamide gel is also recommended since it gives a better resolution of SSR bands than agarose gel applied for current study.

- The population genetics parameters be extended to cover F_{ST} and analysis of molecular variance (AMOVA)

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APPENDICES

Appendix A1

2% CTAB buffer

2.0 g CTAB (Hexadecyl trimethyl-ammonium bromide)

10.0 ml 1M Tris pH 8.0

4.0 ml 0.5 M EDTA pH 8.0 (Ethylene diamine tetra acetic acid Di-sodium salt)

28.0 ml 5 M NaCl

40.0 ml H_2O

1 g PVP 40 (polyvinyl pyrrolidone)

0.2% β-mercaptoethanol (added just before use)

Appendix A2

Chloroform:isoamyl alcohol (24:1)

960 ml/L Chloroform
40 ml/L Isoamyl alcohol

Appendix A3

Chloroform:Isoamyalcohol (1 :1)

40 ml/L Chloroform
40 ml/L Isoamyl alcohol

Appendix A4

Washing buffer

Prepared by adding 380 ml absolute EtOH, 5 ml of 1 M NH_4OAc and 115 ml of dH_2O

Appendix A5

TE buffer

10 mM Tris-HCl (pH 8.0)

1 mM ETDA (pH 8.0)

B1 Morphological distance matrix

	Obatanpa	TZm-120	TZm-121	TZm-123	TZm-124	TZm-125	TZm-126	TZm-127	TZm-130	TZm-1300	TZm-1304	TZm-132	TZm-134	TZm-137	TZm-1427	TZm-143	TZm-144	TZm-145	TZm-146	TZm-147	TZm-148	TZm-149	TZm-1503
Obatanpa																							
TZm-120	0.46																						
TZm-121	0.56	0.57																					
TZm-123	0.76	0.43	0.67																				
TZm-124	0.36	0.06	0.36	0.35																			
TZm-125	0.49	0.63	0.38	0.38	0.21																		
TZm-126	0.67	0.5	0.49	0.76	0.15	0.56																	
TZm-127	0.01	0.36	0.02	0.09	0.63	0.28	0.46																
TZm-130	0.3	0.07	0.02	0.44	0.5	0.48	0.15	0.07															
TZm-1300	0.32	0.33	0.22	0.4	0.03	0.54	0.6	0.29	0.09														
TZm-1304	0.64	0.63	0.36	0.48	0.01	0.71	0.6	0.27	0.1	0.54													
TZm-132	0.3	0.05	0.1	0.56	0.44	0.37	0.26	0.04	0.85	0.1	0.14												
TZm-134	0.31	0.15	0.26	0.62	0.29	0.09	0.47	0.12	0.51	0.2	0.12	0.56											
TZm-137	0.08	0.05	0.01	0.13	0.46	0.07	0.33	0.73	0.06	0.45	0.14	0.24	0.09										
TZm-1427	0.28	0.68	0.22	0.13	0.28	0.88	0.38	0.25	0.55	0.42	0.75	0.44	0.36	0.06									
TZm-143	0.29	0.3	0.16	0.15	0.34	0.01	0.14	0.32	0.15	0.47	0.13	0.14	0	0.52	0.13								

