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CHARACTERIZATION OF YELLOW-, RED-, AND PURPLE- KERNEL MAIZE (*ZEA MAYS* L.) ACCESSIONS IN GHANA.

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By

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DECLARATION

This thesis is the result of research work undertaken by ANSAH GILBERT in the Department of Nuclear Agriculture and Radiation processing of the School of Nuclear and Allied science, University of Ghana under supervision of Dr. H. M. AMOATEY and Dr. M. S. ABDULAI.

I hereby affirm that except for references which have been duly sited, this work is a result of my own research and that it has not been presented in part or whole for any other degree in this University or elsewhere.

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DEDICATION

This thesis is dedicated to my Mother Mrs. Mary Asare (a.k.a. Ama Otuwa) for being very supportive and helpful throughout my education and my children Nene Yaa Otuwa Ansah, Kofi Asamoah Ansah and Anthony Zaato.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ASI	Anthesis Silking Interval
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CIDA	Canadian International Development Agency
CIMMYT	International Center for Maize and Wheat Improvement
CW	Cob weight
DLS	Days to 50% Leaf Senescence
dNTPs	Deoxynucleotide phosphate
DTA	Days to 50% Anthesis
DTH	Days to Harvesting
DTS	Days to 50% Silking
EG	Ear girth
EH	Ear height
EL	Ear length
GAEC	Ghana Atomic Energy Commission
IITA	International Institute for Tropical Agriculture
KRA	Kernel row arrangement
LL	Leaf length
LW	Leaf width

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MOFA	Ministry of Food and Agriculture
MTC	Maturity Classification
NK	Number of kernels per row
NL	Number of leaves above the uppermost ear
NR	Number of rows per cob
PH	Plant height
PHC	Plant height classification
RAPDs	Random Amplified Polymorphic DNAs
RFLP	Restriction Fragment Length Polymorphism
SARI	Savanna Agricultural Research Institute
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeats
SUK	Shape of the uppermost surface of the kernel
SW	Seed weight per plot
HPLC	High Pressure Liquid Chromatography

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Abstract

Twenty yellow-, red- and purple-kernel maize accessions were collected from three regions in Ghana for the study. The objectives were to characterize the yellow-, red- and purple-kernel maize accessions in Ghana using phenotypic traits in order to determine their identity, using molecular traits for confirmation of their identity and to determine the presence of the opaque -2 gene and β-carotene content of the grains as a way of assessing nutritional quality. A replicated field experiment was conducted to evaluate and characterize the accessions based on 16 quantitative and eleven qualitative traits. The same accessions were characterized based on 16 SSR markers. Variability in β-carotene content was determined by HPLC while presence of opaque 2-gene was determined by a light box. The results revealed that accessions GH4055 and GH4863 are extra early maturing and therefore can be very useful for urban farmers producing fresh maize and for cultivation in the coastal savanna ecological zone. However, they produce smaller cobs (Cob weight = 58.24g) as compared to other accessions. Significant variability in morphological traits was observed among the accessions with cob weight, number of kernels per row, plant height and 1000 seed weight having coefficient of variation of 42.7544, 20.5828, 11.4634, 13.0634 and 26.76 respectively. Few traits contributed to the variations observed as revealed by the principal components analysis and these include days to 50% anthesis, days to 50 % leaf senescence, plant height and cob weight. A dendrogram generated from morphological traits clustered the accessions based on kernel colour, physical structure of the plant and geographical location. Two duplicates were

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identified among the accessions and widest genetic distance was observed between NYR1 and GH4055. Strong correlation exist between most of the morphological traits measured (r = 0.9103) but negative correlation was observed between most important yield parameters and number of row per cob. Accession NYR1 showed promise in terms of yield (1000 seed weight = 281 g) as well as high carotenoid (4.7895 µg/g) and β-carotene content (0.8507 µg/g) and therefore can be very useful in future breeding programmes. Population genetic analysis shows that the accession bear close resemblance to each other with high Nei's genetic identity coefficient 0.9587 and 0.9020 between accessions from Northern and Eastern region and Northern and Greater Accra region respectively with short genetic distances and this is confirmed by the high outcrossing rate (0.6642) and gene flow (0.7387) among the populations and the high heterozygosity (0.4023). A dendrogram generated by SSR markers revealed that the accessions are separate entries with no duplications. Significant variations were found in the β-carotene and carotenoid contents of the accession. Accession DYF had the highest carotenoid and β-carotene concentrations of 12.0327 and 2.0313 respectively. No significant correlation was observed between kernel colour and β-carotene content. Generally the amount of β-carotene increased with increasing carotenoid content but this is not always the case as few exceptions may be found as revealed by the experiment. None of the accessions contained the opaque-2 gene and therefore did not fit the description of Quality Protein Maize. It is recommended that accession DYF had the highest carotenoid and β-carotene concentrations. The accession also performed well in various yield parameters and therefore can be useful in the fresh maize market as well as food for infants and

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accessions GH4055 and GH4863 are extra early maturing and can be useful for cultivation in the coastal savanna ecological zone and for minor season cropping in the forest and forest savanna transitional zones. Information presented may be useful to the breeder, the farmer and the consumer.

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CHAPTER 1

1.0 Introduction

Maize (*Zea mays* L.) belongs to the family Poaceae and it is believed to have originated from Mesoamerica and subsequently spread throughout the world (Matsuoka *et al*., 2002). It is the third most important cereal grown besides wheat and rice, in terms of production and consumption (Al-Swailem, 2005). Worldwide production of maize is estimated to be about 8,834,602 tons (FAOSTATS, 2013) with the USA producing about 316 million tons (FAOSTATS, 2013). Other producing countries include China, Brazil, Mexico, and Argentina (Aquaah, 2007). According to reports from the IITA the area planted to maize in West and Central Africa alone increased from 3.2 million ha in 1961 to 8.9 million ha in 2005 (IITA, 2009). The area planted to maize in Ghana in 2012 was 1,042,083 ha with production of up to 1,949,897 tons (FAOSTATS, 2013).

The crop is a major staple for 1.2 million people in many parts of the developing world including sub Saharan Africa; it is a staple food for an estimated 50% of the population in Africa (IITA, 2009). In Ghana, it is the main ingredient for various popular dishes in all the ten regions of the country especially in Volta, Northern, Upper East and Upper West, Central and Greater Accra regions (Tweneboah, 2000). It is an important source of carbohydrate, vitamins A and B, protein, iron and minerals. The highest per capita consumption of maize is averaged to be 100 kg per year in Malawi, 85 kg /capita/year has been estimated for the southern Africa regions and 27 kg and 25 kg for eastern Africa and West/Central Africa respectively (Smale, 2011) supplying about 40% of the calorie needs in eastern and southern Africa. Maize is a source of starch for the pharmaceutical industry. It is also the main feed ingredient in the poultry industry and can also be used as

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biofuel (Tweneboah, 2000).

Low yields have been a major problem facing farmers in sub Saharan Africa. In Ghana, yields of maize are very low throughout the country; on the average, 1.5 t/ha but could be as low as between 0.3-1.6 t/ha compared to over 5 t/ha in parts of eastern and southern Africa (NARP, 1993; PPMED, 1992). This can be attributed to environmental factors such as pest and diseases, poor soil fertility, high cost of inputs like seeds and fertilizers, underdeveloped markets, inadequate agricultural extension services, poor agronomic practices with low and erratic rainfall distribution also being a major constrain (IITA, 2009).

Equally important are genetic factors mainly inherently low yielding varieties and the lack of commercial maize breeders to breed high yielding varieties. Between 1984 and 1996 scientists at the Crops Research Institute (CRI, Kumasi) developed several varieties which were high yielding, early maturing (“Dorke”, “Kawanzi”), streak resistance with improved protein content (“Obatampa”, “Mamaba”, “Dadaba”, “Cidaba”) (Al-Hassan and Jatoe, 2002; Twumasi-Afriyie *et al*., 1997).

In addition there are several maize landraces in Ghana whose potentials have not been thoroughly exploited for the benefit of farmers and consumers and are getting eroded as farmers tend to adopt and plant newly improved varieties which are relatively high yielding leading to loss of good germplasm for future breeding purposes, these under exploited landraces include yellow-, red- and purple kennel maize landraces which also have high protein and β-carotene content.

Vitamin A deficiency is prevalent in 127 million pre-school children and 7 million children worldwide (West, 2003). According to the WHO over 70 % of pre-school children and 18% of pregnant women suffer from vitamin A deficiency in Ghana though

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most of the symptoms are not visible to the naked eye (WHO, 2009). Protein deficiency is also prevalent in the country with "kwashiorkor" and stunted growth being the most common disease and symptom respectively. Protein deficiency is common among people in northern Ghana where the meat, fish and milk are beyond the means of the average family (Buah *et al*., 2009). Maize is an important source of protein accounting for about 60% of the daily human protein supply (Mbuya *et al*., 2012).

Knowledge of the morphological, molecular and nutritional quality of the yellow-, red and purple maize landraces which are currently underexploited may bring to light hidden qualities. This will help in future breeding programmes such as bio-fortification which will help to alleviate some of the vitamin A and protein deficiency problems in the country as most households are dependent on maize as the main staple.

Morphological studies have been very useful in maize characterization before the advent of molecular characterization as a tool for establishing plant identity and in botanical classification. But a major setback to this method is the interaction of the environment which makes reproducibility of results difficult and developmental stage specificity where the record on certain traits can only be collected at a specific stage of the plants development. In molecular characterization DNA templates are used and the results are highly repeatable since it is not affected by the environment or stage of development of the plant making the method a sure and reliable way to establish plant identity and differences even in closely related plants. Also the nutritional composition of the crop is very important and natural variability in nutrient composition can be useful in identifying promising landraces for future breeding programmes.

Since characterization is fundamental to the development, release and popularization of plant varieties (Chavan, 2010) there is the need to take records of morphological traits

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that are highly heritable and can be expressed in all environments so as to establish their identity and phenotypic diversity. Molecular characterization is necessary to establish the unequivocal genetic diversity of the accessions. Finally, it is important that the nutritional composition in terms of protein and β-carotene content of available varieties and landraces are assessed to determine their potential use in future breeding programmes.

The objectives of the study were;

1. To characterize the yellow-, red- and purple-kernel accessions in Ghana using phenotypic traits in order to determine their identity.

2. To characterize the yellow-, red- and purple kernel maize accessions in Ghana using molecular traits for confirmation of their identity.

3. To determine the presence of the opaque-2 gene and β-carotene content of the grains of the yellow-, red- and purple-kernel maize accessions as a way of assessing nutritional quality.

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CHAPTER 2

2.0 Literature Review

2.1: Origin and Domestication

The origin and domestication of maize has been a controversy in evolution, taxonomy and domestication. Even though most scientists believe the crop originated from Mexico, other scientists have also proposed multiple- origins of the crop; based on research on chromosome knob positions in maize from South and Central America. Kato (1984; 1976) and McClintock (1981; 1978) proposed five independent centers of domestication; four in Mexico and one in Guatemala.

Archeological findings of fossil maize in Mexico have also provided some evidence of the domestication of maize from teosinte (Hernandez, 2009). Pollen grains excavated from the coastal plains of Veracruz, Mexico, demonstrate maize cultivation since 5000 years ago. Cobs of fossil maize excavated at the Guilá Naquitz cave in Oaxaca, Mexico, (1926 m above sea level) directly dated by accelerator mass spectrometry 6250 calendar years before present (y. b. p.) (Piperno and Flannery, 2001). The second oldest fossils maize were found at the San Marcos Cave near Tehuacán, Puebla, also dated by accelerator mass spectrometry to be seven centuries younger (5500 y. b. p.) (Long *et al.*, 1989). However in 2001 pollen grains excavated from Tabasco, Mexico, suggest that maize cultivation occurred 1000 -2000 years earlier than the 7000 years ago previously accepted (Sluyter and Dominguez, 2005).

Genetic and botanical information available identifies teosinte (*Zea mays* spp. *parviglumis*) as the progenitor of the current maize plant (*Zea mays* L.) (Hernandez, 2009; Linnaeus, 1748). This has also been controversial due to the large morphological

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differences between the two crops especially in the female inflorescence. The profound differences in the structure of the maize and teosinte female inflorescences (ears) has posed a challenge to formulating a compelling model for the developmental and genetic steps involved in this evolutionary transition (Doebley, 2004). However, there are natural gene exchanges between maize and teosinte as well as hybridization.

Wang *et al*. (2005) reported that the liberation of the kernel from hardened case of the teosinte is the result of a single gene (teosinte glume architecture; tga1) and that modest genetic changes in single genes can induce dramatic changes in phenotype during domestication and evolution. A change in the amino acid sequence of the tga1 gene can result in a dramatic change as observed in the modern maize.

2.2 Distribution

Matsouka *et al.* (2002) proposed that maize was dispersed from its proposed center of origin via two routes; the northward route which traces from Mexico's highlands through northern Mexico into south western United States and the southward route from Mexico's highlands to western and southern lowlands of Mexico into Guatemala and the Caribbean islands. Maize was brought to Europe in the 16th century by Columbus who took the seeds from Cuba to Spain and later spread to Italy and other parts of the world (GTZ/MOFA, 2006). The Portuguese introduced maize to Africa for planting in the Congo basin and the crop spread throughout the continent due to its high yield and numerous uses. Maize was sent to Asia through the Indian Ocean route and Mediterranean trade route.

Through years of intensive breeding and selection the crop can now be grown in tropical, subtropical and temperate climates simply by selecting the right variety and scheduling

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the planting time to avoid harsh environmental conditions including low temperatures below 19 ^{O}C or mean summer temperatures of 23 ^{O}C and high temperatures above 32 ^{O}C; the crop does not tolerate frost and water logging (Ikisan, 2013).

In Ghana, maize is grown in all the ten regions of the country and all the ecological zones from the Coastal savanna through the Forest and Forest savanna transitional zone to the Guinea savanna and Sudan savanna with the Forest savanna transitional zone being the highest producing area. The crop is also grown under a wide range of conditions ranging from soil type, soil fertility, moisture level, temperature and cultural practice (Abdulai *et al.*, 2007)

2.3 Botanical Description

Maize belongs to the Family Poaceae (Gramineae), subfamily panicoideae, tribe andropogoneae, Genus Zea, and Species *Zea mays* (Chemeurope, 2013; Agrisnet, 2013). It is a monocot with an erect, solid, cylindrical stem with well-defined nodes and internodes of 20-30 cm. Internodes are short and thick at the base of the plant. Plant height varies considerably due to varietal differences or environmental conditions from 0.6 m in sweet corn to 5 m in other genotypes. The stem thickness ranges between 3 and 4 cm and attached to the nodes are flag leaves which are arranged spirally on the stem and opposite to each other.

The leaves are long with prominent midribs and tapper towards the end. As in all other monocots the plant has a fibrous root system extending up to 1.5 m laterally and about 2 m deep depending on the soil physical characteristics and plant genotype. The first few nodes close to the surface usually have adventitious and prop roots. Tillers may also develop

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on these nodes. The lateral shoot bearing the main ear develops from a bud on the eighth node above the soil surface, the other five to six buds directly below give rise to rudimentary lateral shoots one or two of which develop to produce ears (Jean du Plessis, 2003). The maize ear is born on the lateral branch of the stem and is covered by special leaves called bracts which completely enclose the ear. The silk appear first before the ear and the silk is receptive in the first 3 weeks. Receptivity however, decreases after first 10 days. The top of the stem ends in a flower, called the tassel. For each silk on which viable pollen lands, one kernel of corn is produced.

The root is profusely branched with a fine rooting system that can grow 1.5 m laterally and up to 2.0 m deep. The permanent root system has adventitious roots which function to support the plant and also to take up nutrients. The plant also produces numerous root hairs which increase the surface area for water and nutrient absorption. The adventitious roots, usually 3 - 5, grow from the nodes above the ground, downwards at the time of germination and help to anchor the plant firmly while the secondary roots, which are 15-20 times as numerous as the adventitious roots, develop from the first few nodes at the base of the stem playing a major role in the uptake of water (Jean du Plessis, 2003).

Unlike other grasses, which produce perfect (bisexual) flowers, the male inflorescences (tassels) emerge at the apex of the stem and female inflorescences (ears) at the apex of condensed lateral branches protruding from leaf axils. The male (staminate) inflorescence, a loose panicle, produces pairs of free spikelets each enclosing a fertile and a sterile floret. The female (pistillate) inflorescence, a spike, produces pairs of spikelets on the surface of a highly condensed rachis (central axis, or "cob"). Each of the female spikelets encloses two fertile florets, one of whose ovaries will mature into a maize kernel once sexually fertilized by wind-blown pollen (Jean du Plessis, 2003). .

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The kernel of corn has a pericarp of the fruit fused with the seed coat. The grains adhere in regular rows round a white pithy substance, which forms the ear. An ear contains a few to four hundred grains, and is from 10 - 25 cm in length. They are of various colors, blackish, red, white and yellow, purple etc. (Jean du Plessis, 2003).

2.4: Crop Diversity

Diversity is very important in any breeding programme. Maize is the most diverse cultivated crop in the world, making the crop the number one choice for diversity studies. The diversity in maize emerged through years of breeding and selection for traits such as high yields, disease resistance and ability to grow in different ecological zones. Human selection has led to the genetic diversity of maize found in Mexican fields today; there are as many as 60 maize landraces in Mexico (Sanchez *et al*., 2000). Several maize cultivars are grown in the world today, the CIMMYT maize genetic resource center and maize breeding programme has over 17000 inbred lines and populations of maize (Warburton *et al.*, 2001).

Maize diversity is both phenotypic and genotypic. Phenotypically, the plants are usually classified into types largely based on the characteristics of the seed (the shape of the kennel, the colour of the kernel) and maturity. They can be allocated to one of seven groups or types. These seven groups are, dent maize, flint maize, sweet corn, soft or flour maize, popcorn, waxy maize and pod maize (Mullen, 1979). Based on kernel colour the plants are grouped as white, red, purple, yellow, variegated, mottled orange and brown. These colours represent the colour of the pericarp (red, colourless), aleurone layer (blue, colourless) and endosperm (yellow, white) (Mullen, 1979).

Genotypically, a lot of studies have been carried out by scientists in molecular genetics to

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determine the genetic diversity in maize. Currently it is known that the degree of diversity in maize is greater than between humans and other primates. The maize genome is complex with striking intraspecific variation in gene order, repetitive DNA content, and allelic content exceeding the levels observed between primate species (Llaca *et al.*, 2011).

Ranatunga *et al*. (2009) studied the genetic diversity in 43 maize accessions and concluded that there were significant differences between the accessions after morphometric and molecular analysis using SSR markers.

Beyene *et al.* (2005) did a comparative study of 62 maize accessions from the highlands of Ethiopia; the experiment revealed that there were significant differences among accessions after molecular analysis and suggested that there was high degree of phenotypic diversity among the accessions.

Abuali *et al.* (2011) assessed the genetic diversity in 27 Sudanese maize genotypes using Random Amplified Polymorphic DNAs (RAPDs) markers and concluded that the degree of genetic polymorphism ranged between 0 and 100% indicating that the genotypes were genetically very diverse and possess a high degree of polymorphism.

Badu-Apraku *et al.* (2006) worked on multivariate analysis of genetic diversity of forty seven *Striga* resistant tropical early maturing maize genotypes using morphological traits and concluded that the mean square among the genotypes were significant indicating a large genotypic variation.

The discovery of transposable element in the maize genome gave an explanation to the cause of high diversity found in maize. Maize genome complexity is primarily driven by polyploidization and explosive amplification of Long Terminal Repeat retrotransposons, with the counteracting effect of unequal and illegitimate crossovers. (Llaca *et al.*, 2011).

The production of hybrid maize varieties with good agronomic qualities requires the

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breeding of inbred lines that are homozygous, the practice have been found to narrow the genetic diversity among maize cultivars resulting in genetic erosion.

2.5 Importance and Uses of Maize

Maize is a very important crop in the world economy as a staple for millions people in the developing world and as animal feed, starch and biofuel in the developed world. It is the third most important cereal grown besides wheat and rice, in terms of production and consumption (Al-Swailem, 2005). According to reports from the IITA the area planted to maize in West and Central Africa increased from 3.2 million ha in 1961 to 8.9 million ha in 2005 (IITA, 2009). The area planted to maize in Ghana in 2012 was 1,042,083 with production of up to 1,949,897 metric tons (FAOSTATS, 2013). It is an important source of carbohydrate, vitamin A and B, protein, iron and minerals. The per capita consumption of maize is averaged to be 100 kg per year supplying about 40% of the calorie needs in Eastern and Southern Africa. The crop has been a great source of food for the animal production industry providing both grains and silage for feed production. The grains are used for the production of quality starch and recently the emphasis is gradually shifting towards the production of biofuel especially in the United States. The crop is of great importance in Africa as a major crop for improving food security on the continent.

2.6 Constraints to production

Despite the wide area under cultivation and high rate of consumption both by humans and animals, farmers are faced with a lot of challenges with the ultimate being low yields. This low yields can be attributed to Environmental factors such as moisture, soil fertility, pest and diseases, Genetics and related factors such as low yielding cultivars and lack of

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private investment in maize breeding programmes for the production of high yielding varieties.

2.6.1 Pest and diseases

The crop is affected by several pest and diseases at various stages of its development. It is attacked by various insect species at different stages in their life cycle; these insects include stem borers such as *Chilo* spp, *Busseola* spp. Other borers attack the ear on the field and at storage *Diatrea* spp. for example and can cause 20-40% loss during cultivation and 30-90% loss during postharvest and storage. Some insects feed on the leaves and can cause high level of destruction to maize plants. *Sitophilus spp* and *Protephanus spp* are the most devastating pests of maize during storage. Another important pest in maize production is weeds which compete with the crop for the limited nutrients and reduce crop yield .The parasitic weed. *Striga* has being a major constraint to increased maize production in West and Central Africa (Badu-Apraku *et al*., 2006). In fact, weed-related yield losses ranging from 65 to 92% have been recorded in the Nigerian savanna.

Remarkable success however was achieved with the development of Striga-resistant varieties that suppress the weed, and other pest-resistant varieties that were released for cultivation in endemic areas of Nigeria and Cameroon (Badu-Apraku *et al*., 2006). Some of the diseases that affect the maize plant are ear rot, stalk rot, rust, downy mildew, leaf blight, smut, tassel smut, tar spot and viral diseases such as corn stunt, corn streak and maize dwarf virus mosaic (GTZ/MOFA, 2006).

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2.6.2 Low soil fertility and input requirement

Maize is a crop that requires large quantities of nutrients especially Nitrogen, Phosphorus and Potassium (Buah *et al*., 2009). In recent years its cultivation has become a problem as large quantities of fertilizers are required before reasonable yields can be obtained.

2.6.3 Low and erratic rainfall distribution

Most of the maize production systems in Africa are rain fed and failure of rain results in severe losses. Especially in the savanna regions, drought has been a major problem facing maize farmers.

2.7 Maize Improvement

Maize was brought to Africa by the Portuguese in the 16th century but actual scientific research on the continent started in the early part of the 20th century around the 1930's when White settler farmers in Eastern and Southern Africa especially in Kenya and Zimbabwe set up research stations in maize producing regions before independence to research into maize production and improvement (Gilbert *et al.*, 1994). The establishment of the International Center for Maize and Wheat Improvement (CIMMYT) in Kenya boosted maize research by being the main source of germplasm to National and Regional Research Institutes like the IITA. In western and central Africa maize research started in the colonial period with the establishment of the International Institute Tropical Agriculture (IITA) in Ibadan, Nigeria with the mandate to produce improved maize varieties that are high yielding, disease resistant and improved nutritional composition (Manyong *et al*., 2000). The IITA has since then supported national maize breeding programmes in West

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and Central Africa by providing improved germplasm and also in human resource development (Manyong *et al*., 2000). The establishment of the West and Central Africa Collaborative Maize Research Network (WECAMAN) which works in collaboration with the IITA and national research systems released a lot of new improved varieties for farmers through national extension services. A total of 379 modern varieties have been released in western and central Africa over the period 1965-2006 with 267 varieties released during 1965-1996 (Alene *et al*., 2009)

Early, intermediate, and late maturing varieties were developed with yields up to twice as much as traditional varieties. Early maturing varieties enabled maize production to expand into new areas, especially to the Sudan savannas where the short rainy season had adversely affected maize cultivation in the past. IITA's postharvest researchers developed effective and simple machines and tools that reduce processing time and labor as well as production losses.

Recently, IITA engaged in research to enhance the nutrient content of maize to combat malnutrition and diseases caused by micro-nutrient deficiency. They are also developing mycotoxin-resistant varieties in collaboration with advanced laboratories to minimize the health hazards of these toxins. In West and Central Africa, IITA has contributed significantly to the capacity building of the national maize research systems.

In Ghana maize research had a boost when the Ghana Grains Development Project (GGDP) was launched in 1979. A collaboration between the Crops Research Institute (Fumesua, Ghana), the government of Ghana and the government of Canada through Canadian International Development Agency (CIDA). The project was mandated to breed improved maize varieties that are high yielding , pest and disease resistant, adapted to local environment and resistant to lodging (Al-Hassan and Jatoe, 2002). The GGDP

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ended in 1997 with the release of 12 varieties including 4 Quality Protein Maize varieties (Twumasi-Afriyie *et al*., 1997).

Recently, maize breeders are interested in breeding varieties that can withstand modern threats to food security including climate change, low soil fertility, pest and diseases. Abiotic stress is as important as biotic stress in limiting maize production in Africa (Freisen *et al*., 2001). Maize production in Africa is seriously affected by biotic and abiotic factors such as drought, pests, disease and other environmental factors such as soil fertility. Moisture stress affect yields on an estimated 40% of the maize sown in lowland tropical environment (Lagoke *et al*., 1991; Edeames *et al*., 1989).

The breeding of drought resistant varieties by CIMMYT and *striga* resistant varieties by the IITA is helping to combat the effect of climate change (Badu-Apraku *et al*., 2005; Freisen *et al*., 2001). Future crop improvement effort should therefore be directed towards the breeding of varieties that are resistant to these deleterious environmental effects with improved nutritional qualities and high yields.

2.8 Characterization

Characterization consists of records on characters or traits that are highly heritable and can be observed by the eye and expressed in all environments. These traits could be phenotypic, genotypic or bio-chemical and therefore characterization can be carried out using morphological or agronomic, molecular and bio-chemical methods. Morphological characterization is based on the physical outward appearance of the organism whilst molecular characterization is based on the DNA polymorphism using molecular markers on genomic DNA templates and bio-chemical characterization is based on the chemical

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composition of the organism. The choice of method depends on the objective of the researcher but these methods tend to complement each other in establishing diversity among plant germplasm.

2.8.1 Morphological Characterization

The use of morphological traits in characterizing plants has been practiced by plant breeders for several years. Morphological characterization has advantages as well as disadvantages as with other methods used in plant diversity studies. The traits usually measured are economically important traits that are well correlated with yield which is important to the farmer. These traits are observed by the farmers and consumers and make it possible for evaluation of the performance of the crop in different environments (Abdulai *et al*., 2007; Sallah *et al*., 2004). The major disadvantages are that the results are influenced by the environment (soil, moisture, solar energy etc.) making the results location specific, therefore the result obtained in one environment will not be observed in another environment and also low polymorphism, low heritability and late expression of traits (Smith and Smith, 1992) .

In morphological characterization descriptors are used. A descriptor is defined as attribute, characteristic or measurable traits that is observed in an accession. A descriptor list shows a set of individual traits that are attributed to a particular species and ways to measure them. It contains information on the germplasm from registration, characterization, evaluation, management and usage of the plant. The descriptor list serves as a standard for data collection on morphological trait and can therefore be used as basis for botanical classification of plant species. A comprehensive descriptor list for

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maize was developed by CIMMYT and IBPGR in 1991 to standardize recording, documentation, data classification, storage and use in research.

2.8.2 Molecular Characterization

Molecular characterization is the use of genotypic traits to characterize germplasm instead of phenotypic traits as in morphological characterization. This method makes use of genetic markers which identify genes at a given locus (Stansfield, 1986). The locus is the location of a gene on a chromosome and at a given locus several forms of the gene known as alleles may be present on homologous chromosomes. Identical allelic forms are homozygous whilst non-identical allelic forms are heterozygous. The genetic markers are therefore related to the DNA at these loci.

The advent of molecular markers has made diversity studies in maize very fast as compared to using morphological traits. Molecular markers have several advantages over conventional methods in diversity studies in view of their high precision in detecting variation with greater potential to explore genetic relationship among populations (Shah *et al*., 2010). Genetic differences between closely related genotypes can be detected (Beyene *et al.*, 2005) and it takes a short period to study large populations as compared to morphological methods.

