HYBIDIZATION STUDIES IN OKRA **(*Abelmoschus spp* (L.) Moench)**

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AMITAABA THEOPHILUS (ID: 10444179)

B Sc. Agricultural Technology (2010)
(University for Development Studies, Tamale)

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DECLARATION

This thesis is the result of research work undertaken by AMITAABA THOPHILUS in the Department of Nuclear Agriculture and Radiation Processing, School of Nuclear and Allied Sciences, University of Ghana, under the supervision of PROF. H.M AMOATEY and DR. SAMUEL AMITEYE

..... Date.....
AMITAABA THEOPHILUS
(CANDIDATE)

…….. Date ……....
PROF. H.M AMOATEY
(PRINCIPAL SUPERVISOR)

..... Date.....
DR. SAMUEL AMITEYE
(SUPERVISOR)

DEDICATION

To JEHOVAH God Almighty be all the Glory

This work is dedicated to my guardians and family especially my belovered wife Rebecca for their immeasurable support, inspiration, guidance, love and prayers.

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LIST OF ABBREVIATION

spp	species
et al	and others
NARP	National Agricultural Research Programme
IBPGR	International Board for Plant Genetic Resources
IPGRI	International Plant Genetic Resources Institute
BSc.	Bachelor of Science
MSc.	Master of Science
MPhil.	Master of Philosophy
AVRDC	Asian Vegetable Research and Development Centre
PGRRI	Plant Genetic Resources Research Institute
CSIR	Council for Scientific and Industrial Research
VEPEAG	Vegetable Exporters Association of Ghana
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
ISSR	Inter-Simple Sequence Repeats
AFLP	Amplified Fragment Length Polymorphism
SRAP	Sequence Related Amplified Polymorphism
QTL	Quantitative Traits Loci
SSR	Simple Sequence Repeat
MAS	Marker Assisted Selection
RAPD	Random Amplified Polymorphic DNA

PER	Protein Efficiency Ratio
PCR	Polymerase Chain Reaction
CTAB	Cetyltrimethyl Ammonium Bromide
ECHO	Educational Concerns for Hunger Organisation
WAT	West African Taxon
FAO	Food and Agriculture Organisation
UNESCO	United Nations Educational, Scientific and Cultural Organisation
USDA	The United States Development Agency
NIHORT	National Horticultural Research Institute
n	Basic chromosome number
sub sp	sub species
IPM	Integrated Pest Management
FAOSTAT	Food and Agriculture Organisation's Statistical Database
PCA	Principal Components Analysis
YVMV	Yellow Vein Mosaic Virus
WCA	West and Central Africa
BNARI	Biotechnology and Nuclear Agriculture Research Institute
GAEC	Ghana Atomic Energy Commission
HPGe	High-Purified Germanium
ANOVA	Analysis of Variance
RCBD	Randomised Complete Block Design

CI	Crossability Index
DMRT	Duncan’s multiple range tests
LoS	Level of significance
Lsd	Least significance difference
CIRAD	Centre for International Research in Agricultural Development
MT	Metric tonnes
m	Metre (s)
cm	Centimetre (s)
mg/g	milligram per gram
ug/g	microgram per gram
NARS	National Agriculture Research Systems

ABSTRACT

Okra (*Abelmoschus spp*. L. Moench) is an important multi-purpose vegetable crop cultivated and consumed across all tropical and temperate regions of the world. In Ghana, it is popular in all ten regions and increasing quantities are exported to Europe in the fresh form. The crop has received little attention by way of breeding to produce varieties combining the most desirable qualities to boost local cultivation and export. Ten accessions of *Abelmoschus spp*., comprising two species, *A. esculentus* (T1, T2, T3, VT, ID and AG) and A. callei (KB, AM, YL and T4) collected from six geographical regions of Ghana were crossed in all possible combinations to assess inter-specific as well as intra-specific hybridisation between and within species. Reciprocal crosses were also carried out and the performances of their F_1 offspring were evaluated against the respective parents for expression of heterosis for key quantitative traits including days to 50% germination, days to 50% flowering, plant height, fresh fruit weight, length of pod and number of seeds per pod. Genetic relatedness among the accessions and their progeny was established by way of a dendrogrom based on furthest neighbour method (Euclidean). All six accessions of *Abelmoschus esculentus* were able to hybridize with one another in both direct and reciprocal cross combinations with high degree of crossability index (CI) (45.71% to 90.32%). On the other hand, cross-compatibility among *A. esculentus* and *A. callei* was successful only in one direction when *A. esculentus* was used as females also with a CI between 34.48% and 60%. Parental lines T3 and T1 emerged as the most compatible female and male respectively. Crossability success was relatively high during early hours of the day but decreased continuously in

