EVALUATION OF GENETIC DIVERSITY OF COTTON (*Gossypium hirsutum*) L. LANDRACES IN NORTHERN NIGERIA USING AGRO-MORPHOLOGICAL, MOLECULAR AND CYTOLOGICAL TOOLS

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN BOTANY (APPLIED PLANT GENETICS AND BREEDING)

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ABSTRACT

Cotton (*Gossypium hirsutum)* is a leading non-food Agricultural and industrial fibre crop grown in many countries. In Nigeria, it is one of the main sources of foreign exchange and raw material with nutritional purpose and health benefit. In an attempt to improve the cotton plant, diversity study was carried out using agro morphological, cytological and molecular tools. Collection of cotton landraces was conducted in major growing zones in Northern Nigeria. The collected cotton land races were grown in an experimental bag of 20 litre bucket size, using a complete randomized design (CRD). Morphological as well as yield parameters such as; plant height (cm), number of monopodial branches per plant, number of bolls per plant, boll size, boll weight (g), seed index (g) and weight of lint in (g) were determined using standard protocol. Fibre quality of the cotton genotypes were determined using high volume instrument (HVI900A). Phytochemical analysis and molecular characterisation of each of the cotton genotypes were determined following already established protocols. Results obtained revealed significant variations in plant height across all weeks with Samcot 13 having the highest plant height (106.10 cm) at maturity followed by Samcot 9 (103.00 cm). The highest number of monopodial branches was observed in Samcot 8 (21.40) while the highest number of sympodial branches was observed in Samcot 11 (16.40). The highest number of bolls was recorded in Samcot 9 (31.30) while the least was recorded in Samcot 12 (19.30). Samcot 8 had the highest boll size (11.25 cm) while Samcot 11 had the least boll size (9.60 cm). The significantly highest weight of lint per plant was observed in Samcot 8 (13.66 g) while the least was recorded in Samcot 13 (6.34 g). Correlation of the agro-morphological parameters revealed significant and positively correlation of plant height with weight of bolls per plant (r = 0.628). Number of bolls per plant was significant and positively correlated with number of monopodial and sympodial branching (r = 0.579), (r = 0.709) respectively. The estimated genetic parameters of the cotton genotypes showed high genotypic and phenotypic coefficient variance (> 30). The heritability value had the values of 46.00 and 66.57 respectively and weight of lint per plant (38.17 and 50.06 respectively). Proximate and phytochemical composition also varied significantly among the genotypes; the highest crude fat was recorded in Samcot11 (18.00 %) and the highest protein was observed in Samcot 8 (22.17 %).Samcot13 recorded the highest palmitic acid (10.74 %) while Samcot 8 had the lowest (0.85 %) palmitic acid and arachidic acid (1.76 %).There was significant variation among the pollen size and mitotic chromosome count of Samcot 8 and Samcot 9 indicated tetraploid nature of chromosome 2n=4x=52. The Widest genetic dissimilarities exist between Samcot 9 and Samcot 10; with the cotton genotype being clustered into two (2) major groups. Cluster I and II, were further subdivided into A_1 (Samcot 9); A_2 (Samcot 8 and Samcot 11) and B_1 (Samcot 12); B_2 (Samcot 10 and Samcot 13) respectively. These results indicated that each identified genotype had unique and distinct characteristics. Exploitation of these identified traits in the cotton genotypes in breeding processes could aid in the improvement of the crop for the maximization of its production in Nigeria.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
DMRT	Duncan Multiple Range Test
IITA	International Institute for Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
GC-MS	Gas Chromatography Mass Spectroscopy
IAR	Institute of Agricultural Research
PCR	Polymeric Chain Reaction
ASTM	American Society for Testing Materials
HVI	High Volume Instrument
HU	Hydroxyl Urea
FISH	Fluorescence Insitu Hybridization
GISH	Genomic Insitu Hybridization
DNA	Deoxyribonucleic Acid

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Cotton is known to be the leading industrial fibre and non-food agricultural crop cultivatedin many countries of the world (Shakeel *et al*., 2011; Vitale, 2018). From the time immemorial, man has utilized cotton for his benefits since ancient times (Goyal *et al*., 2014). The word "cotton" was derived from the Arabic word "al-qatan" (Chaudhary and Guitchounts, 2003) meaning 'fine textile'. In the report of Montes *et al.* (2017), there are four domesticated species of cotton, two tetraploid cultivars from America, *Gossypiumhirsutum* (L.) and *G. barbadense* and two diploid cultivars from Africa and Asia, namely *G. arboreum* and *G. herbaceum*. Among these four species, *G. hirsutum* (upland cotton) has dominated world cotton commerce, being responsible for about 95 % of the annual cotton, with approximate annual plantation of 35 million ha worldwide and grown in over 50 countries. According to Vivek *et al.* (2020) cotton is the world's most important natural textile fibre and an important source of feed, foodstuff, and oil, with approximate world consumption put at 27 million metric tons per year (Vivek *et al.,* 2020). The seed has approximately 25,000 cotton fibres, which are specialized single-celled trichomes that occur on the epidermal layer of the ovule (Xueying *et al.,* 2011).

The local names are Auduga in Hausa, *Ela– Owu* in Yoruba, *Na Owu* in Igbo, *wufu* in Gbagi, and *Lulu Fuka* in Nupe. The crop is used for so many purposes which include production ofincome for approximately 180 million people with the fibre industry producing $30 billion worth of raw cotton and its economic impact estimated to be approximately $500 billion/year worldwide (Vivek *et al.,* 2020). India is the largest producer of cotton which produces cotton at an average of 5,770 thousand metric tones

every year. Followed by the United States, which produces 3,999 metric tones per year (Wright, 2020) United State remains the heighest exporter of cotton and a major economic driver for some developing countries like India, Pakistan, and Nigeria among others.

Cottonseed oil is used in the manufacture of salad and cooking oils, and margarine. To a lesser extent, it is used in the packing of fish and cured meat. A small percentage of cottonseed oil is utilized to produce non-edible products like soap (Sekhar and Rao, 2011). Hence, it was rational that the genetic improvement of this high-valued multi-purpose crop would not only enhance the nutrition and lively hoods of millions of people in food challenged economies but also that its natural fibre would be competitive with petroleum in the world cotton market (Chen *et al*., 2007). The current cotton production in Nigeria as reported by National cotton Association of Nigeria (NACOTAN, 2019) is between 240,000 and 300,000 metric tons in the year 2019. According to Nigeria Raw Material Research Development Council (2004), consumption of cotton lint by textile industry in Nigeria is about 100, 000 metric tons plus or minus 15 %. Textile mills are therefore forced to import 15,000 metric tons of cotton in order to cover the shortfall in local supply and for certain specific requirement for finer yarns such as 30 - 40 inches, which is not grown locally. It is therefore, clear that the local supply is not enough to meet the demand of the product. The report further stressed that against a total demand of about 80, 000 MT of lint per annum (240, 000 MT of seed cotton) by textile mills, the total production of lint has been less, thus resulting in a gap between demand and supply. It is expected that this gap will widen further as step taken by the government to revive the textile industry may lead to increase in capacity utilization, thus resulting in higher demand forcotton.

Annual business revenue stimulated by cotton in the Nigerian economy has an excess of one hundredbillion Naira and contributed over one million jobs (Adeoti *et al*., 2020). This therefore, makes cotton one of the major value-added crops in Nigeria.

In Nigeria, cotton growing areas are divided in to three ecological zones namely Kano, Kaduna, Katsina, Sokoto, Kebbi, Zamfaraand Jigawa States.These states contribute 60-65 % of the cotton produced. Also, the eastern cotton zone comprises of Adamawa, Bauchi, Borno, Gombe, Yobe and Taraba States. Gbadegesin and Uyovinsere (1994) these states contribute 30 – 35 % of the cotton production in Nigeria. The third ecological zone also known as Southern cotton zone is made up of Kwara, Niger, Kogi, Oyo, Osun, Ondo and Edo states; it contributes 5 % of the total cotton production in Nigeria (Odedokun *et al*., 2015).

Adeneji (2011), reported that cotton production in Northern cotton zone is on the increase compared to other zones. In Nigeria, cotton (*Gossypium* spp.) is an important cash crop. It was once the fifth most important export crop and major source of foreign exchange for the country (Alam *et al*., 2013). Unfortunately, the total production of cotton presently is quite behind the national requirement of the textile industries and oil mills. Seed cotton production in Nigeria between 2001 and 2003 ranged from 381,000 metric tons to 403,000 metric tons between the early 1980s and the peak of 2005, cotton production keep increasing progressively in sub-Sahara Africa when compare to other part of the world. The net area under cotton production in Nigeria during the 2001 season was 542,000 hectare which increased to 611,000 hectares in 2002, but later dropped slightly to 610,000 hectare in 2003. Nigeria's cotton lint production as reported by Ali *et al.* (2009) increase to 100,000 metric tons in 2004 from 85,000 metric tons in 2003 due to attractive producer price and favorable rainfall pattern during the 2004 season.

The yields are relatively very low when compared to yields obtained in developed countries e.g. U.S.A (Odedokun *et al*., 2015). The low yields could be attributed to lack of seeds of improved varieties adapted to the growing zones, low level of technology in the cultural practices and low plant population.

1.2 Statement of the Research Problem

Despite the economic values of cotton, as one of the key sources of foreign exchange and raw materials with nutritional benefits and health purpose, the plant still suffers the challenges of yield reduction and low fibre quality. Update of the dominant cotton planting areas in Nigeria is found wanting in the literature. In Nigeria for instance, reduction in cotton production has been attributed largely to lack of adequate improved varieties that can cope with the ever dynamic climate condition. The lack of improved varieties could be attributed to dearth of information on the crops genetic variability particularly in the local land races.

Besides, reduction in the genetic variability of the cultivated genotypes has been due to lack of stability as well as continuous cultivation and selection leading to homogeneity in the crop. The scarcity of published information on the physical and chemical characteristics of the indigenous cotton seed oil has hindered classical breeding for the improved cotton seed oil (Michael *et al.,* 2010). The identification and selection of land races with very low palmitic acid and high nutritional content has not been well exploited. Therefore, there is need for more improvement in cotton yield and fibre qualities that could stand the taste of time.

1.3 Aim and Objectives of the Study

The aim of the study was to evaluate the genetic diversity existing among landraces of cotton in Northern Nigeria, using agromorphological, cytological and molecular tools.

The objectives of the research were to

i. Determine the agro-morphological parameters among the cotton genotypes in Northern Nigeria
ii. determine the yield and fibre qualities of the genotypes
iii. evaluate the nutritional and phytochemical composition of the genotypes
iv. characterize cotton seed oil and fatty acid composition in the various genotypes
v. determine the cytological indices of the cotton genotypes.
vi. determine the genetic diversity of the selected cotton genotypesusing polymerase chain reaction (PCR) techniques.

1.4 Justification for the Study

Cotton is an important cash crop with great economic value in Nigeria which produces lint and seed that serves as raw materials for the local textile and seed crushing industries with great economic value. Increase in breeding potential of the crop through selection will enhance and boost Nigeria economy in order to reduce over dependence on oils. Assessment of the genetic diversity has been reported to be essential breeding strategiesof cotton cultivars for characterization of individual genotypes and selectionof parental genotypes in breeding programs. Collection of desirable genotypes through characterization of germplasm for genetic variability could help in bringing up improved varieties with high yielding properties, good quality fibre and reduced the content of palmitic fatty acid; thereby encourage its cultivation and increase its production in the country. Identification and selection of genotypes with low palmitic acid content will form baseline information for breeding of cotton variety (Michael *et al*., 2010).

Cytological analysis could also reveal variations among the study genotype for the purpose of prediction of the hybridization success within the genotypes (Abubakar *et*

al., 2015). Characterization of the cotton seed oil could provide detailed information onthe oil quality, percentages of fatty acids of various genotypes thereby increasing its usability and market values. Molecular techniques had been deployed in estimating the genetic diversity in germplasm of various crops including cotton plants Surgun *et al*. (2012). This technique will provide accurate results on the genetic diversity of the crop without the influence of environmental factors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and Origin of Cotton

Cotton is a native plant to Mexico and India, cultivation began more than 6000 years ago in the Harappan cultural region of SouthEast Asia (Jack and Mc carty, 2019). The word Cotton, describes cultivated species of *Gossypium*, which comes from the Arabic word *qutum* (Ade-Ademilua and Okpoma, 2018) and canbe seen in Rig-Veda hymn, written about the fifteenth century BC. The use of cotton traced down to about 800 BC recorded in Manu's―Dharmashastra‖. Cotton production attained major development since the end of the Second World War (WWII). First cultivation of cotton happened in the old world 7,000 years ago (5th millennium BC) by the inhabitants of western Pakistan (Malik and Ahsan, 2016). Then wide spread cotton cultivation took place during the Indus valley civilization which covered the present north-western part of the South Asia which are the parts of eastern Pakistan and north-western India.

Until the modern industrialization era of India, the Indus cotton industry was developed well and several methods of cotton spinning and fabrication were being utilized. Between 2000 and 1000 BC cotton became widespread in India with the evidence found at the site of Hallus in Karnataka around 1000 BC. Domestication of cotton is claimed to Mexico approximately 5000 to 3000 BC (Eeckenbrugge and Marc, 2014). Although the exact place of origin is unknown, the primary centres of diversity are west central and southern Mexico (18 species), north-east Africa and Arabia (14 species) and Australia (17 species). DNA sequence data from the existing *Gossypium* species suggests that the genus arose about 10 - 20 million years ago (Chen *et al*., 2007).

Gossypium species are distributed in arid to semi-arid regions of the tropics and sub-tropics. Generally, shrubs or shrub like plants, the species of this genus are

extraordinarily diverse in morphology and adaptation, ranging from fire adapted, herbaceous perennials in Australia to tress in Mexico (Balock *et al*., 2014). Most wild cottons are diploid, but there is a group of five species from America and Pacific Islands which are tetraploid, apparently due to a single hybridization event around 1.5 to 2 million years ago (Jatoi *et al*., 2012). The tetraploid species are *G. hirsutum, G. tomentosum. G. mustelinum, G. barbedense* and *G. darwinii*. According to report of the Organization for Economic Cooperation and Development {OECD, (2010)}, genus *Gossypium* consists of 50 species, only four (*G. hirsutum, G. barbadense, G. arboreum, and G. herbaceum*) of which are domesticated and produce spinnable fibre. *G. hirsutum and G. barbadense* are New World tetraploids (2n = 4x = 52; AD genome species), and *G. arboreum and G. herbaceum* are Asian-African diploids (2n = 26; A) genome species. India is the only country where all four speciesare grown and where about 20 - 30 % of cotton produced consists of *G. arboreum. G. hirsutum* is considered the most important of the cotton-yielding plants, providing about 87 % of commercial cottons (Montes *et al.,* 2017). Wild cotton is a perennial shrub or occasionally as small tree up to 5 m tall. In nature it grows mainly in coastal and river overflow areas, along roads and other disturbed habitats.

2.2 Botanical Classification of Cotton

2.2.1 Systematic classification of cotton plant

Kingdom Plantae
Division Spermatophyta
Class Dicotyledonae
Order Malvales
Family Malvaceae
Genus *Gossypium*
Species *hirstutum*

Seelanan *et al.* (2010)

2.3 Botany of Cotton Plant

Cotton plant has a taproot system that grows rapidly and has the ability to reach a depth of 20 – 25 cm before the seedling emergence. After emergence and unfolding of cotyledons, lateral roots begin to develop; they first grow sideways and then downwards. The taproot continues to grow rapidly. Final depth of root system depends on soil moisture, aeration, temperature and variety but is usually about 180 - 200 cm. Under dry growing conditions, cotton roots have been known to reach a depth of 3 – 4 m. The growth of tap root as well as lateral root is affected by excessive moisture, hard dry soil layer and degree of soil alkalinity. Lateral roots adjust their quantum to the plant spacing and soil moisture regimes {Office of the Gene Technology Regulator (OGTR, 2002)}.

Cotton plant consists of an erect main stem and a number of lateral branches. The stem has a growing point at its apex, with an apical bud. As long as this bud remains active, lateral buds situated below it remain dormant. The main stem carries branches and leaves but no flowers. Length and number of internodes determine the final height of the plant. As a rule, plant with short internodes is early maturing. Length of internodes is determined mainly by the moisture supply while the number of internodes is usually a function of nitrogen supply to the plant. At the axil of each leaf there are two buds, the axillary bud from which most vegetative and fruiting branches (sympodia) develop and a lateral bud on one side of axillary bud. The lateral bud normally remains dormant; but if the axillary bud aborts, it may develop into a branch. Vegetative branches (monopodia) are morphologically similar to the main stem (Ahmad *et al*., 2008). They do not bear flowers or fruits directly, but carry secondary branches (fruiting branches), that are characterized by their sympodial growth habit (James, 2008).

Leaves of cotton are generally hairy and some varieties have glabrous leaves. Hairy leaves causefewer difficulties in mechanical harvesting and are more tolerant to jassids, but bear larger proportions of white fly which apparently finds more sheltered conditions among the leaf hairs. The leaves have variable lobes. Size, texture, shape and hairiness of leaves vary a great deal. Nectaries are present on leaf calyx and bracts. Each leaf has two buds at its axis (Vennila *et al.,* 2000).

Lateral branches arise from the axils of the leaves of main stem and consist of two types *viz*. vegetative and fruiting. Vegetative branches are more vertical and ascending. Fruiting branches are nearly horizontal. The internodes on the fruiting branches, referred to as sympodial branches are not straight as in main stem but have a zig zag appearance with the leaves alternately placed. The flowering and fruiting are dependent on the initiation of sympodial branches. The timing of the crop for harvest is also determined by the early or late production of such sympodial branches on the plant body. Very early varieties have their fruiting branches even at first or second node to the total exclusion of vegetative branching from leaf axils. Similarly, very late varieties go on producing a very large number of monopodial before sympodial divergence appear. Relative proportion of vegetative and fruiting branches is dependent on temperature, day-length, plant density and the rate of boll shedding (Jatoi *et al*., 2012).

Floral bud is enclosed inside three triangular protected bracts, the whole structure is called a "square". The five petals of the corolla are wrapped tightly around one another. Within the corolla is a tube formed of numerous stamen filaments, surrounding the pistil. The ovary at the base of the pistil consists of generally three to five carpels, containing as many locules. Each locule contains 8 - 12 ovules (Oosterhuis and Cothren, 2012).

Flower is large, axillary, terminal and solitary. On account of the sympodial development of fruiting branches, the flower opening follows a spiral course in acropetal and centrifugal succession. The innermost bud of the lowest and oldest branch is the first to open while the outermost bud of the highest and youngest branch is the last to do so. When the flower opens it is white or creamy white or yellow in the American varieties, changing to pink towards the end of the day and becoming red the following morning; on the third day the petals wither and fall (Oosterhuis and Cothren, 2012).

2.3.1 Bolls

In *G. hirsutum* bolls are large, generally ranging from 4 - 5 g. The general variation in boll weight is 3 - 5 g, however in some varieties it can weigh upto 8 g. The bolls are pale green, smooth skinned and with few oil glands. In contrast, bolls of *G. arboreum* are smaller (1.5 – 3 g), dark green and covered with numerous glands. Cotton plants by its remarkable auto-regulatory mechanism shed the bolls that are in excess of the load capacity of the plant under given environmental conditions. As a result, the ratio of bolls to total vegetative growth is fairly constant (Shakeel *et al*., 2011). In general, varieties or strains with large bolls do not adjust so well to change in environment and to stress as do types with smaller bolls. Hence, shedding occurs more readily and to a large extent in the former than in the latter case. Bolls developing under falling temperature need more days to mature than those growing under rising temperature (Oosterhuis and Cothren, 2012). The big-bolled American types in India take about 40 - 50 days while the Asiatic cottons require 35 days to mature. In *G. hirsutum* the boll consists of four to five locules each of which contains about 7 mature seeds. A fair percentage of the ovules remain undeveloped due to non-fertilization, heredity and environment, these are called “motes”. The size and shape of the bolls differ in diploid and tetraploid cotton (Khadi and Kulkami, 2001).

The full-grown seed of cotton is irregularly pear-shaped, varying in size depending on the variety and conditions of growing. It may be naked or bear short hairs called “fuzz”. All cultivated cotton seeds bear long fibres named “lint” and a majority of them also have fuzz on the same seed. The lint is removed by gins while the fuzz remains attached. The colour of fibres is generally white, but may also be brown or green and that of the seed is usually grey, brownish or black (Ahmad *et al*., 2008).

The mature seed has two cotyledons folded up that occupy the entire portion of its cavity. The cotyledons are broad and kidney shaped. Delayed germination in some of the species and varieties may be due to hard seed coat, closed micropyle and partially filled cotyledonary-cum-embryonic contents. The germination increases when the seed coat thickness is reduced by various methods of delinting. The seeds account for about 65 to 70 per cent of the total yield by weight. The seeds are rich in protein (10 – 20 %) and oil (up to 25 %). The oil content in *G. barbadense* is higher than *G. hirsutum* (Bhangu *et al*., 2017).

2.3.2 Seed hairs/Lint

Lint and fuzz represent the outgrowths of epidermal cells on seeds. Some cells continue to lengthen, as in tetraploid cotton while others stop growing after a time. The former is known as tetraploid cotton. The lint hair is unicellular and its development is phased in two stages, the first phase is a period of elongation and the second phase is increase in thickness. A lint cell bulges first, the protoplasm inside turns granular, and the nucleus moves towards the bulge. The swelling enlarges until it is twice the diameter of the original cell and the nucleus moves to or near the tip. The elongation of cell may take upto 40 days. There is no change in thickness. The growth is not regular; slow at first but fast from about the 15th day. The rate slackens during days and quickens during nights. The cell wall thickens in the second half of boll maturation. Deposits of cellulose

are formed on the inside of primary wall. They are laid in layers as seen from some fibres showing as many as 25 concentric layers. As soon as the boll dehisces, the hairs dry, collapse and flatten the cylindrical form, assuming ribbon like shape and go into spirals. The mature hair is uniform in diameter up to ¾ the length and then gradually tapers to a point. The length of lint is a varietal character and varies from 15 – 50 mm indifferent varieties (Bourgou *et al.*, 2018). Fibre quality traits such as length, fineness and strength are important as spinning are dependent on these characteristics (Bourland and Gubr, 2017).

2.4 Factors Contributing to the Variability of Cotton Fibre Quality Attributes

The quality of cotton fibres depends on many factors, including the growth and development of the cotton plant, the indeterminate growth habit of the cotton plant, developmental stages of the cotton fibres, genetics, and environmental conditions during a growing season, and handling during harvest and processing (Faulkner *et al.*, 2012). Even though Upland cotton (*Gossypium hirsutum*) is a perennial shrub with indeterminate growth habit, it has been adapted to the annual cropping systems globally. However, because cotton has an indeterminate growth habit, it produces both reproductive and vegetative biomass at the same time in a sequential pattern. In a season, cotton plants set bolls at different positions on the plant. The number of bolls set is uniquely affected by growth conditions, which ultimately influences the within-plant variability of cotton fibre quality. Although cotton fibre quality such as length, length uniformity, strength, micronaire, and color and leaf grade are determined after the crop is harvested and ginned, it is influenced by several factors throughout the season. Management practices such as plant population density (Bednarz *et al.*, 2006), soil nutrition, and irrigation influence the growth of the cotton plant Feng *et al.* (2010), which also has a large impact on within-plant lint yield and fibre quality variability.