2.8.3 Types of molecular markers

The discovery of the central dogma of molecular genetics which made scientists aware that genes encode proteins started the use of proteins isozymes and allozymes as markers for diversity studies in populations. There are several merits and demerits of this method; the relatively low abundance, low polymorphism and low automation are some of the

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demerits whilst the merits include the high species transferability, medium reproducibility, medium technical complexity and co-dominant inheritance (Semagn and Njiondjop, 2006). The method is not also constrained by tissue and developmental stage specificity.

DNA marker systems were invented in the 1980s (Park *et al*., 2009) and were found to have several advantages over the morphological and protein based systems; the first was that unlimited number of primers could be generated for any organism; the second is that the results are not influenced by the environment and therefore have high reproducibility and transferability as compared to morphological characterization and the third is that the studies are not constrained by tissue or developmental stage specificity and therefore the time needed for diversity studies has reduced drastically since molecular markers were introduced.

Restriction Fragment Length Polymorphism (RFLP) was the first to be developed employing southern blot based markers. RFLP results in point mutations of restriction enzyme recognition sites. Chromosomal mutations such as deletions, translocations, inversion and insertions can all result in RFLP. This technique employs the molecular hybridization of cDNA and genomic DNA probes with genomic DNA fragments using restriction enzymes. The developmental cost and technical complexity of the RFLP is high and high quality DNA is required. DNA sequence information is not required and it has high reproducibility (Semagn and Njiondjop, 2006).

Another, southern blot based marker system employs the use of minisatellites in fingerprinting in humans. Minisatellites are short stretches of DNA that are found in tandem repeats in the eukaryotic genome. They are highly abundant and can be detected as variable number of tandem repeats by PCR amplification (Jeffreys *et al*., 1988).

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The next generation of markers which were Polymerase Chain Reaction (PCR) based revolutionized plant genetic studies by having two important advantages over the southern blot system. First they require only a small amount of DNA at early stages of the plants development and are less expensive and easy to apply allowing the characterization of large populations. RAPD (Randomly Amplified Polymorphic DNA) AFLP (Amplified Fragment Length Polymorphism) and SSR (Simple Sequence Repeats) are the most commonly used PCR based markers (Vos *et al.*, 1995; Welshand McClelland, 1990; Mullis *et al.*, 1986).

RAPD markers are the most efficient and simplest technique of all molecular marker systems. A single arbitrary primer of 10-12 nucleotides long is used in the PCR reaction and therefore prior information on the nucleotide sequence is not a requirement. Genomic DNA is digested with two different restriction enzymes ligated with specific adopted sequences. The adopter ligated restriction fragments are then amplified with adopter complementary primers that have selective nucleotides at the 3' end. The amplified fragments are separated by running a gel electrophoresis in a polyacrylamide gel and then stained with silver. The method has high species transferability and medium reproducibility ((Semagn and Njiondjop, 2006; Jones *et al.*, 1997; Lynch and Milligam, 1994).

The next generation of molecular markers systems are the SNPs (single nucleotide polymorphism) and micro arrays which can be carried out on non- gel based assays. The polymorphism of single base difference can be assessed by thorough-put analysis and hybridization with allele specific oligonucleotide, primer extension, oligonucleotide ligation assay and invasive cleavage (Semagn and Njiondjop, 2006; Sobrino *et al.*, 2005 and Saybanen, 1999).

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2.8.4 SSR Markers

Simple sequence repeats or Microsatellites are polymorphic loci present in the genome of organisms and consist of repeating sequences of 1 to 6 base pairs. Weber and May (1989) demonstrated that SSR polymorphism could be detected by PCR using two flanking primers (forward and reverse) resulting in the development of SSR markers in mammals. Gutpa *et al.* (1996) published that diverse plant genomes have several simple sequence repeats.

PCR based microsatellites have been used extensively by maize breeders for fingerprinting maize germplasm (Beyene *et al*., 2005; Warburton *et al*., 2001; Senior *et al*., 1998; Smith and Smith, 1992) because of several advantages it has over other methods. Beyene *et al.* (2005) did a comparative study on molecular and morphological methods of plant diversity studies on Ethiopian highland maize using SSRs and AFLPs for the molecular studies and suggested that SSRs may be a better choice for marker traits association studies of open pollinated maize varieties.

All methods of plant diversity studies have advantages and disadvantage but SSRs have several advantages, these include the high level of polymorphism; SSRs can detect high allelic variation even in closely related varieties due to its hyper variable nature. According to Powel *et al*. (1996) the number of alleles varied from 1 to 37 and diversity indices ranges between 0.29-0.95 in major crop species. Powell *et al.* (1996) examined the utility of RFLP, RAPD, AFLP and SSR markers on soya bean germplasm and observed that SSRs have the highest expected heterozygosity while AFLPs have the highest effective multiplex ratio.

Another advantage of SSR markers is the high reproducibility, that is; it will produce the same profiles regardless of the state and amount of the DNA template and therefore very

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useful in dealing with specimens that are contaminated, dry, mummified and fossils (Boder *et al*., 2006). RFLP also have high reproducibility but requires restriction enzymes which produces spurious bands when impure DNA templates are used.

Research has revealed that SSRs are abundant and well distributed in the genome of both prokaryotic and eukaryotic organisms; this makes it a very important in the area of gene mapping and DNA sequencing apart from DNA fingerprinting.

2.9 Maize Kernel

There are differences in the chemical composition of the main parts of the maize kernel. The seed-coat or pericarp is characterized by a high crude fiber content of about 87 percent, which is constituted mainly of hemicellulose, cellulose and lignin. The endosperm contains a high level of starch about 87.6 percent and protein levels of about 8 percent. Crude fat content in the endosperm is relatively low. Finally, the germ is characterized by a high crude fat content, averaging about 33 percent (Table 2.1). The germ also contains a relatively high level of protein and minerals. The aleurone is relatively high in protein content as well as in crude fiber. The endosperm contributes the largest amount, followed by the germ, with only small amounts from the seed-coat. About 92 percent of the protein in teosinte comes from the endosperm. Protein in the maize kernel has been reported by a number of researchers.

It is evident that the carbohydrate and protein contents of maize kernels depend to a very large extent on the endosperm crude fiber and to a lesser extent protein and minerals on the germ. Crude fiber in the kernel comes mainly from the seed-coat. The weight distribution among parts of the maize kernel and their particular chemical composition

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and nutritive value are of great importance when maize is processed for consumption. Germ oil provides relatively high levels of fatty acids. Considering the whole kernel, the essential amino acid content is a reflection of the amino acid content in the protein of the endosperm, in spite of the fact that the amino acid pattern in the germ protein is higher and better balanced. Germ proteins nevertheless contribute a relatively high amount of certain amino acids, although not enough to provide a higher quality of protein in the whole kernel. The germ provides some lysine and tryptophan, the two limiting essential amino acids in maize protein. Endosperm proteins are low in lysine and tryptophan, as is the whole grain protein. The deficiencies in lysine tryptophan and isoleucine have been well demonstrated by numerous animal studies (Burgoon *et al*., 1992) and studies on humans (Akuamoah-Boateng, 2002).

Table 2.1: Proximate Chemical composition of main parts of maize kernels (%)

Chemical component	**Pericarp**	**Endosperm**	**Germ**
Protein	3.7	8.0	18.4
Ether extract	1.0	0.8	33.2
Crude fiber	86.7	2.7	8.8
Ash	0.8	0.3	10.5
Starch	7.3	87.6	8.3
Sugar	0.34	0.62	10.8

Source: Watson, 1987

2.11 Quality Protein Maize

Maize is an important source of protein accounting for about 60% of the daily human

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protein supply (Mbuya *et al.*, 2012). In Ghana maize accounts for 23% of the national protein needs (Twumas-Afriyie *et al.*, 1997). The normal maize cultivars lack two essential amino acids lysine and tryptophan. The discoveries of a mutant maize plant with high lysine and tryptophan content and the subsequent discovery of the *opaque-2* gene boosted research into the production of maize with high lysine and tryptophan content. The gene has been found to double the lysine and tryptophan content of the grain (Krivanek *et al.*, 2007). Several natural mutants were also found to confer high lysine and tryptophan content in the grain. These are the *floury-2 (fl2), floury-3(fl3), opaque -7 (07) and opaque-6 (06)* but only the *opaque-2* was found to be suitable for maize breeding programmes. Initially, the grains developed were dull, chalky, 10 % to 15 % less weight than normal maize and less resistant to pest and diseases (Vivek *et al.*, 2008)

Scientists at CIMMYT were able to remove all these unwanted traits to develop the Quality Protein Maize (QPM) which has 70 to 100% more lysine and tryptophan than normal maize cultivars (Buah *et al.*, 2009).

Since the development of QPM by CIMMYT many national research institutions have developed QPM varieties, these include Ghana, South Africa, India and China. Twenty African countries have adopted and inculcated QPM breeding into their national programmes (Mbuya *et al.*, 2012). QPM has been found to reduce protein deficiencies in humans (Akuamoah-Boateng, 2002) and other monogastrics (Burgoon *et al.*, 1992). The Crops Research Institute of Ghana has produced four Quality Protein Maize varieties; “Obatampa”, “Mamaba”, “Dadaba” and “CIDAba” (Twumasi-Afriyie *et al.*, 1997).

“Obatampa” is the most popular and has been adopted by most Ghanaian farmers; “Mamaba” is the second variety that farmers have adopted but “Dadaba” and “CIDAba” are not so popular with the farmers in the country.

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2.11 β-Carotene

Cereals, vegetables and fruits contain several phenolic compounds including carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin), flavonoids (anthocyanin) and other phenolic compounds. These compounds usually impose bright colours on leaves, fruits, grains and other plant parts. β-carotene usually confers yellow colour whilst anthocyanin usually confers red and purple colours on plant parts (Ford, 2000).

Phenolic compounds have been found to have high free radical scavenging activity thereby reducing the deleterious effect of free radicals on the human body. During the past few decades research findings have shown that polyphenols have effects on the human body and that they are anti-carcinogenic, anti-mutagenic, anti-inflammatory, antioxidant, anti-viral, anti-microbial, anti-proliferative, anti-allergic and these enables the prevention of certain chronic diseases including cardiovascular disorders (Ghosh and Konishi, 2007).

Carotenoids have been found to be of great importance to human health. β-carotene, α-carotene and α-cryptoxanthin are pro-vitamin A. The chemical structure of the vitamin A (retinol) molecule is essentially one half of the β-carotene molecule which is the most potent pro-vitamin A and also the most widespread (Rodriguez-Amaya and Kimaru, 1998). Carotenoids have been found to enhance the immune system and prevent regenerative diseases (Olson, 1999) and prevent irreversible blindness and cataract in the elderly (Moeller *et al.*, 2000). In the maize kernel, carotenoids are formed in the endosperm whilst flavonoids are formed in the aleurone layer. The colour of the aleurone layer usually dominates and covers that of the endosperm and therefore yellow and white

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maize have colourless aleurone layers and the colour of the endosperm can be seen. Red and purple coloured maize have the red or purple pigment accumulated in vacuoles of aleurone cells and therefore the colour of the endosperm cannot be seen. The β-carotene content of cereals and other crops are affected by several environmental, mechanical and physiological factors such as soil type, light and temperature, stage of development, processing and storage.

Zeaxanthin and lutein are the major carotenoids with β-carotene and β-cryptoxanthin being present in much smaller amounts (Moros *et al.*, 2002). In order to determine the β-carotene content without the results being masked by zeaxanthin and lutein, β-carotene is separated from the other carotenoids before spectrophotometric measurements.

2.11.1 β-carotene extraction

β-carotene is extracted form powdered grain samples by grinding the sample with a pestle and mortar using cold acetone. The extract is then partitioned using petroleum spirit in a separating funnel. Acetone is washed away using distilled water by draining the aqueous layer. Anhydrous sodium chloride is used to dry the petroleum spirit and the extract is stored at 5 ^{o}C for HPLC phase.

2.11.2 HPLC equipment

The HPLC equipment is made up of 5 major components, these are; (1) the detector (2) the pump, (3) the recorder, (4) the reverse phase column and (5) the mobile phase. The main principle is that peaks are identified by their retention time or elution time with the help of a standard. If the peak of the standard was observed at 1 min then the peak of the

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sample must be ±0.2 of the standard with the assumption that the peak is standardized. The concentration of the β-carotene content in the sample can then be calculated knowing the area of the standard and its corresponding concentration.

2.12 CIMMYT, IBPGR Descriptor List for Maize Characterization.

The maize plant has been vividly described in the section on botanical description of the plant. The CIMMYT, IPBGR descriptor identifies measurable traits on vegetative, ear and kernel. The vegetative traits include; Days 50% tasselling, Days to 50% silking, Days to 50% ear leaf senescence, plant height, ear height, foliage, number of leaves above the uppermost ear, tillering Index, stem colour, sheath pubescence and tassel type.

2.12.1 Days to 50% tasselling

This refers to the number of days from the day of sowing to the day when fifty percent of the plants shed pollen. The tassel which is the male flower is borne on the apex of the stem with spikelet's bearing pollen grains. Pollen matures in two to three days after the tassel have been observed. This trait is very important in crossbreeding programmes.

2.12.2 Days to 50% silking

This is recorded when the silk emerges on fifty percent of the plants and it is counted from the day of sowing to the day when 50% of the plants produce silk. The silk emerges from the female flower which later develops into the ear. This trait is also important in crossbreeding.

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2.12.3 Days to 50% ear leaf senescence

This describes the number of days from sowing to the day when 50% of the plants have dry ear leaf. This indicates physiological maturity of the plant.

2.12.4 Plant height

This trait is measured in centimeters from the ground to the base of the tassel after milking stage. The trait is usually affected by environmental factors such as soil fertility and moisture.

2.12.5 Ear height

Ear height is measured in centimeters from the ground to the node bearing the uppermost ear. Since the force required to break a cereal plant varies with height (Moshie, 1973) the location of the ear on the stem is very important to prevent lodging which is a major problem in maize production (Twumasi-Afriyie *et al.*, 1981)

2.12.5 Folliage

The total leaf surface rated as small, intermediate or large at milking stage. This is based on measurements on leaf length and leaf girth.

2.12.5 Number of leaves above the uppermost ear including the ear leaf

The number of leaves above the uppermost ear including the ear leaf is counted and recorded after milking stage. Data is taken from at least 20 representative plants.

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2.12.6 Leaf length

This trait is measured in centimeters from the ligule to the apex of the leaf preferably on the mid rib. Measurement is taken on the leaf which subtends the uppermost ear and at least 20 representative plants are measured.

2.12.7 Leaf width

This is also measured in centimeters mid-way along the length of the leaf that subtends the uppermost ear. Measurements are taken on at least 20 representative plants.

2.12.8 Tillering index

The number of tillers per plant is counted on an average of more than 20 representative plants after flowering. Most of the maize varieties in Ghana do not produce tillers.

2.12.9 Stem colour

The stem colour is observed between the two topmost ears at flowering. Five classes of stem colour are recognized and these are (1) green, (2) sun red, (3) red, (4) purple and (5) brown. Up to three stem colours are indicated in order of frequency.

2.12.10 Sheath pubescence

This is observed at flowering and refers to the dispersal and density of pubescence on the leaf sheath. They are classified as (1) sparse, (2) intermediate or (3) dense. Thorough observation of several plants is carried out to arrive at the class.

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2.12.11 Tassel type

This is measured at the milking stage and classified as (1) primary, (2) primary - secondary or (3) primary-secondary-tertiary based on branches on the tassel. The initial branch is classified as primary and any branch on the initial branch is the secondary and a branch on the secondary is classified as tertiary ramification. This is done by careful observation of tassels from several plants. Other descriptors for ear traits include husk cover, kernel row arrangement, and number of kernels per row.

2.12.12 Husk cover

This is a very important trait since a good husk cover can reduce ear damage due to insects, birds and aflatoxin. The trait is observed after harvest, using all ears on at least 20 representative plants per accession the husks are rated as (1) poor, (2) intermediate or (3) good.

2.12.13 Kernel row arrangement

The trait is observed on the uppermost ear and is classified as (1) regular, (2) irregular, (3) straight, or (4) spiral based on the arrangement of the kernels on the cob.

2.12.14 Number of kernel rows

The number of kernel rows are counted in the center of the uppermost ear and recorded. This is observed on at least 20 representative plants per accession.

2.12.15 Ear length

This is observed when applicable on at least 20 representative plants per accession. The

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measurement is taken in centimeters from the base of the ear to the tip of the ear. This is a very important trait when yield is being considered.

2.12.16 Ear diameter

This is measured in centimeters at the central part of the uppermost ear preferably using Venier calipers on at least 20 representative plants per accession.

2.12.17 Shape of the uppermost ear

The shape of the uppermost ear is classified into (1) cylindrical (2) cylindrical-conical (3) conical or (4) round. This is done by careful observation of 20 representative ears.

The descriptor for kernel traits includes the following; kernel type, kernel colour, 1000 kernel weight, shape of the uppermost surface of the kernel and Aleurone colour.

2.12.18 Kernel type

This is recorded after harvest and up to three colours are indicated in order of frequency.

The kernel types are classified into eleven types which are; (1) floury, (2) semi-floury, (3) dent, (4) semi-dent, (5) semi-flint, (6) flint, (7) pop, (8) sweet, (9) opaque/QPM, (10) tunicate and (11) waxy.

2.12.19 Kernel colour

Up to three colours are indicated based on frequency that is if the kernels are not uniformly coloured. The kernel colour is classified into one of these nine classes;(1) white (2) yellow, (3) purple, (4) variegated, (5) brown, (6) orange, (7) mottled, (8) white cap and (9) red. White kernel maize is the most consumed in sub Saharan Africa (Smale *et*

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al., 2011) followed by yellow maize. The other kernel colours are produced on very small scale.

2.12.20 Shape of the uppermost surface of the kernel

The shape of the uppermost ear is classified into (1) shrunken, (2) indented, (3) level, (4) rounded, (5) pointed and (6) strongly pointed.

2.12.21: Aleurone colour

The Aleurone is the single cell layer between the pericarp and the endosperm. The presence of colour in the Aleurone usually masks the colour of the endosperm. The colour of the Aleurone is classified as (1) colourless, (2) bronze, (3) red and (4) purple.

2.13: DNA extraction

DNA extraction is carried out using specially designed protocols. These protocols include those designed Doyle and Doyle (1990) and Dellaporta *et al*. (1983). All the protocols use different methods to extract DNA but most of the protocols are based on the CTAB (cetyltrimethyl ammonium bromide=hexadecyltrimethylammonium bromide) and therefore uses certain reagents in common. Protocols can be used with slight modifications based on experimental objectives and nature and type of sample. DNA extraction kits are available for use in extraction and these make the process very easy and rapid without the use of hazardous reagents such as chloroform and phenols. The DNA binds onto a silica gel based membrane and cleansed from all contaminants before washing with distilled water to obtain pure uncontaminated DNA.

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2.14 PCR Cocktail and Master Mix

The PCR cocktail contains distilled water, $MgCl_2$, 10x PCR buffer, 0.4 µl Tag polymerase, dNTPs (dCTPs, dATPs, dGTPs, dTTPs), template DNA, reverse and forward primers. Master Mix is available in the market and this saves time and could be used for different primers. The DNA temperate and primers are added to the PCR master mix.

2.15 DNA amplification

Amplification refers to the production of many copies of a section of the DNA naturally or by artificial means. This is achieved through Polymerase Chain Reaction (PCR) which uses thermo cycler machine. The PCR process starts by denaturing the DNA template at a high temperature which results in the production of single strands. Primers are then annealed to each strand and DNA polymerase recognizes the double stranded DNA and starts to add complementary dNTPs in the 5'to 3' direction extending the primer until a full complementary strand is synthesized. This is achieved at a lower temperature of 72 ^{O}C. At one PCR cycle two molecules of DNA strands are produce form one template DNA. Several cycles could be run depending on the type of research and the quantity of DNA required. This process does not affect the quality of the DNA.

2.16 Gel electrophoresis

The PCR products or amplicons are loaded into wells created in a gel and allowed to run under a certain voltage and time in an electrophoretic tank. Two types of gel are usually used; agarose gel and polyacrylamide gel. Agarose gels are easy to prepare and can give good and accurate result.

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CHAPTER 3

MORPHOLOGICAL CHARACTERIZATION OF TWENTY YELLOW-, RED- AND PURPLE-KERNEL MAIZE *(ZEA MAYS L.)* ACCESSIONS IN GHANA

3.1 Introduction

Maize (*Zea mays* L.) belongs to the Family; Poaceae and it is the most important cereal in Ghana in terms of production, consumption and area under cultivation (Buah *et al*., 2009). The plant is one of the most diverse plant species in the world (Shah *et al*., 2010) and can be cultivated in temperate and tropical regions. Worldwide production in the year 2012 was 8,834,602 tons (FAOSTATS, 2013).

Ghana produced 1,949,897 tons in 2012 (FAOSTATS, 2013). The crop is a major staple for people in many parts of the developing world including sub Saharan Africa, where it is a major staple for an estimated 50% of the population (IITA, 2009). Maize is also important as feed for farm animals and starch for the pharmaceutical industry. Despite its importance, yield of the crop in Ghana has been low amounting to about 1.2 ton/ ha as compared to the world average of 5.5 tons/ha (NARP, 1993).

In the past, efforts have been made by research scientists to develop varieties that are high yielding with high nutritional quality and resistance to pest and diseases. These led to the development of new varieties in most African countries. These varieties were developed from germplasm collected from the CIMMYT and the IITA.

The Ghana Grains Development Project (GGDP) was launched in 1979 as collaboration between the Crops Research Institute of Ghana, the Government of Ghana and the Government of Canada through CIDA. The project was mandated to breed improved maize varieties that are high yielding, pest and disease resistant, adapted to the local

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environment and resistant to lodging (Al-Hassan and Jatoe, 2002). The GGDP ended in 1997 with the release of 12 varieties including 4 quality protein maize varieties "Mamaba", "Dadaba", "CIDAba" and "Obatampa" (Twumasi- Afriyie *et al*., 1997)

Over reliance on white-kernel improved varieties have left several maize landraces in Ghana uncharacterized and at risk of genetic erosion. These under-exploited landraces include yellow-, red- and purple-kernel maize cultivars which are useful as food for humans, food for infants, feed for farm animals, sources of vitamin A and proteins. These landraces are also sources of germplasm for future breeding works such as breeding for resistance to biotic and abiotic stress factors and bio-fortification.

Characterization is fundamental to the development, release and popularization of plant varieties (Chavan, 2010). There is the need to characterize these landraces so as to establish their identity and diversity for future improvement programmes and conservation. Morphological characterization has advantages as well as disadvantages as with other methods used in plant diversity studies. The traits usually measured are economically important traits and are traits that are well correlated with yield which is of importance to the farmer. The major disadvantages are that the results are influenced by the environment (soil, moisture, solar energy etc.) making the results location specific. As such, results obtained in one environment will not be observed in another environment and also exhibit low polymorphism, low heritability and late expression of traits (Smith and Smith, 1992). The major advantages are that the traits are observed by the farmers and consumers and make it possible for evaluation of the performance of the crop in different environments (Abdulai *et al*., 2007; Sallah *et al*., 2004).

The morphological traits measured are qualitative traits such as stem colour, kennel colour, kernel row arrangement and quantitative traits such as plant height at maturity, yield

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at harvest and seed weight. Qualitative traits are highly heritable and least affected by environmental factors and therefore good parameters for diversity studies and classification. Quantitative traits on the other hand are affected by environmental factors, but exceedingly important in plant breeding and agronomy as most of parameters measured during characterization is yield related and yield is a very important to the farmer.

The objective of this experiment was to;

Evaluate and characterize the yellow-, red- and purple-kernel maize accessions in Ghana using morphological traits.

3.2 Materials and Methods

3.2.1 Experimental site

The experiment was conducted at the experimental farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) which is located at 05º 40’ N, 0º 13’ W and an elevation of 76 meters above sea level. The area falls within the Coastal Savanna Agro-ecological Zone. The soil type is the Haatso series which is a well-drained Savanna Ochrosol derived from Quartzite schist (FAO/UNESCO, 1994). Annual rainfall figures in this area range between 700 and 1000 mm.

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3.2.2 Weather Conditions

The weather conditions observed at the experimental site are indicated in the table below

Table 3.1 Prevailing Weather Conditions in the Experimental Site (June-September 2012)

Climatic condition	Minimum	Maximum
Relative humidity (%)	59.73	91.03
Wind speed (m/s)	3.16	4.270
Temperature (°C)	24.59	31.50
Solar radiation (wh/ms)		5156.326
Precipitation (mm)/month		77.28

3.2.3 Collection of Germplasm

Twenty maize accessions were collected from three regions of Ghana for the experiment as indicated in Table 3.2 below.

Table 3.2: Collection Sites and Sources of the 20 maize (*Zea mays* L.) Accessions used for studies.

Accession	Region	Source
GH3351, GH4863, GH3975, GH3981, GH40557, GH2359, GH2351.	Eastern Region	Plant Genetic Resource Research Institute in Bunso
NYR1, NYR2, NYR3, NYR4, NYRW1, NPR1, NYR5, NPR2, NYRW2, NPR3.	Northern Region	Farmers in Yendi
Dorke, DYF,	Northern region;	Savanna Agricultural Research Institute
NYY1	Greater Accra Region.	Farmers at Korle Bu

3. 2.4 Experimental plot and design

The field was ploughed and demarcated into four main blocks (replicates) with 80

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subplots of 4.8 m × 2.4 m in a randomized complete block design. The replicates were separated by 2.0 m paths while subplots were separated by 1.0 m paths. The planting distance used was 0.8 m × 0.4 m giving 46 plants per plot including border plants.

3.2.5 Agronomic Practices

The accessions were planted on 1st June 2012; all replications were planted on the same day. First and second weeding were done manually on the 3rd and 6th week respectively after planting with hoe as the field was heavily infested with *Cyperus rotundus*. NPK (15-15- 15) fertilizer was applied on the 8th of July 2012 at a rate of 7 g per hill based on the recommendation of the Ministry of Food and Agriculture (GTZ/MOFA, 2006).

3.2.6 Data Collection

Data were collected on the following vegetative and yield parameters based on a Descriptor list developed by the International Board for Plant Genetic Resources and CIMMYT;

(i) Planting date,
(ii) Number of plants per plot,
(iii) Days to silking (50% of the plants per plot),
(iv) Days to Anthesis (50% of the plants per plot),
(v) Tassel type (primary or secondary or tertiary branches),
(vi) Pubescence on culm (presence or absence),
(vii) Plant color (stalk) (green, purple, sun red, red),

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(viii) Plant height (cm),

(ix) Ear height (cm),

(x) Number of leaves above the uppermost ear,

(xi) Leaf length,

(xii) Leaf girth,

(xiii) Plant senescence (days from planting to (50%) ear leaf senescence).

Kernel data descriptor list include the following data recorded after harvest

(i) Kernel row arrangement (straight, regular, irregular, spiral)
(ii) Kernel colour (white, yellow, purple, orange, variegated, brown, red, sun red, mottled),
(iii) Number of kernels per row, was counted from the base of the cob to the tip,
(iv) Number of rows per cob,
(v) Kernel type [floury, morocho, dent, flint, pop, sugary, waxy, white cap, QPM (Opaque -2),
(vi) Aleurone colour (colourless, bronze, red purple)

Data were also collected on the following parameters associated with yield:

(i) Husk cover was rated as good, intermediate, poor,
(ii) Harvest date,
(iii) Grain yield/plot (g),
(iv) Ear length (cm),
(v) Ear diameter (cm) was measured using a Vennier caliper
(vi) Seed weight and 1000 seed weight was weighed at 13.5 % moisture content

3.2.7 Data Analysis

Data was recorded using Microsoft excel and statistical analysis was done using Genstats 12 edition, Microsoft Excel and statsgraphics centurion XVI. Statistical analysis and analysis of variance were carried out on the data and means were separated by least significant difference and error bars with standard error. Correlation analysis was done to

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determine the degree of association between the traits and principal component analysis was done to determine the percentage contribution of traits to the total genetic variation. Qualitative traits were scored based on the descriptor developed by CIMMYT and IBPGR. Cluster analysis was performed on the data based on similarity matrix.

