

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

DEPARTMENT OF CROP AND SOIL SCIENCES

FACULTY OF AGRICULTURE

COLLEGE OF AGRICULTURE AND RENEWABLE NATURAL RESOURCES

STUDY OF GENETIC DIVERSITY AMONG IMPROVED MAIZE (*Zea mays*)
VARIETIES IN GHANA USING MORPHOLOGICAL TRAITS AND SIMPLE
SEQUENCE REPEAT MARKERS

BY

CHARLES NELIMOR

BSc. (Hons) AGRICULTURE

August, 2015

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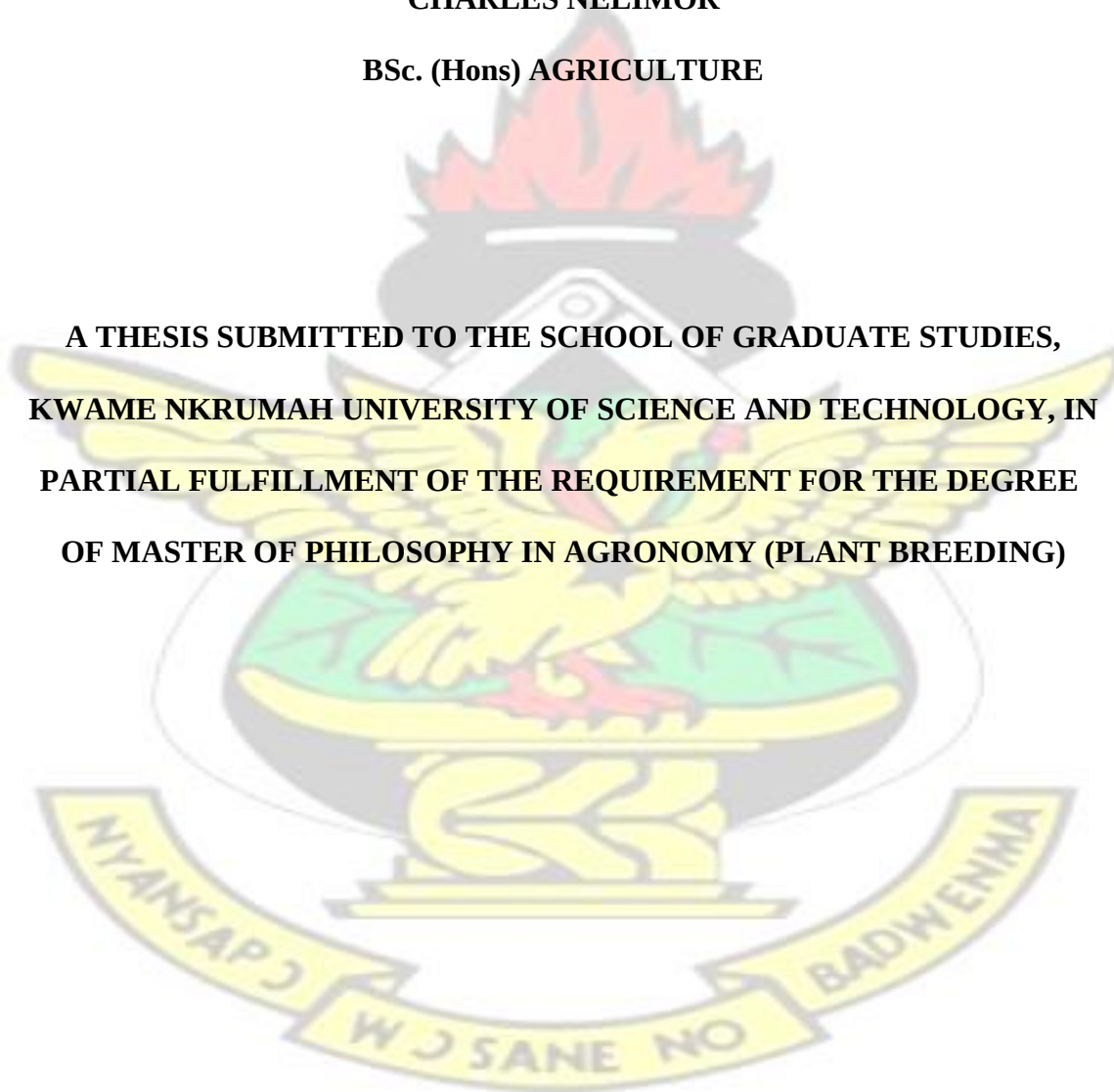
KNUST

BY

CHARLES NELIMOR

BSc. (Hons) AGRICULTURE

**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES,
KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN
PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE
OF MASTER OF PHILOSOPHY IN AGRONOMY (PLANT BREEDING)**



August, 2015

DECLARATION

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

.....
CHARLES NELIMOR
(Student)

.....
DATE

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

.....
DR. (Mrs) ANTONIA TETTEH
(Supervisor)

.....
DATE

.....
DATE
(Co-Supervisor)

..... PROF. RICHARD AKROMAH

.....
DR. ENOCH A. OSEKRE
(Head of Department)

.....
DATE

ABSTRACT

Information on genetic diversity among Ghana maize cultivars is not known. Genetic diversity is important for germplasm enhancement, heterotic breeding and for prevention of losses that may arise due to large-scale cultivation of genetically uniform cultivars. This research was carried out to determine the extent of genetic diversity among 17 maize cultivars comprising populations, Open-pollinated Varieties and hybrids developed between 1987 and 2012 in Ghana using morphological, agronomic and molecular evaluations. Morphological and agronomic measurements were analysed by means of analyses of variance, correlations, heritability estimates, UPGMA cluster analysis, and principal components.

Molecular evaluations were determined using Simple Sequence Repeats profiling. Wide variability was detected among OPVs while populations and Hybrids demonstrated little variation. There were no significant differences among the three classes of genotypes for earliness, anthesis-silking interval, plant and ear height, stay green, as well as ear and kernel characteristics. However, significant ($P \leq 0.05$) differences were observed for tassel length, ear leaf characteristics and also for number of kernels per row, hundred kernel weight and grain yield. Cultivar

‘Akposoe’ was most early with the smallest values for ear leaf characteristics, plant height, ear length and stalk diameter while cultivar ‘Obatanpa’ had the largest ear dimensions and grain yield. The least number of days to anthesis and silking was 49 and 51 days, respectively. Highest grain yield was 3.36 Mgha^{-1} . Earliness was negatively correlated to grain yield while weak to moderate and highly significant correlations were found between tassel length, ear leaf length and ear leaf width.

Heritability estimates were low among hybrids and populations but high in OPVs.

Earliness demonstrated high heritability estimates, plant characteristics showed moderate estimates in OPVs, grain yield exhibited null heritability estimates across the genotypes.

UPGMA morphological cluster analysis grouped the accessions into three main groups. 'Okomasa' and 'Abontem' were the most dissimilar accessions while accessions 'M0826-7F' and 'M0826-12F' were identical. The first three components, with eigenvalues higher than 1.0, accounted for 74.91 % of total the variance. The first component alone explained 49 % of the total variation and was positively associated with AD, SD, TL, ELL, ELW, PLHT, EHT, NL, EP, StD, EL, KL, NKR, NRE, CD, EWT and YLD. Marker genotyping of the 17 maize accessions using 12 SSR markers revealed a total of 31 alleles, detected at 11 polymorphic loci and one monomorphic locus. The number of alleles identified by each marker ranged from 2 to 4 with a mean of 2.82. A total of seven rare alleles were revealed in five accessions. Polymorphism information content ranged from 0.21 to 0.64 with an average of 0.43. Dice genetic similarity coefficient among the accessions based on the molecular data ranged from 0.36 to 0.94 with an average of 0.61. 46.67 % of the estimated coefficients had values greater than 0.61, reflecting a high degree of genetic similarity among the cultivars while 31.11 % of the coefficients were equal to or less than 0.50, suggestive of considerable diversity. Dendrogram constructed based on SSR data revealed three main clusters of maize accessions with distinctive genetic profiles. The cluster patterns in most instances, revealed evidence of associations related to their pedigree records. The information generated in this study might be useful to breeders in Ghana for maize improvement through selective and cross breeding programs. It is thus recommended that accessions which revealed rare alleles such as 'Obatanpa', 'Enibi', 'Etubi', 'Honampa' and 'Kwadaso Local' be incorporated into breeding programs.

DEDICATIONS

I dedicate this piece of work to Professor Richard Akromah and Mr. Alexander Wireko Kena, for their words of encouragement and serving as sources of inspiration for a better me tomorrow.

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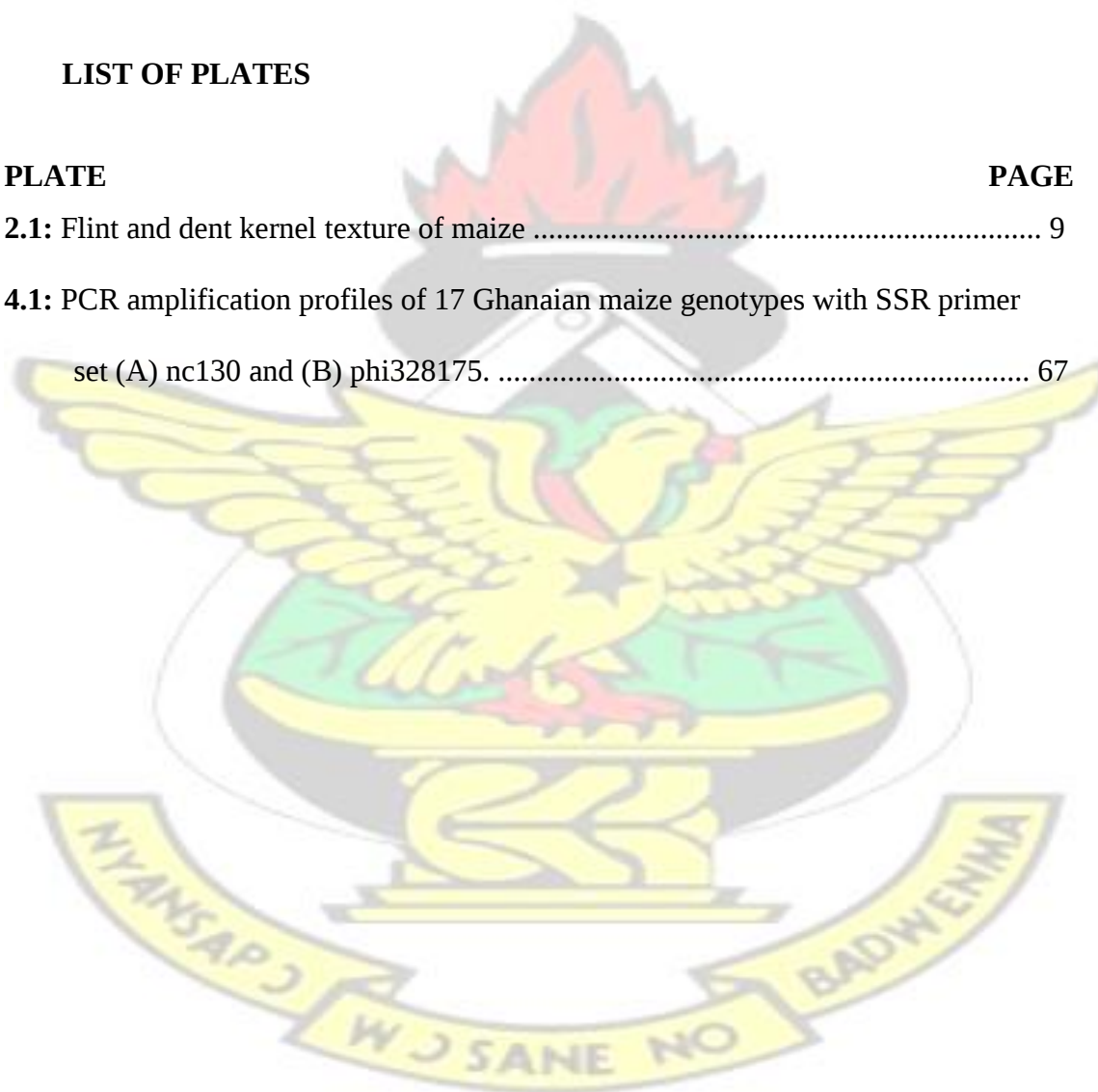
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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
CA	Cluster Analysis
CIMMYT	International Maize and Wheat Center
CRI	Crops Research Institute of Ghana
CSIR	Council for Scientific and Industrial Research
CTAB	Cetyl Trimethylammonium Bromide
DTMA	Drought Tolerant Maize for Africa
ED	Euclidean Distance
EMS	Expected Mean Square
GCV	Genotypic Coefficient of Variation
GD	Genetic Distance
GDP	Gross Domestic Product
IBPGRI	International Board for Plant Genetic Resource Institute
IITA	International Institute of Tropical Agriculture
MDS	Multidimensional Scaling
MRD	Modified Rodger's Genetic Distance
NTSYS-pc	Numerical Taxonomy and Multivariate Analysis System
OPV	Open Pollinated Variety

PC	Principal Component
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
PIC	Polymorphism Information Content
QPM	Quality Protein Maize
RAPDS	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
rpm	revolutions per minute
SAHN	Sequential Agglomerative Hierarchical Nested
SAS	Statistical Analysis System
SIMINT	Similarity for Interval Data
SIMQUAL	Similarity for Qualitative Data
SSR	Simple Sequence Repeats
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L.) is one of the most important economic and staple food crops for a large population of the world. Together with rice and wheat, maize is an essential source of at least 30 % of the food calories of more than 4.5 billion people in about 94 developing countries (Shiferaw *et al.*, 2011). In Africa, maize is the most widely grown and consumed staple crop for more than 300 million people (ABSF, 2010). Maize is produced on nearly 100 million hectares of land in 125 developing countries and is one of the most widely grown crops in 60 % of those countries (FAOSTAT, 2010). In Ghana, maize is the number one cereal crop in terms of area and total production (DTMA, 2013). About 1 million hectares of land is planted to maize in Ghana with a projected annual increase in demand of 1.83 % (DTMA, 2013). Maize plays key role in the agricultural economy of Ghana, ranking as the second largest commodity crop, contributing about 30 % to Gross Domestic Product (ISSER, 2011).

Maize production technology in Ghana varies greatly with agro-ecology, cultural background, resource availability, farmer's preferences and stresses but is generally traditional (Agyare *et al.*, 2014). Average yield of maize in Ghana has consistently been about 1.7 t/ha (Adu *et al.*, 2013; Oppong *et al.*, 2014) for over 20 to 30 years though a higher yield potential is possible. This value is far lower than maize yield of the developed countries such as U.S.A., China and South Africa (MoFA, 2010).

Genetic improvement of crops depends on the extent of genetic variation present in breeding materials (Thanga, 2015). The amount of genetic diversity present in a crop germplasm depends on the extent of recombination, mutation, selection and random genetic drift. Whereas mutation and recombination bring new variations to a

population, selection and genetic drift remove some alleles (Pervaiz *et al.*, 2010). The ability to widen genetic base of a crop germplasm to develop desirable varieties depends on efficient discovery of diverse sources of beneficial alleles and their successful introgression into existing genetic backgrounds (Meseka *et al.*, 2015).

In attempts to breed high yielding and desirable cultivars, plant breeders prefer to use very limited number of germplasm (Wang *et al.*, 2011). Consequently, genetic diversity of breeding materials decreases, leading to narrow genetic base and accompanying genetic vulnerability to new diseases and retardation of breeding progress (Yadav and Indra, 2010). The Irish potato famine of 1845 to 1852 and the Southern corn leaf blight epidemic in U.S.A. of the 1990s are two devastating historical events caused by large-scale cultivation of genetically homogeneous varieties of potato and maize, respectively (Govindaraj *et al.*, 2015). Similarly, the maize streak virus epidemic in the West African Corn Belt in 1983 in which Ghana was most affected arose from the cultivation of homogeneous maize varieties bred from few parents originating from Mexico (Oppong, 2013). In recent times, breeding of new maize cultivars is characterized by use of restrictive and limited number of key inbred lines (Goodman, 2005). Therefore, genetic base of these cultivars is certainly limited, in comparison to large original genetic diversity that is available in landraces and wild relatives (Choukan *et al.*, 2004; Goodman, 2005) which have not yet been exploited. Maize breeding, therefore, faces unique challenges due to the narrow genetic background of commercial cultivars (Choukan *et al.*, 2004).

Knowledge of the amount and distribution of genetic variation and relationships between and within plant populations is indispensable for classifying parental lines and predicting future hybrid performance (Acquaah, 2007). Moreover, information on genetic diversity would facilitate germplasm classification into heterotic groups, their

management in Genetic Resource Centers and enhance the identification of useful alleles for incorporation into breeding programs (Mohammadi and Prasanna, 2003; Kanagarasu *et al.*, 2013). Heterotic breeding takes advantage of transgressive segregation (Sajib *et al.*, 2012) while mating of closely related parents leads to inbreeding depression with loss of vigor and productiveness (Tembo, 2007).

Methods for determining genetic variability of breeding materials include pedigree analysis, morpho-phenological and molecular marker evaluation (Pejic *et al.*, 1998; Mohammadi and Prasanna, 2003; Govindaraj *et al.*, 2015). Morphological markers have been of great value in revealing differences in maize germplasm (Galarreta and Alvarez, 2001; Lucchin *et al.*, 2003; Babić *et al.*, 2008; Obeng-Antwi *et al.*, 2012). Genotypic and phenotypic coefficients of variation are important statistics used in detecting the extent of variability present in a germplasm. Heritability estimates provide information on the genotypic component that is contributing to variation. Morphological evaluation though relatively easy to carry out (Shiri *et al.*, 2014; Govindaraj *et al.*, 2015) are however labour intensive, time-consuming and fraught with environmental influences and as such do not reliably portray genetic relationships between genotypes (Kanagarasu *et al.*, 2013; Shiri *et al.*, 2014). Additionally, morpho-phenological markers express limited polymorphism and late expression of traits (Smith and Smith, 1992). Conversely, DNA-based molecular markers are independent on environment (Ignjatovic-micic *et al.*, 2015). They provide direct measurements of genetic diversity and go beyond indirect measures associated with phenotypic markers (Drinic *et al.*, 2012) as they detect variation down to the DNA or protein level and have proven to be effective tools for distinguishing between closely related genotypes (Beyene *et al.*, 2005; Babić *et al.*,

2012). Among the DNA-based molecular markers, SSRs which are short sequences containing tandemly repeated copies of one to six nucleotide fragments (Rafalski *et al.*, 1996) circumvents the limitations and drawbacks of other molecular profiling techniques. The SSR technique is not only simple but also; highly informative, reproducible, co-dominant, locus-specific and has the advantage of being amenable to PCR automation (Acquaah, 2007). SSRs have therefore become a method of choice in plant research for the study of genetic diversity (Missihoun *et al.*, 2015).

Previous maize diversity studies in Ghana focused on morphological and or molecular characterization of landraces with limited number of improved genotypes (Obeng-Antwi, 2007; Obeng-Antwi *et al.*, 2011, 2012; Oppong *et al.*, 2014). In retrospect, these studies revealed the presence of large reservoir of genetic diversity expressed as variety of alleles in the landraces while four improved cultivars were devoid of most of these alleles, an evidence of a narrow genetic base. Because these evaluations were conducted on few genotypes, it is important to increase the number of genotypes to ascertain the genetic diversity in the entire collection of improved varieties available in Ghana to cover a wide variety of breeding materials such as inbred lines, hybrids, synthetic varieties as well as open-pollinated varieties. The overall goal of this study was, therefore, to analyze, determine and describe the extent and magnitude of genetic variation present in improved maize genotypes in Ghana for the benefit of future breeding programs. The specific objectives were to;

1. Evaluate and characterize improved maize genotypes in Ghana on the basis of morpho-phenological traits.
2. Assess the extent of phenotypic and genotypic coefficients of variation and heritability estimates (broad sense) of important agronomic traits in maize.
3. Assess the genetic diversity in Ghana maize cultivars by means of SSR

profiling.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of maize

Maize is an important commodity which serves as food for a large population of the world. As a primary source of feed for livestock and poultry (Prasanna, 2012), a raw material for many industrial products including starch, thermoplastics, paints, and pharmaceutical products, maize contributes substantially to the world's economy. It provides food and nutritional security in some of the world's poorest regions especially in Africa, Asia, and Latin America making it one of the most important crops in the world. In the developed world, maize is mostly used for animal feed (70 %) and only a small percentage (5 %) is consumed by humans. Undoubtedly, maize is the most preferred staple in the African region where over 300 million people depend on it as their main food source (ABSF, 2010). The developing countries consume about 62 % of maize as food and 34 % is used as feed. The way in which maize is processed and consumed varies greatly from country to country, with maize flour and meal being the two most popular products (USAID, 2002). In Africa, the per capita consumption of maize ranges from 52 to 328 g per person per day (FAOSTAT, 2012). The per capita consumption of maize in Ghana between 2007 and 2009 was estimated as 53 g per person per day (FAOSTAT, 2012).

Maize production provides livelihoods for millions of subsistence farmers in West and Central Africa. It accounts for about 45 % of agricultural production which remains the main source of livelihood for most Ghanaians, providing employment to more than 60 % of the population and contributes about 30 % to GDP (ISSER, 2011). According

to Acquaaah and Kyei (2012), maize production contributes over 20 % of the income earned by smallholder farmers in Ghana.

It is estimated that by 2050, the demand for maize in the developing world will double (Rosegrant *et al.*, 2009). The growth in demand for human consumption of maize in the developing world is predicted to be 1.3 % per annum until 2020. Moreover, rising incomes are expected to result in a doubling of consumption of meat across the developing world (Naylor *et al.*, 2005), consequently leading to an estimated growth in demand for feed maize by 2.9 % per annum.

Among the cereal crops grown, maize has the highest average yield per ha and remains third only after wheat and rice in total area of production in the world (FAOSTAT, 2012). Globally, 765 metric tons (MT) of maize were harvested in 2010 from just less than 153 million hectares (ha). The world area of maize production in 2012 was 176 million ha while that of wheat and rice were 216 and 184 million ha, respectively. Maize however, surpasses both wheat and rice in terms of productivity. In 2012 for instance, the world maize production was 875 million tons, while wheat and rice were 606 million tons and 635 million tons, respectively (FAOSTAT, 2012).

In several of the developing countries, especially in Sub-Saharan Africa, where maize is a highly important staple food crop, yields are still below 1 t/ha, while many countries have only 1-2 t/ha due mainly to climate change, poor soil fertility, frequent occurrence of droughts, high incidence of insect-pests, diseases and weeds, farmers' limited access to fertilizer, and lack of access to improved maize seed (Shiferaw *et al.*, 2011, Cairns *et al.*, 2012; Adu *et al.*, 2013). The average yield of maize in Ghana for instance is about 1.7 t/ha (MoFA, 2011; Adu *et al.*, 2013), which is among the lowest globally especially in comparison to countries such as U.S.A, China and South Africa.

With rising population, increasing per capita income, urbanization, growing poultry and fish sectors in Ghana, maize demand is expected to rise steadily at a projected compound annual growth rate of over 1.83 % (DTMA, 2013). It is estimated that demand will exceed production especially in developing countries in the coming years (FAO, 2013). As a net-importer of maize, Ghana imported an average of nearly 33,000 MT of maize at the cost of about US\$ 8.32 million per year between 2001 and 2010 (DTMA, 2013). The projected maize import in Ghana is estimated to be 267,000 MT in 2015 (FAO, 2013). Interventions are needed to increase maize productivity in Ghana on limited land resources.