subsequent hours. Ten parental accessions and 61 F_1 progenies of *A. esculentus* and *A.callei* evaluated for 15 qualitative and 8 quantitative traits exhibited significant variations in all quantitative traits studied. Clustering pattern based on quantitative traits largely revealed no duplicates and clustering pattern especially among parental accessions, appears to reflect relationship based upon speciation as parental accessions belonging to *A. caillei* are clustered towards one end of the dendrogram, while members belonging to *A. esculentus* clustered towards the opposite end. Contributions of the three principal components were 45.98 %, 23.31 %, and 14.46% for the first (PC1), second (PC2) and third (PC3) respectively, with corresponding Eigen values of 3.21837, 1.63171 and 1.01212 respectively, cumulating into maximum of 83.75 % of total variance. These results demonstrate possibility of producing superior hybrids of okra through artificial cross-pollination. Key recommendations based on these findings include i) use of molecular markers to confirm results of morphological characterisation and also to better understand inheritance of qualitative traits. ii) Genes linked to agronomically important traits in okra should be genetically mapped through Quantitative Traits Loci (QTLs) to serve as a baseline data platform for researchers and breeders.iii) Further studies on inheritance of qualitative traits stretching to the F_2 or even F_3 generations should be carried out, preferably using molecular markers to fully understand the pattern of segregation with appropriate ratios.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Cultivated okra (*Abelmoschus spp,* (L) Moench) also called okra, is an important vegetable crop in the tropical and subtropical regions of the world (Bisht and Bhat, 2006; Siemonsma and Kouame, 2004). The crop is dicotyledonous, belonging to the order Malvales and the family Malvaceae (Schippers, 2000) which consist of many important species including a number of other food, fibre, and medicinal crops such as okra, cotton, and kenaf (Anderson and Pharis, 2003). The plant is robust, erect, and an annual herb, ranging between 1 to 2 m in height, with simple leaves, which are altenate and palmately veined. The flowers are regular and solitary, with superior ovaries and numerous stamens. The fruit is a pod, variable in colour when fresh.

It is one of the oldest cultivated crops and presently grown in many countries and is widely distributed in Asia, Africa, Southern Europe and America (Oyelade *et al*., 2003; Ariyo, 1993).It is known to have originated in tropical Africa (Akanbi *et al.*, 2010; Saifullah and Rabbani, 2009) and also in tropical Asia (Grubben, 1977).The center of origin remains unclear, but centers of genetic diversity include West Africa, India and Southern Asia (Hamon and Van Stolen, 1989).

The crop is easy to cultivate and suited to regions with moderate rainfall. Okra is a warm season crop, requiring ample moisture for germination (Peet, 1992). Okra has high nutritional, medicinal and industrial value (Reddy *et al*., 2013) and high financial value (Sawadogo *et al*., 2009). The seeds are also a good source of vitamins, minerals and medically important compounds (Kumar *et al*., 2010). Mucilage occurs in most parts of the plant, and is associated with other substances such as tannins (Sengkhamparn *et al.,* 2009; Woolfe *et al.,* 1977). It usually occurs in the roots, bark, and seeds, but is also found in the flowers, leaves and cell walls (Kumar *et al.,* 2010).The edible part of okra is the immature pod, which is harvested when tender. The leaves, buds and flowers are also edible. Dried okra can also be stored and used later for soup or stew. In West Africa, okra is utilized mainly because of its high mucilage content which is used in the thickening of soup (Schippers, 2000; Uzo and Ojiakor1980)

The name okra is of West African origin, but most often used in the United States and the Philippines. It is known in many English-speaking countries as lady's fingers, okra, or gumbo, quiabo in Portuguese, gumbo in French, bhinde or bhendi in India (AVRDC, 2010; Obeng-Ofori *et al.,* 2007). In Ghana, it has different names depending on the region and dialect. For instance, it is called *nkruma* by the Akan speaking communities and *fetiri* by the Ewe (National Academies Press, 2006). In West and Central Africa (WCA), okra is called Gombo (French), Miyan-gro (Hausa), La (Djerma), Layre (Fulani), Gan (Bambara), Kandia (Manding), and is among the most frequently and popularly consumed traditional vegetables.