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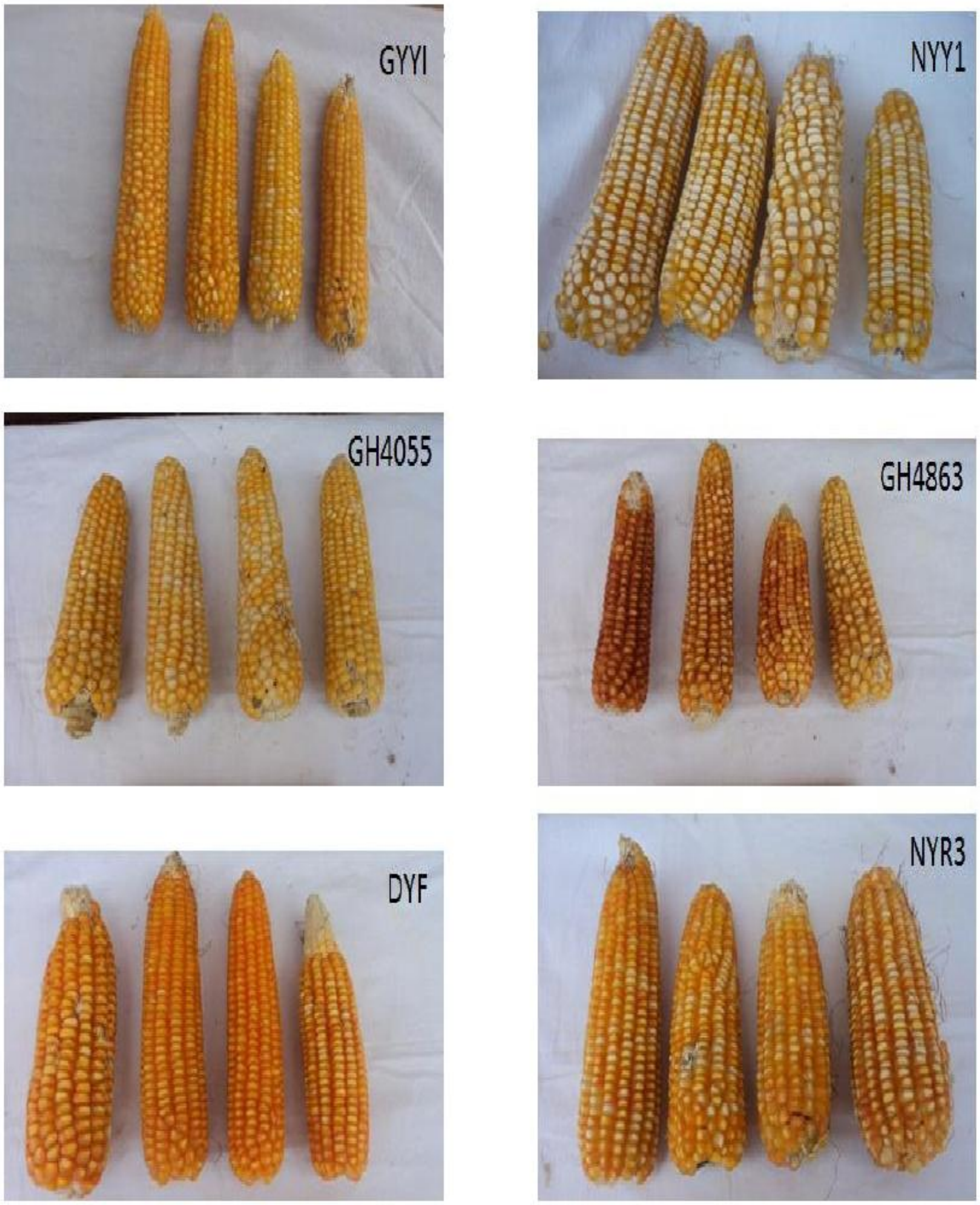
RESULTS

The plates below show the variation in ear traits among the 20 maize accessions after harvest.

Plate 3.1: Variation in ear traits among the 20 maize accessions.



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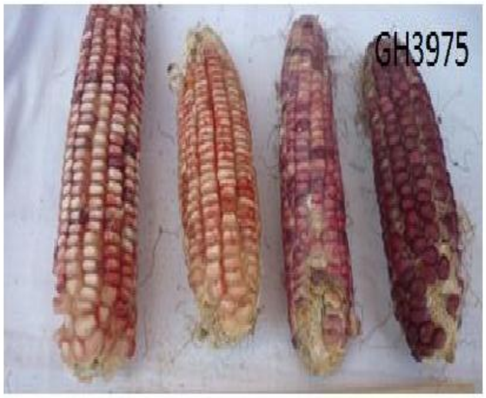

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3.3.1 Days to 50% anthesis (DTA), Days to 50% silking (DTS), Days to 50% leaf senescence (DLS), Days to Harvesting (DTH) Plant height classification (PHC) and Maturity period (MT).

Table 3.3 shows the Days to 50% anthesis (DTA), Days to 50% silking (DTS), Days to 50% leaf senescence (DLS), Days to harvesting (DTH), Plant height classification (PHC) and Maturity period (MT) of the 20 maize accessions. GH4055 was the earliest to mature with DTA, DTS and DLS of 38 day, 43 days and 66 days respectively. The accession was harvested at 76 days. GH4863 was also early maturing with DTA, DTS, DLS, and DH of 41days, 46 days, 70 days and 90 days respectively.

DYF and “Dorke” are medium maturing with DTA, DTS, DLS, and DH of 51 days, 54 days, 84 days and 109 respectively but “Dorke” had a DLS of 83 days. NYR5, NPR2, NYRW2 and GH2359 are late maturing with DTA of 54, 59, 59 and 74 days respectively. DLS was 91, 91, and 91 and 98 days respectively. All the 4 accessions were harvested on the 116th days after sowing. Accessions NYR1, NYR3, NYR2 and GYYI were tall whilst accession NYRW1, GH3981, GH4055, GH3351 and GH4863 were short. Eleven other

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accessions were found to be medium in height. The mean Anthesis Silking Interval (ASI) was 5.3 days.

Table 3.3: Days to 50% anthesis, Days to 50% silking, Days to 50% leaf senescence, Days to harvesting, Plant height classification and Maturity Classification of the 20 maize accessions.

Accession	DTA	DTS	ASI	DLS	DTH	PH C	MT C
Dorke	51	54	3	83	109	Medium	Medium
DYF	51	54	3	84	109	Medium	Medium
NYR1	59	64	5	86	109	Tall	Medium
NYR2	59	64	5	99	116	Medium	Late
NYR3	52	59	7	86	105	Tall	Medium
NYR4	54	61	7	86	109	Medium	Medium
NYRW1	65	69	4	91	116	Short	Late
NPR1	48	52	4	86	109	Medium	Medium
NYR5	54	59	5	91	116	Medium	Late
NPR2	59	66	7	91	116	Tall	Late
NYRW2	59	66	7	91	116	Short	Late
NYY1	45	51	6	81	109	Medium	Medium
NPR3	59	66	7	91	116	Tall	late
GH3981	65	69	4	94	116	Short	Late
GH4055	38	43	5	66	76	Short	Early
GH3975	59	63	4	91	116	Medium	Late
GH3351	62	66	4	91	116	Short	Late
GH4863	41	46	5	70	90	Short	Early
GH2359	74	79	5	98	116	Medium	Late
GYY1	42	51	9	81	109	Tall	Medium
Mean	**55**	**60.1**	**5.3**	**86.85**	**109.7**		

Table 3.3: DTA: Days to 50% anthesis, DTS: Days to 50% silking, ASI: Anthesis Silking Interval, DLS: Days to 50% leaf senescence, DTH: Days to harvesting, PHC: Plant height classification, MTC: Maturity Classification.

3.3.2 Variability in quantitative traits among the population

Table 3.4 shows the mean, standard deviation, the minimum, maximum, range, variance and coefficient of variation for eleven agronomic traits. The mean plant height

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(PH) was 173.034 cm, the minimum was 105.975 cm and the maximum of 208.687 cm and the mean ear height (EH) was 103.641 cm with a minimum value of 49.8 cm and a maximum of 150.5 cm, the coefficient of variation was 13.0634 % and 20.8101 % respectively. The mean cob weight (CW) and 1000 seed weight (1000 SW) were 88.8404 g and 219.9 g with coefficient of variation of 42.7564 % and 26.76 % respectively. The mean ear girth (EG), ear length (EL), number of kernels per row (NK) and number of rows per cob (NR) were 3.037 cm, 13.877 cm, 30.054 and 13.459 respectively.

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Table 3.4: Variation in quantitative traits among population

Measurements	*EH*	*CW*	*EL*	*NK*	*EG*	*NL*	*NR*	*PH*	*LL*	*LW*	*1000* SW
Mean	103.641	88.840 4	13.877	30.054	3.036	6.742	13.459	173.034	103.9	9.83	219.9
Standard deviation	21.569	37.984	2.152	6.186	0.348	0.441	0.749	22.604	86.9	6.8	58.84
Minimum	49.8	19.635	9.2	19.142	2.355	5.75	11.0	105.975	110.5	11.7	137.4
Maximum	150.50	169.71	20.00	46.00	3.68	7.66	14.92	208.69	23.60	4.90	321.50
Range	100.70	150.07	10.80	26.86	1.33	1.91	3.92	102.71	5.32	1.04	184.10
Variance %	510.9	465.2	0.121	4.631	38.37	0.195	0.562	1443	28.29	1.091	3462
Coefficient of variation	20.810	42.756	15.5076	20.582	11.463	6.542	5.570	13.063	5.121	10.620	26.76

EH: ear height, CW: Cob weight, EL: ear length, NK: Number of kernels per row, EG: Ear girth, NL: Number of leaves above the uppermost ear, NR: Number of rows per cob, PH: Plant height, LL: leaf length, LW: leaf width, 1000 SW: 1000 seed weight.

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3.3.3 Variability in quantitative traits between the accessions

Fig. 3.1 shows the distribution of seed weight per plot, 1000 seed weight and mean cob weight, of the 20 maize accessions. NPR3 had the highest 1000 seed weight of 321.54 g followed by NPR1 with 299.18 g. NPR1 had the highest seed weight per plot of 1.112 kg and heaviest cob which weighed 143 g. GH2359 recorded the lowest 1000 seed weight of 137.41 g and the lowest Cob weight was recorded by GH 3975 which weighed averagely 36.82 g; GH 3975 had the lowest seed weight per plot of 172 g. There were significant differences among the accessions for all traits as indicated by the error bars.

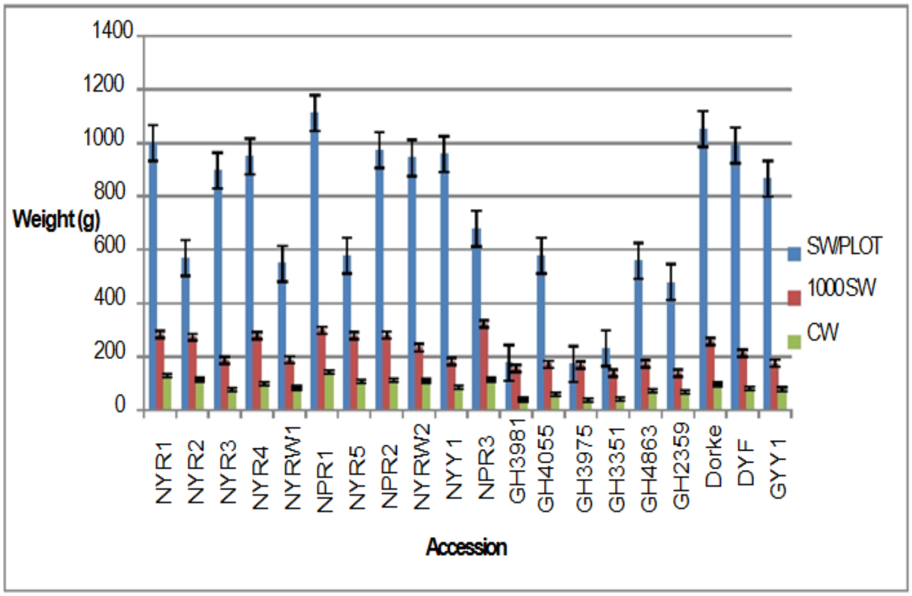

Fig. 3.1: Distribution of mean cob weight, 1000 seed weight and seed weight/plot of the 20 maize accessions. Errors bars that intersect are not significantly different. SW/PLOT: seed weight per plot, 1000 SW: 1000 seed weight, CW: mean cob weight.

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Fig. 3.2 shows the distribution of the Mean plant height and Mean ear height of the 20 accessions. NPR2 was the tallest with plant height of 190.6 cm; the shortest plant GH3981 recorded 117.6 cm. The highest ear height was 127.1 recorded by GH2359 and the shortest was 56.42 recorded by GH4055 error bars indicates significant differences among the accession.

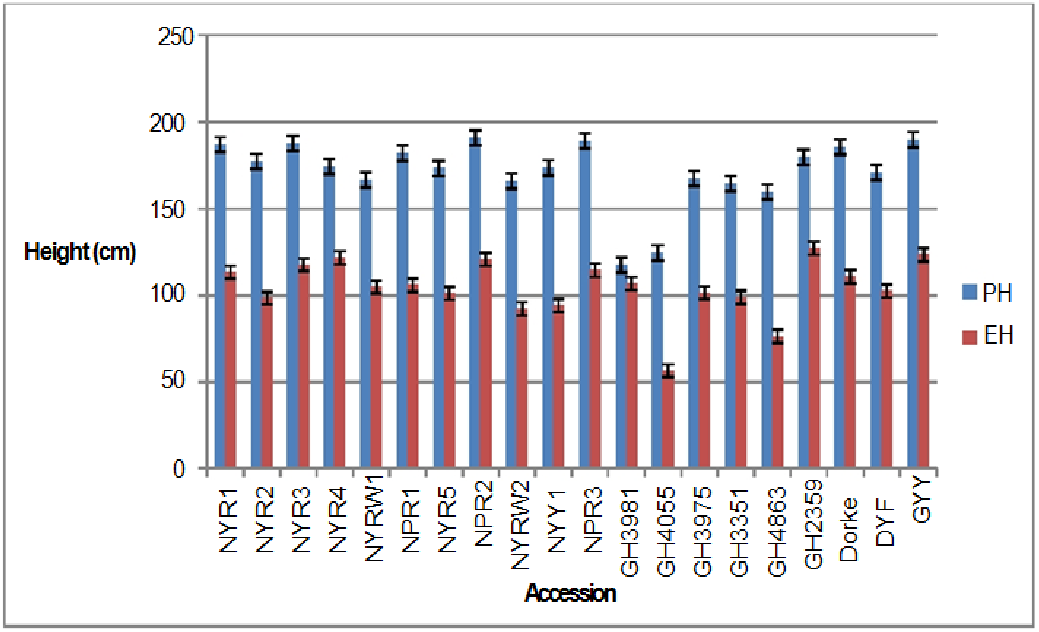

Fig. 3.2: Distribution of the mean plant height and mean ear height among 20 accessions of maize. Errors bars that intersect are not significantly different. PH: mean plant height, EH: mean ear height

Fig. 3.3 shows the distribution of the mean ear length and mean ear girth among the 20 accessions of maize. NPR3 had the longest ear length with an average of 16.92 cm while the shortest ear was 10.18 cm recorded by GH3351 cm. The widest ear girth of 3.386 cm was recorded by NPR1 while GH3975 recorded the shortest width of 2.411 cm. Significant difference were found to exist among the accessions with respect to the two traits.

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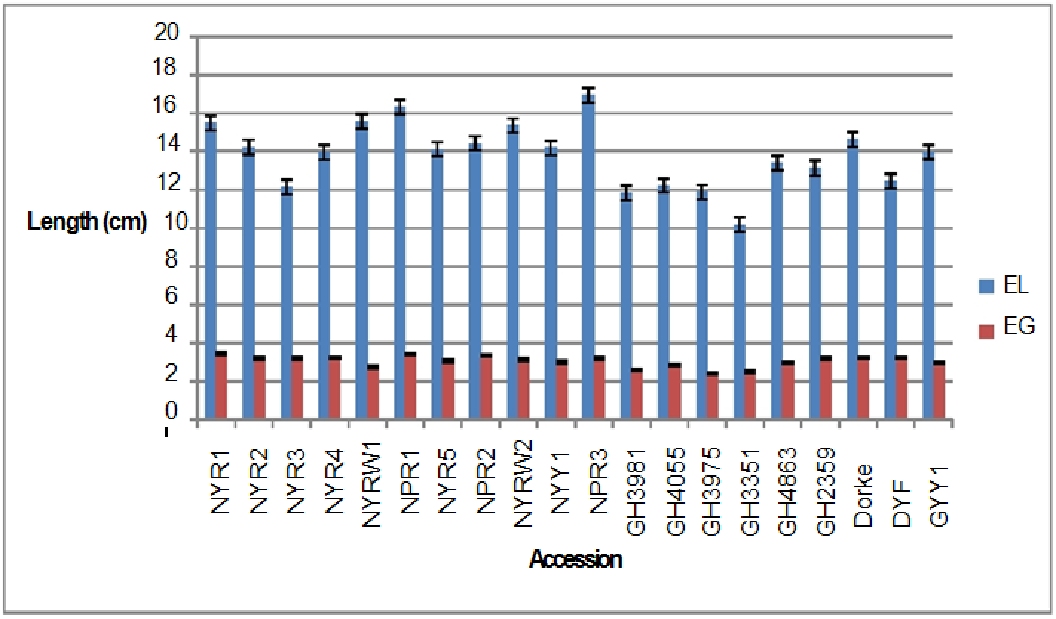

Fig. 3.3: Distribution of the mean ear length and Mean ear girth of the 20 accessions of maize. EL: mean ear length, EG: mean ear girth

Fig 3.4 shows the distribution of the mean leaf length and leaf width for the 20 maize accessions. NPR3 had the longest leaf of length 110.5 cm the shortest leaf was recorded for GH4055 with leaf length of 86.9 cm. NYRW1 had the widest leaf of 11.7 cm and shortest width was also recorded by GH 4055 with 6.8 cm.

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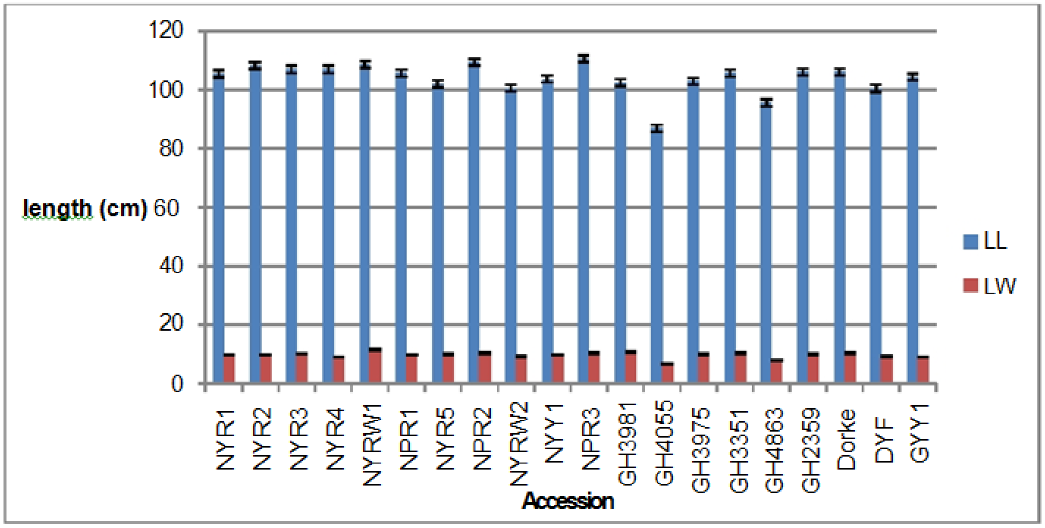

Fig. 3.4: Distribution of mean leaf length and mean leaf width of the 20 accessions of maize. Error bars indicates significant differences among the accession. LL: mean leaf length, LW: mean leaf width

Fig.3.5 shows the distribution of the mean number of kernels per row, mean number of rows on a cob and the mean number of leaves above the uppermost ear. NPR1 recorded the highest mean number of kernels per row of 36.77 and NYRW1 recorded the lowest number of kernels per row with 19.14 kernels. The mean number of row per cob ranged between 14.36 cm obtained by GH4863 and 12.50 by NYRW1. NPR3 had the highest number of leaves above the uppermost ear (7.22) whilst GH4055 recorded the least mean number of leaves with 6.86 leaves. Error bars show that there are significant differences among the accessions with respect to the three traits.

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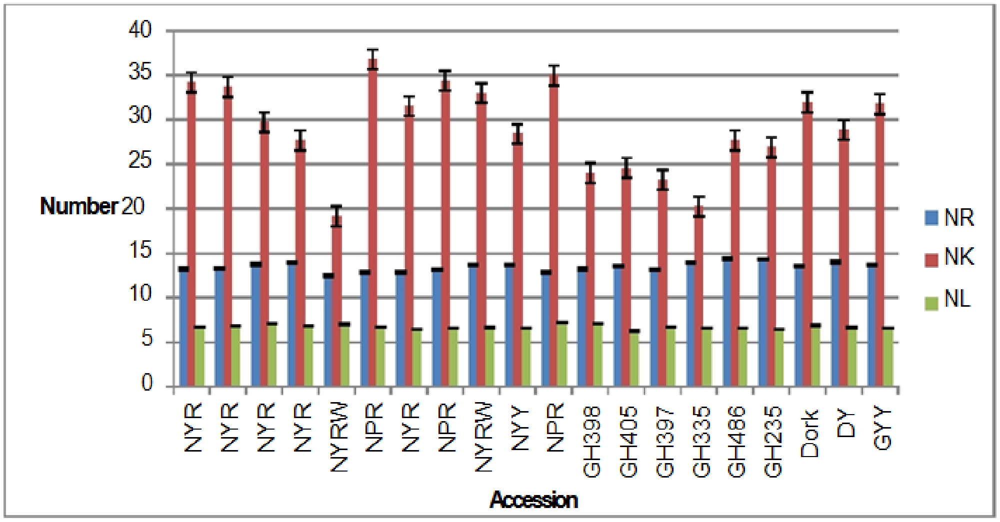

Fig. 3.5: Distribution of the mean number of kernels per row, mean number of rows per cob and the mean number of leaves above the uppermost ear of the 20 accessions of maize. NR: Number of rows per cob, NK: Number of kernels per row, NL: Number of leaves above the uppermost ear.

3.3.4 Variability in qualitative traits among the Accessions

Table 3.4 shows the kernel colour, kernel type, ear shape and kernel row arrangement of the 20 accessions. “Dorke” is white in colour, DYF, GH4055, NYR3, GH4863, GYY1 and NYY1 are all Yellow, NYR2, NYR4, NPR2 and NPR3 are all red, GH3981, GH3975, GH3351 GH2359 are purple while NYR1, NPR1, NYR5, NYRW2 are orange in colour and NYRW2 is mottled. NYR2, NYR4, NPR2 and NPR3 have dent kernel type with the upper surface of the kernel indented. They are also cylindrical in shape and the tassel type is primary as it is for all the other accessions.

All the yellow maize accessions are flint except NYY1 which is a dent type. The shape of the upper surface of the kernel is all rounded except NYY1 whose upper surface is indented. With regards to tassel type all the accessions had the primary tassel with no secondary ramifications. Most of the accessions have purple pigmentation of the stem

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resulting in a green and purple colour of the stem except NYR2 and NPR1.The husk cover rating shows that most of the accessions have good husk cover; only five of the accessions has intermediate husk cover.

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Table 3.5: Variability in qualitative traits among the Accessions

Accession	Kernel Color	Kernel Type	Ear Shape	KRA	SUK	Stem colour	Tassel Type	Sheath Pubescence	Husk	aleurone colour
NYR1	Orange	Dent	Cylindrical	Regular	indented	Purplish	Primary	Dense	Good	Colourless
NYR2	Red	Dent	Cylindrical	Regular	indented	Green	Primary	Dense	Good	Red
NYR3	Yellow	Flint	Conical	Regular	level	Purplish	Primary	Dense	Intermediate	Colourless
NYR4	Red	Dent	Cylindrical	Regular	indented	Purplish	Primary	Intermediate	Good	Red
NYRW1	variegated	Dent	Cylindrical	Straight	level	Purplish	Primary	Dense	Good	Mottled
NPR1	Orange	Dent	Cylindrical	Regular	Indented	Green	Primary	Dense	Good	Colourless
NYR5	Orange	Dent	Cylindrical	Regular	Level	Purplish	Primary	Dense	Good	Colourless
NPR2	Red	Dent	Cylindrical	Straight	Indented	Purplish	Primary	Dense	Intermediate	Red
NYRW2	Orange	Dent	Cylindrical	Regular	Level	Purplish	Primary	Dense	Good	Colourless
NYY1	Yellow	Dent	Cylindrical	Regular	Indented	Purplish	Primary	Dense	Good	Colourless
NPR3	Red	Dent	cylindrical	Straight	Indented	Purplish	primary	Dense	Good	Red
GH3981	Purple	Flint	Conical	Regular	Pointed	Purplish	Primary	Dense	Good	Purple
GH4055	Yellow	Flint	Conical	Spiral	Rounded	Purplish	Primary	Dense	Intermediate	Colourless
GH3975	Purple	Flint	Cylindrical	Straight	Rounded	Purplish	Primary	Dense	Intermediate	Purple
GH3351	Purple	Flint	Conical	Regular	Pointed	Purplish	Primary	Intermediate	Good	Purple
GH4863	Yellow	Flint	Conical	Spiral	Rounded	Purplish	Primary	Sparse	Intermediate	Colourless
GH2359	Purple	Flint	Conical	Regular	Rounded	Purplish	Primary	Dense	Good	Purple
Dorke	White	Dent	Cylindrical	Straight	Indented	Purplish	Primary	Intermediate	Intermediate	Colourless
DYF	Yellow	Flint	Round	Regular	Rounded	Purplish	Primary	Dense	Good	Colourless
GYY1	Yellow	Flint	Conical	Regular	Rounded	Purplish	Primary	Intermediate	Good	Colourless

SUK: Shape of the uppermost surface of kernel, KRA: Kernel row arrangement

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3.3.5 Genetic Relationship among 20 accessions of maize using Morphological Traits

Fig 3.6 below shows the genetic relatedness of the accessions based on twenty three morphological traits. The accessions were separated into two main clusters at 86.2% similarity with the first cluster containing the extra early maturing accessions (GH4055 and GH4863) the second cluster contained all the other accessions.

The second cluster separated into 2 sub-clusters one containing the four purple coloured accessions from Eastern region (Bunso) and the other all the remaining accessions at 88% similarity distance. At 90.2 % similarity distance the remaining 14 accessions separated further into sub-clusters distinguishing the white and yellow accessions from the red and orange accessions. Accessions NYR1 and GH4055 recorded the widest genetic relationship at 100% similarity. Two sets of duplicates were revealed among the accessions. These are NYR1/NYRW2 and GH3981/GH3351 which separated only after 98.3 % similarity.

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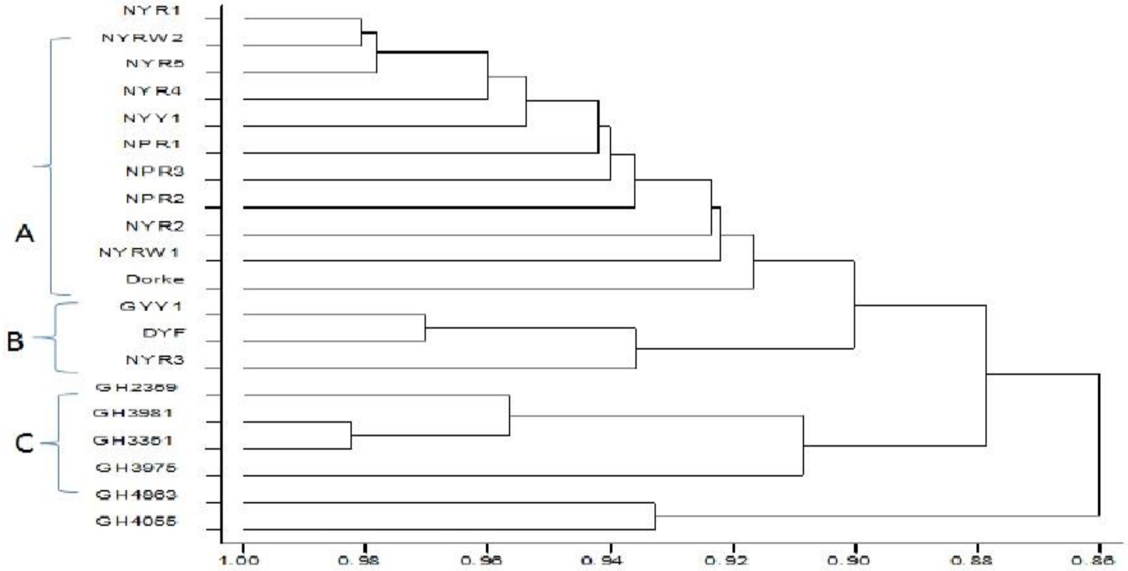

Euclidean coefficient

Fig 3.6: A Dendrogram showing genetic relationship among 20 maize accessions based on morphological traits using Euclidean coefficient single link similarity matrix.