Genetic factors, environmental conditions and the complex interaction between genetic and environmental conditions influence each fibre development stage. In cotton fibre development, initiation determines the number of fibres per unit surface area, fibre elongation determines the length of the fibre, and the secondary cell wall synthesis determines the cellulose deposition, which leads to fibre maturity (Wakelyn *et al*., 2007). The maturation stage during fibre development determines further cellulose deposition and reorganization of the cellulose. Thus, this stage of fibre development has a potential impact on the strength of the cotton fibres. Changes in any environmental conditions throughout the growing season will contribute to the within-plant variability of cotton fibre quality that could affect yarn quality (Kelly and Hequet, 2013).

2.5 Fibre Qualities

The order of importance of fibre properties in open-end spinning is strength, fineness, length, cleanliness, friction, and micronaire, while the order for ring and vortex is fibre length, strength, fineness, friction, and uniformity (Bhangu *et al*., 2017). The predominate global production of ring spun yarns and the expected future dominance of vortex spinning (Eric Hequet, Fibre and Biopolymer Research Institute, Texas Tech University, personal communication) mandates that U.S. cotton breeders consider fibre length and strength, as well as other fibre properties, during the selection process if the U.S. is to maintain a competitive edge in the global fibre market. The minimum requirements of upland cotton in the world market are upper half mean length (UHML) of 28.2 mm, 263 kN m kg-1 fibre bundle strength (Str), and micronaire (Mic) between 3.6 and 4.8; whereas UHML of 26.7 mm, Str of 250 kNm kg-1, and Mic of 3.5 to 4.9 are of non-discount quality upland cotton in the U.S. (Bourland and Jones, 2012). Upland cotton has to compete with international standards and with man made fibres, which provide stable and predictable fibre characteristics. Ayele *et al*., (2017) suggested

that genotype stability for fibre quality was an important selection criterion for cotton breeders, while Bowman (2000) suggested that such stability was important in the selection of parents to initiate breeding cycles. SAS (2015) and Paterson *et al*. (2003) noted that genotype x environment interactions impact the selection of superior fibre genotypes. Bhangu *et al*. (2017) noted that repeatability, as an indicator of stability, was first developed in the early 1900s Cotton fibre is a source of natural textile, and (Yu *et al.,* 2012; He *et al.,* 2013).

2.6 Demands for Higher Quality Upland Cotton Fibre

Advancement in textile manufacturing technologies such as ring spinning requires higher quality cotton fibre to produce better quality products. Furthermore, even though cotton fibres produced in the U.S. is currently trading well on the export markets, it faces increasing competition from synthetic fibres. The global production of man-made fibres has experienced considerable growth since 2000. In 2000, some 31 million metric tons of manmade fibres were produced. Within a fifteen-year period (2000 to 2015), the production of manmade fibre was increased from 31 million metric tons to 66.88 million metric tons (USDA, 2019). The challenge from manmade fibres is seen not only in price but also in quality as perceived by customers. Because of a lack of fibre variation within a given lot of synthetic fibres, its performance in the clothing industry is becoming a benchmark for cotton spinning (Ayele *et al.*, 2017).

Conversely, the high natural variability of cotton fibre quality is a challenging issue for both improvement programs and manufacturers. The major problem associated with cotton is the high natural variability of fibre quality. Cotton fibre quality is variable within a single seed, within in a single boll, within-plant, within the field and among genotypes (Bednarz *et al*., 2006; Kothari *et al*., 2015). Among all these different levels of variability in fibre quality, within-plant variability is expected to be the highest and

could be one of the largest contributors to the within-bale fibre-to-fibre variability. The within-plant variability of fibre quality could be impacted by the variation of fibre quality occurring on a single seed. One reason for the variation of fibre quality on a single seed is the non-uniformity of fibre initiation on the ovule (Ayele *et al.*, 2017). Cotton is an indeterminate growth plant, this could influence the within-plant variability of fibre quality. Bolls set at a different position on the plant are uniquely affected by the change in environmental conditions. Each boll at different positions on the plant consists of several seeds that are naturally variable in fibre quality. The sum of the variability in fibre quality within-seed and within-boll contributes to the within-plant variability of fibre quality. The different stages of fibre development, which are very sensitive to environmental variations, the genetic potential of different cotton genotypes, the interaction between genotype and the environmental variations could also contribute to within-plant variability, which ultimately affects the within-bale fibre-to-fibre variability of cotton fibre quality. Yarn made from a bale with variable fibre quality will result in poor finished product and increased the cost of production (Ayele *et al.*, 2017).

Domestic and foreign mills customers for U.S. and foreign cotton are demanding higher quality fibre. Traditionally, cotton pricing is largely determined by factors such as staple length, grade, color, and micronaire (Ayele *et al.*, 2017) Spinners do not overlook the importance of fibre properties as it affects the spinning performance and yarn quality, and ultimately the finished products. The textile industry has been striving to improve the quality of the product and efficiency through automatic high-speed machinery. New technology demands cotton with better fibre strength, uniformity, maturity, fineness, and lower neps and short fibre content that are very determinant in spinning performance and dyeing ability (USDA, 2019; Faulkner *et al.*, 2011). Contaminants such as foreign matter, stickiness and seed coat fragments continue to be among the

most serious problems affecting the cotton industry worldwide and need to be minimized.

2.7 Cytogenetic Studies in Cotton Plant

The genus gossypium is composed of 45 diploid and 5 tetraploid species. Among them, four species are cultivated: *G. hirsutum* (2n = 4× = 52), *G. barbadense* (2n = 4× = 52), G. *arboreum* (2n = 2× = 26), and G. *herbaceum* (2n = 2× = 26) (Wenbo *et al*., 2016). Cytogenetic analysis and chromosome manipulation are essential to genetic and genomics analysis, as well as germplasm introgression (Andres and Kuraparthy, 2013). Molecular cytogenetic techniques, especially fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH), are excellent tools to study the structure and function of genomes, chromosome land marks, distribution of recombination across the chromosomes, polyploidy, aneuploidy, alien gene introgression and genome evolution. However, efficiency and success of these capabilities depends upon good chromosome preparations (Andres and Kuraparthy, 2013).

Higher mitotic indexes and well-spread or dispersed chromosomes whose morphology was well preserved during the preparation process are an essential requirement for metaphase chromosome spreads to be used in FISH and GISH experiments. Efficient chromosomal spreads with less contamination and/ or hindrance from cell wall debris are even more important for performing FISH with single copy probes in plants. This is especially true in cotton due to the copious and diminutive disposition of the chromosomes, which plagues the identification of physical landmarks such as centromeric constrictions and the discrimination of sister chromatids in mitotic metaphase cells. Besides having numerous small chromosomes, it has been observed that cotton is a particularly troublesome species in regard to chromosome aggregation (Halfmann *et al*., 2007). Furthermore, due to its dicotyledonous nature, cotton has only

the main taproot available for mitotic chromosomal studies. In the absence of efficient chromosome preparation this often becomes a limiting factor for analyzing single plants using FISH and GISH techniques, especially when chromosome morphology and cytological landmarks are pivotal for such analysis. To date, the majority of cotton cytogenetic work has used the squash method procedures to spread chromosomes (Wang *et al.*, 2008; Peng *et al.,* 2012). The squash technique, which involves the placement of a cove slip over a dissected root tip or previously digested cells to flatten and spread the chromosomes, can cause damage and loss of chromosomes and requires a high degree of technical skill. Therefore, there is a critical need to develop a protocol that could increase the mitotic index and improve the efficiency of cell synchronization and chromosomal spreads, while limiting contamination from cell wall debris in the preparations used for in situ hybridizations.

Several methods have been used to increase cell synchronization and mitotic index in plants such as ice water, hydroxyl urea (HU), mimosine, aphidicolin, and nutrient deprivation (Halfmann *et al.*, 2007). Historically, compounds such as colchicines and amiprophos-methyl (APM) are then used to arrest root cells at metaphase (Halfmann *et al.*, 2007). Systematic analyses incotton were done by (Halfmann *et al.*, 2007). They investigated procedures for cell synchronization of root tips using hydroxyl urea as well as the efficacy of several known chemicals for metaphase accumulation. Anti-tubulin compounds provided average metaphase indices of 0.3 or higher on synchronized root tips and were preferable to colchicine (Halfmann *et al.*, 2007). In animals, cells have long been treated with a hypotonic solution to improve the spread of chromosomes through swelling and bursting of the cells. However, this approach is not aseffective in plants since the presence of the cell wall inhibits lysis. Halfmann *et al.* (2007) noted that nitrous oxide has the highest dispersing effect in cotton, though the mitotic index

observed in these spreads was less than expected using the traditional squash method of chromosome preparation. However, the best chromosome spreads were achieved when a nitrous oxide treatment was combined with the drop method in corn and wheat (Danilova *et al.*, 2012). Procedures for the isolation of meiotic pachytene and interphase chromosomes in cotton have been described by Peng *et al.* (2012) but a protocol which can yield efficient mitotic metaphase chromosome preparations is not available in cotton.

2.8 Phytochemical Composition in Cotton

The major chemical constituents of *Gossypium herbaceum* are flavonoids, tannins, carbohydrates, saponins, steroids, terpenoids, glycosides, resins, phenols and proteins (Patel and Mishra, 2017). The leaf extract of *G. herbaceum* were screened to have condensed tannins i.e Proanthocyanidins which can undergo hydrolysis yield Cyanidin and 0 - 75 % del-phinidin. The tannins combination with flavan-3-ols, mainly (+)-catechin and (+)-gallocatechin which are associated with lesser concentrations of (-)-epicatechin and (-)-epigallocatechin (Kumar *et al.,* 2011).

The seed extract of *G. herbaceum* were screened to have glycosides, steroids, resins, carbohydrates, saponins, proteins and phenolic compounds tannins. The main chemical constituents of *G. herbaceum* seed is gossypol. It is a polyphenolic compound present to 0.4 - 2.0 % in the kernels. The presence of two aldehydic groups and six phenolic hydroxyl groups makes gossypol chemically reactive. Gossypol can participate in oxidation, methylation, ozonolysis, Schiff base formation to produce gossypol derivatives (Hemant *et al.,* 2012). Carbohydrates, flavonoids, tannins, steroids, saponins, terpenoids, resins, phenols and proteins are screened from flower extract of *G. herbaceum*.

2.9 Glands in Cotton Plant

Aerial parts of cotton contain internal glands which in different species vary in size, number, distribution and pigmentation. These glands secrete a volatile oil i.e. gossypol and related compounds. Gossypol is a polyphenolic yellow pigment and is toxic to non-ruminants. The presence of gossypol makes cotton seed cake toxic and hence, glandless varieties have been bred in recent years. However, it has been found that glandless varieties are susceptible to a wide range of pests over the glanded varieties (James, 2008).

2.10 Mineral Composition of Cotton

The mineral composition is an important parameter in evaluating cottonseed quality for diff erent applications. Genetic, environmental, and agronomic approaches have been used to alter cottonseed composition (Yang *et al.*, 2017). Cottonseed oil and protein contents can vary from 17 to 27 % and 12 to 32 %, respectively, among genetic variations (Dowd *et al.*, 2010; Yu *et al.*, 2012). Yang *et al.* (2017) found that N fertilization increased N levels in cottonseed with altered amino acid concentration. Pettigrew and Dowd (2011) reported that varying planting dates or irrigation regimes altered cottonseed composition in terms of protein, oil, gossypol, and soluble carbohydrates.

Poultry litter (PL) a mixture of manure and other external materials, such as bedding materials) is a byproduct of the poultry industry and contains valuable plant nutrients. Managed appropriately, land application of PL as a fertilizer is an efficient and environmentally-acceptable method of recycling nutrients and organic matter. Long-term application (up to 20 years) of PL into pasture soil continuously increased the hay yield in the Sand Mountain region of Alabama (He *et al.,* 2008). Similarly, fertilizing cotton plant with PL often results in a yield increase (Endale *et al.*, 2002; Reddy *et al.*,

2007; Tewolde *et al*., 2011). However, the information on the effect of PL application on mineral composition of plants is very limited even though PL contains many mineral elements (Schroder *et al*., 2011). Citak and Sonmez (2010) evaluated the effects of the types of fertilizers (farmyard manure, chicken manure, blood meal, and chemical fertilizer) on nutrient content of the edible part of cabbage (*Brassica oleracea*) plants during two consecutive seasons. They found that the mineral contents of cabbage receiving organic applications tended to be higher than cabbage receiving chemical fertilizers, and on the whole, cabbage responded the best to farmyard and chicken manure as a mixture or separately. Demir *et al*. (2010) measured 17 elements (N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mn, Mo, Cl, Si, Br, Rb, Sr, and Ba) in tomato (*Lycopersicon esculentum* Mill.) leaves and fruits grown in soil fertilized with poultry manure. These researchers concluded that the increased fruit yield, the increased concentration of Zn (an element required in the human diet) and the lower concentration of potentially harmful Br in the fruit make poultrymanure a valuable soil amendment for tomato production. Tewolde *et al*. (2011) comparatively evaluated mineral nutrients of mid-season cotton plants fertilized with PL and ammonium nitrate. Their data suggested that the better yield performance of cotton fertilized with PL compared to ammonium nitrate fertilization was due to a more ideal soil pH, favorable tissue Mn concentration, and improved K and B nutrition. They did not report effect of PL application on the mineral composition of cottonseed (He *et al*., 2013)

2.11 Lipid and Fatty Acid Composition of Cotton Seed Oil

Cottonseed oil is the second most common oil being used today besides sunflower oil. As the most important vegetable oil source in Turkey, sunflower is first ranked with 1.38 million tons, followed by cottonseed with 1.28 million tons, soybean 180,000-ton, peanut 141,000 tons and rapeseed 101,000 tons (Kolsarici *et al*., 2015). Cottonseed oil

is usually used in vegetable oil mixtures (Mert *et al*., 2015), cooking and saladoil, in the preparation of margarine, shortening, mayonnaise and sauces, also to less extend in canned fish and smoked meat (Sekhar and Rao, 2011). Crude cottonseed oil, which has an aroma resembling peanut and walnut, has a blurry appearance (Parali, 2003). Colour of crude cottonseed oil can vary from brunette yellow to dark red dueto significant amount of colour pigment passing to oil during extraction (Orhevba and Efomah, 2012). In addition to triglycerides, nonglyceride components such as gossypol, phospholipids, sterols, pigments, tocopherols and carbohydrates are found in this oil with the amount of about 2 %. Cottonseed oil also has a rich source of minerals, it includes vitamin B andoil soluble vitamins such as A, D, E, K (Lukonge, 2005; Sawan *et al*., 2006). Cotton is regarded as one of the important conventional oilseed crops with potential to bridge the existing gap between the supply and domestic demandof vegetable oils (Sekhar and Rao, 2011).

Cotton as a major crop covers about 2.5 % of the worlds cultivated lands and is also recognized to be a dual-purpose crop, used both for its natural fibre Konuskan *et al.* (2017) as well as contributes to almost 4 % of the world's vegetable oil production. In Pakistan, it contributes 65 – 70 % in the local edible oil industry. Cotton seeds are directly or indirectly employed inhuman food and the livestock (Konuskan *et al*., 2017). The seeds are a good source of oil and protein (Saxena *et al*., 2011). Technically, Cotton seed oil (CSO) is suitable as ingredient for baked goods and cake icings andis being employed for domestic frying and preparation of margarine (butter substitute) and hydrogenated vegetable oil/vegetable ghee (Sekhar and Rao, 2011). CSO is a good source of an essential fatty acid (linoleic acid, C18:2) as well as contains reasonable amount of oleic acid. The oil has good oxidative stability due to its high antioxidant tocopherol contents and oleic acid (Sekhar and Rao, 2011). As far as the industrialuses

of cotton seed oil are concerned, this oil is in use for preparation of alkyl resins for interior paints, special bio-lubricants and soft soaps (Saxena *et al*., 2011). The oil has also been explored as a feedstock for biodiesel production (Reddy and Aruna, 2009).

The physicochemical properties and attributes of vegetable oils, in particular, the fatty acids profile, is animportant factor in establishing their commercial uses and market value (Akubor, 2008; Okonkwo and Okafor, 2016). It is commonly accepted that the yield, chemical composition, nutritive and functional properties of plantseed oils are significantly affected by the genetic/varietal, agroclimatic and geographic factors.

The important component of cottonseed oil is tocopherols and natural antioxidants. However, the amount of tocopherols that present in oil declines significantly during the refining process. Therefore, crude cottonseed oil when compared to refined cottonseed oil and soybean oil is rich in termsof amount of tocopherol and, more resistant to oxidation (Saxena *et al*., 2011; Sekhar and Rao, 2011). Fatty acid composition of cottonseed oil is one of important properties (Lukonge, 2005; Ping *et al*., 2009). Cottonseed oil has a 2:1 ratio of polyunsaturated to saturated fatty acid. It is described as naturally hydrogenated because its fatty acid profile generally consists of 70 % unsaturated fatty acids, including 18 % mono-unsaturated (oleic) and 52 % poly-unsaturated (linoleic), and 26 % saturated (primarily palmitic and stearic) acids. These make the oil stable for frying without the need for additional processing or the formation of trans-fatty acids (Sekhar and Rao, 2011). As with other vegetable oils quality of cottonseed oil usually comesfrom fatty acid composition and unsaponifiable matters mentioned. Their amount and oil yield vary depending on genotype, ecological conditions of region process and storage conditions (Reddy and Aruna, 2009). Result obtained from the related literature showed that cotton seed oil contained about 31.33 % and in the characterization of the oil, percentage unsaturated fatty acid is more of oleic

and linoleic acid (74 %) and the results ofphysicochemical properties obtained, showed that the oil has high industrial value (Yan, 2010). Ojewola *et al.* (2006) evaluated the physicochemical composition and nutritional value of cotton seed cake. The results showed that cottonseed cake can be used as substitute for soybean meal in broiler and these will go a long way to reduce the price of poultry meat products and the competition between man and animals for soybean as plant protein source there by reducing the market price of soybean.

Cottonseed oil is among the most unsaturated oils, others being safflower, corn, soybean, rapeseed and sunflower seed oils. Oil cakes/oil meals are by-products obtained after oil extraction from the seeds. Oil cakes are of two types, edible and non-edible. Edible oil cakes have a high nutritional value; especially having protein contents ranging from 15 – 50 % (Ramachandran *et al*., 2007). Cottonseed oil has a ratio of 2:1 of polyunsaturated to saturated fatty acids and generally consists of 65 – 70 % unsaturated fatty acids including 18 – 24 % monounsaturated (oleic) and 42 – 52 % polyunsaturated (linoleic) and 26 – 35 % saturated (palmitic and stearic) (Agarwal *et al*., 2003). Cottonseed oil performs better than other oil as it lasts for a long time and stores well by withstanding higher temperature for food items due to its high quality,cotton seed is a source of oil for human consumption, cotton meal and minerals for livestock feed antioxidant content (Sekhar and Rao, 2011). Therefore, maintaining high quality cotton seed oil is critical. Cottonseed oil is the second major product from the cotton plant after fibre which serves as raw material for oil extraction or animal feed production (Hamilton *et al*., 2004; Ashokkumar and Ravikesavan, 2011).

2.12 Genetic Diversity Using RAPD and SSR Markers in Cotton

The existence of genetic diversity in an ecosystem or genepool ensures the adaptation of species to environmental changes and thus its survival. The existence of genetic variability is a pre requisite for the evolution of superior cotton varieties through selection and hybridization. The variety specific DNA markers in a cotton breeding program for variety registration, plant patents, and confirmation of the parentage of hybrids, breeder's right protection and early detection of agronomic and economic traits as an aid to marker assisted selection are very much needed and arewidely employed (Asif *et al.*, 2009). Therefore, it is imperative to locate and utilize the diversity present in cotton. During the most part of the last century, cotton breeding had made significant contributions to increase cotton lint yield, improve fibre quality and enhance biotic tolerance. However, the desirable and genetic variations for breeders are limited or lacking or difficult to dissect. Due to the narrow genetic base of cotton germplasm that cotton breeders have been utilizing and low efficiency of traditional selection methods, cultivar improvement in cotton hasslowed down.

During the past few decades, a number of molecular techniques have been recruited to complement traditional methods for the evaluation of biodiversity, estimation of relatedness and genotype identification (Surgun *et al*., 2012). With the development of the polymerase chain reaction (PCR) technology, various methods are in practice to locate and quantify the extent of existing genetic diversity. Some DNA markers such as RAPD, RFLP, AFLP, SSR and EST-SSR are among the most reliable and sophisticated tools in achieving this feat (Ahmad *et al.*, 2008). In genetic analysis of traits, DNA markers have special advantages over morphological or cytological basis because DNA markers (i) are highlpolymorphic (ii) have no pleiotropic or epistatic effects (iii) enable employment of nondestructive methods and require small amount of plant tissue (iv) are

independent ofenvironmental stresses and (v) provide easy access high reproducibility and high genetic resolution.

The use of molecular markers is very promisingfor detailed chromosome mapping, gene cloning and tailoring new plant cultivars (Gostimsky *et al.*, 2005). The introduction of DNA markers has offered new possibilities for studying varietal/genotypic relationships (Rana and Bhat, 2004; Dongre and Kharbikar, 2004). DNA markers can also be used for the confirmation of the true to type plants regenerated through tissue cultural techniques (Sun *et al*., 2005; Malabadi *et al*., 2006). SSRs are simple tandemly repeated di- to tetranucleotide sequence motifs flanked by unique sequences. PCR primers to the flanking regions have been used to identify chromosomal location of SSRs by genetic mapping (Liu *et al*., 2000). Once generated, they are useful in cloning specific genes of interest and synteny mapping of functional genes invarious related organisms. SSRs are popularly used infull genome sequencing and mapping programmes for a number of organisms and for identifying activegenes helping in identification of diagnostics markers as has been demonstrated in many plant species including cotton (Nguyen *et al*., 2004). SSRs are employed in genetic analysis of cotton due to their polymorphic nature (Liu *et al.*, 2000; Reddy *et al*., 2001). EST-derived SSRs have been developed for many plants, such as *Triticum aestivum* (Gao *et al*., 2004) and *Gossypium* L. (Saha *et al*., 2003). The RAPD technique has already been used in cotton for genetic diversity studies. Random Amplified Polymorphic DNA (RAPD) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers nucleotide sequence. RAPD have greatly facilitated linkage mapping in cotton (Khan *et al*., 2000).

Genetic markers are important tools in the determination of genetic diversity, which is the first step for breeding projects and the protection of genetic resources (Velioglu *et al.*, 2002). Researchers denote specificlocations on chromosomes that constitute

important clues for genome analysis. Molecular markers can be divided into 2 groups: biochemical and DNA markers (Farzaneh *et al*., 2010). DNA markers indicate the differences in the sequences of nucleic acid at a particular location or locations in the genome. In addition, they are independent of external factors, and the rates of polymorphism in these variable regions are high (Parmakus, 2004). One of the DNA marker techniques, random amplified polymorphic DNA (RAPD), is based on the amplification of unknown DNA sequences and uses a single short and random oligonucleotide primer. This technique, which is both fast and inexpensive, can detect DNA variations even at the single-base level. Hence, it has become a commonly used technique for exploring intraspecies and interspecies diversity (Carlson *et al.*, 2001).

