MORPHO-ARGRO-PHYSIO-KARYOTYPIC CHARACTERIZATION OF WILD COTTON (*Gossypium* spp.) GERMPLASM FROM SELECTED COUNTIES IN KENYA

BY

NJAGI ANTHONY KIRANGA (B.Ed Sci)
REG. No. I56/12600/09

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

Njagi Anthony Kiranga

Signature..... **Date.....**

We confirm that the candidate carried out the work reported on this thesis, under our supervision.

Dr. Fredrick M. Njoka

Plant and Microbial Sciences Department

Kenyatta University

Signature..... **Date.....**

Dr. Charles W. Nderito

Kenya Agricultural Research Institute, Thika

Signature..... **Date.....**

DEDICATION

I dedicate this work to my family, parents, all my primary school teachers and friends.

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AND Acronyms and abbreviations

ANOVA	Analysis of Variance
CPU	Central Processing Unit
DAPI	4, 6-diamidino-2-phenylindol
FITC	Fluorescein Isothiocyanate
HFI	Horizontal Flowering Interval
KARI	Kenya Agricultural Research Institute
NFB	Node of the First Fruiting Branch
NFRC	National Fibre Research Centre
NORS	Nucleolar Organiser Regions
r	Correlation
r p m	Revolution per minute
VFI	Vertical Flowering Interval

Definition of terms

Chromosome Rod-shaped structure in a cell's nucleus containing an organism's genetic code

Diploid Cell containing a double set of chromosomes arranged in homologous pairs
s
Distal satellite The constriction located at the terminal, position of the chromosome.
Fixation without distorting - To preserve the material in a way that decay and distortion of parts caused by swelling
and shrinkage or other changes is reduced to a minimum.
Genotype Genetic constitution of an organism
Genome Total number of genes within a haploid cell.
Hybridization Crossing of two plants from different species,
Karyotype The number, form, shape and size of chromosomes specific to a given species.
Lint Bits of cotton thread.
Maceration The mechanical breakdown of the middle lamella by shipping of material in
Morphology Study of form and structure; science which deals with structure and form of
organisms.
Phenotypic .
characteristic Outward appearance of an organism

Polyploid An individual having more than two or more sets of chromosomes

Species Group of organisms that interbreed to give rise to a fertile offspring.
Square Flower bud
Squash Method of separating cells pressing.
Staining The colouration of a chromosome in a killed tissue .
using various dyes.

Subtending Leaves arising from fruiting branch.

Leaves
Tetraploid s Cell having four times the usual number of chromosomes.

NJAGI ANTHONY KIRANGA .REG. No. I56/12600/09

MORPHO-PHYSIO-KARYOTYPIC CHARACTERIZATION OF WILD COTTON (*Gossypium* spp.) GERMPLASM IN KENYA

Abstract

Cotton (*Gossypium* species) occupies a prime position as a fibre crop of the world. Today cotton is used as a natural fibre, while seeds are used for making cooking oil, soup and seed cake for animal feed industry. Wild cotton germplasm performance in Kenya has not been studied. While current cultivated variety (HART 89M) has a narrow genetic base, the wild cotton germplasm provides a gene pool that is required for its improvement. Understanding the correlation of traits influencing seed cotton yield is pre-requisite for effective improvement of HART 89M. The study helps in the

identification of yield components, significant correlation of boll weight with plant height, number of fruiting branches and number of nodes per plant. The study was aimed at assessing the differences and relationship between domesticated cotton HART 89M with wild cotton and karyotypic analysis. Data on agronomic traits collected from a sample of forty-five plants, selected at random per studied genotypes were analysed using t-test, ANOVA and Pearson correlation coefficiency. Data on germination were collected on weekly basis from emergent of the first seedling. The data on the rate of flowering was also collected on weekly basis from emergent of the first flower. At maturity, data was collected on height, number of fruiting branches, length of the longest fruiting branch, internodal length, number of nodes on longest fruiting branch and yield per plant. Data on boll traits involved random collections of thirty three bolls per genotype. Each boll was weighed, ginned, seed and lint weighed separately. On karyotypic analysis, young shoot apex were treated and observed under leica microscope. The study revealed a significant difference in agronomic performance in all traits among the studied genotypes, height ($F = 98.842$, $df = 4$, $p \leq 0.05$), number of fruiting branches ($F = 25.973$, $df = 4$, $p \leq 0.05$), length of fruiting branches ($F = 58.726$, $df = 4$, $p \leq 0.05$), number of nodes on the fruiting branches ($F = 27.726$, $df = 4$, $p \leq 0.05$) and yield ($F = 178.85$, $df = 4$, $p \leq 0.05$) per bush. On boll result displayed a significant difference among the studied genotypes, boll weight ($F = 25.938$, $df = 4$, $p \leq 0.05$), seeds weight ($F = 21.181$, $df = 4$, $p \leq 0.05$), lint weight ($F = 26.593$, $df = 4$, $p \leq 0.05$) and lint- seed weight ratio ($F=2.264$, $df = 4$, $p \leq 0.05$). It also revealed a significant difference between the standard genotype (HART 89M) and studied wild genotypes. *G. barbadense* was significant differently higher in height (102.42 cm), ($t = 20.24$, $P \leq 0.05$), number of fruiting branches, 30.47 ($t = 9.18$, $P \leq 0.05$), length of the longest fruiting branch 34.34 ($t = 8.14$, $P \leq 0.05$), number of nodes on the longest fruiting branch, 2.49 ($t = 7.46$, $P \leq 0.05$) and yield 133.73 ($t=17.73$, $P \leq 0.05$) compared with others genotypes. On boll traits the highest significant difference with HART 89M was found with *G. kirkii* on boll weight, 0.73g ($t = 7.72$ $P \leq 0.05$), seed weight 0.52g, ($t = 8.67$ $P \leq 0.05$), and lint weight 0.20g ($t =3.21$, $P \leq 0.05$). The results revealed a significant and positive correlation between height, number of fruiting branches, number of nodes on fruiting branch and number of seeds per boll with seed cotton yield. Within the studied genotype, there was a significant positive correlation between yield and other boll traits. The ploidy study revealed that the three cotton species had tetraploid chromosomes of complement $2n = 52$ and two species had diploid chromosome of complement $2n = 26$. All the five cotton species revealed the presence of metacentric, acrocentric and telocentrics chromosomes. *G. kirkii* nuclei revealed both parallel and ant-parallel chromosome location. During future breeding program, the yield related traits might be kept in mind during selecting, as they were the major attributes of the seed cotton yield. Cotton development agency, need to develop the farming of *G. harbaceum* and *G. kirkii* for seed cotton production for Cooking oil extraction and animal feed industries to avoid competition between lint and oil production.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Cotton (*Gossypium* species) occupies a prime position as a fibre crop of the world. Commercial cotton fibre is produced from only four species. Two diploids are *G. arboreum* and *G. herbaceum* while the two tetraploid, are *G. barbadense* and *G. hirsutum* (Vafaie-tabar, 2004). The species *G. hirsutum* is however usually the early-maturing and higher yielding (Lacape *et al.,* 2005). In Kenya *G. hirsutum* was introduced in the year 1902 by the British colonial administration (Ikotoo *et al.,* 1989). It is grown in several agro-ecological zones, mainly in the semi-arid regions of Eastern, Central, Coast and Western regions of Kenya.

The cotton variety HART 89M was developed at the National Fibre Research Centre (NFRC), Mwea. It is now the most extensively cultivated variety in Kenya (Ikitoo *et al.,* 1989). The level of genetic diversity is low in *G. hirsutum* as revealed by (Gutefrrez *et al.,*, 2002). Hybridization of new varieties of cotton plant, happen by crossing plants of different gene types selected amongst the resultant hybrids. Desirable characteristics are introduced from wild species relative to the cultivated cotton (Kameswara, 2004).

1.2 Historical background

In Kenya, cotton growing started in 1902 (Ikitoo *et al.,* 1989). Cotton was promoted as a suitable cash crop in areas where other cash crops did not fare well (Ikitoo *et al.,* 1989). Hola irrigation scheme was established. Bura irrigation scheme was established in 1981 – 82. By 1987 – 88 the Government was operating both Hola and Bura irrigation schemes, which accounted for 39 % of the national lint production (Ikitoo *et al.,* 1989). The Hola scheme collapsed in 1991-92, when Tana River changed its course. Cotton is also grown in Pekera and Mwea under irrigation (Ikitoo *et al.,* 1989). These schemes were managed by the National Irrigation Board, which closed down

after civil unrest by rice farmers in 1992. Today small-scale famers in areas grow cotton suitable for rain-fed farming (Ikitoo *et al.,* 1989).

1.3 Production of cotton

Cotton is the most important natural fiber in the world for textile manufacturing accounting for 50% of all fiber used in textile industry (George, 2007). It is grown in over 80 countries, China being the leading producer country (George, 2007). Ten largest producers of seed cotton by 2011 were China, India, United States, Pakistan, Brazil, Uzbekistan, Australia, Turkey, Turkmenstan and Greece (Table 1).

Table 1.1: Eleven Cotton p**roducers** Counties in **the year 2012**

County		Million bales
1	People's Republic of China	33.0
2	India	27.0
3	United States	18.0
4	Pakistan	10.3
5	Brazil	9.3
6	Uzbekistan	4.3
7	Australia	4.2
8	Turkey	2.8
9	Turkmenistan	1.6
10	Greece	1.4
11	Kenya	0.3

Source: United States Department of Agriculture 2013

Since the starting of the 2000s, China recorded higher yields compared to other countries with an average of 3.5 tons per hectare for seed cotton and 1.1 tons per hectare for cotton fiber.**1:**min.

Since its introduction, the crop has been characterized by fluctuating production trends. Between 1965 and 1984, the annual national lint production grew from 22,000 to 70,000 bales and the textile-apparel industry became the country's leading manufacturing industry in both size and employment. In mid-1990's the lint production declined to an average of 30,000 bales annually until 2012 (United State Department of Agriculture, 2012), this was far below the country's potential of 300,000 bales annually (Ikitoo *et al.,* 1989). In Kenya, up to 4345 Kg per hectare and 900 Kg per hectare of seeds have been recorded in research centre's and farmer's fields respectively (Ikitoo *et al.,* 1989). This wide difference is probably due to poor agronomic practices, low soil fertility, rainfall patterns, diseases, pest and recycling of seed (Munro, 1987; Ikitoo *et al.,* 1989). The future improvement of cotton relies on introgression of genetic variability from wild cotton resources and understanding of wild cotton germplasm collection (Van Esbroeck *et al.,* 1998). Some of the species of wild cotton in Kenya are *G. barbadense,* var. brasiliense, *G. kirkii*, G. *herbaceum,* and *G. hirsutum* (Waturu, per. Comm).

1.4 Major economic importance

Evidence from Mexico, has shown that man for making string and clothes, since at least 200 BC (Iziko Museum of Cape Town, 2002), and has used wild cotton. Gossypol is a sesquiterpinoid present in vegetative parts of wild cotton as well as cultivated varieties, which acts as a natural deterrent to insects, but not toxic to man and non ruminant animals. Today, cotton is widely used as a natural fiber while seeds of cultivated cotton form the basis for important oil, which can be used for cooking as salad oil and for making soup (Centre for new crops and plant products, 2002). Nutritious Seed cake remains after extraction of the oil and it is used in animal food industry (Tade *et al.,* 1994). It has been shown to have strong anti-fertility, anti-tumor, and anti-HIV properties in human being (Bajaj, 1998). In herbal medicine, wild seed cotton and roots are used to treat nasal polyps, asthma, diarrhea, uterine fibroids and certain cancers (Center for new

crops and plant products, 2002). In Kenya, cotton is largely grown on small land holding averaging about 1 hectare, estimated to have 140,000 small-scale cotton farmers, compared to over 200,000 in mid-1980 when the industry was at its peak. The Cotton Board of Kenya estimates that, the country could have 400,000 hectares suitable for rain-fed cultivation. In Kenya cotton production has a potential to produce about 260,000 bales of lint annually. The board estimated that there is a potential of 34,500 hectares of irrigated cotton that can produce 108,000 bales of lint annually (Ikitoo *et al.,* 1989).

1.5 Statement of the problem

Wild cotton germplasm although collected over time by the National Museum of Kenya and KARI Thika, has not been properly characterized. They possess an important gene pool, especially for adaptation to drought, resistance to diseases and pests. These traits are important in the improvement of cultivated cotton. Cotton is among the few crops where the farmer cannot retain his seeds for planting. All seeds go to the ginneries, which then sell them back to the farmers. In the ginnery, the cotton seeds are most likely to be of unknown genetic origin (Ikitoo *et al.,* 1989). This recycling of seed has led to deterioration of yield and fiber quality. The lack of genetic diversity or narrowness in genetic base creates a potential threat to sustain productivity, due to rapid vulnerability of genetically uniform cultivars caused by new biotic and abiotic stresses (Van Esbroeck *et al.,* 1998). A wider genetic diversity of crop species ensures potential to protect crops from new pathogens, pest epidemics and global environmental changes. This provides an opportunity for further improvement of complex traits of interest, by combining or pyramiding genetic variation within populations (Van Esbrock *et al.,* 1998). The current cultivars that grow today in Kenya have a narrow genetic base. For this reason, it is important and necessary to take advantage of the available gene pool, hence important to identify and study the wild cotton germplasm to provide guidance for evaluation and selection of traits that are

superior.

1.6 Justification of the study

Cotton is cultivated in different soil-climate areas, each having entomofauna and microfrora of its own. Due to adaptive variation of pathogens and pests of cotton under these conditions, agronomists, genetist and breeders should keep on searching for new resources and donors of cotton resistance and adaptation. The varieties losing disease and pests resistance could be replaced with the ones blend for resistance and possessing a series of economically valuable traits, along with the high yielding ability. Modern varieties should have resistance to such abiotic environment factors, such as temperature stress and water deficiency. The understanding of the correlation of factors influencing yield is a pre-requisite for designing an effective plant-breeding program. This helps in the identification of the yield components. Such as correlations between of cotton seed weight per plant and plant height, number of fruiting branches and number of nodes per plant (Soomro, 2005; Igbal *et al*., 2006). Cotton cultivars have been developed from crosses between related ancestors, but so far, only limited increase in productivity has been realised. Pressure for high productivity in cotton farming has stimulated the search for wild cotton germplasm. Morphological and physiological understanding of wild cotton germplasm is important for future improvement of cotton agronomic traits, and study of its genetic potential. The wild cotton germplasm provides a gene pool that requires manipulation beyond simple crossing to obtain fertile hybrids (Van Esbroeck *et al.*, 1998). The future improvements of cotton rely on introgression of genetic variability from wild cotton resources to ensure fiber quality, yield, fiber length, fiber strength properties and resistance to pests, diseases and adaptation to changing environment (Kameswara, 2004; Lacape *et al.*, 2005; Soomro, 2005).

1.7 Hypotheses

Ho = Morphological diversity among the four different species of wild cotton in Kenya, doesn't differ significantly.

i. Ho = There are no significant observable differences in the morphology of chromosomes of wild cotton species.Ho=There are no significance superior traits in wild cotton compared with cultivated one.

ii. Ho= There is no correlation between cotton seed yield with other agronomic traits of wild

1.7 Objectives

1.7.1 General objective

To characterize wild cotton varieties, grown in Kenya using morphological, physiological and karyotypic techniques.

1.7.2 Specific objectives

To determine the morphological, physiological and genetic diversity found in four wild cotton species

To determine correlation coefficiency between cotton seed yield and other quantitative traits.