2.2 The biology of maize

Maize is a tall and monoecious annual grass varying in height from 1 to 4 meters (Sleper and Poehlman, 2006). The main stem is made up of clearly defined nodes and internodes. Internodes are wide at the base and gradually taper to the terminal inflorescence at the top of the plant. Leaf blades are borne alternatively along the length of the main stem. The main stem terminates in a tassel, which bears spikelets. Tasseling begins immediately after knee height growth which generally occurs at 35 to 45 days after emergence.

As the tassels open, spikelets (bearing anthers) are pushed out by elongating filaments and pollen grains are emptied from the extruded anthers (Sleper and Poehlman, 2006). Wind dispersed pollen usually remains viable for 10 to 30 minutes but can be preserved under favorable conditions (Simmond and Smartt, 1999). The reproductive phase begins when one or two axillary buds, present in the leaf axils, develop and form the pistillate inflorescence or female flower (Purseglove, 1972). The axillary buds undergo transformation forming cluster of leaves called the ear at a joint on the stalk on which flowers are borne (Acquaah, 2007). From each flower a style begins to elongate

towards the tip of the cob in preparation for fertilization. These styles form long threads, known as silks which may appear in different colours depending on the genotype. Silk emergence may be affected by temperature, soil moisture, and soil fertility. Adverse weather such as severe drought may also delay or cause complete cessation of silk emergence.

As pollen receptors, each individual silk must be pollinated in order to produce a caryopsis. Pollen shed occurs over a 14 day period and peaks during the first 5 days of shed (Sears *et al.*, 2000). Silks are receptive soon after emergence and remain receptive for up to about 10 days. Generally for each plant, pollen shed usually precedes silk emergence by about 1-3 days (Sleper and Poehlman, 2006). However in prolific genotypes, silks may emerge before tassel begins to shed pollen (Hitchcock and Chase, 1971). A fertilized ear will always come in different shapes with an even number of kernel rows, usually eight or more rows arranged in different patterns (regular, irregular, mixed, straight and spiral) depending on the genotype (Acquaah, 2007). The maize kernels consist of the embryo endosperm and the pericarp and may differ in colour, structure and chemical composition. The most common kernel colours are yellow and white though some landraces may have red, purple and black colours. Different colours on the same ear are often due to the outcrossing nature of the crop.

Based on endosperm and glume characteristics, maize can be grouped into seven types, including dent, flint, flour, pop, sweet, waxy, and pod corns. Depending on the farming area, different kernel textures are preferred by different groups of farmers. In West Africa, the flint and the dent (Plate 2.1) types are the most widely grown and the most consumed. Dent maize (*Zea mays indentata*) is the most widely distributed maize type in the world. It is characterized by a depression (dent) in the crown caused by rapid drying and shrinkage of the soft starch at the crown. The grain is characterized by an

indentation at the distal end. Of the multiple colours available, the yellow or white dent kernels dominate commercial production. Flint maize (*Z mays indurate*) on the other hand is predominantly comprised of corneous or hard starch that encloses the soft starch at the center. The kernels are smooth, hard, and usually rounded at the top. The starch composition gives the kernel a shiny surface. Flint varieties mature earlier, and its seeds store and germinate much better than dent varieties.

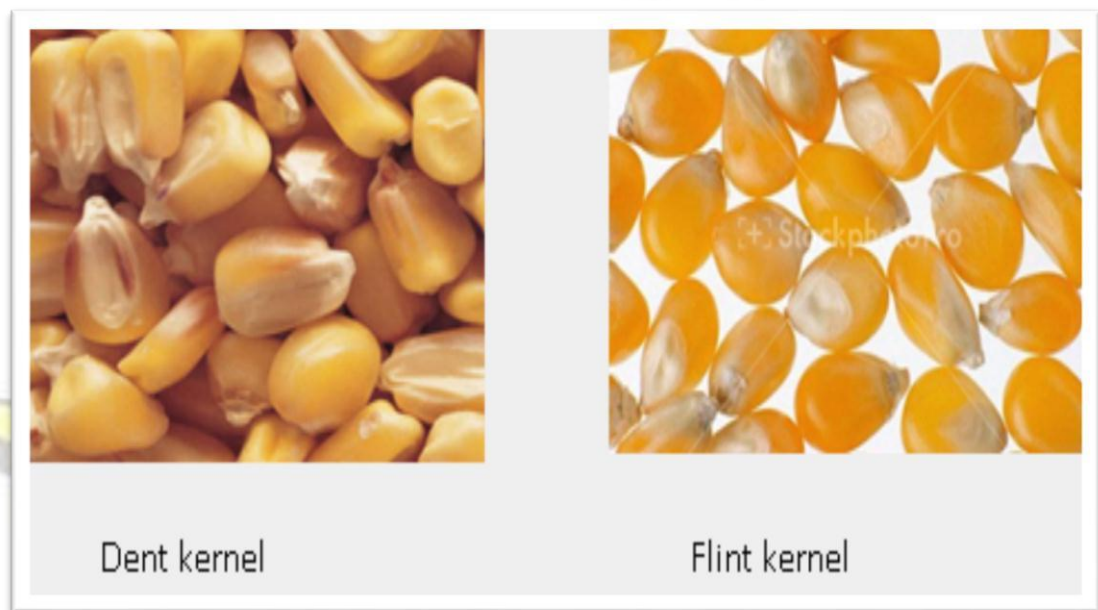


Plate 2.1: Flint and dent kernel texture of maize

2.3 History and status of maize breeding in Ghana

Maize breeding in Ghana started in the early 1930s with the primary focus on development of high and stable yielding varieties for all the agro-ecologies in the country (GGDP, 1986). The first record of varieties in the country dated between 1939-1942 when T. L Williams introduced varieties such as C50 and 'Tsolo' from South Africa (GGDP, 1984; Sallah, 1986). Between 1954 and 1961, two yellow maize varieties namely 'Nyankariwana Number 1' and 'Nyankariwana Number 2' were released in the Northern part of the country by J. McEwen. Effort by some local Ghanaian breeders, especially M. K. Akposoe led to the development of three composite

varieties: Composite 1, 2 and 3, in addition to 'La Posta CRI' and 'Golden Crystal' between 1968 and 1972.

The inception of the maize improvement program by the Ghana Grains Development Project (GGDP) in 1979 revolutionized maize breeding in the country (Sallah, 1986). Under the GGDP, the Ghanaian national maize breeding program was reorganized and collaborations between Crops Research Institute, Ghana, (CRI) and International Maize and Wheat Improvement Center (CIMMYT) in which lowland tropical and subtropical varieties adapted to Ghana were introduced. Following their introduction, variety trials were conducted at the CRI, selection was made for the most promising materials, after which seeds were distributed to farmers throughout the country for on-farm testings. This collaborative process involving CIMMYT breeders, CRI breeders and Ghanaian farmers eventually led to the release of eleven varieties between 1984 and 1997 (Table 2.1). Maize breeding in Ghana during the period of the project initially concentrated on the development of open-pollinated varieties (OPVs) because of socio-economic reasons such as lack of efficient seed production and marketing systems. The need to enhance lysine and tryptophan levels in normal maize had earlier led to the development of Quality Protein Maize (QPM) varieties through introgression of *opaque-2* gene (Mertz, 1974). The QPM and hybrid varieties were found to be more productive than the OPVs, hence the commencement of QPM and hybrid maize development program in 1991. The first successful QPM variety released in Ghana in 1992 was 'Obatanpa GH' which has since remained the dominant OPV in the country, accounting for about 95 % of all maize seed planted in Ghana (DTMA, 2013). This led to the development of three-way QPM hybrid varieties, namely, GH110-5 ('Mamaba'), GH132-28 ('Dadaba'), and GH2328-88 ('CIDA-ba') having yields between 6.3 and

7.3 t ha⁻¹ on experimental stations, which represented an increment in yield of about 19 to 38 % over ‘Obatanpa

GH’ (DTMA, 2013). Moreover, infants livestock and poultry which were fed on these QPM varieties grew faster and healthier compared to those fed on normal maize varieties. These QPM hybrids were, therefore, released for production in 1997 (Twumasi-Afriyie *et al.*, 1997).

Table 2.1: Maize varieties developed by the Ghana Grains Development Project

Name of Variety	Year of Release	Grain colour	CIMMYT Germplasm
‘Aburotia’	1984	White	Tuxpeño PBC16
‘Dobidi’	1984	White	Ejura (1) 7843
‘Golden Crystal’	1984	Yellow	Tocumen (1) 7931
‘Safita-2’	1984	White	Pool 16-SR ^a
‘Okomasa’	1988	White	EV8343-SR ^a
‘Abelechi’	1990	White	Ikenne 8149-SR ^a
‘Dorke SR’	1990	White	Pool 16-SR ^a
‘Obatanpa’	1992	White	Pop 63-SR ^a
‘Mamaba’ ^b	1997	White	Pop. 62, Pop. 63-SR ^a
‘Dadaba’ ^b	1997	White	Pop. 62, Pop. 63-SR ^a
‘Cidaba’ ^b	1997	White	Pop. 62, Pop. 63-SR ^a

Source: GGDP. ^a Developed jointly with IITA. ^b Three-way cross hybrid. SR= resistant to maize streak virus. Source: Morris *et al.* (1999).

The GGDP operated for 18 years before concluding in 1997 following the termination of CIDA funding. Since then, the emphasis has been on developing QPM open pollinated varieties (OPVs) and hybrids (Ragasa *et al.*, 2013). Improved maize varieties were not released in Ghana between 1999 and 2006 (DTMA, 2013). For the past decade, the CSIR-CRI (Fumesua) and CSIR-SARI (Nyankpala) in close collaboration with IITA and CIMMYT have developed and released several maize varieties, including four varieties each in 2007 and 2010, whereas in 2012, six varieties were officially released (Table 2.2).

Today, the main varieties released in the world involve a restricted number of key inbred lines. In Ghana for instance, majority of the germplasm explored by breeders in maize improvement programs have CIMMYT and IITA origins (Sallah, 1998; Regasa *et al.*, 2013). There could, therefore, be a reduction in the genetic base of improved maize cultivars in Ghana. In order to broaden genetic variation for use in future maize breeding, the genetic diversity of maize germplasm in Ghana needs to be investigated.

Table 2.2: Improved maize varieties developed in Ghana in the past decade

Name of Variety	Year of release	Source/ origin
‘Aziga’	2007	CIMMYT
‘Akposoe’	2007	CIMMYT/IITA
‘Etubi’	2007	CIMMYT
‘Golden Jubilee’	2007	CIMMYT
‘Aburohema’	2010	IITA
‘Enibi’	2010	CIMMYT/IITA
‘Abontem’	2010	IITA
‘Omankwa’	2010	-
‘Honampa’	2012	-
‘Aseda’	2012	-
‘Tintin’	2012	-
‘Ewul-Boyu’	2012	-
‘Sanzal-Sima’	2012	-
‘Bihilifa’	2012	-
‘Wang Dataa’	2012	-

Source: Compiled from Regasa *et al.* (2013); DTMA, (2013) and personal communication with scientists in the Council of Scientific and Industrial Research-CRI Kumasi Ghana.

2.4 Genetic diversity

Crop genotype is one of the most important factors governing crop improvement (Aremu *et al.*, 2007). Crop genotypes encompass different crop forms including inbred or pure lines, hybrids, landraces, wild races, varieties and cultivars. These genotypes have wide and diverse origin and genetic background known as genetic diversity. Genetic diversity as defined by Hallauer *et al.* (2010) refers to the probability that two randomly

chosen alleles are different in a sample chartered by the forces of evolution, including mutation, recombination, gene flow, genetic drift, selection, and migration in heterogeneous environments in space and time (Acquaah, 2007). Mutations can occur in the coding region of genes, or the spacer regions within and between genes. A small portion of changes owing to mutation may translate into protein variation, marker polymorphisms, physiological and morphological variation in agronomic characters and ultimately into varieties (Brown, 2008).

Information about genetic diversity in plant species is very important for germplasm enhancement, hybrid breeding and preventing environmental damage that may occur due to genetic uniformity of cultivars grown on large areas. Unless there is sufficient genetic diversity in a germplasm, it is practically impossible to increase yield and other desirable characters via hybridization. Generally, genetically diverse parents produce high heterotic effects and yield desirable segregates.

Maize has been described as one of the most diverse plants on earth and this diversity occurs at both the phenotypic and molecular levels (Buckler *et al.*, 2006). The molecular diversity of maize for instance is approximately three to tenfold higher than that of other domesticated grass species (Buckler *et al.*, 2001). Several factors have been suggested as reasons for the diversity in maize, including (1) variability of growing environments, domestication for various production systems and types of consumption preferences (Aguirre *et al.*, 1998); (2) continuous exchange of genes among maize populations, and in some instances, with their wild relatives owing to its predominant out-crossing nature (3) the maize genome undergoes extensive chromosomal duplications, providing new mutational opportunities that lead to phenotypic variability (Helentjaris *et al.*, 1998) and (4) transposons and

retrotransposable elements have also played an important role in the creation of the wide variation among maize (Bennetzen *et al.*, 2005).

Studies have elucidated that repeated use of key inbred lines in maize breeding programs is narrowing down genetic base of commercial cultivars of national gene pools in Chinese hybrids (Li, 1998; Yu, 2007) and in American hybrids (Goodman, 2005). To date, little or no information is available on the phenotypic and genetic diversity of modern maize varieties available in Africa in general (Magorokosho, 2006) and particularly in Ghana. A more comprehensive analysis of the genetic diversity of improved cultivars is therefore required to reach a definitive understanding of the genetic base and contribute to crop improvement programs in Ghana.

2.5 Methods of assessing genetic variation

Historically, methods used to assess genetic variability and interrelationships in crop germplasm have included morpho-agronomic, pedigree, biochemical and DNAbased molecular techniques (Pejic *et al.*, 1998; Mohammadi and Prasanna, 2003; Govindaraj *et al.*, 2015). Each of these approaches has its own strengths and drawbacks and hence their combined utilization is recommended to increase the resolving power of genetic diversity analyses (Singh *et al.*, 1991).

2.5.1 Morphological descriptions in genetic diversity assessment

Morphological markers are characters manifested on the outside of an organism as a product of interaction of genes and the environment (Acquaah, 2007).

Characterization of genetic diversity has long been based on crop morphology (Sturtevant, 1984) and has remained the mainstay of maize racial taxonomy (Ortiz *et al.*, 2008).

Morphological descriptions are important in the study of genetic diversity and relationships in plant breeding programs because (1) statistical procedures are readily available for analysis of morphological traits; (2) morphological descriptors allow quick and easy discrimination between phenotypes and (3) explanations of heterosis may be enhanced if morphological measures of distances are included.

Diverse taxonomic characteristics have been used to separate and assess patterns of phenotypic diversity and relationships of accessions (Rabbani *et al.*, 1998). Choosing characters that are least subject to environmental biases and are highly heritable are key considerations in phenotypic diversity analysis (Sevilla and Holle, 1995). In maize, the most commonly used descriptors are those related to earliness, plant architecture and yield (Lucchin *et al.*, 2003; Beyene, 2005; Obeng-Antwi *et al.*, 2012; Shrestha, 2013). The International Plant Genetic Resources Institute (IPGRI) has developed a descriptor list for characterization of maize.

Lucchin *et al.* (2003), reported low genetic differentiation within 22 populations of maize landraces grown widely in Spain on the basis of morphological description and attributed the low variation to gene flow and seed exchange among farmers. In their study, ear length of hybrids averaged 17.3 ± 0.2 cm whereas ear and cob diameter averaged 4.47 ± 0.18 cm and 2.67 ± 0.07 cm, respectively. In a study to determine the phenotypic diversity for morphological and agronomic traits in maize, Beyene *et al.* (2005) reported high phenotypic variability among traditional Ethiopian highland accessions. Their study revealed a wide range of expression across the accessions for agro morphological traits including 28 day range in male flowering, 155 cm range in plant height and 25.1 g range in 100 seed weight. Grain yield in their study ranged from 0.44 to 7.3 Mgha^{-1} . In a similar study, Ranawat *et al.* (2013) reported significant differences among parental QPM and non QPM lines in India. Shrestha (2013)

reported in Nepal maize accessions variation in number of leaves per plant as 8.33 to 13.33, plant height ranged of 95 cm to 211 cm, ear height range of 25 cm to 111 cm and tassel length of 27.66 to 46.00 cm. Silk colour was observed to be 53 % semi purple, 25 % white and 22 % purple.

2.5.1.1 Heritability of agronomic traits

Progress in crop improvement programs does not only depend on the amount of genetic variation present in the population but also on the extent to which the desirable characters can be transmitted from one generation to the other (Hussain *et al.*, 2011; Wang *et al.*, 2011). Heritability is the measure of phenotypic variance attributable to genetic causes and is relevant for prediction of gain from selection and determination of the relative importance of genetic effects (Kashiani *et al.*, 2010; Laghari *et al.*, 2010). Characters with high heritability can easily be fixed with simple selection thus resulting in quick progress.

Heritability estimates greater than 60 % of for plant and ear heights has been reported in maize by Rebourg *et al.* (2001). Beyene *et al.* (2005) reported high heritability estimates for days to tasselling (78.50 %), days to silking (77.80 %), plant height (70.10 %), number of leaves per plant (86.90 %) and number of kernels per row (69.50 %). In addition, they reported moderate heritability estimates for ear height (53.00 %), leaf length (45.80 %) ear diameter (44.70 %) and number of rows per ear (46.40 %), while low values of 17.00 %, 17.70 %, 18.10 % and 21.60 % were recorded, for grain yield, leaf width, 1000-seed weight and ear length, respectively, among 180 traditional highland maize accessions in Ethiopia. In a study to investigate genetic variability for vegetative and yield traits in maize genotypes, Idris and Abuali (2011) observed heritability estimates lower than 50 % for plant height, ear length, number of kernels per row, hundred kernel weight and grain yield and higher than 50.00 % for stem

diameter. In estimating heritability and genetic advance for grain and yield and its component characters in maize, Bello *et al.* (2012) reported high heritability estimates of 77.54 %, 84.32 %, 61.79, 98.64 %, 92.54 %, 96.45 % and 96.45 % for days to 50 % pollen shed, days to 50 % silking, anthesis-silking interval, plant height, ear height, number of grains per ear and grain yield, respectively.

Langade *et al.* (2013) reported on genetic variability and seasonal interaction for yield and quality traits in 10 maize inbred lines to include high estimates of heritability of 99.50 % and 99.40 % for days to 50 % tasselling and days to 50 % silking, respectively. They also reported moderate estimates of 74.80 %, 66.50 %, 58.90 %, 55.70 % and 54.60 % for number of kernels per row, plant height, ear height, and ear length and ear diameter, respectively. However, they recorded low estimates of 37.90 % and 34.60 % for number of rows per ear and 100-seed weight, respectively. Moderate estimates of heritability for plant height (59.1 %), and low levels of heritability estimates for number of rows per ear (42.70 %), hundred seed weight (41.60), ear diameter (40.50 %), ear height (39.50 %), ear length (33.70 %) and days to 50 % silking (23.20 %) were reported among 24 F₁ hybrid maize in India (Atnafua and Rao, 2014). In yet another study, Rahman *et al.* (2015) reported low heritability estimates for the agronomic traits, days to tasselling, days to silking, and days to anthesis, anthesis-silking interval, plant height and ear height in Pakistani maize accessions.

2.5.2 Molecular profiling of genetic diversity

A molecular marker can be defined as a segment of DNA whose characteristics can be measured and inferences made on the ecology and evolution of individuals, populations and species or simply, differences at the genotype level that can be used to answer and explain questions of genetics (Lokko *et al.*, 2005). DNA-based molecular markers have provided breeders with new tools to understand and efficiently

select for complex traits during breeding programs (Akinbo *et al.*, 2007). Molecular markers circumvent the limitations and drawbacks associated with both morpho-agronomic and biochemical diversity assessments. Their expressions, unlike morphological markers, are independent on environmental factors; thus they reflect the actual level of genetic difference existing between genotypes (Shiri *et al.*, 2014; Govindaraj *et al.*, 2015).

Various systems are used to assay molecular markers. On the basis of method of detection, DNA markers may be broadly divided into three classes:

1. Hybridization-based e.g. Restriction Fragment Length Polymorphisms (RFLPs)
2. Polymerase Chain Reaction (PCR)-based e.g. Amplified Fragment Length Polymorphisms (AFLPs), Random Amplified Length Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSRs), and
3. DNA sequence-based e.g. Single-Nucleotide Polymorphism (Tanksley and McCouch, 1997).

PCR-based molecular markers have the advantage of requiring small amounts of DNA and being relatively quick to assay. On the basis of genetic characteristics, molecular markers may be grouped into two main categories:

1. Single-locus, multi-allelic, co-dominant markers e.g. RFLPs and SSRs.
2. Multi-locus, single-allelic, dominant markers e.g. AFLPs and RAPDs (Acquaah, 2007).

DNA-based molecular markers such as; RFLPs, RAPDs, AFLPs and SSRs have successfully been employed for maize genetic diversity analyses. RFLPs have high discriminative power (Rebourg *et al.*, 2001; Rebourg *et al.*, 2003). The RFLP technique is however cumbersome and time consuming; needs large quantities of DNA

and is difficult to automate (Drinic *et al.*, 2012). RAPDs on the other hand which overcome some of the limitations of RFLPs have shown some problems with reproducibility of amplification and scoring of error data (Demeke *et al.*, 1997; Karp *et al.*, 1997). SSRs and AFLPs, the most informative among the DNA-based markers, seem to be the ideal for analyzing genetic diversity in crop species (Lubberstedt *et al.*, 2000; Kanagarasu *et al.*, 2013), since they overcome the problem of reproducibility and error data associated with RAPD markers, are relatively simpler to apply than RFLPs, are highly polymorphic, can be automated and yet provide results consistent with those from RFLP analysis and are easily detected on high resolution gels (Fufa *et al.*, 2005). However, AFLPs and SSRs present major distinctions. While AFLP markers are genomic fragments detected after selective PCR amplification, SSR markers consist of tandemly repeated units of short (1-6 bp) nucleotide motifs that show variation between individuals (Rafalski *et al.*, 1996); AFLP markers are dominant and biallelic, while SSR markers are co-dominant and multi-allelic. Furthermore, SSRs are more informative (Acquaah, 2007).

2.6 Statistical analysis of genetic diversity

Mohammadi and Prasanna (2003) presented comparison of some salient statistical tools for analyzing genetic data and recommended, depending on the objective of the study, the adoption of appropriate sampling strategies depending on the nature of the genetic material in question; utilization of diverse data set to encompass morphological, biochemical, or molecular since each data set has its own strengths and constraints, as well as choice of the genetic distance measure, clustering procedure, and other multivariate methods in analysis of the data.

Genomic data may be analyzed using various statistical concepts such as determining polymorphism (total number of alleles revealed); estimating allele frequency, allelic

diversity and measuring informativeness of a locus or Polymorphic Information Content (PIC). PIC provides an estimate of the discriminatory power of a locus or loci and may be estimated by a formula suggested by Smith *et al.* (1997) as

$$PIC = 1 - \sum f_i^2 \dots (1)$$

where ' f ' is the frequency of the marker or locus ' i '.