Comparative studies of RAPD and simple sequence repeat (SSR) markers in a wide range of crop species, including corn, soybean, barley, sorghum, rice, bluegrass, and wheat, have generally revealed good congruence between the genetics patterns shown by the 2 genetic markers. Ali *et al*. (2008) utilized RAPD analysis in order to investigate hybrid authentication in upland cotton in Pakistan. Comparing the RAPD band pattern of the parents with that of the respective hybrids resulted in the clear identification of genuine hybrids (Maleia *et al.,* 2010). Farzaneh *et al.* (2010) performed a cytogenetic and molecular study using 10 tetraploid cotton cultivars (*Gossypium hirsutum* L.), including parental genotypes and F1 progenies. Based on cytogenetic and RAPD data, the results indicated that the parental genotypes and their hybrids were different from each other (Tahir 2010). Inyet another RAPD study involving African and American cultivars (*Gossypium hirsutum* L. race *latifolium* H.) and 8 inbred lines, approximately 90 % of commercial cultivars originally identified as African were placed in 1 clusster while the remaining American cultivars were clustered into other groups.

2.13 Important Characteristics for Cotton Improvement

Cotton cultivars may react differently to different production areas and have different characteristics but mainly are hairy or non-hairy, normal or okra leaf shaped, frego bractor normal bract, reddish or green coloured varieties. Hairy plant types are used to resist jassidsin Africa and Asia. Cotton varieties without hairs (glabrous) offer resistance to *Helioths* spp.and pink bollworm by incurring decreased egg laying that is associated with decreased trashcontent of fibre (Bourland and Jones, 2012). Okra leaf shaped plants have a more open canopy which permits 70 % more light penetration. This reduces the numbers of boll weevil, pinkbollworm and boll rot. Okra leaf shape is associated with accelerated fruiting rates, earlymaturity and production of fibre with less trash than normal leaf cultivars. Normal leaf cottonhas taller plants than okra leaf cotton and lower fruit loss at 43 % compared to 59 % for okra leaf plants. The frego bract trait is associated with a high level of resistance to boll weevil and can reduceboll weevil damaged squires up to 50 % compared with normal bract Frego bract is associated with delayed fruiting in maturity in addition to reduced yield. Red plant colour confers significant degrees of non-preferenceto the boll weevil and cotton aphid damage. Varieties with the smooth-leaf traitgenerally give higher fibre grades than those with normal or densely hairy leaves (Bourland and Jones, 2012).

2.13.1 Yield of cotton

Yield refers to the total seedcotton and lint yield. Yield is a composite of many other traits, each influenced by many genes that have variable effects and are modified by environmental conditions and cultural methods Cotton varieties vary in yield potential, therefore varieties producing high seed cotton and highlint yield are important to the client. It was reported that lower yields with okra leaf cotton compared to normal leaf cotton. Lint and seed yield are highly significantly positively related. As one tends to

increase, so does the other. Selections should not be based on seedcotton yield, instead selections should be based on lint yield as lint yield depend on seedcotton and ginning outturn (Bourland and Jones, 2012).

2.13.2 Number of bolls and boll size

The boll is the unit package of yield, thus high yield is achieved when the size and number of bolls per unit area is maximised. Ibrahim *et al.* (2012) suggested that prolificacy (boll number per plant or per unit area) is an important factorto consider during selection for yield improvement. Selection for boll size and seed size could positively influence lint yield, if a breeder selects for medium boll size, small seeds per boll and maintaining high ginning percentage (Ibrahim *et al*., 2012).

2.13.3 Plant height

Plant height is important as a contributor to yield and can determine vegetative and fruiting branches of the plant. Breeding for plant height variation is influenced by both yield potential and harvesting methods. Balock *et al.* (2014) observed plant heights between 0.95 - 1.07 m while Dangana *et al.* (2017) reported that the plantheight of cotton ranged from 1.0 - 2.0 m which is within closer range. Generally, plant height is highly affected by the environment.

2.13.4 Hairiness

Hairiness of the leaf and other parts of the plant is heritable and varies between varieties. Hairiness is important for insect pest (jassid) resistance. The hairs on leaves and stems interfere with the oviposition and laying of eggs, thus reducing the rate of damage. Cotton leaf hairs are stellate and vary both in length and density from sparse to densely hairs (Bourland and Gbur, 2017).

2.13.5 Ginning outturn (GOT)

This is the percentage of lint obtained from a sample of seedcottonand varies between cotton varieties and for upland strains ranges between 30 – 40 %. It was reported that the range of varieties with regard toginning percentages was shown to change little from year to year and from place to place. Lukonge (2005) studied genetic control of ginningout turn and indicated that ginning outturn is controlled by additive genetic effects.

2.13.6 Seeds per boll

It was reported that seeds per boll and the number of locules is acharacteristic of the species or a variety. In *G. hirsutum* there is mainly eight seeds per loculeand the locule per boll varies between three to five (Ibrahim *et al.,* 2012) Seeds are the units of production and fibres grow from the outer cells of seed surfaces. The higher the number of seeds per boll, the morelint is produced because it increases the amount of surface area for lint production. Therefore, breeding for increased bolls per unit land area, more seeds per boll, large seed surface area per unit seed weight and increased weight per unit seed surface areimportant. Report from Lukonge (2005) indicates that seeds per boll are thesecond largest contributor to yield.

2.13.7 Seed and lint index

Seed index refers to 100 seed weight. Seed index is important indetermining the yield, especially in seed cotton. It varies between varieties and is highlyaffected by population density. Cotton seed measures about 10 x 6 mm and weighs about 80 mg (5 - 10 g per 100 seed) (Lukonge, 2005). Lint index represents the absolute weight of lint borne by a single seed (or more often 100 seeds). Lint index has a direct relationship with the yield potential of a genotype but is affectedby population density (Lukonge, 2005). It is a compound characteristic being a function of meannumber of hairs per seed and

meanhair weight. Basbag and Gencer (2007) reported that lint indexis governed by two genetic systems, a single pair of factors having pleiotropic effects and acomplex of modifiers, which have minor effects on lint production. Lint index is controlled byadditive genetic effects (Lukonge, 2005).

2.13.8 Fibre strength

This fibre quality trait is useful for spinners and processors. The inherentstrength of individual cotton fibres is an important factor in the strength of the thread spun fromthem. High tensile strength of fibres is necessary for good spinning properties, especially with modern fast spinning machines Fibre strength is affected by environmentalfluctuations (Bourland and Gbur, 2017).

2.13.9 Fibre length

This is the staple length that is universally recognized as the premier fibre property, because it is closely associated with the processing efficiency in manufacturing and determining the quality of the yarn produced. Fibre length variation can occur from boll to bolland plant to plant. Even on a single seed the hairs are not of the same length (Bourland and Gbur, 2017).

2.13.10 Fibre fineness (Micronaire)

This is the measure of soft or silky feel. It is an important quality trait of cotton associated with long hairs and smaller cell diameter in combination with wall thickness. Fibre fineness determines the texture of cotton fibre into soft and silky or coarse and harsh and is affected by the environment. Bourland and Gbur (2017) reported that coarse lint is dominant over fine lint and is quantitatively inherited. Micronaire is acceptable anywhere within the base range of 3.5 - 4.9 units inclusive. The premium range is between 3.5 - 4.2, with values below 3.5 too fine and above 4.9 too course.

2.13.11 Uniformity of fibre length

It is an important fibre quality characteristic determining the maturity of the fibres. The value is important in determining the spinning performance and utility of the lint. Higher values are an indication that the yarn spun from such fibres was uniform in size and strength, with less wastage of fibres. Uniformity varies between varieties and it is affected by environmental factors (Bourland and Gbur, 2017).

2.14 Estimation of Genetic Variability and its Association

Development of any crop improvement program essentially depends on nature and magnitude of genetic variability, genetic advance, characters association, direct and indirect effects on yield and yield attributes (Ibrahim and Hussein, 2006). Genetic variability for agronomic traits is the key component for broadening the gene pool of crops thereby, providing a wider scope for selection. The diversity of gene pool is one of the most important prerequisites for any crop improvement (Manjit and Shwet, 2011).

Therefore, genetic variability, character association and path among selected lines are of viatal importance to breeding programs that aimed at selection of superior genotype with specific desired trait (s) for marginal growing environment (Krishnaveni *et al.*, 2006).

The genetic improvement of any crops for quantitative traits requires accurate estimates of genetic variability, heritability and genetic advancement in respect to the breeding crop for effective planning and efficient breeding program (Chand *et al.,* 2008).

Therefore, Medagam *et al.* (2015) reported that estimates of the variability for yield, yield contributing characters and their heritable components in any crop are important for the improvement program of the crop. Also, the knowledge of heritability had been

reported as an essential for selection-based improvement, as it indicates the extent of transmissibility of a character into future generations (Sabesan *et al*., 2009).

Sivasubramanian and Menon (1973) recommended that PVC and GCV values should be classified as low for estimate less than (< 10 %), moderate for (10 – 20 %) and high for (> 20 %). According to the suggestion of Johnson *et al.* (1955), heritability values less than (< 30 %) should be considered as low, moderate for (30 – 60 %) and high for (> 60 %). It was further stated that genetic advance with the value (< 10 %) should be considered as low, moderate for (10 – 20 %) and high for (> 20 %). Record of low heritability values signifies the contribution of environmental component in the expression of the character. Therefore, selection of a specific trait by direct method would be faithless; hence indirect selection comes in play for successful improvement programme (Rajyalakshmi *et al*., 2013).

2.15 Pearson's Linear Correlations

Correlation gives information regarding association among various traits which is animportant part for the initiation of any breeding program as it provides an opportunity for the selection of genotypes having desirable traits simultaneously (Ali *et al*., 2009). Knowledge of correlations between traits of economic importance is not only of interest from a theoretical consideration of quantitative inheritance of characters, but of practical value since selection is usually concerned with changing two or more traits simultaneously (Yeye, 2000). The degree of phenotypic and genotypic correlations between characters is therefore very important. According to Aghughu (2001) significant positive phenotypic correlation between characters indicates that they can be predicted from each other and that genotypic correlation between them will determine whether this relationship was passed on to the offspring or not. Hence, this will

determine whether or not genetic improvement of one will lead to a corresponding change in the other. The values for the correlation coefficient range from -1 to +1.

2.16 Cluster Analysis

Cluster analysis presents patterns of relationships between genotypes and hierarchicalmutually exclusive grouping such that similar descriptions are mathematically gathered intosame cluster (Aremu *et al*., 2007). Cluster analysis has four methods namely unweighted pairedgroup method using centroids (UPGMA), Single Linkages (SLCA), Complete Linkage (CLCA) and Median Linkage (MLCA). UPGMA and UPAMC provide more accurate grouping information on breeding materials used in accordance with pedigrees andcalculated results found most consistent with known heterotic groups than the other clusters (Aremu *et al*., 2007)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Cotton Landraces

The cotton landraces used during the experiment were collected from the major cotton growing zones of Northern Nigeria, which includes: Kano, Kaduna, Katsina, Sokoto, Kebbi and Zamfara states and Institute of Agricultural research (IAR) Ahmadu Bello University (A. B. U), Zaria. Questionnaires were administered through an interpreter and seed samples of the available cotton landraces under husbandry were collected. The seeds collected were packed and sealed in thick paper envelopes, each of which were given a collecting code number, local name, and locality. They were tested for viability and stored in a dry container in a refrigerator of about 0- 4˚C (Falusi *et al.,* 2014).

3.2 Description of the Study Area

The study was carried out at the experimental garden; Department of Plant Biology, Federal University of Technology, Minna Niger State, Nigeria. Geographically, Minna is located in the North Central geopolitical zone of Nigeria, found within latitude 9°36 North and longitude 6°34 East. It covers a land relative humidity and rainfall of 20 – 30°C area of 88 square kilometres with an estimated human population of 348,788 (Niger State MAAH Bulletin, 2008). The area has a tropical climatic condition with mean annual temperature, 61 % and 1334 cm respectively. The climate presents two distinct seasons: a raining season between May and October and dry season between November and April each year. The vegetation is a typical Guinea Savanna type consisting majority of grassland with scattered trees.

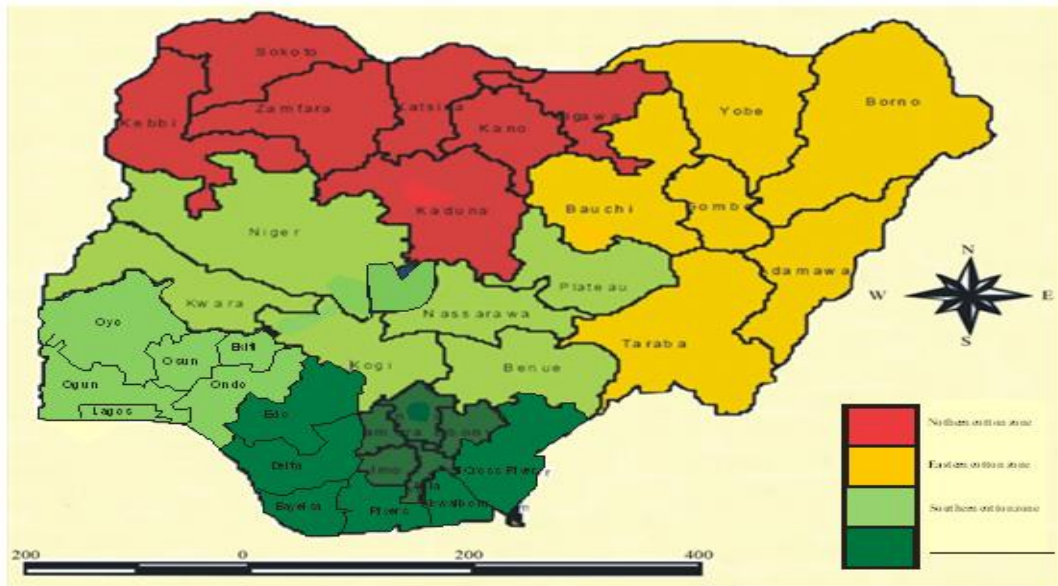


Figure 3.1: Cotton Growing States in Nigeria

3.3 Certification of Seed Viability

The seed viability test was carried out before planting for characterization using germination test method (Stephen, 2009). Ten seeds from each genotype were spread on separate Petridishes containing moist filter paper and partially covered. The experimentalset-up was monitored on daily basis. Seven days after the experimental set-up, the germination percentages were calculatedand used to certain the viability of the seeds (Stephen, 2009).

$$\% \text{ germination} = \frac{Totalnumberofseedsgerminated}{Totalnumberofseedsused} \times 100$$

3.4 Analysis of Soil Samples

Soil samples were collected randomly and analysed for pH, Ca, Mg, K, Na and N using standard methods Bhaswatee *et al.* (2012), this gave the insight of the nutrient and mineral composition of the study field.

3.5 Experimental Design and Sowing of Seeds

All the different genotypes collected were raised using a complete randomized design (CRD) with five replicates (Figure 2). Five seeds of each genotype were sown at the depth of 1-2cm inan experimental bag of 20 litre size of bucket. At 2 weeks after sowing, the emerged (seedlings) were thinned to two seedlings per plant per stand. The planting spacing werekept at 75cm in between the rows and 40 cm in between the plant (Baloch *et al.,* 2014) (Plate 1) Watering and insecticide application were carried out at the appropriate time. All other cultural practices including weeding were uniformly adopted in whole experiment throughout the growing period to minimize the environmental variation (Abdullahi, 2015).

Plate I: Experimental Design for Six Nigerian Cotton genotypes

Source: Field Photograph

SAMCOT-8	SAMCOT-9	SAMCOT-10	SAMCOT-11	SAMCOT-12	SAMCOT-13
SAMCOT-8	SAMCOT-9	SAMCOT-10	SAMCOT-11	SAMCOT-12	SAMCOT-13
SAMCOT-8	SAMCOT-9	SAMCOT-10	SAMCOT-11	SAMCOT-12	SAMCOT-13
SAMCOT-8	SAMCOT-9	SAMCOT-10	SAMCOT-11	SAMCOT-12	SAMCOT-13
SAMCOT-8	SAMCOT-9	SAMCOT-10	SAMCOT-11	SAMCOT-12	SAMCOT-13

Figure3.2: Experimental Design for Six Nigerian Cotton Genotypes

3.6 Measurement of Morphological and Yield Parameters

Morphological parameters data were collected from fiverandomly tagged plants were pergenotype. All the agronomic and yield parameters data were taken at weekly interval following the standard procedures of Akinyele and Osekita (2006) as describe below.

1. Plant height (cm) – the height was measured as the distance from the base of the plant to the apex using a metre rule
2. Number of monopodia branches per plant - the number of monopodia branches on main stemwere determine by direct counting (Plate II).
3. Number of sympodia branch per plant - the number of branches which areextra-axillary in position and normally horizontal with zig-zag pattern of fruiting points at maturity were recorded as the sympodia branches (Plate II).
4. Numbers of bolls per plant - number of bolls per plant weredetermine by direct counting at maturity.

5. Boll size- fully matured bolls per plant were used to determined boll size using tape rule.
6. Boll weight (g) - fully matured and well dried boll per plant obtainedand measure in grams using electrical sensitivebalance. (Type; Citizen electric balance, Model: MP 600)
7. Seed index (g) –are recorded as the absolute weight of 100 seeds in grams usingelectrical sensitive balance (Type: Citizen electric balance, Model: MP 600).
8. Weigh of Lint (g) –are measured and recorded as the absolute weight of lint obtained from 100 seeds in grams. (Type Citizen electric balance, Model: MP 600.

PlateII: Monopodial and sympodial branches

Sourse: Field photograph

3.7 Determination of Fibre Qualitties

The fibre quality analysis was carried out at Cotton Samples Testing Laboratory Cotton SA, Silverton, South Africa. The cotton lint samples were conditioned for 24 hours in standard atmospheric conditions (Temperature = 20˚C ± 2 and R.H. % = 65 % ± 2) before testing. The fibre parameters was determined using High Volume Instrument (HVI 900A). The procedure of testing was adopted as described in America Society for Testing Material (ASTM) Standards (2002).

3.8 Guidelines used forFibre Quality Data Improvement in the Laboratory

The laboratory was conditioned to ASTM standard for 24 hours, 7 days a week during cotton classing season. The air conditioning system was checked regularly within the laboratory for accuracy and consistency. Temperature and relative humidity should be maintained throughout the use of ASTM in the laboratory. There should be no draft in any place in the laboratory due to the air conditioning system, especially not anywhere close to the HVI instrument. The micronaire balance is susceptive to air draft, which is the reason it is already protected by a plastic shield.

Samples must be stored openly in the conditioned laboratory, not paper or plastic bags. Open – wire shelves are preferred for samples conditioning since the air needs to be able to penetrate the samples from all sides. Plastic mesh baskets were used for sample storage as long as they are stored in mesh wire racks. Again, it is important that the conditioned air can penetrate the cotton sample from all sides. Calibration cottons must be stored within the same laboratory under the same conditions as the test samples and where they will be tested (where the HVI instrument is installed). Just take one of the two calibrations at a time to the instrument when you calibrate the HVI. This helps avoiding mixing the calibration cottons accidentally.

Calibration cottons must be replaced when there is any chance that they have been mixed up or are otherwise contaminated. This happens even in the best laboratories, it is better to discard mixed or contaminated calibration cotton immediately to avoid any problems with the calibration. Calibration cotton also needs to be replaced when they have been used very frequently. This is especially true the USDA micronaire calibration cottons that become easily "clumpy" due to frequent use. It is recommended that the micronaire calibration cotton sample not be reused more than three times. There is an expiration date on the new USDA calibration cottons for length and strength. Calibration cottons should be discarded after this expiration date. Cotton testing involves a lot of dust and dirt. Read and follow the maintenance instructions in the instruction manual thoroughly. Excessive dirt accumulation will affect the data reliability of the instrument, and will eventually reduce the instrument's lifetime.

3.9 Steps used for Fibre Quality Test

In order to obtain maximum efficiency in operating an HVI, it is necessary to properly organize the sample handling for testing. It is recommended that sample trays be used for organizing cotton lots. Either metal wire or plastic open mesh trays may be used (see comment above). The bale samples in a lot should be placed in as many trays as required to contain them. These trays then provide a convenient container in which the samples are conditioned. When testing is started, the first tray should be placed to the right of the HVI and an empty tray to the left to receive the samples as they are tested. Usually, the following test set – up is selected for classing upland – type cottons.

Micronaire 1 repetition per sample

Length /strength 2 repetitions per sample Color/trash 2 repetition per sample

The different tests performed by the HVI may be done in different sequences. However, performing the tests in the same sequences each time will minimize errors and

maximize throughout. The most efficient sequence may vary for different operators but the suggested sequence is the following.

1. Pick up the bale sample from the right-hand tray and remove the bale tag.
2. Either scan the bale tag or enter it manually via the keyboard.
3. Place approximately 10 grams on the balance for the micronaire measurement.
4. Place two samples with a thickness greater than 2 inches (app.5cm) in the center of the color/trash trays and press the color/trash start button.
5. Remove two 8 - 12 grams samples for the length/strength measurement. Place length /strength samples in the sample drums and press the length/strength start button.
6. This will have allowed the balance time to stabilize. If the balance mass is not within the allowed range, adjust the mass of the cotton on the balance.
7. Check that the mass on the balance agrees with the mass displayed on the HVI monitor.
8. Insert all the cotton on the balance completely into the micronaire chamber and close the micronaire door.
9. When all tests are completed and no limits are encountered, collect the cotton from each module, fold the sample around the bale tag, and place the sample in the completed sample tray to the left.

PlateIII: Sample storage in plastic-mech baskets in open- wire shelves

Plate IV: Wire basket and Plastic-mesh basket for shelving cotton smples for analysis

3.10 Determination of Phytochemical Constituent of the Cotton Genotypes

The phytochemical analysis was carried at the centre for genetic engineering and Biotechnology, Federal University of Technology, Minna.

3.10.1 Test for flavonoids (Sofowora *et al.*, 2013).

A portion of powdered seeds of each genotype was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate

was shaken with 1 ml of dilute ammonia solution. Change in coloration of the sample to yellow indicates the presence of flavonoids.

3.10.2 Test for tannins (Sofowora, 2008).

A total of 0.5 g of the dried powdered sample was boiled in 20ml distilled water in a test tube and filtered. 0.1 % ferric chloride ($FeCl_3$) solution was added to the filtrate and the appearance of brownish green or a blue-black colouration indicate the presence of tannins in the test sample

3.10.3 Test for saponins (Harborne, 1998)

A portion of 2.0 g of the powdered sample was boiled in 20ml of distilled water in a test tube and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to the formation of stable persistent froth. The frothing was mixed with drop of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

3.10.4 Test for alkaloid (Harborne, 1998).

Extract (0.5 ml) of the dried powered of each genotype was stirred with 5cm^3 of 1 % aqueous HCI on a steam bath. Few drops of picric acid solution was added to 2 cm^3 of the extract and the formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids.

3.11 Quantitative Phytochemical Screening of Cotton leaves genotypes

3.11.1 Determination of total phenol (Edeoga *et al*., 2005)

A total of 2.00 g of each sample were defatted with 100ml of diethyl other using a soxhlet apparatus for 2 hours. the fat free sample was boiled with 50 ml of petroleum ether for the extraction of the phenolic component for 15 minutes. 5 ml of the extract was piptted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of

ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505nm. Tannic acid was used to establish the calibration curve.

3.11.2 Determination of total flavonoid

Total flavonoid was determined using aluminum chloride colorimetric method. Quercetin was used to establish the calibration curve. Exactly 0.5 ml of the diluted sample was added into test tube containing 1.5 ml of methanol. 0.1 ml of 10 % $AlCl_3$ solution and 0.1 ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10 % $AlCl_3$ was substituted by the same amount of distilled water in blank.