Appendix

TZm-144	0.18	0.17	0.32	0.56	0.14	0.22	0.51	0.54	0.15	0.52	0.28	0.47	0.38	0.83	0	0.38						
TZm-145	0.18	0.72	0.37	0.18	0.11	0.37	0.22	0.47	0.14	0.06	0.54	0	0.34	0.09	0.56	0.34	0.17					
TZm-146	0.28	0.1	0.28	0.17	0.45	0.09	0.05	0.65	0.18	0.4	0.18	0.07	0.29	0.8	0.1	0.57	0.58	0.17				
TZm-147	0.03	0.14	0.3	0.15	0.05	0.16	0.3	0.25	0.44	0.24	0.35	0.24	0.54	0.14	0.33	0.28	0.13	0.4	0.05			
TZm-148	0.61	0.29	0.58	0.84	0.08	0.41	0.69	0.35	0.22	0.39	0.56	0.47	0.4	0.46	0.18	0.06	0.73	0.35	0.22	0.06		
TZm-149	0.33	0.75	0.29	0.22	0.23	0.55	0.2	0.18	0.14	0.11	0.41	0.27	0.17	0.62	0.67	0.45	0.35	0.43	0.45	0.11	0.09	
TZm-1503	0.41	0.29	0.41	0.57	0.12	0.36	0.65	0.15	0.14	0.39	0.32	0.04	0.21	0.19	0.2	0.11	0.33	0.01	0.05	0.06	0.51	0.13
TZm-1504	0.4	0.15	0.07	0.17	0.08	0.12	0.25	0.17	0.17	0.08	0.33	0.05	0.1	0.25	0.09	0.22	0.22	0.12	0.01	0.02	0.22	0.07	0.22
TZm-1505	0.66	0.44	0.6	0.69	0.02	0.48	0.8	0.2	0	0.41	0.62	0.21	0.27	0.19	0.31	0.12	0.44	0.33	0.02	0.07	0.71	0.2	0.73
TZm-1507	0.36	0.54	0.4	0.59	0.1	0.72	0.69	0.33	0.17	0.52	0.52	0.02	0.41	0.16	0.58	0.07	0.45	0.4	0.05	0.16	0.57	0.43	0.41
TZm-152	0.44	0.41	0.28	0.23	0.17	0.35	0.58	0.05	0	0.05	0.25	0.2	0.18	0.35	0.33	0.08	0.27	0.12	0.47	0.2	0.01	0.49	0.28
TZm-1522	0.61	0.2	0.26	0.58	0.14	0.33	0.61	0.06	0.14	0.27	0.59	0.44	0.51	0.07	0.26	0.36	0.22	0.22	0.12	0.04	0.56	0.12	0.36
TZm-1526	0.43	0.03	0.4	0.28	0.62	0.16	0.13	0.76	0.02	0.27	0.03	0.07	0.22	0.71	0.05	0.59	0.4	0.1	0.73	0.29	0.03	0.42	0.01
TZm-1531	0	0.27	0.25	0.18	0.13	0.1	0.15	0.4	0.03	0.54	0.4	0.11	0.22	0.38	0.27	0.23	0.17	0.15	0.4	0.1	0.12	0	0.13
TZm-1534	0.28	0.07	0.41	0.14	0.41	0.01	0.13	0.4	0.02	0.26	0.36	0.21	0.29	0.36	0.23	0.31	0.26	0.25	0.57	0.06	0.04	0.06	0.11
TZm-155	0	0.1	0.24	0.2	0.29	0.05	0.31	0.12	0.24	0.05	0.02	0.07	0.28	0.33	0.15	0.13	0.35	0.05	0	0.11	0.34	0.31	0.62
TZm-157	0.17	0.45	0.39	0.09	0.04	0.74	0.05	0.03	0.66	0.08	0.25	0.64	0.26	0.23	0.63	0.17	0.09	0.36	0.13	0.33	0.13	0.55	0.22
TZm-167	0.18	0.34	0.04	0.11	0.1	0.16	0.11	0.24	0.01	0.05	0.3	0.24	0.27	0.59	0.37	0.06	0.58	0.07	0.45	0.15	0.34	0.57	0.03
TZm-183	0.25	0.61	0.45	0.28	0.01	0.49	0.15	0.14	0.41	0.09	0.31	0.24	0.23	0.04	0.49	0.47	0.17	0.5	0.06	0.33	0.26	0.45	0.44
TZm-185	0.31	0.21	0.51	0.34	0.15	0.4	0.23	0.24	0.25	0.08	0.42	0.06	0.09	0.24	0.39	0.26	0.02	0.43	0.16	0.49	0.42	0.33	0.22
TZm-190	0.28	0.27	0.22	0.5	0.25	0.04	0.51	0.29	0.18	0.02	0.07	0.35	0.53	0.47	0.24	0.25	0.54	0.07	0.15	0.14	0.71	0.53	0.44
TZm-295	0.68	0.71	0.64	0.52	0.06	0.69	0.59	0.08	0.28	0.36	0.77	0.08	0.08	0.03	0.63	0.25	0.18	0.47	0.15	0.39	0.47	0.53	0.65
TZm-3	0.07	0.29	0.02	0.19	0.06	0	0.21	0.43	0.31	0.03	0.25	0.21	0.21	0.07	0.13	0.19	0.1	0.47	0.18	0.04	0.32	0.1	0.08
TZm-310	0.16	0.05	0.02	0.26	0.22	0.45	0.15	0.4	0.28	0.52	0.17	0.15	0.27	0.16	0.26	0.12	0.06	0.41	0.12	0.09	0.1	0.27	0.41
TZm-342	0.24	0.33	0	0.42	0.37	0.42	0.19	0.11	0.56	0.15	0.09	0.69	0.47	0.23	0.61	0.15	0.33	0.39	0.02	0.32	0.37	0.45	0.43
TZm-343	0.16	0.22	0.1	0.06	0.23	0.2	0.07	0.03	0.45	0.04	0.28	0.44	0.06	0.05	0.1	0.11	0.06	0.12	0.23	0.2	0.25	0.27	0.46
TZm-386	0.36	0.84	0.32	0.24	0.33	0.64	0.59	0.66	0.24	0.41	0.73	0.09	0.24	0.28	0.72	0.07	0.26	0.72	0.21	0.14	0.31	0.44	0.3
TZm-398	0.03	0.56	0.01	0.15	0.22	0.36	0.15	0.41	0.27	0.46	0.44	0.23	0.53	0.23	0.48	0.2	0.1	0.27	0.27	0.18	0.13	0.27	0.1
TZm-43	0.1	0.21	0.02	0.22	0.3	0.2	0.12	0.18	0.23	0.14	0.04	0.03	0.37	0.41	0.23	0.1	0.15	0.08	0.36	0.34	0	0.49	0.28
TZm-46	0.01	0.22	0.32	0.07	0.27	0.33	0.28	0.29	0	0.37	0.27	0.22	0.21	0.13	0.45	0.35	0.2	0.05	0.13	0.33	0.1	0.36	0.02
TZm-49	0.09	0.32	0.08	0.2	0.3	0.3	0.07	0.09	0.5	0.11	0.48	0.24	0.47	0.04	0.47	0.01	0.04	0.29	0.13	0.6	0.12	0.23	0.23

Appendix B1 cont'd.

	TZm-1504	TZm-1505	TZm-1507	TZm-152	TZm-1522	TZm-1526	TZm-1531	TZm-1534	TZm-155	TZm-157	TZm-167	TZm-183	TZm-185	TZm-190	TZm-295	TZm-3	TZm-310	TZm-342	TZm-343	TZm-386	TZm-398	TZm-43	TZm-46
TZm-1505	0.42																						
TZm-1507	0.04	0.61																					
TZm-152	0.32	0.48	0.35																				
TZm-1522	0.17	0.58	0.55	0.28																			
TZm-1526	0.13	0.07	0.05	0.15	0.26																		
TZm-1531	0.15	0.03	0.04	0.03	0.07	0.58																	
TZm-1534	0.18	0.15	0.11	0.09	0.06	0.69	0.55																
TZm-155	0.09	0.56	0.27	0.12	0.34	0.1	0.18	0.14															
TZm-157	0.09	0.21	0.48	0.11	0.03	0.44	0.31	0.36	0.02														
TZm-167	0.41	0.11	0.03	0.65	0.05	0.12	0.27	0.16	0.28	0.03													
TZm-183	0.38	0.22	0.3	0.09	0.14	0.24	0.1	0.18	0.17	0.67	0.24												

Appendix

TZm-185	0.3	0.57	0.47	0.24	0.48	0.55	0.47	0.11	0.32	0.51	0.02	0.26											
TZm-190	0.26	0.53	0.29	0.07	0.44	0.08	0.36	0.02	0.62	0.14	0.44	0.14	0.27										
TZm-295	0.18	0.78	0.5	0.43	0.5	0.27	0.1	0.04	0.34	0.5	0.28	0.61	0.52	0.08									
TZm-3	0.36	0.18	0.13	0.04	0.1	0.4	0.18	0.43	0.13	0.15	0.27	0.1	0.1	0.11	0.01								
TZm-310	0.13	0.19	0.42	0.07	0.24	0.37	0.1	0.07	0.06	0.41	0.05	0.19	0.2	0.02	0.26	0.37							
TZm-342	0.24	0.3	0.12	0.25	0.28	0.06	0	0.09	0.38	0.51	0.28	0.19	0.19	0.46	0.03	0.11	0.17						
TZm-343	0.5	0.02	0.2	0.19	0.09	0.06	0.15	0.24	0.35	0.43	0.14	0.54	0.1	0.16	0.23	0.44	0.34	0.11					
TZm-386	0.09	0.48	0.53	0.42	0.32	0.33	0.55	0.37	0.09	0.28	0.3	0.43	0.1	0.07	0.68	0.33	0.17	0.31	0.13				
TZm-398	0.14	0.06	0.04	0.09	0.23	0.47	0.77	0.43	0.19	0.07	0.43	0.22	0.4	0.47	0.38	0.24	0.06	0.23	0.12	0.7			
TZm-43	0.01	0.11	0.48	0.22	0.12	0.26	0.02	0.14	0.4	0.13	0.28	0.15	0.08	0.21	0.17	0.24	0.24	0.23	0.14	0.01	0.16		
TZm-46	0.46	0	0.24	0.37	0.1	0.37	0.35	0.42	0.44	0.02	0.58	0.29	0.23	0.21	0.05	0.57	0.1	0.24	0.17	0.33	0.38	0.45	
TZm-49	0.02	0.29	0.07	0.2	0.1	0.15	0.32	0.51	0.29	0.17	0.39	0.19	0.25	0.2	0.55	0.11	0.04	0.26	0.16	0.51	0.56	0.46	0.01