The power of SSR in revealing genetic diversity depends on the number of loci examined and the total number of alleles revealed per locus. Efficiency of detection of polymorphisms depends on the number and extent of variability of the operative taxonomic units (OTUs) and the level of resolution of the method, that is agarose versus polyacrylamide gel electrophoresis or automated or manual scoring of gels) employed for detection of the markers (Kanagarasu *et al.*, 2013, Shiri *et al.*, 2014). Obeng-Antwi (2007) reported over 96 % heterogeneity within 90 landraces in Ghana by means of AFLPs and found several alleles not present in four improved maize cultivars grown in Ghana. Choukan *et al.* (2006) found 2 to 11 alleles with 4.9 alleles per locus using 46 SSR loci. Legesse *et al.* (2007) reported 3.85 alleles per locus and average PIC of 0.58 using 27 SSR loci. Van Nguyen *et al.* (2012) and Shah *et al.* (2009) reported a mean of 2.07 and 1.56 alleles using 20 and 28 polymorphic SSR loci, respectively. Kanagarasu *et al.* (2013) also reported 2.3 alleles per locus and mean PIC of 0.45 with 10 SSR loci. Shiri *et al.* (2014) obtained a mean of 3.33 alleles in Iranian maize accessions using 12 SSR primers. The mean PIC recorded by Van Nguyen *et al.* (2012) SSR was 0.44. Shiri *et al.* (2014) in estimating genetic diversity in Iranian maize hybrids also obtained a mean PIC of 0.44 and concluded that the lower PIC value obtained may be due to a narrow genetic background of the studied genotypes. Silva *et al.* (2015), in a study to determine the population structure and genetic

diversity of Brazilian popcorn germplasm reported 2 to 8 alleles per SSR locus and a mean PIC of 0.53.

2.6.1 Similarity and Dissimilarity (Genetic Distance) Measures

Information concerning genetic distances is necessary for identifying parental combinations in heterotic breeding programs (Reif *et al.*, 2005). Depending on the type of data, the properties of the marker system employed, the germplasm genealogy, and the study objectives, many distance measures are available. The Euclidean or straight-line distance measure is the most commonly used method for estimating genetic distance between accessions or populations for morphological data (Mohammadi and Prasanna, 2003). Euclidean distance between two individuals (say I and j), may be calculated using the formula described by Weir (1996) as,

$$d_{ij} = \left[(x_1 - y_1)^2 + (x_2 - y_2)^2 + \dots (x_p - y_p)^2 \right]^{1/2} \dots (2)$$

Where i and j is the ED between two individual lines having morphological traits (p) $x_1, x_2, \dots, x_p$ is the traits for i individuals and $y_1, y_2, \dots, y_p$ is the traits for j individuals. Obeng-Antwi (2007) evaluated 90 landraces in Ghana and reported Euclidean distance ranging from 2.30-13.61.

The advent and explorations in molecular genetics has led to a better measurement of genetic distance. For DNA-based data where amplification products are equated to alleles, allele frequencies can be calculated and genetic distance between two individuals, say i and j may be estimated based on allelic informative data as

$$d_{ij} = 1 - \left[\sum^n (X_{ai} - X_{aj}) \right]^{1/r} \dots (3)$$

where X_{ai} is the frequency of the allele 'a' for individual 'I' and 'n' is the number of alleles per loci; 'r' is the constant based on the coefficient used. In its complex form

where $r = 2$, d_{ij} is referred to as Rogers (1972) measure of distance (RD), in which case the formula becomes

$$RD_{ij} = 1/2 \left[\sum (x_{ai} - x_{aj})^2 \right]^{1/2} \dots (4)$$

Allelic data can be converted into presence or absence binary matrix from which genetic distance or dissimilarity estimated. Mohammadi and Prasanna (2003) state that the most commonly used measures of genetic distance for binary data are (i) Nei and Li's (1979) coefficient (GD_{NL}), (ii) Jaccard's (1908) coefficient (GD_J), and (iii) simple matching coefficient (GD_{SM}) (Sokal and Michener, 1958). Genetic distance between any two individuals (say i and j) determined by the above measures may, respectively be estimated as follows:

$$GD_{NL} = \frac{2A}{2A+B+C} \dots (5)$$

$$GD_J = \frac{A}{A+B+C} \dots (6)$$

$$GD_{SM} = \frac{A+D}{A+B+C+D} \dots (7)$$

where A is the numbers of bands/alleles present in both individuals; D is the number of bands/alleles absent in both individuals; B is the number of bands/alleles present only in the individual i and C is the number of bands/alleles present only in the individual j .

The Nei and Li coefficient is sometimes also referred to as the Dice (1945) similarity coefficient and is similar to Jaccard's (1908) similarity coefficient except that double weight is given to the positive co-occurrences (A) in the former approach.

Studies in genetic diversity have shown high probability of non-amplification of DNA fragments (Duarte *et al.*, 1999; Reif *et al.*, 2005). Thus, similarity coefficients that consider the common absence of bands (Simple matching coefficient) should be

avoided since the absence of amplification of a determined band in two genotypes does not necessarily represent genetic similarity between them. This makes those coefficients that exclude negative co-occurrences from their expression of similarity such as Jaccards (1908) and Dice (1945) more adequate for binary data (Duarte *et al.*, 1999).

Commendable number of researchers have compared the efficiency of similarity coefficients for binary matrix data (Duarte *et al.*, 1999; Reif *et al.*, 2005; Balestre *et al.*, 2008). In a study to compare similarity coefficients in Common Bean using RAPD markers, Duarte *et al.* (1999), observed that the dendrograms generated using Jaccards (1908) and Dice (1945) similarity coefficients were very identical. Balestre *et al.* (2008), in a similar study observed identical clustering pattern of maize with Jaccards and Dice similarity coefficients with SSR markers. Nonetheless, these studies found that the efficiency of Dice similarity coefficient was higher. The Dice similarity coefficient is therefore considered as the most adequate for estimating genetic similarity or dissimilarity using binary data (Duarte *et al.*, 1999; Balestre *et al.*, 2008). Estimate of genetic distance on the basis of allele frequency and binary data matrix sets have previously been reported in maize (Obeng-Antwi *et al.*, 2011; 2012; Nidhal *et al.*, 2014). In assessing the intra-landrace variability in Ghanaian maize, ObengAntwi *et al.* (2011) reported genetic distances ranging between 1.90 to 18.80 and 2.30 to 14.14 for two landraces. In similar studies, Magorokosho (2006) and Nidhal *et al.* (2014) reported values of genetic distance ranging from 0.34 to 0.94 among 99 Southern Africa maize accessions and 0.24 to 0.79 in a set of 20 maize accessions in Iraq, respectively.

2.6.2 Grouping techniques in measuring genetic diversity

The use of established multivariate statistical algorithms is important in classifying breeding materials from germplasm, accessions, lines, and other races into distinct and variable groups depending on the genotype performance. Before subjecting to statistical grouping techniques, it is advisable to transform units of measurements of characters (agronomic) into standardized units. This will eliminate the impact of the unit in differences of measurement of each variable on variances and covariance.

Different multivariate approaches are available for analyzing the dissimilarity or similarity of genotypes based on variables recorded; cluster analysis (CA), Principal coordinate analysis (PCoA), Principal component analysis (PCA), Canonical Correlation and Multidimensional Scaling (MDS) (Aremu, 2012). CA and PCA are, however, the two commonly used approaches.

Cluster analysis is a group of multivariate techniques whose primary purpose is to group individuals or objects based on their characteristics, so that individuals with similar descriptions are mathematically gathered into the same cluster (Aremu, 2005). The resulting clusters of individuals would then exhibit high internal (within cluster) homogeneity and high external (between clusters) heterogeneity. Thus, with a successful classification, individuals within a cluster are similar or related to one another and different or unrelated to those in other groups. Distance-based clustering methods can either be hierarchical or nonhierarchical. The former is more commonly used in analysis of genetic diversity in crop species. Among various hierarchical methods, the UPGMA (Unweighted Paired Group Method using Arithmetic averages) is the most commonly adopted clustering method.

Principal components are used to derive a 2 or 3 dimensional scatter plot of individuals such that geometrical distances among individual genotypes reflect the genetic distances among them. It is done using standardized values to explore the contribution of each trait to the total variability (Obeng-Antwi *et al.*, 2011). The goal of PCA is to extract important information from a table and represent it as a set of new orthological variables. The first step in PCA is to calculate Eigen values which explain the amount of total variation displayed on the component axes. Eigen values greater than one are worthy of interpretation. The rationale is that an Eigen value less than one implies that the scores on the component would have negative reliability. It is expected that the first 3 axes will explain a large sum of the variations captured by the genotypes. The first PC summarizes most of the variability present in the original data relative to all remaining PCs. The second PC explains most of the variability not summarized by the first PC and uncorrelated with the first PC and so on. Generally each PC reveals different properties of the original data and as such is interpreted independently.

A combination of cluster analysis and principal component analysis have been used to classify and explain variation among maize genotypes (Lucchin *et al.*, 2003; Beyene, *et al.*, 2005; Obeng-Antwi *et al.*, 2011; 2012; Hafiz *et al.*, 2015). In characterizing Italian maize landraces, Lucchin *et al.* (2003) grouped 20 accessions into seven main principal axes on the bases of quantitative traits. The first four component axes with Eigenvalues greater than 1 explained 83.61 % of the total variation. The first PC alone contributed 46.09 % of the variation in their study. Beyene *et al.* (2005), using agro-morphological traits grouped traditional highland maize accessions from Ethiopia into five main principal components. The five principal components together in their study explained 75.10 % of the total variation, with the first three components, with Eigen values higher than 1.0, accounting for 69.30 % of the total variation. Morphological traits

such as days to tasseling and silking, plant and ear height, leaf length and days to maturity, were the major discriminatory traits associated with the first principal component axis, which accounted for 40.40 % of the total variation, while agronomic traits (number of kernels per row, number of rows per ear, 1000 seed weight, ear diameter and yield) were important traits associated with the second principal component, which accounted for 15.00 % of the total variation. The third PC, which explained 7.40 % of the total variation, was dominated by number of leaves per plant, leaf width and grain yield. In a related study, Obeng-Antwi *et al.* (2012) using 13 agromorphological traits grouped maize landraces in Ghana into four main principal component axes with Eigen values greater than unity explaining 69.82 % of the total variation. Agronomic traits such as grain yield per ear, ear diameter, cob diameter and number of kernels per ear which together contributed 27.60 % of the total variation loaded in PC₁. Vegetative traits such as plant height, ear height, days to anthesis and days to silking were loaded in PC₂ and accounted for 22.10 % of the total variation. PC₃ accounted for 11.50 % of the total variation and the traits involved included; ears per plant, Kernel rows, Kernel width and hundred kernel weight. Hafiz *et al.* (2015), in a study to evaluate the genetic diversity among maize genotypes using PCA and Cluster analysis grouped 40 accessions into four main clusters. Clusters I, II, III and IV accounted for 32.30 %, 27.00 %, 17.90 % and 14.10 %, respectively in their study.

In summary, the above review shows that there have been extensive breeding efforts to improve yield and other desirable attributes of maize in Ghana. These efforts have over the years led to the development of several varieties, especially in the past decade. With the increasing demand for maize in Ghana, it is expected that breeding efforts would be intensified in the coming years. For such efforts to be successful, it is highly imperative that the genetic variability of existing genotypes be assessed

accurately; hence the basis of the current study.

KNUST



CHAPTER THREE

3.0 MATERIALS AND METHODS

The study was conducted under two broad categories (1) Morpho-phenological diversity of maize genotypes and (2) SSR diversity of maize genotypes.

3.1 Morpho-phenological diversity of maize genotypes

3.1.1 Plant material

A set of seventeen improved maize genotypes produced in Ghana were investigated in the current study (Table 3.1). The genotypes were grouped into the following classes: (a) Open-pollinated varieties (OPVs), (b) hybrids, and (c) Populations. One CIMMYT population, 'Pool 16 SR' and one landrace, 'Kwadaso Local', were included as checks. 'Pool 16 SR' is a tropical lowland intermediate white dent and maize streak resistant genotype. The genotypes were obtained from the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR), Fumesua, Ghana. Each of the 17 materials was regenerated in the previous year by sowing of 13 rows with 12 hills per row. For the OPVs, female plants were detasseled and ears were pollinated by male rows. All ears harvested per population were bulked and shelled to form composite samples from which 30 individual seeds were randomly chosen and planted for evaluation of genetic diversity as well as molecular diversity based on simple sequence repeats. The accessions were evaluated in field trials at the Anwomaso Agricultural Experiment Station, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, from March to July and from August to November 2014.

Table 3.1: Pedigree information of the 17 maize accessions used in this study

Number	Name/variety	Status/Type	Pedigree/germplasm source
1	‘Abontem’	OPV	TZEE-YPOP.STR QPM CO
2	‘Aburohemaa’	OPV	EVDT-Waa STR QPM CO
3	‘Akposoe’	OPV	EV9990 QPM
4	‘Dodzi’	OPV	TZEEW SR BC3 (EV9990)
5	‘Dorke SR’	OPV	POOL 16 SR
6	‘Enibi’	Hybrid	GH110× Entry 75
7	‘Etubi’	Hybrid	GH110× Entry 85
8	‘Golden Jubilee’	OPV	Obatanpa/GH9866 SR
9	‘Honampa’	OPV	PVA SYN 6
10	‘Kwadaso Local’	Landrace (OPV)	Landrace
11	‘Mamaba’	Hybrid	GH110× Entry 5
12	‘M0826-12F’	Population	M0826
13	‘M0826-7F’	Population	M0826
14	‘Obatanpa’	OPV	CIMMYT POP. 63
15	‘Okomasa’	OPV	CIMMYT POP 43 SR
16	‘Omankwa’	OPV	TZE-W POP STR QPM C4
17	Pool 16 SR	CIMMYT OPV	CYMMYT Population

Source: CRI; OPV= Open-pollinated variety

3.1.2 Location of Experimental Sites

The accessions were tested in field trials at the Kwame Nkrumah University of Science and Technology Agricultural Experimental Stations at Anwomaso and Ayeduase in the Kumasi Metropolis in the Ashanti region of Ghana, from May to August and from August to December, 2014, respectively. Anwomaso is located at latitude 6° 41’ 28.4’’N and longitude 1° 30’ 58.8’’ W while Ayeduase is located at latitude 6° 41’N and 1° 33’ W. Soil type at the Anwomaso site is well-drained sandy loam with pH and organic matter content of 5.20 and 1.80 %, respectively while that of Ayeduase is 4.70 % and 2.13 %, respectively.

Both Anwomaso and Ayeduase experience an annual bimodal rainfall pattern with high relative humidity. The major rainy season begins from middle of March and ends

in July with a relatively short dry spell in August. The minor rainy season begins from September and ends in November. The mean annual rainfall at Anwomaso is 1500 mm and an average monthly temperature of not less than 20 °C

(20 °C - 25 °C). Information on the weather conditions during the study period at Ayeduase is presented Table 3.2.

The vegetation of the research sites is a semi-deciduous forest zone type characterized with thick grass cover commonly dominated with Guinea grass (*Panicum maximum*) on a weed frequency scale, with a fairly flat topography.

Table 3.2: Mean monthly weather conditions during the growing period at Ayeduase

Month	Temperature (°C)		Rainfall mm	Relative (%)Maximum	
	Minimum	Maximum			
August	20.9	27.7	7.42	80	90
September	21.5	28.0	13.58	69	89
October	21.7	30.3	12.56	64	86
November	22.4	32.1	13.40	61	83
December	21.8	32.1	5.40	77	52

Source: Ghana Meteorological Agency, Ayeduase, Animal Science Department-KNUST, 2014.

3.1.3 Land Preparation, Planting and Experimental Design

Land preparation involved ploughing and harrowing, followed by pre-emergence weed control with Roundup (glyphosate, 360 g/L) applied at 5.0 L/ha and Gramoxone (Paraquat) applied at 3.5 L/ha). All entries were planted in May and August 2014 in a randomized complete block design with three replications. Each entry was sown in single row plots measuring 7.5 m × 1.0 m. Plots were separated by 1.0 m alley and blocks were separated by 2 m. Hills were spaced at 0.5 m within rows. Three seeds were planted per stand and later thinned to one plant per stand after establishment, giving a target population of approximately 20,000 plants ha⁻¹.

Recommended crop management techniques were applied. The trials were irrigated throughout the growing seasons to supplement the low natural rainfall. Fertilizer equivalent to 120:60:40 kg ha⁻¹, of N-P₂O₅-K₂O plus sulphate of ammonia (125 kg/ha) was applied at 21 days after planting and at ear emergence. Post-emergence weeds were controlled by application of Atrazine (4.5 L/ha) and weeding by hand hoeing. Maize stem borers (*Buseola fusca*, *Sesamia calamistis*) and cutworms (*Agrotis spp.*) were controlled using Conpyrifos 48 % (1-1.5 L/ha) and Cymethoate Super (1-1.5 L/ha). All agro-chemicals were applied using a knapsack sprayer.

3.1.4 Collection and measurement of Morphological traits

Thirty morphological parameters consisting of 5 qualitative and 25 quantitative traits covering earliness, plant form, ear and kernels were collected following the maize descriptor list developed by IBPGRI and CIMMYT (1991). Quantitative traits were taken with meter rule, micrometre screw gauge, vernier calliper and weighing scale as appropriate with each data involved while qualitative data were taken using arbitrary scales. Additional variables calculated from direct measurements were: grain yield (YLD) calculated as shelled grain weight per plot adjusted to 125 g kg⁻¹ moisture and converted to Mg ha⁻¹, Anthesis-silking interval (ASI) calculated as SD less AD, number of ears per plant calculated as number of ears (NE) with at least one fully developed grain divided by number of plants harvested and ear position calculated as EHT divided by PLHT. Ten plants from each plot were evaluated for the morpho-phenological traits.

Table 3.3: List of the 30 morphological descriptors of maize used in the study

	Trait	Code	Measurement procedure/ description
1	Anthesis date	AD	Number of days from sowing to when 50 % of plants sheds pollen in a plot.
2	Silking date	SD	Number of days from planting to 50 % of the plants in a plot having silks at least 1 cm long
3	Anthesis-silking interval	ASI	Calculated as SD-AD (days)
4	Number of leaves per plant	NL	Count total number of leaves on a plant after silking
5	Tassel length	TL	Measured from the point of origin to the tip of the central spike at blister stage.
6	Ear leaf length	ELL	Length of Leaf which subtends the uppermost ear at blister stage (cm)
7	Ear leaf width	ELW	Width of Leaf which subtends the uppermost ear (mm) at blister stage
8	Plant height	PLHT	Height from soil level to the flag leaf insertion node (cm) at milk stage
9	Ear height	EHT	Height from soil level to upper ear insertion node (cm) at milk stage
10	Stalk diameter	StD	Diameter of stem measured on the second internode (cm) at milk stage
11	Stay green	SG	Estimation of % dead leaves at milk stage
12	Ear position	EP	Calculated as EHT divided by PLHT at physiological maturity
13	Number of ears per plant	EN	calculated as number of ears (NE) divided by number of plants harvested per plot
14	Ear length	EL	Length of ears located on the highest point on plant (cm) on plot basis after harvest
15	Ear weight	EWT	Weight of harvested cobs (kg) per plot after harvest
16	Ear diameter	ED	Diameter of ears located on the highest point on plant (cm) on plot basis after harvest.

17	Number of rows per ear	NRE	Number of kernels around the cob at a height of 5 cm from the shank of uppermost ear.
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Table 3.3 continued

	Trait	Code	Measurement procedure/ description
18	Number of kernels per row	NKR	Average number of kernels in two rows on opposite sides of cob after harvest.
19	Kernel length	KL	Length of five randomly selected kernels (mm) per plot
20	Kernel width	KW	Width of five randomly selected kernels (mm) per plot
21	Kernel thickness	KT	Diameter of five randomly selected kernels (mm) per plot
22	Cob diameter	CD	Diameter of cob at a height of 5 cm from the shank (cm) on plot basis
23	Shelled grain weight	GWT	Weight of grains of all shelled cobs (kg) on plot basis after harvest
24	Grain yield	YLD	Shelled grain weight per plot adjusted to 12.0 g/kg moisture and converted to Mg ha ⁻¹
25	One hundred kernel weight	HKWT	Mass of 100 kernels adjusted to 12.5 % moisture content
26	Silk colour	SC	Pigmentation of the silk, Pale yellow=1 and red=2
27	Kernel arrangement	KA	1=regular, 2=irregular, 3=straight and 4=spiral
28	Cob colour	CC	Score 0=red and 5=white
29	Kernel texture	KTEX	Score 1=flint, 3=mixed and 5= dent
30	Principal grain colour	PGC	Score 0=white and 1=other colours

Adapted from IBPGR, 1991: Descriptors for Maize, International Maize and Wheat Improvement Centre, Mexico City/ International Board for Plant Genetic Resources, Rome

3.1.5 Statistical analyses of morphological data

Means, standard deviation, minimum and maximum values, as well as frequencies for the two environments were computed for qualitative and quantitative traits, using PROC FREQ and PROC MEANS procedures, respectively, of SAS version 9.3 (SAS Institute, Cary, 2011). Combined analysis of variance was performed for the metric traits by the SAS General Linear Model (GLM) Procedure. Differences and means were located by Duncan multiple range test (DMRT) means. Variance components were extracted from the expected mean squares (EMS) of main and interaction effects. The format of ANOVA and generation of the expected mean squares are presented in Table 3.4.

Table 3.4: Expected mean squares and extraction of variance components Format of analysis of variance for obtaining estimates of variance

Source	df	MS	Expected Mean Square
Environment	$e-1$	M_e	$\sigma^2_e + r\sigma^2_{ge} + g\sigma^2_{r(e)} + rg\sigma^2_e$
Rep (Environment)	$e(r-1)$	M_{re}	$\sigma^2_e + g\sigma^2_{r(e)}$
Genotype	$g-1$	M_g	$\sigma^2_e + r\sigma^2_{ge} + re\sigma^2_g$
Genotype*environment	$(e-1)(g-1)$	M_{ge}	$\sigma^2_e + r\sigma^2_{ge}$
Error	$e(g-1)(r-1)$	M_e	σ^2_e

where, g , e and r , are numbers of genotypes, environment and replicates, respectively.

σ^2_e = environmental variance component σ^2_g =

genotypic variance component σ^2_e = variance

component associated with environment σ^2_{ge} = variance

component associated with $g \times e$ $\sigma^2_e = M_e =$

environmental or error variance component $\sigma^2_g = M_g =$

$(M_g - M_{ge})/r_e =$ genotypic variance component $\sigma_y^2 = \{(M_e$

$+ M_e) - (M_{re} + M_{ge})\}/r_g$ variance component associated

with environment $\sigma_{ge}^2 = (M_{ge} - M_e)/r =$ variance

component associated with $g \times e \sigma_{r(e)}^2 = (M_{ry} - M_e)/g =$

variance component of replication within environment

Broad-sense heritability of traits H_B^2 was calculated on plot basis as the ratio between the genetic variance σ_g^2 and the phenotypic variance, σ_p^2 . The phenotypic variance is given by

$$\sigma_p^2 = \sigma_g^2 + \sigma_{ge}^2 + \sigma_{r(e)}^2 \dots (8)$$

The broad sense heritability is given by
$$H_B^2 = \frac{\sigma_g^2}{\sigma_p^2}$$
 which translates into

$$H_B^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2 + \sigma_{r(e)}^2} \dots (9)$$

The phenotypic coefficient of variation (%) was calculated as

$$PCV = \frac{\sigma_p}{\bar{p}} \times 100\% \dots (10)$$

and the Genotypic coefficient of variation (%) as

$$GCV = \frac{\sigma_g}{\bar{g}} \times 100\% \dots (11)$$

where σ_P and σ_g are the phenotypic and genotypic standard deviations, respectively, and $\bar{X}$ is the population mean of the trait under consideration. All computations were performed by SAS.

3.1.5.1 Genetic distance measure and relationship among accessions

Genetic distances among accessions were based on calculation of Euclidean distance on the standardized agro-morphological data using SIMINT option of NTSYS (Rohlf, 2009). Relationships among the accessions and traits were determined from a tree plot generated by the hierarchical Unweighted Pair Group Method with Arithmetic Average (UPGMA) by means of SAHN option of NTSYS.