3.3.5 Spearman Rank correlation coefficients for eleven quantitative traits.

Table 3.5 depicts Spearman Rank correlation coefficients for eleven quantitative traits which show the degree of association between the different traits of the twenty accessions. Cob weight had high positive correlation with ear girth (r = 0.8361), ear length (r = 0.8061) and number of kernels per row (r = 0.9103). Low positive correlation with ear height (r = 0.1170) and leaf width (r = 0.085) and negatively correlated with number of leaves above the uppermost ear (r = -0.0308) and number of rows per cob (r = -0.2712). Seed weight was moderately positively correlated with cob weight (r = 0.6251), ear girth (r = 0.6449), number of kernels per row (r = 0.5665) and negatively correlated

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with leaf width (r = 0.0500) and number of rows per cob (r = -0.0639).

Positive correlation was found between ear length and all the other traits measured except number of rows on a cob. The trait correlated positively with yield related traits such as cob weight (r = 0.8061), seed weight (r = 0.4578), ear girth (r = 0.5749) and number of kernels per row (r = 0.8197) but had negative correlations with the number of rows per cob (r = -0.3015). Ear girth also had positive association with most traits measured and correlated well with yield parameters such as cob weight, seed weight ear length and number of kernels per row. The trait had negative correlation with number of leaves above the uppermost ear and number of kernels per row.

Plant height had positive correlation with all other parameters measured the highest association was with ear height (r = 0.7546) and leaf length (r = 0.5832) while the other association recorded low coefficients. Number of rows per cob was negatively correlated with the traits that are associated with yield such as seed weight and cob weight but positively correlated with ear height, leaf length and number of leaves above the uppermost ear.

Leaf length was positively correlated with all other traits measured and had moderate positive correlation for yield related trait s such as cob weight (r = 0.4152) and plant height (r = 0.5832). Leaf width also showed moderately positive association with other parameters such as number of leaves above the uppermost ear (r = 0.5935) plant height (r = 0.4656), ear height (r = 0.4705) and leaf length (r = 0.5413) but negatively correlated with number of rows per cob and seed weight per plot. The number of leaves above the uppermost ear had positive correlation with all traits associated with vegetative growth and negative correlations for yield related parameter such as cob weight and seed weight.

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Table 3.6: Spearman Rank correlation coefficients for eleven quantitative traits

	CW	EG	EH	EL	LW	LL	NK	NL	NR	PH
CW										
EG	**0.8361**									
EH	0.1170	0.2069								
EL	**0.8061**	0.5749	0.1723							
LW	0.0854	0.0738	0.4705	0.1585						
LL	0.4152	0.3124	0.3635	0.3790	0.5413					
NK	**0.9103**	0.7521	0.1808	**0.8197**	0.0993	0.3573				
NL	-0.0308	-0.0015	0.4570	0.0448	0.5935	0.3715	0.0394			
NR	-0.2712	-0.0905	0.1312	-0.3015	-0.2320	0.0583	-0.3119	-0.1656		
PH	0.3419	0.3071	0.7546	0.4229	0.4656	0.5832	0.4674	0.4777	0.0312	
SW	0.6251	0.6449	0.1731	0.4578	-0.0500	0.1618	0.5665	-0.0639	0.1464	0.2714

CW: cob weight, EG: Ear girth, EH: ear height, EL: ear length, LW: leaf width, LL: leaf length, NK: Number of kernels per row, NL: Number of leaves above the uppermost ear, NR: Number of rows per cob, PH: Plant height, SW: Seed weight per plot. ($P \leq 0.05$) is considered significant.

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3.3.6: Principal Components Analysis for Agronomic Traits.

Table3.6: shows the Eigenvalues, percent of variation and cumulative percentage of sixteen morphological traits. The three principal components had Eigenvalues greater than or equal to 1.0. Together they account for 82.279 % of the variability among the accessions. PC1 with Eigenvalue of 6.6782 explained 41.739 % of the total observed variation and PC2 with Eigenvalue of 4.9836 accounted for 31.148 % of the total observed genetic variation while PC3 had Eigenvalue of 1.5028 and accounted for 9.393 % of the total variation. In PC1 the predominant traits that contributed to most of the variation among the accessions are days to 50% leaf senescence, days to date of harvesting, plant height, leaf length and leaf girth. The most predominant traits contributing to PC2 are the days to date of 50% anthesis, days to date 50% silking and leaf length. The traits that dominated PC3 were number of rows per cob, ear height and plant height.

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Table 3.7: Principal components analysis showing the contribution of sixteen agronomic traits among 20 maize accessions, Eigenvalues, percent of variation and cumulative percentage accounted for by three major principal components.

Trait	***Component 1***	***Component 2***	***Component 3***
1000 SW	0.240856	-0.274022	-0.245681
CW	0.239002	-0.326619	-0.112316
NR	-0.176358	0.00513176	0.661664
NK	0.193558	-0.333515	0.0498956
NL	0.211294	0.119569	-0.252395
PH	0.263481	-0.168103	0.334765
EH	0.303082	0.0699489	0.348865
EL	0.232618	-0.255739	-0.285038
EG	0.193288	-0.33514	0.208377
SW	0.121432	-0.368891	0.184747
DTA	0.227752	0.309691	0.0425144
DTS	0.243164	0.292665	0.0801502
DLS	0.294019	0.242545	0.0257736
DTH	0.310226	0.208842	0.0333634
LL	0.352511	0.0854513	0.105633
LG	0.293607	0.233275	-0.0963994
Eigenvalue	**6.6782**	**4.9836**	**1.5028**
Percentage of variance	**41.739**	**31.148**	**9.393**
Cumulative percentage	**41.739**	**72.886**	**82.279**

3.7.1: Principal components analysis based on quantitative traits

Fig. 3.7: depicts principal components analysis based on quantitative traits among the 20 accessions. The major contributing traits to the PC1 are leaf parameters such as number of leaves above the uppermost ear and leaf girth. EG, CW and NK contributed to most of

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variation observed on the PC2 axis. The predominant trait in PC3 axis is the number of kernels per row.

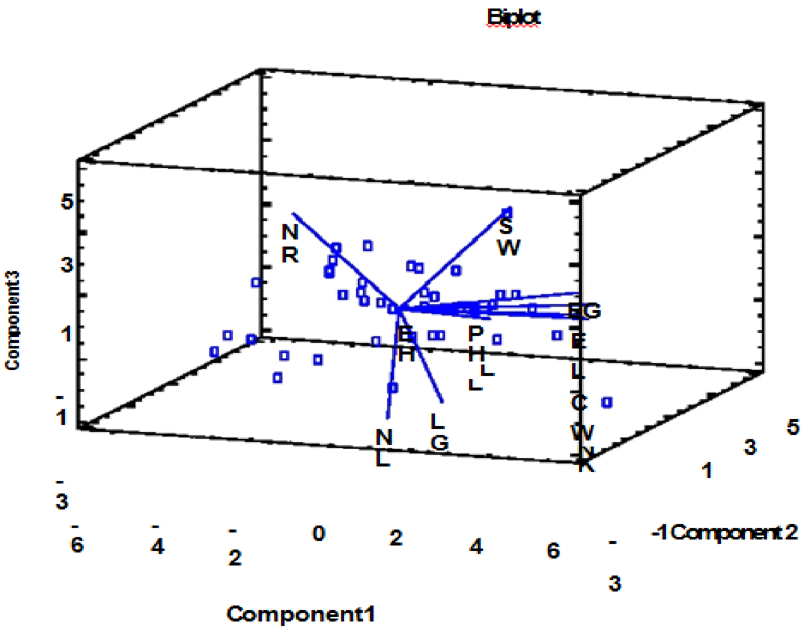

Fig. 3.7: principal components analysis based on quantitative traits

PH: Plant height, EH: Ear height, EL: Ear length, EG: ear girth, NK: Number of kernels per row, NR: number of rows per cob, CW: cob weight, LL: leaf length, LW: leaf width, SW: Seed weight per plot, NL: number of leaves above the uppermost ear

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DISCUSSION

3.4.1 Days to 50% anthesis (DTA), Days to 50% silking (DTS), Anthesis Silking Interval (ASI), Days to 50% leaf senescence (DLS), Days to harvesting (DTH) and Maturity period (MT).

Three maturity durations were classified in the results; early, medium and late maturing. Maturity is determined by the days to 50% anthesis, the Anthesis Silking Interval (ASI) and days to 50% silking. The mean days to 50 % anthesis (DTA) (55 days), days to 50 % silking (DTS) (60.1 days), ASI (5.3), days to 50 % leaf senescence (DLS) (86.85) and date of harvesting (DTH) (109.7) is lower than the results obtained by Badu-Apraku *et al*. (2010) who obtained DTA (64.8) DTS (65.7) and ASI (0.9). However, the results were consistent with what was obtained by Shah *et al*. (2010) and Ranatunga *et al*. (2009) who obtained mean DTA of 56 and 57.28 days, DTS of 59 and 63.04 and ASI of 2.9 and 5.76 days respectively. High anthesis silking interval is undesirable (Badu- Apraku *et al*., 2010) and when maize flowers are under drought, one universal phenomenon observed is the delay of silking in relation to pollen shed, giving rise to the anthesis-silking interval (ASI) whose duration is highly correlated with kernel set (Edmeades *et al*., 2000) this phenomenon can be observed on some of the cobs on plate 3.1. Leaf senescence indicates physiological maturity but fresh maize users usually harvest before physiological maturity. Plant height also plays a major role in determining maturity, as shorter plants tend to mature earlier than tall plants. This is because the duration of vegetative phase of growth and development is reduced in short plants whilst new nodes and internodes are being formed by tall plants; therefore the taller the plant the longer the maturity period. The height of the plant is influenced by both genetic (polygenic control) and environmental factors such as nutrients and moisture. The difference in maturity is a very important

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adaptive mechanism which allows the growing of maize in different ecological zones by avoiding environmental stress conditions. Accession GH4055 and GH4863 are extra early maturing and will be very useful for urban maize farmers whose objective is to produce fresh maize.

3.4.2 Variation in quantitative traits among accession

The 20 accessions showed significant variation in all quantitative traits studied (Appendix 1). The results obtained were reliable and repeatable in all replications given the low coefficient of variation obtained by all traits measured. The result is in agreement with findings by Badu-Apraku *et al.* (2010; 2006) who worked on diversity studies in *striga* resistant varieties and Quality Protein Maize and Abdulai *et al.* (2007) working on grain yield stability in full season lowland maize in Ghana who had coefficient of variation of 11.94 for plant height and 14.94 for ear height and Beyene *et al*. (2005) working on 62 Ethiopian highland maize accessions.

3.4.3 Genetic relationship among the accessions based on cluster analysis

The cluster analysis based on morphological traits grouped the accessions based on duration to maturity, geographical location, colour and physical structure. The extra early maturing accessions GH4055 and GH 4863 separated at 86.2 % similarity from all at the other accessions due to their uniqueness in terms of structure, they are short (PH= 124.5 cm), with small leaves (LL = 86.9 cm) and small cobs (CW = 58.24 g) and early maturing. The two accessions may be useful to urban farmers who usually produce fresh maize for the urban market. They are also recommended for production in the coastal and

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Sudan savanna zones with short rainfall duration and the forest and forest savanna transitional zones during the minor season (Sallah *et al.*, 2004). At 88 % similarity the accessions separated into two sub clusters made up of accessions from the Eastern Region (Bunso) in one cluster and the other accessions in another cluster. These can be attributed to the fact that the accessions are purple kernelled with large leaves and had purple coloured stem. The second cluster further separated into two sub-clusters containing accessions from Northern region (SARI) and those from Northern region (farmers). These could be explained by their colour and geographical location similar results were obtained by Nelson *et al.* (2004) working on Molecular characterization of maize inbreeds with expired US plant variety protection.

The results obtained indicates that less variation exists among accessions collected from the same geographical location with the same kernel colour but high variation exists between accessions collected from different geographical location with different kernel colours. The results are contrary to those obtained by Beyene *et al*. (2005) working on 62 Ethiopian highland maize accessions.

3.4.4 Correlations among quantitative trait

Correlation is a measure of the degree of association between two variables or characters.

Based on the degree of association selection of a particular trait may or may not imply the selection of another trait, this is based on the correlation coefficient. The high positive correlation between cob weight and ear girth (r = 0.8361), ear length (r = 0.8061) and number of kernels per row (r = 0.9103) this is consistent with the results obtained by Badu-Apraku *et al*. (2006). There was a low but positive correlation between plant height and yield parameters such as cob weight and seed weight. The number of rows per cob

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recorded negative correlation with all other yield parameters studied and low but positive correlation for traits like ear height, leaf length and number of leaves above the uppermost ear. The negative correlations observed can be attributed to the fact that the number of kernels per row decreases with increasing grain size and therefore large cobs with bigger grains produce less number of rows per cob than cobs with smaller grains. The results also showed that the number of rows/cob are usually even numbered unless there is some defect on the cob implying that rows on the cob are usually in pairs.

3.4.5 Contribution of traits to genetic variance through principal component Analysis

The factor score for the sixteen agronomic traits Days to 50% leaf senescence, Days to harvesting, Plant height, Leaf length, Leaf girth Days to 50% Anthesis, Days to 50% silking, number of rows per cob and Ear height were mostly correlated with the three major principal components of the principal component axis. The results obtained is in agreement with results obtained by Beyene *et al.* (2005) where traits such as Days to 50% anthesis, Days to 50% silking and ear height were the major traits contributing to the variation.

The 82.279 % cumulative variance obtained by the three major principal components is higher than what was observed by Beyene *et al.* (2005) who obtained 71.79 % and Badu - Apraku *et al*. (2006) where 79 % of the total phenotypic variation was accounted for by 4 major principal components. This result implies that the traits that contributed significantly to the variation must be considered in selection for future breeding programmes.

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CHAPTER 4

MOLECULAR CHARACTERIZATION OF TWENTY YELLOW-, RED- AND PURPLE-KERNEL MAIZE *(ZEA MAYS L.)* ACCESSIONS IN GHANA

4.1 Introduction

Characterization is fundamental to the development, release and popularization of plant varieties (Chavan, 2010) and knowledge of the genetic relatedness among various breeding materials is fundamental to the success of any breeding program. It gives account of the variability and potential use in plant breeding and provides evidence on loss of genetic diversity, offers evidence of evolutionary force shaping plant diversity and selection of genotypes to be given priority for conservation (Thorman *et al.*, 1994).

Morphological traits have been used in characterization of crop plants for many years before the advent of molecular markers. Morphological characterization is carried out based on observable traits on the plant; it gives information on the vegetative, reproductive and yield traits and help in the selection of superior genotypes. This method of characterization is influenced by the environment making their reproducibility difficult and data collection on traits are affected by developmental stage specificity since data on certain parameters are measured at specific stage of development of the plant.

Molecular markers on the other hand are not affected by the environment and developmental stage specificity. Some of them have very high reproducibility making them a more reliable and rapid tool for diversity studies than morphological characterization. Molecular markers have several advantages over conventional methods in diversity studies in view of their high precision in detecting variation with greater potential to explore genetic relationship among populations (Shah *et al.*, 2010).

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In this system the DNA is used instead of morphological traits and since the DNA sequence of even closely related plants is different, genetic differences between closely related genotypes can be detected (Beyene *et al*., 2005).

Several molecular markers have been developed. These include PCR-based marker systems such as AFLP, RAPD and SSRs and non PCR-based systems such as RFLP and SNTs. The choice of a marker system depends on the objective of the experiment. SSR markers have been found to have the highest heterogeneity whilst AFLPs have the highest effective multiplex ratio (Powell *et al*., 1996).

SSR markers were used in this experiment because of the advantages they have over other PCR based methods. These include the co-dominance inheritance which enables the results to be used in genetic analysis of segregating F_2 generation and the interpretation of population structure, the high reproducibility, high automation and relative abundance (Scott *et al*., 2000).

SSRs have been used by several researchers in maize diversity studies. Ranatunga *et al*. (2009) studied the genetic diversity in 43 maize accessions and concluded that there were significant differences between the accessions after morphometric and molecular analysis using SSR markers. Warburton *et al*. (2001) analysed 57 maize inbred lines from CIMMYT populations using SSR markers.

Over reliance on white kernelled improved varieties have left several maize landraces in Ghana uncharacterized and at risk of genetic erosion. These under-exploited landraces include red- , purple- and yellow-kernel maize cultivars which are useful as food for humans, food for infants, feed for farm animals, sources of vitamin A and proteins and a source of germplasm for future breeding programmes. There is the need to characterize

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these landraces so as to accurately establish their identity and diversity for future improvement programmes.

Due to the influence of the environment on morphological traits and their polygenic control which reduces the reproducibility of results, molecular characterization has become important in drawing meaningful conclusions since diversity in even close relations can be detected.

The objective of this study was to investigate the genetic diversity among the 20 yellow-, red- and purple-kernel maize accessions and their populations over the locations where they were collected using sixteen SSR markers.

Materials and Methods

4.2.1 Germplasm Collection

Twenty maize accessions were collected from three regions of Ghana for the experiment. Two from Savanna Agricultural Research Institute in the Northern Region ("Dorke", DYF), seven from the Plant Genetic Resource Research Institute in Bunso in the Eastern Region (GH3351, GH4863, GH3975, GH3981, GH40557, GH2359, GH2351). Nine were collected from farmers in Yendi Municipality in the Northern Region (NYR1, NYR26, NYR3, NYR4, NYRW1, NPR1, NYR5, NPR2, NYRW2, and NPR3).YY1 was collected from a vegetable farmer at Korle Bu in the Greater Accra Region.

4.2.2 Experimental Site

The experiment was conducted at Biotechnology and Nuclear Agriculture Research Institute (BNARI) and the Biotechnology Laboratory of the College of Agriculture,

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University of Ghana, Legon. Seeds were sown in the heat chamber of BNARI on 27th December 2012 and leaf samples were collected on the 10th of January 2013 and transferred to the Biotechnology Laboratory on ice cubes for DNA extraction and analysis.

4.2.3 DNA Extraction

DNA was extracted from 0.1g young leaf tissue using the DNeasy plant mini kit bench protocol. 0.1 g of young leaves from each accession was grinded in liquid nitrogen with the addition of 400 µl of buffer AP1 and 4 µl Rnase and vortexed vigorously. The mixture was incubated at 65 ˚C for 10 minutes and mixed 3 times by inverting the tube. 130 µl of buffer AP2 was added to the lysate, mixed and incubated for 5 minutes on ice.

The lysate was applied to the QIAshredder Mini Spin Column in a 2 ml collection tube and centrifuged at 13,000 rpm for 3 minutes. The flow-through fraction was then transferred to a new tube without disturbing the cell-debris pellet. 1.5 µl volume of the buffer AP/3 was added to 450 µl of the cleared lysate and mixed by pipetting. 650 µl of the mixture including any precipitate that may have formed was transferred to the DNeasy Mini Spin Column sitting in a 2 ml collection tube and centrifuged for 1 minute at 8000 rpm.

The flow-through was discarded. This step was repeated with the remaining sample. The flow-through and collection tube were discarded.

The DNeasy Mini Spin Column was placed in a new 2 ml collection tube and 500 µl of buffer AW added and centrifuged for 1 minute at 8000 rpm. Flow-through was discarded and collection tube-reused in the next step. 500 µl of buffer AW was added to the DNeasy Mini Spin Column and centrifuged for 3 minutes at 13,000 rpm to dry the membrane. The

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DNeasy Mini Spin Column was then transferred to a 2 ml micro-centrifuge tube and 40 µl of pre-heated (65 ˚C) buffer AE pipetted directly onto the DNeasy membrane. The mixture was incubated for 5 minutes at room temperature and centrifuged for 1 minute at 8000 rpm. This step was repeated.

4.2.4 Quality assurance

Samples of the extracted DNA was verified by running an electrophoresis on a 2% agarose gel and polymorphic bans were obtained indicating very good results.

4.2.5 DNA Amplification

Polymerase chain reaction was performed on the DNA of all genotypes using sixteen (16) SSR markers obtained from BIORON GmbH, Hubertusstraβe, 67065 Ludwigshafen, Deutschland. The amplification was carried out in a 25 µl reaction mixture containing 16.7 µl distilled water, 1 µl $MgCl_2$, µl 2.5 of 10x PCR buffer, 0.4 µl Tag polymerase, 0.4 µl dNTPs, 2 µl template DNA, 1 µl reverse primer and 1 µl forward primer on a thermal i cycler form BIO RAD.

DNA amplification was done under the following conditions. Denaturing at 95 °C for 1 min; followed by 10 cycles of 95 °C for 1 min; 55 °C for 1 min; 72°C for 1 hour 30 min; followed by 30 cycles of 95 °C for 1 min; 55 °C for 1 min; 72°C for 1 hour 30 min. The PCR products were then verified for amplification on a 2% agarose gel. The amplicons were then stored at 4 °C till electrophoresis.

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4.2.6 Gel Electrophoresis

The amplification products and a 100kb ladder marker were run on a 2% agarose gel prepared by dissolving the agarose in a 1× TAE buffer. 400ml of 5% Ethedium bromide was added. To ensure that the agarose was well dissolved it was kept in the microwave for 5 min and allowed to cool and solidify. The gel was then place in an electrophoretic tank and 4 µl of amplicons from each accession was loaded into wells with the 100 bp DNA marker to estimate the size of the generated bands.

Samples were made to run at 65 volts for 2 hours and visualize under UV light. Photographs were taken by a BioDoc-it gel documentation system.

4.2.7 Data collection and analysis

Due to the co-dominant nature of SSR markers the DNA bands (alleles) generated were scored as different genotypes or accessions. Bands were scored as homozygous genotypes (AA, BB, CC,) or heterozygous genotypes (AB, AC, BC,). Genetic parameters such as average observed number of alleles (Na), percentage polymorphic loci (P), observed heterozygosity (Ho), expected heterozygosity (He), Nei's heterozygosity (Nei He) Shannons diversity index (I) and gene flow (Nm) were calculated to estimate the level of diversity. F-test was carried out to test for departure from Hardy-Weinberg equilibrium and to estimate genetic differentiation among the maize population under study.

The outcrossing rate (t = (1-Fit)/ (1+Fit) was calculated based on Fit values to estimate indirectly the mating pattern of the maize population. Data were analyzed using POPGENE version 1_32. Molecular analysis of variance (AMOVA) was performed on the accessions to partition genetic diversity within and among regions. The banding patterns were converted

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into a 0 (absence) and 1 (present) and subjected to hierarchical multivariate cluster analysis using Gentat software 12 edition, based on Jaccard coefficient using Single Linked Similarity Matrix Method.

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RESULTS

4.3.1 Overview of amplification products

Fig. 4.1 is a profile generated by Primer umc2251 for the 20 maize accessions from DNA amplicons. The figure shows the polymorphic nature of the bands obtained. Twenty SSR primers were used in the study. However, only sixteen primers produced bands. The figure reveals that the primers were very informative due the generation of polymorphic bands.

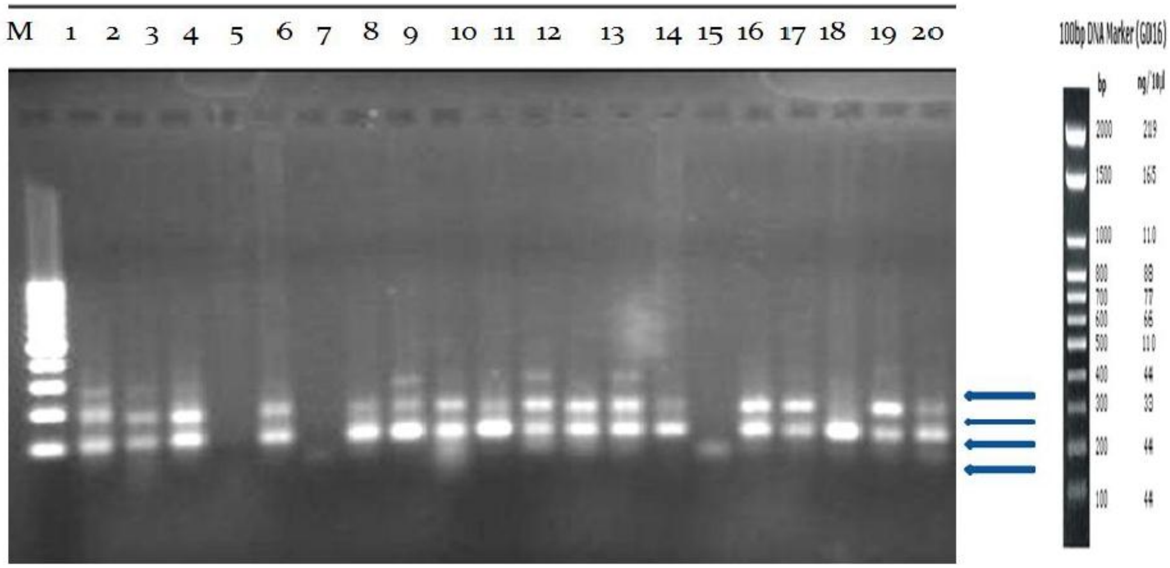

Fig. 4.1: Profile generated by Primer umc2251 for accessions 1 to 20 from DNA amplification of the 20 maize accessions.

Forward primer: 5'-cct gaa tcg ctc att cgc tc-3', reverse primer: 5'-gtc gag ggt ttg gag gag aga-3'

1= “Dorke”, 2= GH3981, 3 = DYF, 4 = NYR1, 5 = NYR2, 6 = GH4055, 7 = NYR3,
8 = NYR4, 9 = GH3975, 10 = NYRW1, 11 = GH3351, 12 = GH4863, 13 = NPR1,
14 = NYR5, 15 = NPR2, 16 = NYRW2, 17 = GH2359, 18 = NYY1, 19 = GH2359,
20 = NPR3

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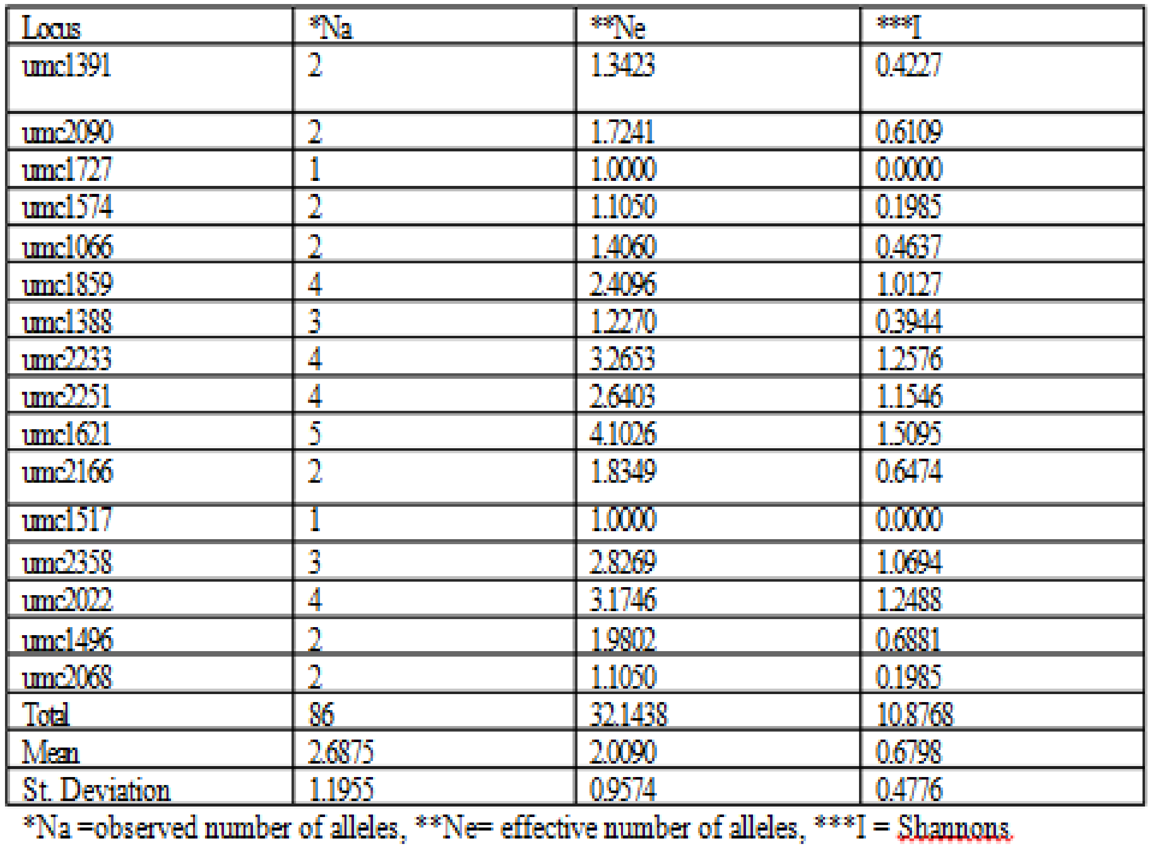
4.3.2 Microsatellite variation statistics for all loci

Sixteen loci generated a total of 86 alleles with a mean of 2.6875 alleles per locus. Locus ucm1621 produced the highest number of alleles whereas locus ucm1727 and ucm1517 produced the lowest number of alleles. Locus ucm1621 recorded the highest variability with a Shannon Information Index of 1.5095, Expected Heterozygosity of 0.7756 and Nei's Heterozygosity of 0.7562. All but two primers produced polymorphic bands and these were locus ucm1727 and locus ucm1517.