3.11.3 Determination of total alkaloids (Harborne, 1998)

A total of 0.5 g of the sample was dissolved in 96 % ethanol -20 % H_2SO_4 (1:1). One ml of the filtrate was added to 5 ml of 60 % tetraoxosulphate (VI), and allowed to stand for 5min. Then 5ml of 0.5 % formaldehyde was added and allowed to stand for 3hr. The reading was taken at absorbance of 565 nm. The extinction coefficient (E296, ethanol {ETOH} = 15136M^{-1} cm^{-1}) of vincristine was used as reference alkaloid.

3.11.4 Determination of saponins (Oloyede *et al.*, 2013).

A total 0.5 g of the sample was added to 20 ml of 1N HCl and was boiled for 4 hours. After cooling it was filtered and 50ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 ml of each was taken into 3 different test tubes. 6ml of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after

10 minutes and the absorbance was taken at 490 nm. Standard saponin was used to establish the calibration curve.

3.11.5 Determination of tannins (AOAC, 2019)

A total of 0.2 g of sample was measured into a 50 ml beaker. 20 ml of 50 % methanol was added and covered withparafilm and placed in a water bath at 77 - 80 ^{o}C for 1 hour which was shaked thoroughly to ensure a uniform mixture. The extract was quantitavely filtered using a double layered whatman No.41 filter paper into a 100 ml volumetric flask, 20 ml water added, 2.5 ml Folin-Denis reagent and 10ml of Na_2CO_3 were adderd and mixed properly. The dissoved sample was allowed to stay in order to mixed properly for 20 minutes for the development of a bluish-green colour. The absorbance of the tannic acid as well as samples standard solutions was read after colour development on a UV-spectrophotometer model 752 at a wavelength of 760 nm.

3.11.6 Determination of phytic acid content

The phytic acid content was determined using a modified method of (Wu *et al*., 2009). The method depends on an iron phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample. Five grams of the sample was extracted with 20 ml of 3 % trichloroacetic acid and filtered and 5 ml of the filtrate was used for the analysis. The Phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 ml of 1 M NaOH. The precipitate was dissolved with hot 3.2 M HNO_3 and the absorbance was read immediately at 480 nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different Fe $(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 iron: phosphorus molar ratio.

3.11.7 Determination of oxalate

Oxalate in the sample was determined by permanganate titrimetric method as described by Liu *et al*. (2009). Two grams of the sample flour was suspended in 190ml of distilled water in 250 ml volumetric flask, 10ml of 6M HCI was added and the suspension digested at 100 ºC for 1hr, cooled, then made to the mark before filtration. Duplicate portion of 125 of the filtrates were measured into beakers and 4 drops of methyl red indicator added. This is followed by the addition of cone. NH_4OH solution drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion is then heated to 90 ºC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90 ºC and 10ml of 5 % $CaC1_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5 ºC. The solution was then centrifuged at 2500 rpm for 5 mins, the supernatant decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution. The total filtrate resulting from the digestion was made up to 300 ml. aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persisted for 30 seconds. The calcium oxalate content is calculated using the formula:

$$T \times \frac{(Vme)(Df) \times 10^5}{(ME) \times Mf} \quad (mg/100g)$$

Where T is the titre of $KMnO_4$ (ml), Vme is the volume-mass equivalent (1 cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution factor V_T/A (2.5 where V_T is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of flour used.

3.12 Determination of Proximate Analysis of Cotton Seed Oil.

3.12.1 Percentage (%) moisture content

The moisture content of the processed samples was determined by vacuum oven method. Exactly 3 g of sample were weighed into crucibles of known weight. The crucibles with the samples were placed into an oven operated at 105 ^oC for 3 hours. They were carefully removed from the oven and allowed to cool in desiccators before re-weighing. The crucible with the sample were returned to the oven and dried further and re-weighed. The process of drying, cooling and re-weighing was continued until a constant weight was obtained (Bolek *et al*., 2016).

3.12.2 Determination of ash content

Total of 2 g of the sample were weighed into crucible of known weight. The crucible with its content was placed in a furnace set at 550 ^oC for 6 hours after which it was carefully removed from the furnace and allowed to cool before being re-weighed. The process was continued until a constant weight was obtained.

3.12.3 Determination of crude protein

The crude protein was determined by Kjeldahl method. 2 g of processed sample were taken and placed into 100 ml Kjeldahl digestion flasks. Few grams of Kjeldahl catalyst mixture (Na_2SO_4 + $CuSO_4$) and 15 ml of concentrated H_2SO_4 were added. The mixtures were mixed thoroughly and heated in a fume cupboard for about 2 hours until complete digestion was reached. This was identified when a clear solution was obtained. The cool digest was diluted to 100 ml and only 10 ml of the digest was mixed with equal volume of 10 mol/dm^3 NaOH. The mixture was placed in micro- Kjeldahl distillation apparatus which was distilled by steam and the distillate was collected into a conical flask containing 10 ml of 4 % Boric acid. Few drops of mixed indicator (5 g bromocrysol green and 1 g methyl red in 100 ml of ethanol) were added into 50 ml of the distillate

and titrated against 0.1 M H_2SO_4 solution. A blank was conducted simultaneously under similar experimental condition (Bolek *et al.,* 2016).

3.12.4 Extraction of oil from the sample

Three grams of the processed samples were weighed into a known weight of thimble. A clean dry extraction flask (250 ml capacity) was weighed and sufficient quantity of 400 ml – 600 ml acetone was poured into it. The thimble with the sample was placed into the flask and oil was extracted as already described above. At the end of extraction, the resulting mixture containing oil was heated to recover the solvent and the weight of the round bottom flask with oil was noted after cooling.

3.13.5 Determination of iodine value

The iodine values of the oils were determined using Wijs's iodine method. 20ml of carbon tetrachloride (solvent) was added into 0.2 g sample that was placed into 500ml flask and the mixture was swirled. Exactly 25 ml of Wijs's iodine solution was pipette into flask containing the sample and covered with glass stopper and swirled to ensure an imitate mixture. A blank was prepared simultaneously under similar experimental condition. The flasks were stored in the dark for 30 minutes at a temperature of 25 $^{o}C \pm 5\ ^{o}C$ after which 15 ml of potassium iodide (KI) solution was added, followed by 100 ml of distilled water. The solutions were titrated with 0.1 M sodium thiosulphate solution using starch as an indicator with constant and vigorous shaking.

3.12.6 Determination of saponification value

A total of 2 g of the samples was measure into conical flask and 25 ml of 0.1 M alcoholic potassium hydroxide was added. The contents which were constantly stirred were allowed to boil gently for 1 hour with reflux condenser placed on the flask containing the mixture. Few drops of phenolphthalein indicator was added to the warm

solution and then titrated with 0.5 M HCI to end point (until color of the indicator disappeared). The same procedure was used for the blank (Okwokon and Okafor, 2016).

3.12.7 Determination of acid value (A.V.)

Ten gram of oil sample was weighed in a conical flask. 50 cm^3 of ethyl alcohol and few drops of phenolphthalein indicatorwere added to the conical flask with the oil sample. The mixture was heated for 5 minutes and then titrated with 0.05 M KOH to a faint pink color end point with constant shaking (Okwokon and Okafor, 2016).

3.12.8 Determination of moisture content and volatile matter

The moisture content and the volatile matter of oil was determined using air oven method. 15 g of oil sample wasweighed into a known weight of tarred Petri dish. The Petri dish was placed in the oven for approximately 2 hours. The dish was carefully removed from the air oven, cooled and was re-weighted. The process of drying, cooling andreweighing was continued until a constant weight was obtained.

3.12.9 Determination of mineral element composition of cotton seed oil

Among all elements only Sodium (Na) and Potassium (K) were estimated by using flame photometer while Calcium (Ca), Magnesium (Mg), Iron (Fe), Phosphorus (P), Copper (Cu) and Manganese (Mn) in cottonseed oil were analyzed by using Atomic Absorption Spectrophotometer (AAS) which was equipped with flame and graphite furnace. For the experiment, air acetylene flame mode was used. The condition fixed with acetylene 1.8 l/min and air 15 l/min, argon gas flow for inert atmosphere. The instrumental default temperature parameters were automatically fixed for each element analysis (Aziz *et al.,* 2018).

3.13 Cytological Studies of Cotton Genotypes

3.13.1 Pollen grain viability test

Pollen grain viability test were carried out by collecting ten matured flowers whose anthers have not dehisced from each genotype. The pollen grains from each flower were dusted on a clean glass slide one after the other. They were stained using lactophenol cotton blue. Each slide was carefully covered with coverslip and observed under light microscope (Daudu *et al*., 2015). Properly stained pollengrains containing nuclei were regarded as being viable and those that were empty or lack nucleus were considered as aborted (Dangana *et al*., 2017). Percentages of pollen grain viability were calculated as the proportion that absorbed the stain to the total count. Percentage pollen fertility and sterility were calculated using the formulae below (Sharmin, 2012).

$$\text{Pollen fertility (\%)} = \frac{No.of fertile(stained) pollengrains}{TotalNo.of pollengrains} \times 100$$

$$\text{Pollen sterility (\%)} = \frac{No.of sterile(unstained) pollengrains}{TotalNo.of pollengrains} \times 100$$

3.13.2 Mitotic analysis

The cytological analyses were carried out according to the modified technique adopted by Abubakar *et al.* (2015). Mitotic studies, the seeds of each genotype plant were air-dried and plated on moist filter paper in petridishes. The root tips of young seedlings were excised and pre-treated in aqueous solution of paradichlorobenzene for one hour with a view to producing clear and thick chromosome. The root tips was rinsed in water and fixed 1.3 v/v of glacial acetic acid and 95 % ethanol for 24 hours. For squash preparation, the root tips was rinsed in water and hydrolyzed in 18 % hydrochloric acid for five minutes at room temperature. The root tipswere rinsed in distilled water once again to get rid of the acid off the root tips. The root cap was carefully removed and the root tip squashed in FLP orcein (Abubakar *et al*., 2015).

3.14 Genetic Parameters Estimates.

Broad Sence heritability (h) was estimated using the formular below

$$h^2=\frac{\sigma^2 g}{\sigma^2 ph}$$

Where $\sigma^2 g$ is the genotypic variance; $\sigma^2 ph$ is the phenotypic variance. Phenotypic and Genotypic variances wasobtained from the analysis of variance table using equations 2 and 3 as follows;

$$\sigma^2 g=\frac{MS1-MS2}{rXs}$$

$$\sigma^2 ph=\frac{MS1}{rXs}$$

(Where r: replication, s: season, MS1: mean square for cultivar, MS2: mean square for cultivar X season).

The mean value was used for genetic analyses to determine Genotypic Coefficient of Variation (GCV) and Phenotypic coefficient of Variation (PCV), using equations 4 and 5 as follows:

$$\text{GCV (\%)} = \frac{\sqrt{GenotypicVariance}}{GrandMean} \times 100$$

$$\text{PCV (\%)} = \frac{\sqrt{PhenotypicVariance}}{GrandMean} \times 100$$

Genetic advance (GA) was calculated with the method suggested by Singh and Chaundry (1985) using equation 6 as follows:

$$GA= K.\ \sigma ph.\ h^2$$

Where K: constant = 2.06 at 5 % selection intensity, σph: square root of phenotypic variance, h^2: Heritability

GA as percentage of mean (GAM) = $\frac{GA}{GrandMean} \times 100$

3.15 Molecular Characterization using RAPD Molecular Markers

The molecular analysis was carried out at Bioscience centre, International Institute of tropical Agriculture (IITA) Ibadan, Nigeria.

3.15.1 DNA extraction.

The DNA was extracted from young leaves of each of the genotypes. Extraction was done using the method of (Daudu *et al.,* 2015) with little modifications.

Approximately 100 mg of cotton leaf was grinded with Della porta extraction buffer and DNA extracted as described briefly. Each sample was grinded in 1000 µl of the buffer in a sterilized sample bag. Mix was collected in sterile eppendorf tube and 40 µl of 20 % SDS was then added, this was followed by brief vortexing and incubated at 65 ^{o}C for 10 minutes. At room temperature, 160 µl of 5 M potassium acetate was then added vortexed and centrifuged at 1000 g for 10 minutes. Supernatant where collected in another eppendorf tube and 400 µl of cold iso propanol was added mixed gently and kept at -20 ^{o}C for 60 minutes. Centrifugation was at 13000g for 10 minutes to precipitate the DNA after which supernatant was gently decanted and ensured that the pellet was not disturbed. DNA was then washed with 500 µl of 70 % ethanol by centrifuging at 10000 g for 10 minutes. Ethanol was decanted and DNA air-dried at room temperature until no trace of ethanol was seen in the tube. Pellet was then re-suspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA.

3.15.2 RAPD PCR protocol and bands separation

Polymorphic ten (10) RAPD universal markers were used for genotyping the entire gemone of the 6 cotton genotypes. Total PCR reaction was optimized to be 15 µl and this included 2 µl of about 100 ng DNA template, 7.0 µl DreamTaq PCR master mix (Thermos Scientific Inc.), 2 µl of RAPD universal primer, and 4.0 µl nuclease free

water. PCR protocol was followed; an initial denaturation at 94 ˚C for 5 minutes followed by 35 cycles of denaturation of 94˚C for 15seconds. Annealing was done at 37 ˚C for 30 seconds and extension of 72 ˚C 60 minutes then a final extention of 72˚C for 7 minutes.

Table 3.1 Primers used for the Molecular Analysis

S/N	Primer name	Primer sequence
1	T01	GGGCCACTCA
2	H08	GAAACACCCC
3	T08	AACGGCGACA
4	H04	GGAAGTCGCC
5	H05	AGTCGTCCCC
6	T05	GGGTTTGGCA
7	T10	CCTTCGGAAG
8	B04	GGACTGGAGT
9	T17	CCAACGTCGT
10	H10	CCTACGTCAG

3.15.3 Band separation

The separation of bands as produced by each primer was done in a 1.5 % Agarose gel. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Seven µl of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded

into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel (Oluwajobi *et al.,* 2016)

3.16 Fatty Acid Determination in Cotton Seed Oil

3.16.1 Seed preparation

Seeds were dehulled in a 1-L stainless-steel blender operated at variable speed. Some quantity of seedswas collected in the blender then poured over stacked #4, #12, and baseplate sieves. Hull pieces (with linters) and uncracked seeds were then be retained on the surface of the #4 sieve; whole kernels and large pieces were retained on the surface of the #12 sieve, and fines (both hull andkernel pieces) passed through the #12 sieve. The top fractions were then re-milled increasing the durationand intensity of the milling and re-sieved. The process was repeated until all the kernels are completely cracked, and most of the larger kernel pieces are recovered on the #12 sieve. Recovered kernels was grinded for approximately1min with a Braun hand-chopper to pass a #20 sieve, after which it be freeze-dried, and stored at -20 °C until used for oil recovery (Michael *et al.,* 2010).

3.16.2 Oil extraction and trans-methylation chemistry

The oil extraction was done according to the methods of Michael *et al.* (2010) with little modifications. Approximately 100 mg of the ground seedmaterial was weighed into a 2-mL microcentrifuge vial. Two 2.3 mm diameter chrome-steel bolls and 1 mL of hexane was added. The tubes were sealedand the contents pulverized on a Beadbeater-8microcentrifugemill (Bio-spec Products, Bartlesville, OK) operated at 90 % maximum speed for 2 minutes. Tubes were shaken on a platform shaker overnight at room temperature to extract the oil. After extraction, each tube was centrifuged at 90 %

maximum speed for 2 minutes toseparate seed debris, and the crude (solvent plus crude oil) was transferred to a test tube.

To form fatty acid methyl esters, 200 µL of 0.5 N methanolic base (Supelco, Inc., Bellefonte, PA) will added to the crude and the tube was cappedand heated to 70 °C for 10 minutes with periodic vortexmixing. Upon cooling, 1 mL of brine and 1 mL of hexane was added, and the contents wasvortex mixed again. After allowing the phases to separate, 1 mL of the organic phase containing the methyl esters was transferred to a gas chromatographyautoinjector vial.

3.16.3 Determination of percentage fatty acid using GC-MS

Percentage fatty acid was determined by weighing 0.2 g of oil sample into 250 ml conical flask and methylated with 6 ml of sodium methyloxide. The mixture was refluxed for 10 minutes on steam bath; thereafter 10ml of chloride was added andrefluxed for another 10 minutes. Ten ml (10 ml) ofhexane was added and refluxed for more 2 minutes after which the solution was allowed to cool. 10 ml of distilled water was added and poured into a separating funnel, organic layers collected and dried over $CaCl_2$. The samples were injected into the GC-MS (QP2010 PLUS, SHIMADZU JAPAN) to identify the fatty acid present in the oil samples (Bolek *et al.,* 2016).

3.17 Data Analysis

The data collected was subjected to analysis of variance (ANOVA) (SAS Institute Inc. 2015). Mean separation was done by Least Significant Difference (LSD). Coefficient ofvariation was computed and then used to compare variability of each character studied. The statistical model that was used for the analysis was based on the linear model for randomized complete design. The statistical package Agrobase (version 2000) was also used to do simple descriptive analysis and correlation coefficients. Simple

percentages were used to estimate certain parameters like germination percentage and pollen viability test. All mean values were considered significant at $p \leq 0.05$.

Binary data was then generated for each primer sets using 1 (presence of positive amplification at a particular band size) and 0 (absence of positive amplification at a particular band size) the generated binary data was the used to create a data matrix which was analyzed using the Powe rmarker V2.35 software. Genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content where then generated using the power marker software. The geneticrelationship among treated samples were also estimated by constructing a dendrogram through unweighed pair group method with arithmetic means [UPGMA] using the mega6 softwareand genetic distance where computed also using the mega6 software.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Collection of cotton landraces

The exploration of six states Kaduna, Katsina, Kebbi, Kano, Sokoto and Zamfara states and Institute for Agricultural Research, Ahmadu Bello University, Zaria in Northern cotton region of Nigeria gives a total of twelve genotypes. Preliminary pilot study of the collected genotypes using some qualitative and quantitative characters such as plant height, number of bolls per plant, weight of bolls per plant, Number of seeds per boll, weight of 100 seeds, weight of lint per plant flower colour, seed length and pollen shapes revealed that most of the genotypes were replicated (Table 4.1). Clustering of the genotypes using some qualitative and quantitative morphological parameters resulted in six distinct genotypes (clade) at genetic distance of 1.5. Cluster II, IV, V and VI were made up of one distinctgenotypes, ie SAMCOT-9, SAMCOT-10, SAMCOT-11 and SAMCOT-13, respectively. Cluster I is composed of two genotypes Zamfara and SAMCOT-8 and cluster III is made up of six genotypes SAMCOT-8, SAMCOT-9, SAMCOT-10, SAMCOT-11, SAMCOT-12 and SAMCOT-13 (fig 4.3). The clustering of the genotypes revealed their geographical similarities, all the genotypes in cluster III were similar in geographical region and border town to each other. Thus, for further characterisation on the Nigeria cotton, six distinct genotypes were selected from the initial twelve (12) genotypes that were collected across the Northern states of Nigeria. These six genotypes are SAMCOT-8, SAMCOT-9, SAMCOT-10, SAMCOT-11, SAMCOT-12 and SAMCOT-13.

Table 4.1: Preliminary Pilot Studies of Twelve Nigeria Cotton Accessions

Genotypes	Weight of dry boll	Number of seeds per boll	Weight of 100 seed	Weight of lint
KT 001	27.75 ± 4.45^{b}	26.14 ± 1.64^{b}	8.14 ± 0.34^{b}	9.86 ± 1.64^{c}
KN 001	21.17 ± 1.63^{a}	22.89 ± 1.43^{a}	8.25 ± 0.37^{b}	7.13 ± 0.81^{a}
SK 001	34.42 ± 7.39^{c}	26.44 ± 3.45^{b}	7.63 ± 0.50^{a}	12.13 ± 2.18^{d}
KAD 001	23.66 ± 3.01^{a}	27.98 ± 1.92^{b}	7.75 ± 0.45^{a}	7.25 ± 1.26^{a}
KEB 001	25.47 ± 4.54^{a}	28.90 ± 3072^{c}	8.00 ± 0.46^{a}	10.75 ± 0.74^{c}
ZF 001	25.82 ± 2.22^{a}	27.59 ± 1.44^{b}	7.89 ± 0.6^{a}	8.67 ± 0.87^{b}
SAMCOT-8	11.90 ± 1.27^{a}	27.66 ± 2.41^{b}	9.74 ± 0.67^{c}	13.66 ± 1.10^{d}
SAMCOT-9	32.17 ± 5.49^{d}	26.82 ± 2.49^{a}	8.33 ± 0.47^{b}	10.23 ± 1.19^{c}
SAMCOT-10	26.08 ± 5.27^{cd}	30.35 ± 1.40^{c}	8.64 ± 0.45^{bc}	10.23 ± 1.27^{c}
SAMCOT-11	25.67 ± 4.15^{c}	30.68 ± 1.50^{c}	8.05 ± 0.66^{a}	9.40 ± 1.03^{b}
SAMCOT-12	23.15 ± 2.74^{bc}	28.06 ± 2.46^{bc}	10.29 ± 0.56^{c}	7.78 ± 1.07^{ab}
SAMCOT-13	21.07 ± 3.19^{b}	30.40 ± 1.37^{c}	9.93 ± 0.57^{c}	6.34 ± 0.89^{a}

Values follows by the same superscript alphabet on the same column and not significantly different at $p > 0.05$

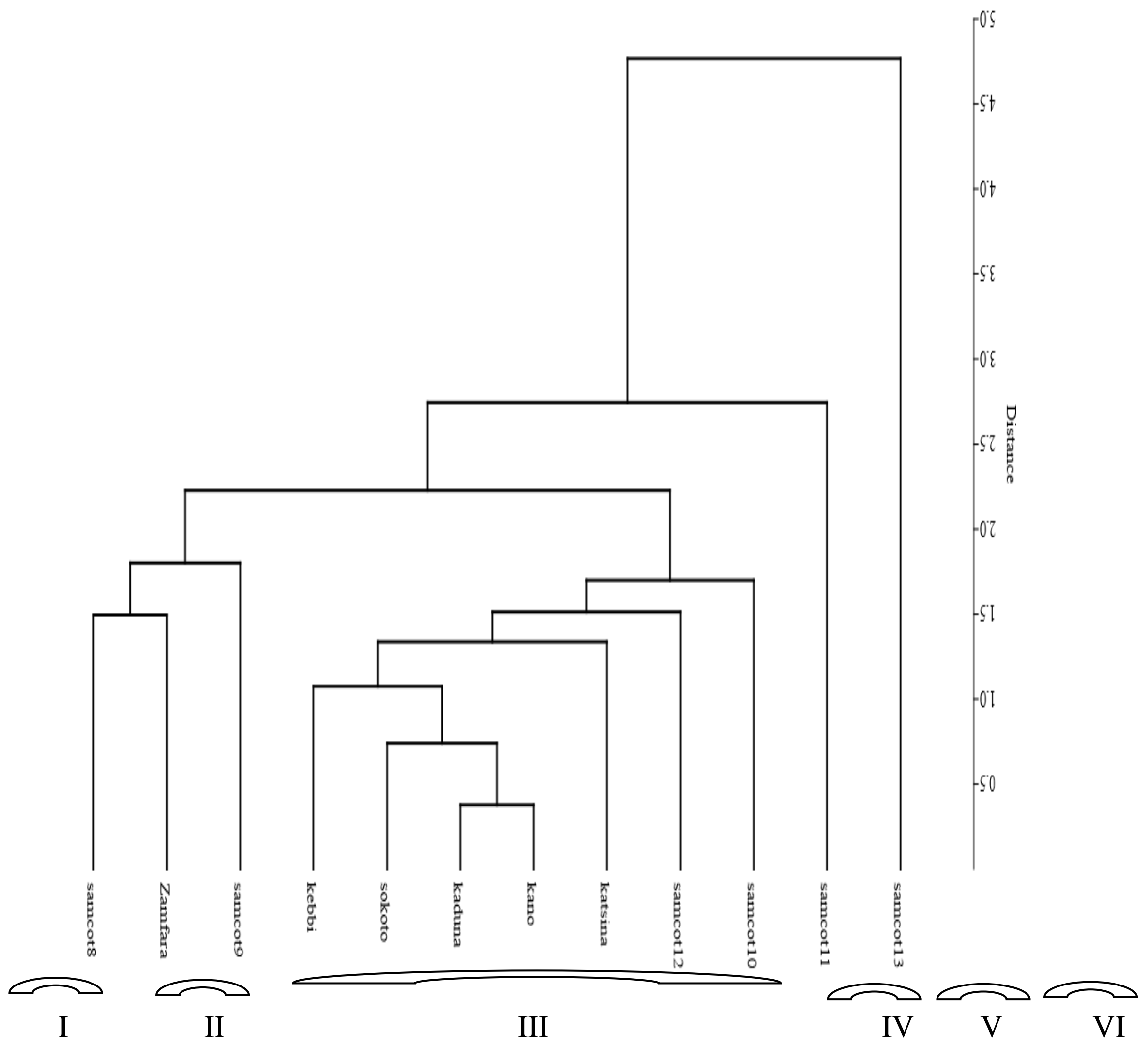


Figure 4.1: Dendrogram Showing Twelve (12) Cotton Genotypes Collected In Northern Cotton Zone Of Nigeria

4.1.2 Weekly plant height of six Nigeria cotton genotype

The result of analysis of variance (ANOVA) showed that there were significant differences in plant height among the genotypes in all the sampling period (Table 4.2). At week 1 after germination, significant highest plant height was observedin SAMCOT-8 with the value of 11.50 cm followed by SAMCOT-9 and SAMCOT-10 10.55 cm each. The shortest plant height (8.20 cm) was obtained in SAMCOT-12. This lowest value was significantly different from the value of all other genotypes. However, from week 4 to 10, the lowest plant height was recorded in SAMCOT-8 with the value of 17.80, 49.00, 69.00 and 82.50 cm at week 4, 6, 8, and 10, respectively (Table 4.2). These lowest values were significantly different from the values of all other genotypes, except at week 8 and 10 where SAMCOT-12 had 76.90 and 85.40 cm, respectively. Similarly, at week 4, 7, 9, and 10 to SAMCOT-13 had the heighest plant height 38.70, 81.10, 103.00 and106.00 cm, respectively. This highest plant height at week 4, was not significantly different from the value of SAMCOT-9, 10, and SAMCOT-11 each with the values of 38.70 cm. At week 10 the plant height of SAMCOT-9 (103.00 cm) was not significantly different with the highest height of 106.00 cm in SAMCOT-13.