B2: Molecular distance matrix

	TZm-33	TZm-1503	TZm-1507	TZm-343	TZm-127	TZm-157	TZm-1505	TZm-149	TZm-132	TZm-310	TZm-124	TZm-144	TZm-143	TZm-399	TZm-394	TZm-185	TZm-1522	TZm-295	TZm-134	TZm-1297	TZm-1300	TZm-1534	TZm-3	TZm-1449	TZm-1508	TZm-1531	TZm-1509	TZm-126	TZm-404
TZm-33																													
TZm-1503	0.75																												
TZm-1507	0.82	0.73																											
TZm-343	0.82	0.77	0.97																										
TZm-127	0.75	0.82	0.80	0.80																									
TZm-157	0.75	0.68	0.77	0.80	0.81																								
TZm-1505	0.77	0.65	0.75	0.79	0.89	1.00																							
TZm-149	0.71	0.61	0.83	0.80	0.62	0.83	0.79																						
TZm-132	0.68	0.72	0.70	0.77	0.70	0.76	0.80	0.76																					
TZm-310	0.77	0.64	0.79	0.83	0.70	0.90	0.91	0.90	0.91																				
TZm-124	0.61	0.52	0.61	0.61	0.57	0.76	0.70	0.79	0.68	0.71																			
TZm-144	0.81	0.81	0.83	0.89	0.83	0.90	0.90	0.76	0.72	0.79	0.70																		
TZm-143	0.86	0.73	0.79	0.86	0.70	0.77	0.77	0.77	0.86	0.81	0.68	0.88																	
TZm-399	0.55	0.64	0.67	0.75	0.65	0.83	0.86	0.77	0.81	0.75	0.64	0.78	0.72																
TZm-394	0.64	0.82	0.75	0.82	0.85	0.77	0.77	0.63	0.78	0.66	0.68	0.81	0.81	0.81															
TZm-185	0.82	0.64	0.83	0.88	0.55	0.73	0.68	0.73	0.75	0.78	0.68	0.75	0.84	0.66	0.75														