Tble 4.1: Genic variation statistics for all loci



Locus	*Na	**Ne	***I
umc1391	2	1.3423	0.4227
umc2090	2	1.7241	0.6109
umc1727	1	1.0000	0.0000
umc1574	2	1.1050	0.1985
umc1066	2	1.4060	0.4637
umc1859	4	2.4096	1.0127
umc1388	3	1.2270	0.3944
umc2233	4	3.2653	1.2576
umc2251	4	2.6403	1.1546
umc1621	5	4.1026	1.5095
umc2166	2	1.8349	0.6474
umc1517	1	1.0000	0.0000
umc2358	3	2.8269	1.0694
umc2022	4	3.1746	1.2488
umc1496	2	1.9802	0.6881
umc2068	2	1.1050	0.1985
Total	86	32.1438	10.8768
Mean	2.6875	2.0090	0.6798
St. Deviation	1.1955	0.9574	0.4776

*Na =observed number of alleles, **Ne= effective number of alleles, ***I = Shannons

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4.3.3 Differentiation of maize populations

The accessions showed high genetic diversity with respect to the overall genetic parameters with I of 0.6798, Nei's He of 0.3923 and He of 0.4023.The population collected from farmers in the Northern region had the highest level of genetic diversity with I of 0.6715, Nei's He of 0.3953 and He of 0.4161, followed by the accession from Bunso (I = 0.6091, Nei's He = 0.3552, He = 0.3826) while Greater Accra population recorded the lowest genetic diversity with I of 0.1733 Nei's He of 0.1250 and He of 0.2500. The overall percentage polymorphism was 87.50 %.

The F-statistics shows a very low negative F_{is} value of -0.0683 and a positive but low F_{it} value of 0.2018. These low values indicate the high heterozygosity among the populations and a significant departure from Hardy-Weinberg equilibrium. The F_{st} value obtained indicates that only 25.29 % of the total genetic variations exist among the population. The gene flow among the population is very high (73.87 %) with overall outcrossing rate of 0.6642.

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Table 4.2: Estimated Genetic diversity among 20 maize accessions populations from four locations based on polymorphisms of 16 SSR loci

Region	N_a	N_e	***PL***	***P (%)***	H_o	H_e	***Nei's*** He	***I***	F_{is}	F_{it}	F_{st}	***t***	***Nm***
Eastern (Bonso)	2.4375	1.8274	13	81.25	0.3125	0.382	0.3552	0.6091					
Northern (Farmers)	2.5000	2.0109	12	75.00	0.3313	0.416	0.3953	0.6715					
Northern (SARI)	1.375	1.3542	5	31.25	0.2500	0.218	0.1641	0.2383					
Greater Accra	1.250	1.2500	4	25.00	0.2500	0.250	0.1250	0.1733					
Over all	2.6875	2.0090	14	87.50	0.3125	0.402	0.3923	0.6798	-0.0683	0.2018	0.2529	0.6642	0.7387

Na =number of alleles Ne =average number of effective alleles (Kimura and Crow 1964); number of polymorphic loci; P = percentage of polymorphic loci; Ho observed heterozygosity (Levene, 1949); Nei's He = Nei,s Expected heterozygosity (Nei 1973); I = shannon diversity index (LEWONTON 1973); F_{is} F_{it} *and* F_{st} *= estimate of F statictics of regional populations (Hartl and Clark 1989); t, out crossing rate =* $(1- F_{it})/ (1+ F_{st})$ *(Coa et al., 2006); Nm = gene flow estimated from* $F_{st}= 0.25(1- F_{st})/ F_{st}$.

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4.3.4 Genetic diversity of the accessions population.

Table 4.3 shows the genetic identity at the population level, the genetic identity ranges between 0.9409 and 0.7727 indicating that the populations are very close in identity. Eastern region (Bunso) population and Northern region (farmers) shows the closest identity with genetic identity of 0.9409. The least genetic identity was recorded between Eastern region population and the Greater Accra population with genetic identity of 0.7727. The genetic distance among the population ranges from 0.0609 and 0.2579. The closest distance was recorded between accessions from Eastern region (Bunso) and the Northern region (farmer) populations and the widest genetic distance was recorded between Eastern region (Bunso) and the Greater Accra population.

Table 4.3: Nei unbiased measures of genetic identity and genetic distance (Nei, 1978)

Region	Eastern (Bunso)	Northern (farmers)	Northern SARI	Greater Accra
Eastern (Bunsu)	****	0.9587	0.8647	0.7999
Northern (farmers)	0.0422	****	0.8835	0.8213
Northern SARI	0.1454	0.1239	****	0.9020
Greater Accra	0.2232	0.1968	0.1032	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

3.4.5 Genetic similarity and cluster analysis

Figure 4.2 shows the Dendrogram based on Nei's (1978) genetic distance using Unweighted Pair Group Method modified from NEIGHBOR procedure of PHYLIP Version 3.5. Eastern (Bunsu) population and Northern (farmer) showed the closest similarity in cluster 1. Northern (SARI) and populations from Greater Accra formed the

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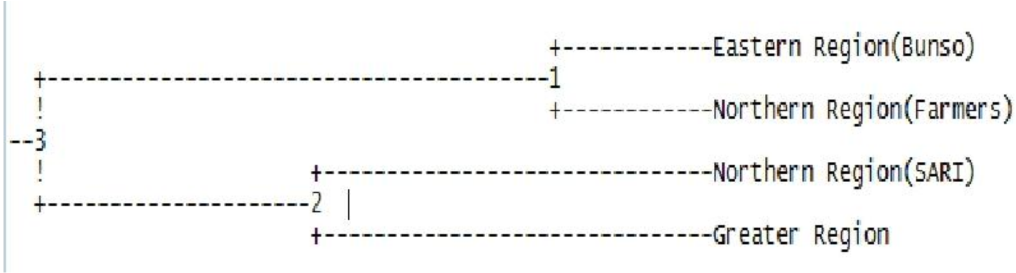
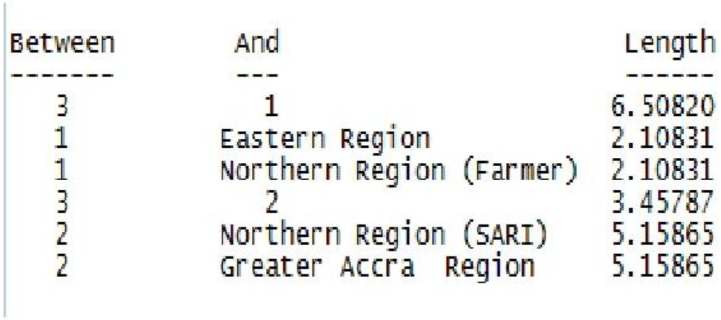
second cluster with genetic distance of 8.61652.



```
                                             +------------Eastern Region(Bunso)
  +--------------------------------------------1
  !                                          +------------Northern Region(Farmers)
--3
  !                      +------------------------------Northern Region(SARI)
  +---------------------2  |
                         +------------------------------Greater Region
```

Figure 4.2: Genetic Distance Dendrogram Using 16 SSR Markers

Table 4.4: Length of Genetic Distance between Populations



```
Between          And                          Length
-------          ---                          ------
   3               1                          6.50820
   1             Eastern Region               2.10831
   1             Northern Region (Farmer)     2.10831
   3               2                          3.45787
   2             Northern Region (SARI)       5.15865
   2             Greater Accra  Region        5.15865
```

4.3.6 Cluster analysis based on 16 SSR markers

Figure 4.3 shows the maximum genetic relationship among the accessions as revealed by the 16 SSR markers base on Jaccard coefficient, using single linked similarity matrix method. Two major clusters were formed at a genetic similarity distance of 54.1 % with accession NYR1 separating from all the other accessions. Accession GH4055 also separated from all the other 18 accessions at 58.1 % genetic distance. The 18 accessions were further separated into two sub-clusters at 65.7 % similarity. NYR3 and GYY1 showed the closest resemblance at 83.6% similarity while the widest genetic distance was recorded between accessions NYRI and GH3351. No duplications were found among the

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accessions.

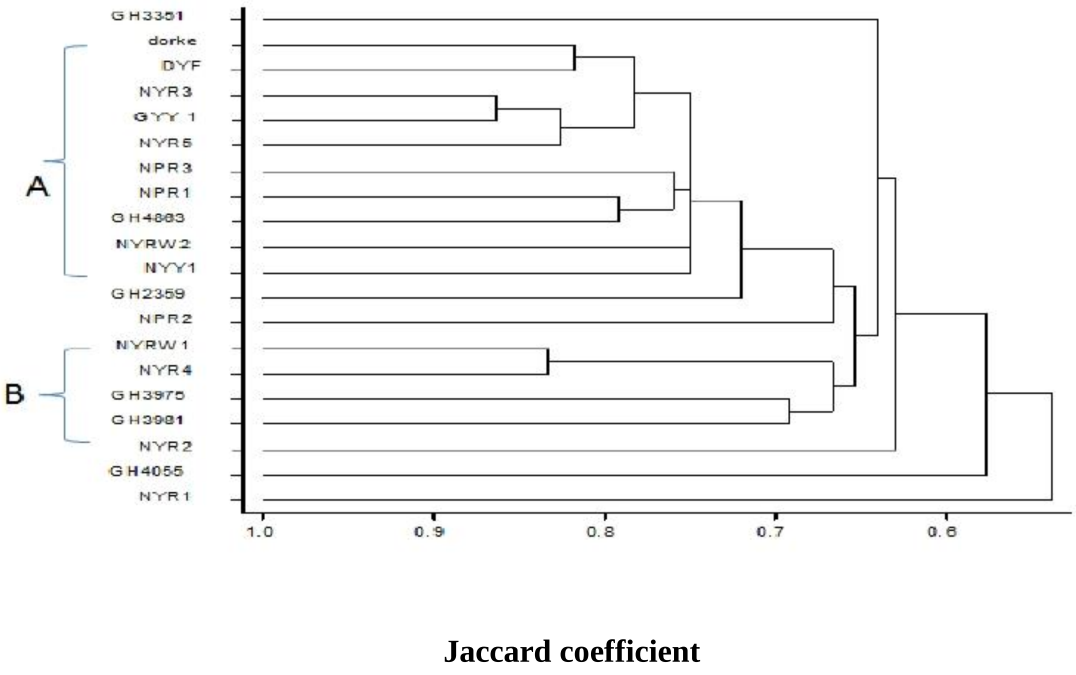

Figure 4.3: Dendrogram showing the genetic relationship between 20 maize accessions generated by 16 SSR markers based on Jaccard coefficient using Single Link Similarity Matrix method

DISCUSSION

4.4.1 Microsatellite variation statistics for all loci and population

Sixteen SSR primers were used to produce 86 alleles with an average of 2.68 alleles per primer and high percentage allelic polymorphism of 87.5 % indicating that SSR primer are very informative and good for diversity studies in maize. This is however lower than the results obtained by Beyene *et al.* (2005) who observed 100% polymorphism using SSR markers on Ethiopian highland maize accessions and Abuali *et al*. (2011) who also obtained 100% polymorphism using RAPD markers on Sudanese maize accessions.

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However, lower percentages were obtained by Mokharib *et al.* (2010); Chen *et al.* (2000) and Lanza *et al*. (1997).

The observed heterozygosity, the expected heterozygosity and Nei's heterozygosity values of 0.3125, 0.4023 and 0.3923 respectively with a Shannons information index of 0.6798 indicate low variability among the accessions. A similar result was obtained by Barry *et al*. (2007). By contrast Doku *et a*l. (2013) and Coa *et al.* (2006) using SSR markers to assess genetic diversity among some accessions of *Oryza glaberrima* rice in Ghana obtained an *I* value of 1.178 and *He* and *Nei's He* of 0.625 and 0.608 respectively.

Genetic diversity among the accessions can be due the geographical location where the accessions were collected, the genotype and the population structure of the accessions. Genotypic diversity in a population is influenced by selection, mutation, migration, population size and genetic drift. The SSR makers reveal that only 25.29 % of the genetic variation is due to variation between the populations. The high outcrossing rate (0.6642) and low F_{is}(-0.0683), F_{it} (0.2018) and F_{st} (0.2529) and the very high gene flow of 0.7387 confirm that this accessions are open pollinated and there is high level of gene transfer among the populations. The population analysis shows a departure from Hardy-Weinberg equilibrium.

4.4.2 Genetic divergence

Nei unbiased measure of genetic identity and genetic distance confirms the low variation among the populations. A short genetic distance and a high degree of genetic identity among accessions indicate that the accessions are not too divergent genetically and hence there is close resemblance in the populations. The population from the Plant Genetic Resource Institute (Bunso) in the Eastern region and the population collected from

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farmers in the Northern region showed the closest resemblance with a genetic identity of 0.9587 and a genetic distance of 0.042. This result is lower than what was obtained by Drimic MlaDenovic *et al.* (2012) working on correlation of yield and heterosis of maize hybrids and their parental line with genetic distance based SSR markers with an average genetic distance of 0.58 and range between 0.55-0.61 and Mokharib *et al.* (2010) working on molecular diversity of selected maize inbred lines who had a minimum genetic distance of 0.128 and a maximum of 0.297. The close resemblance may be attributed the fact that most of the accessions from the Northern (farmers) population are red coloured and most of those from eastern (Bunso) are purple both of which are caused by anthocyanin pigmentation in the aleurone layer which is a single cell layer between the pericarp and the endosperm. The most diverse populations were the population from Greater Accra and Eastern regions.

4.4.3 Cluster analysis

Most of the genetic gains that have been made in maize breeding can be attributed to heterosis. In order to take full advantage of this effect more distant parents are required since the wider the genetic distance between the parents the better the heterosis.

The cluster analysis also confirms the low genetic variability and high genetic similarity between the populations with Northern (farmer) and Eastern region showing the greatest relationship.

4.4.4 Genetic relationship between accessions based on 16 SSR markers.

The cluster analysis indicates that the accessions are separate entries with no duplications. The Dendrogram reveals that the accessions did not cluster based on

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geographical location or colour except yellow maize accessions which formed one cluster. Accession NYR1 which promise to be high yielding separated from the other accessions at 54.3 % and accession GH4055 which is the extra early accession separated from the other accessions at 58.1 % indicating its uniqueness from all other accessions.

GH4055 may be very useful in future breeding programmes; for the production of drought tolerant varieties due to its ability to escape drought conditions as result of its earliness (Banziger *et al.*, 2002) and is recommended for cultivation in coastal and Sudan savanna zone with short rainfall seasons and also for minor season planting in the forest and forest savanna transitional zones (Sallah *et al.,* 2004) the dendrogram developed from the morphological characterization revealed two duplicates among the accessions but the one generated by molecular characterization revealed that the accessions were separate entries with no replications, this confirms the efficiency of SSR primers in distinguishing between even closely related genotypes as reported by Beyene *et al.* (2005) and Shah *et al*. (2010).

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CHAPTER 5

DETERMINATION OF OPAQUE-2 GENE AND BETA CAROTENE CONTENT IN TWENTY YELLOW-, RED AND PURPLE-KERNEL MAIZE *(ZEA MAYS L.)* ACCESSIONS IN GHANA.

5.1 Introduction

Maize is a major staple for most people in Ghana (Buah *et al.*, 2009) and it is an important source of calories in the diets of the people. It is also a source of protein accounting for about 60% of the daily human protein supply in some high maize-consuming communities (Mbuya *et al.,* 2012). In Ghana maize accounts for 23% of the national protein needs (Twumasi-Afriyie *et al.,* 1997). The traditional maize cultivars, however, lack two essential amino acids lysine and tryptophan and therefore have poor protein quality.

Maize has also been found to be a good source of vitamin A due to the presence of β-carotene in the endosperm. The β-carotene molecule is the most potent pro-vitamin A and also the most widespread (Kimura *et al.,* 1991). Zeaxanthin and lutein are the major carotenoids in maize with β-carotene and β-cryptoxanthin being present in much smaller amounts (Moros *et al*., 2002). The amount of β-carotene in the maize grain is influenced by both genetic and environmental factors (Kimura *et al*., 1991). Carotenoids have been found to enhance the immune system, prevent regenerative diseases (Olson, 1999) and prevent irreversible blindness and cataract in the elderly (Moeller *et al*., 2000).

Malnutrition is a very important health problem in the developing world including Ghana. According to the WHO over 70 % of pre-school children and 18% of pregnant women suffer from vitamin A deficiency in Ghana though most of the symptoms are not

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visible to the naked eye (WHO, 2009). Though most people in Ghana depend on animal protein and legumes for their protein needs, maize also plays an important role in supplying some of the essential amino acids required for proper growth and development. Protein deficiency diseases such as Kwashiorkor and symptoms such as stunted growth are prevalent in the country and consumption of Quality Protein Maize with high lysine and tryptophan content can help to alleviate this problem (Akuamoah-Boateng, 2002).

The discovery of the opaque 2 gene which is responsible for increasing the lysine and tryptophan content and the further development of Quality Protein Maize varieties by CIMMYT has helped to double the lysine and tryptophan content of the grain (Krivanek *et al*., 2007). The opaque-2 gene is one of several mutant genes conferring high lysine and tryptophan content in maize. The trait is expressed in the homozygous recessive o2o2 gene and not in the heterozygous o2O2 and the homozygous dominant O2O2 (Crow and Kernicle, 2002).

Quality Protein Maize (QPM) has 70 to 100% more lysine and tryptophan than normal maize cultivars (Buah *et al*., 2009). The Crops Research Institute (Fumesua, Ghana) has produced four Quality Protein Maize varieties; “Obatampa”, “Mamaba”, “Dadaba” and “CIDAba” (Al-Hassan and Jatoe, 2002; Twumasi-Afriyie *et al.*, 1997) which are currently being used by farmers.

Since maize is produced and consumed by most families in Ghana especially in the rural areas there is the need to determine the QPM status and β-carotene content of local maize accessions in order to make recommendations to breeders, farmers and consumers.

The QPM status and β-carotene content of the red -, yellow- and purple kernel maize accessions which are good food for humans and quality feed for farm animals are currently not documented.

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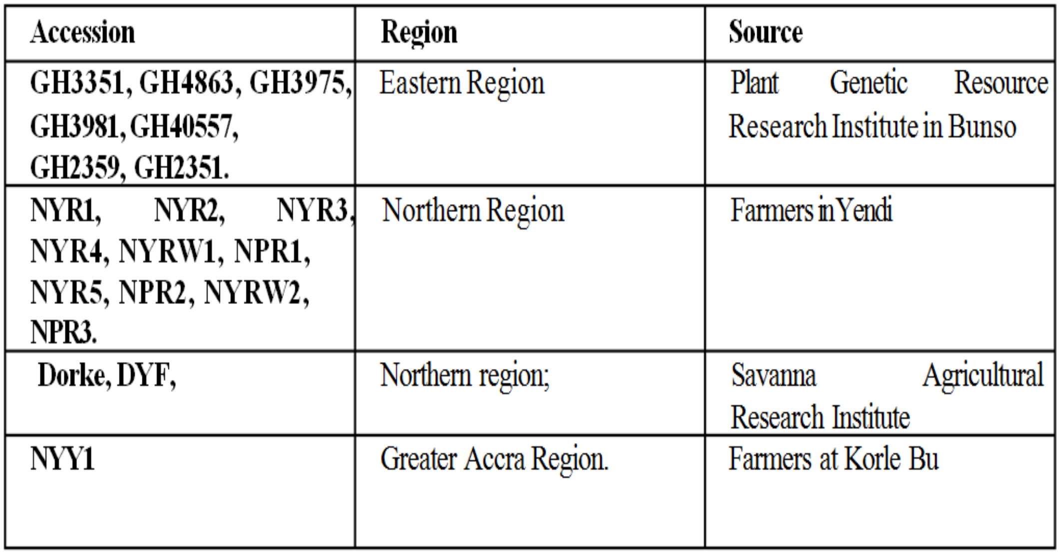
The objective of this experiment was to determine the presence of the opaque-2 gene and β-carotene in the 20 maize accessions

Materials and methods

5.2.1 Collection of Germplasm

Twenty maize accessions were collected from four locations in Ghana for the experiment as indicated in Table 5.1 below.

Table 5.1: Collection Sites and Sources of the 20 maize (*Zea mays* L.) Accessions used for the studies.



Accession	Region	Source
GH3351, GH4863, GH3975, GH3981, GH40557, GH2359, GH2351.	Eastern Region	Plant Genetic Resource Research Institute in Bunso
NYR1, NYR2, NYR3, NYR4, NYRW1, NPR1, NYR5, NPR2, NYRW2, NPR3.	Northern Region	Farmers in Yendi
Dorke, DYF,	Northern region;	Savanna Agricultural Research Institute
NYY1	Greater Accra Region.	Farmers at Korle Bu

5.2.2 Experimental Site

The experiment was conducted at the experimental field of the Biotechnology and Nuclear Agriculture Research Institute and the Nutrition laboratory of the Noguchi Memorial Institute for Medical Research.

5.2.3: Sampling

Grains from individual plots were bulked into one and the quartering method was used to

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reduce the sample to laboratory sample size. Grains were heaped and flattened into a circle. A cross was made dividing the circle into four equal parts. Two parts were discarded and the process was repeated until about 200 g of grains were obtained.

5.2.4: Sample preparation

The sample from each accession was grinded using an electric blender and packaged into 50 g of maize powder. The samples were packed in aluminum foil and stored in a desiccator.

5.2.5: Beta carotene extraction

Six to 8 g of the powdered samples were weighed and transferred into a mortar for grinding. 40 ml of acetone was added and grinded using a pestle. The mixture was then filtered using a filter paper and the corn place back into the mortar for grinding with acetone. The process was repeated until the sample was devoid of colour. The extract was then partitioned in 20 ml petroleum spirit in a 200 ml separating funnel. The extract was washed several times with 300 ml of distilled water until aqueous layer was clear. The aqueous layer (lower phase) was then drained away leaving the extract in the separating funnel. The extract was then dried by passing it through anhydrous sodium sulphate settled at the base of a funnel placed in a measuring cylinder. The extract was the collected and volume was recorded (Rodriguez -Amaya and Kimura, 1998).

5.2.6 Preparation of standard

Three mg of the refined standard was dissolved in petroleum spirit. The standard with the blank sample were placed in a Hitachi spectrophotometer for reading of absorption. This was done at a wave length of 450 nm.

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5.2.7 Preparation of mobile phase

The mobile phase was prepared by mixing 6 parts of Hexane with 1 part of Benzene. 300 ml of hexane was added to 50 ml of Benzene to give a total of 350 ml. The mixture was then sonicated for 3 minutes.

5.2.8 High Pressure Liquid Chromatography (HPLC)

A Shimadzu HPLC equipment was used for the determination of the beta carotene. The equipment has five components (1) the Shimadzu pump = LC-6A, (2) Shimadzu detector = SPD-6AV, (3) Shimadzu recorder (4) Shimadzu reverse phase column=ODS 025M×4.6. At the HPLC, 250 ml of the extract was evaporated under steam of nitrogen gas and then reconstituted with 50 µl of the mobile phase made of hexane and benzene.

20 µl of the reconstituted sample was injected into the HLPC machine and the readings were recorded.

5.2.9 Data analysis

Based on the data obtained from the HPLC procedure the following calculations were done to arrive at the total carotenoid concentration and the β-carotene concentration.

Formula for calculating carotenoids in the sample

$$concentration\ of\ \beta - carotene = \frac{A \times volome(mL) \times 10^3}{A^{1\%}\ cm \times sample\ weight\ (g)}$$

Where,

A = absorbance

Volume = total volume of extract

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A1%1cm= absorption coefficient for b carotene. In petroleum ether it is 2592.

$$Total\ carotenoid\ concentration = \frac{Cx\,\frac{\mu g}{g} \times Cs\,\frac{\mu g}{g} \times TV_{extract}\ (mL)}{As\ \times sample\ weight\ (g)}$$

Where,

Cx = concentration of carotenoid x

Cs = concentration of the standard

Ax = peak area of carotenoid x

As = peak area of standard

TV= Total volume

Where no detectable peaks were formed the area under the curve was calculated and used to calculate the actual area based on simple proportion with an accession with a known HPLC area and area under the graph.

5.2.10: Determination of Opaque -2 gene in grains of 20 maize accessions

Thirty grains were collected from each of the 20 accession for the experiment. The grains were placed on a light box (Vivek, *et al*., 2008) and observed for; translucent, <25 % opaque, 25-50 % opaque and 100 % opaque grains. Grains with 25 to 50 % have the Opaque 2 genes.

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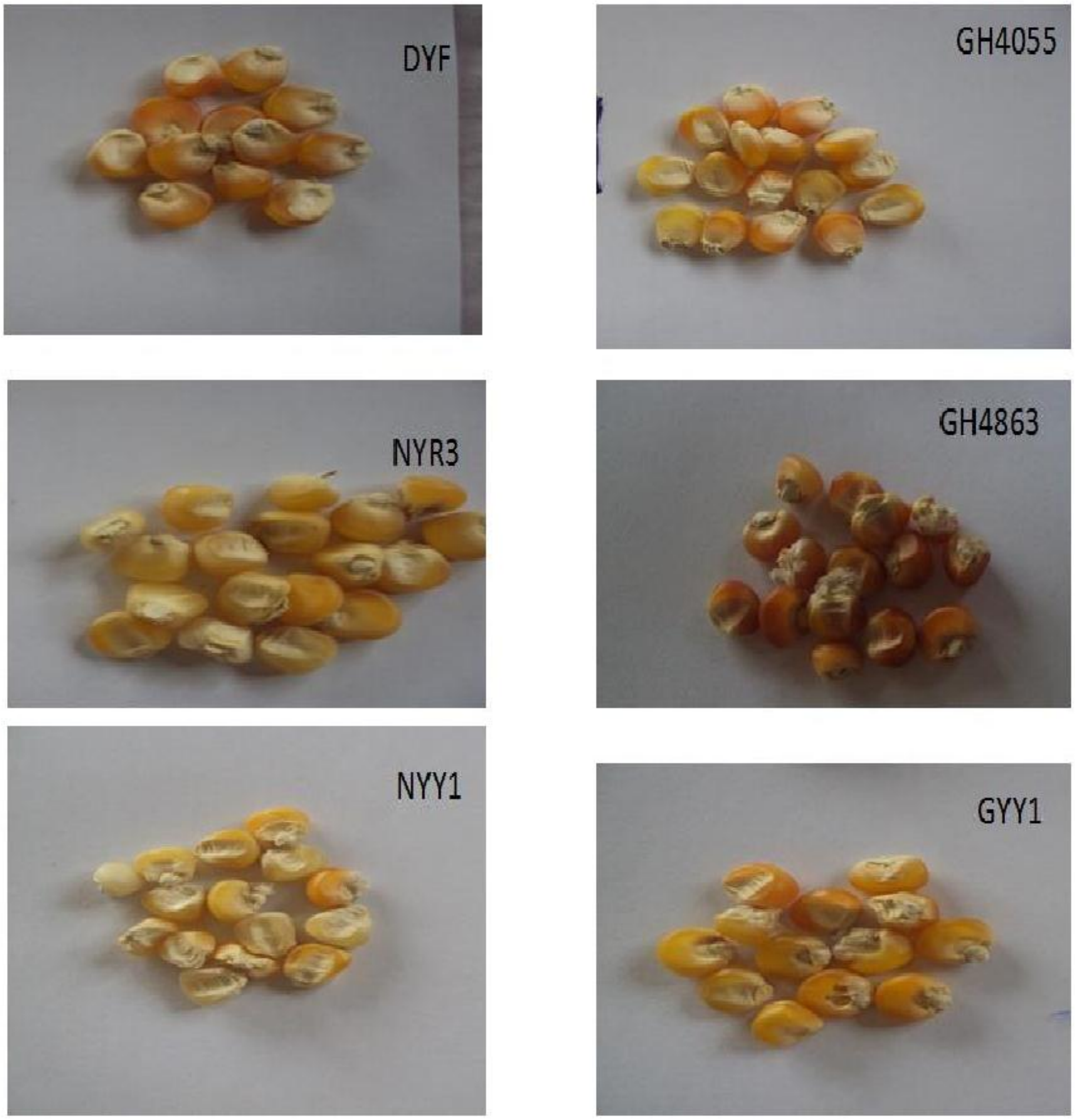
5.3: RESULTS

Plate 5.1 below shows the variation in kernel colour among the 20 maize accessions after harvest.