To compare agronomic traits, between HART 89M and four genotypes of wild cotton in Kenyan.

i. To determine karyotypic characteristics of five genotypes of cotton in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 General description of wild cotton

Wild cotton population grows as shrubs while some grow as small trees of 1.5m to 5m in height and 15 cm in stem diameter. They are generally deep rooted with a taproot and lateral roots. Some wild cotton have single trunk, unless disturbed, but have branches arising just above the ground (Balock, 2004). The bark is grey, smooth, tough and stingy, while the stem wood of older plants is medium density and brittle. The twigs are relatively stout, and leaves on young plants are usually without lobes, older plants usually have three lobes while some are occasionally deeply incised with five lobes (Mauney, 1968). The flowers are large and showy with cream to

yellow petals. The capsules have three to five vales with a smooth surface and embedded dots and yield white or brown lint with embedded seeds, which vary from wrinkled to smooth with tightly adhering fuzz (Howard, 1989; Liogier, 1994). In certain instances today, wild cotton has been misidentified as *G. barbadense* (Little *et al.*, 1974), which occurs as a residual from cultivation, with a wide range of leaves, bract shapes, seed characteristics, and lint length and color. Cotton is a perennial plant with indeterminate growth, having vegetative and reproductive phases occurring at the same time. Six main growth stages can be distinguished as germination, seedling establishment, leaf area and canopy development, flowering, boll development and maturation (Dosterhuis, 1992).

2.2 Taxonomy of cotton

Cotton belongs to the division Manohoplyta, class Magnollopsida, order Malvales, family Malvaceae, genus *Gossypium* (Hutchinson, 1962). The genus *Gossypium*, includes *G. hirsutum, G. barbadense, G. harbaceum, G. kirkii* among others (Watt, 1907; Hutchinson *et al.*, 1947; Fryxell, 1969 ; Brubaker *et al*., 1999).

2.3 Morphological pattern and properties of some wild *Gossypium* species

There are different morpho-physiological patterns and properties among species, which are imperative for both positive and negative response to the rapid environmental changes and consequent survival of species. Narrow genetic diversity is caused by extensive use of one or more closely related genotypes in breeding programs (Van Esbrock *et al.*, 1999). Cotton productivity and future cotton breeding efforts, as in many other agronomic crops depend on genetic diversity of the cotton gene pools. The existing and predicted problem associated with narrowness of the genetic basis, of cultivar germplasm, has been caused by genetic bottleneck,

through historic domestication and selection of few genotypes (Igbal *et al.*, 2006). Also largely due to cross breeding challenges and the lack of innovative tools, used to mobilize useful genetic variation, from diverse exotic cotton species of *Gossypium* genus to the breeding cultivars. The *Gossypium* genus encompasses a wide geographical and ecological niche, which has large amplitude of morphobiological and genetic diversity. This can be exploited effectively, in particular reservoir of potentially underutilized genetic diversity of exotic wild cotton germplasm, which are 'golden' resources to improve cotton cultivars and solve many problems associated with fibre quality, resistance to insect, pathogens and tolerance to abiotic stress (Jenkins *et al*., 1992).

2.3.1 *G. herbaceum*

This type of cotton traditionally grew in open forests and grasslands of the African continent. Compared to the domesticated shrub, it has smaller fruit and thicker seed coats. Unfortunately, no clearly domesticated remains of *G. herbaceum* have been recovered from archeological contexts. However, the distributions of its closest wild progenitor suggest a northward distribution, North Africa and near East (Hancock, 2004). It is one of the wild cotton species, short lived and fast growing perennial shrub, found throughout Asia and in many parts of Africa elsewhere it can be grown as an annual plant This species has a thick, rigid stem, which grows up to 1.5 m tall . It has a few vegetative branches and alternatives. Leaves are relatively deeply lobed and more or less hairy (George, 2007). The bracts of the epicalyx do not enclose the flower bud closely, but spread widely from it and are round and broadly triangular with six to eight definite teeth. The bolls are more rounded but have a 'beak' at the apex. They have a smooth, few pit or oil glands which are divided into three or four locules each containing up to eleven fused seeds having both fuzz and lint. Bolls do not open widely when they are ripe (Munro, 1966).

2.3.2 *G. barbadense*

The species *G. barbadense* is commercially an important cotton species of tropical South American origin. It is presently grown in many regions of the world. The species is morphologically diverse, consisting of a wide range of wild, commercial landrace and highly improved commercial forms (Wendel *et al.*, 2010). It is an annual shrub up to 3m tall with several strong ascending branches. The leaves are fairly deeply divided into three to five lobes and are usually glabrous. The flowers are large and showy but the petals do not spread so widely, they are deep yellow color and have a red or purple spot at their bases. The bracts of the epicalyx are large and partially enclose the flower with each having ten to fifteen long pointed teeth at its apex. The bolls had deeply pitted with oil glands that are dark green. The species *G. barbadense* Var brasiliense has three to five locules, each containing five to eight kidney shaped seeds, with fuzz being concentrated in a single tuft at the end of the seed. The hair covers the seed as long, lustrous lint hairs and staples, which varies from 5 mm to 50mm (Brown 1975; Wendel *et al*., 2010).

2.3.3 *G. hirsutum*

The species *G. hirsutum* was first cultivated in Mexico. The oldest evidence of *G. hirsutum* came from the Tehuacan Valley and has been dated between 3400 BC to 2300 BC (Mauer, 1954). In different caves of the area, archeologists attributed to the project of Richard MacNeish found remains of freely domesticated examples of this cotton (Hancock, 2004). The first clear evidence of domestication of this type of cotton came from Ancon, a site on the Peruvian coast, where archaeologists found remains of cotton bolls dating back to 4200 BC (Stephens *et al.*, 1974). This species exhibits diverse types of morphological forms including wild primitive to domesticated accessions (Ulloa *et al.*, 2006). Generally, it is an annual shrub and stem is green or brown woody of about 1.5m tall when grown under good husbandry, and have few vegetation

branches. Leaves are large, broad with three or five lobes, densely hairy and nectarines are present. The flowers have large corollas, which are yellow, while petals have no spots, and stigmas are generally none protruding. Bolls are long and rounded with oil glands and there are three to five locules each with up to eleven seeds with fuzz and lint hairs covering the entire seed coat (Brown, 1975; Wendel *et al.*, 2003). The staple length varies from one to one eighth inches (Kirby, 1963).

2.3.4 *G. kirkii*

This species is found in the Indian Ocean islands along the East African coast. It is occasionally a small tree of 2 to 3 m height. The stem and branches are quadrangular in outline and bracts are coarsely separated splitting into three to expose dull brown flowers. The flowers are produced towards the end of branches and branchlets (Kirby, 1963).

2.3.5 HART 89M

The cotton variety HART 89M was developed at National Fibre Research Centre (NFRC) Mwea in 1983. It is the commercial variety recommended as suitable for production in the cotton growing zones of the Rift Valley, Eastern, Central and Coast of Kenya (Kambo *et al*., 2005).

2.4 Cotton plant

2.4.1 Germination of cotton seed

The cotton growing season varies from 100 to over 190 days depending on climatic conditions and plant species**.** Seed Cotton germination is epigeal and is sown at between 2 cm and 4 cm depth and rather deeper in dry conditions and shallower when soil is wet. Cotton has a taproot that grows quickly and it can reach a depth of 20-25 cm before the emergence of seedling above the ground (Govila *et al*.1969; Balock, 2004). The depth to which it penetrates is roughly equal

to the height of the stem. For the adult plants, the lateral roots arise freely from the upper thicker part of the taproot and run more or less horizontal and constitute the bulk of the feeding roots when soil is moist, but penetrate much more deeply when soil is dry (Bird, 1981 and Krakhimalev, 1991). Excessive moisture, soil pH and soil hardness (Farbrother, 1972) affect the development of the root systems.

2.4.2 Stem and branches

Cotton plant consists of vertical stem carrying two kinds of branches; fruiting branches and vegetative branches similar to the mainstream in the primary axis and continues to grow from the same point developing into fruiting branches that terminate with a flower bud. Vegetative branches and mainstream stems do not bear flowers directly but produce fruiting branches at the tip (Mauney, 1966). The fruiting branches usually occur at nodes five and seven. A cotton plant usually produces 16-18 fruiting branches with two to five fruiting nodes on each branch, (Jenkins *et al.*, 1990). Of the total possible fruiting sites only small fractions matures and are harvested. In general, cotton mature boll is on position one more often than on other positions. The boll at position one is larger than those in other positions (Jenkins *et al.*, 1990). The fruiting branch at nodes nine through fourteen produces the bulk of lint and only a small percentage of bolls on nodes higher than fruiting branch position sixteen matures (Buxton K,*et al*., 1981).

2.4.3 Leaves

Cotton has two types of leaves, those which arise from the main stem are referred to as main stem leaves, while those that arise from the fruiting branch are referred to as subtending leaves (Rakhimov *et al*., 1976). Leaves are arranged in a spiral of 3/8 way round the circumference of the stem. They are broad and lobed with three to five lobes. The vegetative branches show the same direction of its spiral as the main stem (George, 2007). The development of the buds at

each node largely depends on environmental conditions, close spacing, low fertility and drought. The first of the buds to develop at each main stem node is the axially bud producing either fruiting branches or vegetative branches which depends on conditions sometimes before the branches appears (Mauney, 1966). The second and third bud at each node rarely develops below the Node of the first Fruiting Branch (N F B) but if conditions are favorable, a second bud will develop. This often produces a short fruiting branch or vegetative branch, independent of species and variety. High temperatures increase the NFB (George, 2007). The main leaf is palmate but the leaf shape and size depends on species and varieties. The cotyledons are between an opposite pair of simple leaves with short stalks. They are rather fleshy, glabrous and usually expand to a width of four centimeters within three days. The terminal bud of loosely furled leaves protects the growing point as it develops from between the cotyledons (George, 2007).

2.4.4 Reproduction biology of *Gossypium* species

Gossypium species have a complete hermaphroditic, solitary, axial flower that begins to form four to five weeks after planting (Macfarlane *et al.*, 2002). The floral buds form epically and flowers approximately 25 days after they first appear. Flowering follows a distinct pattern with the first flower opening low on the plant at first position of the fruiting branch and approximately three days later a flower appears and opens on the next higher fruiting branch at the same position. About six days after the first flower on that branch, the second flower will open. This same pattern continues provided the plant continues to grow actively. Flowers open at dawn and remain open for a single day (Liogier, 1994).

2.4.4.1 The flower

The flower bud, called square, begin to form at the initiation of the fruiting branch. The first square produced on a fruiting branch is referred to as a first- position square. As this square

develops the portion of the fruiting branch between the main stem and the square elongates. An auxiliary meristem also develops adjacent to the square. The auxiliary meristem produces a second position square and subtending leaf (Qayyum *et al. 1992*). The flower first becomes visible as a small green cone closely hard pressed to the fruiting branch. Three bracts or bracteoles, joined at their bases, surround the buds. The bracteoles are heart shaped forming a pointed canopy over the bud (Austin, 1998). Within the bracts is an inconspicuous green calyx. The day before the flowering, the conical bud starts to enlarge rapidly (Mauney, 1968; Merideth *et al*., 1984). The petals double their length in 24 hours and early on, the following day the corolla opens widely to expose the stamina column and the stigma. The floral parts are epicalyxes (E). 3 - 4, calyx (K) 5 undulate, petals (P) 5, androecia's (A) 100 - 150, gynoecia's (G) 3 - 5 (Macfarlane *et al.*, 2002). The corolla is white or slightly cream color found in upland cotton. The day after anthesis the petal turns pink and the next day or two it drops off to reveal the young green boll (Iziko, 2002).

2.4.4.2 Flowering pattern

The flowering data provides a convenient method of studying the sequence of development of the cotton plant. The flower opens early in the day and petals start to turn pink by evening (Mitchison, 1971); Macfarlane *et al*., 2002). The next day they can easily be distinguished by their red color from nearly all open flowers. Both the vertical flowering interval (VFI`), between successive fruiting branches on the main stem and horizontal flowering interval (HFI), between successive flowers on the fruiting branch follow an orderly pattern in the same cotton plant (Tharp, 1960). Seasonal average of HFI range from 5.26 - 8.5 days in the USA and VFI is about 3 days (Mauney, 1968). West Africa are between 2.0-3.0 days in the VFI and 5.5-6.0 days for HFI. Malawi at Makanga and Makaya is 8.8 and 5.6 days for HFI respectively (Mauney, 1968).

The differences are caused by difference in temperature. The ratio between HFI and VFI is a

useful rule of thumb but not precisely accurate (Buxton *et al*., 1981; Liogier, 1994). The ratio varies between 2.1 and 2.6 (McClelland and Needy, 1931). The ratio increases during the season from between 2.1 for the early flowering to 2.6 for the late flowering. This ratio appeared to be specific for genetically different cottons. In Uganda the ratio is 2.0, American upland 2.5 and African upland for example Tanga's G. *barbadense* 3.0 (Farbrother, 1961). The day of the first flower indicates the earliness of the crop and marks the start of the flowering period (Iziko, 2002). It is a resultant of number of factors besides sowing data, temperature, water supply and NFB, species, varieties and day and night temperatures. At Namulonge, Uganda the comparatively cool days (maximum temperature 28 °C and a consistent 12 hours day), the first flower on a typical upland variety appears 65-70 days after planting. At Makanga, Malawi (maximum temperature 33 °C), the first flower is produced in 45 days. In Sudan, G. *barbadense* produce first flower in 80 days (Brown, 1975; Iziko, 2002).

2.4.4.3 Pollen grain and pollination

Soon after anthesis, the anthers of cotton flowers dehisce, discharging pollen. Cotton pollen is relatively large, heavy and not easily dispersed by wind (Jenkins, 1992). Cotton is a facultative self-pollinator and an opportunistic out crosser, when insect pollinators are present, (Dosterhuis, 1992; Macfarlane *et al*., 2002). Cotton pollen remains viable for about 12 hours (Govila and Rao, 1969). Fertilization of ovules occurs about 12 to 30 hours after pollination. The great majority of seeds are as a result of self pollination (Johnand David, 1995).

2.4.4.4 Fruit development

The growth and development of cotton fruit, known as bolls begins immediately following fertilization although the most rapid period of growth occurs between 7 and 18 days (Jenkins *et al.,* 1990). During development, the bolls are spherical to ovoid and pale green. Maximum boll

size is achieved about 25 days after fertilization with full maturity later. Mature bolls are thick, leathery and dry rapidly to become brittle and brown (Igbal, 2006; Bariola *et al.,* 1981). Such fruit split open revealing the seeds and associated fibres. The cotton boll is a dehiscent schizocarp with 3-5 locules each containing about 8 seeds (Percy,, 2006).

About 100 bolls or more may be needed to gather a reliable average. The fruit usually ripens from 50-70 days after fertilization, with the time varying with species, variety, temperature and humidity. In field there are greater variations from plant to plant and from boll to boll which appears to be independent of date of flowering and position of the boll on the plant. Temperature accounts for 96 % of the variation in the boll period. Night temperatures have greater effect than day temperature (Gipson and Johan, 1970).

Table 2.2: Number of locules **and seeds of species and varieties**

Species	Locules per boll	Seeds per locule
HART 89M	3 – 4	6 – 9
G. herbaceum	3-4	Not more than 11
G. hirsutum	3 -5	5-11
G. barbadense	3 – 4	5-8

Source (Munro, 1987).

The boll reaches full size half way through the boll period. At the same time, the seeds in it reach full size and the lint grown to their full length with thickening of the fibres well started. The boll wall is pitted at outside with gossypol glands sucked as the boll dries out at maturity, with the outer wall of each loculus opening outwards exposing the lint and the seeds (Ahmad *et al*., 2008).