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TZm-1522	0.75	0.63	0.69	0.70	0.65	0.81	0.83	0.81	0.83	0.93	0.85	0.77	0.81	0.72	0.63	0.75														
TZm-295	0.84	0.70	0.76	0.72	0.74	0.65	0.70	0.55	0.56	0.52	0.65	0.71	0.69	0.55	0.72	0.72	0.68													
TZm-134	0.79	0.79	0.73	0.70	0.81	0.75	0.75	0.69	0.73	0.73	0.84	0.70	0.80	0.63	0.83	0.85	0.75	0.81												
TZm-1297	0.84	0.84	0.85	0.81	0.83	0.79	0.80	0.79	0.83	0.81	0.68	0.74	0.86	0.69	0.78	0.84	0.80	0.75	0.97											
TZm-1300	0.76	0.80	0.78	0.74	0.83	0.79	0.80	0.73	0.78	0.75	0.68	0.74	0.81	0.69	0.83	0.84	0.74	0.72	0.97	0.95										
TZm-1534	0.71	0.68	0.67	0.68	0.73	0.69	0.68	0.64	0.67	0.69	0.74	0.71	0.72	0.72	0.75	0.78	0.76	0.77	0.89	0.82	0.82									
TZm-3	0.72	0.68	0.74	0.74	0.65	0.73	0.68	0.73	0.69	0.75	0.81	0.77	0.78	0.72	0.75	0.84	0.77	0.69	0.82	0.77	0.77	0.90								
TZm-1449	0.75	0.61	0.70	0.76	0.69	0.75	0.79	0.64	0.72	0.69	0.61	0.77	0.78	0.78	0.81	0.84	0.66	0.71	0.78	0.77	0.82	0.81	0.79							
TZm-1508	0.61	0.61	0.57	0.59	0.65	0.69	0.71	0.58	0.70	0.70	0.62	0.58	0.56	0.62	0.56	0.67	0.67	0.51	0.64	0.68	0.68	0.55	0.54	0.65						
TZm-1531	0.61	0.71	0.70	0.71	0.69	0.69	0.64	0.69	0.59	0.67	0.66	0.73	0.71	0.65	0.71	0.70	0.67	0.51	0.69	0.76	0.76	0.65	0.73	0.65	0.65					
TZm-1509	0.64	0.77	0.63	0.67	0.70	0.70	0.64	0.63	0.56	0.63	0.62	0.73	0.67	0.59	0.70	0.67	0.62	0.57	0.69	0.70	0.73	0.67	0.73	0.73	0.60	0.97				
TZm-126	0.54	0.71	0.69	0.70	0.65	0.63	0.54	0.56	0.54	0.46	0.67	0.69	0.59	0.56	0.72	0.64	0.53	0.55	0.58	0.54	0.60	0.61	0.63	0.61	0.61	0.72	0.77			
TZm-404	0.58	0.71	0.54	0.60	0.77	0.69	0.63	0.56	0.66	0.68	0.70	0.71	0.69	0.59	0.75	0.71	0.63	0.48	0.72	0.69	0.74	0.71	0.71	0.71	0.75	0.75	0.81	0.79		
TZm-147	0.57	0.61	0.63	0.60	0.73	0.64	0.57	0.53	0.57	0.59	0.55	0.68	0.59	0.53	0.63	0.69	0.59	0.52	0.63	0.63	0.69	0.63	0.63	0.63	0.72	0.61	0.63	0.76	0.79	
TZm-146	0.54	0.68	0.63	0.65	0.69	0.69	0.64	0.58	0.56	0.56	0.68	0.69	0.61	0.61	0.69	0.66	0.58	0.49	0.69	0.67	0.72	0.62	0.69	0.67	0.70	0.80	0.80	0.79	0.79	
TZm-1301	0.54	0.68	0.57	0.59	0.70	0.60	0.57	0.53	0.67	0.62	0.56	0.66	0.67	0.57	0.67	0.73	0.63	0.52	0.73	0.76	0.82	0.69	0.70	0.78	0.76	0.76	0.83	0.75	0.88	
TZm-130	0.75	0.67	0.77	0.82	0.77	0.68	0.70	0.54	0.64	0.64	0.67	0.68	0.64	0.68	0.77	0.77	0.68	0.74	0.82	0.76	0.76	0.89	0.84	0.79	0.68	0.64	0.64	0.71	0.79	
TZm-378	0.75	0.71	0.92	0.97	0.73	0.69	0.71	0.69	0.76	0.73	0.68	0.79	0.77	0.73	0.80	0.77	0.72	0.68	0.69	0.76	0.70	0.69	0.73	0.69	0.61	0.72	0.65	0.72	0.64	
TZm-1548	0.58	0.63	0.77	0.80	0.73	0.88	0.88	0.88	0.73	0.88	0.76	0.82	0.67	0.83	0.70	0.62	0.81	0.55	0.61	0.67	0.67	0.61	0.70	0.61	0.64	0.69	0.62	0.58	0.61	
TZm-1302	0.56	0.68	0.56	0.59	0.61	0.73	0.64	0.67	0.69	0.72	0.79	0.82	0.69	0.69	0.69	0.69	0.77	0.61	0.72	0.72	0.75	0.71	0.81	0.71	0.61	0.67	0.73	0.61	0.71	
TZm-305	0.71	0.90	0.84	0.89	0.74	0.68	0.65	0.68	0.77	0.68	0.72	0.82	0.84	0.68	0.95	0.79	0.64	0.78	0.84	0.95	0.95	0.72	0.82	0.76	0.60	0.68	0.73	0.64	0.64	
TZm-342	0.72	0.68	0.85	0.89	0.65	0.64	0.64	0.64	0.70	0.70	0.62	0.73	0.67	0.67	0.70	0.74	0.66	0.65	0.69	0.77	0.73	0.67	0.73	0.73	0.64	0.58	0.60	0.62	0.62	
TZm-1525	0.71	0.81	0.87	0.91	0.74	0.76	0.71	0.69	0.69	0.78	0.73	0.88	0.74	0.70	0.83	0.83	0.72	0.63	0.69	0.77	0.81	0.66	0.81	0.66	0.66	0.72	0.69	0.76	0.76	
TZm-381	0.50	0.63	0.50	0.57	0.56	0.55	0.67	0.60	0.65	0.64	0.69	0.71	0.57	0.71	0.57	0.43	0.70	0.39	0.55	0.65	0.71	0.65	0.71	0.65	0.60	0.65	0.65	0.50	0.70	
TZm125	0.76	0.74	0.67	0.60	0.78	0.78	0.74	0.67	0.53	0.70	0.67	0.73	0.67	0.63	0.63	0.74	0.70	0.61	0.81	0.80	0.83	0.82	0.80	0.76	0.68	0.81	0.83	0.64	0.79	
TZm-1504	0.67	0.89	0.80	0.79	0.80	0.69	0.61	0.62	0.62	0.56	0.54	0.78	0.72	0.62	0.76	0.68	0.56	0.64	0.63	0.66	0.69	0.59	0.66	0.66	0.67	0.70	0.69	0.81	0.72	
TZm-1176	0.64	0.61	0.69	0.60	0.63	0.68	0.57	0.68	0.52	0.62	0.65	0.64	0.56	0.60	0.52	0.62	0.61	0.50	0.62	0.64	0.64	0.64	0.71	0.61	0.69	0.73	0.64	0.64	0.68	
TZm-145	0.80	0.50	0.58	0.50	0.42	0.56	0.30	0.72	0.54	0.65	0.69	0.63	0.67	0.50	0.46	0.70	0.67	0.60	0.59	0.63	0.58	0.54	0.63	0.50	0.64	0.68	0.61	0.58	0.63	
TZm-398	0.67	0.61	0.65	0.67	0.80	0.73	0.67	0.58	0.50	0.52	0.67	0.72	0.62	0.66	0.72	0.56	0.50	0.57	0.60	0.52	0.55	0.63	0.66	0.69	0.53	0.67	0.70	0.78	0.72	
TZm-43	0.65	0.70	0.64	0.59	0.78	0.68	0.68	0.54	0.53	0.48	0.56	0.57	0.56	0.59	0.74	0.52	0.46	0.77	0.67	0.59	0.63	0.53	0.48	0.60	0.61	0.61	0.68	0.69	0.58	
TZm-155	0.65	0.65	0.63	0.65	0.67	0.72	0.65	0.76	0.57	0.58	0.74	0.67	0.61	0.71	0.68	0.54	0.56	0.52	0.56	0.55	0.58	0.61	0.68	0.71	0.59	0.76	0.80	0.78	0.74	
TZm-1304	0.54	0.64	0.75	0.71	0.69	0.70	0.64	0.67	0.55	0.62	0.67	0.76	0.67	0.70	0.73	0.73	0.64	0.55	0.65	0.67	0.73	0.72	0.88	0.72	0.56	0.79	0.79	0.72	0.69	