The standardized mean values of morpho-phenological traits were subjected to principal component analyses (PCA) to obtain information on the most discriminating traits. From the eigenvectors, eigenvalues, individual and cumulative proportions of the total variation expressed by the traits were calculated and the traits which contributed most to the variation were revealed. The first two components displaying the maximum variance were selected for further ordination analysis to generate biplots. All analyses were carried out using NTSYS-software (Rohlf, 2009).

3.2 SSR diversity in maize genotypes

3.2.1 Sampling of leaves, DNA Extraction and DNA quality assessment Young fresh leaves at the third and fourth leaf stage after planting was used. For each accession, 1 cm² leaf disc was harvested from 15 plants and bulked to form composite samples. The leaves were cut, wiped with ethanol and immediately transferred into plastic bags and transported to the laboratory on ice cubes for storage at -80 °C until

further processing. DNA was extracted from 200 mg of bulked leaf tissue of each accession using the CTAB procedure (Saghai-Maroo *et al.*, 1984) with little modifications by Kirkhouse Trust Mobile Laboratory of the Cocoa Research Institute of Ghana. Leaf samples were ground to fine powder with liquid nitrogen in 2.0 ml Eppendorf tubes and to it was added 500 μ l of extraction buffer incubated in water bath at 65 °C for 30 minutes with intermittent gentle rocking. The tubes were cooled to room temperature and 1 % β -mercaptoethanol and 33 μ l of 20 % SDS were added in a fume hood. Samples were mixed thoroughly by several inversions of the tubes for 5 to 10 minutes and centrifuged at 14,000 rpm for 10 minutes. To each mixture was added equal volumes of chloroform: isoamyl alcohol solution were added to and shaken by gentle inverting for 5 to 10 minutes and centrifuged at room temperature to separate the aqueous phase from the organic phase. The top aqueous phase containing the nucleic acids was transferred into clean 1.5 ml tubes containing 4 μ l of 10 mg/ml RNase A (pre-boiled) and gently mixed by inversion followed by incubation at 37 °C for 15 minutes. To the mixture was added two thirds volume of ice cold isopropanol and kept overnight at -20 °C to enhance DNA precipitation. The precipitated DNA was centrifuged at 14,000 rpm for 5 minutes to obtain DNA pellets and the isopropanol was carefully decanted. The DNA pellets were then washed with 70 % ethanol on a rocking surface and centrifuged at 5,000 rpm for 3 minutes. The ethanol was decanted and pellets dried at room temperature. The washed DNA was then transferred to a 2 ml microfuge tube containing 200 μ l of 1 $\times$ TE buffer. The tube was capped and rocked gently overnight at room temperature to dissolve DNA. The quality of each DNA isolate was checked by electrophoresis on 0.8 % agarose gel. Samples were finally stored at 4 °C until required for use.

3.2.2 Primers and PCR amplification

The maize DNA samples were amplified using a set of SSR primer pairs selected from the public maize Database (<http://www.agron.missouri.edu/ssr-probes/ssr.htm>) based on their polymorphism information content from previous studies (Warburton *et al.*, 2002; Choukan and Warburton, 2005) and chromosome location with at least one SSRs per chromosome. A total of 15 primers were first tested for their ability to reveal easily scorable polymorphism and only those that showed amplifications were selected after optimizing the PCR conditions. Twelve (12) out of the 15 primers were used in all. There was no representation for chromosomes 4 and 6 (Table 3.5).

The SSR markers were amplified by PCR in a 15 μ l reaction mix consisting of 1 μ l DNA template, 0.5 μ l each of forward and reverse primer, 0.10 μ l of Taq DNA polymerase and 7.5 μ l of 10 $\times$ PCR reaction buffer topped up with 5.4 μ l deionized water in 0.2 ml PCR tubes on ice. The reaction mixture was mixed thoroughly by spinning and the PCR tubes were loaded in a thermal cycler (Master cycler, Hamburg, Germany). The PCR was programed at a temperature of 94 $^{\circ}$ C for 5 min for an initial denaturation (profile 1) followed by 94 $^{\circ}$ C for 45 sec for denaturation (profile 2), X $^{\circ}$ C for 45 sec for annealing (profile 3), 72 $^{\circ}$ C for 1 minute for extension (profile 4), all run over 35 cycles. This was followed by one final extension cycle at 72 $^{\circ}$ C for 5 min and an indefinite hold at 4 $^{\circ}$ C. The X $^{\circ}$ C refers to the annealing temperature which varied from 52 $^{\circ}$ C to 60 $^{\circ}$ C for the primers used (Table 3.5).

Table 3.5: Names of the 12 SSR markers showing their location on a chromosome, repeat unit and annealing temperature for evaluation of diversity among Ghana maize cultivars

SSR locus	Repeat unit	Bin number	Annealing temperature ($^{\circ}$ C)
phi002	AG/CT	1.08	60
phi109642	ACGG	2.00	54

phi453121	ACC	3.00	54
nc133	GTGTC	3.00	54
umc1399	CTAG	3.07	54
nc130	AGC	5.00	54
phi328175	AGG	7.04	54
phi100175	AAGC	8.03	54
umc1161	GCTGGG	8.06	54
umc1279	CCT	9.00	54
phi065	CACTT	9.03	54
umc1196	CACACG	10.07	52

3.2.3 Electrophoresis and visualization of amplified products

PCR products were electrophoresed on 2 % agarose gel system. Ten (10) µl of a mixture of the PCR product and O'Gene 6× blue loading dye (Thermo Scientific) were loaded into a 1.0 mm wide gel well with a 100 bp (100 ng/µl) DNA ladder (KAPA Biosystems, Cape Town, South Africa). A comb with 50 wells was used with the first well loaded with 5 µl of the 100 bp ladder, followed by a negative control (purified water instead of DNA) and then the bulked PCR products of each of the 17 genotypes. The gels were run in 1× TAE buffer stained with gel red for 90 minutes at 120 V. After electrophoresis, gels were visualized using a gel documentation system (Digi DOC-It imaging system, UVP Inc.).

3.2.4 Statistical analysis of molecular diversity data

Allele sizes of the SSR bands were determined by comparing with 100 bp molecular weight ladder (KAPAbiosystems, Cape Town, South Africa). Each SSR primer was considered as a locus, and each band as an allele. DNA bands were scored as presence or absence data to produce a 17×12 binary matrix where 1 indicated presence of a specific allele and 0 indicated absence. The numeral 9 was given to absence of amplified products. Only clear and unambiguous bands were scored.

Genetic diversity was analysed based on three genetic diversity measurements, viz., number of alleles per locus (allelic richness), genetic distance and polymorphic

information content (PIC) which gave an estimate of the discriminatory power of a locus by taking into consideration the number and frequency of occurrence of each allele (Smith *et al.*, 1997). Polymorphic information content (PIC) of each marker was calculated by applying the formula,

$$PIC = 1 - \sum_{i=1}^n f_i^2 \dots (11)$$

Where, f is the frequency of the i th allele (Smith *et al.*, 1997). The binary data matrix, being binomial and not a normal distribution did not require standardization.

Genetic similarity or distance between all pairs of genotypes was estimated by the DICE coefficient (Dice, 1945) as,

$$\frac{2a}{2a + b + c} \dots \dots (12)$$

using the SIMQUAL procedure of NTSYS (Rohlf, 2009), where a is the number of bands/alleles present in both individuals; b is the number of bands/alleles present only in one individual i and c is the number of bands/alleles present only in the other individual, j . Subsequently, the similarity matrix generated was used to generate a dendrogram based on UPGMA so as to visualize the genetic relationships among the accessions. To determine the desired number of clusters, the dendrogram was cut at where the largest distinction was created by using the formula proposed by Shiri *et al.* (2014) as,

$$\text{Number of clusters} = \sqrt{\frac{n}{2}} \dots \dots (13). \text{ where } n \text{ is the number of genotypes}$$

CHAPTER FOUR

4.0 RESULTS

4.1 Morpho-phenological variability

4.1.1 Variability in qualitative traits

The frequencies of classes of genotypes among the 17 accessions on the basis of qualitative traits are shown in Table 4.1. In all, 1,024 individual plants were evaluated. All traits demonstrated wide variability expressed in varying percentages of the various classes except cob colour in which the predominant colour was white (100 %). The general description of the ears of the improved Ghana maize populations was red silks (83.40 %) which produced white kernels (77.20 %) having mixed (54.30 %), kernel texture in straight kernel arrangement (78.38 %) borne on white cobs (100 %) (Table 4.1).

Table 4.1: Distribution of qualitative traits in 17 maize cultivars grown in Ghana.

NO.	Trait	Description	Class/Score	Number of Plants/Cobs	Percentage (%)
1	SC	Pale yellow	1	170	16.60
		Red	2	854	83.40
2	KA	Straight	1	377	78.38
		Irregular	2	26	5.41
		Spiral	3	78	16.22
3	KTEX	Flint	1	340	32.17
		Mixed	3	574	54.30
		Dent	5	143	13.54
4	PGC	White	0	816	77.20
		Others	1	241	22.80
5	CC	Red	0	0	0.00
		White	5	1024	100.00

SC= Silk colour, KA= kernel arrangement, KTEX= Kernel texture, PGC= Principal grain colour, CC= Cob colour

4.1.2 Variation in quantitative traits

Variability in quantitative traits was assessed by coefficient of variability and significance of the mean squares from analyses of variance. The descriptive statistics

of all traits observed across the 17 accessions are shown in Table 4.2. The accessions were significantly ($P < 0.05$) different for all the traits except KT and KW. The coefficient of variation (CV) varied from a minimum value of 9.48 % for AD to a maximum of 125.86 % for ASI. Plant architectural traits exhibited the widest ranges of variation compared to other traits. Generally, wider coefficient of variation was observed across the accessions for traits viz. ASI, YLD, SG, GWT and EHT. EP and ED recorded the lowest standard error especially with respect to their means.



Table 4.2: Means, standard deviations, range, coefficient of variation and standard error of 25 agro-morphological traits in 17 maize varieties across two environments in Ghana.

Trait	Mean	StD	Min.	Max.	CV (%)	S.E	Mean square (Accession)
AD (days)	55.34	5.25	40.00	76.00	9.48	0.17	76.36**
SD (days)	56.72	5.62	40.00	77.00	9.91	0.18	87.87**
ASI (days)	1.36	1.71	-6.00	12.00	125.86	0.05	1.78**
TL (cm)	47.48	6.07	19.20	68.00	12.79	0.19	55.40**
ELL (cm)	85.69	10.58	47.60	120.00	12.35	0.34	211.60**
ELW (cm)	9.66	1.41	5.00	14.80	14.58	0.04	4.10**
PLHT (cm)	153.99	29.01	65.00	258.00	18.84	0.92	1837.33**
EHT (cm)	66.95	20.64	10.25	141.00	30.83	0.65	878.77**
StD (mm)	20.35	2.76	10.23	25.49	13.55	0.09	13.67**
NL	12.36	1.42	7.00	17.00	11.50	0.04	2.74**
SG (%)	14.83	7.76	0.00	50.00	52.30	0.26	29.30*
EP	0.43	0.08	0.06	0.84	19.31	0.00	0.01**
ED (cm)	4.73	0.44	3.60	6.10	9.52	0.02	0.44**
EL (cm)	16.79	2.67	9.60	24.00	15.88	0.12	11.59**
KL (mm)	10.70	1.10	7.26	14.04	9.96	0.04	1.91**
KW (mm)	9.45	0.92	7.00	11.44	9.72	0.04	0.54
KT (mm)	4.63	0.69	3.04	7.38	14.84	0.03	0.20
NRE	14.09	1.96	10.00	20.00	13.99	0.09	4.08**
CD (cm)	2.81	0.31	1.70	3.90	11.03	0.01	0.22**
NKR	34.27	5.94	16.00	53.75	17.33	0.27	52.33**
EN	1.07	0.12	1.00	1.45	11.44	0.01	0.03*
EWT (kg)	1.75	0.85	0.30	4.10	48.23	0.08	1.93**
HKWT(kg)	26.2	4.07	17.46	35.94	15.53	0.41	34.10**
GWT (kg)	1.33	0.66	0.20	3.30	49.91	0.07	1.04**
YLD (t/ha)	2.31	1.29	0.30	6.82	55.7	0.13	2.86**

AD = Anthesis date, SD = Silking date, ASI = Anthesis–silking interval, TL = Tassel length, ELL = Ear length, ELW = Ear length width, PLHT = Plant height, EHT = Ear height, StD = Stem diameter, NL = Number of leaves, SG = Stay green, EP = Ear position, ED = Ear diameter, EL = Ear length, KT = Kernel thickness, KW = Kernel width, NRE = Number of rows per ear, NKR = Number of kernels per row, EN = Ear number, EWT = Ear weight, HKWT = Hundred kernel weight, GWT = Grain weight, YLD = Yield. CV = Coefficient of variation. * $p \leq 0.05$, ** $p \leq 0.01$

4.1.3 Analyses of variance of the three classes of genotypes

The overall mean square results showed that earliness was not significant across the three classes of genotypes (Table 4.3). Across the classes, TL, ELL, ELW, SG, EP, NKR and grain YLD were significant ($P < 0.05$). The populations recorded the highest values for TL (51.61 cm), ELL (92.77 cm) and ELW (10.33). The population was however not significantly different from the hybrid group for traits *viz.*, SG, NKR and grain YLD.

Results of the analyses of variance supported the low variabilities among the cultivars. The mean square results of the three classes of genotypes (Tables 4.4 to 4.6) were variable. In the populations, the mean squares were significantly ($P < 0.05$) different for only EN. The main effect of cultivar and environment were the most important sources of variation. The interaction effect was not important. Among the OPVs, mean squares were significantly different ($P < 0.05$) for only grain YLD but highly significant ($P < 0.01$) for all traits except SG, ED, EL, KW, and KT. The main effect of cultivar as well as interaction effects were important. Among the hybrids, ASI, TL, ELW, EP, StD, and KW were significantly different ($P < 0.05$) but highly significant ($P < 0.01$) for PLHT and EHT. In the hybrid varieties, mean squares were significantly ($P < 0.05$) different for ASI, TL, ELW, EP, StD, and KW, and highly significantly ($P < 0.01$) different for PLHT and EHT. Variation in earliness traits was observed only in the OPVs.

Table 4.3: Combined Analyses of variance for various traits across three classes of maize genotypes in Ghana

Trait	OPVs	Populations	Hybrids	Means square
AD	54.92 ^a	55.23 ^a	57.08 ^a	33.86
SD	56.37 ^a	56.11 ^a	58.31 ^a	29.54
ASI	1.43 ^a	0.91 ^b	1.19 ^a	1.68
TL	46.87 ^b	51.64 ^a	46.54 ^b	133.80 ^{**}
ELL	84.94 ^b	92.77 ^a	83.94 ^b	373.60 ^{**}
ELW	9.51 ^b	10.33 ^a	9.83 ^b	4.03 [*]
PLHT	146.74 ^a	159.02 ^a	154.92 ^a	669.70
EHT	69.01 ^a	67.83 ^a	59.73 ^a	623.28
SG	15.84 ^a	12.43 ^b	12.82 ^b	106.40 ^{**}
EP	0.44 ^a	0.43 ^{ab}	0.41 ^b	0.01 [*]
ED	4.69 ^a	4.90 ^a	4.74 ^a	15.98 [*]
EL	16.39 ^a	17.36 ^a	17.75 ^a	0.25
KL	10.63 ^a	10.91 ^a	10.78 ^a	0.50
KW	9.50 ^a	9.50 ^a	9.25 ^a	0.46
NRE	14.02 ^a	14.06 ^a	14.35 ^a	0.77
CD	2.80 ^a	2.92 ^a	2.77 ^a	0.100
NKR	33.26 ^b	36.02 ^a	36.29 ^a	91.50 ^{**}
HKWT	26.33 ^{ba}	27.57 ^a	24.64 ^b	34.7 [*]
YLD	2.11 ^b	3.02 ^a	2.58 ^{ba}	5.38 [*]

Traits abbreviation and units as given in Table 4.1. *p≤0.05, **p≤0.01

Table 4.4:

Mean squares for traits of two maize populations evaluated across two environments (Env) in Ghana.

Source	df	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	StD	NL	SG	EP	ED
Env	1	0.90	4.50	1.69*	13.70	0.01	0.18	85.79	80.60	5.38**	2.75*	26.92	0.00	0.78
Rep(env)	2	3.48	3.97	0.15	2.47	33.72	0.43	148.81	22.93	5.37**	0.04	38.18	0.00	0.04
Acc	1	7.80	10.98	0.42	3.86	0.50	0.15	47.81	61.36	0.25	0.28	1.23	0.00	0.04
Acc*Env	1	9.97	7.14	0.13	0.02	0.10	0.00	42.93	38.48	0.64	0.00	2.38	0.00	0.12
Error	4	5.78	4.13	0.18	6.48	9.51	0.07	91.42	64.75	0.09	0.20	23.10	0.00	0.11
% CV		4.33	3.61	46.8	4.90	3.32	2.51	6.03	11.87	1.39	3.67	39.13	5.93	6.81
R ²		0.61	0.70	0.78	0.52	0.75	0.85	0.59	0.48	0.98	0.83	0.57	0.48	0.70
<u>Source</u> Env	<u>df</u>	<u>EL</u>	<u>KL</u>	<u>KW</u>	<u>KT</u>	<u>NRE</u>	<u>CD</u>	<u>NKR</u>	<u>EN</u>	<u>EWT</u>	<u>HKWT</u>	<u>GWT</u>	<u>YLD</u>	
	1			0.00	0.03	0.12	0.18	0.12	0.01	3.41	25.67*	2.00	4.64	
		7.08	0.49											
Rep(env)	2	5.15	0.48	0.43	0.36	2.08*	0.02	2.08*	0.09*	0.06	0.36	0.02	0.42	
Acc	1	2.17	0.01	0.37	0.01	0.01	0.15	0.01	0.05*	0.08	7.22	0.01	0.52	
Acc*Env	1	3.43	0.89	0.01	0.06	1.61	0.15	1.61	0.02	0.21	0.11	0.10	0.99	
Error	4	2.33	0.42	0.29	0.16	0.29	0.04	0.29	0.00	0.60	3.44	0.48	0.70	
% CV		8.80	5.87	5.60	8.80	3.84	7.18	3.84	5.63	30.27	6.65	36.82	27.46	
R ²		0.74	0.59	0.59	0.63	0.90	0.79	0.90	0.95	0.67	0.74	0.57	0.72	

AD = Anthesis date, SD = Silking date, ASI = Anthesis-silking interval, TL= Tassel length, ELL= Ear length, ELW = Ear length width, PHT= Plant height, EHT= Ear height, StD = Stem diameter, NL = Number of leaves, SG = Stay green, EP = Ear position, ED = Ear diameter, EL = Ear length, KT = Kernel thickness, KW=Kernel width, NRE = Number of rows per ear, NKR = Number of kernels per row, EN = Ear number, EWT = Ear weight, HKWT = Hundred kernel weight, GWT= Grain weight, YLD= Grain Yield. *p≤0.05, **p≤0.01. Env = Environment; Rep (env) = Rep within environment; Acc = Accession, Acc*Env = Accession by environment interaction.

Table 4.5:

Mean squares for traits of 12 OPVs evaluated across two environments in Ghana.

Source	df	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	StD	NL	SG	EP	ED
Env	1	0.20	15.79*	9.35**	41.26**	342.69**	6.04**	3544.03**	1196.03**	0.69	0.00	49.85*	0.00*	0.78
Rep(env)	2	11.16*	15.46*	0.29	8.89	84.05**	1.29*	1346.68**	799.27**	7.69**	0.81*	100.14**	0.01**	0.04
Acc	11	108.45**	121.46**	2.11**	46.72**	214.96**	4.42**	2361.76**	1006.02**	15.83**	3.92**	20.31	0.01**	0.04
Acc*Env	11	1.95	2.79	0.35	5.69	21.28	0.47*	163.96	123.82**	3.11**	0.70*	18.44	0.00**	0.12
Error	44	3.16	3.34	0.33	3.56	11.48	0.22	114.08	48.44	0.70	0.27	12.10	0.00	0.11
% CV		3.24	3.24	40.08	4.04	4.00	5.01	6.92	10.16	4.18	4.17	21.95	5.70	6.81
R ²		0.90	0.91	0.72	0.80	0.86	0.87	0.87	0.88	0.88	0.84	0.58	0.34	0.70
Source	df	EL	KL	KW	KT	NRE	CD	NKR	EN	EWT	HKWT	GWT	YLD	
Env	1	7.08	1.55	0.55	0.45	0.00	0.03	0.00	0.04*	0.83	45.48*	0.32	1.00	
Rep(env)	2	5.15	0.22	0.25	0.13*	0.18	0.00	0.18	0.00*	0.39	0.54	0.33	3.59	
Acc	11	2.17	2.54**	0.47	0.24	5.70**	0.24**	5.70**	0.02**	1.29**	34.42**	0.73**	2.32*	
Acc*Env	11	3.43	0.24	0.50	0.37	1.64	0.07*	1.64	0.02	0.85*	29.73	0.47	2.84*	
Error	44	2.33	0.62	0.42	0.18	1.33	0.03	1.33	0.01	0.34	12.81	0.29	1.16	
% CV		8.80	7.42	6.82	9.14	8.24	6.20	8.24	7.94	38.56	13.66	46.41	52.32	
R ²		0.74	0.56	0.41	0.48	5.60	0.74	5.60	0.62	0.64	0.58	0.55	0.57	

Table 4.6:

AD= Anthesis date, SD= Silking date, ASI =Anthesis–silking interval, TL= Tassel length, ELL= Ear length, ELW= Ear length width, PLHT= Plant height, EHT= Ear height, StD= Stem diameter, NL= Number of leaves, SG=Stay green, EP= Ear position, ED= Ear diameter, EL=Ear length, KT= Kernel thickness, KW=Kernel width, NRE=Number of rows per ear, NKR=Number of kernels per row, EN= Ear number, EWT= Ear weight, HKWT= Hundred kernel weight, GWT= Grain weight, YLD= Grain Yield. * $p \leq 0.05$, ** $p \leq 0.01$ Env=Environment; Rep (env) =Rep within environment; Acc=Accession, Acc*Env=Accession by environment interaction.

Mean squares for traits of 3 hybrid maize varieties evaluated across two environments (Env) in Ghana.

Source	df	AD	SD	ASI	TL	ELL	ELW	PLHT	EHT	StD	NL	SG	EP	ED
Env	1	0.79	0.49	2.09**	56.33**	256.05**	1.33**	1496.75**	688.43**	0.68	0.21	15.10	0.00	0.00
Rep(env)	2	18.55	23.58	0.34	3.93	61.35	1.41**	608.33**	372.60**	4.62**	2.60**	34.14*	0.01*	0.01
Acc	2	1.21	1.70	1.06*	11.15*	33.70	0.49*	219.38**	447.11**	2.73*	0.02	9.48	0.01*	0.08
Acc*Env	2	3.32	4.55	0.24	9.03	20.94	0.28	91.57*	37.19	1.72	0.25	18.65	0.00	0.01
Error	8	8.88	8.11	0.19	2.53	14.44	0.09	19.53	32.91	0.54	0.17	7.63	0.00	0.03
% CV		5.22	4.88	36.52	3.42	4.53	3.08	3.01	9.60	3.59	3.35	21.56	8.41	3.93
R ²		0.41	0.49	0.79	0.84	0.84	0.93	0.96	0.92	0.85	0.89	0.72	0.82	0.50
Source	df	EL	KL	KW	KT	NRE	NKR	EN	EWT	HKWT	GWT	YLD		
Env	1	0.25	0.33	0.14	0.22	0.05	0.05	0.01	0.16	3.78	0.07	0.15		
Rep(env)	2	4.04*	0.15	0.11*	0.18	3.55*	3.55*	0.06*	0.58	0.65	0.46	3.93*		
Acc	2	1.78	0.69	0.88	0.17	0.65	0.65	0.00	0.74	9.07	0.49	1.09		

Table 4.7:

Acc*Env	2	0.68	0.03	1.22	0.09	2.11	2.11	0.03	0.71	3.01	0.48	2.76*
Error	8	0.66	0.57	0.21	0.14	0.76	0.76	0.01	0.25	12.28	0.15	0.61
% CV		4.57	7.02	4.92	8.22	6.08	6.08	10.49	25.00	14.22	25.40	30.29
R ²		0.73	0.47	0.74	0.51	0.70	0.70	0.66	0.68	0.30	0.72	0.77

AD = Anthesis date, SD = Silking date, ASI = Anthesis–silking interval, TL = Tassel length, ELL= Ear length, ELW = Ear length width, PLHT= Plant height, EHT= Ear height, StD = Stem diameter, NL = Number of leaves, SG = Stay green, EP = Ear position, ED = Ear diameter, EL = Ear length, KT = Kernel thickness, KW = Kernel width, NRE = Number of rows per ear, NKR = Number of kernels per row, EN = Ear number, EWT= Ear weight, HKWT = Hundred kernel weight, GWT= Grain weight, YLD= Grain Yield. * $p \leq 0.05$, ** $p \leq 0.01$. Env=Environment; Rep (env)=Rep within environment; Acc=Accession, Acc*Env=Accession by environment interaction.