The world's leading producers of the crop are India, leading in production with 70%, Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%) and Iraq (1.7%) (FAOSTAT, 2012; Gulsen *et al*., 2007). In Ghana, Brong-Ahafo, Northern, Volta, Greater Accra and Central regions are the leading producers (NARP, 1993).

1.1.1 PROBLEM STATEMENT

Okra is one of the crops that have the potential of improving food security, malnutrition and poverty alleviation in tropical and the sub-tropical regions of the world. It is considered a prized vegetable due to its high nutritive value (Dabire-Binso *et al.*, 2009). The crop is high-yielding under improved production practices, with yields varying from 4,480 to 5,500 kg ha^{-1} of green pods (Ayodele, 1993). Its usefulness has enhanced world production by an estimated 6.35 million tonnes per year (FAOSTAT, 2013). There are about 2,283 reported accessions of okra in the world (Hammon and Van Slotten, 1989), 2,029 of these are from the African continent, with 1,769 from West Africa (Hammon and Van Slotten, 1989). Okra, therefore, is far more heavily represented in West Africa than any other parts of the world (Omonhinmin and Osawaru, 2005).

The crop is easy to cultivate, especially by women in agriculture, providing source of income and employment. More so, it is cultivated in traditional agriculture with little or no attention on yield (Ariyo, 1993). The yield potential of okra has been grossly affected by lack of improved agronomic practices, poor soil, pests and diseases (Siemonsma *et al.*, 2004).

In Ghana, the introduction of exotic varieties of okra has led to a much reduced production of the well adapted indigenous okra (*Abelmoschus caillei*), as genetic enhancement has not been made in the latter. Okra is considered a minor crop and until recently no attention was paid to its improvement in international research programmes (Duzyaman, 1997).Varieties cultivated over the years, in the various regions across the country are mainly landraces (Ahiakpa, 2013). These landraces are however, vulnerable to biotic and abiotic stresses such as pests, diseases and nematodes (Oppong-Sekyere *et al.*, 2012). They also take a long time to fruit. Hence, the need for hybridization studies to provide information on the proportion of phenotypic variance that is due to genetic factors for different traits. Hybridization studies have been done in a wide range of crops in other breeding programmes, example kenaf (Mostofa *et al.*, 2002), roselle (Ibrahim *et al.*, 2006), tomato (Foolad *et al.*, 2006), cowpea (Aremu *et al.*, 2007) and eggplant (Islam *et al.*, 2009).

The success of any crop improvement programme largely depends on the magnitude of genetic variability, genetic advance, character association, direct and indirect effects on yield and yield attributes. Genetic diversity is important for selection of parents to recover transgressive segregants (Kiran *et al.*,2004).The value of heritability estimates is enhanced when used together with the selection differential or genetic advance (Ibrahim and Hussein, 2006). Information on the amount and direction of association between yield and yield related characteristics is important for rapid progress in selection and genetic improvement of a crop (Asish *et al.*, 2008). Unfortunately, very little research

attention by way of breeding has been directed to the study of genetic diversity and breeding behaviour of existing local landraces in order to facilitate improvement of the crop. Consequently, the full economic potential of okra production has not been realised in Ghana (Ahiakpa, 2012).

1.1.2 JUSTIFICATION AND RELEVANANCE OF THE STUDY

The crop is extensively grown for its tender pods, which are used as a very popular, tasty and gelatinous vegetable. It is a powerhouse of valuable nutrients. Its fruits are rich in vitamins, calcium, potassium and other mineral (Camciuc *et al.,* 1981). The mature okra seed is a good source of oil and protein (Oyelade *et al.,* 2003; Martin and Ruberte, 1979) and has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid (Savello *et al.,* 1980), which is essential for human nutrition. Its mature fruits and stems contain crude fibre, which is used in the paper industry.