TZm-167	0.71	0.87	0.65	0.67	0.76	0.61	0.62	0.61	0.73	0.63	0.61	0.85	0.83	0.65	0.74	0.68	0.69	0.60	0.63	0.69	0.73	0.66	0.73	0.76	0.67	0.74	0.80	0.79	0.79
TZm-1427	0.73	0.75	0.67	0.68	0.78	0.63	0.63	0.63	0.78	0.70	0.67	0.78	0.88	0.67	0.71	0.75	0.70	0.65	0.71	0.78	0.70	0.73	0.78	0.77	0.71	0.71	0.78	0.73	0.80
TZm183	0.65	0.73	0.79	0.74	0.85	0.63	0.59	0.63	0.67	0.61	0.65	0.71	0.72	0.61	0.78	0.83	0.67	0.71	0.84	0.81	0.86	0.81	0.86	0.81	0.79	0.84	0.81	0.86	0.86
TZm-137	0.67	0.86	0.70	0.74	0.78	0.66	0.62	0.66	0.71	0.61	0.78	0.65	0.75	0.57	0.82	0.71	0.65	0.71	0.93	0.84	0.84	0.77	0.77	0.71	0.69	0.79	0.77	0.74	0.81
TZm-190	0.72	0.60	0.61	0.61	0.50	0.39	0.38	0.44	0.65	0.65	0.44	0.60	0.82	0.47	0.59	0.82	0.60	0.61	0.61	0.65	0.60	0.60	0.65	0.55	0.56	0.61	0.56	0.55	0.60
TZm-1526	0.61	0.75	0.60	0.65	0.64	0.75	0.72	0.85	0.84	0.84	0.72	0.64	0.68	0.79	0.58	0.68	0.82	0.50	0.75	0.77	0.73	0.73	0.77	0.68	0.80	0.75	0.70	0.55	0.73
TZm-49	0.63	0.50	0.54	0.50	0.50	0.58	0.58	0.65	0.68	0.64	0.71	0.56	0.64	0.64	0.50	0.68	0.71	0.62	0.65	0.64	0.64	0.64	0.68	0.61	0.77	0.54	0.55	0.64	0.68
TZm-1506	0.62	0.74	0.43	0.43	0.70	0.70	0.74	0.67	0.73	0.65	0.67	0.69	0.70	0.74	0.65	0.52	0.69	0.59	0.70	0.73	0.77	0.62	0.62	0.62	0.74	0.59	0.63	0.55	0.69
TZm-46	0.67	0.70	0.67	0.73	0.57	0.67	0.63	0.83	0.79	0.73	0.75	0.59	0.63	0.74	0.58	0.67	0.68	0.55	0.59	0.59	0.55	0.59	0.64	0.64	0.77	0.64	0.61	0.73	0.68
TZm-152	0.73	0.80	0.85	0.82	0.77	0.74	0.55	0.74	0.60	0.62	0.76	0.76	0.72	0.60	0.76	0.71	0.56	0.57	0.65	0.60	0.60	0.60	0.68	0.64	0.65	0.78	0.79	0.92	0.80
TZm-120	0.57	0.61	0.63	0.65	0.60	0.82	0.79	0.91	0.70	0.74	0.83	0.73	0.65	0.87	0.65	0.58	0.69	0.42	0.58	0.58	0.62	0.69	0.73	0.73	0.62	0.69	0.68	0.73	0.73
TZm-386	0.79	0.94	0.94	0.80	0.90	0.73	0.71	0.64	0.61	0.53	0.61	0.73	0.65	0.61	0.83	0.68	0.54	0.75	0.81	0.81	0.85	0.69	0.65	0.65	0.69	0.77	0.68	0.81	0.73
TZm-123	0.88	0.86	1.00	0.87	0.89	0.76	0.82	0.76	0.69	0.68	0.67	0.76	0.73	0.69	0.77	0.68	0.69	0.83	0.83	0.86	0.83	0.83	0.76	0.72	0.59	0.62	0.59	0.62	0.55
TZm-121	0.76	0.85	0.90	0.84	0.88	0.81	0.79	0.67	0.63	0.60	0.71	0.74	0.67	0.67	0.79	0.75	0.70	0.86	0.80	0.78	0.78	0.74	0.70	0.70	0.68	0.72	0.67	0.78	0.63
TZm-1543	0.93	1.00	1.00	0.94	0.94	0.80	0.83	0.75	0.81	0.71	0.78	0.83	0.86	0.71	0.95	0.82	0.63	0.75	0.92	0.92	0.92	0.75	0.75	0.79	0.67	0.71	0.65	0.71	0.75
TZm-148	0.83	0.78	0.80	0.88	0.60	0.73	0.61	0.73	0.78	0.83	0.77	0.80	0.89	0.70	0.81	0.96	0.70	0.64	0.73	0.73	0.70	0.73	0.80	0.73	0.57	0.67	0.65	0.70	0.70

Appendix B2 cont'd

	TZm-404	TZm-147	TZm-146	TZm-1301	TZm-130	TZm-378	TZm-1548	TZm-1302	TZm-305	TZm-342	TZm-1525	TZm-381	TZm125	TZm-1504	TZm-1176	TZm-145	TZm-398	TZm-43	TZm-155	TZm-1304	TZm-167	TZm-1427	TZm183	TZm-137	TZm-190	TZm-1526	TZm-49	TZm-1506	TZm-46	TZm-152	TZm-120	TZm-386	TZm-123	TZm-121	TZm-1543	TZm-148
TZm-404																																			
TZm-147	0.79																																		
TZm-146	0.79	0.74																																	
TZm-1301	0.88	0.75	0.83																																
TZm-130	0.79	0.79	0.79	0.73																															
TZm-378	0.64	0.63	0.61	0.60	0.82																														
TZm-1548	0.61	0.56	0.64	0.57	0.61	0.75																													
TZm-1302	0.71	0.60	0.66	0.76	0.56	0.62	0.72																												
TZm-305	0.64	0.60	0.68	0.79	0.67	0.80	0.68	0.80																											
TZm-342	0.62	0.67	0.73	0.74	0.84	0.86	0.69	0.67	0.88																										
TZm-1525	0.76	0.76	0.76	0.70	0.76	0.86	0.83	0.76	0.84	0.86																									