4.1.4 Mean performance of the three classes of genotypes

4.1.4.1 Earliness of the three classes of genotypes

Earliness in maize is desirable for drought tolerance. On a plant basis, AD varied from 40 days for ‘Dodzi’ and ‘Aburohemaa’ to 76 days for ‘M0826-7F’, with a mean of 55.34 ± 5.25 days, SD of 40 days for ‘Aburohemaa’ to 77 days for ‘Okomasa’ with a mean of 56.72 ± 5.62 days, and ASI of -6 days in ‘Kwadaso Local’ to 12 days in ‘Okomasa’ with a mean of 1.36 ± 1.71 days. The large standard deviation of ASI demonstrates wide variability in ASI depicted by 79 (8 %) individual plants demonstrating development of silks 1 to 6 days earlier than emergence of the tassel, a phenomenon termed protogyny. On accession mean basis, the earliest cultivar was ‘Akposoe’ with 49.55 ± 0.76 days to anthesis and 51.03 ± 0.99 days to silking. Though ‘Akposoe’ was most early-maturing, its earliness was not significantly different from that of ‘Dodzi’ (49.87 ± 2.83) and ‘Pool 16 SR’ (51.62 ± 1.93) in days to anthesis, and ‘Dodzi’ (51.60 ± 2.67), ‘Pool 16 SR’ (52.25 ± 2.04) and ‘Aburohemaa’ (53.24 ± 2.28), respectively in days to silking. The most late-maturing cultivar was ‘Okomasa’ with AD of 63.58 ± 1.24 days and SD of 65.60 ± 2.02 days (Tables 4.7 to 4.9).

4.1.4.2 Plant architecture of the three classes of genotypes

Plant architectural traits encompass TL, PLHT, ELL, ELW, EHT, StD, NL, SG and EP. The range and mean of the plant architectural traits were on a plant basis, TL 6.07 to 68.00 cm with a mean of 47.48 ± 19.20 cm, PLHT 65.00 to 258.00 cm with a mean of 153.99 ± 29.01 , ELL 47.60 to 120.00 cm with mean of 85.69 ± 10.58 cm, ELW 5.00 to 14.80 cm and mean of 9.66 ± 1.41 cm, EHT 10.25 to 141.00 cm and mean of 66.95 ± 20.64 cm, StD 10.23 to 25.49 mm and mean of 20.35 ± 2.76 , NL 7.00 to 17.00, mean of 12.36 ± 1.42 , and finally SG 0 % to 50 % and mean of 14.83 ± 7.76 .

While majority of the cultivars showed variable plant architectural traits, ‘Akposoe’ and ‘Obatanpa’ consistently exhibited the lowest and highest values of ELL, ELW, PLHT, EHT, and StD, respectively. ‘Akposoe’ had mean values of 76.43 ± 6.51 cm, 7.97 ± 1.06 cm, 130.23 ± 17.41 , 51.34 ± 10.08 , and 16.85 ± 1.87 while ‘Obatanpa’ had 95.00 ± 4.31 cm, 10.85 ± 1.04 cm, 194.89 ± 16.43 cm, and 89.18 ± 10.52 cm and 22.48 ± 1.09 mm, respectively (Table 4.7-4.9).

On accession mean basis, TL ranged from 42.74 ± 1.68 cm for ‘Honampa’ to 53.07 ± 2.76 cm for ‘Obatanpa’, both of which are OPVs. The populations had long TLs similar to that of ‘Obatanpa’ while TL of the hybrids were relatively lower. Long TL are beneficial for efficient pollen shed and is expected to correlate with yield components. In the current study, TL had positive correlation ($r=0.47$, R^2 of 0.23 to $r=0.74$, R^2 of 0.55) with ELL, ELW, PLHT, EHT, and StD indicating that plants with long TL also had long ELL, ELW, PLHT, EHT and wide stalks and that 23 % to 55 % of variation in TL is explained by these traits. High values of plant architectural traits associate with biomass and is expected to have positive correlation with yield components and grain yield. In this study, ELW had positive correlation with all yield and yield components ($r=0.28$, R^2 of 0.08 to $r=0.52$, R^2 of 0.27) except HKWT indicating that plants with wider ELW also had high values for yield and yield components and that 8 % to 27 % of the variation in ELW is explained by yield and yield components (Table 4.10).

4.1.4.3 Yield and Yield components of the three classes of genotypes

Yield components are important traits as they may influence biomass and grain yield. Yield components on a plant basis varied from 3.60 cm to 6.10 cm for ED, 9.60 cm to 24.00 cm for EL, 7.26 to 14.04 mm for KL, 7.00 to 11.44 mm for KW and 3.04 to

7.38 mm for KT. NRE and NKR ranged from 10.00 to 20.00 and 16.00 to 53.75, respectively. On mean plot basis, EN, EWT, HKWT and GWT varied, respectively from 1.00 to 1.45 (mean: 1.07 ± 0.12), 0.30 to 4.10 Kg (mean: 1.75 ± 0.85), 17.46 to 35.94 g (mean: 26.20 ± 4.07), and finally 0.20 to 3.30 Kg (mean: 1.33 ± 0.66).

Grain yield of the Ghana maize classes ranged from 0.30 to 6.82 with a mean of 2.31 ± 1.29 Mgha⁻¹. These values were not unexpected as many reports indicate grain yield of 1.7 Mgha⁻¹ for other Ghana genotypes (Adu *et al.*, 2013; Oppong *et al.*, 2014). On accession mean basis, grain yield spanned values of 1.65 ± 0.66 for ‘Golden Jubilee’ to 3.36 ± 0.72 for ‘Obatanpa’ (Table 4.7). The genotypes exhibited variable expression in grain yield and yield components. Grain yield in ‘Obatanpa’ was derived from five yield components, EL, KL, KW, NKR, EWT, and HKWT but not directly from ED, EN nor KT. The large ED of ‘Obatanpa’ arose from the CD (3.24 cm), which was the largest of all the cultivars. Additionally, ‘Obatanpa’ had the lowest EN and was among the cultivars with the smallest KT (Table 4.7). The low EN and small KT were circumvented by the largest HKWT values, hence the highest grain yield. The HKWT is a measure of compactness of maize arising from properties such as starch and protein contents of kernels. Being a QPM with high protein level of 10 %, ‘Obatanpa’ was expected to exhibit such high grain yield. Values of yield components of ‘Obatanpa’ were ED (5.27 ± 0.26 cm), EL (18.38 ± 2.44 cm), CD (3.21 ± 0.23 cm), KL (12.04 ± 0.85 mm), NKR (39.22 ± 3.84), HKWT (30.82 ± 3.73 g), GWT (1.73 ± 1.06 kg) and finally grain YLD (3.36 ± 0.72 Mgha⁻¹) while traits such as KW, KT, NRE and EN were highest in ‘Okomasa’ (9.96 ± 0.69 mm), ‘Pool 16 SR’ (5.09 ± 0.54 mm), ‘Kwadaso Local’ (16.33 ± 1.83) and ‘M0826-12F’ (1.21 ± 0.12), respectively (Table 4.7-4.9). Observation on the three classes of genotypes shows that the average yield of the two populations is 3.04 Mgha⁻¹ with a

range of 2.84 Mgha⁻¹ to 3.25 Mgha⁻¹, hybrids is 2.58 Mgha⁻¹ with a range of 2.23 Mgha⁻¹ to 3.05 Mgha⁻¹ and that of OPVs is 2.00 Mgha⁻¹ with a range of 0.48 Mgha⁻¹ to 3.36 Mgha⁻¹. The high yield of the populations were derived from long ears, multiple number of ears, and large grain weight.

Information on association between earliness and grain yield is particularly important for breeding for drought tolerance in maize. In the current study, AD and SD had low positive correlation ($r=0.20$, R^2 of 0.04 to $r=0.35$, R^2 of 0.12) with EL, NKR and EWT indicating that plants with long AD and SD also had long EL, high NKR and large EWT and that 4.0 % to 12 % of the variation in AD and SD is explained by EL, NKR and EWT (Table 4.10).

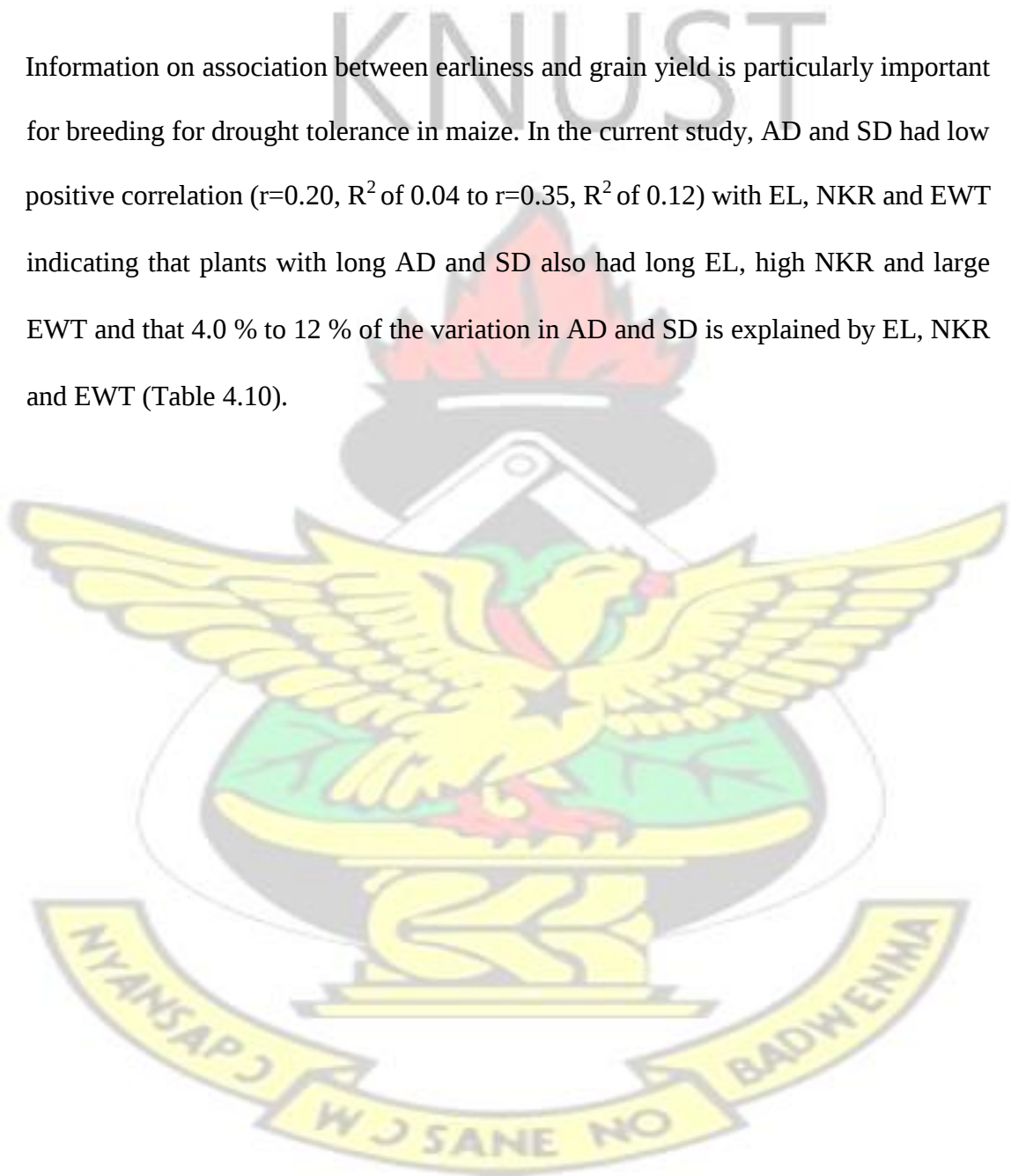


Table 4.7: Combined mean performance (Range), S.E of two maize populations in Ghana evaluated across two environments

Trait	M0826-12F			M0826-7F		
	Mean	Range	S.E	Mean	Range	S.E
AD	54.69 ^a	52.87-56.21	1.49	56.31 ^a	51.10-58.69	3.05
SD	55.40 ^a	53.93-57.36	1.37	57.32 ^a	52.50-59.71	2.93
ASI	0.71 ^a	0.10-1.36	0.55	1.08 ^a	0.45-1.71	0.47
TL	51.38 ^a	46.35-53.40	2.67	52.51 ^a	48.00-54.30	2.31
ELL	93.04 ^a	85.02-99.55	4.93	92.63 ^a	89.90-95.68	2.29
ELW	10.39 ^a	9.91-11.21	0.46	10.16 ^a	9.80-11.00	0.42
PLHT	160.44 ^a	144.11-177.29	11.29	156.44 ^a	146.90-166.00	7.02
EHT	70.08 ^a	57.56-81.80	8.13	65.56 ^a	58.85-70.70	4.44
StD	21.82 ^a	20.07-23.89	1.57	21.54 ^a	20.21-22.75	1.03
NL	12.43 ^a	11.55-13.50	0.70	12.12 ^a	11.10-14.00	0.91
SG	11.96 ^a	3.21-18.38	5.59	12.60 ^a	7.06-16.94	3.19
ED	4.87 ^a	4.66-5.30	0.23	4.99 ^a	4.36-5.58	0.48
EL	17.80 ^a	16.74-18.50	0.59	16.95 ^a	12.74-19.84	2.31
KL	10.99 ^a	10.61-11.42	0.34	11.04 ^a	9.62-12.32	0.93
KW	9.44 ^a	9.25-9.83	0.23	9.79 ^a	8.24-10.42	0.84
KT	4.53 ^a	4.20-4.95	0.28	4.47 ^a	3.55-5.04	0.50
NRE	14.13 ^a	13.20-15.60	0.90	14.07 ^a	12.80-15.60	1.11
CD	2.84 ^a	2.74-2.94	0.10	3.06 ^a	2.58-3.60	0.38
NKR	36.76 ^a	31.95-42.90	3.86	34.95 ^a	25.80-39.15	4.55
EN	1.21 ^a	1.00-1.45	0.12	1.08 ^b	1.00-1.25	0.11
EWT	2.63 ^a	1.60-3.80	2.63	2.47 ^a	1.70-3.70	0.79
HKWT	27.11 ^a	25.00-30.59	2.03	28.67 ^a	23.68-32.23	2.79
GWT	1.90 ^a	1.10-2.90	0.67	1.85 ^a	1.20-2.70	0.64
YLD	3.25 ^a	1.71-4.48	1.12	2.84 ^a	1.85-3.98	0.75

AD = Anthesis date, SD = Silking date, ASI = Anthesis–silking interval, TL= Tassel length, ELL= Ear length, ELW = Ear length width, PLHT = Plant height, EHT = Ear height, StD= Stem diameter, NL= Number of leaves, SG = Stay green, EP = Ear position, ED = Ear diameter, EL = Ear length, KT = Kernel thickness, KW= Kernel width, NRE= Number of rows per ear, NKR= Number of kernels per row, EN= Ear number, EWT= Ear weight, HKWT= Hundred kernel weight, GWT= Grain weight, YLD= Yield. S.E= Standard Error. Means within traits followed by the same letter do not differ significantly (Duncan’s multiple range test, 5 %).

Table 4.8: Combined mean performance (Range) S.E of 12 open pollinated maize varieties in Ghana evaluated across two environments

Trait	Okomasa	Honampa	Golden Jubilee	Kwadaso Local	Obatanpa	Dorke
AD	a 63.58 (62.7-65.7) 1.24	b 58.72 (55.60-63.57) 3.57	b 58.57 (54.1-63.57)3.57	b 58.47 (57.45-60.09)0.97	c 56.21 (52.90-58.00)1.62	d 56.01 (52.20-55.40)1.09
SD	a 65.60 (63.5-68.9)2.02	b 60.25 (56.50-62.20)2.05	b 59.68 (54.70-64.88)3.63	b 60.25 (58.70-62.09)1.28	b 58.72 (55.00-61.72)2.02	c 55.73 (53.40-57.70)1.57
ASI	ba 2.14 (0.8-3.33)0.97	bcd 1.53 (0.9-2.2)0.54	ecd 1.30 (0.00-2.14)0.83	bc 1.78 (0.90-2.40)0.58	a 2.51 (1.00-3.73)1.00	bc 1.73 (0.90-3.00)1.73
TL	b 47.69 (44.22-47.69)2.43	d 42.74 (40.72-44.80)	cd 44.72 (40.14-46.56)2.44	b 47.52 (45.50-51.00)1.92	a 52.08 (50.89-59.00)2.76	a 50.58 (46.75-54.70)2.73
ELL	dc 87.54 (79.28-92.00)4.36	e 79.44 (77.56-81.80)1.36	bc 90.53 (84.25-95.33)3.86	d 84.97 (82.51-87.20)1.78	a 95.00 (91.70-103.00)4.31	ba 92.85 (80.67-99.10)6.61
ELW	bcd 9.97 (9.00-10.68)0.77	fe 8.97 (8.39-9.66)0.49	bcd 9.95 (8.70-11.00)0.81	ba 10.38 (10.24-10.55)0.12	a 10.85 (9.48-13.00)1.04	bc 10.21 (8.86-11.06)0.87
PLHT	a 183.11 (153.32-202.50)18.03	ed 142.39 (136.88-147.50) 4.43	cb 157.97 (131.86-177.67) 17.20	b 166.53 (150.09-177.80) 10.42	a 194.89 (169.49-218.10)16.43	cb 160.84 (139.5-180.3)17.14
EHT	ba 85.04 (64.2-98.85)14.00	c 64.26 (55.55-76.84)7.06	c 67.47 (50.25-81.17)11.69	a 85.82 (72.18-100.70) 12.91	a 89.18 (73.91-103.00)10.52	b 77.03 (58.50-91.25)12.22
StD	bc 21.37 (20.28-22.69)0.93	d 20.26 (18.65-21.75)1.09	de 20.16 (18.59-21.86)1.23	ba 22.25 (21.18-23.43)0.80	a 22.48 (21.21-23.87)1.09	dc 20.74 (18.38-22.05)1.29
NL	a 14.05 (13.10-14.89)0.59	cb 12.60 (12.20-13.30)1.68	b 12.90 (12.00-13.33)0.52	b 13.22 (12.80-13.64)0.35	cb 12.69 (11.00-13.44)0.81	cd 12.04 (11.00-12.90)0.73
SG	ba 15.56 (13.18-19.57)2.90	b 13.50 (10.33-16.38)2.46	ba 15.05 (9.73-18.79)3.10	ba 18.17 (11.94-22.86)3.54	ba 14.80 (9.33-23.60)4.66	ba 15.55 (8.90-21.46)4.60
EP	cb 0.46 (0.41-0.49)0.05	cebd 0.44 (0.40-0.52)0.04	efd 0.42 (0.38-0.46)0.04	a 0.51 (0.46-0.57)0.05	cbd 0.45 (0.42-0.51)0.03	b 0.48 (0.43-0.50)0.03
ED	bcd 4.83 (4.58-4.94)0.145	e 4.34 (4.02-4.72)0.22	e 4.30 (3.86-4.68)4.30	ba 5.09 (4.86-5.20)0.13	a 5.27 (4.78-5.54)0.26	bcd 4.85 (4.42-5.10)0.24
EL	ba 18.18 (15.60-20.72)2.13	bdac 16.32 (15.30-17.70) 0.85	ebdac 16.03 (12.64-18.30) 2.05	bdac 16.45 (14.68-18.44)1.52	a 18.38 (16.06-21.92)2.44	bac 17.22 ((14.20-20.88)2.74
KL	ba 11.41 (10.49-13.04)0.99	dc 10.09 (9.06-11.39)0.76	d 9.95 (9.03-11.18)0.89	bdac 11.03 (10.35-11.83)0.54	a 12.04 (10.57-13.33)0.85	bac 11.14 (10.15-11.96)0.71
KW	a 9.96 (9.25-11.19)0.69	a 9.31 (8.63-10.13)0.62	a 9.10 (8.11-10.21)0.71	a 9.22 (8.43-9.95)0.64	a 9.80 (8.88-10.52)0.60	a 9.54 (8.94-10.08)0.39
KT	bac 4.68 (4.20-5.43)0.51	bac 4.64 (4.04-5.09)0.45	c 4.22 (3.81-4.43)0.23	bac 4.71 (4.35-5.95)0.62	bac 4.60 (4.06-5.22)0.44	bac 4.67 (4.16-5.10)0.36
NRE	dc 13.35 (12.40-15.0)0.96	dc 13.40 (11.20-16.40)1.80	dc 13.32 (12.00-15.60)1.33	a 16.33 (14.00-18.00)1.83	ba 15.27 (14.00-16.00)0.78	bac 14.87 (14.40-15.60)0.59
CD	bcd 2.85 (2.73-3.00)0.11	fe 2.57 (2.28-3.00)0.25	f 2.52 (2.26-2.94)0.27	ba 3.00 (2.86-3.12)0.09	a 3.21 (2.92-3.50)0.23	bc 2.92 (2.70-3.12)0.17
NKR	ba 36.86 (30.81-42.55)4.74	bac 35.21 (30.33-39.95) 3.69	edc 31.37 (27.80-35.95)2.93	bdc 34.53 (27.10-40.10) 4.28	a 39.22 (32.06-43.75) 3.84	bedc 32.73 (27.70-39.80)4.29
EN	b 1.00 (1.00-1.00)0.00	b 1.00 (1.00-1.00)0.00	a 1.15 (1-1.38)0.18	a 1.18 (1.00-1.40)0.18	b 1.02 (1.00-1.10)0.05	ba 1.10 (1.00-1.30)0.13

EWT	1.67 ^{bac} (0.8-2.9)0.75	1.40 ^{bc} (0.60-1.70)0.42	1.32 ^c (0.60-2.20)0.58	2.13 ^{ba} (1.70-2.60)0.33	2.23 ^a (1.10-4.10)1.34	2.23 ^a (0.90-3.20)1.03
HKWT	26.40 ^{bdac} (22.93-32.63)3.63	22.14 ^d (17.46-27.06)3.54	23.39 ^{dc} (19.40-28.03)3.63	23.45 ^{dc} (19.61-30.28)3.75	30.33 ^{ba} (26.27-35.94)3.73	28.03 ^{bac} (21.44-33.06)5.15
GWT	1.17 ^{ba} (0.6-1.9)0.47	1.00 ^{bac} (0.40-1.30)0.32	0.97 ^{bac} (0.40-1.50)0.37	1.58 ^{ba} (1.20-2.10)0.34	1.73 ^a (0.80-3.30)1.06	1.72 ^a (0.60-3.00)0.97
YLD	2.01 ^{ba} (0.93-3.37)0.98	1.66 ^{ba} (0.57-2.30)0.59	1.65 ^{ba} (0.59-2.37)0.66	2.82 ^a (1.85-3.86)0.74	3.36 ^a (1.11-6.82)0.72	3.13 ^a (0.91-6.05)2.12