2.4.4.5 The seed morphology

Cotton is grown primarily for its fibre, which are produced by epidermal cells of the seed coat. Prior to ginning and delinting the seed coat bears two types of fibres, long lint fibres valued by the textile industry and short fuzz fibres known as linters used in various products. The cotton seed measures about 10 mm to 4 mm and weighs about 80 mg (George, 2007). It has a hard seed coat covered by a slightly waxy cuticle (Tharp, 1960). Beneath the epidermal cells, which produce the seed hairs, there is a thin outer seed coat with an inner epidermis. This has a different origin from that of the inner seed coat but the two are fused together (Christiansen *et al*., 1960; Merideth ,*et al*., 1984).

2.5 Occurrence of intraspecific or interspecific hybrids

Gene transfer between adjacent G. *hirsutum* individuals does occur but at relatively low frequencies (Llewellyn and Fitt, 1996). It is estimated that cross-pollination between cotton plants in adjacent rows accounted for only 1 to 2 % of seeds. Fertile progeny are also produced when *G. hirsutum* is cross-pollinated with *G. barbadense* (Brubaker *et al.,* 1999). This potentially provides another ready means by which G. *hirsutum* genes, may be spread in the environment. Although cross-pollination can occur, cotton is considered as self-pollinated crop (Niles, 1970). However, there are no morphological barriers to cross-pollination based on flower structure. Hybrid vigor in cotton has been observed in interspecific crosses as well as in crosses between varieties within the species. (Fryxell *et al.,* 1969; Jenkin *et al*., 1990; Marani, 1967; Stroman, 1961), in particular showed that crosses between *G. barbadense* and *G. hirsutum* were much more productive than either parent. Because of the differences in the characteristics of the lint of the two species, it frequently has objectionable qualities in the

hybrid. This problem are less likely to arise in intraspecific hybrids where considerable hybrid vigor has also been shown.

2.6 Genome of *Gossypium* species

The genus *Gossypium* includes approximately 45 diploid species (2n = 26) and five tetraploid species (4n = 52). Diploid species fall into eight genomic groups A-G and K. The African clade, comprising the A, B, E and F genome (Wendel and Crown, 2003), occurs naturally in Africa and Asia, while the D genome clade is indigenous to America. A third diploid clade C, G and K genome are found in Australia. All the tetraploid 4n = 52 *Gossypium* including *G. hirsutum* and *G. barbadense* are classic allotetraploids that arose in new world from interspecific hybridization between genomes (Nadeem *et al*., 1998). The ancestral Africa species and D genome are like the American species (Endrizzi,, 1985). The closest existing relative of the original tetraploid progenitors are the A genome species *G. herbaceum* (A1) and *G. arboreum* (A2) and the G genome species *Graindii* (D5) among others (Brubaker *et al.*, 1999). Polyploidization is estimated to have occurred 1 - 2 million years ago (Wendel and Crown, 2003) which gives rise to five existing allotetraploid species.

The A genome species produce spinnable fibre and are cultivated on limited scales, whereas the D genome species do not produce spinnable fibres. Understanding the contribution of the A and D subgenome to gene expression in the allotetraploids may facilitate improving fibre traits (John and David, 1995; Saha *et al.*, 2006) All the wild species in both old and new world and cultivated species, which originated in the old world are diploids with 26 somatic chromosomes whereas the cultivated species from the new world are tetraploid with 52 chromosomes. Depending on origin and relationships, they are divided into three groups wild lintless diploid species (2n = 26), old world linted diploid species (2n = 26) and new world tetraploid species (4n = 52) (Hutchinson and Joseph, 1962). The wild lintless diploid (2n = 26) *Gossypium* species probably

evolved in Southern Africa and spread to arid regions of Arabia, South East Australia and America. With geographical isolation, perhaps during the Mesozoic era, different groups of species, which are now genotypically distinct subsequently, evolved in each of these areas. Some of the wild cottons like *G. somalense* are lintless perennial xerophytic shrubs or small trees often found growing on desert fringes, in dry river-beds or on rocky hillsides, where vegetation is sparse (Wendel *et al*., 2010). They all have strong, deep taproot and though their leaf area is decreased as adaptation to the arid climates, their leaves are commonly hairy or leathery, enabling the plant to survive in such rigorous localities. Although, the wild cotton are phenotypically distinct they produce small dehiscent capsules. They contain seeds covered to varying degrees, in most species with short dark colored surface hairs (fuzz) which cannot be spun to make yarn (Wendel *et al*., 2010).

2.7 Evolution of cotton

The old world linted diploid species (2n = 26) are *G. herbaceum* and *G. arboreum.* Each has perennial and annual races. The mutation of gene controlling the development and structure of the seed coat hair, led to the production of spinnable lint, in addition to fuzz occurred in *G.*. *herbaceum* race africanum, which is found growing in Southern Africa. It may be the only truly wild diploid linted cotton. It is believed that all diploid, linted cotton evolved from race africanum and that the ancestral form was probably domesticated in Ethiopia or Arabia (Hutchinson *et al.*, 1947; Nadeem *et al*., 1998). The new world linted tetraploid species (2n = 52 are *G. hirsutum* and *G. barbadense* among others.. They arose from South America, following a natural hybridization of an old world A genome, linted diploid of *G. herbaceum* and New world D genome lintless diploid (*G. raimondii* and *G. thurberi* or both) (Wendel and Crown, 2003). The diploid genome AD hybrid from this cross was infertile until its chromosome number was doubled. The new world linted cotton is thus allotetraploid with the genome formula AADD

(Adams *et al.,* 2004). At meiosis, normal pairing occurs between thirteen pairs of homologous D genome chromosome. Whether the cross between old and new world diploid occurred, only one of *G. barbadense* or *G. hirsutum* arose independently from two or more natural crosses (Schubert , 1995). There is no general agreement about the ways old linted diploid cotton reached South America (Hutchinson, 1962).

2.8 Microscopic study of plant's chromosome

Development of interspecific hybrid due to crossing of two different species of the same genus is possible if a breed is equipped with knowledge on ploidy. In cotton interspecific hybrid are fully fertile in a cross between *G. barbadense* and *G. hirsutum*. Chromosomes analysis is necessary to establish the ploidy of studied *Gossypium* genotypes. In the study of chromosomes, light microscopes are required. The squash or smear method is used which involves several steps such as pre-treatment and hypotonic treatment, fixation, staining and mounting.

2.8.1 Squash and smear preparations

This method has a great advantage, in that the entire process is rapid and also much more suitable for critical observation. In properly prepared smears or squashes, one can carry out observation on separate single cells. Moreover, the cell, being released of its compactness, undergoes much enlargement in volume, affording wider space for the chromosomes to become scattered. The only disadvantage of this method, when specially applied to somatic chromosomes is that the individual cells being released from one another shift from their original site, and the original topography is altered.

The term 'smear' and 'squash' are often loosely used, resulting to an impression that the two processes are identical, but strictly speaking, they are not so.

In smear, the cells are directly spread over slide prior to fixation and no treatment is necessary

to secure cell separation (Kao, 1975). In squashes, special treatment are needed for dissolution of the pectic salts of the middle lamella, so that separation of individual cells can be obtained, from a compact mass of cells, this treatment being carried out after staining. After passing through the required steps, the softened material or small tissue can be neatly squashed on a slide by generally applying pressure or tapping with a needle over the cover-glass. In squashes, the most important step is the softening of tissue. The different schedules can be divided into two categories, namely, softening performed prior to staining, clearing and staining accomplished in the same fluid. The most important agent needed for softening the tissue is dilute hydrochloric acid, in Feulgen stain, this step is essential. The normal hydrochloric acid at 60 ºC serves two important purposes; dissolution of pectic salts of middle lamella thus helping in cell separation and clearing of the cytoplasm (Bezbaruah, 1968). If dilute hydrochloric acid (10%) is used, for best result, the treatment should be carried out in a slightly warm temperature such as 60 ºC for 4-5 min until softening, the acid being washed off either in 45% acetic acid solution. Softening and maceration of the tissue can be achieved during fixation by the use of mixture of equal parts of 95% ethanol and concentrated hydrochloric acid as a fixative.

No warming is needed, after 5 minutes of treatment; the tissue becomes fixed and softens. If necessary, hardening of the materials, ethanol and hydrochloric acid mixed in the proportion of 3:1 is more effective (Kao, 1975).

2.8.2 Pre-treatment and hypotonic treatment

Pre-treatment for the study of the chromosomes is performed for clearing the cytoplasm, separation of the middle lamella causing softening of the tissue, bringing about scattering of

chromosomes with clarification of constricted region (Lavan,, 1965). Pre-treatment for clearing the cytoplasm and softening the tissue, clearing the cytoplasm from heavy contents is achieved by short treatment, in normal hydrochloric acid, it brings about transparency of the cytoplasm background. Such treatments, however, require washing for the removal of excess acid and acid soluble materials. For the separation of chromosomes and clarification of constrictions, change in cytoplasm viscosity brings about destruction of spindle mechanism with chromosome remaining free, not attached to any binding force within the cell. Pressure applied during squashing result in the scattering of chromosome throughout the cell surface. The change in cytoplasm viscosity causes differential hydration in its segments, due to this differential effect, constricted regions in chromosomes appear well clarified, 3-amino-1, 2, 4, triazole has limited application in leaf and shoots tip squash (Bezbaruah, 1968).

2.8.3 Fixation

Fixation can be defined as a process by which tissues or their components are fixed selectively at a particular stage to a desired extent. The purpose of fixation is to kill the tissues without causing them any distortion of the components to be studied as far as it is practicable. Fixation increases visibility of the chromosome structure and clarifies the details of chromosome morphology, such as the chromatic and heterochromatic region and the primary and secondary constrictions. Although a number of fixing reagents have been devised, all of them possess certain common characteristic, which are essential for a fixative. Each fixing chemical is lethal in its action, (Sharma, 1956). The structure integrity of the chromosome must be maintained intact, precipitation of the chromatin matter is essential to render the chromosome visible and to increase its basophilic nature in staining, as the purpose of chromosome study is to observe the minute details of chromosome morphology, the staining should be perfect. Even with the best fixative, the chemical changes undergone by the nuclear bodies can not be ignored as there

is certain inherent disadvantage in the entire process, for example the tissue shows a tendency to shrink on coming into contact with the chemical. Acetic acid used as fixative fluid can be mixed in all proportion with water and ethanol it can be used from very low concentration of 1% to even ‘glacial’ form. The term glacial is derived from the fact that it freezes to a form resembling ice at very cold temperature. Acetic acid is an ideal fixative for chromosomes, as it has been observed to maintain the chromosome structure intact and ideal for chromosome count (Krikorian *et al.,* 1983).

2.8.4 Staining

The structure of chromosomes can be studied only after they are made visible under the microscope. A certain chemical agent that is insoluble in the chromosome substance causes the colouration of a chromosome in a killed tissue. The principal kinds of dye that are used to stain chromosomes are synthetic organic dyes. The colour of a dye is due to certain chemical configuration, known as chromophores. Feulgen reaction is considered the most effective with regard to chromosome staining. Feulgen and Rossenbeck devised a method based on the Schiff’s reaction for aldehydes which stains the nucleic acid of the chromosomes specifically, as such has been effectively employed for the visualization of chromosomes (Schubert, 1995).

2.8.5 Mounting

After staining, the tissue is mounted in a suitable medium for observation under the microscope, if the tissue is observed under the microscope without mounting, it usually dries up and rendered opaque. The chief aim of mounting is to render the tissue transparent, to increase its visibility under the microscope, to hold it within the protecting cover-glass firmly in place and to preserve it for a period of time or indefinitely. McClintock (1929) first devised the acetic-alcohol schedule. This method is based on the principle of detaching the cover-glass

in a solvent, dehydrating and clearing before mounting in the desired medium. For tissues stained by the Feulgen reaction, 45% of acetic acid-ethanol 1:1 is a good solvent. The paraffin seal around the cover-glass of the temporary preparation is removed carefully by wiping the top of the cover-glass with xylol. The slide is then inverted in a covered tray containing the solvent. The cover-glass detaches and falls to the bottom of the tray after some time. The slide and cover-glass are transferred to a mixture of the acid and absolute ethanol 1:1 and treated for 15 minutes, later they are passed through acetic-ethanol mixture of 1:3 and two grades of absolute ethanol keeping it for 5 minutes each. In spite of careful handling, the material is liable to be washed away while transferring the slides. An airtight container lined with absorbent paper that is saturated with ethanol for 24 hours can be employed for dehydration (Snow, 1963).

2.9 Karyotypic analysis

The importance of chromosomes in heredity has created inevitable search for detailed information regarding their structure and behavior. Apart from this and any other general consideration, the visible form and intricate maneuvers of the chromosome, during cell division have presented many fascinating problems of intrinsic interest. As a result, the chromosome unquestionably became the most thoroughly investigated cellular organelle (Swanson, 1957). It is usual to compare the number, form, shapes and sizes of chromosomes by their morphology as observed at metaphase. During this stage, chromosomes reach their maximum contraction, attaining a length that under ordinary environmental condition remains remarkably constant from cell to cell (Krikorian *et al.,* 1983). In gross structure, the mitotic chromosome is usually a rod like body with one constriction at the centromere called the primary constriction. The centromere itself is generally not visible at somatic metaphase as definable entity, but its structure causes a constriction in the chromosomes. The constriction caused may be located at the terminal, sub-terminal or median positions causing the chromosome as it proceeds poleward in anaphase to

assume respectively a rod j and v shape (Swanson, 1957). Chromosome with a terminal constriction has been referred to as telocentric. The shape of chromosome can be altered by pericentric inversion. This involves a breakpoint at different distance on either side of centromere, or parametric inversion, this involves a breakpoint on one arm at distances on either side of a Nucleolus Organizing Region (NOR) which marks the chromosome on either at terminal or intestinal position. This inversion gives rise to a secondary constriction and a distal satellite. The shape and size of chromosome can also be altered by reciprocal translocation. This result to exchange of unequal part between the chromosomes involved (Schubert *at et.*, 1995). Beside centromere, secondary constriction can also be observed in chromosomes, which if present in a distal region of the arm would pinch off as a small fragment called satellite. The satellites remain attached to the rest of the chromosomes by thread chromatin. The secondary constriction are allays constant in their position and hence can be used as a marker. The chromosomes having satellite are markers chromosome and are called Sine Acid Thymonucleinico chromosome (SAT). On staining, SAT reveals a relative deficiency of DNA in the NOR (Zhang *et al*., 2008).

2.9.1 Chromosome comparison and chromosome count

Chromosome comparison and chromosome count would be used to find the evolution connection they provide "similarities" analysis (Gill *et al.*, 1991). The chromosome number represent the first data set that lead to an understanding of genetics of any species, each species has a specific number of chromosomes in each cell. Chromosome numbers is probably more constant than any other single morphological characteristic that is available for species identification (Singleton, *et al.*, 1953). Cotton has 2n = 26 chromosomes for diploid and 2n = 52 for tetraploid (Beasley, 1942). In an extensive number of cases polyploidy have on the contrary been shown to exhibit better stability and are described for various characteristics not found in diploids (Rose, 1981).

For example in tea *Camellia* polyploids exhibit higher and more prolific growth rate than diploids (Fedak *et al*., 1987). Chromosome counts and observations of reproduction for 55 taxa of *Cactaceae* indicated that polyploidy was correlated with self fertility, adventure embryo profuse branching and vegetative reproduction (Wendel *et al.*, 2010).