Ghana is the ninth largest producer of okra in the world (FAOSTAT, 2013). Okra is the fourth most important vegetable crop in the country after tomato, pepper, and garden eggs. It has huge socio-economic potential for enhancing livelihoods in both rural and urban areas. It offers a possible route to prosperity for small, medium, and large-scale producers alike.

Okra is a vegetable which one finds in a fresh state in almost all markets in Ghana, during the rainy season and in a dehydrated form during the dry season, particularly in Northern Ghana due to its strong commercial value for poor women farmers (Oppong-Sekyere *et al.*, 2011), and its vital importance as food component among the inhabitants of the cities and villages. Notwithstanding the potential of the crop, there are no improved local varieties for cultivation by okra farmers in Ghana. The accessions under cultivation, over the years are mainly landraces or exotic varieties imported by agro-input dealers. However, these landraces are associated with challenges such as high susceptibility to diseases, pests and nematode (Sinnadurai, 1992).

In addition, these landraces have long maturity periods yet short harvesting duration. They are of poor nutritional quality, non-standard in shape, colour and size, making them unfit for the export market (Oppong-Sekyere, 2011). By contrast, the exotic varieties have desirable quality characteristics but are not adapted to local growing conditions. The net effect is low national output, even though the crop can be produced in all ten geographic regions of Ghana. It is important to develop improved varieties of okra, for adoption by Ghanaian vegetable farmers and for the export market. Varieties that are perennial in growth habit and at the same time combine higher yields and early maturity with longer harvest duration as well as resistance to diseases and pests, would be ideal to the vegetable industry in Ghana (Oppong-Sekyere *et al.*, 2012). Such improved varieties must also meet standards regarding fruit size, shape and colour to meet the requirement desired in the Ghanaian okra export market (Boateng, 2011.percomm.).

Artificial sexual hybridisation is a conventional breeding approach which involves crossing of different genotypes in order to introduce genetic variability for generating new or novel varieties with improved qualities (Sharma, 1994). This is mostly aimed at incorporating genes for desirable traits such as disease resistance and high yield present in one genotype into the genetic background of the other genotype to produce superior hybrids. It is a very useful approach in quantitative genetic analysis such as studies of combining ability and expression of heterosis towards improvement of targeted traits.

Heterosis breeding has been the most successful approach in increasing the productivity in cross-pollinated vegetable crops. The presence of heterosis was demonstrated for the first time by Vijayaraghavan and Warier (1946). Since then, heterosis for yield and its components has been extensively studied.

Okra is often self-pollinated but cross-pollination is possible. Several research workers have reported on occurrence of heterosis in considerable quantities for fruit yield and its various components (Jindal *et al*., 2009; Weerasekara *et al*., 2007; Mehta *et al*., 2007; Elangovan *et al*., 1981). The ease of emasculation and very high percentage of fruit set indicates the possibilities of exploitation of hybrid vigour in okra. The presence of sufficient hybrid vigour is an important prerequisite for successful production of hybrid varieties. Therefore, hybridization studies can provide the basis for the exploitation of valuable hybrid combinations in a future breeding programme towards production of commercial varieties.

1.1.3 OBJECTIVES OF THE RESEARCH

The main objective of this study is to determine hybridization success among 12 selected accessions of okra (*Abelmoschus spp* (L.) Moench) and to estimate heterosis among F_1 progenies with respect to yield and yield-determining traits.

The specific objectives of the research are;

1. To make pairwise crosses among selected accessions of okra and determine yield and yield-determining traits among F_1 progenies
2. To establish level of heterosis among F_1 progenies for yield and yield-determining traits in comparison to parents.
3. To determine genetic diversity among parents and different sets of F_1 progenies towards selection of individuals for use in future hybrization programmes.

REFERENCES

References for **CHAPTER ONE** are merged with those for **CHAPTER TWO** as directed by University of Ghana thesis guideline.