Plate 5.1: Variation in kernel colour among the 20 maize accessions



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5.3.1: Standardization of Spectrophotometer

Table 5.2 shows the spectrophotometer reading for the standard at a wave length of 450 nm. Three readings were taking and the average was found to be 0.387.

Table 5.2: shows the spectrophotometer reading for the standard

Absorbance reading	**blank**	**standard**
1st reading	0.00	0.382
2nd reading	0.00	0.386
3rd reading	0.00	0.394
Avearge	0.00	0.387

5.3.2 Sample weight, Total volume of extract, Elution time and Area

Table 5.2 shows the Sample weight, Total volume of extract, Elution time and Area obtained from the extraction and HPLC. Some of the samples did not produce any peak at the expected elution time whilst others produced peaks that were not within the detectable limits of the Shimadzu detector. In the latter case the area under the graph was calculated and used to determine the area by simple proportion using a sample with a known area and elution time. In this study NYY1 was used. Accession DYF had the widest area and the best elution time compared to the standard

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Table 5.3: Sample weight, Total volume of extract, Elution time and Area.

Accession	Sample weight (g)	Total volume of extract (ml)	Elution time (s)	Area (mm^2)
GH3981	0.7821	8	1.167	4422
GH4055	0.7884	8.4	0.888	5853
GH3975	0.7709	11	1.173	2147
GH3351	0.7472	6.7	0.1	6974
GH4863	0.7123	7	na	4203
GH2359	0.7538	9.1	na	1697
NYR1	0.7929	7.1	1.192	10509
NYR2	0.7912	5	1.183	4265
NYR3	0.7294	15	1.113	1015
NYR4	0.7251	7	na	1940
NYRW1	0.7804	9	1.163	4019
NPR1	0.7696	6	na	2667
NYR5	0.7737	5	1.177	7556
NPR2	0.584	8.1	na	2910
NYRW2	0.7695	7.3	1.175	9054
NYY1	0.793	7.5	1.18	12125
GYY1	0.7575	4.5	1.167	19699
NPR3	0.6294	6.4	1.175	6097
Dorke	0.7236	9.1	1.167	4706
DYF	0.7693	7.7	1.158	25091
Standard				
reading 1			1.152	372250
reading 2			1.153	362506
reading 3			1.157	371783
Blank			0	0

5.3.3 Variability in β- carotene among the maize accessions

The accessions show variability in β-carotene content with significant differences between accessions as indicated by the error bars in figure 5.1. DYF from Northern (SARI) population has the highest β-carotene content of 2.0313 µg/g followed by accession GYY1 with 1.5923 µg/g. The lowest β-carotene content was observed in accession NYR3 with 0.082 µg/g.

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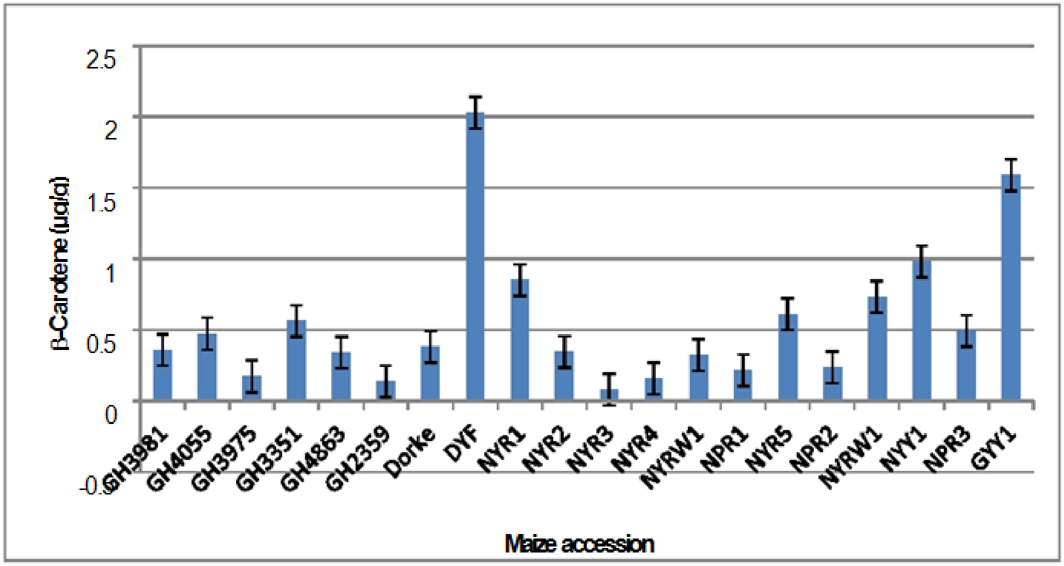

Fig 5.1: Variability in β-carotene among 20 maize accessions

5.3.4 Variability in Total carotenoid content among 20 maize accessions

The accessions shows a wide range of variability in terms of the carotenoid content with accession DYF having the highest carotenoid content of 12.0327 (µg/g) followed by "Dorke" a white maize accession. The lowest concentration of carotenoids was obtained by NYR4 with a value 0.7971 µg/g.

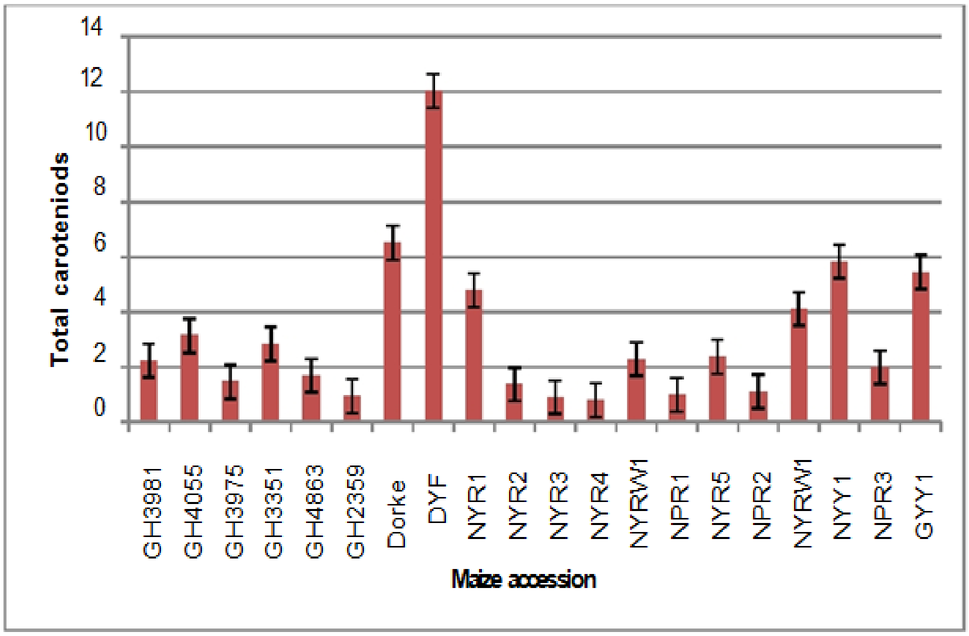

Fig 5.3: Variability in total carotenoid content among 20 maize accessions

5.3.5 Degree of association between β-carotene and kernel colour score

Figure 5.3 shows the results of fitting a linear model to describe the relationship between β-carotene and kernel colour score. Since the P-value in the ANOVA table (appendix 5) is greater or equal to 0.05, there is not a statistically significant relationship between β-carotene and kernel colour score at the 95.0% or higher confidence level. The R-Squared statistic indicates that the model as fitted explains 7.78865% of the variability in β- carotene. The correlation coefficient equals -0.279082, indicating a relatively weak relationship between the traits.

The standard error of the estimate shows the standard deviation of the residuals to be 0.490748. The mean absolute error (MAE) of 0.352354 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur in the data file. Since the P-

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value is greater than 0.05, there is no indication of serial autocorrelation in the residuals at the 95.0% confidence level.

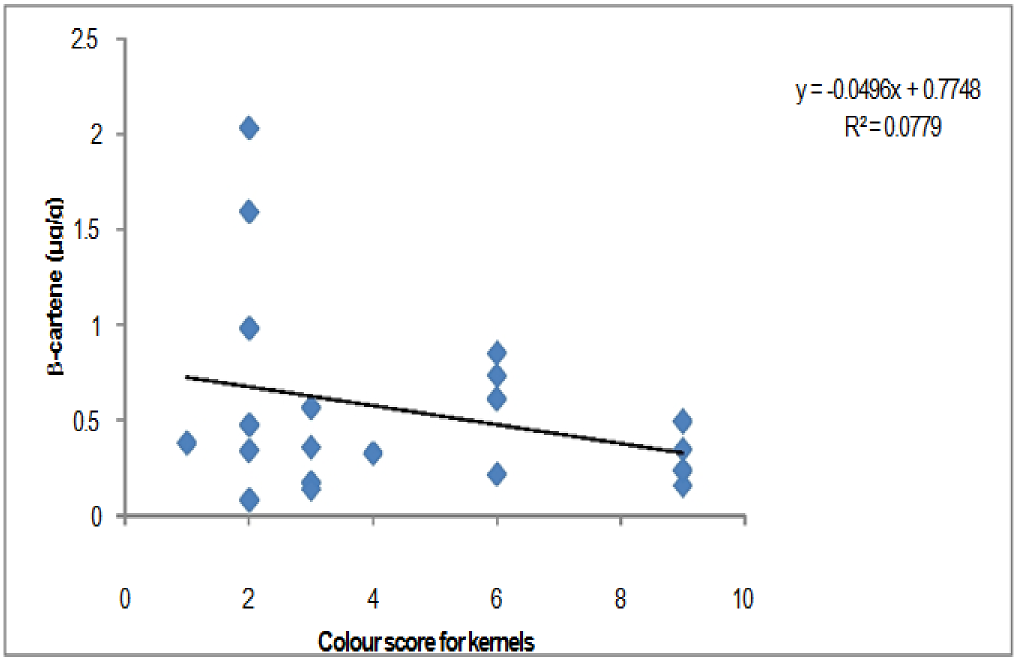

Fig 5.3: Regression plot for kernel colour vs β-carotene content (µg/g).

Correlation Coefficient = -0.279082, R-squared = 7.78865, percent R-squared (adjusted for d.f.) = 2.6658, percent Standard Error of Est. = 0.490748. Mean absolute error = 0.352354 Durbin-Watson statistic = 1.5694 (P=0.1563)

5.3.6: Degree of association between carotenoids and kernel colour

Figure 5.4 shows the results of fitting a linear model that describes the relationship between carotenoids and kernel colour score. The R-Squared statistic indicates that the model as fitted explains 18.4504% of the variability in carotenoids. The correlation coefficient equals -0.429539, indicating a relatively weak relationship between the traits. The standard error of the estimate shows the standard deviation of the residuals to be 2.53926. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any

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significant correlation based on the order in which they occur in your data file. Since the P-value is greater than 0.05, there is no indication of serial autocorrelation in the residuals at the 95.0% confidence level.

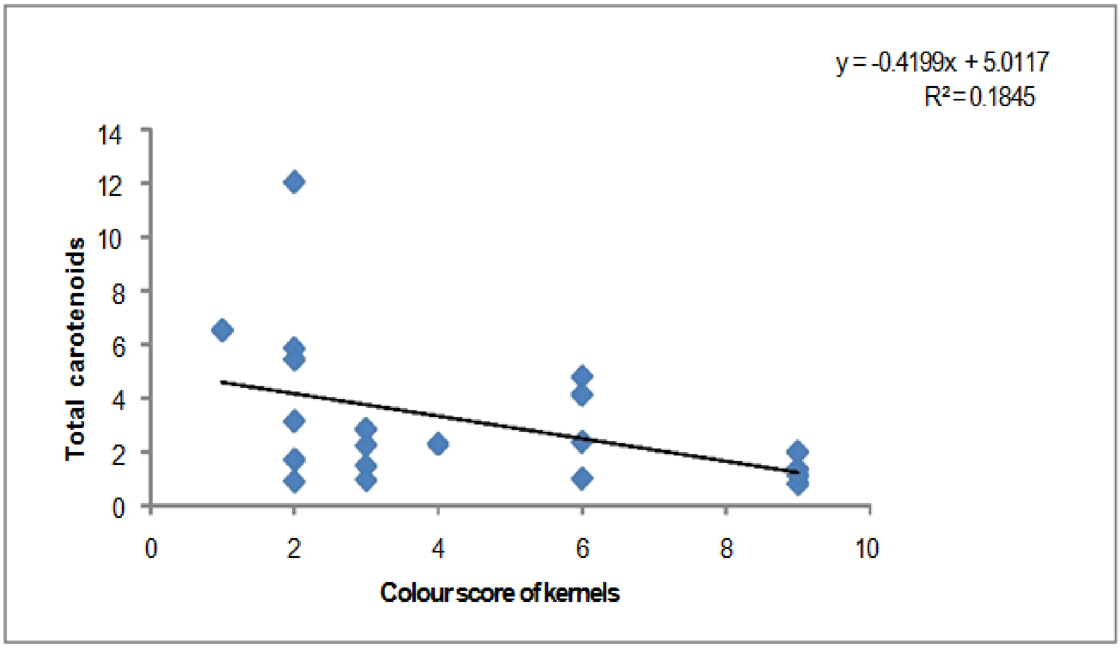

Fig 5.4: Regression plot for kernel colour vs Total carotenoid content (μg/g).

Correlation Coefficient = -0.429539, R-squared = 18.4504, percent R-squared (adjusted for d.f.) = 13.9199, percent Standard Error of Est. = 2.53926, Mean absolute error = 1.75292, Durbin-Watson statistic = 1.7495

5.3.7 Degree of association between Beta-carotene (μg/g) and Total carotenoids (μg/g)

Figure 5.6 shows the results of fitting a linear model to describe the relationship between Beta-carotene (μg/g) and Total carotenoids (μg/g). The equation of the fitted model is Beta-carotene (μg/g) = 0.0520983 + 0.159732*Total carotenoids (μg/g) Since the P-value in the ANOVA table (appendix 5) is less than 0.05, there is a statistically significant relationship between Beta-carotene (μg/g) and Total carotenoids (μg/g) at the 95.0%

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confidence level. The R-Squared statistic indicates that the model as fitted explains 77.2402% of the variability in Beta-carotene (µg/g). The correlation coefficient equals 0.878864, indicating a moderately strong relationship between the variables.

The standard error of the estimate shows the standard deviation of the residuals to be 0.24381.

The mean absolute error (MAE) of 0.125397 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur in your data file. Since the P-value is less than 0.05, there is an indication of possible serial correlation at the 95.0% confidence level.

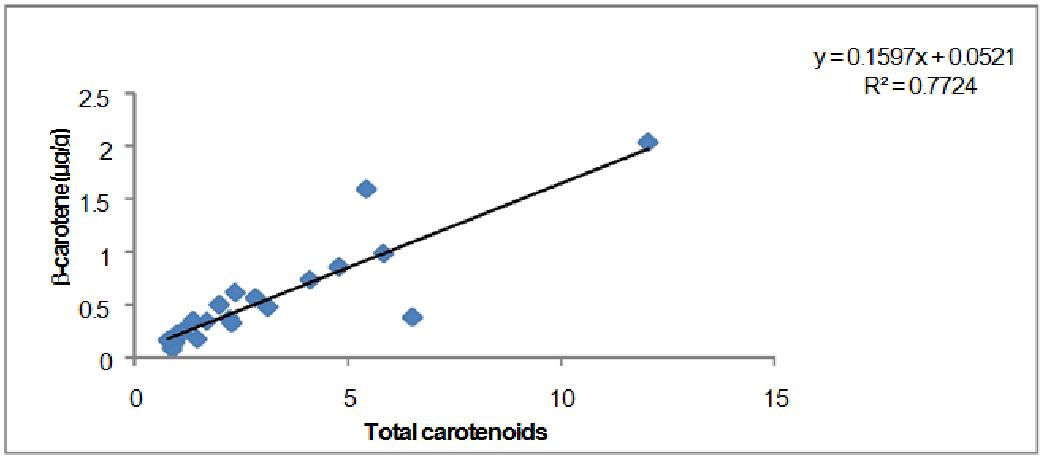

Fig 5.5: Regression plot for β-carotene vs Total carotenoid content (µg/g)

Correlation Coefficient = 0.878864, R-squared = 77.2402 percent, R-squared (adjusted for d.f.) = 75.9758 percent, Standard Error of Est. = 0.24381, Mean absolute error = 0.125397, Durbin-Watson statistic = 1.26004 (P=0.03

5.3.8 Presence of opaque -2 gene.

Table 5.3 shows the presence of opaque two genes among the accessions. None of the

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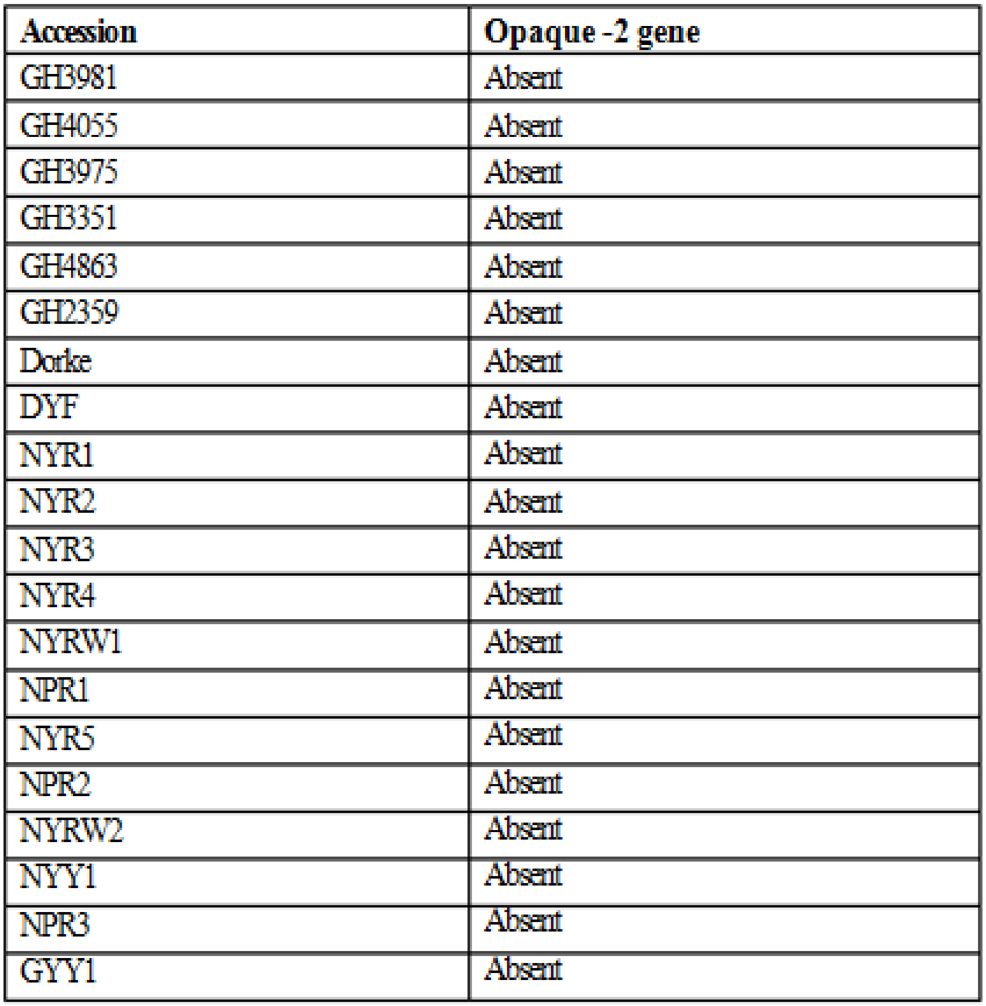
accessions was found to have the gene.

Table 5.4: Presence of *opaque-2* gene among 20 accessions.



Accession	**Opaque -2 gene**
GH3981	Absent
GH4055	Absent
GH3975	Absent
GH3351	Absent
GH4863	Absent
GH2359	Absent
Dorke	Absent
DYF	Absent
NYR1	Absent
NYR2	Absent
NYR3	Absent
NYR4	Absent
NYRW1	Absent
NPR1	Absent
NYR5	Absent
NPR2	Absent
NYRW2	Absent
NYY1	Absent
NPR3	Absent
GYY1	Absent

DISCUSSION

The experiment revealed high carotenoid and beta carotene contents in the maize accessions with some accessions recording as high as 12.0327 (µg/g) and 2.0313 (µg/g) respectively. However, this results is lower than what was obtained by Safawo *et al.* (2010) working on the exploitation of natural variability in maize for β-carotene content using HPLC who had as high as 63 µg/g for some accessions.

Yellow maize had the highest total carotenoid and β-carotene content but reports indicates

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that the β-carotene content of most yellow maize grown and consumed in the world has 0.5-1.5 µg/g (Safawo *et al.*, 2010). The total carotenoid content of 12.0327(µg/g) and β-carotene content of 2.0313 (µg/g) obtained by accession DYF is high and within the range (10.17-29.1 µg/g) and (0.33-9.2 µg/g) respectively, obtained by Dhyneswaran in 2012 screening maize germplasm for β-carotene content. Accession DYF therefore can be very useful as food for infants.

However, white maize recorded a high carotenoid content with a relatively low β-carotene concentration. Orange kernel accessions also had relatively high levels of carotenoids and β-carotene compared to purple and red coloured accessions. The accessions showed statistically significant variation in β-carotene and carotenoid contents.

The regression analysis indicates the low association between kernel colour and carotenoid contents. There was also a negative correlation between the two traits indicating that selection for high carotenoid content cannot be done based on kernel colour. This has been reported by Harjes *et al.* (2003) who stated that comparism between shades of yellow kernel colour and total carotenoid has poor correlation and attributed this to the presence of other carotenoid molecules. Similar correlation was observed between β-carotene concentration and kernel colour. This confirms the results obtained by Safawo *et al.* (2010) with r^2 values of 0.184 and 0.033 for carotenoids and β-carotene respectively.

High level of association was found between the total carotenoid content and the β-carotene content with 87.88 % correlation indicating that maize with high levels of carotenoids are more likely to high levels of β-carotene. As demonstrated accession "Dorke" for instance had high levels of carotenoids but low level of β-carotene while with all other accession the β-carotene concentration increases with increasing carotenoid concentration.

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With respect to the presence of the opaque 2 gene in the accessions, all the accessions were translucent except the purple accessions which were difficult to determine due to the grain size and colour. None of the accessions fulfills the descriptor for quality protein maize.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

Three separate experiments; Morphological characterization using qualitative and quantitative traits, molecular characterization using SSR markers and variation in nutritional quality in terms of β-carotene using HLPC and quality protein using the light box were carried out on yellow- , red- and purple kernel maize (*Zea mays* L.) accessions .

Based on the results obtained the following conclusions can be drawn;

a1) Accessions GH4055 and GH4863 are extra early maturing and therefore can be very useful for urban farmers producing fresh maize for sale. However, they produce smaller cobs (Cob weight = 58.24g) as compared to other accessions.

b1) Significant variability in morphological traits was observed among the accessions with cob weight, number of kernels per row, plant height and 1000 seed weight having coefficient of variation of 42.7544, 20.5828, 11.4634, 13.0634 and 26.76 respectively.

c1) Few traits contributed to the variations observed and these include days to 50% anthesis, days to 50 % leaf senescence, plant height and cob weight.

d1) A dendrogram generated from morphological traits clustered the accessions based on kernel colour, physical structure of the plant and geographical location. Two duplicates were identified among the accessions and widest genetic distance was observed between NYR1 and GH4055.

e1) Strong correlation exist between most of the morphological traits measured (r = 0.9103) but negative correlation was observed between most important yield parameters and number of row per cob.

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f1) Accession NYR1 showed promise in terms of yield (1000 seed weight = 281 g) as well as Carotenoid content (4.7895 µg/g) high β-carotene content (0.8507 µg/g) and therefore can very useful in future breeding programmes.

a2) The population genetic analysis shows that the accession bear close resemblance to each other with high Nei's genetic identity coefficient of 0.9587 and 0.9020 between accessions from Northern and Eastern region and Northern and Greater Accra region respectively with short genetic distances and this is confirmed by the high outcrossing (0.6642) rate and gene flow (0.7387) among the populations and the high heterozygosity (0.4023).

b2) A dendrogram generated by SSR markers revealed that the accessions are separate entries with no duplications.

a3) Significant variations were found in the β-carotene and carotenoid contents of the accessions.

b3) Accession DYF had the highest carotenoid and β-carotene concentrations of 12.0327 and 2.0313 respectively;

c3) No significant correlation was observed between kernel colour and β-carotene content.

d3) Generally the amount of β-carotene increased with increasing carotenoid content but this is not always the case as few exceptions may be found as revealed by the experiment.

a4) None of the accessions contained the opaque-2 gene and therefore did not fit the description of Quality Protein Maize. This information may be useful to the breeder, the farmer and the consumer.

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6.2 Recommendation

Accession DYF had the highest carotenoid and β-carotene concentrations. The accession also performed well in various yield parameters and therefore can be useful in the fresh maize market as well as food for infants.

Accession GH4055 and GH4863 are extra early maturing and can be useful in the coastal savanna zone and for minor season cropping in the forest and forest savanna transitional zones.