Appendix

TZm-381	0.70	0.50	0.70	0.79	0.60	0.60	0.70	0.70	0.69	0.75	0.70																	
TZm125	0.79	0.69	0.79	0.81	0.78	0.55	0.61	0.72	0.61	0.59	0.67	0.61																
TZm-1504	0.72	0.82	0.69	0.69	0.68	0.70	0.63	0.64	0.77	0.62	0.77	0.41	0.73															
TZm-1176	0.68	0.67	0.75	0.73	0.67	0.54	0.62	0.75	0.55	0.59	0.64	0.54	0.88	0.75														
TZm-145	0.63	0.55	0.58	0.67	0.50	0.50	0.55	0.65	0.50	0.50	0.56	0.43	0.77	0.67	0.90													
TZm-398	0.72	0.61	0.78	0.58	0.73	0.60	0.60	0.54	0.50	0.54	0.62	0.41	0.80	0.76	0.76	0.63												
TZm-43	0.58	0.53	0.60	0.54	0.65	0.54	0.50	0.53	0.65	0.54	0.54	0.40	0.63	0.74	0.58	0.61	0.73											
TZm-155	0.74	0.52	0.74	0.64	0.59	0.56	0.68	0.67	0.59	0.56	0.62	0.65	0.80	0.70	0.83	0.67	0.96	0.73										
TZm-1304	0.69	0.69	0.81	0.77	0.77	0.68	0.71	0.69	0.70	0.70	0.78	0.67	0.82	0.80	0.85	0.68	0.80	0.58	0.80									
TZm-167	0.79	0.68	0.72	0.79	0.63	0.67	0.59	0.72	0.68	0.61	0.70	0.79	0.78	0.85	0.73	0.72	0.73	0.60	0.81	0.78								
TZm-1427	0.80	0.69	0.67	0.73	0.75	0.71	0.54	0.65	0.65	0.62	0.62	0.67	0.80	0.83	0.75	0.75	0.75	0.60	0.79	0.71	0.93							
TZm183	0.86	0.86	0.81	0.86	0.93	0.79	0.58	0.67	0.75	0.75	0.81	0.57	1.00	1.00	0.83	0.80	0.80	0.69	0.77	1.00	0.94	0.95						
TZm-137	0.81	0.58	0.81	0.80	0.80	0.72	0.59	0.64	0.86	0.69	0.69	0.65	0.77	0.71	0.63	0.60	0.72	0.74	0.74	0.76	0.77	0.83	0.86					
TZm-190	0.60	0.50	0.50	0.60	0.64	0.61	0.33	0.45	0.50	0.56	0.56	0.33	0.70	0.65	0.63	1.00	0.65	0.60	0.47	0.65	0.75	0.78	0.75	0.65				
TZm-1526	0.73	0.50	0.64	0.73	0.69	0.65	0.80	0.68	0.63	0.60	0.60	0.73	0.80	0.64	0.72	0.71	0.53	0.47	0.71	0.75	0.73	0.80	0.78	0.77	0.60			
TZm-49	0.68	0.64	0.57	0.64	0.68	0.54	0.54	0.56	0.47	0.52	0.52	0.57	0.70	0.68	0.72	0.93	0.50	0.60	0.65	0.62	0.76	0.81	0.81	0.68	0.70	0.86		
TZm-1506	0.69	0.55	0.59	0.70	0.52	0.44	0.63	0.66	0.61	0.44	0.52	0.67	0.72	0.69	0.68	0.78	0.58	0.67	0.71	0.62	0.83	0.81	0.75	0.73	0.60	0.90	0.91	
TZm-46	0.68	0.50	0.59	0.59	0.71	0.73	0.73	0.56	0.57	0.61	0.61	0.56	0.64	0.73	0.72	0.79	0.68	0.62	0.88	0.64	0.73	0.80	0.79	0.78	0.61	0.94	0.94	0.83

TZm-152	0.80	0.71	0.80	0.63	0.73	0.74	0.65	0.62	0.68	0.63	0.79	0.40	0.74	0.88	0.68	0.70	0.92	0.79	0.90	0.78	0.84	0.88	0.87	0.86	0.77	0.60	0.60	0.58	0.80						
TZm-120	0.73	0.64	0.73	0.65	0.59	0.58	0.81	0.73	0.56	0.55	0.64	0.71	0.75	0.69	0.73	0.61	0.78	0.53	0.95	0.75	0.80	0.78	0.75	0.64	0.36	0.88	0.81	0.80	0.89	0.79					
TZm-386	0.73	0.82	0.81	0.80	0.77	0.73	0.65	0.64	0.89	0.73	0.82	0.53	0.75	0.85	0.73	0.67	0.70	0.88	0.62	0.75	0.75	0.72	1.00	0.82	0.57	0.56	0.63	0.70	0.61	0.79	0.62				
TZm-123	0.55	0.64	0.66	0.61	0.76	0.72	0.69	0.68	0.86	0.82	0.77	0.62	0.79	0.77	0.65	0.50	0.70	0.71	0.67	0.70	0.75	0.76	0.79	0.76	0.57	0.63	0.58	0.65	0.61	0.70	0.68	0.86			
TZm-121	0.63	0.70	0.74	0.59	0.88	0.88	0.76	0.65	0.93	0.89	1.00	0.56	0.71	0.71	0.60	0.44	0.76	0.73	0.68	0.70	0.63	0.64	0.74	0.74	0.50	0.50	0.52	0.50	0.59	0.76	0.61	0.89	0.95		
TZm-1543	0.75	0.70	0.71	0.72	0.81	0.83	0.67	0.75	0.95	0.80	0.85	0.55	0.71	0.83	0.67	0.63	0.71	0.73	0.68	0.67	0.78	0.81	0.86	0.90	0.57	0.64	0.57	0.70	0.67	0.76	0.65	0.95	0.83	0.83	
TZm-148	0.70	0.62	0.63	0.58	0.73	0.80	0.67	0.73	0.77	0.73	0.81	0.47	0.64	0.70	0.58	0.68	0.70	0.57	0.64	0.64	0.67	0.73	0.69	0.73	0.78	0.60	0.55	0.50	0.68	0.87	0.65	0.65	0.69	0.73	0.79

B3: Binary scoring of SSR amplification products of 64 African lowland maize accessions

	TZm-335	TZm-1503	TZm-1507	TZm-343	TZm-127	TZm-157	TZm-1505	TZm-149	TZm-132	TZm-310	TZm-124	TZm-144	TZm-143	TZm-399	TZm-394	TZm-185	TZm-1522	TZm-295	TZm-134	TZm-1297	TZm-1300	TZm-1534	TZm-3	TZm-1449	TZm-1508	TZm-1531	TZm-1509	TZm-126	TZm-404	TZm-147	TZm-146	TZm-1301
bnlg1597a	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
bnlg1597b	1	1	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
bnlg1597c	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	1	1	9	1	0	0	1	1	1	1	1
bnlg1597d	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1	1	1	9	0	0	0	0	0	0	0	0
bnlg1597e	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	9	1	1	0	1	1	1	1	1
bnlg1597f	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
bnlg1597g	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0	1	0	1	0	0	0	0	0	9	1	1	1	0	0	0	0	1
phi002a	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	1

Appendix

phi002b	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1
phi002c	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1
phi002d	0	0	0	0	9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1
phi002e	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
nc133a	1	1	0	0	0	0	1	1	1	1	9	9	1	1	0	0	0	0	0	1	0	1	1	1	0	0	0	9	9	0	0	0
nc133b	0	0	0	0	0	0	0	0	0	0	9	9	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	9	9	0	0	0
nc133c	1	0	1	1	1	1	1	1	1	1	9	9	1	0	0	1	0	0	0	1	1	0	0	1	1	0	0	9	9	0	1	1
nc133d	1	0	0	1	0	1	1	1	1	1	9	9	0	1	1	1	1	0	0	1	1	1	1	1	0	1	1	9	9	1	0	0
nc133e	0	0	0	1	0	1	0	1	0	0	9	9	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	9	9	0	0	0
phi453121a	9	9	9	9	9	9	9	9	0	0	1	0	0	0	0	0	0	9	9	0	0	0	0	0	9	9	9	1	0	0	1	0
phi453121b	9	9	9	9	9	9	9	9	1	1	0	1	1	1	0	1	1	9	9	1	1	1	1	1	9	9	9	0	0	1	0	1
phi453121c	9	9	9	9	9	9	9	9	0	0	0	0	0	0	1	0	0	9	9	0	0	0	0	0	9	9	9	0	1	0	0	0
phi453121d	9	9	9	9	9	9	9	9	1	0	0	1	1	1	1	1	1	9	9	1	1	1	0	1	9	9	9	0	1	1	0	1
phi072a	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
phi072b	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1237a	0	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1237b	1	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1237c	1	9	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0