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN, DISTRIBUTION AND UTILIZATION

Okra (*Abelmoschus spp.* (L.) Moench) is a member of the family Malvaceae. The crop is native to Africa (ECHO, 2003; Purseglove, 1987; Kochhar, 1986). It originated somewhere around Ethiopia, and was cultivated by the ancient Egyptians by the 12th century BC. Its cultivation spread throughout Middle East and North Africa (Lamont 1999; Tindall 1983). The Nile Basin seems to have been the route by which this crop spread through North Africa, the Eastern Mediterranean, Asia, and to India. Okra reached the new world by the way of Brazil and Dutch Guinea. African slaves brought okra to North America by way of New Orleans (Bish *et al*., 1995 and Hamon *et al*., 1990). The crop is grown in many parts of the world, especially in tropical and sub-tropical countries (Kumar *et al.,* 2010; Saifullah and Rabbani 2009; Arapitsas 2008). It is grown on a large scale in Africa, especially in Nigeria, Egypt, Ghana and Sudan, (Joshi *et al*., 1974). It is also very important in other tropical areas including Asia, Central and South America (FAOSTAT, 2008; Joshi *et al*., 1974).

There are a number of species, both wild and cultivated. Some of these are *A. esculentus, A. caillei*, *A. moschatus, A. manihot, A. ficulneus* and *A. tetraphyllus*. Two main species in the genus *Abelmoschus* are cultivated; A. *manihot* L. and A. *moschatus* L. (Siemonsma, 1991 and Stevels, 1988).

Okra is an amphidiploid-having a complete diploid set of chromosomes derived from each parent form (Siemonsma, 1982a) with varieties displaying a tremendous variation in plant size, shape, fruit type and colour. Okra plant is a semi-woody, fibrous herbaceous annual with an indeterminate growth habit (Nonnecke, 1989). The plant forms a deeply penetrating taproot with dense shallow feeder roots reaching out in all direction in the upper 45 cm of the soil. The stems reach heights from 3 m in dwarf varieties to 7 m to 8 m in others (Anonymous, 2010).

The seeds are dicotyledonous and they vary in shape, which is either round, kidney or spherical with epigeal germination (Ariyo, 1993; Hamon *et al.,* 1991). The monoic flowers of okra are self-compatible (Hamon *et al*., 1990; Martin, 1983).The leaves are dark green in colour (Kumar *et al.,* 2010). About 35-60 days after emergence, the plant begins to flower. The flowers are axillary and solitary, borne on a peduncle 2.0 – 2.5 cm long within the leaf axil (Valeriana, 2011). The flower usually remains open for a day. It is mostly self-fertilized; however, insects such as honeybees and bumble bees can cross-pollinate. Okra is self-compatible, and passive self-pollination can take place in its hermaphrodite flowers (Al-Ghzawi *et al*., 2003).

Its pollen grains are very large and echinate, 156 μm in diameter with spines over 20 μm in length (Vaissière and Vinson, 1994) so that pollination with both self-pollen and cross-pollen is possibly achieved by insects (Al-Ghzawi *et al.,* 2003; Hamon and Koechliu, 1991). Anthesis takes place at dawn, and the flower remains open all morning and closes

by noon or early afternoon. Cross pollination can occur in okra up to a maximum of 42.2% (Mitidieri and Vencovsky, 1974). The extent of cross-pollination in a particular place will depend upon the cultivar, competitive flora, insect population and season. (Al Ghzawi *et al.,* 2003) reported that, no significant differences were found between insect- and self-pollinated plants for the number of flowers. Immature fruits of 8-9 cm long are ready for harvest 4-6 days after anthesis.

Harvesting is recommended at least every other day for size and quality (Ramu, 1976). About 35-40 days are required from anthesis to seed maturity. If fruits are allowed to mature, plant growth declines and few flowers develop, but with continuous harvesting, the plant continues to set fruit (Norman, 1992). Mature fruits should be removed and discarded as they reduce the plant growth and decrease yield (Ramu, 1976). The rate of allogamy differs according to type of variety and ecological conditions (Hamon *et al*., 1991). When ripe the fruit becomes fibrous and splits longitudinally in five parts, showing 5 rows of seeds, with 50 – 100 seeds per fruit (Norman, 1992).