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APPENDICES

Appendix 1

Analysis of variance

Variate: CW

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	3		9398.5	3132.8	6.47	
rep.*Units* stratum						
Accession	19		79155.1	4166.1	8.61	<.001
Residual	49	(8)	23708.5	483.8		
Total	71	(8)	102442.7			

==

Least significant differences of means (5% level)

Table	accession
rep.	4
d.f.	49
l.s.d.	31.26

==

Stratum standard errors and coefficients of variation

Variate: CW

Stratum	d.f.	s.e.	cv%
rep	3 12.52	14.4	
rep.*Units*	49	22.00	25.3

Analysis of variance

Variate: EG

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	3		0.55961	0.18654	4.10	
rep.*Units* stratum						
accession	19		6.53837	0.34412	7.57	<.001
Residual	46	(11)	2.09032	0.04544		
Total	68	(11)	8.24109			Least

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significant differences of means (5% level)

```
Table           accession
rep.            4
d.f.            46
l.s.d.          0.3034
 ===========================================================
```

Stratum standard errors and coefficients of variation

```
Variate: EG

Stratum                    d.f.        s.e. cv%
rep                        3 0.0966    3.2
rep.*Units*                46          0.2132        7.1


=============================================================
```

Analysis of variance

```
Variate: EH

Source of variation     d.f. (m.v.)          s.s.        m.s.       v.r.    F pr.

rep stratum               3                 893.3       297.8      1.04

rep.*Units* stratum
accession                19               19322.6      1017.0      3.54    <.001
Residual                 56     (1)       16108.8       287.7

Total                    78     (1)       36285.9
==============================================================
```

Least significant differences of means (5% level)

```
Table           accession
rep.            4
d.f.            56
l.s.d.          24.02

==========================================================
```

Stratum standard errors and coefficients of variation

```
Variate: EH

Stratum                    d.f.        s.e.          cv%
rep                        3 3.86      3.7
rep.*Units*                56          16.96         16.4
```

Analysis of variance

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```
Variate: EL
_____________________________________________________________________
Source of variation      d.f. (m.v.)        s.s.       m.s.     v.r.   F pr.
_____________________________________________________________________

rep stratum                 3              41.370     13.790    7.18

rep.*Units* stratum
accession                  19             223.625     11.770    6.13   <.001
Residual                   45    (12)      86.469      1.922
_____________________________________________________________________
Total                      67    (12)     310.282

=====================================================================
```

Least significant differences of means (5% level)

```
Table          accession
rep.           4
d.f.           451
.s.d.          1.974

 ====================================================================
```

Stratum standard errors and coefficients of variation

```
Variate: EL
_____________________________________________________________________
Stratum                  d.f.          s.e.          cv%
rep                      3 0.830       6.0
rep.*Units*              45            1.386         10.0
=====================================================================
```

Analysis of variance

```
Variate: LG
_____________________________________________________________________
Source of variation      d.f. (m.v.)        s.s.       m.s.     v.r.   F pr.
_____________________________________________________________________

rep stratum                 3               0.978      0.326    0.26

rep.*Units* stratum
accession                  19              80.657      4.245    3.45   <.001
Residual                   52     (5)      64.060      1.232
_____________________________________________________________________
Total                      74     (5)     142.074
=====================================================================
```

Least significant differences of means (5% level)

```
_____________________________________________________________________
Table          accession
rep.           4
d.f.           52
l.s.d.         1.575
=====================================================================
```

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Stratum standard errors and coefficients of variation

```
Variate: LG

Stratum                    d.f.          s.e.          cv%
rep                        3 0.128       1.3
rep.*Units*                52            1.110         11.3

=======================================================
```

Analysis of variance

```
Variate: LL

Source of variation      d.f. (m.v.)         s.s.       m.s.     v.r.   F pr.

rep stratum                3              816.62     272.21     4.89

rep.*Units* stratum
accession                 19             2218.57     116.77     2.10   0.018
Residual                  52     (5)     2891.75      55.61

Total                     74     (5)     5726.56
=======================================================
```

Least significant differences of means (5% level)

```
Table            accession
rep.             4
d.f.             52
l.s.d.           10.581

=======================================================
```

Stratum standard errors and coefficients of variation

```
Variate: LL

Stratum                    d.f.          s.e.          cv%
rep                        3             3.689         3.5
rep.*Units*                52            7.457         7.2
=======================================================
```

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Analysis of variance

Variate: NK

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	3		56.99	19.00	1.13	
rep.*Units* stratum						
accession	19		2215.17	116.59	6.93	<.001
Residual	31	(26)	521.82	16.83		
Total	53	(26)	2028.15			

Least significant differences of means (5% level)

Table	accession
rep.	4
d.f.	31
l.s.d.	5.917

==

Stratum standard errors and coefficients of variation

Variate: NK

Stratum	d.f.	s.e.	cv%
rep	3 0.975	3.3	
rep.*Units*	31	4.103	14.0

==

Analysis of variance

Variate: NL

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	3		0.7863	0.2621	1.45	
rep.*Units* stratum						
accession	19		4.6653	0.2455	1.36	0.191
Residual	49	(8)	8.8346	0.1803		
Total	71	(8)	13.8150			

==

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Least significant differences of means (5% level)

```
Table           accession
rep.            4
d.f.            49
l.s.d.          0.6034

=============================================================
```

Stratum standard errors and coefficients of variation

```
Variate: NL

Stratum                 d.f.        s.e.        cv%
rep                     3           0.1145      1.7
rep.*Units*             49          0.4246      6.3

============================================================
```

Analysis of variance

```
Variate: NR

Source of variation     d.f. (m.v.)       s.s.        m.s.     v.r.   F pr.

rep stratum               3             2.2315      0.7438     1.69

rep.*Units* stratum
accession               19             22.6647      1.1929     2.71   0.008
Residual                28    (29)     12.3177      0.4399

Total                   50    (29)     28.1098

=============================================================
```

Least significant differences of means (5% level)

```
Table           accession
rep.            4
d.f.            28
l.s.d.          0.9607

========================================================
```

Stratum standard errors and coefficients of variation

```
Variate: NR

Stratum                 d.f.        s.e.        cv%
rep                     3           0.1929      1.4
rep.*Units*             28          0.6633      4.9
============================================================
```

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```
Analysis of variance

Variate: PH

Source of variation         d.f. (m.v.)           s.s.       m.s.     v.r.   F pr.

rep stratum                   3                 3485.2     1161.7     3.38

rep.*Units* stratum
accession                    19                17144.7      902.4     2.63   0.003
Residual                     56     (1)        19226.4      343.3

Total                        78     (1)        39853.6

=====================================================================

Least significant differences of means (5% level)

Table          accession
rep.           4
d.f.           56
l.s.d.         26.25

===================================================================
 Stratum standard errors and coefficients of variation

Variate: PH

Stratum                   d.f.          s.e.          cv%
rep                       3 7.62        4.4
rep.*Units*               56            18.53         10.7

===================================================================
Analysis of variance

Variate: SW

Source of variation         d.f. (m.v.)           s.s.       m.s.     v.r.   F pr.

rep stratum                   3                 0.6369     0.2123     1.94

rep.*Units* stratum
accession                    19                 6.7227     0.3538     3.24   <.001
Residual                     48     (9)         5.2443     0.1093

Total                        70     (9)        11.7823
=========================================================================
```

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Least significant differences of means (5% level)

Table	accession
rep.	4
d.f.	48
l.s.d.	0.4699

===
=====

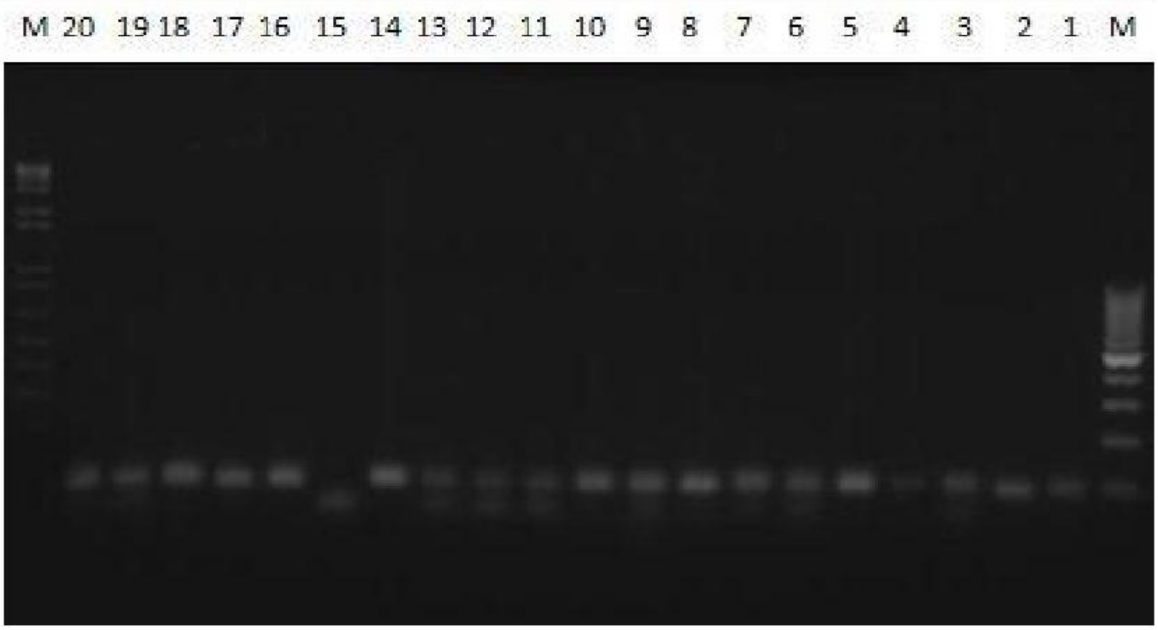
Stratum standard errors and coefficients of variation

Variate: SW

Stratum	d.f.	s.e.	cv%
rep	3	0.1030	14.4
rep.*Units*	48	0.3305	46.3

Appendice II

Primer 10 Fig. 1a Profiles generated by Primer umc1391 for accessions 1 to 19 (from right to left) from DNA amplification of maize



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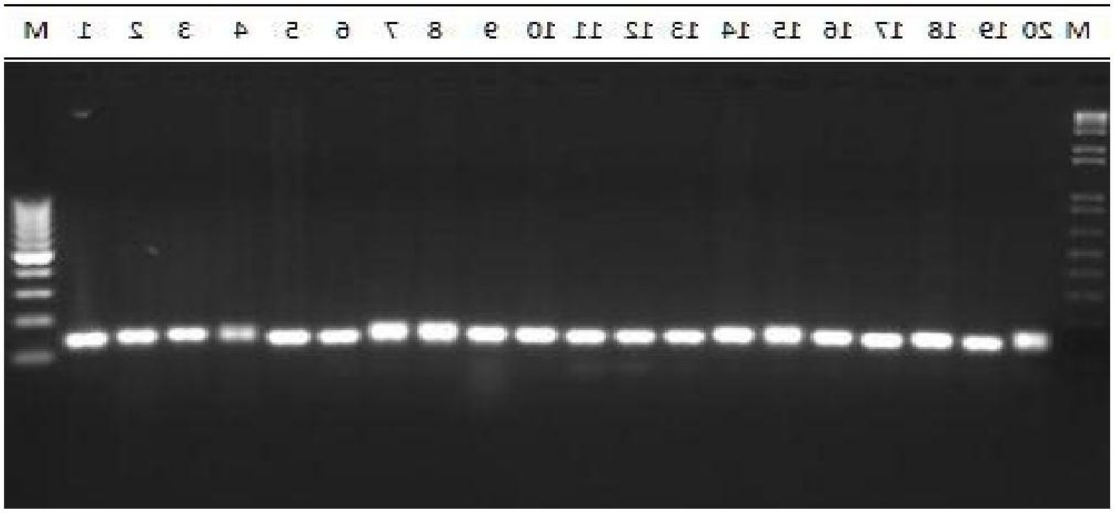
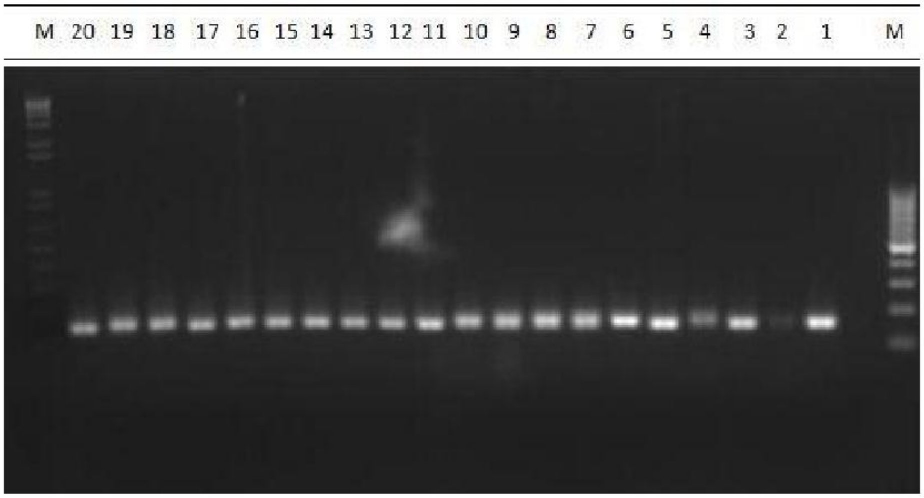
Primer 12 Fig. 1a Profiles generated by Primer umc2090 for accessions 1 to 19 (from right to left) from DNA amplification of maize



Primer 15 Fig. 1a Profiles generated by Primer umc1727 for accessions 1 to 19 (from right to left) from DNA amplification of maize



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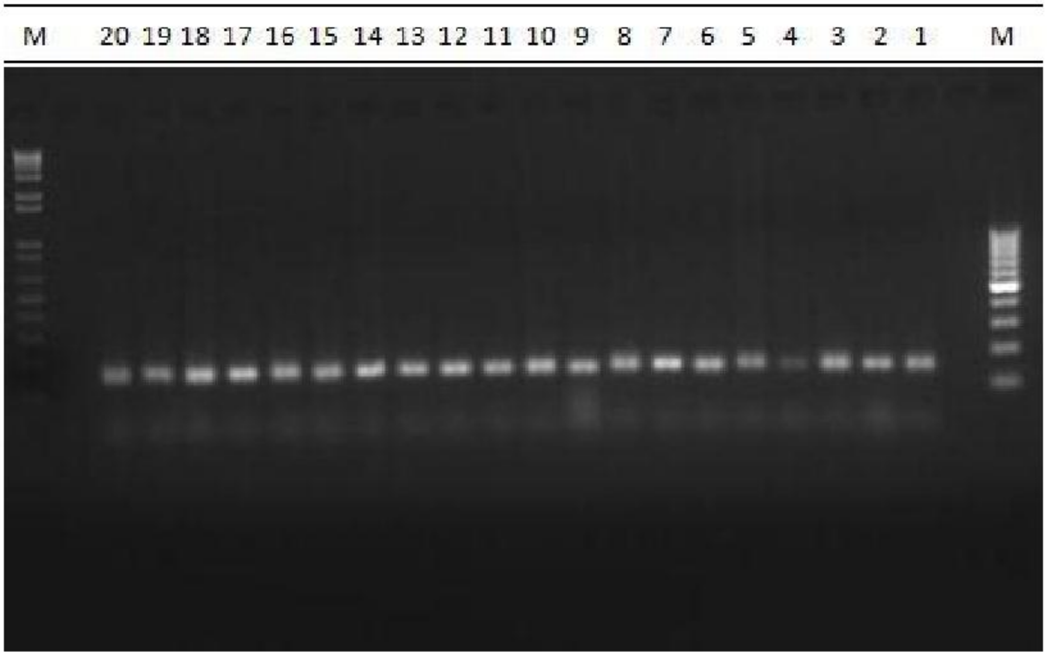
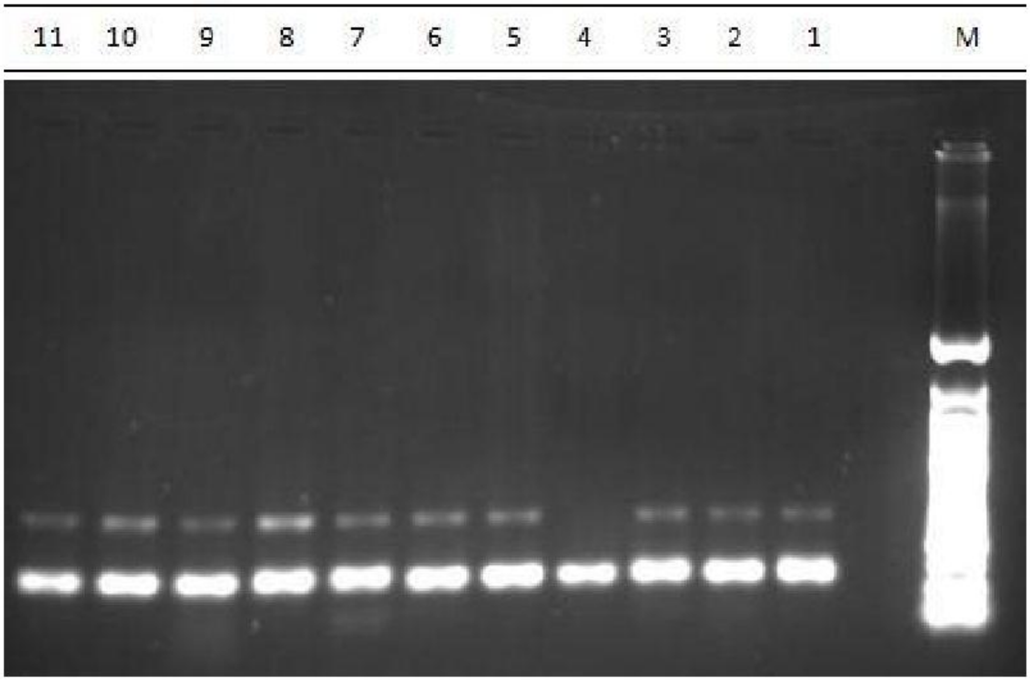
Primer 19 Fig. 1a Profiles generated by Primer umc1574 for accessions 1 to 19 (from right to left) from DNA amplification of maize



Primer 8 Fig. 1a Profiles generated by Primer umc1496 for accessions 1 to 19 (from right to left) from DNA amplification of maize



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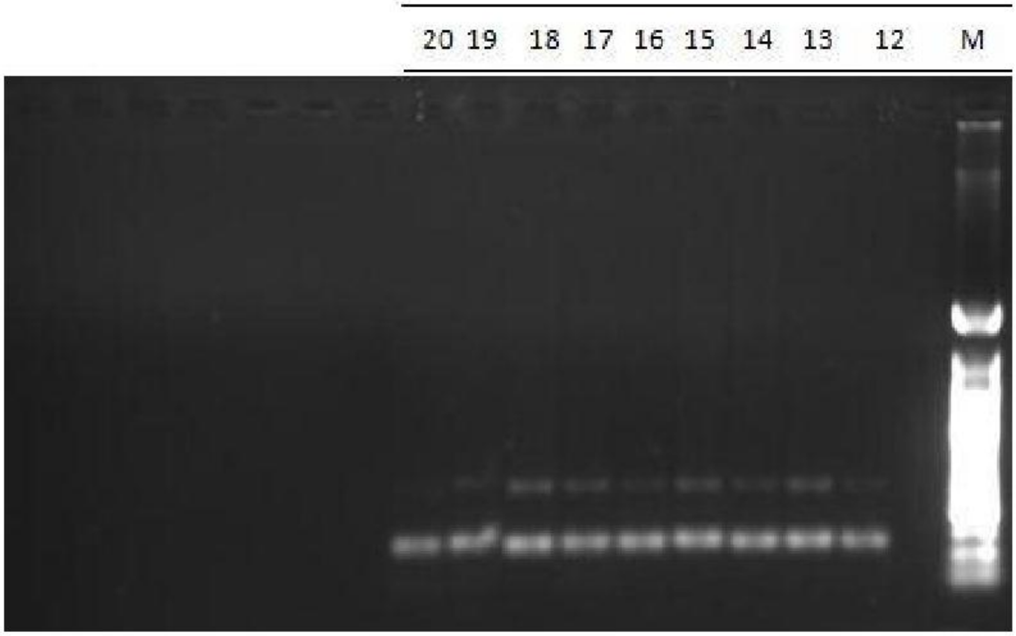
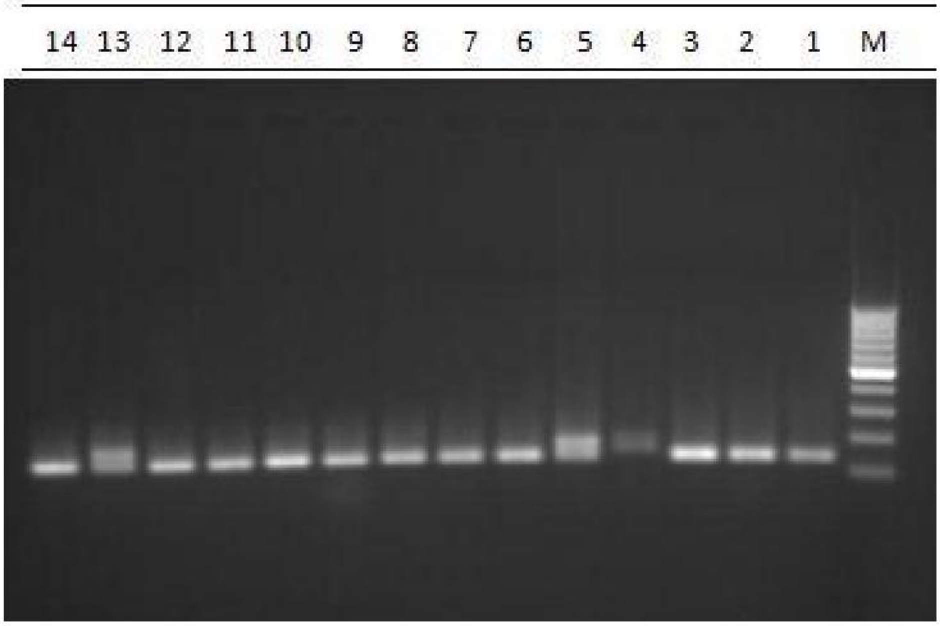


Primer 24 Fig. 1a Profiles generated by Primer umc1066 for accessions 1 to 14 (from right to left) from DNA amplification of maize



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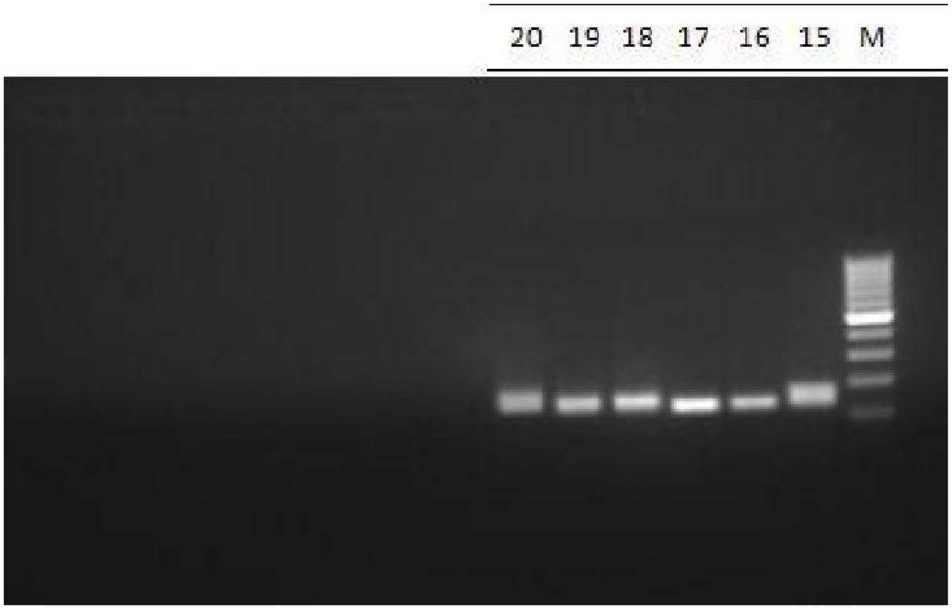
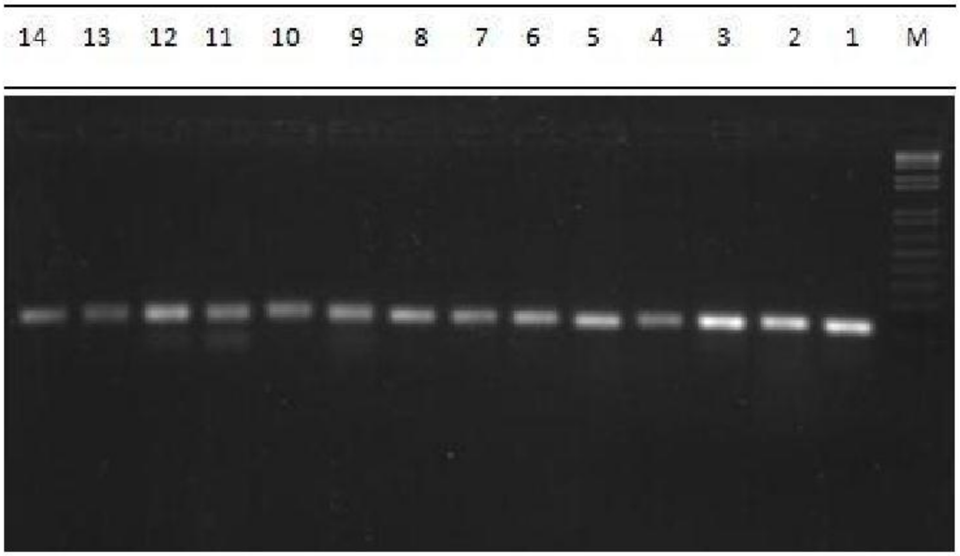


Primer 6 Fig. 1a Profiles generated by Primer umc2068 for accessions 1 to 14 (from right to left) from DNA amplification of maize



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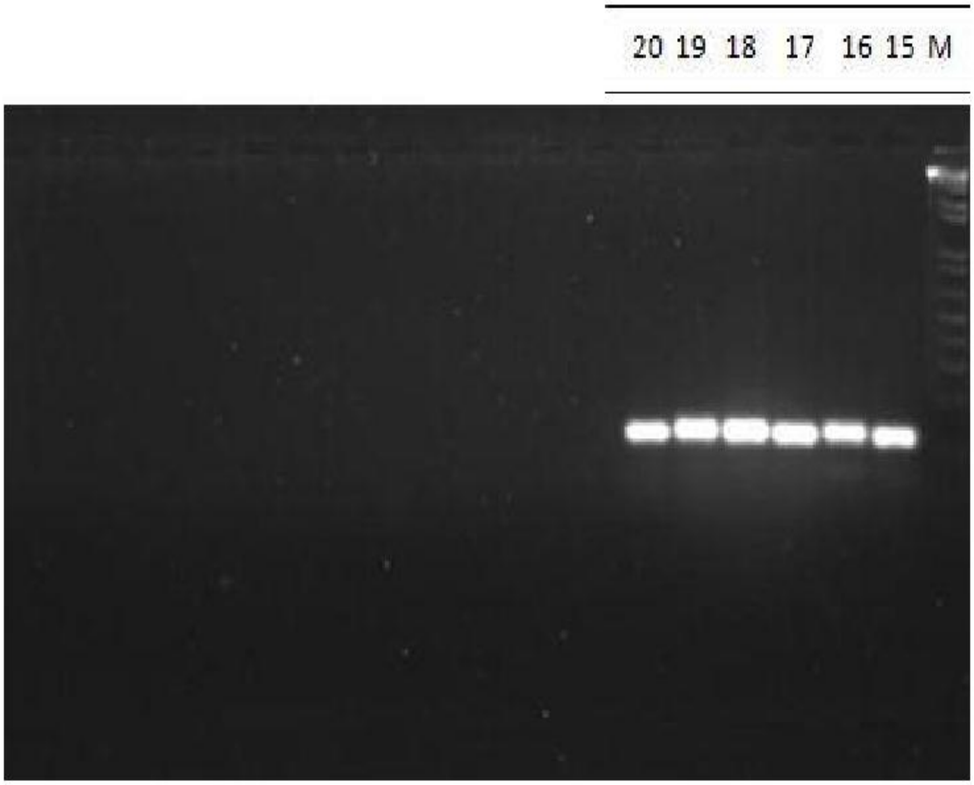
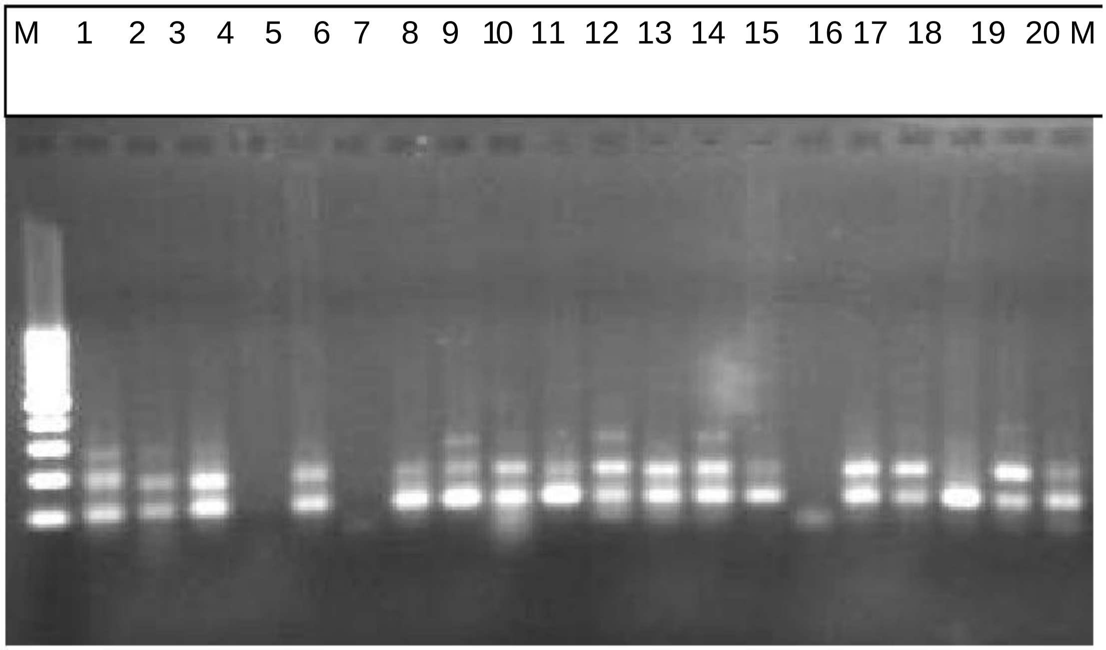


Primer 29 Fig. 1a Profiles generated by Primer umc2251 for accessions 1 to 20 (from right to left) from DNA amplification of maize



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PRIMER 20 Fig. 1a Profiles generated by Primer umc2233 for accessions 1 to 20 (from right to left) from DNA amplification of maize