bnlg1237d	0	9	0	0	1	0	1	0	0	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	0	0	0	1	1	1
bnlg1237e	0	9	0	0	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
bnlg1237f	0	9	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0
blng1695a	0	0	9	9	0	0	9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
blng1695b	0	0	9	9	0	0	9	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
blng1695c	0	0	9	9	0	0	9	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0
blng1695d	0	0	9	9	1	0	9	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	0	1	0
blng1695e	0	1	9	9	1	1	9	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	1	0	1	0	0	1	0
blng1695f	1	0	9	9	0	1	9	1	1	1	0	0	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0

Appendix B3 cont'd

	TZm-335	TZm-1503	TZm-1507	TZm-343	TZm-127	TZm-157	TZm-1505	TZm-149	TZm-132	TZm-310	TZm-124	TZm-144	TZm-143	TZm-399	TZm-394	TZm-185	TZm-1522	TZm-295	TZm-134	TZm-1297	TZm-1300	TZm-1534	TZm-3	TZm-1449	TZm-1508	TZm-1531	TZm-1509	TZm-126	TZm-404	TZm-147	TZm-146	TZm-1301
umc1066a	0	0	0	0	0	0	0	0	9	9	0	0	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
umc1066b	0	0	0	0	0	0	0	0	9	9	0	0	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
umc1066c	0	0	0	0	0	0	0	0	9	9	0	0	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
umc1066d	0	0	0	0	0	0	0	0	9	9	0	0	9	9	9	9	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
umc1066e	0	0	0	0	0	0	1	1	9	9	0	0	9	9	9	9	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
umc1066f	1	1	1	1	1	1	0	0	9	9	1	1	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phi080a	9	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	0	0	9	0	0	0	0	0	0	0	1	0	0	0	0	1
phi080b	9	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	0	0	9	0	0	0	0	0	1	1	1	1	0	0	0	1
phi080c	9	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	9	0	0	0	0	0	1	1	0	0	0	0	0	1
phi080d	9	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	0	0	1	1	1	1	1
phi080e	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1525a	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1525b	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1525c	0	0	0	1	0	1	1	1	0	1	1	0	1	1	0	0	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0
bnlg1525d	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
bnlg1525e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
bnlg1525f	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0
phi022a	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	0	0	9	0	0
phi022b	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	1	0	0	0	0	0	0	0	0	0	9	0	0	9	0	0
phi022c	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	1	0	0	0	0	0	0	0	0	0	9	0	0	9	0	0
phi022d	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	0	0	0	0	0	0	0	0	1	0	9	0	0	9	0	0
phi022e	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	0	0	0	0	0	0	1	0	0	0	9	0	0	9	0	0
phi022f	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	1	1	1	0	1	1	0	0	0	0	9	0	0	9	0	0
phi022g	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	0	0	0	1	0	0	1	1	1	1	9	1	1	9	1	1
phi022h	9	0	9	1	0	9	9	9	1	9	9	1	1	1	1	9	1	1	1	1	1	1	1	1	0	0	9	1	1	9	0	0
phi022i	9	0	9	0	0	9	9	9	0	9	9	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9	1	1	9	0	0
phi022j	9	1	9	1	1	9	9	9	1	9	9	1	1	1	1	9	0	0	0	1	0	0	0	1	1	0	9	0	0	9	1	1
umc1367a	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
umc1367b	9	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
umc1367c	9	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0
umc1367d	9	0	0	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1
umc1196a	1	0	0	0	0	0	1	9	1	9	9	9	0	1	9	9	0	0	1	9	9	0	9	0	0	0	0	0	0	0	1	1
umc1196b	0	0	0	0	0	0	0	9	0	9	9	9	0	0	9	9	0	0	0	9	9	0	9	0	0	0	0	0	0	0	0	1
umc1196c	0	1	1	1	0	0	0	9	0	9	9	9	0	0	9	9	0	0	0	9	9	0	9	0	0	1	1	0	0	0	0	0
umc1196d	1	0	0	0	1	1	1	9	1	9	9	9	0	0	9	9	0	0	0	9	9	0	9	1	1	0	0	1	1	1	0	1
umc1196e	1	0	0	0	1	1	1	9	0	9	9	9	1	1	9	9	1	1	1	9	9	1	9	1	1	1	0	0	1	1	1	1