Table 4.2: Weekly Plant Height (cm) of Six Nigeria Cotton Genotypes

Genotypes	Sampling period (week)				
	2	4	6	8	10
Samcot-8	11.50±0.92^{c}	17.80±0.53^{a}	49.00±1.99^{a}	69.00±2.50^{a}	82.50±2.85^{a}
Samcot-9	10.55±0.45^{bc}	36.70±1.08^{c}	76.00±2.50^{cd}	89.70±3.50^{b}	103.00±2.39^{bc}
Samcot-10	10.55±0.22^{bc}	36.70±1.08^{c}	62.05±1.42^{b}	87.90±3.03^{b}	97.00±2.07^{b}
Samcot-11	9.30±0.56^{ab}	36.70±1.08^{c}	71.50±2.17^{c}	89.90±2.56^{b}	95.90±2.71^{b}
Samcot-12	8.20±0.92^{a}	27.20±3.98^{b}	72.90±2.99^{c}	76.90±2.23^{a}	85.40±2.45^{a}
Samcot-13	10.02±0.57^{abc}	38.70±0.72^{c}	81.10±3.77^{d}	103.00±3.42^{c}	106.00±3.83^{c}

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at P > 0.05

4.1.3 Monopodial branches of six Nigeria cotton genotypes

The result of monopodial branches of the six Nigerian cotton genotypes is presented in Table 4.2. Analysis of variance (ANOVA) showed that there were significantly differences ($P < 0.05$) among all the genotypes in monopodial branches. At week 8 SAMCOT-11 and SAMCOT-12 both had the highest mean of number of branches (12.30). The lowest number of branches was due to SAMCOT-10 (10.80). This value was significantly different ($P < 0.05$) from all other genotypes except SAMCOT-8 (11.08). At week 9 the least number of branches was recorded in SAMCOT-12 (13.10) and Samcot10 (13.10). These values were significantly different statistically at ($P < 0.05$) from the value of SAMCOT-11 (15.20), but not different from the value of all other genotypes. The highest mean value was due to SAMCOT-11 with (15.20) branches which was significantly different from all other genotypes. However, SAMCOT-13 and SAMCOT-9 had their values to be 14.40 and 13.70, respectively. These values were not significantly different from each another. At Week 10 the highest monopodial branch was due to Samcot-11 with (16.40) followed by SAMCOT-13.00 (15.70), SAMCOT-9 (15.50) and SAMCOT-8 (15.40). These values were not significantly different ($P>0.05$) from one another. The significant lowest number of monopodial branches was due to SAMCOT-10 with the value of 13.70.

Table 4.3: Monopodial Branches of Six Nigeria Cotton Gynotypes

	Sampling period (week)		
GENOTYPES	8	9	10
SAMCOT-8	11.10 ± 0.43^{ab}	13.30 ± 1.03^{a}	15.40 ± 0.45^{c}
SAMCOT-9	11.80 ± 0.68^{b}	13.70 ± 0.59^{ab}	15.50 ± 0.94^{c}
SAMCOT-10	10.80 ± 0.09^{a}	13.10 ± 0.98^{a}	13.70 ± 0.96^{a}
SAMCOT-11	12.30 ± 0.63^{bc}	15.20 ± 0.57^{b}	16.40 ± 0.86^{d}
SAMCOT-12	12.30 ± 0.34^{bc}	13.10 ± 0.60^{a}	14.50 ± 0.62^{b}
SAMCOT-13	12.20 ± 0.66^{b}	14.40 ± 1.01^{ab}	15.70 ± 1.11^{c}

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at $P > 0.05$

4.1.4 Sympodial branches

Results revealed significant differences in the number of sympodial branches among the genotypes. SAMCOT-9 had the highest sympodial branches in all the weeks with the value of 17.60, 20.70 and 19.60 at week 1, 2, and 3, respectively. These highest mean values were significantly different from the values of all other genotypes in the same week. The lowest value was recorded in SAMCOT-13, (11.60) at week 1. This was significantly different from SAMCOT-8, 10, 11, and 12 with the value of 12.20, 12.10, 2.30 and 11.80, respectively. With the exception of SAMCOT-8 (18.80); which was not significantly different (P > 0.05) from SAMCOT-9, there was significant different between the highest value (20.70) in SAMCOT-9 and the values from all other genotypes (Table 4.4). At week 2, SAMCOT-12 had the lowest sympodial branches (13.80), which was not significantly different from the values of SAMCOT-10 (14.60), SAMCOT-11 (15.70) and SAMCOT-13 (15.80).

Table 4.4: Sympodial Branches of Six Nigeria Cotton Genotypes

	Sampling period (week)		
GENOTYPES	8	9	10
SAMCOT-8	12.20 ± 0.86^{a}	18.30 ± 0.70^{b}	21.40 ± 0.87^{d}
SAMCOT-9	17.60 ± 0.88^{b}	20.70 ± 1.39^{b}	19.60 ± 1.05^{b}
SAMCOT-10	12.10 ± 0.91^{a}	14.60 ± 0.73^{a}	18.70 ± 0.78^{a}
SAMCOT-11	12.30 ± 0.36^{a}	15.70 ± 0.63^{a}	20.80 ± 0.57^{cd}
SAMCOT-12	11.80 ± 1.53^{a}	13.80 ± 0.77^{a}	18.00 ± 0.81^{a}
SAMCOT-13	11.60 ± 0.74^{a}	15.80 ± 0.77^{a}	20.20 ± 0.95^{c}

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at P > 0.05

4.1.5 Number of bolls

The result of statistical analysis showed that there were significant differences ($P > 0.05$) in the number of bolls produced among all the genotypes. The highest number of bolls was recorded in SAMCOT-9 (31.30) bolls per plant. This value was not significantly different from the value of SAMCOT-8 (31.00) bolls per plant. The lowest bolls value was recorded in SAMCOT-12 (19.30 bolls per plant). This value was significantly different from all the values recorded for all other genotypes. The number of bolls obtained for SAMCOT-10 and 11 with the values of 20.00 and 26.40 bolls per plant, respectively were not significantly different from each another.

4.1.6 Boll size

Analysis of variance (ANOVA) revealed that there were significant differences ($P < 0.05$) for the boll size in all the genotypes. The biggest boll in terms of size was obtained in SAMCOT-8 with the value of 11.25 cm. This value is significantly different from the value of all other genotypes. SAMCOT-11 had the smallest boll size value (9.60 cm). This smallest value is not significantly different from the boll size of SAMCOT-12 (9.70 cm), but differ significantly from other value of all other genotypes. Similarly, SAMCOT-9 and Samcot10 with boll size of 10.85 cm and (10.90cm), respectively,which are not significantly different ($P > 0.05$) from each other, but differ from the boll size of all other genotypes.

Table 4.5: Number of Bolls Per Plant and Bolls Size of six Nigerian cotton Genotypes

GENOTYPES	Number of Bolls	Boll Size (cm)
SAMCOT-8	31.00 ± 5.50^{c}	11.25 ± 0.25^{c}
SAMCOT-9	31.30 ± 3.23^{c}	10.85 ± 0.30^{b}
SAMCOT-10	20.00 ± 2.27^{b}	10.90 ± 0.31^{b}
SAMCOT-11	26.40 ± 2.23^{bc}	9.60 ± 0.28^{a}
SAMCOT-12	19.30 ± 3.03^{a}	9.70 ± 0.45^{a}
SAMCOT-13	22.10 ± 3.40^{ab}	10.10 ± 0.35^{ab}

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at $P > 0.05$

4.1.7 Yield parameters of six Nigeria cotton gynotypes.

The highest weight of boll per cottongenotype was due to SAMCOT-9 (32.17 g/per plant), this value was significantly different from the value of all other all genotypes at $P < 0.05$. The least in the weight of the boll per plant was due toSAMCOT-8 (11.90 g/per plant), this value was significantly different from the values of other genotypes. The number of seeds per boll showed significant difference among the genotypes (Plate III). The least number of seeds per boll was due to SAMCOT-9 (26.82 seeds per boll), this value was significantly different ($p < 0.05$) from the values of all other genotypes. The highest number of seeds per boll was recorded in SAMCOT-11 (30.68 seeds per boll), followed by SAMCOT-13 (30.40 seeds per boll) and SAMCOT-10 (30.35 seeds per boll). These values were significantly different from one another and with the value of all other genotypes.

Weight of 100 seeds per plant (seed index) was significantly different among the genotypes. Significant highest weight of 100 seeds was due to SAMCOT-12 with the value of 10.29 g per genotype followed by SAMCOT-13 (9.93 g/genotypes) and SAMCOT-8 (9.74 g/genotypes). The least in the weight of seeds per genotype was recorded in SAMCOT-11 (8.05 g). This value was significantly different from the value of all other genotypes. The weight of lint per plant showed significant differences among the genotypes. The lowest weight of lint per plant was obtained in SAMCOT-13 with the value of (6.34 g), this value was significantly different from the value of all other genotype. The highest weight of lint per plant was due to SAMCOT-8 (13.66 g/per plant). SAMCOT-9 and SAMCOT-10 had equal weight of lint per plant with the value of 10.23 g/per plant, which was significantly different from the value of all other genotypes (Plate III).

Table 4.6: Yield Parameters of Six Nigeria Cotton Genotypes

Genotypes	Weight of Bolls per plant (g)	Number of Seeds Per Bolls	Weight of 100 Seeds Per genotypes (g)	Weight of Lint per plant (g)
SAMCOT-8	11.90±1.27[a]	27.66±2.41[b]	9.74±0.67[c]	13.66±1.10[d]
SAMCOT-9	32.17±5.49[d]	26.82±2.49[a]	8.33±0.47[b]	10.23±1.19[c]
SAMCOT-10	26.08±5.27[cd]	30.35±1.40[c]	8.64±0.45[bc]	10.23±1.27[c]
SAMCOT-11	25.67±4.15[c]	30.68±1.50[c]	8.05±0.66[a]	9.40±1.03[b]
SAMCOT-12	23.15±2.74[bc]	28.06±2.46[bc]	10.29±0.56[c]	7.78±1.07[ab]
SAMCOT-13	21.07±3.19[b]	30.40±1.37[c]	9.93±0.57[c]	6.34±0.89[a]

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at P > 0.05

Plate V: Boll and Lint of Cotton

4.1.8 Fibre qualities of six Nigeria cotton genotypes.

The fibre qualities of six gynotypes ofcotton are presented in table 4.7. The result indicated that there is significant difference ($p < 0.05$) in fibre qualities among the six cotton genotype. Spinabilityindex (SCI) varied from 116.50 in SAMCOT-10 to 152.25 in Samcot-11. These values are significantly differently from each other. However, there was no significant difference ($P > 0.05$) in the SCI values obtained from SAMCOT-12 (121.75), SAMCOT-13 (125.00), SAMCOT-8 (131.00) and Samcot9 (129.25).

The fineness, or Micronaire (MIC) measurement for cotton genotype varied from fine (3.00 – 3.90 µg/inch^{-1}) to averagely fine (4.00 – 4.90 µg/inch^{-1}) with the value of 3.96 in SAMCOT-9 and 4.38 in SAMCOT-10. This highest MIC value in SAMCOT-10 (4.38 µg/inch^{-1}) was significantly different from the value of all other genotypes. However, there were no significant difference in the MIC values in SAMCOT-11 (4.02 µg/inch^{-1}), SAMCOT-12 (4.05 µg/inch^{-1}) and SAMCOT-8 (4.13µg/inch^{-1}). With the exception of SAMCOT-12 with the lowest average maturity (MAT) values of 0.85 %, there was no significant difference among the maturity value of all the other genotypes with the MAT value of 0.86 %.

The upper half mean length (UHML) which is also referred to as the average fibre length were low (< 21 mm) in all the genotypes; ranging from (1.09 mm) in SAMCOT-12 to (1.19 mm) in SAMCOT-11. Similarly, there were no significant difference in the UHML recorded for SAMCOT-10 (1.10 mm), SAMCOT-12 (1.09 mm), SAMCOT-13 (1.11 mm) and SAMCOT-9 (1.11 mm), respectively. The fibre uniformity index (UI), otherwise called length uniformity of the cotton examined genotypes revealed that there were significant difference ($p < 0.05$) among the genotypes for this trait. The highest (UI) was due to SAMCOT-11 (86.58 %), followed by SAMCOT-8 (84.45 %); while the

least recorded in SAMCOT-9 with the value of (82.95 %). Significant highest length (7.40 %) of short fibre (SF) was due to SAMCOT-9 and the lowest was obtained in SAMCOT-11 with the value of (5.03 %). These values were significantly different from each other and from the values of all other genotype. However, there were no significant difference ($p > 0.05$) in the SF values recorded for SAMCOT-8 (6.30 %), SAMCOT-10 (6.70 %) and SAMCOT-13 (6.43 %). The strength value (STR) of the cotton genotypes ranges from (24.18 $kNmkg^{-1}$) in SAMCOT-10 to (29.08 $kNmkg^{-1}$) in SAMCOT-11. The STR value recorded for SAMCOT-11 was significantly higher than the value of all other genotype. Also, there was no significant difference in the STR values recorded for SAMCOT-12 (25.30 $kNmkg^{-1}$) and SAMCOT-8 (26.10 $kNmkg^{-1}$). The values of fibre elongation (ELG) varied significantly among the cotton genotypes. The highest ELG was recorded for Samcot12 with the value of (6.30 %). This value was not significantly different ($p < 0.05$) from the ELG values recorded forSAMCOT-11 which had the lowest fibre elongation of (6.15 %) with the lowest value of (5.78 %) in SAMCOT-8.

The values of brightness (RD) (% reflectance) of the genotypes varied significantly from (76.08 %) in SAMCOT-9 to (72.30 %) in SAMCOT-13. However, there was no significant difference ($p > 0.05$) in the RD values recorded for SAMCOT-10(74.75 %) and SAMCOT-8 (75.70 %), respectively. The hunters scale (HS) values of the fibre of six genotypes of cotton showed that SAMCOT-12 has a significantly lowest value (7.80) than all other cotton fibre. On the other hand, there was no significant difference in the values of hunters scale recorded for other cotton Genotypes (Table 4.6). The value of colour grade code (CGRD) of the cotton genotypes was significantly highest in SAMCOT-12 (43.65). Also, colour grade code (CGRD) of SAMCOT-11 (36.35) and SAMCOT-8 (34.15) were not significantly different.

Table 4.7a: Fibre Qualities Composition of Six Nigeria Cotton Genotypes.

Genotypes	SCI	MIC ($\mu g/inch^{-1}$)	MAT (%)	UHML (mm)	UI (%)	SF (%)	STR ($kNmkg_{-}^{1}$)
SAMCOT-8	131.00±10.22[b]	4.13±0.04[ab]	0.86±0.00[ab]	1.13±0.02[ab]	84.43±0.86[b]	6.30±0.53[b]	26.10±1.78[ab]
SAMCOT-9	129.25±3.94[ab]	3.96±0.07[a]	0.86±0.00[ab]	1.11±0.02[a]	82.95±0.19[a]	7.40±0.18[c]	27.73±0.85[bc]
SAMCOT-10	116.50±4.56[a]	4.38±0.01[c]	0.86±0.00[ab]	1.10±0.02a	83.55±0.66[a]	6.70±0.14[b]	24.18±0.17[a]
SAMCOT-11	152.25±2.50[c]	4.02±0.04[ab]	0.86±0.00[ab]	1.19±0.02[b]	86.58±0.58[c]	5.03±0.40[a]	29.08±0.18[c]
SAMCOT-12	121.75±2.56[ab]	4.05±0.02[ab]	0.85±0.00[a]	1.09±0.01[a]	83.70±0.39[a]	7.03±0.41[bc]	25.30±0.21[ab]
SAMCOT-13	125.00±7.49[ab]	4.09±0.03[b]	0.86±0.00[ab]	1.11±0.03[a]	83.69±0.74[ab]	6.43±0.32[b]	26.43±0.95[abc]
USDA	-	3.50-4.90	0.75-0.95	1.11-1.36	77-87	6-18	22-32

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at P > 0.05

SCI: Spinability index MIC: micronaire MAT: Maturity UHML: Upper Half Mean Length UI: Uniformity Index SF: Short Fibres STR: Strength

Table 4.7b: Fibre Qualities Composition of Six Nigeria Cotton Genotypes

Genotypes	ELG (%)	RD (%)	+B	CGRD	TrCnt	TrAr (%)	TrID
SAMCOT-8	5.78±0.10a	75.70±0.57^{d}	9.18±0.24^{b}	34.15±2.32^{b}	20.25±3.15^{b}	0.43±0.09^{ab}	3.50±0.65^{ab}
SAMCOT-9	6.15±0.09bc	76.08±0.26^{d}	8.78±0.22^{b}	31.25±0.03^{a}	10.75±0.63^{a}	0.18±0.02^{a}	1.50±0.29^{a}
SAMCOT-10	6.05±0.09abc	74.75±0.30^{cd}	9.15±0.06^{b}	31.58±0.21^{a}	27.25±2.46^{bc}	0.47±0.08^{ab}	3.75±0.48^{ab}
SAMCOT-11	5.90±0.04ab	74.08±0.54^{bc}	8.65±0.10^{ab}	36.35±2.86^{b}	25.50±3.71^{bc}	0.45±0.10^{ab}	3.75±0.48^{ab}
SAMCOT-12	6.30±0.11c	73.00±0.52^{ab}	7.80±0.32^{a}	43.65±2.48^{c}	33.75±1.93^{cd}	0.51±0.09^{b}	3.75±0.48^{ab}
SAMCOT-13	6.00±0.08ab	72.30±0.61^{a}	9.18±0.30^{b}	39.43±2.42^{bc}	38.25±2.95^{d}	0.62±0.08^{b}	4.50±0.50^{b}
USDA	5.0-7.77						

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at P > 0.05

Keys: ELG: Fibre Elongation (%) RD: Brightness b: hunters scale CGrd: Colour grade code TrID: Indication of trash particles 0.01 inch in diameter or larger TrAr: The (%) of the sample area occupied by trash.
USDA: United state Department of Agriculture Standard

4.1.9 Cross correlation coefficient of the morphologicaland yield parameters of six Nigeria cotton genotypes

Person correlation of the morphological parameters is detailed in Table 4. Significant and positive correlation of plant height with Number of bolls per plant (r = 0.356) and weight of boll per plant (r = 0.628) recorded at p < 0.05 indicate that plant height has direct influence on yield of the crop. Similarly, number of bolls per plant was significantly and positively correlated with number of monopodial branching (0.576) and sympodial branching indicating that improvement for any of these traits would simultaneously enhance the other characters. Strong positive and significant correlation of Lint weight per plant with sympodial (r = 0.465) and monopodial (r = 0.668) braching, number of seeds per plant (r = - 0.437) at p < 0.05 and number of bolls per plant (r = 0.747, p < 0.01), showed that this trait (Lint weight) could be breed for, by selection and improvenet on these traits. However, weight of lint per plant was significantly and negatively correlated to plant height (r = -0.538) and weight of boll per plant.

Table 4.8 Cross correlation Coefficient of the Morphological and yield Parameters of Six Nigeria cotton Genotypes

Pameters	Plh	MPB	SYPB	NBoll/p	Bollsize	WtB/P	NS/Boll	Wt100S/g	WL/P
Plh	1.00								
MPB	0.228	1.00							
SYPB	-0.02	0.747**	1.00						
NBoll/p	-0.042	0.579**	0.709**	1.00					
Bollsize	-0.103	-0.322	0.239	0.495*	1.00				
WtB/P	0.628*	-0.061	-0.494*	-0.087	-0.233	1.00			
NS/Boll	0.356*	0.053	0.069	-0.554*	-0.44	0.012	1.00		
Wt100S/g	-0.417	-0.244	-0.195	-0.373	-0.085	-0.631*	-0.193	1.00	
WL/P	-0.538*	-0.045	0.465*	0.668*	0.747**	-0.378*	0.437*	-0.242	1.00

** Significant at $p \leq 0.01$ * Significant at $p \leq 0.05$

Keys: Plh – Plant Height MPB – Monopodial Branch SYPB – Sympodial BranchNBoll/p – Number of Bolls per Plant WtB/P – Weight of Boll per PlantNS/Boll – Number of Seed per Boll Wt100S/g – Weight of Hundred SeedsWL/P - Weight of Lint per Plant

4.1.10 Estimated genetic parameters for morphologicaland yield parameters of six Nigerian cotton genotypes

Analysis of genetic parameters for the six cotton genotypes showed with the exception of plant height and weight of lint per plant, the genotypic variance (GV) of most traits studied were lower than their corresponding environmental variance (EV). This indicates that the expressions of most of the character wereinfluenced by evironmnental factors. The higher GV (263.34, 13.45) for plant height and weight of lint per plant, respectively obtained revealed the reliability of these traits for selection.Hiigh genotypic and phenotypic coefficient of variance (> 30) recorded for weight of dry bolls (46.00 and 66.57, respectively) and weight of lint per plant (38.17 and (50.06), respectively) imply that the selection based on these characters would help successful isolation of desirable genotypes due to the resulted high genetic advance. However, the PCV and GCV were low for all other traits studied.