Table 4.8 continue

Trait	Omankwa	Abontem	Aburohema	Pool 16 SR	Dodzi	Akposoe
AD	53.21 ^{ed} (51.30-56.10)1.75	52.95 ^{ed} (52.64-54.00)0.52	51.93 ^{edf} (49.90-54.60)1.71	51.62 ^{egf} (49.85-55.00)1.93	49.87 ^{gf} (46.80-54.20)2.83	49.55 ^g (48.6-50.3)0.76
SD	54.20 ^{dc} (52.00-56.40)1.82	53.60 ^{dce} (52.50-55.10)1.01	53.24 ^{dfe} (49.70-55.56)2.28	52.25 ^{dfe} (49.80-55.00)2.04	51.60 ^{fe} (48.70-55.60)2.67	51.03 ^f (49.6-52.3)0.99
ASI	0.80 ^{ed} (0.00-1.40)0.58	0.65 ^e (-0.40-1.55)0.80	0.73 ^e (-0.2-1.4)0.73	0.83 ^{ed} (0.00-1.60)0.54	1.73 ^{bc} (1.40-2.20)0.32	1.49 ^{bcd} (0.40-2.11)0.68
TL	46.70 ^{cb} (42.86-49.10)2.07	48.04 ^b (44.82-50.70)2.33	46.17 ^{cb} (43.75-49.70)2.03	46.68 ^{cb} (43.67-48.00)1.90	43.08 ^d (39.80-45.50)1.92	44.42 ^{cd} (41.30-48.50)2.89
ELL	84.45 ^d (78.96-89.60)4.17	84.84 ^d (79.20-88.70)3.670	79.18 ^e (73.50-85.94)4.42	83.52 ^d (74.96-92.00)6.83	77.53 ^e (67.11-84.30)5.95	76.43 ^e (70.80-86.90)6.511
ELW	9.46 ^{ed} (8.95-10.58)0.58	8.95 ^{fe} (8.42-9.44)0.42	8.72 ^f (8.29-9.02)0.30	9.70 ^{cd} (8.56-10.40)0.64	8.39 ^{fg} (7.76-9.02)0.46	7.97 ^g (6.73-9.58)1.06
PHT	147.98 ^{cd} (136.86-166.25)11.36	148.53 ^{cd} (135.43-157.9)9.77	130.52 ^e (114.25-140.70)12.61	150.21 ^{cd} (122.96-175.50)20.46	137.84 ^{ed} (113.62-157.25)16.28	130.23 ^e (113.65-130.23)17.41
EHT	67.07 ^c (57.95-77.45)8.87	58.87 ^{dc} (53.17-67.73)5.22	51.73 ^d (42.90-56.60)5.52	63.63 ^c (46.00-75.50)12.58	60.93 ^c (49.60-75.35)10.70	51.34 ^d (39.4-67.1)10.08
StD	20.14 ^{de} (18.05-21.86)1.32	19.17 ^{de} (17.78-19.73)0.71	19.04 ^f (17.33-20.70)1.22	19.87 ^{def} (18.31-21.46)1.10	18.06 ^g (16.13-19.22)1.01	16.85 ^h (14.11-19.15)1.87
NL	11.88 ^{ed} (11.30-12.60)0.44	11.76 ^{ed} (10.70-12.67)0.76	11.27 ^{ef} (10.60-12.10)0.76	11.54 ^{ed} (10.18-12.00)0.48	10.77 ^f (10.50-11.20)0.38	11.80 ^{ed} (11.10-12.30)0.62
SG	19.10 ^a (11.12-26.77)6.75	15.08 ^{ba} (10.82-22.60)4.96	17.78 ^{ba} (15.99-19.85)1.94	14.33 ^{ba} (8.33-17.69)3.94	14.03 ^{ba} (12.16-17.53)3.03	18.71 ^{ba} (16.21-20.80)2.32
EP	0.45 ^{cbd} (0.41-0.49)0.03	0.39 ^{gf} (0.38-0.43)0.02	0.39 ^{gf} (0.37-0.42)0.02	0.41 ^{ef} (0.35-0.50)0.05	0.43 ^{ced} (0.37-0.48)0.04	0.38 ^g (0.34-0.42)0.03
ED	4.54 ^{ed} (4.32-4.82)0.17	4.51 ^{ed} (4.03-4.82)0.29	4.50 ^{ed} (4.24-4.72)0.19	4.90 ^{bc} (4.40-5.40)0.71	4.63 ^{ecd} (4.3-5.04)0.30	4.52 ^{ed} (4.32-4.78)0.18
EL	15.72 ^{ebdc} (13.60-18.00)1.59	17.48 ^{bac} (13.88-19.70)2.06	13.79 ^e (11.38-15.36)1.75	16.80 ^{bdac} (14.80-18.80)2.83	14.53 ^{ed} (12.56-17.52)1.95	15.14 ^{edc} (12.12-18.46) 2.53
KL	10.27 ^{dc} (9.91-10.61)0.32	10.53 ^{bdc} (9.89-11.31)0.55	10.28 ^{dc} (9.47-10.96)0.51	10.23 ^{dc} (9.99-10.56)0.40	10.21 ^{dc} (9.12-11.88)0.97	10.01 ^{dc} (9.19-11.19)0.70
KW	9.49 ^a (9.15-9.76)0.23	9.41 ^a (8.25-10.52)0.93	9.24 ^a (8.72-10.10)0.60	9.24 ^a (8.16-10.32)1.53	9.41 ^a (8.73-9.84)0.53	9.94 ^a (8.99-11.04)0.70
KT	4.48 ^{bc} (3.91-4.80)0.35	4.91 ^{ba} (3.80-5.35)0.58	4.69 ^{bac} (4.38-5.03)0.24	5.09 ^a (4.71-5.48)0.54	4.76 ^{bac} (3.81-5.32)0.57	4.82 ^{bac} (4.40-5.40)0.40
NRE	13.04 ^d (11.60-14.00)0.97	13.53 ^{dc} (12.00-15.20)1.06	13.83 ^{bdc} (12.80-15.00)0.88	14.00 ^{bdc} (14.00-14.00)0.00	13.60 ^{dc} (12.40-14.40)0.67	13.63 ^{dc} (12.00-14.80)1.01
CD	2.62 ^{fed} (2.46-2.76)0.09	2.67 ^{fed} (2.38-2.88)0.17	2.69 ^{fecd} (2.38-2.88)0.17	3.00 ^{ba} (2.60-3.40)0.57	2.78 ^{becd} (2.50-3.06)0.23	2.76 ^{bedc} (2.58-2.98)0.15

NKR	32.02 ^{edc} (27.55-34.75)2.87	33.92 ^{bdc} (26.50-38.15)4.17	29.04 ^e (25.00-34.85)4.17	31.00 ^{edc} (31.00-31.00)0.00	30.59 ^{edc} (25.60-30.59)3.06	29.73 ^{ed} (23.19/35.60)4.65
EN	1.03 ^b (1.00-1.20)0.08	^b 1.00 (1.00-1.00)0.00	^b 1.04 (1.00-1.22)0.09	^b 1.00 (1.00-1.00)0.00	^b 1.00 (1.00-1.00)0.00	^b 1.02 (1.00-1.14)0.06
EWT	1.27 ^c (1.10-1.40)0.14	1.30 ^c (0.30-1.90)0.56	1.13 ^c (0.50-1.80)0.55	0.35 ^d (0.30-0.40)0.07	1.28 ^c (0.60-1.80)0.51	^{dc} 1.07 (0.40-1.60)0.43
HKWT	^{bdac} 27.06 (23.74-32.27)3.10	^{bdac} 26.18 (21.21-32.18) 4.23	^{bdac} 26.92 (20.69-32.95)4.84	^a 30.82 (26.38-35.26)6.28	^{bdc} 25.47 (20.35-30.59)4.18	^{bdac} 27.24 (21.71-31.63)3.29
GWT	^{bac} 1.02 (0.80-1.30)0.19	^{ba} 1.05 (0.20-1.60)0.51	^{bc} 0.90 (0.30-1.40)0.49	^c 0.25 (0.20-0.30)0.07	^{bac} 1.00 (0.40-1.60)0.48	^{bc} 0.87 (0.30-1.20)0.35
YLD	^{ba} 1.74 (1.02-2.54)0.52	^{ba} 1.95 (0.30-3.20)1.17	^{ba} 1.63 (0.39-2.78)0.97	^b 0.48 (0.38-0.57)0.13	^{ba} 1.86 (0.54-3.33)1.02	^{ba} 1.69 (0.42-2.56)0.87

Trait abbreviations and units as given in Table 4.1.

Table 4.9: Combined mean performance (Range) S.E of three hybrid maize varieties in Ghana evaluated across two environments

Traits	Enibi	Etubi	Mamaba
AD	56.68 ^a (51.67-59.75)1.09	57.57 ^a (52.90-61.86)3.15	57.00 ^a (53.44-60.00)2.78
SD	58.39 ^a (53.40-57.70)1.57	58.79 ^a (53.70-63.00)3.28	57.74 ^a (54.10-60.00)2.20
ASI	1.23 ^{ba} (0.80-1.43)0.23	1.59 ^a (0.40-2.71)0.76	0.76 ^b (0.00-1.70)0.63
TL	45.22 ^a (41.89-49.90)3.44	47.94 ^{ba} (45.14-50.29)1.73	46.45 ^a (43.64-49.70)2.53
ELL	81.22 ^a (69.50-90.70)7.93	85.51 ^a (77.63-92.60)5.83	85.10 ^a (75.31-90.50)5.79
ELW	9.64 ^b (8.43-10.90)0.89	9.68 ^b (8.97-10.36)0.65	10.16 ^a (9.14-11.14)0.74
PLHT	141.61 ^b (117.02-167.3)17.38	153.40 ^a (137.23-174.70)13.64	145.30 ^b (124.92-160.50)13.08
EHT	54.13 ^b (40.08-73.15)11.39	69.67 ^a (57.36-90.20)15.21	55.40 ^b (40.90-65.95)8.97
StD	19.85 ^b (18.66-21.05)0.84	21.19 ^a (19.10-22.53)1.25	20.65 ^{ba} (18.77-22.75)1.56
NL	12.23 ^a (11.33-13.70)3.08	12.35 ^a (11.14-13.70)12.35	12.29 ^a (11.10-13.56)0.92
EP	0.39 ^b (0.34-0.44)0.04	0.45 ^a (0.38-0.53)0.07	0.38 ^b (0.33-0.41)0.03

StD	0.36	0.33*	-0.02	0.51**	0.71**	0.81**	0.71**	0.71**	1.00									
NL	0.48**	0.48**	0.21*	0.02	0.40**	0.55**	0.56**	0.61**	0.65**	1.00								
SG	-0.16	-0.11	0.13	-0.16	-0.12	-0.11	0.02	0.11	-0.08	0.04	1.00							
ED	0.12	0.15	0.21*	0.46**	0.40**	0.49**	0.44**	0.37**	0.39**	0.17	-0.02	1.00						
EL	0.28*	0.25	0.01	0.34**	0.45**	0.51**	0.40**	0.29*	0.36*	0.24*	-0.20	0.59*	1.00					
NRE	0.08	0.12	0.17	0.22*	0.20*	0.33*	0.25*	0.32*	0.28**	0.20	0.03	0.52**	0.15	1.00				
NKR	0.35*	0.34	0.09	0.33*	0.41	0.46**	0.43	0.31**	0.40*	0.23*	-0.20	0.51**	0.80**	0.08	1.00			
EN	0.15	0.12	-0.08	0.18	0.28*	0.28*	0.17	0.21*	0.24*	0.09	-0.20	0.16*	0.21**	0.22*	0.22*	1.00		
EWT	0.25*	0.24*	0.11	0.40**	0.45**	0.52**	0.31*	0.27*	0.38**	0.17	-0.22	0.64**	0.69**	0.29*	0.66**	0.42**	1.00	
HKWT	-0.15	-0.16	-0.04	0.30*	0.23*	0.20	0.18	0.09	-0.01	0.18	0.01	0.46**	0.40**	-0.08	0.26	-0.01	0.32*	1.00
YLD	0.14	0.14	0.10	0.32*	0.36*	0.40**	0.22*	0.20*	0.25*	0.07	-0.21	0.54**	0.69**	0.21*	0.60**	0.42**	0.918*	0.47**

* $p \leq 0.05$, ** $p \leq 0.01$, Traits abbreviations and units as given in Table 4.1

4.1.5 Variance components and heritability estimates of agronomic traits

Estimates of phenotypic and genotypic coefficient of variations and heritability estimates (broad sense) of all traits across the different classes are presented in Table 4.11. Except for plant and ear heights for OPVs and hybrids, the phenotypic coefficients of variation (PCV) were higher than their corresponding genotypic coefficients of variation (GCV) for all the traits studied across the various classes. Both were generally low; ranging from 0.00 to 114.08 for PCV and from 0.00 to 366.30 for GCV. PCV was highest for PLHTs (76.08) and (114.08) for the two populations and OPV genotypes, respectively and highest for EHT (32.91) in the hybrid genotypes. GCV were highest for EHT (3.54) and (68.32) and PLHT (366.30) in the population, hybrid and OPV genotypes, respectively.

Heritability estimate of all studied traits across all classes of maize ranged from 0.000.86. Generally, heritability estimates were higher in the OPVs with SD (0.86) and AD (0.85), PLHT (0.74), ELL, ELW (0.69) EHT (0.67), TL (0.62) and ED (0.57) showing high and moderate heritability estimates. EN (0.38), ASI (0.27) and HKWT (0.27) were the traits which showed the highest heritability (though very low) in the population. Traits such as EL and PLHT had moderate heritability estimates of 0.67 and 0.51, respectively in the hybrid varieties. The remaining traits recorded very low heritability estimates in the three classes of maize genotypes. Some traits displayed null heritability values (0.00), across all the classes; interestingly, YLD was a classic example.

Table 4.11: Phenotypic and Genotypic coefficient of variation and heritability ($\pm$ standard error) estimates for various traits in some maize populations in Ghana.

Trait	Phe Pop.	Phenotypic coefficient of variation		P p.	Genotypic coefficient of variation		Broad Sense Heritability $\pm$ (S.E)		
		OPVs	Hybrids		OPVs	Hybrids	Pop.	OPVs	Hybrids
AD	5.56	20.51	6.67	0.39	17.59	0.00	0.07 $\pm$ (0.35)	0.85 $\pm$ (0.06)	0.00 $\pm$ (0.00)
SD	5.58	22.94	6.45	0.86	19.01	0.00	0.15 $\pm$ (0.44)	0.86 $\pm$ (0.06)	0.00 $\pm$ (0.00)
ASI	0.18	0.63	0.34	0.05	0.29	0.14	0.27 $\pm$ (0.47)	0.46 $\pm$ (0.14)	0.40 $\pm$ (0.36)
TL	4.05	11.11	5.05	0.00	6.84	0.35	0.00 $\pm$ (0.00)	0.62 $\pm$ (0.13)	0.07 $\pm$ (0.47)
ELL	6.44	47.02	18.73	0.00	32.28	2.13	0.00 $\pm$ (0.00)	0.69 $\pm$ (0.12)	0.11 $\pm$ (0.34)
ELW	0.07	0.98	0.19	0.02	0.67	0.04	0.23 $\pm$ (0.43)	0.69 $\pm$ (0.12)	0.19 $\pm$ (0.45)
PLHT	76.08	114.08	19.53	0.00	366.30	21.30	0.00 $\pm$ (0.00)	0.74 $\pm$ (0.10)	0.33 $\pm$ (0.49)
EHT	40.10	48.44	32.91	3.54	147.03	68.32	0.08 $\pm$ (0.32)	0.67 $\pm$ (0.13)	0.67 $\pm$ (0.27)
StD	0.09	0.70	0.55	0.00	2.14	0.20	0.00 $\pm$ (0.00)	0.59 $\pm$ (0.16)	0.19 $\pm$ (0.42)
EP	0.00	0.00	0.00	0.00	0.00	0.00	0.00 $\pm$ (0.00)	0.48 $\pm$ (0.18)	0.51 $\pm$ (0.33)
ED	0.07	0.14	0.04	0.00	0.08	0.01	0.00 $\pm$ (0.00)	0.57 $\pm$ (0.13)	0.25 $\pm$ (0.30)
EL	2.49	5.41	0.85	0.00	0.74	0.19	0.00 $\pm$ (0.00)	0.14 $\pm$ (0.18)	0.22 $\pm$ (0.30)
KL	0.37	0.87	0.50	0.00	0.35	0.04	0.00 $\pm$ (0.00)	0.40 $\pm$ (0.14)	0.08 $\pm$ (0.23)
KW	0.26	0.43	0.40	0.02	0.00	0.00	0.09 $\pm$ (0.34)	0.01 $\pm$ (0.11)	0.01 $\pm$ (0.47)
KT	0.12	0.21	0.13	0.00	0.00	0.01	0.00 $\pm$ (0.00)	0.00 $\pm$ (0.00)	0.05 $\pm$ (0.21)
NRE	0.44	2.05	0.94	0.00	0.73	0.00	0.00 $\pm$ (0.00)	0.36 $\pm$ (0.15)	0.00 $\pm$ (0.00)
CD	0.08	0.07	0.01	0.00	0.03	0.00	0.00 $\pm$ (0.00)	0.41 $\pm$ (0.18)	0.25 $\pm$ (0.37)
NKR	6.14	20.77	7.11	0.73	5.04	1.37	0.12 $\pm$ (0.36)	0.24 $\pm$ (0.18)	0.19 $\pm$ (0.28)
EN	0.01	0.01	0.01	0.01	0.00	0.00	0.38 $\pm$ (0.63)	0.15 $\pm$ (0.18)	0.00 $\pm$ (0.00)
EWT	0.39	0.59	0.39	0.00	0.08	0.03	0.00 $\pm$ (0.00)	0.13 $\pm$ (0.21)	0.09 $\pm$ (0.36)
HKWT	3.09	19.13	8.28	0.83	12.18	0.16	0.27 $\pm$ (0.43)	0.02 $\pm$ (0.18)	0.02 $\pm$ (0.19)
GWT	0.24	0.40	0.25	0.00	0.04	0.02	0.00 $\pm$ (0.00)	0.10 $\pm$ (0.17)	0.09 $\pm$ (0.37)
YLD	0.58	1.63	0.98	0.00	0.00	0.00	0.00 $\pm$ (0.00)	0.00 $\pm$ (0.00)	0.00 $\pm$ (0.00)

Trait abbreviations and units as given in Table 4.1. OPVs = Open pollinated varieties, Pop.= population, PCV= Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation.



4.1.6 Cluster analysis

Genetic distances among the cultivars were determined as dissimilarity distance measures calculated as correlation coefficients (Rohlf, 2009) to generate a correlation matrix of 25 morphological traits of 17 maize cultivars. Cluster analysis of the distance matrix based on UPGMA was performed to reveal relationships among traits and among accessions. A tree diagram of the cluster analysis is shown in Figure

4.1. The distance matrix ranged from -0.22 to 0.72 and revealed three main clusters I, II and III at a genetic similarity of 35 %. The most distant accessions were 'Okomasa' and 'Abontem', which occupied the first and last position of the dendrogram. On the other hand, the lowest genetic distance was found between the two populations used in the study. Accession pairs such as 'Honampa'×'Okomasa'; 'Aburohemaa'×'Akposoe' and 'Mamaba'×'Enibi' were very similar. Percentage similarity was, however, appreciable in the former accession pairs.

Main cluster I was heterogeneous and had the fewest membership of five accessions, constituting close to 30 % of the total population studied. This cluster was subdivided into two sub-groups, 'Honampa' and 'Okomasa' in one sub-group and 'Golden Jubilee', 'Kwadaso Local' and 'Etubi' in the second at a genetic similarity of 53 %. The closest accessions in this group were 'Okomasa' and 'Honampa' with a genetic similarity of 93 %. This group (Cluster I) was characterized by late male and female flowering (AD and SD), delayed ASI, tall plants, high prolificacy (EN), thick stems and intermediate yield potential accessions such as 'Okomasa' (2.01 Mha^{-1}), 'Etubi' (2.23 Mha^{-1}) and 'Kwadaso Local' (2.82 Mha^{-1}) (Table 4.12).

Cluster II was highly heterogeneous and contained six accessions, 'Obatanpa', 'Mamaba', 'Dorke', 'Enibi', and the two populations, 'M0826-12F' and 'M08267F'. These genotypes make up two each of the OPVs, hybrids and population and

constituted 35 % of the studied genotypes. Two hybrid accessions, ‘Mamaba’ and ‘Enibi’ clustered separately at a genetic distance of 0.53 from the rest of the accessions and had a genetic similarity coefficient of 0.95. Cluster II constituted the best yielding varieties including ‘Obatanpa’, ‘Mamaba’, ‘Dorke’, ‘Enibi’ and the two populations, ‘M0826-12F’ and ‘M0826-7F’ with the highest prolificacy such as the two populations (Table 4.7).

Cluster III was composed of six OPV genotypes. ‘Akposoe’ and ‘Aburohemaa’ were the most similar accessions in this group (with genetic similarity coefficient of 0.91). This group was characterized by early anthesis and silking dates, short ASI and tassels, short plants, small seed sizes, thin stems, short and narrow ear leaves, least NRE, NKR, EWT and GWT. This group also had accessions with the lowest yield potential such as ‘Akposoe’, ‘Dodzi’ and ‘Abontem’ (Table 4.8).

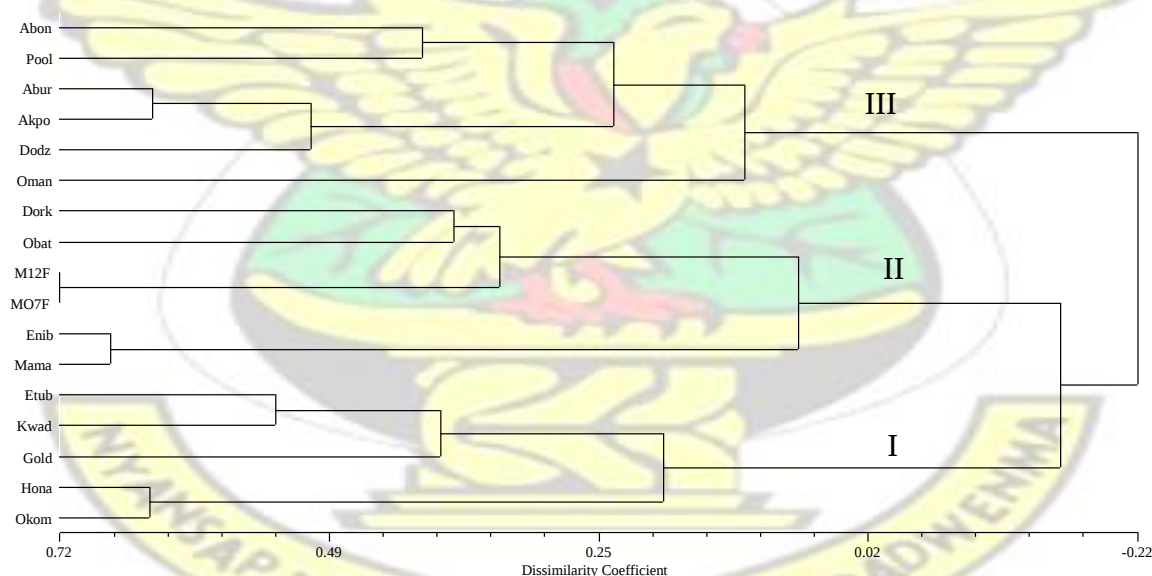


Figure 4.1: Dendrogram derived using UPGMA cluster analysis based on 25 agromorphological characters of 17 maize accession. I, II and III indicate major cluster groups. Okom = Okomasa, Hona = Honampa, Gold = Golden Jubilee, Kwad = Kwadaso Local, Etub = Etubi, Mama= Mamaba, Enib = Enibi, M07F = M0826-7F, M12F= M0826-12F, Obat = Obatanpa, Dodz = Dodzi, Dork= Dorke, Oman = Omankwa, Akpo = Akposoe, Abur = Aburohemaa, Pool = Pool 16 SR, Abon = Abontem.