CHAPTER THREE

Materials and methods

3.1 Study site

The reported work, was carried out in a farm, at the Kenya Agricultural Research Institute (KARI) Thika centre, beginning May 2010 to. The Centre is located at 0 59 S and 37 04 E. It is 5 Kms from Thika Town in Kiambu County and 43 Kms from Nairobi. The Centre is located at an altitude of 4800 ft above the sea level. The soils are classified as sandy loam to clay and depth ranges from deep to shallow with good to poor drainage.

3.2 Materials

KARI - Thika, provided wild cotton germplasm and domestic cotton. The germplasms, which were studied, were collected from various regions in Kenya, including Lamu, Kilifi, Kwale and Siaya counties. The domestic cotton used in the study was HART 89 while four wild cotton species, *G. kirkii, G. hirsutum*, *G. barbadense*, *G. harbaceum* were included

3.3 Experimental design and field practice

The four wild cotton species and HART 89M were planted in three experimental blocks, each measuring 6 m by 30 m. There were three replications per experimental block per species, the distance between blocks was 2 m. The experimental blocks were then divided into 5 plots of 5 m by 5 m, with 1m separating plots. The cultural practices such as application of fertilizer, hoeing, irrigation and pest control were done at the same time. Crops were grown under uniform conditions to minimize environmental variation. Picking of bolls extended from months of December 2011 to April 2012. The cotton seeds were planted in rows of 1m by 0.5m at depth of 2 cm.

3.4 Data collection

The data collected included monthly rainfall, average monthly temperature, germination

percentage, flowering, bush height, internodes length, and number of branches in the main stem, lint and seed weight, lint and seed weight ratio.

The percent germination was recorded every seven days per plot from the date of the first germination. About 15 bushes from each plot were randomly marked and used for all subsequent sampling as proposed by Bird (1981).

3.5. Weather during vegetative period

The data on daily temperature and rainfall were collected from the month of September 2010 to October 2011. Mean monthly temperature and rainfall were calculated on monthly basis. Rainfall was measured in millimeters (mm) and temperature in Celsius (°C).

3.6 Physiological characteristics of cotton species in the field experiment

3.6.1 Seed germination described

The data on germination was collected on weekly basis from the emergence of the first seedling. Data collection continued until germination counts were constant. The percent germination rates were calculated from the final data, by the formular:

Percent germination= No. of germinated seeds / Total seeds planted × 100.

3.6.2 Flowering of **Gossypium** species

The data on flowering rate was collected on weekly basis from the emergence of the first flowering plant to the time when all the plant flowered. The percent flowering was calculated per the final data on flowering per species and varieties as described by Soomro (2000).

3.7 Agro-morphological performance per genotype

The data was collected from experiment conducted at the experiment farm at Thika K.A.R.I. and

a sample of forty five bushes was selected at random per studied species. The collected data included plant height, number of fruiting branches, length of the longest fruiting branches, and number of nodes on the longest fruiting branch and inter nodal length as described by (Soomro, 2000).

3.7.1 Plant height

At maturity plant height was measured from ground level to tip of the tallest branch. The height was measured using centimeters.

3.7.2 Number of fruiting branches

At maturity, the number of fruiting branch was counted from the lowest fruiting branch to the highest. The fruiting branch counts were recorded per plant.

3.7.3 Inter nodal length

At maturity, samples of three successful internodes per plant were picked from the longest fruiting branches. Measure of each inter nodal length were taken in centimeters. The average inter nodal lengths were calculated per plant.

3.7.4 Length of the longest fruiting branch

At maturity, four longest fruiting branches were selected, their length measured in centimeters and the length of the longest branch was recorded per plant.

3.7.5 Number of nodes on the longest fruiting branch

The data on nodes count was collected from the longest fruiting branch. Each node count was recorded per plant.

3.7.6 Yield per plant per species

Data on plant yield was collected by weighing all the cotton seeds per plant from each genotypes. All cotton seeds per plant were picked and weighed using electronic balance. Mean weight in grams, was obtained by dividing the total cotton seed yield per plant by the total number of plants.

3.8 Boll traits analysis

The data was collected on boll traits, this included boll weight, number of seeds per boll, seed weight, and percent seed weight, lint weight, percent lint weight and calculation on lint-seed ratio. Thirty three bolls were picked from the sample plant, placed into paper envelops per plant. The bolls were hand ginned, seeds and lint placed in separate envelops per boll.

3.8.1 Boll weight

Data was collected by weighing one hundred bolls from each species. At least two bolls were picked from sample plant at random. Each boll was weighed using electronic balance. Mean boll weight was obtained by dividing the total cotton seed weight by the total number of bolls picked.

3.8.2 Seed weight per boll

The bolls were ginned. Seeds were placed into a separate labeled kirk envelop on boll basis. The seeds from each boll were weighed using an electronic balance and each result was recorded in grams. The average seed weights was calculated per genotype, by dividing the total seed weight by the number of seeds.

3.8.3 Number of seeds per boll

After ginning, seeds obtained from the entire boll were pooled, counted and divided by the total number of bolls to obtain the number of seeds per boll.

3.8.4 Lint weight per boll

Clean and dry samples of cotton seed were weighed and then ginned separately by use of hands. The lint obtained from each sample was weighed by electronic balance and the average lint yield per boll was calculated by dividing the total lint weight by the number of bolls.

3.8.5 Percent lint weight

Clean and dry samples of cotton seed were weighed and ginned separately. The lint obtained from each boll was weighed and lint percentage was calculated by the following formular. Lint percent = weight of lint in a boll / weight of cotton seed in a boll.

3.8.6 Percent seed weight

Clean and dry samples of cotton seed was weighed and ginned separately. The seeds obtained from each boll were weighed and percent seed weight was calculated by the following formular. Seed percentage = weight of seeds in a boll/weight of seed cotton in a boll.

3.8.7 Seed lint ratio

The data on seed lint ratio was calculated by dividing the weight of seed by the weight of lint per boll.

3.9 Relationships between agronomic traits and yield per species

The phenotypic correlation coefficient and variation between agronomic traits was determined using Pearson correlation coefficient as described by Azhar *et al.,* (1999).

3.9.1 Genotypes correlation between yield and other traits

The correlation coefficient and variation between yield and other agronomic traits such as height, number of fruiting branches, length of the longest fruiting branches and number of nodes on the longest fruiting branch for studied genotypes were determined using Pearson correlation coefficient. (*G. herbaceum, G. hirsutum*, HART 89M, *G. barbadense* and *G. kirkii*) as described by Ansari *et al.,* (1989) and Arshad *et al.,* (1993).

3.6 Laboratory microscopic work

Laboratory work was carried out at Plant and Microbial Sciences Department laboratory (Kenyatta University) and Genetics laboratory (University of Nairobi).

3.10.1 Collection of materials and fixation

Cotton seeds from the five genotypes were collected from the seed bank at Thika KARI centre. The seed coats were peeled off to improve the rate of germination. They were planted in potted sand in the green house. After germination, the seedlings were transplanted to potted red soil, where they were left to grow until the shoot had three to four leaves. About 2 mm young shoot apex of cotton plant was obtained from the experimental plants. A bud at the apex was cut using forceps and a surgical blade, the apex tip together with the last two millimeters of stem were cut and placed in a Petri dish containing FAA mixed at ratio of 9:5:5 formalin, alcohol and acetic acid respectively for fixation. Since chromosomes were sticky, the buds were treated with 0.2% colchicine for 30 minutes to stop chromosomes from clamping together. Second fixations were done using a mixture of Acetic acid and Acetyl alcohol at a ratio of 3:1 for seven minutes as described by Peacock (1966).

3.10.2 Hydrolysis

Hydrolysis was done using normal hydrochloric acid at 55 ºC for 7 minutes in a water bath. Five bottles containing hydrolysis fluids were placed in water bath for acclimatization then buds were added into a bottle containing normal hydrochloric acid as described by Singleton (1953) and Sharma (1956).

3.10.3 Staining of buds

The buds were removed from hydrolysis fluid, chopped at the tip, then placed on slides and macerated to expose the chromosomes. The macerated buds were stained using feulgen for one hour. Feulgen only stains the parts that contain chromosomes as described by Dyer (1963). On the slide a drop of 45% acetic acid was put to remove excess stain. A small tissue was neatly squashed on a slide, by applying gentle pressure on the cover-glass as described by La-cour (1954). Slides were examined for proper spread, labeled and sealed using clear nail varnish, the prepared slides were observed under leica Microscope,magnification X scanned vertically and horizontally around the coverslip for chromosomes analysis. Karyotypic analysis of five cotton species was done as described by (McClintock, 1929). The ploidy study was carried out to determine the chromosomes complement. Microscopy photograph were taken using photomicroscope (Leica Icc 50) which has software that connects to the Computer Processing Unit (CPU) and the images observed from the microscope were transmitted to computer monitor.

3.11 Data analysis

Oneway Analysis of Variance (ANOVA) was used to determine the significance difference among the studied species and varieties at $P \leq 0.05$. T-test was used to determine the agronomic traits and boll traits significance difference between the standard species HART 89 M and wild

cotton at $P \leq 0.05$ (Steel and Torrie, 1980). The t-test was also used to determine agronomic and boll performances at $P \leq 0.05$. Correlation coefficient and coefficient of determination were used to determine the relationship between the cotton boll yield with other agronomic traits such as height, number of fruiting branches, length of the longest fruiting branch and number of nodes on the fruiting branch at $P \leq 0.05$ as described by (Fonseca and Peterson 1968). The graphical presentation was used to demonstrate different agronomic traits and boll traits among the studied species.

CHAPTER FOUR

RESULTS

4.1 Weather conditions during the vegetative period

The mean monthly temperatures were high during sowing 21.6°C (October) (Figure 4.1).

Temperatures were low, between 18.6°C on months of June 2011 and August 2011. The mean monthly rainfall was unevenly distributed, ranging from 1 mm in January 2011 and July 2011 to 155.4 mm in November 2010 and March 2011, during vegetative period. There was high rainfall from November to June at the time of flowering and ripening of bolls (Figure 4.1). This resulted to rotting of seeds and fibres hence affecting yield in some genotypes especially HART 89M.

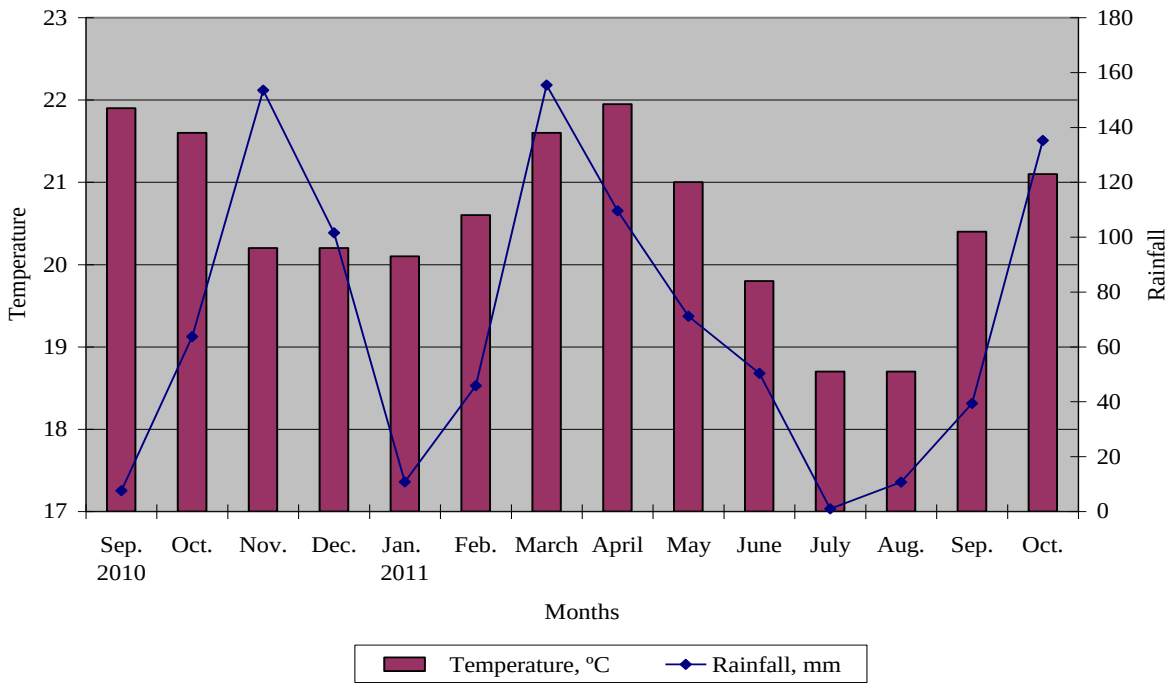

Fig. 4.1: Mean monthly temperature and rainfall during the vegetative period.

4.2 Physiological characteristics of cotton species in the field experiment

The study determined different agronomic and boll performances of four wild *Gossypium* genotypes of *G. herbaceum*, *G. barbadense*, *G. kirkii* and *G. hirsutum* in comparison with locally cultivated genotype HART 89M.

4.2.1 Seed germination

The results in figure 4.2 revealed that during sowing, there was water deficit therefore,

germination was initiated by irrigation until all the viable seeds germinated. While later, development depended on rain water. On 29th October 2010, fifty cotton seeds were sown per plot, by the 8th week they differed in germination percent ranging from *G. barbadense* having the highest percent of 74.3%, G. *herbaceum* 64%, HART 89M 56.7 %, *G. hirsutum* 53.3% and lowest was *G. kirkii* 35.3%, which never attained 50% germination. The results revealed that, all the other genotypes took eight weeks to attain 50% of seeds to germinate (Figure 4.2). There was delayed germination in all the studied genotype, which can be associated with hard seeds found among the genotypes. The results in figure 4.2 revealed that, between 27% for *G kirkii* and 66% *G. barbadense* seeds never germinated, which can be associated with low viability and non-swelling of seeds, caused by poor absorption of water. % ≤% ≤

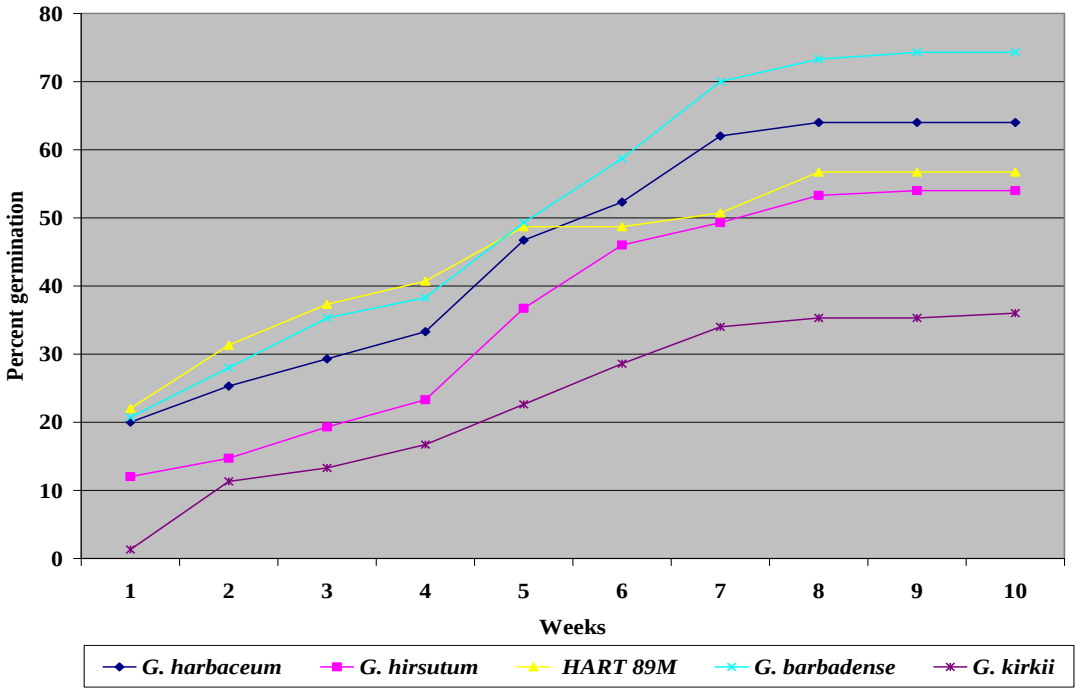

Fig. 4.2: Percent germination per genotype

4.2.2 Flowering of *Gossypium* genotypes

The genotypes differed in flowering duration ranging from the appearance of the first flower (Figure 4.3). Four genotypes took eight week to attain % flowering may be due to duration in germination, except *G. kirkii* which by eighth week had attained 86.7% as shown in Fig 4.3. The study showed an interesting scenario where *G. kirkii* plant which shed the shoot at early stage was the first in flowering compared with the unsheded shoot, which extended flowering from ten to eighteen weeks depending on the time of germination.