Appendix B3 cont'd

	TZm-130	TZm-378	TZm-1548	TZm-1302	TZm-305	TZm-342	TZm-1525	TZm-381	TZm125	TZm-1504	TZm-1176	TZm-145	TZm-398	TZm-43	TZm-155	TZm-1304	TZm-167	TZm-1427	TZm183	TZm-137	TZm-190	TZm-1526	TZm-49	TZm-1506	TZm-46	TZm-152	TZm-120	TZm-386	TZm-123	TZm-121	TZm-1543	TZm-148
bnlg1597a	0	0	0	9	9	9	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0
bnlg1597b	0	0	0	9	9	9	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0
bnlg1597c	0	0	0	9	9	9	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0
bnlg1597d	1	0	0	9	9	9	0	0	9	0	0	0	0	0	0	1	9	0	1	1	0	0	1	0	0	9	0	0	1	0	0	0
bnlg1597e	0	0	0	9	9	9	0	0	9	0	0	0	0	0	0	1	9	1	0	0	0	0	1	0	0	9	0	1	0	0	0	0
bnlg1597f	1	1	0	9	9	9	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
bnlg1597g	1	1	1	9	9	9	1	1	9	1	1	1	1	1	1	1	9	1	0	0	1	1	0	1	1	9	1	0	0	1	1	1
phi002a	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phi002b	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phi002c	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
phi002d	9	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phi002e	9	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
nc133a	9	0	0	0	9	0	9	9	9	9	9	9	9	0	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
nc133b	9	0	0	0	9	0	9	9	9	9	9	9	9	0	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
nc133c	9	0	0	0	9	0	9	9	9	9	9	9	9	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
nc133d	9	1	0	1	9	1	9	9	9	9	9	9	9	0	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
nc133e	9	1	1	0	9	0	9	9	9	9	9	9	9	0	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
phi453121a	9	9	9	1	0	9	0	9	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	9	9	9	0	9	9
phi453121b	9	9	9	0	0	9	0	9	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	9	1	9	9	9	0	9	9
phi453121c	9	9	9	0	0	9	0	9	0	0	1	1	0	0	0	1	1	1	1	0	1	1	1	1	9	0	9	9	9	1	9	9
phi453121d	9	9	9	0	1	9	1	9	1	1	0	1	0	1	0	1	1	1	0	0	0	0	1	0	9	0	9	9	9	0	9	9
phi072a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	1
phi072b	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	9	0	0	0	0	1	0	0	0	0	0	0	0	0	0
bnlg1237a	0	0	0	0	9	0	0	0	0	0	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	9	0	0	0	9	9	0
bnlg1237b	0	0	0	0	9	0	0	0	0	1	9	0	0	0	0	0	0	0	9	0	0	0	0	0	0	9	0	0	0	9	9	0
bnlg1237c	0	0	0	1	9	0	0	1	1	1	9	0	1	0	1	1	1	0	9	0	0	0	0	0	0	9	1	0	0	9	9	1
bnlg1237d	0	0	0	0	9	0	0	0	0	0	9	1	0	0	0	0	1	1	9	0	1	0	0	1	0	9	0	0	0	9	9	0
bnlg1237e	1	1	0	0	9	1	1	0	0	0	9	1	0	1	0	1	0	0	9	0	0	0	1	0	0	9	0	0	1	9	9	0
bnlg1237f	1	1	1	0	9	1	1	0	0	1	9	0	0	0	0	0	0	1	9	1	0	1	1	0	1	9	0	1	0	9	9	0
bing1695a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	9	0
bing1695b	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	9	9	0	0	0	0	9	0
bing1695c	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0	9	9	9	9	9	9	9	9	9	0	1	0	1	9	0
bing1695d	1	1	0	0	0	0	0	0	1	1	1	1	0	1	0	1	1	9	9	9	9	9	9	9	9	9	0	0	0	0	9	0
bing1695e	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	1	1	9	9	9	9	9	9	9	9	1	0	0	0	9	0	0
bing1695f	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	1	9	9	9	9	9	9	9	9	0	0	1	0	9	1	1

Appendix B3 cont'd

	TZm-130	TZm-378	TZm-1548	TZm-1302	TZm-305	TZm-342	TZm-1525	TZm-381	TZm125	TZm-1504	TZm-1176	TZm-145	TZm-398	TZm-43	TZm-155	TZm-1304	TZm-167	TZm-1427	TZm183	TZm-137	TZm-190	TZm-1526	TZm-49	TZm-1506	TZm-46	TZm-152	TZm-120	TZm-386	TZm-123	TZm-121	TZm-1543	TZm-148
umc1066a	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	0	0	0	1	0	0	0	0	9	0	0	0	0	0	0
umc1066b	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
umc1066c	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
umc1066d	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
umc1066e	1	1	1	1	1	1	1	1	1	1	0	0	9	9	9	0	0	1	1	0	1	0	0	0	0	0	9	0	0	0	0	0	1
umc1066f	0	0	0	0	0	0	0	0	0	0	0	1	9	9	9	1	1	0	0	1	0	0	1	1	1	1	9	1	1	1	1	1	0
phi080a	1	0	0	0	1	1	0	1	0	1	0	9	0	9	0	1	0	0	0	1	0	0	0	9	0	0	0	1	0	1	1	9	1
phi080b	1	1	1	0	1	1	1	0	1	1	0	9	0	9	0	0	0	0	0	1	0	0	0	9	0	0	0	1	0	1	1	9	0
phi080c	1	1	1	0	0	1	1	0	1	1	0	9	0	9	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	9	0
phi080d	1	1	0	1	0	0	0	0	0	0	1	9	1	9	0	1	1	1	1	0	0	1	9	1	1	1	0	0	0	0	0	9	0
phi080e	0	0	0	0	0	0	0	0	0	0	0	9	0	9	1	0	0	0	0	0	0	0	0	9	0	0	0	0	1	0	0	9	0
bnlg1525a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1525b	0	1	1	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bnlg1525c	0	1	1	0	0	0	0	0	0	0	0	0	1	0	9	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	0
bnlg1525d	1	1	1	1	1	1	1	0	1	1	1	1	0	0	9	1	1	1	1	0	0	0	1	1	0	1	0	1	1	1	1	1	1
bnlg1525e	1	1	1	0	1	1	1	0	1	0	1	0	0	0	9	1	1	1	1	0	0	0	1	1	0	1	0	1	0	0	0	0	0
bnlg1525f	1	0	0	0	0	1	1	1	0	1	0	1	0	1	9	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
phi022a	0	0	9	9	0	0	9	9	1	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	0	0	0	0	0	0	0	0
phi022b	0	0	9	9	0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	0	0	0	0	0	0	0	0
phi022c	0	0	9	9	0	0	9	9	0	0	0	0	0	0	0	1	0	0	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0
phi022d	0	0	9	9	0	0	9	9	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0
phi022e	0	0	9	9	0	0	9	9	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0
phi022f	0	0	9	9	0	0	9	9	1	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0
phi022g	0	0	9	9	0	0	9	9	0	0	1	1	1	0	0	1	0	0	9	9	9	9	9	9	0	0	0	0	0	0	1	1	
phi022h	1	0	9	9	0	0	9	9	0	1	0	0	0	0	1	0	1	1	9	9	9	9	9	9	1	1	0	0	1	0	1	1	
phi022i	0	0	9	9	0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	9	9	9	9	9	9	0	0	1	1	0	1	0	0
phi022j	1	1	9	9	1	1	9	9	0	1	0	1	0	1	0	0	1	1	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0
umc1367a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	1	0	9	9	9	9	9	9	
umc1367b	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	9	1	1	9	9	9	9	9	9	
umc1367c	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	1	9	9	9	9	9	9	
umc1367d	1	0	1	0	0	1	1	0	1	1	0	1	1	0	0	1	1	1	0	0	0	0	1	9	0	0	9	9	9	9	9	9	
umc1196a	1	1	0	0	1	0	1	1	0	0	1	9	1	1	9	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	9	1	
umc1196b	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	
umc1196c	0	0	0	0	0	0	0	0	0	0	0	9	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	
umc1196d	0	1	1	1	0	1	0	0	1	0	0	9	0	0	9	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	9	0	
umc1196e	1	1	1	0	0	1	1	0	1	1	0	9	0	0	9	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	9	0	