Table 4.12: Key characteristics of the three groups formed by cluster analysis of 17 maize varieties in Ghana.

Trait	Overall mean	Cluster I		Cluster II		Cluster III	
		mean	Variance	Mean	Variance	Mean	Variance
AD	55.33	59.38	4.05	54.45	-0.88	51.52	-3.81
SD	56.67	60.91	4.24	55.63	-1.04	52.65	-4.02
ASI	1.32	1.60	0.28	1.15	-0.17	1.03	-0.29
TL	47.41	46.12	-1.29	46.91	-0.50	45.85	-1.56
ELL	85.75	85.60	-0.15	84.81	-0.94	80.99	-4.76
ELW	9.67	9.79	0.12	9.47	-0.20	8.87	-0.8
PLHT	154.02	160.68	6.66	147.62	-6.4	140.89	-13.13
EHT	67.25	74.45	7.20	62.72	-4.53	58.93	-8.32
StD	20.36	21.05	0.69	19.98	-0.38	18.86	-1.50
SG	14.80	15.23	0.43	14.47	-0.33	16.39	1.59
EL	16.76	16.94	0.18	16.50	-0.26	15.42	-1.34
NL	12.35	13.02	0.67	12.11	-0.24	11.19	-1.16
EP	0.43	0.46	0.03	0.42	-0.01	0.41	-0.02
ED	4.72	4.63	-0.09	4.64	-0.08	4.56	-0.16
CD	2.81	2.73	-0.08	2.75	-0.06	2.72	-0.09
NRE	14.09	14.21	0.12	13.88	-0.21	13.56	-0.53
NKR	34.17	34.81	0.64	33.47	-0.70	31.06	-3.11
HKWT	26.19	23.72	-2.47	25.95	-0.24	26.84	0.65
EN	1.07	1.07	0.00	1.09	0.02	1.02	-0.05
KL	10.69	10.59	-0.10	10.51	-0.18	10.25	-0.44
KW	9.46	9.30	-0.16	9.40	-0.06	9.48	0.02
KT	4.63	4.53	-0.10	4.63	0.00	4.74	0.11
EWT	1.76	1.64	-0.12	1.67	-0.09	1.16	-0.60
GWT	1.33	1.20	-0.13	1.27	-0.06	0.92	-0.41
YLD	2.31	2.07	-0.24	2.20	-0.11	1.69	-0.62

Trait abbreviations and units as given in Table 4.1; (Cluster mean less overall mean).

4.1.7 Principal component and Biplot analysis

Principal Component Analysis grouped the 25 agronomic traits into 10 components, which accounted for the entire (100 %) variability among the accessions. The first three components, with eigenvalues higher than 1.0, accounted for 74.91 % of the total variance in which PC1 explained 49.00 % (Table 4.13) of the total variation. Based on eigenvectors with values equal to or greater than 0.50, agronomic traits such as AD, SD, TL, ELL, ELW, PLHT, EHT, StD, NL, EP, ED, EL, KL, NRE, CD, NKR, EWT, GWT and YLD are the major discriminatory characters associated with the first PC

while traits such as AD, SD, ED, KT, CD and HKWT are associated with the second PC, which accounted for 15.14 % of the total variance. The third PC, which explained 10.77 % of the total variation, was dominated by ASI and SG.

Table 4.13: Principal component analysis of 25 agronomic traits in 17 maize accessions in Ghana.

Trait	PC1	PC2	PC3
Days to anthesis	0.62	0.65	0.25
Days to silking	0.64	0.62	0.32
Anthesis-silking interval	0.45	0.01	0.65
Tassel length	0.77	-0.40	-0.22
Ear leaf length	0.82	-0.06	-0.18
Ear leaf width	0.92	0.01	-0.11
Plant height	0.88	-0.06	0.35
Ear height	0.81	0.04	0.48
Stem diameter	0.91	0.19	-0.04
Number of leaves	0.69	0.49	0.34
Stay green	-0.30	-0.09	0.56
Ear position	0.55	0.19	0.48
Ear diameter	0.78	-0.53	0.05
Ear length	0.79	-0.02	-0.11
Kernel length	0.90	-0.29	0.05
Kernel width	0.25	-0.49	0.30
Kernel thickness	-0.26	-0.66	0.22
Number of rows per ear	0.60	-0.17	0.01
Cob diameter	0.66	-0.67	0.15
Number of kernels per row	0.84	0.12	-0.15
Number of ears per plant	0.43	0.36	-0.46
Ear weight	0.82	0.04	-0.42
Hundred kernel weight	0.18	-0.88	0.07
Grain weight	0.79	-0.00	-0.45
Grain yield	0.79	-0.06	-0.41
Eigen value	12.04	3.72	2.65
Proportion (%)	49.00	15.14	10.77
Cumulative (%)	49.00	64.14	74.91

To better visualize the relationships between accessions and between measured traits, biplot analyses were carried out. In the biplots, traits and accessions were represented as vectors. While most of the trait vectors were located at the right side of the biplot

diagram indicating that these traits produce positive response in performance of maize; few others were on the left side of the diagram (Figure 4.2).

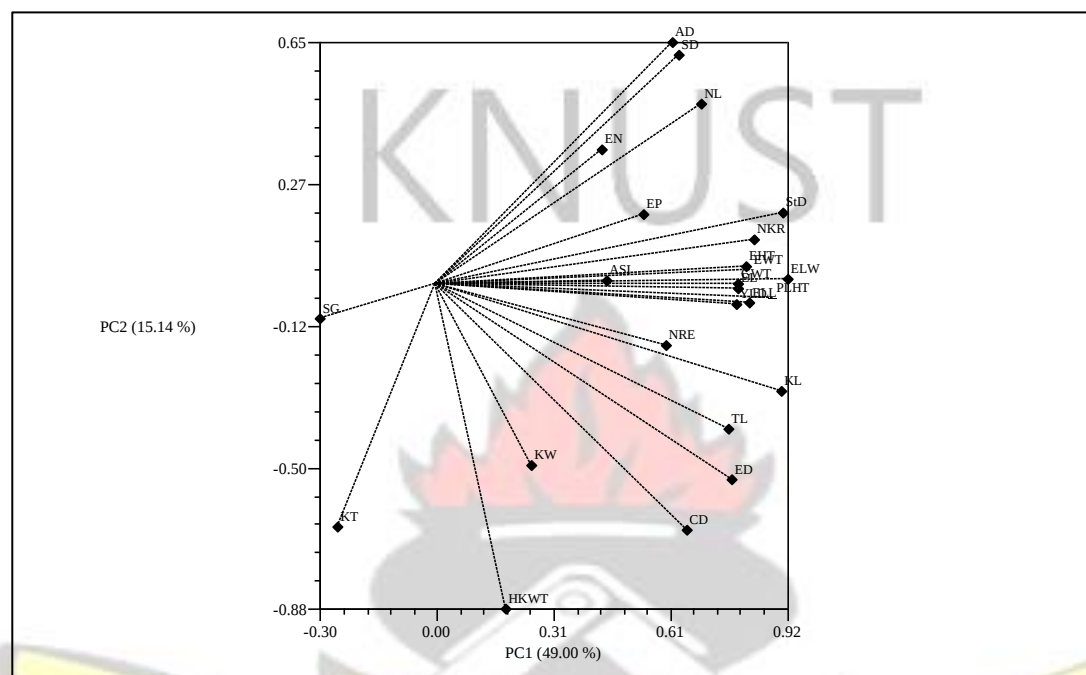


Figure 4.2: PCA biplot of 25 morphological traits of 17 maize accessions in Ghana. Trait abbreviations as given in Table 4.1

Accessions were grouped at different locations in the plot (Figure 4.3). While some accessions showed longer vector distances, shorter vector distances were observed for others. ‘Obatanpa’ recorded the longest vector distance. ‘Pool 16 SR’, ‘Akposoe’ and ‘Golden Jubilee’ recorded moderate distances from the vector origin whiles ‘Mamaba’ and ‘Enibi’ recorded the shortest. ‘Okomasa’ and ‘Kwadaso Local’ and ‘Aburohemaa’ and ‘Dodzi’ had similar vector distances and were separated by a tight acute angle. ‘Omankwa’ was isolated from the rest of the genotypes. The PCA biplot clustered the accessions similarly to the morphological dendrogram with some differences. As observed in Cluster III of the dendrogram (Figure 4.1), accessions with similar morphology also grouped together using the PCA biplot (Figure 4.3). Similar comparisons were observed for other accessions.

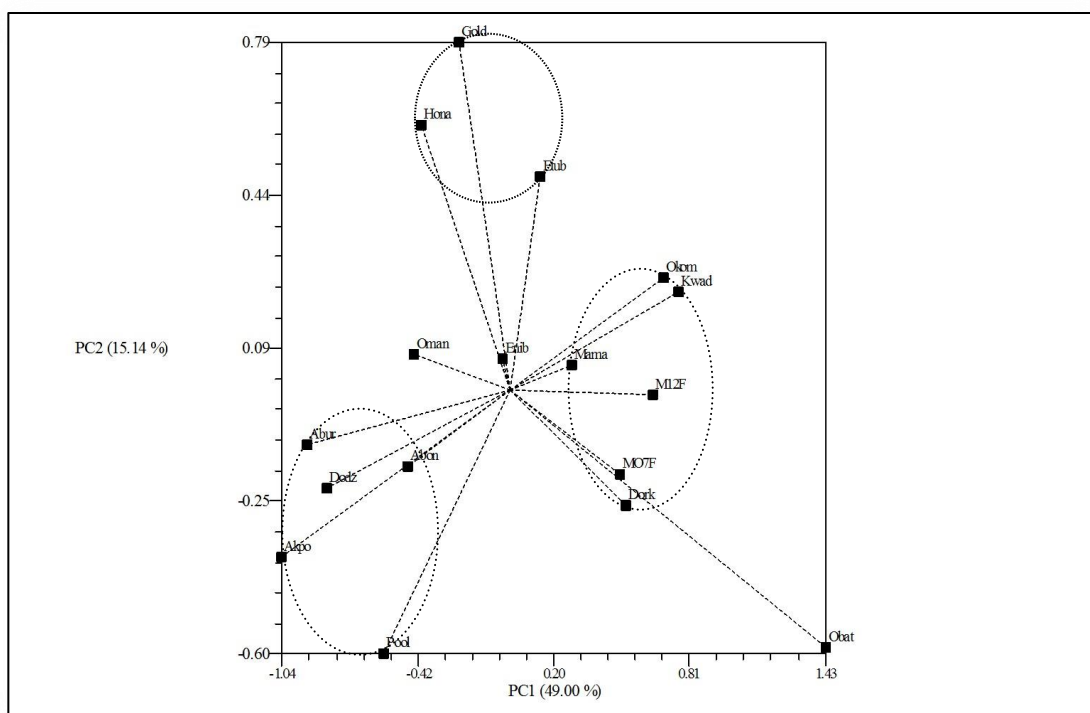


Figure 4.3: Principal component score plot of PC₁ and PC₂ of 17 maize accessions using 25 morphological characters. Accession abbreviations as given in Figure 4.1

4.2 SSR diversity in maize accessions

4.2.1 Genetic information from SSR markers

The genetic diversity study by molecular analysis encompassed evaluation of the 17 Ghana maize cultivars and a check, 'Pool 16 SR' with 12 SSR primer loci. All genotypes including the check produced good quality amplification products consisting predominantly of clear and sharp bands and some stutter bands, typical of SSR gels (Plate 4.1). Stutter bands were avoided during scoring. Eleven of the 12 SSR loci, were polymorphic while one locus nc133 revealed a monomorphic pattern and hence was not considered for further analysis. The SSR loci represented all chromosomes except chromosomes 4 and 6. Table 4.14 shows the genetic information generated by the SSR primers. The number of alleles per locus generated by each marker varied from 2 to 4 alleles. Majority of the SSR loci (63.64 %) had 3 alleles. The highest number of

alleles (4) was detected in locus phi453121 and the lowest (2) in phi109642, phi002 and umc1279. A total of 31 alleles were detected from the 11 amplified loci, with an average of 2.82 alleles per locus. Allele frequency ranged from 0.25 for phi453121 to 0.50 for phi002, phi109642 and umc1279. Polymorphism Information Content (PIC) varied from 0.21 for phi002 to 0.64 for phi100175 with an average of 0.43. The highest PIC value (0.64) was observed for phi100175 followed by phi065 (0.61), umc1196 (0.56) and umc1399 (0.54).

Table 4.14: Genetic information generated by 11 SSR markers in 17 maize accessions

Primer	Primer Sequence (5'-3')	NA	AF	PIC
phi109642	CTCTCTTTCCTTCCGACTTTCC	2	0.50	0.22
phi453121	ACCTTGCCTGTCCTTCTTTCT	4	0.25	0.47
umc1399	GCTCTATGTTATTCTTCAATCGGGC	3	0.33	0.54
phi065	AGGGACAAATACGTGGAGACACAG	3	0.33	0.61
phi328175	GGGAAGTGCTCCTTGCCAG	3	0.33	0.26
umc1161	GGTACCGCTACTGCTTGTTACTGC	3	0.33	0.44
phi100175	TATCTGACGAATCCCATTCCC	3	0.33	0.64
umc1279	GATGAGCTTGACGACGCCTG	2	0.50	0.36
umc1196	CGTGCTACTACTGCTACAAAGCGA	3	0.27	0.56
nc130	GCACATGAAGATCCTGCTGA	3	0.33	0.38
phi002	CATGCAATCAATAACGATGGCGAGT	2	0.50	0.21
Total		31	4.00	4.69
Mean		2.82	0.36	0.43

NA = Number of Alleles; AF = Allele Frequency; PIC = Polymorphic Information Content

A total of 7 (22.58 %) unique (rare) alleles (frequency of occurrence ≤ 0.05) were detected in 6 SSR loci across the accessions analysed; 12 alleles (38.71 %) had frequencies ranging from 0.10-0.29; 2 alleles (6.45 %) were frequent (occurrence frequencies ranging from 0.30-0.50) and finally 10 were highly frequent with their occurrence frequencies ranging from 0.51-0.88. Among the 17 accessions analysed, 5 ('Kwadaso Local', 'Honampa', 'Obatanpa', 'Etubi' and 'Enibi') revealed unique or

rare alleles. ‘Obatanpa’ and ‘Enibi’ each showed a maximum of two unique alleles while ‘Kwadaso Local’, ‘Etubi’, and ‘Honampa’ showed one unique allele each. The SSR loci which detected the ‘rare’ alleles were Phi453121 (1), umc1399 (1), Phi328175 (2), umc1161 (1), umc1196 (1) and nc130 (1).

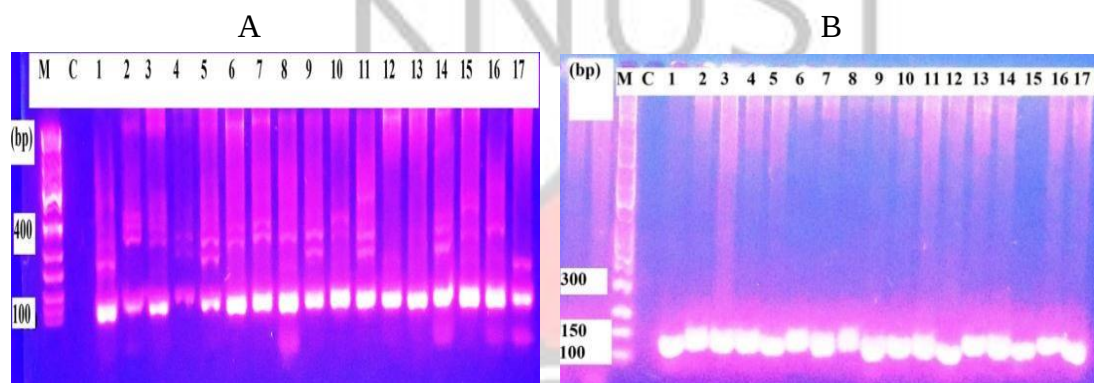


Plate 4.1: PCR amplification profiles of 17 Ghanaian maize genotypes with SSR primer set (A) nc130 and (B) phi328175. M = Molecular size marker (100 bp ladder, 100ng/μl). C = Negative control. (1 = Dodzi, 2 = Enibi, 3 = Okomasa, 4 = Etubi, 5 = M082612F, 6 = Omankwa, 7 = Mamaba, 8 = Abontem, 9 = Aburohemaa, 10 = Kwadaso Local, 11 = Pool 16 SR, 12 = Obatanpa, 13 = Golden Jubilee, 14 = Dorke, 15 = Honampa, 16 = M0826-7F and 17 = Akposoe).

4.2.2 SSR genetic distance / similarity and Cluster analysis

Estimates of genetic similarity matrices based on the SSR molecular data for all pairwise combinations of the 17 maize accessions are presented in Appendix 4.2. The Dice genetic similarity matrix for all accessions ranged from 0.36 to 0.94 with an average of 0.61. The highest similarity coefficient (0.94) was observed between ‘Golden Jubilee’ and ‘Akposoe’ followed by ‘Golden Jubilee’ and ‘Abontem’ (0.90). The lowest coefficient of similarity (0.36) were observed between the accession pairs ‘Dodzi’ and ‘Etubi’, ‘Aburohemaa’ and ‘Okomasa’, ‘Aburohemaa’ and ‘Enibi’, ‘Aburohemaa’ and ‘M0826-12F’ and between ‘Kwadaso Local’ and ‘Dodzi’.

UPGMA cluster analysis grouped the accessions into three distinct groups at 58 % similarity coefficient (Figure 4.4). Twelve accessions were represented in Cluster I whereas 2 and 3 accessions were placed in Clusters II and III, respectively. Cluster I was divided into three sub-clusters IA, IB and IC at 75 % similarity coefficient. The largest genetic distance was between ‘Obatanpa’ and ‘Dodzi’. Sub-cluster ‘IA’ was represented by only one accession (‘Obatanpa’). Sub-cluster ‘IB’ included six varieties, ‘Honampa’, ‘Kwadaso Local’, ‘Pool 16 SR’, ‘M0826-7F’, ‘Dorke’ and ‘Mamaba’ in which ‘Kwadaso Local’ and ‘Honampa’, ‘Pool 16 SR’ and ‘M0826-7F’ were the most similar. Sub-cluster ‘IC’ on the other hand was represented by five varieties, namely, ‘Aburohemaa’, ‘Akposoe’, ‘Golden Jubilee’, ‘Abontem’ and ‘Omankwa’. Among them ‘Omankwa’ was the most diverged accession whereas ‘Akposoe’ and ‘Golden Jubilee’ were identical.

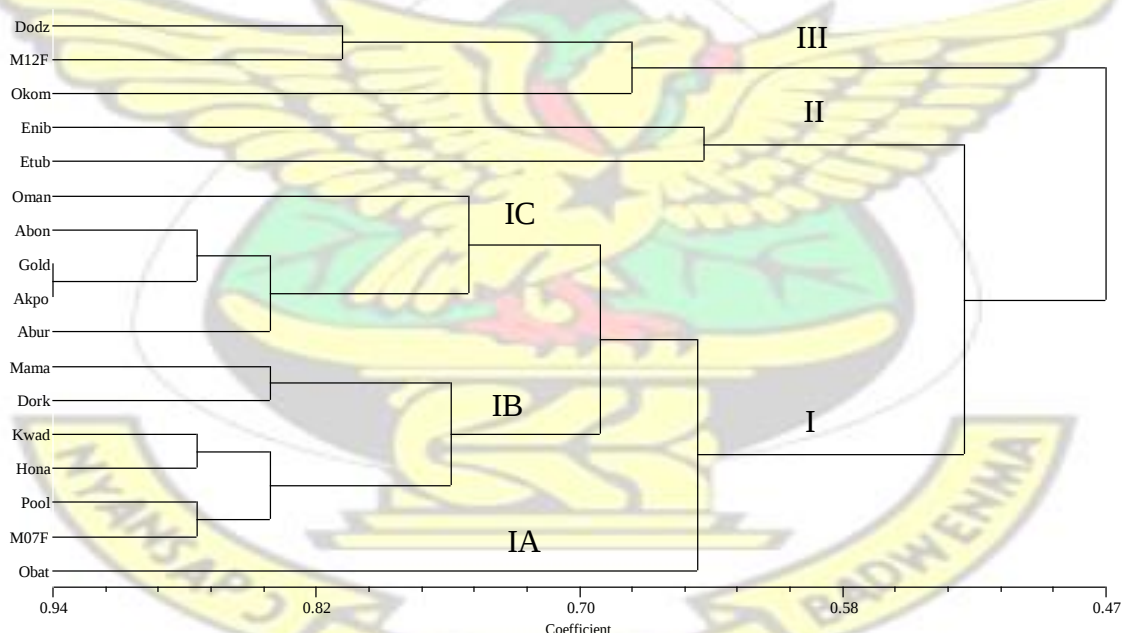


Figure 4.4: Dendrogram of 17 maize accessions generated using 11 SSR markers. I, II and III indicate major cluster groups and IA, IB and IB indicate sub- groups. Accession abbreviations as given in Figure 4.1

CHAPTER FIVE

5.0 DISCUSSION

Information on genetic diversity and relationships in crop germplasm is useful for plant breeders because it assists them in planning crosses (Sun *et al.*, 2001). Such information could be used to design strategies to improve on traits, maintain and manage germplasm in Genetic Resource Centers, or enhance the genetic base of future varieties. Hence, to effectively maintain, evaluate and utilize germplasm, it is imperative to investigate the extent of available diversity. In the present study, a set of maize genotypes (Populations, OPVs and hybrids) in Ghana were subjected to diversity analysis based on variation in morpho-phenological traits and SSR molecular profiles.

5.1 Variation in morpho-phenological traits

In the current study, evaluation on five qualitative and 25 quantitative traits across sixteen Ghana maize cultivars and a check provided a wealth of information needed to understand the existing diversity in the germplasm. The wide variability identified in the qualitative traits including kernel arrangement, kernel texture, moderate variability in silk colour and grain colour, represent diversity that can be harnessed for trait improvement. The single phenotypic variant for cob colour indicates less genetic diversity across the genotypes. Rigorous selection criteria by breeders for unique traits may result in genetic uniformity of breeding materials. Today in Africa, consumers prefer white maize to yellow or any other maize type. The less divergence observed for grain colour might, therefore, be due to exhaustive selection for white maize owing to its preference by consumers. Similar observations have been reported by Rebourg *et al.* (2001) in traditional European maize accessions, Magorokosho (2006) in Zimbabwean, Zambian and Malawian maize varieties, Ranatunga *et al.* (2009) in

Asian accessions and Serpolay-Besson *et al.* (2014) in French and Italian maize accessions in which uniformity in grain colour was documented.