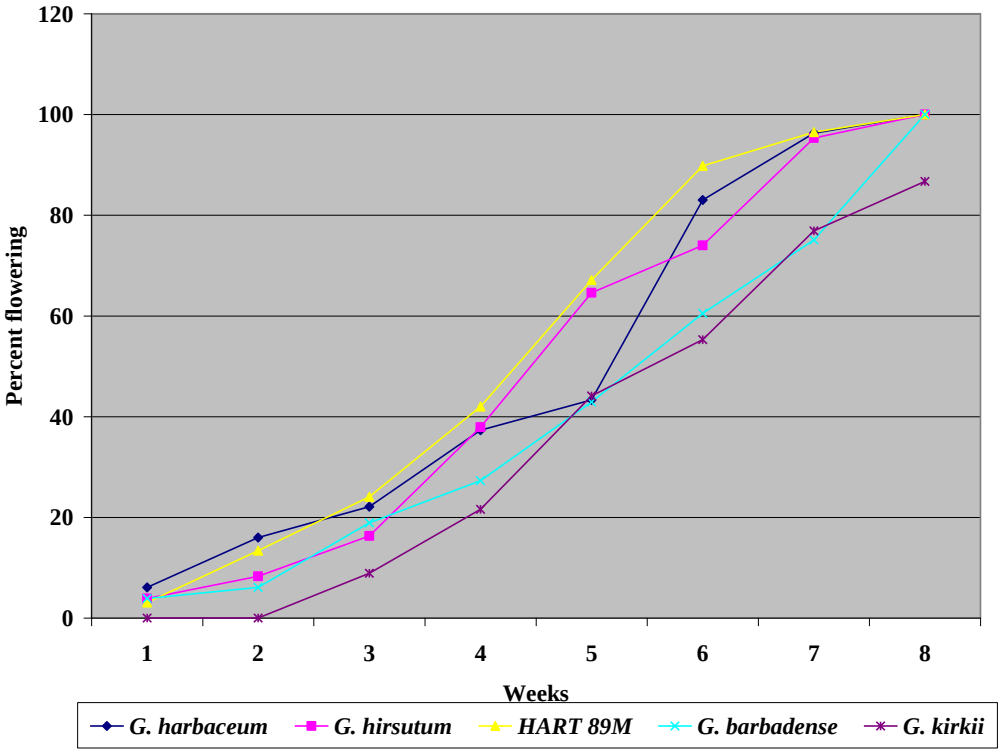

Fig. 4.3: Percent flowering of cotton genotypes per week

4. 3 Agro-morphological performance per genotype
The Analysis of Variance (ANOVA) presented in Table 3, revealed thatall the studied genotypes differed significantly at P≤0.05 for all the phenotypic characteristics 4.1.

Table 4.1: Mean agronomic-morphological **performances of genotypes**

Genotypes						
Parameter	*G. herbaceum*	*G. hirsutum*	HART 89M	*G. barbadense*	*G. kirkii*	F df = 4 P ≤0.05
Plant height, cm	143.89 ± 6.98 a	160.06 ± 9.86 b	103.42 ± 8.08 c	203.37±7.18 b	18.15 ±6.09 e	98.84
Number of fruiting branches	47.93 ± 4.19 a	61.49 ± 5.52 b	36.47 ± 2.81 c	66.93 ± 3.90 b	55.89 ± .85 d	25.97
Inter nodal length, cm	5.22 ±0.22 a	5.78 ±0.48 b	4.07 ±0.23 c	5.50 ± 0.25 b	6.36 ± 0.30 d	26.10
Length of the longest fruiting branch, cm	39.69 ±3.27 a	50.40 ± 4.70 b	34.02 ± 2.98 c	68.36 ±3.34 d	38.60±2.84 a	58.72
Number of nodes of the longest fruiting branch	3.87±0.35 a	4.69 ±0.42 b	2.89 ±0.17 c	5.38 ±0.34 d	3.78 ±0.34 a	27.47
Yield, g	88.38±9.74 a	100.27 ±8.27 b	71.48 ± 3.28 c	205.21±15.61 d	40.29 ±2.29 e	178.80

Parameters marked with the same letter on a row had no significant difference according to Tukeys HSD significant difference at 5 % level

4.3.1 Plant height

There were significant differences in plant height among the studied genotypes (F = 98.84, df =4, P = 0.05) (Table 4.1). The mean height among the genotypes ranged from the lowest height being HART 89M, (103.43 ± 8.08 cm) to the highest being *G. barbadense*, (203.37 ± 7.18 cm). comparison, i 4.3, ≤ ≤.The result revealed that,ed Plant height has a direct influence on number of fruiting branches and therefore cotton seed yield.

4.3.2 Number of fruiting branches per plant

The results in Table 4.1 indicated significant differences in the number of fruiting branches, among the studied genotypes (F = 25.973, df = 4, P ≤ 0.05). The highest mean number of fruiting branches was found in *G. barbadense,* (66.93) ± 3.9 branches and the lowest was found in HART 89M, (36.47) ± 2.81 branches (Table 4.1). ,P ≤,P ≤ (4.3)

4.3.3 Internodal length from the first fruiting branch per plant

The results in Table 4.1 indicated a significant difference among the four studied genotypes (F = 26.10, df = 4, pP ≤ 0.05). In internodal length, G. *kirkii* had the highest internodal mean length (6.36± 0.30 cm) and the lowest was found in HART 89M (4.07 ± 0.23cm) (Table 4.1). on revealed that,ad ofP ≤w()P ≤.

4.3.4 Length of the longest fruiting branch per plant

The results in Table 4.1, indicated a significant difference in mean length of the longest fruiting branch per bush among the studied genotypes (F = 58.726, df = 4, P ≤ 0.05) while *G. barbadense* had the highest mean, (68.35 ± 3.34 cm) and the lowest being HART 89M with a mean, (34.02 ± 2.98 cm). The results in Table 4.3 revealed that, the lyt,P ≤P ≤.

4.3.5 Number of nodes in the longest fruiting branch per plant

The results in Table 4.1 indicated a significant difference among the genotypes in the mean number of nodes in the longest fruiting branch, (F = 27.479, df = 4, P ≤ 0.05). Results in Table 4.3 revealed that, the mean range from the highest inbeing *G. barbadense,* (5.38) ± 0.34 to the lowest mean was in HART 89M (2.89) ± 0.17. The numberbranchwslyt difference of and, whilewas .

4.3.6 Yield in grams per plant

The results in Table 4.1 revealed a significant difference among the genotypes (F= 178.805, df = 4, P ≤ 0.05). while the results in Table 3 revealed that, *G. barbadense* had the highest mean yield per plant (205.21 ± 15.61g) w and *G. kirkii* having the lowest yield per plant with mean yield of 40.29 ± 2.29 g *G. hirsutum* 100.27 ± 8.827, *G. herbaceum* 88.38 ± 9.74 g and HART 89M 71.48 ± 3.27g *i* (4.3)i4.3P ≤28.79 g ≤being G16.9 gP ≤**4.4 Boll traits analysis**

The Analysis of Variance (ANOVA) results presented in Table 4.1 revealed, that all the studied species differed significantly at (Pp ≤ 0.05 for all the characters at phenotypic level (boll traits) among the studied genotypes. Data on different traits was analysed and revealed different potentials. This provides the evidence of phenotypic variability present in traits of the studied genotypes.

Table 4.2: Mean boll traits on studied genotypes

	Genotypes					
Traits	*G. harbaceum*	*G. hirsutum*	HART 89M	*G. barbadense*	*G. kirkii*	F df = 4 P ≤ 0.05
Boll weight, g	3.19 ± 0.16 a	2.77± 0.14 b	2.98 ± 0.15 c	2.98 ± 0.12 c	3.71± 0.14 d	25.938
Seed weight, g	2.18± 0,099 a	1,85± 0.11 b	1.78± 0.09 c	2.05± 0.10 a	2.30± 0.08 d	21.181
Lint weight, g	0.98± 0.05 a	1.03± 0.07 a	1.21± 0.10 b	0.99± 0.047 a	1.41± 0.06 c	26.592
No. of seeds per boll	20.73 ± 0.74 a	20.83±1.05 a	19.01±0.89 b	20.83±0.62 a	21.05±0.59 c	4.309
Lint weight %	32.31±2.10 a	37.82±2.56 b	39.71±2.49 c	34.59±2.00 a	37.82±0.66 b	13.613
Seed weight %	68.68±0.86 a	62.17±0.66 b	60.28±1.85 b	65.46±2.52 a	62.17±0.64 c	16.364
Lint seed ratio	1.65±0.047 a	1.98±0.13 b	1.79±0.13 b	2.31±0.268 c	0.65±0.13 d	2.264

Parameters marked with the same letter on a row had no significant difference accoording to Tukeys HSD significant difference at 5 % level.

4.3

≤.4.4.1 Mean boll weight

The results in Table 4.2 indicate a significant difference among the genotypes (F = 25.938, df = 4, P ≤ 0.05). Results in Figure 4.4 revealed that, *G. kirkii* (3.71, g) had the highest mean boll weight followed by *G. harbaceum* (3.19, g) and the lowest being *G. hirsutum*. In comparison with HART 89M, the results in Table 4.3 revealed a positive significant difference with *G. kirkii* (0.73, g), (t = 7.72, p ≤ 0.05) and *G. harbaceum* 0.73 g (t = ≤ 2.21, p 0.05).

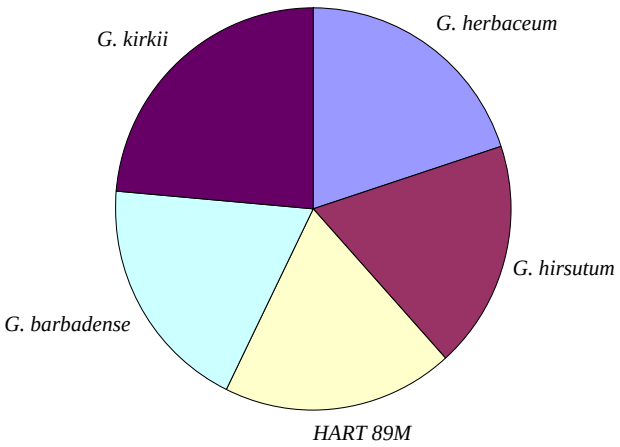

Fig. 4.4: Boll weight, g per genotype

.4.4.2 Lint and seed weight, g per boll

The result in Table 4.2 revealed, a significant difference among the genotypes (F = 21.181, df =4, P ≤ 0.05). Result in Table 4.4 and Figure 4.5, revealed that, *G. kirkii* had the highest mean seed weight, 2.30 ± 0.08 g, *G. herbaceum* 2.18 ± 0.1 g, *G. hirsutum* 1.85 ± 0.11 g while HART 89M had the lowest mean seed weight of 1.768 ± 0.087 g. On lint results revealed a significant difference in mean lint weight among the studied genotypes (F = 26.592, P ≤0.05). While the

results in Figure 4.5, revealed that, *G. kirkii* had the highest mean weight of 1.41 ± 0.06, HART 89M 1.21 ± 0.10, *G. hirsutum* 1.03 ± 0.07, *G. barbadense* (*e*0.99 ± 0.04. The lowest mean lint weight was *G. harbaceum* 0.98 ± 0.05. The results revealed that *G. kirkii* had the highest mean weight for both seed and lint. The results in Table 4.2 revealed a negative significant difference in mean lint weight between HART 89M and other genotypes. Seeds weight had a positive significant difference between HART 89M and other genotype, with *G. kirkii* 0.52 g ($t = 8.67$, $p \leq 0.05$) having the highest while *G. hirsutum* 0.07 g ($t = 1.3$, $p \leq 0.05$) had the lowest.

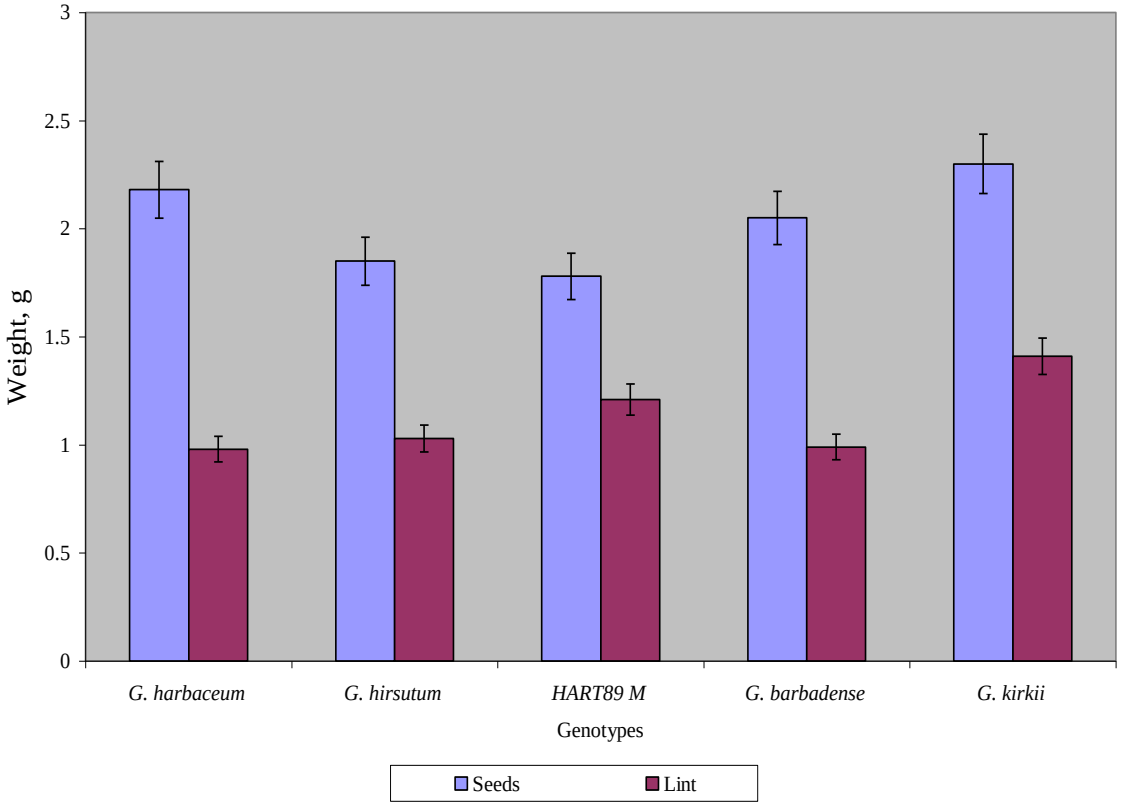

Fig. 4.5: Comparison of mean weight of lint and seeds per boll per genotype

4.4.3 Number of seeds per boll

The results in Table 4.2 show a significant difference among the cotton species and varieties studied ($F = 4.309$, $df = 4$, $P \leq 0.05$). *G. kirkii* had the highest mean of 21.05)± 0.59, followed *G.*

hirsutum 20.83± 1.05, *G. barbadense* 20.83± 0.62, G. herbaceum 20.73± 0.74 and HART 89M had the lowest mean of 19.01 ± 0.89 (Table 4.2).The results in T4.3s a positive 2.04 ($t = 3.74$, $p \leq 0.05$) followed by 1.82, ($t = 3.32$ $p \leq 0.05$), 1.82, ($t = 3.32$ $p \leq 0.05$) *G. harbaceum*, ($t = 2.67$, $p \leq 0.05$) had the lowest mean difference with HART 89M.