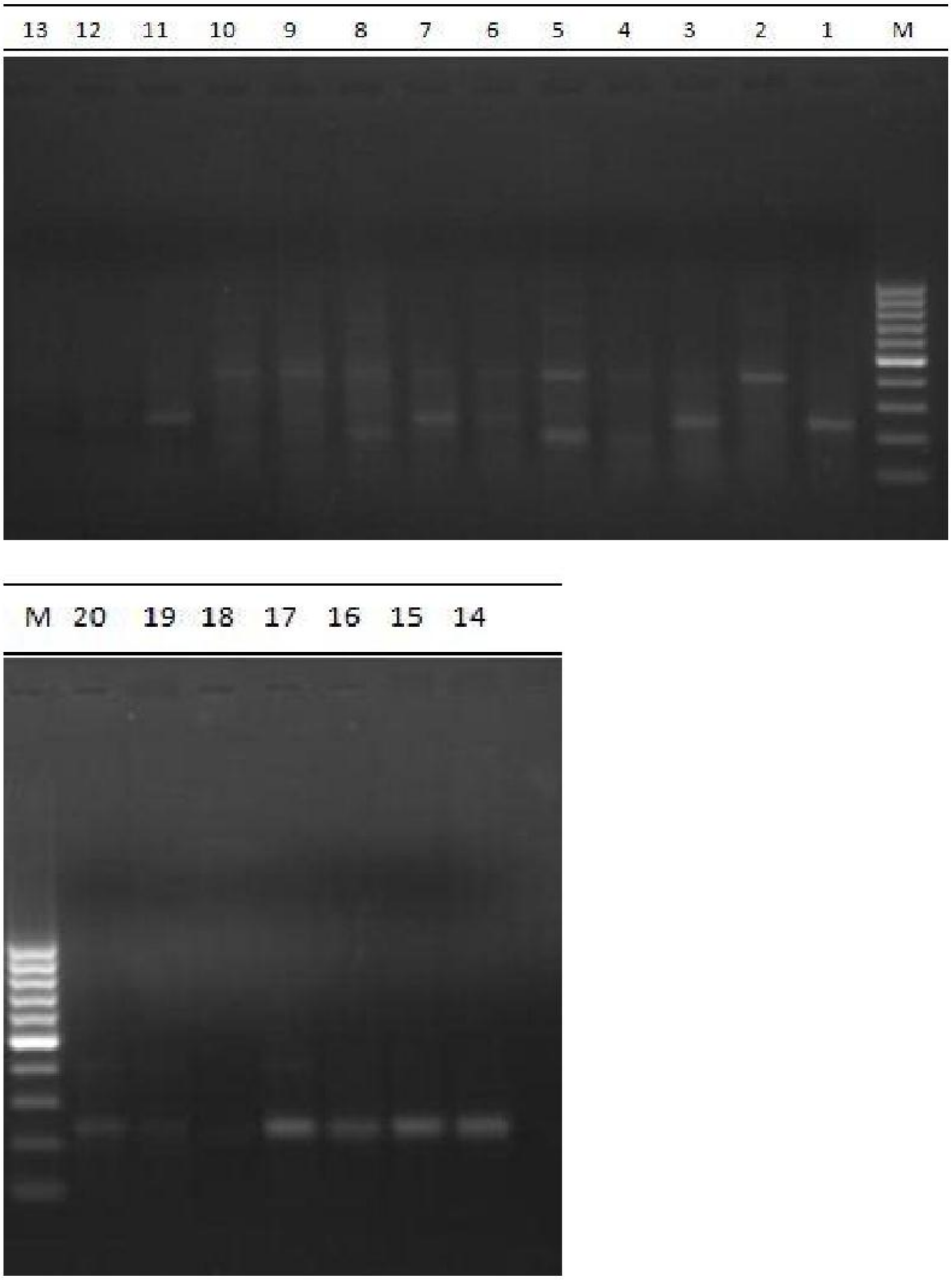

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Fig. 1a Profiles generated by Primer umc1388 for accessions 1 to 19 (from right to left) from DNA amplification of maize

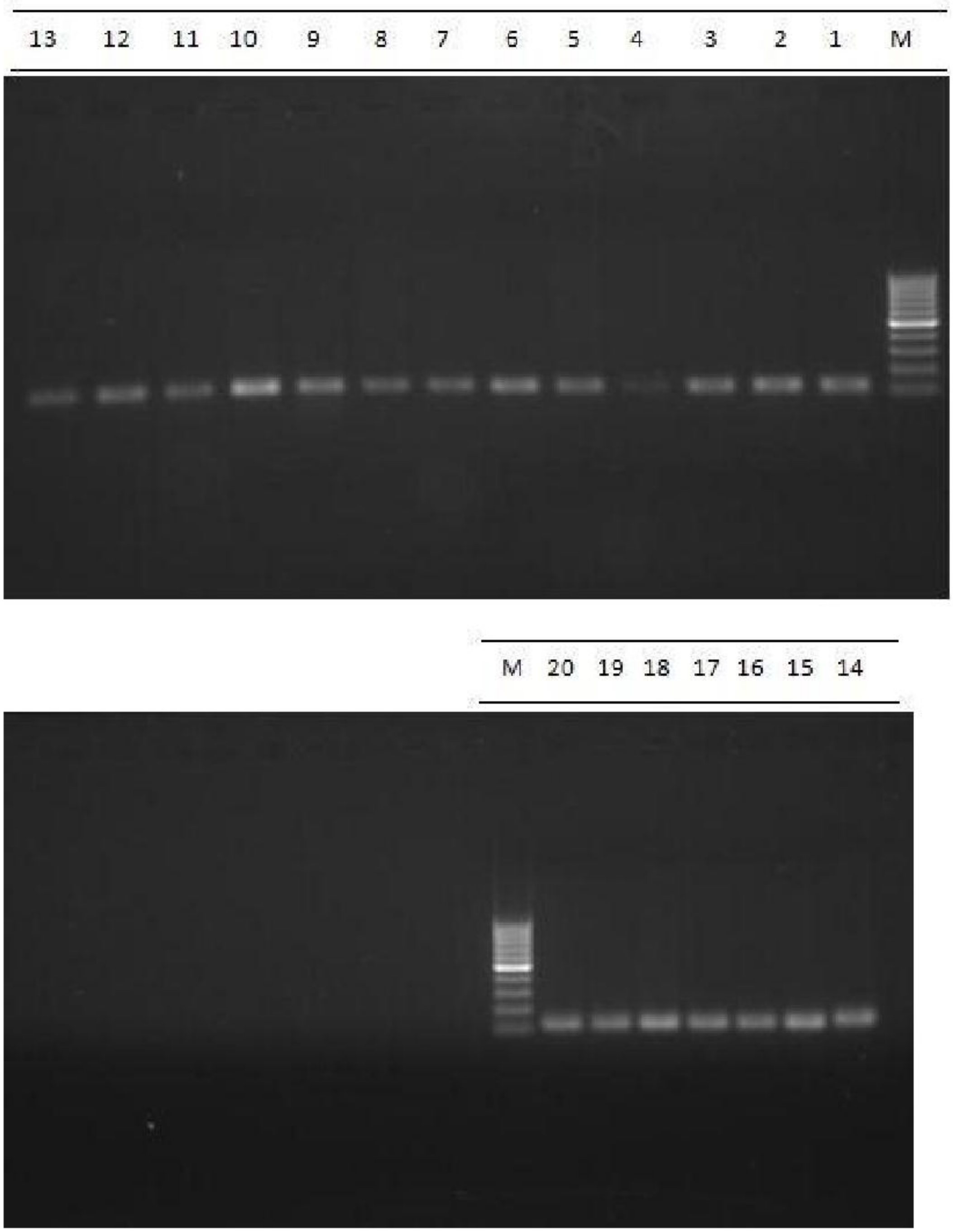

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Fig. 1a Profiles generated by Primer umc2358 for accessions 1 to 20 (from right to left) from DNA amplification of maize

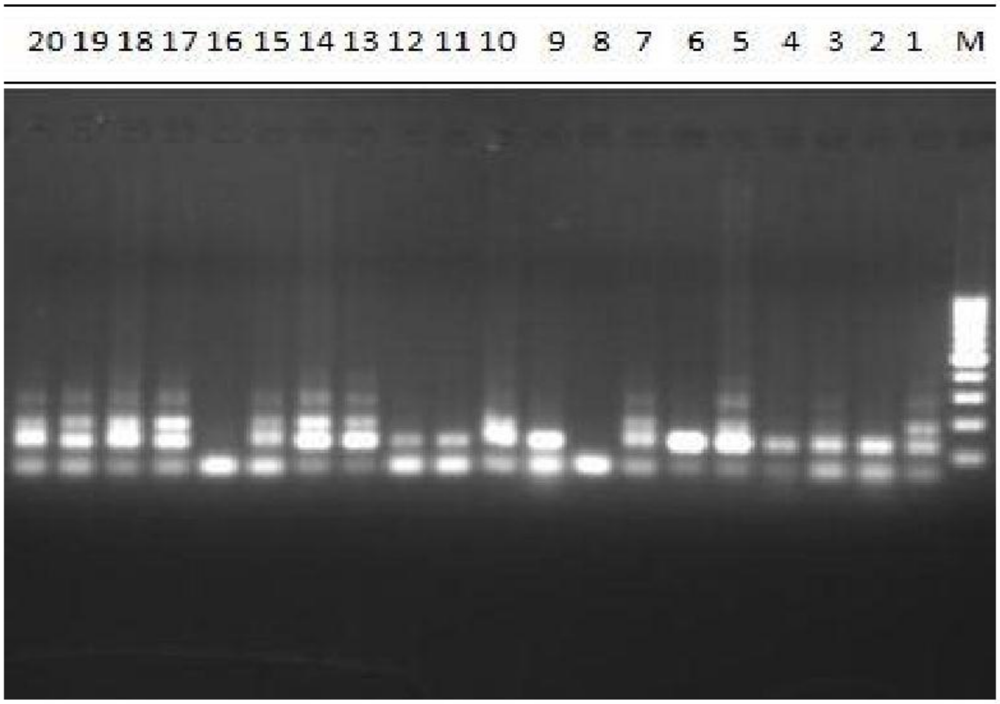

Fig. 1a Profiles generated by Primer umc1517 for accessions 1 to 20 (from right to left) from DNA amplification of maize

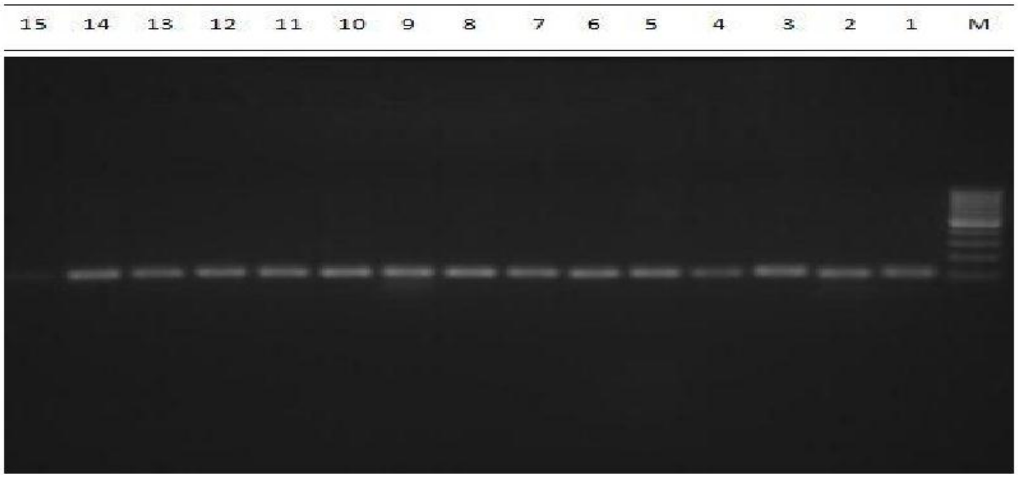

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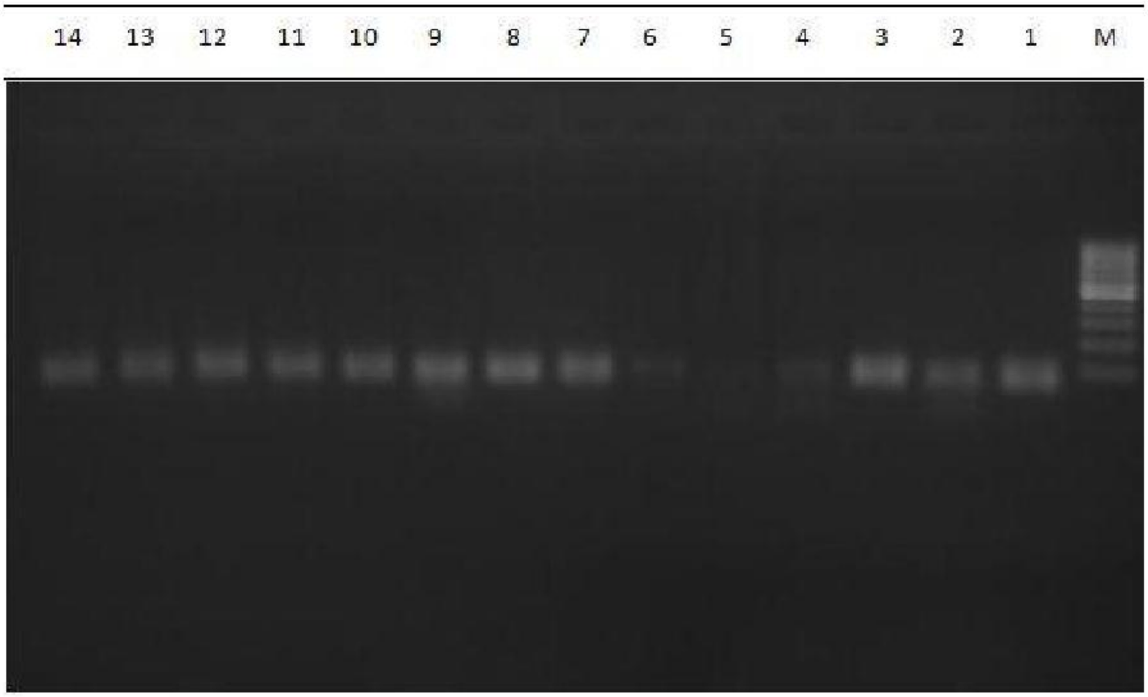
Fig. 1a Profiles generated by Primer umc1859 for accessions 1 to 14 (from right to left) from DNA amplification of maize



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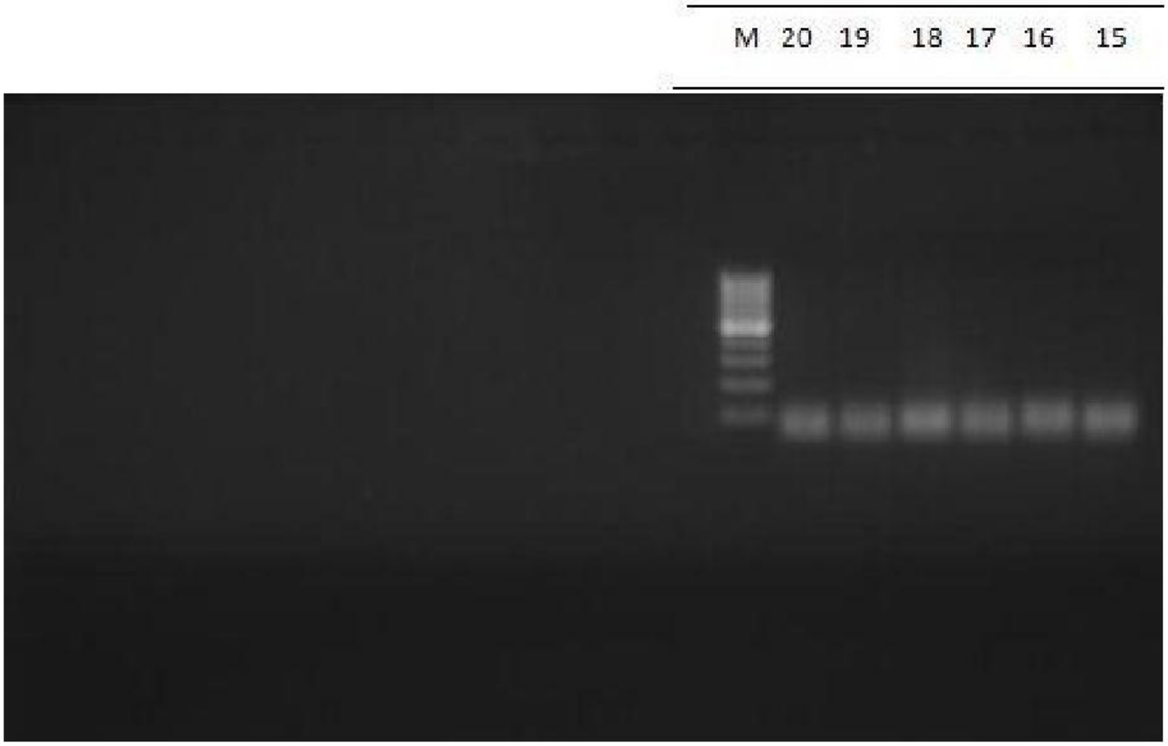
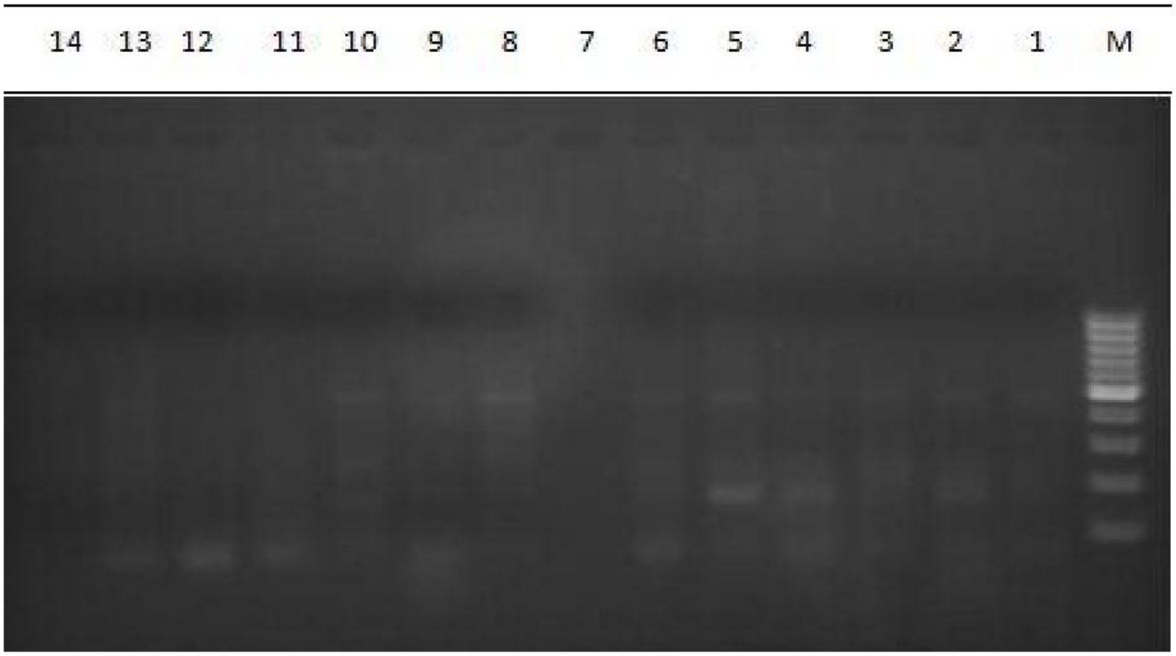


PRIMER 33 Fig. 1a Profiles generated by Primer umc1621for accessions 1 to 19 (from right to left) from DNA amplification of maize



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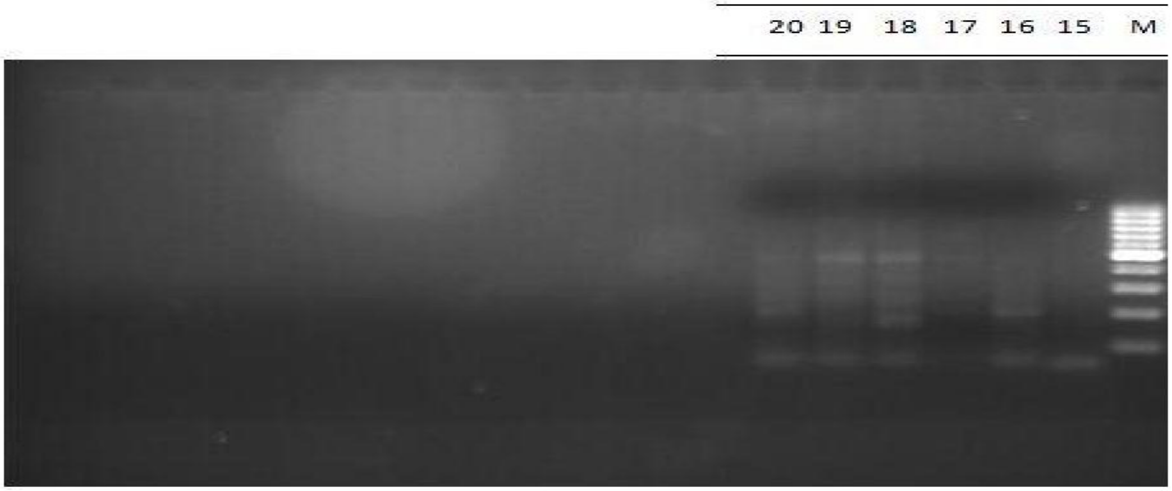

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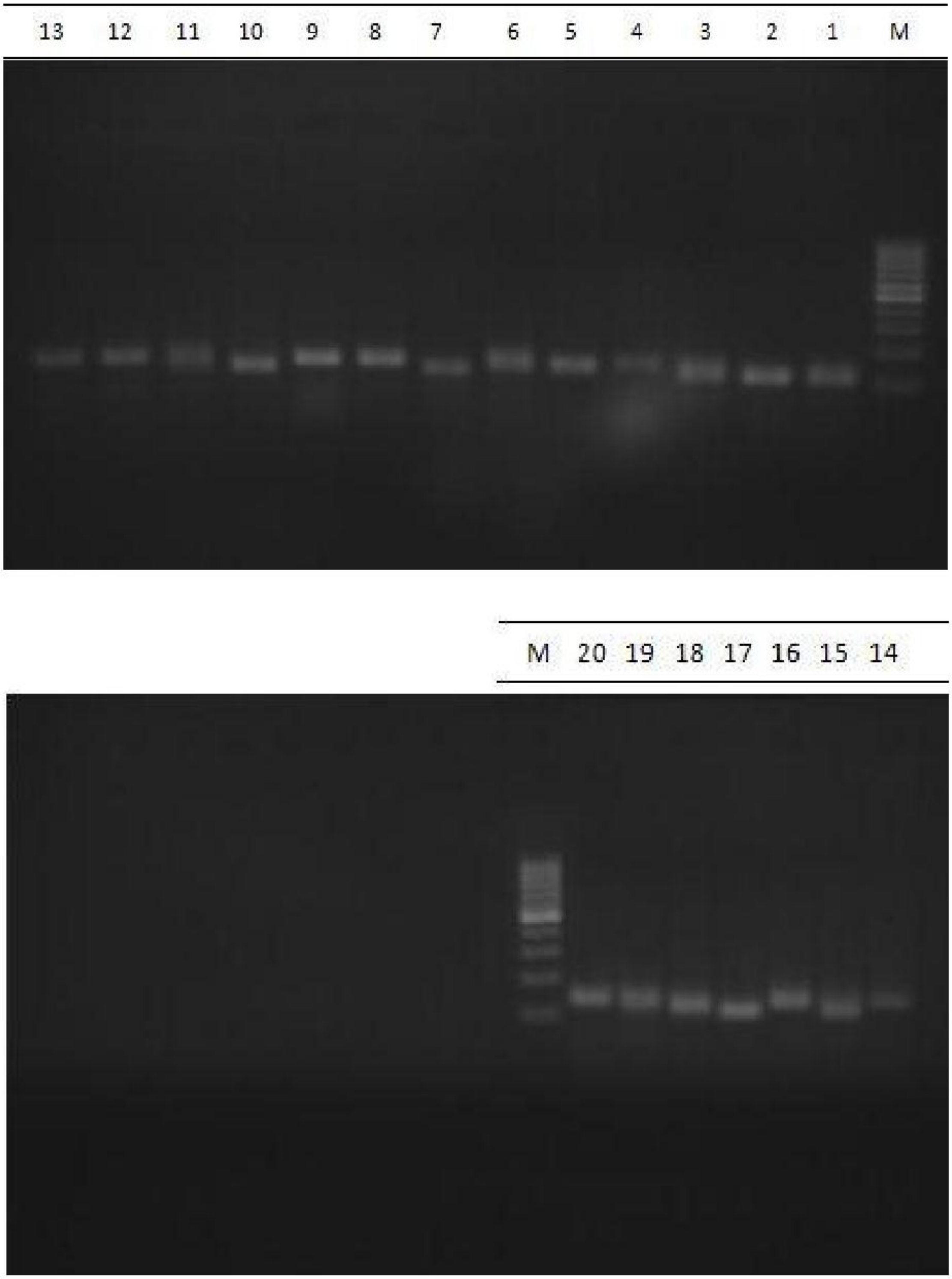
Fig. 1a Profiles generated by Primer umc2166 for accessions 1 to 13 (from right to left) from DNA amplification of maize



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Appendice III

Primer name	Primer sequence
umc1391	Forward:5'-CAA GAA CTC GTA CTC TGA CCA CCA-3' Reverse:5'- GGA TAT GGG GGA TCA TCA GGA TA-3'
umc2068	Forward:5'-CCG CTC CTT CTC CTC CTC ATC-3' Reverse:5'- GAG ACG CCT ACG AGT ACC ACA ACT-3'
ucm1496	Forward: 5'-GAT TAC AAC CCG GAG TTA CAG-3' Reverse: 5'-GCT CTT CCT AGG TGC AGA CAA AGA-3'
ucm2090	Forward: 5'- CAG AAG GAA DTT TCA GAG AGG CAG -3' Reverse: 5'-CAT CAT GAA GGA AGA AGA CGA TGA -3'
ucm2022	Forward: 5'TTA GTC TAA CCG AGT CCA ACC AGT G-3' Reverse:5' ACC AGC AGA CGG AGA GCT TG-3'
ucm1066	Forward: 5'-ATG GAG CAC GTC ATC TCA ATG G-3' Reverse:5'- AGC AGC AGC AAC GTC TAT GAC ACT-3'
ucm2166	Forward: 5'-TAC GTC GTA CAT CGC CCA CC-3' Reverse:5'-GTC GTA GCC ATA TCA GTT GGA ACG-3'
ucm2251	Forward:5'-CCT GAA TCG CTC ATT CGC TC-3' Reverse: 5'-GTC GAG GGT TTG GAG GAG AGA C-3'
ucm2233	Forward:5'-ACA CCA CGC TCT ACC TCC TCC T-3' Reverse: TTA TTT ACA GAA ACC ATG GCG TGC-3'
ucm1388	Forward:5'- CAT GGT TGC TGT AAT CTC CCC TTA -3' Reverse: 5'- TGC CAC TCC CTA CTC TCC ATA CTC- 3'
ucm2358	Forward:5'- GCA CGA GGT TTC CCT TGC TC-3' Reverse: 5'-GAC TCG CGA ATA AGG TCT GGG TT-3'
ucm1517	Forward: 5'-TAA CAC TCG GAA CCT TCC TTC TC-3' Reverse:5'-GAT GGG AAA ATG TGT GGA ATT GGA ATT TAT-3'
ucm1898	Forward:5'-ACG AGC AGC AGT CTC TTG GG-3' Reverse: 5- ACA TGG GGC ACA AGA AGCAAT -3'
ucm1621	Forward:5'-AGA CTC TGA GGA GGT AGA CGC TGA-3' Reverse:5'-GAC TCG GAG GCC TTC TAC ATG AT-3'
ucm1574	Forward: 5'-TTT CAT GTG CTT GCA GAG TTT GAC-3' Reverse: 5'-GTC ATG CAA GTA TCC GCT GTC TT-3'
ucm1727	Forward:5'-GGC TGA TCT TCT CCG CTT TTA AC-3' Reverse: 5' TTA GTC TAA CCG AGT CCA ACC AGT

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Appendice IV

Single linkage cluster analysis

Dendrogram

```
 **  Levels   85.0   75.0   65.0   55.0
 45.0

GH3351     1 ..............
dorke     18 ..)
DYF       19 ..)...)
NYR3      10 ..)
NYR5      12 ..)..) )
NYY       20 .....) )
NPR3      17 .....) )
NPR1      15 .....)       )
GH4863     2 .....)       )
NYRW2     14 .....)       )
GYY1       7 .....).. )
GH2359  6     ........)..     )
NPR2      16 ...........) )
NYRW1     13 ..        ))
NYR4      11   ..)........)   )
GH3975  3    ...........)    )
GH3981  4    ...........)    )
NYR2       9 ...........)..)..
 GH4055 5 .................)..
NYR1       8 ....................)..............
```

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Single linkage cluster analysis

Dendrogram

```
 ** Levels 100.0 90.0

NYR1    1 ..
NYRW2     9 ..)
NYR5      7 ..)
NYR4      4 ..)
NYY1     10 ..)..
NPR1      6 .....)
NPR3     11 .....)
NPR2     8 .....)
NYR2     2 .....)
NYRW1     5 .....)
Dorke     18 .....)
GYY1     20 .. )
DYF      19 ..)..)
NYR3      3 .....)..
GH2359    17 .. )
GH3981    12 ..) )
GH3351    15 ..).. )
GH3975    14 .....)..)
GH4863    16 ..... )
GH4055    13       .....)..)....
```

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