The range of variability observed for the quantitative traits across the three classes of genotypes was found to be significant ($P \leq 0.05$) in the OPVs in majority of the traits (AD, SD, ASI, TL, ELL, ELW, PLHT, EHT, StD, NL, EP, KL, NRE, CD, NKR, EN, EWT, HKWT, GWT and YLD), significant in the hybrids for only eight traits (ASI, TL, ELW, PLHT, EHT, StD, EP and KW), but non-significant in the population group except EN. These observations indicate possibility of grouping the accessions into various groups of good and poor performers and that considerable level of genetic diversity exists among the OPVs. These findings render the OPVs as good candidates for development into improved varieties. This may reflect a sampling bias in this study, because only two populations, compared to three hybrids and 12 OPVs analyzed and/or the fact that OPVs are highly heterogeneous. This observation coincides with earlier reports of genetic diversity studies in nontemperate maize landraces, OPVs and inbred lines where an unexpected high level of genetic diversity in inbred lines than OPVs was attributed to the relatively higher number of inbred lines analyzed (Warburton *et al.*, 2008).

The 25 quantitative traits studied exhibited means and ranges that were consistent or similar to results from other studies. Most of the accessions required nearly 2 months from sowing to male and female flowering (AD of 55.34 ± 5.25 and SD of 56.72 ± 5.62 days). These values are similar to those observed for Nigerian OPVs of AD of 55.00 ± 0.34 and SD of 56.00 ± 5.48 days and QPM genotypes of AD of 55.30 and SD of 57.30 days (Bello *et al.*, 2012, 2014). The wide range of anthesis-silking interval of -6 to 12 with a mean of 1.36 ± 1.71 days was similar to those of Magorokosho

(2006) and Shrestha (2013) who reported mean ASI values 1.35 ± 0.06 days and range of -6 to 9 days in Southern African and Asian accessions, respectively. Earliness in maize is crucial in breeding for drought tolerance (Ngugi *et al.*, 2013). Drought stress affects maize productivity most, when it occurs during flowering periods (Edmeades, 2000) as accumulation of large amount of moisture is required for silk development (Ngugi *et al.*, 2013), as such, silking is delayed until moisture is available, resulting in long anthesis-silking interval. Lengthening of ASI decreases the chance of successful seed set and leads to reduced grain yield (Ngugi *et al.*, 2013). However, Bolaños and Edmeades (1993) and Gonzalez *et al.* (2014) consistently demonstrated that short anthesis-silking interval is a strong measure of drought tolerance in maize. Genotypes in the current study which exhibited short anthesis-silking interval may find uses in drought-tolerance breeding programs. Advances in maize breeding have improved the stress tolerance of modern varieties, including drought tolerance (Barker *et al.*, 2005), in part by reducing ASI and selecting on other traits. The short ASI observed in genotypes of current study may be attributed to their previous breeding enhancement. This observation confirm previous knowledge that modern genotypes especially hybrids have shorter ASI (Bänziger *et al.*, 2000; Campbell *et al.*, 2014). The highest mean values for TL, ELL and ELW together with their positive correlation with grain yield indicated that accessions with higher values of these traits also had high values for grain yield. About 10 to 16 % of grain yield is explained by these traits. It is therefore not surprising that the two populations recorded the highest grain yield in the current study. A similar finding of the association of TL and ELW with grain yield was made among some landraces of lowland regions of Africa (unpublished) demonstrating a correlated response which may be exploited for selection of high yield genotypes at early developmental stages.

The Ghana maize cultivars had smaller ear leaves, in terms of length and width, were relatively short with ears positioned at lower heights but had longer TL and higher number of leaves than those observed for Ethiopian accessions (Beyene *et al.*, 2005) and Southern African landraces (Magorokosho, 2006). However, mean values for yield components such as EN of 1.07 ± 0.12 (range: 1.00-1.45) and EL of 16.79 ± 2.67 cm and ED of 4.73 ± 0.44 cm are similar to the mean values of 1.02 (range: 0.96-1.05) reported by Lucchin *et al.* (2003) and 16.10 ± 13.59 cm and 4.43 ± 2.34 cm reported by Magorokosho (2006), respectively. The Ethiopian and Southern African accessions were landraces and were expected to be taller than improved varieties

5.2 Phenotypic and Genotypic coefficient of variation and heritability estimates

Estimates of genotypic and phenotypic coefficient of variations are useful in detecting the amount of variance present in a germplasm. Knowledge of heritability estimates of a trait guides the plant breeder to predict behavior of succeeding generations and helps to predict the response of a trait to selection. Therefore, availability of good knowledge of heritability of important agronomic traits in maize populations in Ghana could be a pre-requisite for effective improvement exercise. The slightly higher phenotypic coefficient of variation estimates over the corresponding genotypic coefficient of variation estimates for most of the traits in this study indicates the environmental influence on trait expression. This observation is in consonance with previous findings in Ethiopian highland maize accessions (Beyene *et al.*, 2005) and in Indian maize accessions (Langade *et al.*, 2013, Kumar *et al.*, 2014). Close estimates of genotypic coefficient of variation and phenotypic coefficient of variation were recorded for most of the characters. Therefore, selection on the basis of phenotype alone can be effective for the improvement of these traits.

Heritability estimates act as predictive instruments in expressing the reliability of phenotypic value. High broad sense heritability estimates leads to effective selection for a particular character. In the present study, some characters especially in the OPVs exhibited high heritability estimates. For the two populations, all the characters evaluated in this study except PLHT, EHT and SG exhibited very low phenotypic coefficient of variation, genotypic coefficient of variation and broad sense heritability, whereas the OPVs exhibited moderate for TL, ELL, EHT and PLHT. Similar results were obtained by Bello *et al.* (2012) in 10 OPVs in Nigeria. The heritability estimates of 0.62 and 0.74 observed for PLHT and TL, respectively in the present study for OPVs are very close to the 0.64 and 0.71 obtained by Rebourg *et al.* (2001) in traditional European maize populations. Beyene *et al.* (2005) also obtained broad sense heritability estimate of 0.70 for PLHT in traditional Ethiopian highland maize accessions. Moderate heritability estimate of 0.57 obtained for ED in this study was close to the 0.55 reported by Langade *et al.* (2013) in India. The high heritability estimates of anthesis and silking days was also reported in Indian maize (Langade *et al.*, 2013, Kumar *et al.*, 2014) and suggests successful improvement in these traits via simple selection of plants. The low heritability estimates suggest that these characters were highly influenced by the environment and progress in genetic improvement through selection will be very slow or difficult due to the masking effects of environment on genotypic effects, while the moderate heritabilities are indicative of successful trait improvement, but over many cycles of selection. The hybrids exhibited very low phenotypic coefficient of variation, genotypic coefficient of variation and broad sense heritability estimates for all traits similar to that exhibited by the population. These results are in close conformity with those obtained by Atnafua and Rao (2014) in Indian hybrid maize accessions except

PLHT and Rahman *et al.* (2015) in Pakistani maize. Unexpectedly, YLD exhibited very low phenotypic coefficient of variation, genotypic coefficient of variation and virtually null broad sense heritability across all the classes. Similar findings have been reported by Lucchin *et al.* (2003) in Italian flint accessions, Beyene *et al.* (2005) in Ethiopian highland maize accessions and Idris and Abuali (2011) in Sudanese maize.

5.3 Phenotypic relatedness of accessions and implications for improvement

Multivariate statistical tools have been of great value in summarizing and describing the inherent variation among crop genotypes. Principal component analysis (PCA) and hierarchical cluster analyses in particular help to identify plant traits that characterize distinctness among selected genotypes.

In the present study, cluster analysis based on 25 morphological traits grouped the accessions into three main clusters, indicating considerable phenotypic variation among the studied genotypes. The genetic divergence among clusters was well reflected in their cluster means. The different clusters could be regarded as heterotic groups which possess genes for yield and other important characters for hybrid breeding. Cluster I gave high mean values for most of the traits. This observation suggests that Cluster I contained genotypes with more desirable traits, which could be directly selected and utilized in breeding programs. Accessions in cluster II combined intermediate flowering (AD, SD and ASI) with high grain yields. Accessions in this group could therefore be good candidates in breeding for high yielding drought tolerant varieties. The first group included accessions from different parents (Table 3.1). ‘Okomasa’ and ‘Honampa’ separated together within the first group indicating that these accessions were more phenotypically similar than other members of the group. Two hybrids (‘Mamaba’ and ‘Enibi’) and two populations

(‘M0826-7F’ and ‘M0826-12F’) grouped together with two OPVs in cluster II suggesting a possible genetic similarity between the parents of these accessions. ‘Mamaba’ and ‘Enibi’ have the same female parent (GH110) and were derived from the same CIMMYT population (Pop. 63-SR) as ‘Obatanpa’. Because of their genetic similarity derived from common parent, it seems logical that these accessions are in the same group. The two populations used in the study were very much identical, an observation which is suggestive of common parentage. With the exception of ‘Dodzi’, all accessions in cluster III had QPM based parents that could be genetically similar. It is therefore not unexpected that these accessions clustered together.

The existence of phenotypic diversity among studied maize accessions and relationships among traits was further explained graphically by PCA biplots and interpretations made on the basis of length of the vectors (Geleta and Grausgruber, 2013 and the angles between them (Sharifi and Aminpana, 2014; Magorokosho, 2006). On account of the acute angles which existed among the plant architectural traits ELL, ELW, PLHT, with ear and kernel characteristics, as well as grain yield these traits were deemed to be positively correlated. In addition, based on the lengths of the vectors, the most critical traits which enhanced grain performance in a positive direction were AD, StD, ELW, and HKWT. Other equally important traits were SD, NL, TL, ELL, PLHT, and ED. The contribution of ELW was more pronounced than ELL as was also observed in a similar study involving West African maize landraces (unpublished). Traits such as ASI, KW and KT showed shorter vector lengths, suggesting that these traits will enhance grain yield performance in a negative direction. Similar observations have been reported in maize (Beyene *et al.*, 2005; Magorokosho, 2006; Langade *et al.*, 2013; Atnafua and Rao, 2014).

The classification of the accessions into three major groups was confirmed by accession PCA biplot. The biplot scattered the accessions in groups, indicating that the accessions were diverse for the phenotypic characters measured. According to Yan (2005), the length of genotype vectors measures the differences of the genotype from the grand mean. Genotypes with long vectors are the best contributors to total variability. Moreover, the cosine of the angle between the vectors of two genotypes measures their similarity. In the present study, genotypes such as ‘Obatanpa’, ‘Golden Jubilee’, ‘Akposoe’ and ‘Pool 16 SR’ were the most contributors to total variance as depicted by the respective vector lengths (Figure 4.2). ‘Kwadaso local’ and ‘Okomasa’ were highly correlated in their trait performance, as also ‘Abontem’, ‘Akposoe’, ‘Dodzi’, ‘Aburohemaa’ and ‘Pool 16 SR’, ‘Okomasa’ and ‘Abontem’ were not correlated (approximately 180° apart) and as such divergent in their performance. These observations confirm the efficiency of biplots in grouping accessions based on their genetic similarity. It is undeniable the fact that characters with Eigen values closer to unity within the first principal component influence the clustering more than those with Eigen values closer to zero. The first component in particular which explained 49 % of the total variation was positively associated with AD, SD, TL, ELL, ELW, PLHT, EHT, NL, EP, StD, EL, KL, NKR, NRE, CD, EWT and YLD suggesting that these traits significantly weighted the clustering. Lucchin *et al.* (2003) reported similar observation in their study aimed to characterize 20 Italian maize populations. The results of cluster analysis, the biplot, and PCA may be used to design a strategy to maintain or enhance the genetic diversity of future varieties.

5.4 Genetic diversity using SSR markers

SSR markers are currently the molecular marker of choice for genetic diversity studies in maize and other cereals (Aci *et al.*, 2014; Semagn *et al.*, 2014; Choudhary *et al.*,

2015; Ignjatovic-Micic *et al.*, 2015, Oyekunle *et al.*, 2015) due mainly to their high allelic diversity. The efficiency of detection of alleles depends mainly on the number and extent of variability of the examined operative taxonomic units (OTUs). Evaluation of 17 genotypes with 11 SSR loci revealed an average number of 2.82 alleles per locus similar to the 2.70, 1.56, 2.07 and 2.30 alleles per SSR locus reported by Wietholter *et al.* (2008) using 21 SSR primers, Shah *et al.* (2009) with 10 SSR markers, Van Nguyen *et al.* (2012) using 20 SSRs and Kanagarasu *et al.* (2013) using 10 SSR loci to evaluate maize genotypes, respectively. Relatively higher values such as 4.9 alleles with 46 SSR loci (Choukan *et al.*, 2006), 3.85 alleles using 27 SSR loci (Legesse *et al.*, 2007), and 3.33 alleles with 12 SSR loci (Shiri *et al.*, 2014). Some researchers have reported that the kind of resolution technique employed for detecting alleles also influences allelic difference (Bantte and Prasanna 2003; Shiri *et al.*, 2014). In this study, agarose gel electrophoresis was used for detection of easily scorable bands. Enhanced resolution and improved detection of allelic variation may have been achieved on polyacrylamide gel combined with an automated detection system. Nevertheless, to improve the efficiency and quality of scoring, effort and care was taken to identify and eliminate stutter bands, a common problem with SSR gels.

Polymorphism Information Content demonstrates the informativeness of a marker system and its potential to reveal differences among genotypes based on their genetic relationships. In the current study, the average PIC value obtained (0.43) was lower than the 0.54 of Bantte and Prasanna (2003), 0.83 of Ranatunga *et al.* (2009), and 0.53 of Silva *et al.* (2015). It is, however, in close agreement with other investigations in maize (Van Nguyen *et al.*, 2012; Nidhal *et al.*, 2014 and Shiri *et al.*, 2014) all of whom estimated average PIC value of 0.44. The low PIC value obtained in this study suggest a narrow genetic base in the cultivars.

Generally in maize, genetic similarity around 70 % indicate considerable genetic diversity (Wietholter *et al.*, 2008). In the current study, the average genetic diversity existing among all the genotypes was relatively high (61 %), indicating moderate levels of polymorphisms in the genotypes. It was also observed that 46.67 % of the estimated coefficients had values greater than 0.61, reflecting the high degree of genetic similarity among the cultivars used in this study. However, 31.11 % of the coefficients were equal to or less than 0.50, and can therefore be exploited for divergent parent selection. These results are in close agreement with previous findings in maize using SSR markers (Magorokosho, 2006, Legesse *et al.*, 2007). Magorokosho (2006), reported genetic similarity coefficients of between 0.34 and 0.94 with an average of 0.65 in a set of maize genotypes from Zambia, Malawi and Zimbabwe. In a similar study, Legesse *et al.* (2007), reported an average genetic diversity of 59 % among African maize population.

The SSR-based dendrogram constructed using the UPGMA clustering algorithm grouped the accessions into three main clusters. This grouping is in consonance with previous studies in maize (Magorokosho, 2006; Aci *et al.*, 2013; Shiri *et al.*, 2014).

The pedigree information indicates that ‘Golden Jubilee’ was derived from ‘Obatanpa’ and ‘Dorke SR’ was derived from ‘Pool 16 SR’. This suggest similarity between CIMMYT Pop. 63 and Pool 16 SR. Therefore, considering the pedigrees of these accessions, cluster I grouping seems logical. The second main cluster included two hybrid accessions with the same female parent (GH110). Therefore, the fact that these two hybrids are in the same group indicate that the molecular method used was highly efficient in revealing genetic relationships. Genetic relationships among some of the accessions revealed by the cluster analysis were not in consonance with the pedigree information, as was observed in the case of members in cluster III. ‘Mamaba’ for

instance, by pedigree have the same female parent as the two hybrids in Cluster II but was grouped in the first cluster. The failure of this genotype to be grouped in cluster II is most likely as a result of the limited SSR data set (11 primers) in this study. Incongruence such as this have been reported in maize before (Bantte and Prasanna, 2003; Shiri *et al.*, 2014).

In the present study, the number of SSR loci (11) used to screen the genotypes were considerably lower than some earlier reports in maize. Moreover, because two chromosomes were not represented, complete information on the diversity was not obtainable. However complementation of the molecular information with morphological evaluation increased the value of the research, exposed the level of genetic diversity hidden in the cultivars, and provided a guide to planning of maize breeding in Ghana for high yield performance, drought tolerance and any other trait improvement.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

A large number of methods are available for assessing genetic diversity among and within crop species. In the present study, morpho-phenological and molecular techniques were employed to examine the amount of genetic diversity present in a set of 17 maize accessions originating from breeding programs in Ghana.

The ANOVA revealed significant differences among the accessions, particularly for most of the phenotypic characters examined. In addition, cluster analysis and PCA based on phenotypic markers separated and grouped the accessions according to their

genetic distances or similarities. A dendrogram constructed based on phenotypic similarity showed that the accessions may be grouped into three major clusters.

Broad sense heritability of agronomic traits was moderate in OPVs but rather low in hybrids and populations. Based on variability and heritability estimates, it is concluded that improvement by direct selection is possible for traits such as days to anthesis, days to silking and plant height in OPVs, as well as ear length in hybrids.

The molecular marker study revealed moderate reservoir of genetic diversity among the accessions. A total of 31 alleles were detected by 11 SSR primers with average PIC value of 0.43. Approximately 23 % of the alleles were unique and they were revealed in five accessions. Estimates of genetic similarity between all possible pairwise combinations based on SSR data showed that genetic similarity ranged from 36 % to 94 % with an average of 61 %. A dendrogram generated from the SSR data also grouped the accessions into three major clusters. Some genotypes were placed in groups that could not be predicted based on similarity in pedigree data.

Generally, the present study has explained the relevance of employing phenotypic markers together with molecular markers to determine genetic distances and relationships in maize. Moderate level of genetic diversity was observed both at the molecular and phenotypic levels. The overarching question now is how we can use the reservoir of genetic diversity observed in this study to improve maize productivity per unit area supported by moderation in inputs such as water and fertilizer?.

6.2 Recommendations

Considering the presence of large number of traits available for estimating genetic diversity in maize, many other traits could have been measured. Tassel number, tassel

angle, tassel attitude, spikelet density, anther colour and ear and kernel shape could also have been considered. These traits were not added because enough data had been collected from other useful traits. It is recommended that future studies on maize phenotypic diversity should consider these traits.

Based on the null heritability estimates recorded for yield and its components, future studies should consider profiles of individual plants of the same accession to examine the population structure of maize in Ghana and thus determine if the plateau of yield improvement has been reached.

To obtain complete information on the genetic diversity of Ghanaian maize accessions, future studies should employ high number of SSR loci covering all the 10 chromosomes in maize. Moreover, biochemical characterization such as mineral and protein analyses should also be included.

Distinct maize accessions possessing novel alleles that have been identified with a combination of the phenotypic and SSR markers such as ‘Obatanpa’, ‘Kwadaso Local’, ‘Enibi’, and ‘Etubi’ may be included in breeding programs.

In comparison with previous studies on landraces, the diversity revealed in this study is narrow. It is, therefore, recommended that maize breeding programs in Ghana should include new genetically unrelated genotypes in order to broaden the genetic base of Ghanaian maize germplasm.

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LIST OF APPENDICES

Appendix 4.1 Preparation of reagents

1. CTAB
 - a. 2% CTAB (Hexadecyltrimethyl-ammonium bromide)
 - b. 100 mM TrisHCl {pH = 8}
 - c. 20 mM EDTA
 - d. 1.4 M NaCl
 - e. 0.1% (w/v) PVP (polyvinyl polypyrrolidone)
 - f. 0.2% β -mercaptoethanol (added just before use)
 - g. mg/mL proteinase K (added just before use)

2. TE buffer (1000 ml)
 - a. 1 M Tris pH 8.0 10 ml.
 - b. 0.5 M EDTA pH 8.0 2 ml.
 - c. 5 M NaCl 200 ml.
 - d. dH₂O complete volume to 1000 ml
3. Chloroform:isoamyl alcohol (24:1)
 - a. Measure 960 ml/L Chloroform in beaker.
 - b. Add 40 ml/L Isoamyl alcohol into the beaker.
4. Phenol/chloroform (1:1v/v)
 - a. Weigh out 20 g phenol in a glass beaker.
 - b. Add 20 ml chloroform cover with cling film and mix well over a period of a few hours until all the phenol has dissolved.
5. 0.8 % Agarose
 - a. Weigh 0.8 g of agarose and add 100 ml of 1 X TBE and heat in a micro wave to dissolve.
6. 2 % agarose gel
 - a. Weigh 2 g of agarose and add 100 ml of the 1 X TBE and heat in a micro wave to dissolve.
7. 70 % ethanol (100 ml)
 - a. Measure and mix 70 ml of absolute ethanol, and 30 ml distilled water.

Appendix 4.2: Genetic similarity among 17 maize accessions generated using eleven SSR primer combinations based on Dice's similarity coefficient

<u>Acc.</u>	<u>Dodz</u>	<u>Enib</u>	<u>Okom</u>	<u>Etub</u>	<u>M12F</u>	<u>Oman</u>	<u>Mama</u>	<u>Abon</u>	<u>Abur</u>	<u>Kwad</u>	<u>Pool</u>	<u>Obat</u>	<u>Gold</u>	<u>Dork</u>	<u>Hona</u>	<u>M07F</u>	<u>Akpo</u>
Dodz	1.00																
Enib	0.52	1.00															
Okom	0.61	0.62	1.00														
Etub	0.63	0.65	0.48	1.00													
M12F	0.81	0.65	0.74	0.55	1.00												
Oman	0.42	0.39	0.42	0.61	0.48	1.00											
Mama	0.52	0.48	0.45	0.65	0.45	0.65	1.00										
Abon	0.42	0.45	0.42	0.55	0.42	0.81	0.77	1.00									
Abur	0.42	0.36	0.36	0.45	0.36	0.71	0.68	0.84	1.00								
Kwad	0.36	0.45	0.48	0.61	0.42	0.48	0.71	0.68	0.65	1.00							
Pool	0.42	0.52	0.55	0.74	0.55	0.61	0.77	0.68	0.65	0.81	1.00						
Obat	0.48	0.39	0.42	0.48	0.42	0.48	0.58	0.61	0.71	0.68	0.68	1.00					
Gold	0.48	0.48	0.48	0.58	0.48	0.77	0.68	0.90	0.81	0.71	0.71	0.65	1.00				
Dork	0.52	0.45	0.52	0.61	0.45	0.55	0.84	0.61	0.58	0.81	0.74	0.55	0.71	1.00			
Hona	0.48	0.58	0.55	0.74	0.55	0.61	0.84	0.81	0.71	0.87	0.87	0.74	0.84	0.81	1.00		
M07	0.42	0.45	0.42	0.61	0.42	0.61	0.71	0.81	0.71	0.81	0.87	0.74	0.84	0.68	0.87	1.00	
Akpo	0.55	0.48	0.48	0.58	0.48	0.71	0.68	0.84	0.87	0.71	0.71	0.71	0.94	0.71	0.84	0.77	1.00

Acc=Accession, Okom=Okomasa, Dodz=Dodzi, Enib=Enibi, Okomasa, Etub=Etubi, M12F=M0826-12F, Oman=Omankwa, Mama=Mamaba, Abon= Abontem, Abur=Aburohemaa, Kwad=Kwadaso Local, Pool=Pool 16 SR, Obat=Obatanpa, Gold=Golden Jubilee, Dork=Dorke, Hona=Honampa, M07F=M0826-7F and AKPO=Akposoe.

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KNUST



Appendix 4.3: Some phenotypic variations in maize in Ghana

A. Tassel colouration



Obatanpa



Omankwa



Honampa

B. Silk colouration

Pale yellow



Akposoe

Red



Enibi

C. Kernel colouration

Yellow



White



Yellow



Abontem

Obatanpa

Honampa

D. Kernel arrangement

Straight



Spiral



Irregular



Obatanpa

Golden Jubilee

Kwadaso Local

KNUST