4.4.4 Percent mean weight of lint and seed boll

The results in Table 4.2 revealed that, differed significantly among the studied genotypes ($F = 13.613$, $df = 4$, $p \leq 0.05$)

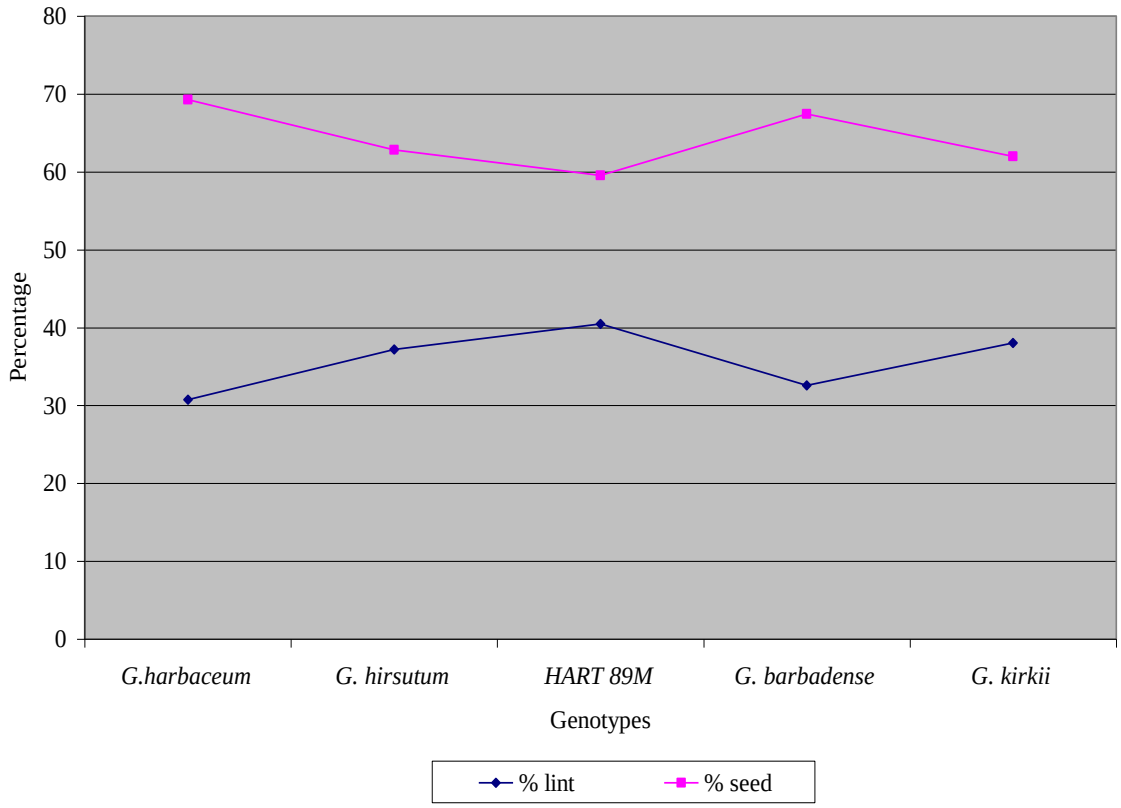

Fig. 4.6: C**omparison of percent mean** weight lint **and** seeds per **boll**

The results on Table 4 a show that HART 89M had the highest % lint weight of 40.46 ± 2.49 followed*G. kirkii* 38.05 ± 0.66, *G. hirsutum* 37.18 ± 2.56, *G. barbadense* (32.56) and *G. herbaceum* thehad the lowest lint weight %30.72 ± 2.10nd (Figure 4.6). The results on mean

seed weight percentage revealed a significant difference among the genotypes (F = 16.364, P ≤ 0.05). *G. herbaceum* had highest percentage of(69.82 ± 0.86 *G. hirsutum* 62.82 ± 0.66, *G. barbadense* 67.44 ± 2.52, *G. kirkii* 61.95 ± 0.64) andand HART 89M 59.54 ± 1.85 (Table 4 Figure 4.6). The result revealed *G. herbaceum* and *G. barbadense* as having a wider difference between lint and seed percentage weight of 28.56% and 34.88% respectively. percentage % respectively

4.4.5 Seeds lint ratio per boll

All genotypes were significantly different (F = 2.264, P ≤ 0.05) in seeds lint ratio (Table 4.2). The average lint-seed ratio ranged from *herbaceum* (2.22 ± 0.047 *G. barbadense* 2.07 ± 0.27, *G. hirsutum* (1.79), *G. kirkii* (1.63) and the lowest HART 89M (1.47) ± -0.13.

4.4.6 Seeds of *Gossypium* genotype

Plate 4.1 revealed different seeds having different morphological traits. All seeds had hard coat covered by slightly waxy cuticle. Seeds of studied genotype exhibited different shapes.

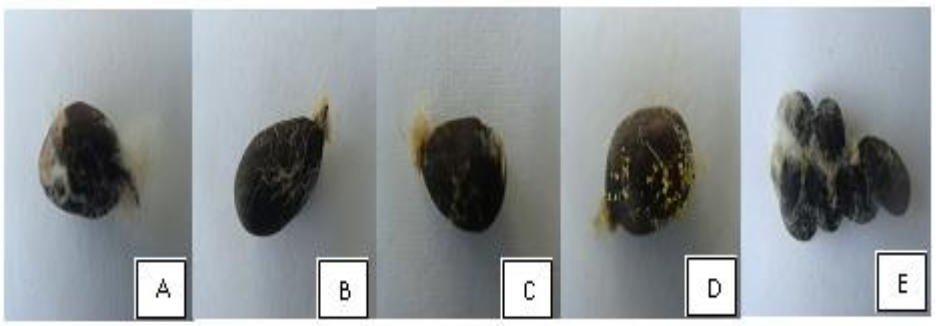

Plate 4.1: Seed of *Gossypium*. *barbadense*seed with long hooklike extension at one end and the fuzz is concentrated in a single tuft at the end of the seed;

Seed of *Gossypium kirkii*

Seed B, with rounded shape on one side and short hooklike extension on the other end with fuzz and lint at the other end;

Plate 3: Seed of HART 89M

The seed with rounded on both ends, with a blunt hairy extension, short hooklike extension, and fuzz and lint at one end;

with ; with  **Plate 4: seed of *Gossypium herbacium***

The seeds are oval shaped are fused and have both fuzz and lint with no pointed end.

 Plate 5: **Seed of *Gossypium hirsutum***

Seed is rounded shape at one end and oval shaped on the other end.

	Genotypes							
Traits	*G. hirsutum*	T p=0.05	*G. harbaceum*	t p=0.05	*G. barbadense*	T p=0.05	*G. kirkii*	t p=0.05
Germination, %	-1.18±2.34 a	-3.96	8.65±2.18 b	0.53	7.29±3.09 c	2.35	-21.48±0.83 d	-25.76
Flowering rate, %	-4.47±1.90 a	-1.29	-3.98±3.069 a	-2.34	-12.66±4.04 b	-3.13	-16.2±4.04 c	-4.15
Plant height	38.71 ± 5.68 a	6.82	40.4 ± 5.72 b	6.86	109.2± 5.4 c	20.20	81.9±4.75 d	17.25
No. of fruiting branches	25.27-1.19 a	4.40	11.4 ± 1.17 b	2.91	30.4± 1,25 c	9.18	19.43 ±5.23 d	3.57
Internodal length	1.71 ± 0.28 b	6.09	1.15 ± 0.17 b	6.90	2.29 ± 0.19 c	9.46	1.37 ± 0.17 b	7.94
Length of the longest fruiting branch	16.2 ± 3.47 b	6.12	5.67. ± 2.72 c	4.17	34.34 ± 2.60 d	8.14	4.58± 2.97 c	6.97
No. of nodes on longest fruiting branch	1.80 ± 0.28 b	8.69	-0.98± 0.17 a	3.89	2.49 ± 0.37 b	7.46	0.89 ± 0.13 a	5.86
Yield per plant	28.79 ± 4.74 b	6.06	16.90±5.19 c	3.25	133.73 ± 7.5 d	17.73	-31.19±1.95 e	-16.66
Boll weight in grams	-0.11± 0.04 a	-2.25	0.17 ± 0.12 b	2.21	0.05 ± 0.18 a	-0.12	0.73 ±0.13 c	-7.72
Lint weight in grams	-0.18±0.06 b	-3.11	-0.23±0.60 c	-3.51	-0.24±0.06 c	-3.61	0.2 ±0.06 d	3.21
Seeds weight in grams	0.07 ± 0.06 a	-1.30	0.40 ± 0.65 b	-6.34	-0.27± 0.06 c	-4.46	0.52 ±0.6 d	8.67
No of seeds per boll	1.82±0.67 a	2.67 b	1.72±0.59 a	2.88	-1.81±0.54 a	3.32	-2.04±0.55 a	-3.74
Lint % per boll	-3.28 ±1.71 a	-1.10	-9.74±1.57 b	-4.72	-7.90 ± 1.64 b	3.11	-2.41±1.33 a	1.42
Seeds % per boll	3.28 ±2.58 a	2.87	9.74 ± 3.13 b	4.57	7.90±2.68 b	3.46	2.41±1.76 a	4.14

Parameters marked with the same letter on a row had no significant dif4.5 Relationship between agronomic traits and yield per genotype

4.5.1 HART 89M

Results in Table 4.4 revealed that, there was a highly significant positive correlation (r = 0.83) between height in cotton yield, which meant that the yield was greatly influenced by height. The correlation coefficient of determination revealed 69.1%, variation in cotton yield per plant was due to its relationship with height of the plant. The number of fruiting branches also displayed a highly significant positive correlation (r = 0.64) with yield. The correlation coefficient of determination revealed 40.9% variation in yield per cotton plant due to its relationship, with the number of branches. The results also displayed a significant positive correlation (r = 0.47) between the cotton yield with the number of nodes on the fruiting branch. The correlation coefficient of determination revealed a 22.1% variation in cotton yield per bush due to its relationship, with the number of nodes on the fruiting branches. There was a significant positive correlation (r = 0.63) between cotton yield and length of fruiting branch per bush. There was significant correlation between seed weight per boll (r = 0.59); lint weight (r = 0.72) with the seed cotton yield. There was negative low significant correlation between the number of seeds and plant yield (r = -0.14) (Table 4.4).

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Table: 4.4 Correlation coefficient (r) and variation percent (v %) in relation to **seed cotton yield of Gossypium genotypes for different traits**

Traits	**Genotypes**									
	HART 89M		*G.herbaceum*		*G. hirsutum*		*G. barbadense*		*G. kirkii*	
	r	V %	r	V %	r	V %	r	V %	r	V %
Height	0.83	68.9	0.92	84.6	0.71	50.4	0.88	77.4	0.81	65.6
No. of fruiting branches	0.64	40.9	0.61	37.2	0.56	31.3	0.56	31.4	0.56	31.4
Length of longest fruiting branches	0.63	39.7	0.71	50.4	0.77	59,3	0.75	56.2	0.49	24.0
No. of nodes on longest fruiting branch	0.47	22.1	0.63	39.7	0.79	62.4	0.86	73.9	0.86	73.9
Seed weight	0.59	34.8	0.48	23.0	0.56	31.4	0.57	32.5	0.96	92.2
Lint weight	0.72	50.4	0.43	18.5	0.51	26.0	0.49	24.0	0.93	86.5
No. of seeds	-0.14	1.96	0.28	7.8	0.48	23.0	0.45	20.3	0.78	60.8

4.5.2 *Gossypium herbaceum*

Results in Table 4.4 displayed a highly significant positive correlation (r = 0.92) between height and cotton yield per plant. The correlation coefficient of determination revealed that height had 84.6% variation in determining the cotton yield. The result also displayed that, the number of fruiting branches had a highly significant positive correlation (r = 0.61) with cotton yield per plant. The correlation coefficient of determination revealed that the number of fruiting branches influenced 37.2% variation of cotton yield, Number of nodes on the fruiting branch displayed a highly significant positive correlation (r = 0.63) with cotton yield per plant. The correlation coefficient of determination revealed that the number of nodes on the fruiting branch had a 39.7

% influence on variation of cotton yield. The result also revealed that the length of fruiting branch had a significant correlation (r = 0.71) with the cotton yield per plant. The correlation coefficient of determination revealed that 50.4% variation of cotton yield per plant was influenced by length of fruiting branch. The results on Table 4.4 revealed a moderate correlation between seed weight and plant yield (r = 0.48), lint weight and plant yield (r = 0.43) and low correlation between number of seeds and plant yield (r = 0.28).

4.5.3 *Gossypium hirsutum*

Results in Table 4.4 revealed that height displayed a highly significant positive correlation (r = 0.71) with cotton yield per plant. The correlation coefficient of determination showed that height was responsible for 50.4% variation in yield per plant On the number of fruiting branches, the result revealed that highly significant positive correlation (r = 0.56) was displayed by number of fruiting branches with yield per plant, which showed that yield was greatly influenced by the number of fruiting branches. The correlation coefficient of determination revealed 31.3% variation in yield per plant was due to its relationship with the number of fruiting branches. The length of the longest fruiting branch revealed a highly significant positive correlation (r = 0.77) with yield per plant. The correlation coefficient of determination showed that the length of the longest fruiting branch was responsible for 59.3% variation in cotton yield per plant. The number of nodes on the longest fruiting branch displayed a highly significant positive correlation (r = 0.79) with cotton yield per plant. The correlation coefficient of determination revealed that 62.9% variation in yield per plant was due to the effect of the number of nodes on the longest fruiting branch. The results also displayed a moderate correlation between seed weight and plant yield (r = 0.56), between number of seed per boll and plant yield (r = 0.51) and between number seeds per boll and plant yield (r = 0.48 (Table 4.4).

4.5.4 *Gossypium barbadense*

Results in Table 4.4 displayed a very strong correlation between height and plant yield (r = 0.88). The correlation coefficient of determination revealed that height was responsible for 77.4% variation in cotton yield per plant. The number of fruiting branches displayed a highly significant positive correlation (r = 0.56) with cotton yield per plant. The correlation coefficient of determination revealed that number of branches was responsible for 31.4% variation in cotton yield. There was also a significant positive correlation (r = 0.75) between cotton yield and length of the fruiting branch per plant. The correlation coefficient of determination revealed that 56.2% variation in cotton yield was due to its relationship with length of fruiting branch. The results also displayed a highly significant positive correlation (r = 0.86) with number of nodes on the fruiting branch and cotton yield per plant. The correlation coefficient of determination revealed that 73.9% variation was due to relationship between cotton yield and number of node on fruiting branch per plant. Results on Table 6, show a moderate correlation between seed weight and plant yield (r = 0.57), between lint weight and plant yield (r = 0.49) and between number of seeds per boll and plant yield (r = 0.45).