The plant height and weight of dry boll recorded a very high percentage of phenotypic value (PV) 340.20 % and 241.40 % respectively. Values observed for Number of seed/boll and weight of lint. (25.68) and (23.13) respectively are equally high; but number of bolls, sympodial branch has moderate values in (PV). The least was due to monopodial branch and weight of 100 seeds 7.85 and 4.06 %. Furthermore, highest value for environmental variance was due to weight of dry boll (126.12) followed by plant height 76.92. The value in the environmental variance as recorded in weight of 100 seed (seed index) was 2.59. It was observed that 22.40 was due to number of seed/boll while other trait such as number of bolls, weight of lint and monopodial branch recorded very low except sympodial branch that recorded moderate value 18.27.

The broad sense heritability was high for plant height (77 %) and moderate for number of bolls per plant (41 %), weight of dry boll (48 %) weight of 100 seeds (36 %) and

weight of lint (58 %). These indicate that such traits are heritable with considerable high to moderate hybridization successs in the next generation. However, number of seeds per boll, monopodial branching and synpodial branching have very low heritability percentage (13.00 %, 8.00 % and 6.00 %), respectively. These traits are greatly influenced by environmental factors, and as such hubridisation success could be low. High genetic advance (GA > 30 %) was recorded for plant height (30.97), weight of dry boll (65.48) and weight of Lint per (59.97). These hight GA coupled with moderate to high heritability obtained in these traits indicate the reliability inselection of these characters for transmission and improvement of next generations.

Table 4.9: Estimated Genetic Parameters for Morphological and Yield Parameters of Six Nigerian Cotton Genotypes

Traits	Means	PV	GV	EV	GCV	PCV	h^2	GA
Plant Height	94.97	340.26	263.34	76.92	17.09	19.42	77.39	30.97
Num of Boll	25.02	12.26	4.98	7.28	8.92	14.00	40.61	11.71
Weight of Dry Boll	23.34	241.40	115.28	126.12	46.00	66.57	47.75	65.48
Num of Seed/Boll	29.00	25.68	3.28	22.40	6.24	17.48	12.76	4.59
Wight of 100Seeds	9.16	4.06	1.47	2.59	13.24	21.98	36.26	16.42
Weight of Lint	9.61	23.13	13.45	9.68	38.17	50.06	58.15	59.97
Monopodial Branch	15.20	7.85	0.63	7.22	5.23	18.44	8.05	3.06
Sympodial Branch	19.78	19.41	1.14	18.27	5.40	22.27	5.88	2.70

KEYS

PV – Phenopytic Variance GV – Genotypic Variance EV–Environmental Variance GCV – Genopytic Coefficient of Variance PCV – Phenopytic Coefficient of Varianceh^2 – Heritability GA – Genetic Advance

4.1.11 Estimated genetic parameters for fibre qualities of six Nigerian cotton genotypes

The results showed that for most of the fibre quality, the genotypic variance (GV)were higher than their corresponding environmental variance (EV), except for STR, TrAt and Amt with EV values of 3.26, 0.03 and 5285.82, respectively when compared with the GV value of 2.95, 0.02 and 887.07, respectively (Table 4.5). This indicated that the expression of these traits was more under the genetic control than the environmental factors. However, for STR, the EV (3.26) was greater than the GV (2.95) indicating high influenced of the environment on the expression of the traits thereby restricting the scope for it improvement through selection.

Highest phenotypic and genotypic variance was due to Amt with the value of 6172.89 and 887.07, respectively and the least was obtained for UHML with the value of 0.00 for both PV and GV. With the exception of Trcnt (41.83, 46.59), TrAt (30.82, 47.67) and TrID (29.79, 41.09) with moderate to high coefficient of variability (value > 20) for GCV and PCV respectively, the values were low for all the fibre qualities. These high PCV and GCV for Trcnt, TrAt and TrID suggest that the selection based on these characters would facilitate successful isolation of desirable genotypes. Also, the narrow gap between the PCV and GCV for most of the traits indicates that environment had little influence in the expression of the traits.

Broad sense heritability estimate was high (> 60 %) for MIC (79.00), RD (74.00), and Trcnt (81.00), but low (< 30) in Amt (14.00).In all other parameters, the broad heritability obtained was moderate (30 – 60 %). Genetic advanced (GA) was high (> 60) for only Trcnt with percentage values of 77.36. The high broad sense heritability and GA obtained in Trcnt indicating that this trait was under the influence of additive gene action and hence selection for this character could be effective in improving fibre quality in cotton. However, the GA value was moderate for TrAt and TrID with the

value of 41.04 and 44.49 respectively; while low in all other traits with value of less than 30 % (Table 4.5).

Table 4.10 Estimated Genetic Parameters for Fibre Qualities of Six Nigerian Cotton Genotypes

Traits	Means	GV	PV	EV	h^2	GCV	PCV	GA
SCL	129.29	158.43	298.28	139.85	53.12	9.74	13.36	14.62
MST	7.79	0.09	0.14	0.05	63.92	3.86	4.83	6.36
MIC	4.11	0.03	0.03	0.01	79.41	4.00	4.49	7.35
UHML	1.12	0.00	0.00	0.00	33.33	2.82	4.88	3.35
UI	84.15	1.68	3.19	1.51	52.69	1.54	2.12	2.30
SF	6.48	0.72	1.23	0.51	58.59	13.11	17.12	20.67
STR	26.47	2.95	6.21	3.26	47.49	6.49	9.41	9.21
ELG	6.03	0.04	0.07	0.03	54.08	3.12	4.24	4.72
RD	74.32	2.63	3.58	0.94	73.63	2.18	2.54	3.86
0B	8.79	0.31	0.52	0.21	59.92	6.34	8.20	10.12
CGRD	36.07	25.22	42.30	17.08	59.62	13.92	18.03	22.15
Trcnt	25.96	117.89	146.26	28.38	80.60	41.83	46.59	77.36
TrAt	0.44	0.02	0.04	0.03	41.79	30.82	47.67	41.04
TrID	3.46	1.06	2.02	0.96	52.56	29.79	41.09	44.49
Amt	598.54	887.07	6172.89	5285.82	14.37	4.98	13.13	3.89

4.1.12 Percentage mineral composition of six Nigeria cotton genotypes

The result obtained for minerals are presented in Table 4.8. SAMCOT-12 had the lowest Calcium (Ca) with a mean of (10.41 %); this value is significantly different from the other genotypes. The highest mean value (21.72 %) was recorded in SAMCOT-10. This was significantly different from the value of all other genotypes, except SAMCOT-8 (21.00 %). SAMCOT-9 (12.70 %) and SAMCOT-11 (12.65 %) are not significantly different from each other. Magnesium content was highest in genotype Samcot12 (72.46 %) and lowest in SAMCOT-9 (35.43 %).These values were significantly different from each other and from the values of all other genotypes. The value due for Samcot11 (63.71 %) and Samcot13 (65.60 %) are significantly the same in magnesium content $P > 0.05$. Other genotypes such as SAMCOT-8 (47.05 %) and Samcot-9 (35.43 %) have their values significantly different from each other.

SAMCOT-13 (1.90 %) had the highest value in sodium (Na) content followed by SAMCOT-12 (1.81 %). These values were not different significantly ($P > 0.05$) from each other but different from the values all other genotypes. The lowest in the sodium content was due to SAMCOT-9 (1.14 %). However, SAMCOT-10 and SAMCOT-11 had the same values 1.30 % and are not significantly different from each other. In terms of potassium the highest mean value was obtained in SAMCOT-12 (56.45 %) follow by SAMCOT-9 (52.52 %). These values were significantly different from one another at $p > 0.05$). The lowest potassium content was due to SAMCOT-12 followed by SAMCOT-10 with the value of (44.43 %) and (44.60 %), respectively. These values were not significantly different from each other. The highest phosphorus content was due to SAMCOT-9 (5.61 %), which was significantly different from the value of all other genotypes. SAMCOT-8 (3.27 %) had the lowest phosphorus content. Phosphorus

content of (4.88 %) and (4.68 %) were due to SAMCOT-11 and SAMCOT-13; these values were not significantly different from each other.

In terms of iron (Fe) the lowest content was due to SAMCOT-9 (2.54 %) which was significantly different from all other genotypes. Significant highest (Fe) content was obtained in SAMCOT-10 (3.84 %). SAMCOT-11 (2.89 %), SAMCOT-12 (2.91 %) and SAMCOT-13 (2.89 %) were significantly different. SAMCOT-8 contains the highest value (2.28 %) of copper (Cu) which was significantly different from the value of all other genotypes. The least in copper content was due to SAMCOT-13 (1.38 %) which was not significantly different from the value of SAMCOT-9 (1.55 %). It was observed that SAMCOT-12 (2.10 %) and SAMCOT-11 (2.18 %) are significantly the same. The highest mean value of manganese was due to SAMCOT-10 (1.73 %) which was significantly different from all other value. SAMCOT-11(1.64 %) and SAMCOT-8 (1.60 %) had their values significantly the same. The lowest in the manganese content was obtained in SAMCOT-12 (1.21 %) which was significantly different from the value of all other genotypes.

Table 4.11: Percentage (%) Mineral Compositionof Six Nigeria Cotton Genotypes Seeds

Genotypes	Ca %	Mg %	Na %	K %	P %	Fe %	Cu %	Mn %
Samcot-8	21.00±0.57[c]	47.50±0.08[c]	1.44±0.06[c]	49.21±0.35[c]	3.27±1.33[a]	2.74±0.05[b]	2.28±0.01[d]	1.60±0.01[d]
Samcot-9	12.70±0.17[b]	35.43±0.40[a]	1.14±0.02[a]	52.52±0.24[c]	5.61±10.01[d]	2.54±0.03[a]	1.55±0.01[b]	1.59±0.0[c]
Samcot-10	21.72±0.16[d]	44.73±0.24[b]	1.30±0.01[b]	44.60±0.31[a]	3.70±0.88[b]	3.84±0.02[d]	2.06±0.03[b]	1.73±0.0[e]
Samcot-11	12.65±0.44[b]	63.71±0.60[d]	1.30±0.02[b]	44.43±0.30[a]	4.88±1.73[c]	2.89±0.05[c]	2.18±0.01[c]	1.64±0.0[d]
Samcot-12	10.41±00.21[a]	72.46±0.32[e]	1.81±0.04[d]	56.45±0.28[b]	3.58±0.50[ab]	2.91±0.02[d]	2.10±0.09[c]	1.21±0.0[a]
Samcot-13	10.74±0.14[ab]	65.60±0.32[d]	1.90±0.01[d]	50.28±0.01[b]	4.68±1.71[c]	2.89±0.03[c]	1.38±0.01[a]	1.31±0.02[b]

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at $P > 0.05$

Ca – Calcium, Mg – Magnesium, Na – sodium, K –Potassium, P – Phosphorus, Fe – Iron, Cu – Copper and Mn -Manganese

4.1.13 Physical propertiesof cotton seed oil of six Nigeria cotton genotypes

The properties of the oil from the genotypes are presented in Table 4.9. The value of peroxide in the oil was highest in SAMCOT-8 (3.17 meq/kg). This value is significantly different ($p > 0.05$) from the value of all other genotypes. The lowest peroxide value is due to SAMCOT-9 (1.26 meq/kg) which differ significantly from all other genotypes. SAMCOT-11 and SAMCOT-12 had peroxide value of2.37meq/kg and 2.38 meq/kg, respectively. Iodine value was lowest in SAMCOT-13 (74.51 g/100g), which is significantly different from the value recorded for other genotypes. Significant highest in iodine value was due to SAMCOT-11 (84.28 g/100g). SAMCOT-10 and SAMCOT-12 had iodine value of 78.40 g/100 g and 78.60 g/100 g, respectively which were not significantly different from one another.

The result for acid values was significantly higher in SAMCOT-8 (2.73 mg/100 g) and the lowest was due to SAMCOT-9 (1.91 mg/100g). Other genotypes such as SAMCOT-10, SAMCOT-11, SAMCOT-12 and SAMCOT-13 had the values of 2.91 mg/100 g, 2.13 mg/100 g, 2.21 mg/100 g, and 2.10 mg/100 g, respectively which were not significantly different from one another at ($p > 0.05$).Saponification value is significantly highest in SAMCOT-9 (2.10 mg/100 g) and the lowest value was obtained in SAMCOT-11 (1.90 mg/100 g). These values are significantly different from one another and from the value of all other genotypes. SAMCOT-10 and SAMCOT-13 each had values of 1.92. Specific gravity of all the genotypeswere the same, each with the value of (0.91).

Viscocity of the oil is significantly highest which wasdue to SAMCOT-10 (17.42 m m/s^2) and lowest value was due to SAMCOT-9 (16.22 m/s^2).These values were significantly different from one another and from the values of all other genotypes.

SAMCOT-11 (16.80 m/s^2) and SAMCOT-13 (16.90 m/s^2) were not significant from each other. Other genotypes such as SAMCOT-8 (16.60) and SAMCOT-12 (17.25 m/s^2) had values that were significantly different. Moisture contents is lowest in SAMCOT-9 (1.44 %), which was significantly different from all other genotypes. SAMCOT-12 andSAMCOT-13 had the values of (1.58 %) and (1.53 %), respectively. These values were significantly not different at ($p < 0.05$). The highest in the moisture contents was obtained in SAMCOT-8 (1.66 %) followed by SAMCOT-10 (1.65 %). These values were not significantly from each other.

Table 4.12: Physical Properties of Cotton Seed oil of Six Nigeria Cotton Genotypes

Genotypes	PV (meq/kg)	IV (g/100/sg),	AV (mg/100g),	SPV (mg/100)	SG	V (m/s^2),	MC (%)
SAMCOT-8	3.17 ± 0.06^d	83.60 ± 0.04^c	2.73 ± 0.15^c	2.04 ± 0.01^d	0.91 ± 0.00^a	16.60 ± 0.01^b	1.66 ± 0.01^c
SAMCOT-9	1.26 ± 0.02^a	76.24 ± 0.02^b	1.91 ± 0.02^a	2.10 ± 0.02^e	0.91 ± 0.00^a	16.22 ± 0.01^a	1.44 ± 0.03^a
SAMCOT-10	2.15 ± 0.01^b	78.40 ± 0.02^c	2.19 ± 0.01^b	1.92 ± 0.01^b	0.91 ± 0.00^a	17.42 ± 0.02^e	1.65 ± 0.01^c
SAMCOT-11	2.37 ± 0.02^c	84.28 ± 0.02^f	2.13 ± 0.01^b	1.90 ± 0.01^a	0.91 ± 0.00^a	16.80 ± 0.01^c	1.77 ± 0.02^d
SAMCOT-12	2.38 ± 0.01^c	78.60 ± 0.01^c	2.21 ± 0.02^b	1.94 ± 0.01^c	0.91 ± 0.00^a	17.25 ± 0.02^d	1.58 ± 0.02^b
SAMCOT-13	2.21 ± 0.01^b	74.51 ± 0.02^a	2.10 ± 0.01^b	1.92 ± 0.01^b	0.91 ± 0.00^a	16.90 ± 0.02^c	1.53 ± 0.02^b

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at $P > 0.05$.

Keys: PV -Peroxide value (meq/kg), IV -Iodine Value (g/100/sg), AV -Acid Value (mg/100g), SPV – Saponification Value (mg/100) SG - Specific gravity, V -Viscocity (m/s^2), MC -Moisture contents (percentage) %

4.1.14 Phytochemical composition of Nigeria cotton genotypes leaves

Significant variation was obtained in all the phytochemical composition (Table 4.10). With the exception of Oxalate, SAMCOT-8 had the lowest phytochemical constituent for Phenol, Flavonoid, Tanmins, Phytate, Saponin, and Alhaloid with the value of 294.34 mg/100 ml, 163.12, 206.18, 315.40, 116.14 and 162.98 mg/100 ml, respectively. These values were significantly different from the value of all other genotypes. Significant highest Phenol (1040.14 mg/100 ml), Flavonoid (342.56 mg/100 ml), Tannins (936.35 mg/100 l), Alkaloid (284.82 mg/100ml) and Saponins (642.26 mg/100 ml) were obtained in SAMCOT-13. SAMCOT-10 had the highest phytate content with the value of 702.91 mg/100ml. These highest values were significantly different from the value of all other genotype. However, there was not significantly different between SAMCOT-11 (185.45 mg/100 ml) and Samecot 12 (184.14 mg/100 ml) for Alkaloid and between SAMCOT-8 (10.310) and Samecot 11 (9.94 mg/100 ml) for Oxalate (Table 4.10).

Table 4.13 Phytochemical Composition of Nigeria Cotton Genotypes leaves

Parameter	Phenols (mg/100 ml)	Flavonoids (mg/100 ml)	Tannins (mg/100 ml)	Phytates (mg/100 ml)	Saponins (mg/100 ml)	Alkaloids (mg/100 ml)	Oxalates (mg/100 ml)
Samcot-8	294.34±1.50[a]	163.12±1.19[a]	206.18±2.46[a]	315.40±1.83[a]	116.14±2.06[a]	162.98±1.80[a]	10.31±0.00[b]
Samcot-9	554.26±1.88[b]	283.34±1.56[b]	354.35±1.46[c]	429.58±1.91[d]	184.96±2.42[b]	177.16±1.69[b]	11.88±0.38[c]
Samcot-10	984.47±4.57[e]	315.36±0.00[e]	684.00±0.70[d]	702.91±3.16[f]	225.53±2.49[c]	225.31±2.36[d]	15.32±0.62[d]
Samcot11	962.62±2.45[d]	334.47±2.37[d]	718.95±2.04[e]	352.82±0.00[b]	243.38±1.78[d]	185.45±2.10[c]	9.94±0.37[b]
Samcot-12	652.08±0.98[c]	291.37±3.12[c]	219.59±1.81[b]	375.09±1.56[c]	505.54±1.90[e]	184.14±2.23[c]	8.00±0.00[a]
Samcot-13	1040.14±0.00[f]	342.56±0.87[f]	936.35±2.16[f]	569.49±2.32[e]	642.26±1.72[f]	284.82±1.16[e]	19.29±0.37[e]

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at $P > 0.05$

4.1.15 Percentage (%) proximate composition of six Nigeria cotton genotypes

Proximate analysis showed that SAMCOT-11 had the highest moisture content of 3.50 %, which was significantly different from the value of all other genotypes. The lowest value of moisture content was due to SAMCOT-13 (2.00 %), this value was significantly different from the value of all other genotypes. Other genotypes such as SAMCOT-10 (2.17 %), SAMCOT-9 (2.33 %), SAMCOT-8 (2.83 %) and SAMCOT-12 (2.67 %) were significantly different from one another ($p < 0.05$). Ash content was lowest in SAMCOT-10 (3.00 %) and highest in SAMCOT-9 (5.33 %). These values were significantly different from each other and from the value of all other genotypes.

The highest crude fat was obtained in SAMCOT-11 (18.00 %) which was significantly different from the value of all other genotypes. SAMCOT-13 (13.83 %) and SAMCOT-8 (14.00 %) had values that were not significantly different from each other. The lowest crude fat was due to SAMCOT-9 (11.33 %) which was significantly different from the value of all other genotypes. Other genotypes such as SAMCOT-12 (5.83 %) and SAMCOT-10 (12.50 %) have their values significantly different from one another. The result of crude protein showed that SAMCOT-8 had the highest value of (22.17 %). This value is not significantly different from the value of SAMCOT-13 (21.79 %), but significantly different from the value of all other genotypes. The lowest value recorded for protein content was due to SAMCOT-9 (11.66 %). This value was significantly different from all other values. Significantly differencein the value of fibre content was obtained among the genotype, with SAMCOT-10 (11.67 %) having the lowest content. This value was significantly different from all other values.

The result for fibre contents in SAMCOT-8 (14.50 %) and SAMCOT-11 (14.67 %) were not significantly different from each other, but differ from all other genotypes. SAMCOT-12 (60.87 %) had the highest carbohydrate value; this value was significantly

different from all other genotypes. Also, SAMCOT-11 and SAMCOT-13 had the value of 55.75 % and 55.62 %, respectively, which were not significantly different one another, but significant to all other genotypes. The lowest in carbohydrate was due to SAMCOT-8 (11.80 %) which was significantly different from other genotypes.

Table 4.14: Percentage (%) Proximate Composition of Six Nigeria Cotton Genotypes

Genotypes	Moisture	Ash	Fat	Protein	Fibre	Carbohydrate
SAMCOT-8	2.83 ± 0.17^{d}	5.00 ± 0.29^{e}	14.00 ± 0.50^{c}	22.17 ± 0.58^{e}	14.50 ± 1.00^{c}	41.80 ± 1.45^{a}
SAMCOT-9	2.33 ± 0.17^{b}	5.33 ± 0.17^{f}	11.33 ± 0.17^{a}	11.66 ± 0.17^{a}	15.50 ± 0.29^{d}	53.88 ± 0.85^{b}
SAMCOT-10	2.17 ± 0.17^{ab}	3.00 ± 0.29^{a}	12.50 ± 0.58^{b}	17.56 ± 0.15^{c}	11.67 ± 0.17^{a}	54.91 ± 1.61^{c}
SAMCOT-11	3.50 ± 0.00^{e}	4.83 ± 0.17^{d}	18.00 ± 0.29^{e}	15.93 ± 0.10^{b}	14.67 ± 0.44^{c}	55.75 ± 0.34^{d}
SAMCOT-12	2.67 ± 0.33^{c}	4.50 ± 0.29^{b}	15.83 ± 0.17^{d}	20.88 ± 0.12^{d}	17.00 ± 0.00^{e}	60.87 ± 0.70^{e}
SAMCOT-13	2.00 ± 0.50^{a}	4.67 ± 0.60^{c}	13.83 ± 0.44^{c}	21.79 ± 0.43^{e}	13.33 ± 0.44^{b}	55.62 ± 0.57^{d}

Values are mean ± standard error of mean. Values of genotypes followed by the same letter(s) along the column are not significantly different at $P > 0.05$

4.1.16 Percentage (%) fatty acid composition of six Nigeria cotton genotypes using Gc-Ms

The result obtained for the fatty acid composition is shown in figure4.4 from the result SAMCOT-13 had the highest palmitic acid content of 10.74 %. SAMCOT-8 recorded the least palmitic value of 0.85 %. Fatty acids are not evenly distributed in all the samples in this study. Lenoleic and Oleic acid are the least distributed which occur in SAMCOT-11 and SAMCOT-9 with the values 12.72 % and 2.17 % respectively.

Arachidic and Margaric acid are not distributed in all the samples as well. SAMCOT-13 had the highest value for arachidic acid with (1.76 %) and the least was SAMCOT-12 (0.58 %). SAMCOT-10 recorded 0.68 % for margaric acid. The lowest value for margaric acid occurred in SAMCOT-11. SAMCOT-9 and SAMCOT-12 have their values 0.62 % and 0.58 % respectively for margaric acid. Oxalic acid had 5.71 % in SAMCOT-11 followed by SAMCOT-8 with 2.90 %. SAMCOT-13 had 0.26 % for meristic acid while SAMCOT-11 (0.19 %) had the least value for meristic acids.