4.5.5 *Gossypium kirkii*

The results in Table 4.4 revealed highly significant positive correlation (r = 0.81), displayed by height with cotton yield, which indicated that cotton yield was greatly influenced by height. The correlation coefficient of determination revealed 65.6% variation in cotton yield per plant was due to its relationship with height. The fruiting branches displayed a significant positive correlation (r = 0.56) with cotton yield per plant. The correlation coefficient of determination revealed that fruiting branches were responsible for 31.4% variation in cotton yield per plant. The length of the longest on the fruiting branch also revealed a significant positive correlation (r = 0.49) with cotton yield per plant. The correlation coefficient of determination indicated that the

length of longest on the fruiting branch influenced 24.0% variation of the cotton yield. Results on Table 6, also revealed a strong correlation between seed weight and plant yield (r= 0.96), between lint weight and plant yield (r = 0.93) and between number of seeds and plant yield.

4.6 Chromosomes analysis
4.6.1 HART 89M

The photomicrograph showing metaphase chromosome of HART 89 M cellsat X1000 magnification obtained from apical meristemactic. The slide preparation for shoot bud revealed a tetraploid with chromosome complement of 4n = 52. colchicinized apical bud nuclear revealed parallel chromosomes location (Plate 4.2). Some chromosomes overlap while some extend beyond the field of view, this presented difficulties in establishing the length of most of chromosomes. Some of chromosomes revealed a rod shaped, as illustrated in Plate 4.2 and some chromosomes showing j-shaped. Several chromosomes revealed a condensed shape which made the chromosomes to be tightly paired throughout there entire length.

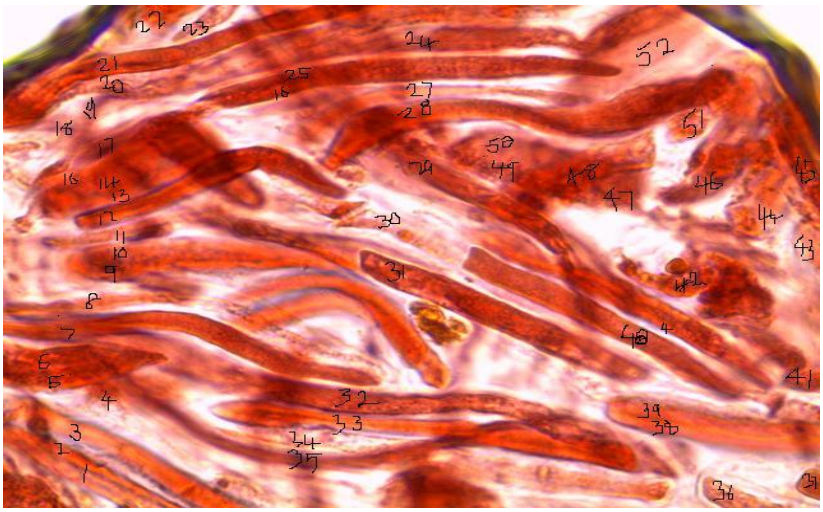

Plate 4.2: *G. hirsutum* "HART 89 M" of apical bud nuclear

4.6.2 *Gossypium herbaceum*

The slide preparation revealed a diploid chromosome complement of 2n = 26 at X1000 magnification. Most of the chromosomes did not reveal their heterochromatic region due to

excessive condensation (Plate 4.3). Colchicinized apical bud nuclear revealed parallel chromosomes location. Some chromosomes overlapped while most extended beyond the region covered in field of view, this presented difficulties of establishing the length of chromosomes. Some chromosomes revealed acrocentric shape, while others revealed metacentric and telocentrics shape. Some chromosomes revealed chromosome constrictions.

4. 3

4.6.3 *Gossypium kirkii*

The slide preparation of apical meristem cells revealed diploid of chromosome complement 2n = 26. At magnification X1000 colchicinized apical bud nuclear revealed parallel chromosomes location (Plate 4.4). Some chromosomes overlapped most of the chromosomes are telocentrics shape, as illustrated in Plate 4.4, while some chromosomes revealed a metacentric shape. Some chromosomes revealed secondary constriction as revealed in chromosomes. This indicated the presence of satellite chromosomes. Chromosomes did not reveal chromatids due to excess condensation.

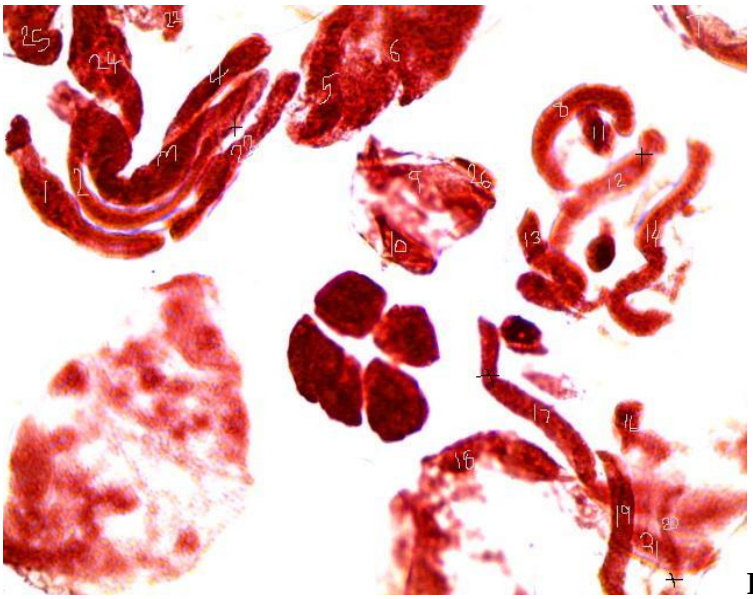

Plate 8: *G. kirkii* apical bud nuc

4.44.6.4 *Gossypium hirsutum*

The microphotograph slide revealed chromosomes, at magnification X1000, of pretreated apical meristem. The studied species revealed tetraploid of chromosomes complement 4n = 52. Colchicinized apical bud nuclear revealed parallel chromosomes location (Plate 4.5). Most of chromosomes overlapped while some extend beyond region of field of view. This made it difficult to establish the chromosomes length, hence numbering of chromosomes was done arbitrary. In addition, most of chromosomes did not reveal heterochromatic region due to excessive condensation, therefore did not reveal individual chromatids. Some chromosomes were tightly paired throughout their entire length, making it difficult to detect the two individual chromosomes, as illustrated in (plate 4.5) some chromosome revealed an acrocentric shaped, while some chromosomes revealed metacentrics and a telocentrics shape. Some chromosomes revealed secondary constrictions.

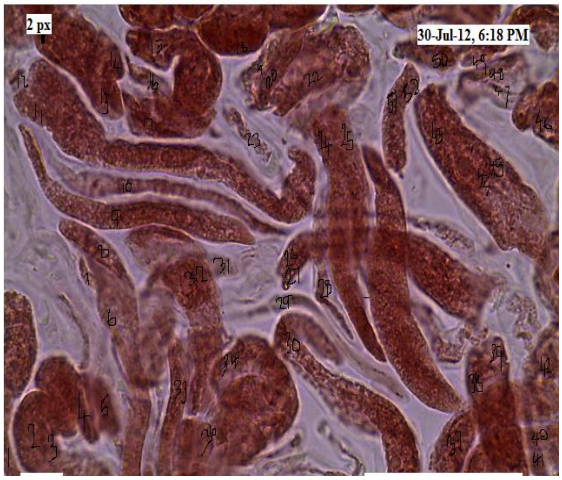

Plate 4. 5: *G. hirsutum* of apical bud nuclear.

4.6.5 *G. barbadense*

The slide was prepared from pretreated taproot tip at magnification X1000. It revealed a tetraploid cell with a chromosomes compliment of 4n = 52. Colchicinized apical bud nuclear revealed parallel chromosomes location (Plate 4.6). Most the chromosomes overlapped while some extended beyond the region of field of view, this presented difficulties of establishing the length of chromosomes (Plate 4.6). The numbering of chromosomes was arbitrary allocated, for the purpose of description of chromosomes. The chromosomes did not reveal their chromatic region due to formation of sticky chromosomes hence recognition of individual chromosomes was with difficulties. Most of chromosomes revealed an acrocentric shape. Some chromosomes revealed telocentrics shape while some chromosomes revealed secondary constriction.

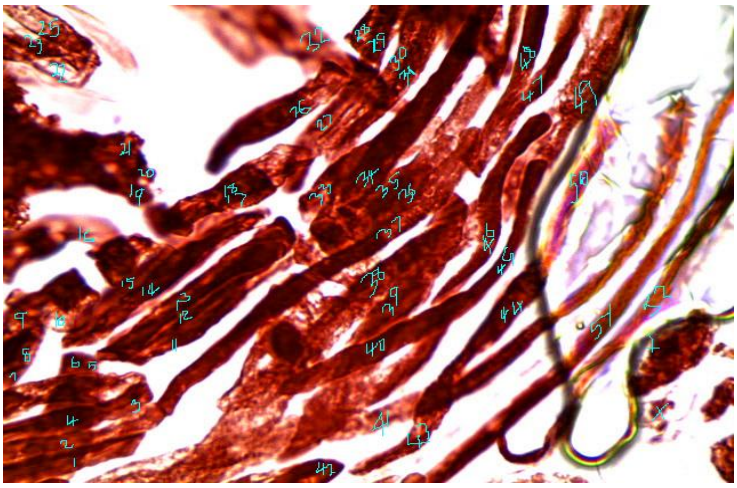

Plate 4. 6: *G. barbadense* apical bud nuclear

CHAPTER FIVE

DISCUSSION

5.1 Qualities of best yielding cotton

The revitalization of cotton industry calls for provisions of quality and high yielding cotton varieties. The maintaining of genetic purity of introduced, promising and existing commercial cotton varieties is an essential practice for all the varieties and cultivars. Before initiating any cotton improvement program, the precise knowledge about the nature and genetic potential of existing germplasm, extent of relationship and association of different morphological trait with yield and contributory traits with seed cotton yield is very important, (Taohua and Haipeng, 2006; Batool *et al*., 2010). Correlation analysis reflected response of a particular trait with its counterpart and provides a good index to predict the corresponding change which occurs in one character at the expanse of the proportionate change in the other (Meena *et al*., 2007). Information on character association between traits and yield is important for breeding material subjected to selection for high yielding. According to Fonseca and Peterson (1968) the correlation coefficient analysis measures the magnitude of the relationship between various plant traits and determines the component characters on which selection is based on, for improvement in cotton seed yield. Different traits had a direct effect of the trait that contributes directly to improved cotton yield (Igabal *et al.*,, 2006; Singh *et al* Tariq *et al.*,, 1992; and Batool *et al.*, 2010). Data on different traits was analysed and revealed different potentials in agronomic performance for different wild cotton traits. This provides the evidence for the genetic variability present for the traits among the studied genotypes.

5.1.1 Plant height

Significant and positive correlation between plant height, seed cotton yield and number bolls per plant was established in the present study. Selection for plant height could therefore be effective in breeding for seed cotton yield. There was a significant difference in height between *G. barbadense* and HART 89M. Development of interspecific hybrid could improve seed cotton yield. Similar results were reported by Suinaga *et al*. (2006), Meena *et al.* (2007) and Khan *et*

al. (2010).

5.1.2 Number of fruiting branches

The result enunciated that, there was significant difference in number of fruiting branches among the genotypes and a significant and positive correlation between number of fruiting branches and seed cotton yield. The number of fruiting branches is desirable and attention should be paid in the selection in the breeding for seed cotton yield. This is in agreement with results by Meena *et al.* (2007) and Khan *et al.* (2010).

5.1.3 Internodal length

The internodal length had an influence on maturity rate on cotton plant. Therefore the cotton plant with short internodal value have mature earlier compared with the one having longer internodal length this was HART 89M (Table 4.2), which had the shortest internodal length and flowered earlier than other studied genotype. Therefore internodal length should be kept in the mind while breeding for early maturity. Badr (2003) and Igbal et al. (2003) who studied the earliness in *Gossypium hirsutum* varieties manifested the same findings.

5.1.4 Length of the longest fruiting branch

The length of fruiting branch has a direct influence on flowering point, in that the longer the length the more the nodes that produces flowering points, which determines the cotton seed yield the more the nodes the flowering point. This was found in *G. barbadense*, which had the longest fruiting branch and the highest number on nodes on the fruiting branch. Similar results were reported by Khan (2003), Khan *et al.* (2007, 2010). Therefore, selection of the length of fruiting branch can be effective in breeding for seed cotton yield when paired with good set other traits that have a significant correlation with seed cotton yield.

5.1.5 Number of nodes in the longest fruiting branch

The number of nodes has direct influence on flowering point hence determine the seed cotton yield. The genotype that had the highest number of nodes per fruiting branch was *G. barbadense*. The number of nodes on fruiting branch showed a significant and positive correlation with seed cotton yield. This play an important role in cotton crop, which should be considered by the breeder while breeding for seed cotton yield. This is in agreement with the results reported by Hussein *et al.* (2000); Meena *et al.* (2007); Khan (2003) and Khan *et al.* (2010).

5.1.6 Boll weight

There is a positive effect of the number of seeds per boll to the seed cotton yield through increasing boll weight. The results revealed a significant and positive correlation of the number of seeds per boll with seed cotton yield. This is in agreement with the results reported by Ahmad *et al*. (2008) and Khan, (2003). The genotypes having high number of seed per boll have a relatively good yield potential in comparison with those with less number of seeds per boll. Number seed per boll is an important yield component, it determines boll weight, seed cotton yield, lint, and cotton seeds for oil. The number of seeds per boll should be considered while breeding for seed cotton yield and cooking oil extraction industry.

5.1.7 Plant yield

The results revealed a significant difference in seed cotton yield, number of fruiting branches, length of fruiting branch and number of nodes of fruiting branch between *G. barbadense* and all the other genotypes. The results were in agreement with those reported by Ansari *et al.* (1989); Quyyum *et al*. (1992); Arshad *et al.* (1993); Larik, (1999); Satange *et al.* (2000) and Soomro,

(2000), Meena *et al* (2007), and Khan *et al.* (2009).

The plant height, number of fruiting branches, the length of fruiting branches and the number of nodes on the fruiting branches had a significant and positive correlation with seed cotton yield, should be considered by breeder to improve seed cotton yield.

5.1.8 Ploidy of genotypes

Hybrid vigor in cotton has been observed in interspecific crosses as well as in crosses between varieties within the species. Marani (1967), Stroman (1961) and Ware (1931) in particular showed that crosses between *G. barbadense* and *G. hirsutum* were much more productive compared with both parents. Because of the differences in the characteristics of the lint of the two species, it frequently has objectionable qualities in the hybrid. The study on ploidy managed to differentiate the studied cotton genotypes in terms of diploids and tetraploids. The ploidy study revealed *G. barbadense, G. hirsutum* and HART 89M as tetraploid chromosomes of complement 4n = 52 while *G. herbaceum* and *G. kirkii* as diploid chromosome of complement 2n = 26. The increase ploidy may impart desirable traits on cotton (Saha *et al.,* 2006). Development of fertile interspecific hybrids between *G. barbadense* and HART 89M is possible. *G. barbadense* and HART 89M have chromosomal homology as it was reported by Wendel and Crown (2003), Adams *et al*. (2004) and Saha *et al.* (2006). In all the studied cotton genotypes, chromosomes length establishment failed, due to overlapping of chromosomes in some genotypes that led to formation of sticky chromosomes because of using of ethyl alcohol. The chromosomes separation technique had failed to yield a pure preparation of single chromosome. Hence, recognition of some individual chromosomes, in some cotton genotypes, was not possible, Schubert and Fuchs (1995)., also observed this Several chromosomes

revealed a condensed shape which made the chromosomes to be tightly paired throughout there entire length. This pairing of some chromosomes was so precise that it was difficult to detect two individual chromosomesthis, which is in agreement with the results reportedSaha *et al.* (2006)

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Genetic variance in most cases was equal to that of phenotypic variance consequently giving high heritability estimates and significant genetic gains. Therefore, the selection based on traits such as height, number of fruiting branches, boll weight, number of nodes on fruiting branch, lint weight and seed weight could be exploited for improvement of yield (Gomma *et al.*,, 1999; Baloch,, 2004; Batool *et al.*, 2010). The analysis of variance (ANOVA) indicated significant difference among the traits of the studied genotypes. The studied genotype revealed significant difference in germination ranging from *G. barbadense* having the highest percent of 74.3 and *G. kirkii* the lowest percent 35.3. On flowering all the studied genotype attained 100 % by 8^{th} week except *G. kirkii* that only attained 86.7 % by the same time. On morphological traits, the result revealed a significant difference among the genotypes with *G. barbadense* being the best performer in height, seed cotton yield, number of fruiting branches and number of nodes on the fruiting branches. In all studied genotypes, results revealed a very high significant correlation among all studied agronomic traits with yield per plant, except for inter nodal length that revealed no significant correlation. Boll traits, displayed a significant correlation between the boll weight, lint weight and seed weight per boll. *G. kirkii* had the highest boll weight, seeds

weight per boll and lint weight per boll, compared to other studied genotypes. *G. hirsutum* had the lowest boll weight, while HART 89M had the lowest seeds weight per boll. *G. herbaceum* had the lowest lint weight per boll. On percentage lint-seeds weight proportions *G. herbaceum* had the highest seed weight percent, while HART 89M had the lowest seed weight percent and the highest lint weight percent. *G. barbadense* had the highest height per plant, seeds-lint ratio and yield per plant. The results in Table 4.4, indicates that *G. herbaceum* had the strongest correlation between height and yield. HART 89M had the strongest correlation between number of fruiting branches and plant yield and *G. barbadense* had the strongest correlation between nodes on the longest fruiting branches and plant yield. *G. kirkii* had the strongest correlation between plant yield and seed weight, Lint weight and number of seeds. This indicated that seeds weight of *G. barbadense* contributed most to the boll weight as compared to other studied genotypes. *G. kirkii* indicated very high positive significant correlation of boll weight, as compared with other boll traits, though it had the lowest yield. *G. barbadense*, which had the highest yield weight per plant, had the lowest lint weight per boll. HART 89M had the highest lint percent and the lowest seed weight percent. Seed cotton yield showed a significant and positive correlation as well as strong dependency upon yield contributing traits such as number of fruiting branches, number of nodes per fruiting branch, boll weight and number of seeds per boll. In future breeding program, these traits should be kept in mind during making selection as they were the major attributes of seed cotton yield. The chromosomes study of the five genotypes revealed difference chromosomes morphology, all the five cotton genotypes revealed the metacentric, acrocentric and telocentrics chromosomes The ploidy study revealed *G. barbadense, G. hirsutum* and HART 89M as tetraploid chromosomes of complement 4n = 52 while *G. herbaceum* and *G. kirkii* as diploid chromosome of complement 2n = 26. All the five studied genotypes revealed secondary constriction in some of their chromosomes. .

6.2 Recommendations

- Selection for plant height, number of fruiting branches, number of nodes per fruiting branch, boll weight and number of seeds per boll should be kept in mind while breeding for seed cotton yield.
- Molecular characterizations of the studied species need be carried out to establish genotypic relationship between HART 89M, *G. barbadense*, *G. hirsutum*, *G. herbaceum* and *G. kirkii* for accurate classification.
- Further study needs to be carried out to determine the effect of shoot loss at seedling stage to the overall performances of *G. kirkii*.

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7.0 APPENDICES

Appendix I:Temperature and rainfall distribution during the vegetative period.

Month	Average temperature (Cº)	Rainfall (mm)
Sep. 2010	21.9	7.6
Oct.	21.6	63.7

Nov.	20.2	153.5
Dec.	20.2	101.6
Jan. 2011	20.1	10.8
Feb.	20.6	45.8
March	21.6	155.4
April	21.9	109.6
May	21	71.2
June	19.8	50.3
July	18.7	1
Aug.	18.7	10.7
Sep.	20.4	39.4
Oct.	21.1	135.2

Appendix II:Germination percent of *Gossypium* genotypes, %

Weeks	*G. harbaceum*	*G. hirsutum*	HART 89 M	*G. barbadense*	*G. kirkii*
1	20.0	12.0	22.0	20.7	1.3
2	25.3	14.7	31.3	28.0	11.3
3	29.3	19.3	37.3	35.3	13.3
4	33.3	23.3	40.7	38.3	16.7
5	46.7	36.7	48.7	49.3	22.6
6	52.3	46.0	48.7	58.7	28.6
7	62.0	49.3	50.7	70.0	34.0
8	64.0	53.3	56.7	73.3	35.3
9	64.0	54.0	56.7	74.3	35.3
10	64.0	54.0	56.7	74.3	36.0

Appendix III:Percent flowering of *Gosspium* genotypes

Weeks	*G. harbaceum*	*G. hirsutum*	HART 89M	*G. barbadense*	*G. kirkii*
1	6.1	3.9	3.1	3.9	0.0
2	16.00	8.3	13.4	6.1	0.0
3	22.10	16.3	24.1	18.9	8.9
4	37.3	37.9	42.0	27.3	21.6
5	43.3	64.6	67.1	42.9	44.1
6	83.0	74.0	89.8	60.5	55.3
7	96.3	95.3	96.5	75.1	76.9
8	100	100	100	100	86.7

		Sum of Squares	Df	Mean Square	F	Sig.
Boll weight	Between Groups	51.268	4	12.817	25.938	.000
	Within Groups	244.600	495	.494		
	Total	295.868	499			
Seeds weight per boll	Between Groups	19.787	4	4.947	21.181	.000
	Within Groups	115.603	495	.234		
	Total	135.390	499			
Lint weight per boll	Between Groups	13.422	4	3.356	26.592	.000
	Within Groups	62.463	495	.126		
	Total	75.885	499			
Number of seeds	Between Groups	278.278	4	69.569	4.309	.002
	Within Groups	7992.182	495	16.146		
	Total	8270.460	499			
Percentage of seeds weight per boll	Between Groups	10090.812	4	2522.703	9.361	.000
	Within	133394.85	495	269.485		

	Groups	0				
	Total	143485.663	499			
Percentage of seeds weight per boll	Between Groups	3513.919	4	878.480	7.990	.000
	Within Groups	54423.986	495	109.947		
	Total	57937.905	499			

Appendix IV:**ANOVA on** boll analysis

Appendix V:Paired samples test on average boll weight

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G .harbaceum* - HART 89M	.2152	.97155	.09716	.0224	.4079	2.215	99	.029
Pair 2	*G. hirsutum* - HART 89M	-.2030	.90022	.09002	-.3817	-.0244	-2.255	99	.026
Pair 3	HART 89M - *G. barbadense*	-.0091	.76490	.07649	-.1609	.1427	-.119	99	.906
Pair 4	HART 89M - *G. kirkii*	-.7343	.95189	.09519	-.9232	-.5455	-7.715	99	.000

Appendix VI:Paired samples test on average seed weight

		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbaceum* - HART 89 M	.4131	.65097	.06510	.2840	.5423	6.346	99	.000
Pair 2	*G. hirsutum* - HART 89 M	.0838	.64349	.06435	-.0438	.2115	1.303	99	.196
Pair 3	HART 89 M - *G.*	-.2838	.63702	.06370	-.4102	-.1574	-4.456	99	.000

	barbadense								
Pair 4	HART 89 M - *G. kirkii*	-.5343	.61647	.06165	-.6567	-.4120	-8.668	99	.000

Appendix VII:Paired samples test on average number of seed

		Paired Differences					t	Df	Sig. (2 tailed)
		Mean	Std. Deviation	Std. Erro Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbaceum* - HART 89M	1.72	5.965	.596	.53	2.90	2.879	99	.005
Pair 2	*G. hirsutum* – HART 89 M	1.82	6.744	.674	.48	3.16	2.696	99	.008
Pair 3	HART 89 M - *G. barbadense*	-1.81	5.440	.544	-2.89	-.73	-3.324	99	.001
Pair 4	HART 89 M - *G. kirkii*	-2.04	5.451	.545	-3.12	-.96	-3.743	99	.000

Appendix VIII:Paired samples test on mean percent lint weight

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbaceum* - . HART 89 M	-7.3970	15.68862	1.56886	-10.5099	-4.2840	-4.715	99	.000
Pair 2	*G. hirsutum*- HART 89 M	-1.8852	17.10881	1.71088	-5.2799	1.5096	-1.102	99	.273
Pair 3	HART 89 M - *G. barbadense*	5.1148	16.44085	1.64409	1.8526	8.3770	3.111	99	.002
Pair 4	HART 89 M - *G. kirkii*	1.8830	13.26094	1.32609	-.7483	4.5142	1.420	99	.159

APPENIX IX:Paired samples test on average lint weight

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. hirsutum* – HART 89 M	-.1848	.59465	.05947	-.3028	-.0669	-3.109	99	.002
Pair 2	*G. harbaceum* - HART 89 M	-.2242	.60421	.06042	-.3441	-.1044	-3.711	99	.000
Pair 3	HART 89 M - *G. barbadense*	.2131	.59009	.05901	.0960	.3302	3.612	99	.000
Pair 4	HART 89 M - *G. kirkii*	-.2000	.62329	.06233	-.3237	-.0763	-3.209	99	.002

Appendix X:Paired samples test on average percent seed weight

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbaceum* - HART 89 M	1.7799	31.28311	3.12831	-4.4273	7.9871	.569	99	.571
Pair 2	*G. hirsutum* - HART 89 M	-1.2586	25.84769	2.58477	-6.3874	3.8701	-.487	99	.627
Pair 3	HART 89 M - *G. kirkii*	7.7592	17.57692	1.75769	4.2716	11.2469	4.414	99	.000

Appendix XI:Paired samples test on percent germination

	Paired Differences						T	df	Sig. (tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the					

					Difference				
					Lower	Upper			
Pair 1	*G. harbacium* - HART 89M	1.176	7.0770	2.2379	-3.887	6.239	.525	99	.612
Pair 2	*G. harsutum* -HART 89M	-8.658	6.9010	2.1823	-13.595	-3.721	-3.967	99	.003
Pair 3	HART 89M - *G. barbadense*	-7.293	9.7985	3.0985	-14.302	-.284	-2.354	99	.043
Pair 4	Hart 89M - *G. kirkii*	21.480	2.6366	.8338	19.594	23.366	25.763	99	.000

Appendix XII:Paired samples test on flowering rate

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interv of the Difference				
					Lower	Upper			
Pair 1	*G. harbacium* - HART 89M	-3.987	8.67977	3.06876	-11.2440	3.2690	-1.299	99	.235
Pair 2	*G. harsutum* - HART 89M	-4.462	5.37612	1.90075	-8.9570	.0320	-2.348	99	.051
Pair 3	Hart 89M - *G. barbadense*	12.662	11.44202	4.04536	3.0967	22.2283	3.130	99	.017
Pair 4	Hart 89M - *G. kirkii*	16.200	11.03617	3.90188	6.9735	25.4265	4.152	99	.004

Appendix XIII:Paired samples test on height of plant

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interva of the Difference				
				Lower	Upper			

Pair 1	*G. herbecium* – HART 89M	39.2222	38.37666	5.72085	27.6926	50.7518	6.856	44	.000
Pair 2	*G.hursutum* - HART 89M	38.7111	38.07446	5.67581	27.2723	50.1499	6.820	44	.000
Pair 3	HART 89M- *G. barbadense*	-109.2222	36.19448	5.39555	-120.0962	-98.3482	-20.24	44	.000
Pair 4	Hart 89M - *G. kiirkiI*	-81.9778	31.87225	4.75123	-91.5533	-72.4023	-17.25	44	.000

Appendix XIV:Paired samples test on number of branches

		Paired Differences					T	df	Sig. (2-tailed
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbacium* - HART 89M	3.40	7.840	1.169	1.04	5.76	2.909	44	.006
Pair 2	*G. harsutum* - HART 89M	5.27	8.029	1.197	2.85	7.68	4.400	44	.000
Pair 3	*HART- G*. barbadense	-11.47	8.382	1.249	-13.98	-8.95	-9.17	44	.000
Pair 4	Hart 89M - *G. kirkii*	-4.73	8.072	1.217	-8.98	-6.95	-7.34	44	.000

Appendix XV:Paired samples test on average length of the longest fruiting branch

		Paired Differences					T	df	Sig. (tailed)
		Mean	Std. Deviatic	Std. Error Mear	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. herbacium* – HART 89M	11.333	18.2296	2.7175	5.857	16.810	4.170	4	.000
Pair 2	*G. hursutum* –HART 89M	21.200	23.2502	3.4659	14.215	28.185	6.117	4	.000
Pair 3	HART 89M- *G. barbadense*	-29.022	17.4623	2.6031	-34.268	-23.776	-11.14	4	.000

Pair 4	HART 89M- *G. kirkii*	-20.711	19.9221	2.9698	-26.696	-14.726	-6.97	4	.000

Appendix XVI:Paired samples test on internodal length

		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbacium* – HAR 89M	1.15	1.123	.167	.82	1.49	6.897	44	.000
Pair 2	*G. hursutum* – HAR 89M	1.71	1.885	.281	1.15	2.28	6.096	44	.000
Pair 3	HART 89M- (*barbadense*	-2.29	1.338	.199	-2.69	-1.88	-11.461	44	.000
Pair 4	HART 89M- G. kirkii	-1.37	1.154	.172	-1.71	-1.02	-7.946	44	.000

Appendix XVII:Paired samples test on number of nodes on the longest branch

		Paired Differences					t	Df	Sig. (2-taile
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	*G. harbacium* – HART 89M	0.98	1.123	.170	.82	1.49	4.173	44	.000
Pair	*G. hursutum* – HART 89M	1.81	1.885	.281	1.15	2.28	6.121	44	.000
Pair	HART 89M-*G. barbadense*	-2.49	1.338	.372	-2.69	-1.88	-8.142	44	.000
Pair	HART 89M- *G. kirkii*	-.29	1.154	.130	-1.71	-1.02	-6.973	44	.000

Appendix XVIII:Paired samples test on yield per plant

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*G. harbacium* – HART 89M	28.7889	31.83864	4.74622	19.2235	38.3543	6.066	44	.000
Pair 2	*G. harsutum* - - HART 89M	16.9133	34.86121	5.19680	6.4399	27.3868	3.255	44	.002
Pair 3	HART 89M- *G. barbadense*	-133.7333	50.58594	7.54091	-148.9310	-118.5356	-7.734	44	.000
Pair 4	HART 89M-- *G. kirkii*	32.6000	13.12276	1.95623	28.6575	36.5425	16.665	44	.000