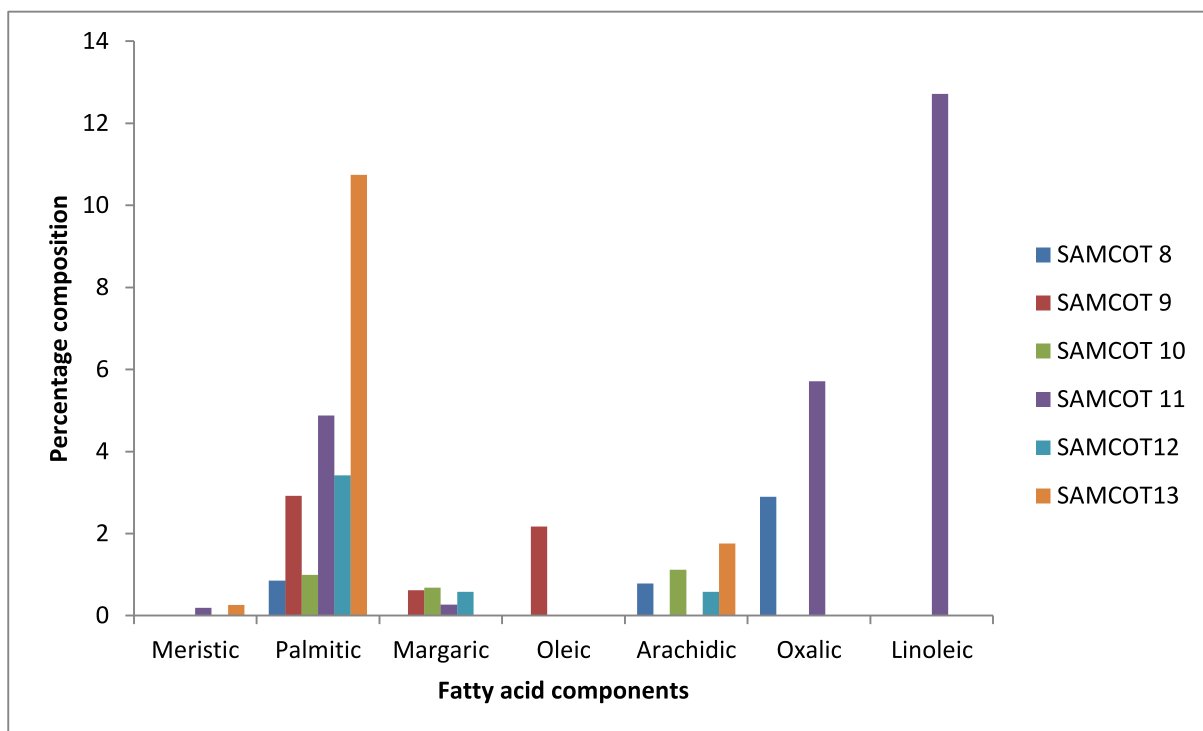


Figure 4.2: **Percentage (%) Fatty Acid Composition of SixNigeria Cotton Genotypes using GC-MS**

4.1.17 Cytological analysis of six Nigeria cotton genotypes.

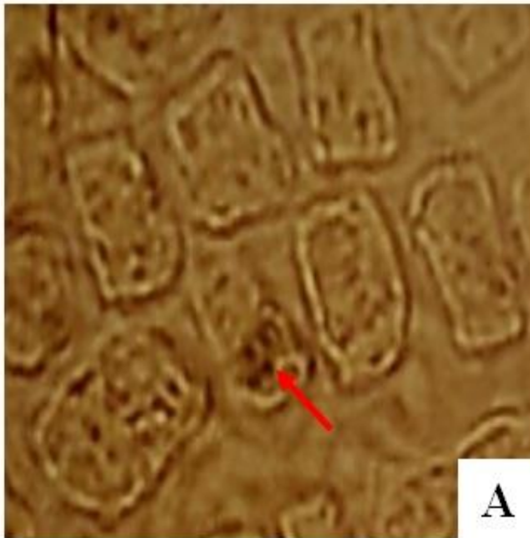
The pollen viability and size of the genotypes were presented in Table 4.11. The results indicated that there were significant variations ($P < 0.05$) in the pollen parameters of the cotton genotypes. The highest percentage viability was recorded in SAMCOT-11 (93.48 %) and the lowest was recorded in SAMCOT-8 with value of 77.98 %. significant variation in the pollen size (both intine and exine) among the genotype was obtained, with the lowest intine (20.50 µm) and exine (27.00 µm) recorded in SAMCOT-11. The highest intine of pollen was obtained in SAMCOT-12 (25.50 µm) and exine (33.00) in SAMCOT-8. However, there was no significant different in the values recorded in the intine for the values of SAMCOT-9 (25.50), 10 (25.50) and 12 (25.50), and between the highest exine (33.00) in SAMCOT-8.

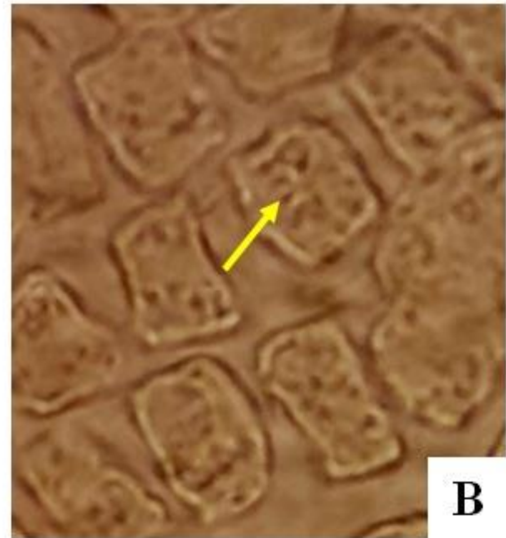
The mitotic chromosome counts of all the genotypes indicated that the plant is tetraploid with $2n = 4x = 52$. Based on distance of the centromere from chromosome centre, some of the genotypes (SAMCOT-8, SAMCOT-9 and SAMCOT-12) revealed acrocentric, metacentric and submetacentric types of chromosome (Plates 6a, 6b and 6c). Regularcytokinesis was observed at the telophase stage of SAMCOT-8 and SAMCOT-12 with the division of cytoplasm into equal part (Plate 7). Two sister chromatids of each chromosome divided equationally and were pulled oppositely. Division of the cells was normal (late telophase stage).

Table4.15: Percentage (%) of Pollen Viability and Non-viability of some Nigeria cotton Genotypes of Flower bud

Genotypes	% Pollen viability	% Pollen non-viability	Intine(μm)	Exine(μm)
SAMCOT-8	77.78±0.75^{a}	22.22±0.62^{cd}	24.25±2.64^{b}	33.00±1.43^{c}
SAMCOT-9	94.25±0.42^{c}	5.75±0.64^{a}	25.25±0.69^{c}	30.50±0.62^{ab}
SAMCOT-10	81.22±0.45^{b}	18.78±0.36^{c}	25.50±0.73^{c}	31.50±0.55^{b}
SAMCOT-11	93.48±0.96^{c}	6.52±0.74^{a}	20.50±1.17^{a}	27.00±1.11^{a}
SAMCOT-12	86.14±0.34^{b}	13.86±1.41^{bc}	25.50±1.17^{c}	31.50±1.19^{b}
SAMCOT-13	90.16±1.26^{c}	9.84±0.43^{b}	24.50±0.82^{b}	30.50±0.73^{ab}

Values with the same superscript along the column are not significantly different from each other at $P \leq 0.05$ according to DMRT





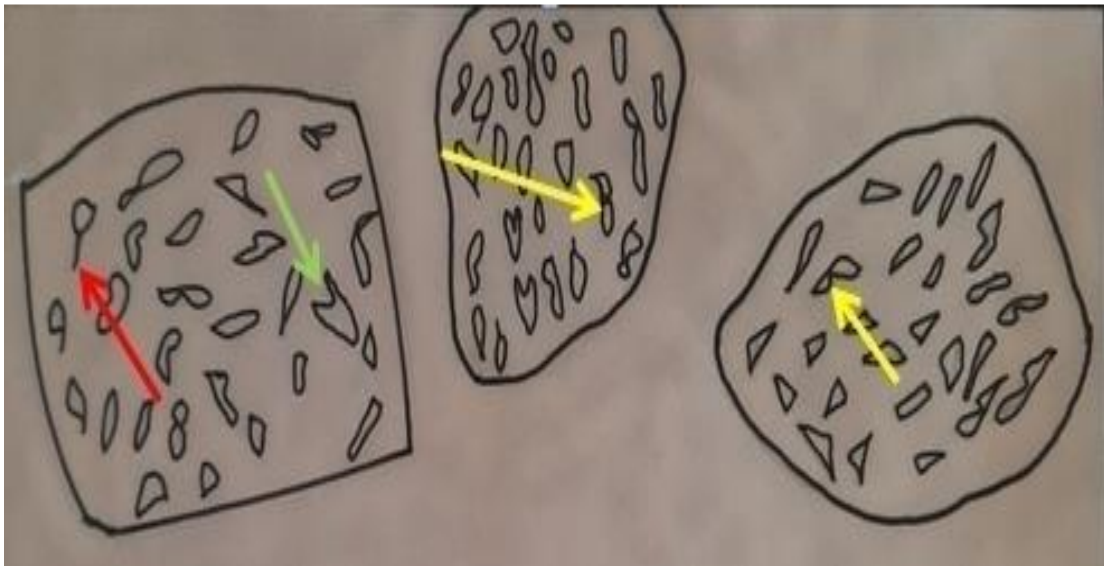


Plate VI: (A) Congregation of chromosomesSAMCOT-8 (B) Denotes presence of some chromosomes SAMCOT-9 (C) chromosomes shapes based on the position of the centromere. Red arrow is telocentric green arrow is acrocentric while yellow arrow is metacentric

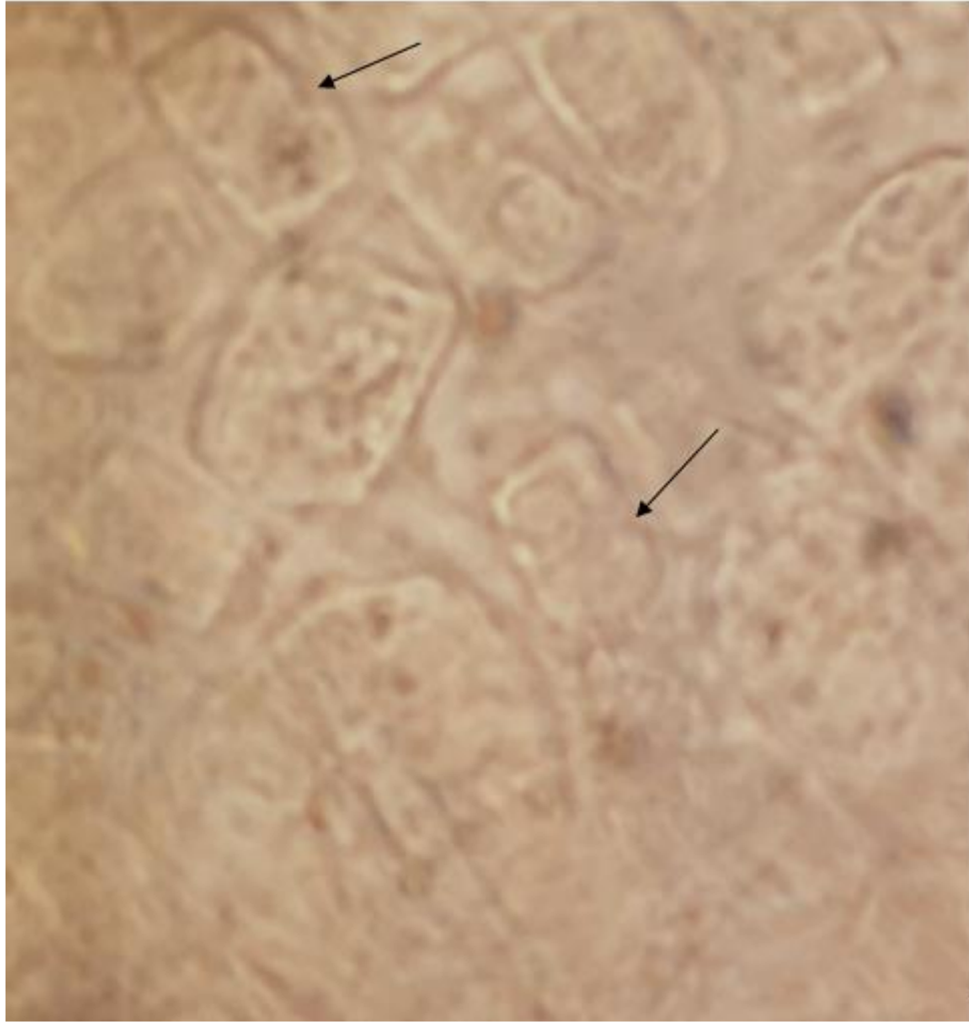
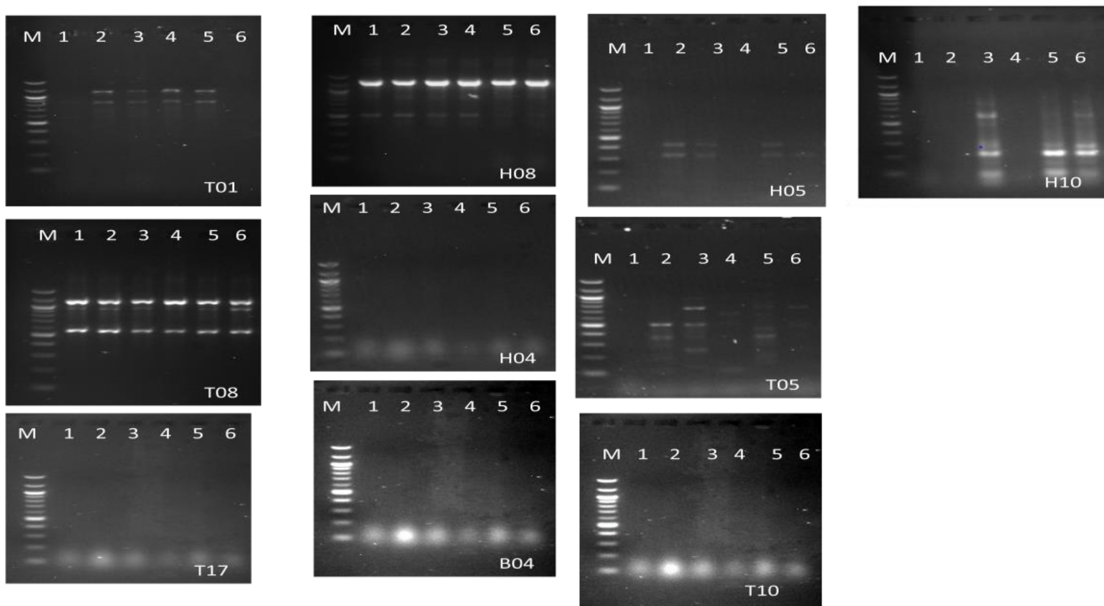


Plate VII: Photomicrograph of SAMCOT-12: Black arrow denotes cytokinesis taking place in the two cells.

4.1.18 Sequencing and polymorphic information of ten RAPD dna markers for diversity study

A total of 60 amplified fragments bands were generated from six (6) cotton genotypes using 10 RAPD primers. Fifty-four (54) of the total bands were monomorphic while six of the bands were polymorphic. Primer TO5 has the highest number of polymorphic bands of five (5) with percentage polymorphism, followed by H10 with percentage polymorphism 16.67. Polymorphic information contents (PIC) range from 0.00 - 0.81 with the highest PIC value of 0.81 recorded in TO5 and the least 0.00 from TO8, HO4, T10, BO4 and T17. Table 4.17 the major Allelic frequency obtained varied from 0.17 in TO5 to 1.00 in TO8, HO4, T10, BO4 and T17 primers. Table 4.17 Primer TO5 has the highest gene diversity of 0.83 followed by 0.61 in H10. However, the gene diversity recorded in TO1, HO8, HO5, H10 were higher than the overall average of 0.28. The highest number of allels 6.00 was obtained in TO5, followed by 3.00 in H10 and 2.00 allels in TO1, HO8 and HO5 (Table 4.17).



PlateVIII: RAPD Amplification patterns of 10 DNA primers

Table 4.17: Allelic Information, Gene Diversity Sequencing and polymorphic information of six cotton genotypes

S/N	Primers	Primer sequence	MAF	Sample Size	No. of obs.	AlleleNo	Availability	Gene Diversity	NMB	NPB	PP	PIC
1	T01	GGGCCACTCA	0.67	6.00	6.00	2.00	1.00	0.44	6.00	0.00	0.00	0.35
2	H08	GAAACACCCC	1.00	6.00	6.00	1.00	1.00	0.00	6.00	0.00	0.00	0.35
3	T08	AACGGCGACA	1.00	6.00	6.00	1.00	1.00	0.00	6.00	0.00	0.00	0.00
4	H04	GGAAGTCGCC	0.67	6.00	6.00	2.00	1.00	0.44	6.00	0.00	0.00	0.00
5	H05	AGTCGTCCCC	0.17	6.00	6.00	6.00	1.00	0.83	6.00	0.00	0.00	0.35
6	T05	GGGTTTGGCA	1.00	6.00	6.00	1.00	1.00	0.00	1.00	5.00	83.33	0.81
7	T10	CCTTCGGAAG	1.00	6.00	6.00	1.00	1.00	0.00	6.00	0.00	0.00	0.00
8	B04	GGACTGGAGT	1.00	6.00	6.00	1.00	1.00	0.00	6.00	0.00	0.00	0.00
9	T17	CCAACGTCGT	0.50	6.00	6.00	3.00	1.00	0.61	6.00	0.00	0.00	0.00
10	H10	CCTACGTCAG	0.77	6.00	6.00	2.00	1.00	0.28	5.00	1.00	16.67	0.54
Mean									5.40	0.60	10.00	0.24

KEYS

MAF – Major Alleilic Frequenc

PIC – Polymorphic Information Contents

NMB - Number of Monopodial Braches

NPB – Number of Polymorphic Band

PPP – Percentage Polymorphism

4.1.19 Dissimilarity index of six cotton genotypes

The genetic dissimilarity among the six (6) cotton genotypes varied between 0.064 and 0.801. The least dissimilarity of 0.064 was due for SAMCOT-8 and SAMCOT-11, and the highest similarity of (0.801) were for SAMCOT-13 and SAMCOT-11. Wide genetic dissimilaritieswas observed between SAMCOT-9 and SAMCOT-10, and SAMCOT-10 and SAMCOT-11 were 0.202 and 0.29 respectively (Table 4.18).

Table 4. 18: Dissimilarity Index of Six Cotton Genotypes

	Samcot-8	Samcot-9	Samcot-10	Samcot-11	Samcot-12	Samcot-13
Samcot-8	0.00					
Samcot-9	0.115	0.00				
Samcot-10	0.801	0.202	0.00			
Samcot-11	0.064	0.087	0.290	0.00		
Samcot-12	0.290	0.087	0.087	0.202	0.00	
Samcot-13	0.151	0.151	0.064	0.801	0.115	0.00

4.1.20 Dendrogram of genetic diversity of six cotton genotypes

The UPGMA dendrogram generated from the six cotton genotypes revealed two (2) major cluster groups I and II. The first Cluster group I was further sub divided in to A1 and A2. A1 is a distinct group which contain only one (1) genotype, (SAMCOT-9) while group A2 comprises of two (2) genotypes (SAMCOT-8 and SAMCOT-11). Similarly, group II comprises of one (1) genotype in its sub division (SAMCOT-12) and two (2) other genotypes in subdivision (B2)(SAMCOT-10 and SAMCOT-13) Figure 4.5.

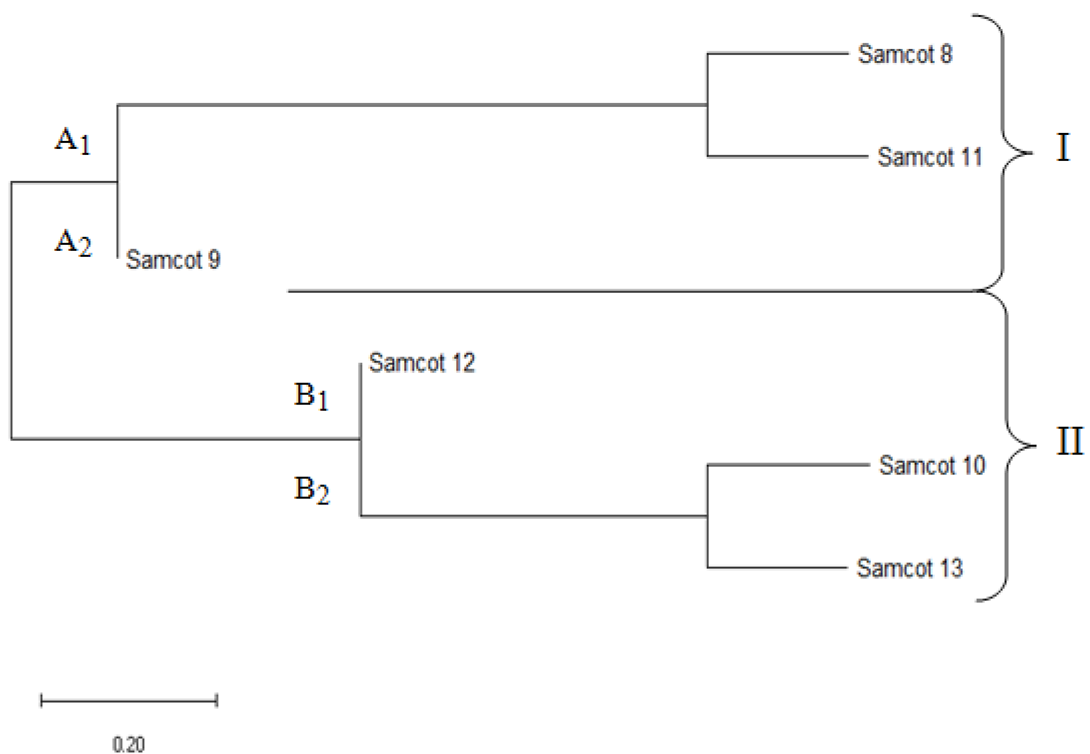


Figure 4.3: Dendrogram of genetic diversity of six cotton genotypes

4.2 Discussion

4.2.1 Morphological parameters of Nigeria cotton gynotypes.

Germplasm lines are often used by plant breeders to develop improved genotypes for the upcoming environmental conditions that completely outclass the previous genotypes in terms of performance (Khan *et al.,* 2015). Genetic variation based upon morphological and agronomic attributes has been exploited in cotton for victorious future breeding (Rathinavel, 2019). The variability in germplasm is vital for acceptable exploitation of the attributes for selection and breeding (Sajjad *et al.,* 2011). The significant variation in height of plant in each of the weeks among the cotton genotypes, are indications of high diversity in the genetic base of the cotton plant. The range of plant height (82.50 – 106.00 cm) obtained at maturity in this study, were lower than the ranged of112.63 cm to 122.47 cmearlier reported by Ashokkumar and Ravikesevan (2011), 1.0 - 2.0 m reported by (Copeland *et al.,* 2017). The variation in the plant height among cotton genotypes could be attributed to the differences in genetic makeup of the crop. Also, the low plant height obtained in the study compared to the earlier authors could be attributed to the variation in soil type, environmental factors, as well as variation in planting season.

Number of branches in cotton plant is highly heritable and influenced by the genetic content of the genotype. Khan and Hassan (2011) reported that morphological traits like sympodia branching are very important in the cotton plant because sympodia are positively correlated with yield and manage the seed cotton yield. Therefore, criteria for selection based on a number of sympodia branch will be helpful for increasing plant yield. The significant differences observed in the number of branches (monopodial and sympodial) are an indication of high genetic variability in the crop. In support of these findings, variation in number of monopodial and sympodial branches had earlier been

reported by Basbag and Gencer (2007), Yahaya *et al.* (2013). The high number of branches observed in some of the genotypes is an indication of the boll bearing ability of the plant which in turn contributed to the yield. Bozorov *et al.* (2018) had earlier reported that sympodial branches are the fruit bearing branchesand positively associated with yield in cotton, this further corroborates this finding.

4.2.2 Yield parameters of cotton gynotypes

The actual number of bolls produced by each plant determines the yield potential of the genotype and it is considered as major yield component with strong relationship to cotton yield. Significant variation in number and size of boll per plant obtained in this study is in conformity with the observation of Wang *et al.* (2004), who reported that cotton cultivars varied significantly for number of bolls per plant and lint index. Also, Basbag and Gencer (2007), Ehsan *et al.* (2008) had earlier reported that number of bolls in hybrid cotton varieties varied from genotype to genotype.

The significant variation observed in weight of boll could be attributed to the diversity in the genetic composition of the genotypes. Similar variation among varieties of cotton for average boll weight had earlier been reported by Hofs *et al.* (2006). In conformity with the result of this study Khan *et al.* (2007) attributed differences in weight of boll to varietal characteristics and environmental conditions of that area. He further, reported that the response of different cotton cultivars to a photoperiod in a region might be one of the environmental factors causing variation in boll weight. However, the range of boll weight (11.90 – 32.17 g) obtained in this study were greater than 2.77 – 4.70 g reported by Khan *et al.* (2007), 17.28 – 19.33 by Nawaz *et al.* (2019). The variation in the results could be attributed to the differences in the difference in the genetic make-up of the genotypes, geographical location and photoperiods of the areas. Also, in conformity with the results Ashokkumar *et al.* (2010) attributed maximum seed cotton

yield with maximum number of sympodia per plant, number of bolls, boll weight, ginning outturn, lint index and seed index.

The variability for economic attributes in the given landraces is vital for exploitation following selection and breeding (Sajjad *et al.,* 2011). Significant differences among all the genotypes, with the heaviest genotype having the value (10.29 g) and the least with the value of 8.05 g reflects the heterogeneity of the cotton genotypes. The mean seed index of 9.85 earlier reported by Ashokkumar *et al.* (2010) was within the range of value obtained in this study. However, these values were greater than the value of 0.17 to 1.44 reported by Khan *et al*. (2015). The variation in the seed index recorded could be attributed to the differences in genetic composition of the crop and environmental factors.

The lint yield has been reported to be an interaction of many factors such as number of bolls, boll weight and seed cotton yield. The high significant differences recorded in weight of lint among the genotypes studied, demonstrated that adequate variability exist in the gerrmplasm; indicating the heterogeneity of the collected cotton genotypes. Lukonge (2005) also reported significant differences in lint yield of cotton cultivars. The highest weight of lint (13.66), coupled with high number of boll (31.00), and boll size (11.25 cm) in SAMCOT-8 indicate the superiority and potentials of the genotype for further improvement of the crop.

4.2.3 Fibre qualities of cotton genotypes

Micronaire is referred to as fineness of the single cell trichome that arise or originate from the seed. The medium (3.00 – 3.90 µg/inch) to moderately (4.00 – 4.90 µg/inch) fineness in fibre quality obtained in the study is an indication of the quality of the genotype Bakhsh *et al*. (2019) reported that the end product of fibre in cotton is affected

by the fineness quality. However, fineness range (3.50 - 4.90) obtained in the study within the ranged value (3.41 – 5.90) reported by Okubazghi *et al*. (2017) but lower than the range value of (4.55 – 5.42) by Chen *et al*. (2018). The slight variation in the ranged of the result could be attributed to variation in the photoperiod and environmental conditions of experimental sites. Variation or increase in mean Micronaire had been attributed to effects of increased temperature during the fibre-thickening period due to quicker crop development arising from increased temperature (Luo *et al.*, 2016).

Fibre maturity, this indicate the degree of cell wall thickness, which is measured in percentage the acceptable range is 80 % and above. According to Luconge (2005), the values recorded in this study lies within the recommended value. The finding of this research is supported with the work of Bourgou *et al.* (2018), who reported similar value for *Gossypium barbadense* it was observed that genotype with this kind of value are diverse.

Fibre strength (g/tex) is a key quality parameter in cotton that has ultimate impact on durability of the fibre during harvesting, ginning and manufacturing of the yarn. The range ofvalues of 24.18 to 27.73 obtained in this study for fibre strength fall within the findings of Sawar *et al.* (2016) who pointed out that fibre strength are more responsive to the growth in the environment. These values were lower than the values of 25.14 – 35.56 earlier reported by Sun *et al.* (2019) and 29.50 to 32.08 by Chen *et al.* (2018). Similarly, the values are slightly lower than the recommended value as measured by high volume instrument. These finding differs greatly with the work of Luconge (2005), and Bhangu *et al.* (2017), perhaps it may be due to differences in the genetic make-up of the genotypes grown under assorted environmental conditions.

Fibre elongation which is a measure of percentage which define strength with which a bundle of fibre incured prior to break. The acceptable ranges of fibre elongation had earlier been reported ranged from 5.20 to 8.00 and above are regarded as high. The values of fibre elongation recorded (5.78 – 6.30) in this study were within the acceptable range. In conformity with this result, Okubazghi *et al.* (2017) reported a range of 4.03 to 6.90. Turley *et al.* (2019) had reported similar acceptable valuesand differ slightly with the findings of Basbag and Gencer (2007) who reported that genotype variation account for differences in fibre elongation.

Uniformity index had been reported to be a vital trait to consider in selection programme of the crop. According to Luo *et al.* (2016), low fibre length uniformity and high short fibre content are associated with increased manufacturing waste and decreased spinning efficiency during yarn processing. The fibre uniformity index obtained (82.95 – 86.58 %) in this study. These values fall within the range of 76.33 – 86.275 % with an average mean of 81.88 % earlier reported by Okubazghi *et al.* (2017). The slight variation in the minimum value could be ascribed to the variation of daily mean and amplitude temperature as reported by Sawar *et al.* (2016).

4.2.4 Mineral composition of Nigeria cotton genotypes

Cotton seed oils contained significant amount of important minerals. The mean concentration of calcium was found to be between (10.41-21.72 %) the value recorded in this study is closed to the value reported by Bellalou *et al.* (2015) that the higher value reported could be that of (Ca) taken up was not used for further physiological and biochemical process involved in lint and fibre development and structure such as the synthesis of cell wall polysaccharides and fatty acid Yang *et al.* (2008), Pang *et al.* (2010). The significant role of Ca in cotton was previously reported and involved in pollen germination and pollen tube Zhang *et al.* (1997) and Bellalou *et al.,* (2015).

Magnesium is an essential mineral for enzyme activity like calcium and chloride, magnesium also plays a role in regulating the acid alkaline balance in the body. The mean value recorded is low compared to the report of Ndangui *et al.* (2010) in *Abelmoschus esculentus*. Perhaps it could be as a result of physiological activities involved in lint and fibre development in cotton. Potassium is an essentialnutrient that plays a very vital role in the synthesis of amino acid and protein. The result obtained in this study is lower compared to what was obtained in the report of Habtamu *et al.* (2018) in *Abelmoschus esculentus*. The differences could be as a result of nutrient up take by the plant and also variation in mineral composition in the soil. According to the report of Sawan *et al.* (2006) improvement in cotton yield and quality resulting from potassium (K) may be reflected in distinct changes in seed weight and quality. Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance (Bellalou *et al.,* 2015). The result from the present study compared to the report of He *et al*. (2013) who reported higher percentagesof phosphorus in cotton seed. The fact that poultry litter can provide more minerals to soil since those minerals have been added in to poultry feed for various purposes and excreted into poultry litter, further confirmed presence of phosphorus in the cotton seeds.

Iron is an essential minerals element that has a direct stimulatory effect in erythroposis (haemoglobin synthesis). The result obtained in this study is between 2.54-3.84g/100g which differs greatly with the report of He *et al.* (2013). Who reported higher value of Fe, in mg/kg, and reported by Muhammad *et al.* (2014) who reported high value inµg/cm. Nutrient up take, translocation, redistribution and accumulation are processes controlling the accumulation of minerals in seeds and most of the genetic basis of these processes are still not known (Ding *et al*., 2010).

4.2.5 Proximate composition of Nigeria cotton genotypes

The moisture content of the cotton (*Gossypium hirsutum*) seeds was between 2.10 - 3.50 percent in this study which is a bit low when compared with legumes which ranges between 7.0 - 11.0 %. This shows that the seed are very high in dry matter content which is an advantage because it reduces microbial content prevent oxidation, reduction reaction, algae and fungi growth and increase their shelf life when properly stored. The value obtained in this study is lower than the result of Etaware and Etaware (2019). The difference in the value could be as result of genetic content of the samples.

The percentage protein obtained is close to the values of seeds rich in protein such as soy beans cowpea and pigeon peas which were between 23.1-33.0 % (Bolek, 2016). The high percentage protein will make them serve as a proper source of amino acid and protein for both man and animal. The value recorded in this study is within the recommended value of FAO (2004) which is 19.8 %. *Gossypium hirsutum* is high in crude fibre when observed from the result. It has enough fibre for dietary nutrition which will help to maintain intestinal digestion, reduces coastipation colon diseases and cancer Njoku *et al.* (2007). The result of this study is close to the commercial standard of 18.49 % as reported by Bhatkalkar *et al*. (2016).

The percentage ash content in this study is within the recommended standard of 4.06 % according to Bhatkalkar *et al.* (2016) which indicates high inorganic matter that could be retained in the body. The value obtained for the fat in this study is between 11.33-18.00 which is lower when compared with the work of Okonkwo and Okafor (2016), perhaps it could be due to differences in their genetic base. But the value is within the standard of *Gossypium hirsutum* which is between 15.8 - 20.2 %. This reveals that the seed is rich in oil and fat, and fat is very important in diet as it promotes absorption of the fat, soluble vitamin and provide high energy nutrient. The value of this seed oil is

quiet promising and suggests obtaining commercial quantities for industry, pharmaceutical cooking and other purposes. The total calculated carbohydrate is considered sufficient for energy the body required.

4.2.6 Phytochemical composition of Nigeria cotton genotypes

Plant provides a large repertoire of phytochemicals such as polyphenols, flavonoids which are ingredient of therapeutic drugs in modern age (Al-Snafi, 2018). The high range (294.34 – 1040.14) of phenolic concentration obtained in the *Gossypium hirsutum* species are quite higher when compare with the values of Ade-Ademilua and Okpoma (2018) and Chandrashekhar *et al.* (2019). Both reported low range value of (4.6-5.3) and 160 mg/100 g in *Gossypium hirsutum*. In agreement with this result Al–Kanani *et al.* (2019) who work on *Albelmoschus esculentus* had values range between 185 – 1460 mg/100 g. Value obtained for the flavonoids in *Gossypium hirsutum* as reported by Ade-Ademilua and Okpoma (2018) (120 mg/100 g) is close to what was obtained in this study. Result for both saponins and alkanoids are higher when compared with what was observed in Chandrashekhar *et al.* (2019) in the species of *Gossypium*. So also, in the report of Habtamu *et al*. (2018) it was observed that Tanins, phytate, and Oxalate, with range values of 0.71 – 3.78, 039 – 0.46, and 0.74 – 075 respectively in *Abelmosschus esculentus* is low when compared to what was observed in this study. The observed variation in the phytochemical contents could be attributed to the different extraction methods used, solvent type, and genetic composition as well as the environmental condition at which the plant is grown.

Most phytochemicals are antoxidant agents which essentially reduce the damages caused in tissues during physiological activities (Ezeonu and Ejikeme, 2016). High value of phytochemicals observed in this study showed that *Gossypium* species possess high medicinal potentials. This assertion is in agreement with the report of Al-snafi

(2018) that *Gossypium* species contain high medicinal value. Chemical properties of oils are one of the most important properties that determined the present quality of oil (Nzikou *et al.,* 2009). Peroxide value which is used as an indicator of deterioration of oils ranges from to be 1.26 – 3.17 (meq/kg) indicating that the oil is fresh. Konuskan *et al.* (2017) earlier reported that oil with less than 10 meq/kg are fresh oils. The result of the present study is in conformity with the statement of Orherba and Efomah (2012) and findings of Konuskan *et al.* (2017) who reported peroxide value of less than 10 meg/kg in cotton seed. The iodine value which indicates the degrees of oil saturation shows that the oil of *Gossypium hirsutum* used is semi – dry up and unsaturated, and makes it suitable for utilization in certain industrial application (Okonkwo and Okafor, 2016). Contrary to the result of this study Konuskan *et al.* (2017) reported high iodine value (102 – 110). The differences in the iodine value obtained may be due to the variation in genotype, climatic factors and oil processing methods. The low acidic value (1.91 – 2.73) is contrary to the findings of (Orhevba and Efomah, 2012) who reported 11.50 mg KOH/g but partially agree with the findings of Okonkwo and Okafor (2016) who reported 3.76 mg KOH/g. The low acid value shows the ability of the oil to resist hydrolytic rancidity (Akubor, 2008). Result of the saponification value (SPV) is quiet very low which disagree with the findings of Okonkwo and Okafor (2016), Orhevba and Efomah (2012) both reported high value in saponification. Low moisture content observed in this study is advantageous in terms of storage stability since the low the moisture contents, the better the storability and the longer the shelf life of the oils.

4.2.7 Genetic parameters of Nigerian cotton genotypes

Medagam *et al.* (2015) reported that estimation of GCV in conjuction with heritability provide a better indication for selection on the phynotypic performance. In confirmation of the earlier statement the high GCV of plant height, weight of dry boll and weight of

lint led to high heritable phynotypic performance. The high heritability coupled with high to moderate genetic advance possessed by plant height, weight of dry boll and weight of lint indicate that these characters were under the influence of additive gene action and hence selection for these characters could be effective in improvent of cotton yield. In affirmation of this statement Johnson *et al.* (1955), reported that heritability in conjuction with genetic advance would give a more reliable index of selection values of characters.

The dispersal, fitness and survival of any plant generation is dependent on the viabilityof the pollen of the species. It is also essential for the plant breeding and consequently crop improvement. Lack of variability in pollen size of different genotypes could be attributed to the similarity in the genetic make up of the genotypes. The range of pollen size 27.00 – 33.00 µm obtained in this study is quite lower than that earlier recorded in the study of Yang *et al.* (2017). The variation in the exine diameter could be as a result of the differences in the varieties used and the environmental factors. Also, slite variation witnessed in this study inboth intine and exine of the genotypes could be due to aberration in microsporogenesis resulting from high degree of meiotic irregularity. These results support the report of Abubakar *et al*. (2015) who recorded variation in the pollen size of *Celosia argentea*.

4.2.8 Fatty acid composition of Nigeria cotton genotypes

Demand of quality oils and fats is increasing all over the world. To cope with the increasing demand of the oils and fats the non conventional sources are getting importance. Palmitic, Oleic, Linoleic, Meristic and Arachidic acid are the principal fatty acid determined for all the genotype analyzed. The major saturated fatty acid present in all the samples tested was palmitic acid which is similar with the findings of Liu *et al* (2002) and Okonkwo and Okafor (2016). The values recorded for palmitic acid in most

of the samples were higher than the findings of Konuskan *et al.* (2017) who have their values less than 1.00 % but slightly agree with the report of Roy *et al.* (2012) who reported 11.73 % in some of the samples studied. Values recorded for myristic acid in this study is low in some of the samples (0.26 %) which is close to the findings of Okonkwo and Okafor (2016). Reports of linoleic and oleic acid are quite very low which disagree with the findings of Roy *et al.,* (2012) and Konuskan *et al*. (2017).

Findings for arachidic acid in this study is higher and it's in contrary with the work of (Konuskan *et al.,* 2017). The variation in the value recorded could be as a result of differences in the genotype and differences in the oil extraction methods. Fatty acid is very important in human food because of the inability of the body to synthesize it. It presence in the body prevents distinct heart vascular diseases in the body (Okwonko and Okafor, 2016).

4.2.9 Cytological analysis of Nigeria cotton genotypes

The 2n=4x=52 obtained in this study is in conformity with the earliar authors results (Andres and Kuraparthy, 2013) and (Wenbo *et al.,* 2016). Report from Haroun (2010) stated that the variations in lengths between chromosomes and centromere position pointed to degree of heterogeneity in the genome structure of the species. How ever the differences in some of the chromosomes in respect of the size could have arisen through segmental interchange involving translocation of unequal sizes (Peng *et al.,* 2012). The low number observed in somatic chromosome number could be as a result of chromosomal mutation that perhaps must have occure when the plant is trying to adopt to changes in the environment.Variations in morphology which may be as a resultof positionof centromere in the chromosomes may also arise due to chromosomal evolution in plant in the natural populations chromosomal re-arrangements such as

translocation, duplications, deletion and inversion occurs in population (Mukherjee and Roy, 2012).

4.2.10 Genetic diversity and similarity index of cotton genotypes

Random Amplified Polymorphic DNA (RAPD) have been earlier reported to be simple and reliable markers to detect DNA polymorphism for cultivars identification and diversity analysis Farzaneh *et al.* (2010) the high percentage polymorphism and polymorphic information content (PIC) generated by primer TO5 indicate its reliability and effectiveness in assessing diversity among cotton species. In confirmation to this statement Ahmad *et al.* (2008) reported that markers is effective if the PIC value is higher than 0.5. Also reported higher percentage polymorphism is Tidke *et al.* (2014) using RAPD in cotton studies with the ranges (0.45- 0.91). The least desimilarity 0.064 between some of the genotypes (SAMCOT-8 and SAMCOT-11 as well as SAMCOT-10 and SAMCOT-13) could be attributed to the similarity in sources of the genotypes and transborder exchange of seeds by farmers during their trading and gining activities. Similar to this statement Chaudhary *et al.* (2010) reported that genetypes with maximum similarity might have been selected from a single population. Further more, breeders mostly share the elite lines of other breeding stations in cotton improvement programs, making the breeding materials identical which ultimately result in close kinship of the varieties.

The high number of amplified DNA band generated by 10 primers in this study was similar to the work of Tidke *et al.* (2014) and Verma *et al*. (2015) who generated high polymorphic percentage using RAPD primers. However, the percentage polymorphism obtained in this research is quite lower than 96.96 % in 26 cotton germplasm reported by Modi *et al.* (2020), 84.95 % polymorphism in 21 cotton germplasm using RAPD primer (Eminur *et al*., 2014) whereas, Rana *et al.* (2004) detected 89.1 % polymorphism

in 23 cotton germplasm comprised of *G. arboreum* and *G. hirsutum* using 26 RAPD primers.

The number of alleles obtained in this study was higher than that reported by Verma *et al.* (2015). The difference could be as a result of variation in genotype. How ever, RAPD techniques had earlier been used to evaluate the genetic relationship among cotton genotypes by different authours. The clustering of the genetypes into two major groups is an indication of frequent selection of preferred elite genotypes for cultivation in the subsequent years which narrow genetic base of the crop. In affirmation to this assertion Chaudhary *et al.* (2010) reported that clustering of varieties might be due to selection of the elite lines from a single population.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

It can be concluded from theresult of this study that *Gossypum hirsutun* landraces have a wide genetic variablity with distinct morphological identities that could be exploited and used in the crop improvement programme. The study revealed that SAMCOT-13 performed well for some morphological parameters such as plant height, monopodial and sympodial branches.

SAMCOT-9 had the best performance for yield parameters like number of bolls, boll size and weight of lint per plant. All the genotypes evaluated had good quality fibres, with the parameters determined within the USDA standard range.

The result from the physical properties of oil revealed that oil from the cotton seed genotypes are of good quality due to low value of peroxide value. The low moisture contents of cotton seeds (1.44-1.77) could be an indication of high storage stability and long shelf life of the genotypes. The lowest peroxide and acid value as well as moisture contents of SAMCOT-9 indicates that the genotype could be selected for exploitation in the cotton seed oil improvement programme. Findings from this research revealed that *Gossypium* genotypes are rich ine phytochemical constituents with high antioxidant contents.

The high polymorphic information (0.81) of primer T05 indicate its reliability and effectiveness in the study of genetic diversity among cotton genotypes. Clustering of the genotypes two distinct groups is an indication of relative variability among the northern cotton.

5.2 Recommendations

The following recommendations were made from the result of this research

1. Mutationbreeding is recommended to create more variation among the genotypes.
2. Oil extracted from SAMCOT-9 is recommended for further analysis and study due to its low level of peroxide value and moisture contents.
3. Further molecular analysis should be carried out using other molecular makers to check the diversity of the crop.

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APPENDIX

Physicochemical properties of the experimental soil

Ph	OC (%)	OM (%)	TN (%)	AP (ppm)	EC (Cmolkg^{-1})				EA (Cmol kg-1	CEC Cmol kg	Clay (%)	Sand (%)	Silt (%)
					Na	k	Ca	Mg					
6.32	0.21	0.36	0.09	8.56	1.67	1.32	5.44	0.90	0.23	9.56	5.20	92.24	2.56

OC = organic carbon, OM = organic Matter, TN = Total Nitrogen, AP = Available Phosphorus

EC = Exchangeable cations, EA = Exchangeable Acidity, CEC = cation exchange capacity.

Pollen Fertility of Cotton Genotypes

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA
DEPARTMENT OF PLANT BIOLOGY
GERMPLASM QUESTIONAIRE FORM

Part A
(Location Details).

1 Date ....

1) Name of farmer ---
2) State --
3) Local government area ---
4) ADP Zone
5) Name of collection locality---
6) Name of accessions grown--
7) Local name of the variety
8) Longitude ---------------------------------- and Latitude ------------------------------

Part B (Famers experience)

Please thick the best option that fits your responds in this part

	Grades		
	1	2	3
1. How long have you been growing the crops?1=Above 10 years, 2=6-10 years, 3= Below 5 years			
2. How did you get your first supply of seeds? 1=from the fellow famers, 2=purchase from market, 3=ADP and others			
3. Which type of cotton variety did you prefer to plant most? ,			
4. Why did you prefer your choice.			
5. Does leaf eaten pest constitute a major hindrance to your production? 1=Yes, 2=No, 3=Neutral			
6. Do you have problems of attack by rodent and other animals? 1=Yes, 2=No, 3=Neutral			

7. Do you require fertilizer/manure application in the cultivation of cotton 1=Yes
8. , 2=No, 3=Neutral

Part C famer’s experience

Please write down your responses to the questions below

1) When do you plant --
2) When do you harvest --
3) How do you handle your harvest---
4) How long can it stay in storage ---
5) Do you store your products or you sell immediately after harvest?
6) If you have to store how do you store your produce? ---------------------
7) What type of Farming system do you use
8) What are the major pest in your farm
9) Which other varieties do you know
10) What are the uses of cotton do you know
11) Is there any cottage industries around you area here?
12) Where do you sell your harvest (if any)
13) How much yield per Hectare
14) What is the market price per hectare