Okra has alternate, palmate broad leaves and the flowers have five large yellow petals with a large purple area covering the base. The fruits, which are harvested immature, are pale green, green, or purplish and in many cultivars are ridged (Hamon *et al*., 1990). When mature, they are dark brown dehiscent or indehiscent capsules. Fruit shapes range from round to ridged and short to long (Siemonsma, 1982). The plant and fruits may have

small spines on them that create allergies in some people (Ariyo, 1993; Düzyaman, 1997).

Okra has several uses which include nutritional, economic and industrial. The nutritive value of okra comprises carbohydrate, protein, fat, iron, calcium, fibre, thiamine, nicotinamide, riboflavin, and ascorbic acid (Fajinmi and Fajinmi, 2010; Arapitsas, 2008; Tindall, 1986). Mature seeds of 100 g okra contain 20% edible oil and 20.23% crude protein due to high lysine content and it is a good source of vitamin C (Berry *et al*., 1988; Siemonsma and Kouame, 2004). Okra seed oil is rich in unsaturated fatty acids such as linoleic acid (Savello *et al*., 1980), which is essential for human nutrition.Okra seeds are used as a substitute or additive in feed compounds (Purseglove, 1974). Dried okra seeds, can be used to prepare vegetable curds, or roasted and ground and used as coffee additive or substitute (Moekchantuk and Kumar, 2004).The pods contain a mucilaginous substance which is used as plasma replacement or blood volume expander (Onunkun, 2012; Siemonsma and Kouame, 2004). It has also been reported to cure ulcers and relief pain from haemorrhoids (Adams, 1975). The roots can be used to cure syphilis (FAO, 1988). Okra is a very important soup condiment that is consumed daily in almost all homes and restaurants.

The tender pods of okra are used in stews or cut into slices, sun dried and then ground as a powder and used as a favourite Sudanese dish called “Weika” (Abdelmageed, 2010). Similarly, the older immature pods and leaves that are yet to develop fiber are also cut into slices, sun dried and ground and used in soups in the dry season when fresh fruits are

scarce (Oppong-Sekyere, 2011; Siemonsma and Kouame, 2004). The mature fruit and stem of okra contain crude fibre which is used in the paper industry. Industrially, okra mucilage is usually used to glace certain papers and also useful in confectionery among other uses such as bioabsorbent (Kumar *et al.*, 2010; Adetuyi *et al.*, 2008; Savello *et al.*, 1980).

2.2 TAXONOMY, CYTOLOGY AND BOTANY

Okra (*Abelmoschus spp.* (L.) Moench) is a dicot, belonging to the order Malvales, family Malvaceae and genus *Abelmoschus* (Schippers, 2000). Okra was previously included in the genus *Hibiscus*. Later, it was designated to *Abelmoschus*, which is distinguished from the genus *Hibiscus* by the characteristics of the calyx; spatulate, with five short teeth, connate to the corolla and caduceus after flowering (Terrell and Winters 1974; Kundu and Biswas, 1973). The section *Abelmoschus* was subsequently proposed to be raised to the rank of distinct genus by Medikus (1987). The wider use of *Abelmoschus* was subsequently accepted in the taxonomic and contemporary literature. Although about 50 species have been described, eight are most widely accepted (IBPGR, 1990; Borssum, 1966).

There is significant variation in chromosome numbers and ploidy levels in *Abelmoschus.* Different authors have variably reported the chromosome number 2n for *Abelmoschus esculentus* L. (Moench). The most frequently observed somatic chromosome number is 2n=130, although Datta and Naug (1968) suggested that the numbers 2n=72, 108, 120,

132 and 144 are in regular series of polyploids with n=12. This makes the existing taxonomical classifications at the species level in the genus *Abelmoschus* quite unsatisfactory. There was a detailed cytogenetical study on Asian okra and related species, which have provided more evidence of the existence of amphidiploids in the genus (Siemonsma, 1982a).

The nomenclature of *Abelmoschus spp* with varied chromosome numbers of both cultivated and wild species of *Abelmoschus* genus as distinctly reported involved most species of the genus. Chromosome number of 2n = 72 has been reported in *Abelmoschus moschatus* for both cultivated and wild type (Chevalier, 1940; Ford, 1938). Van Borssum-Van Borssum Waalkes (1966) observed 2n = 130-1388 in *A. manihot sub sp tetraphyllus var tetraphyllus* for wild species. Siemonsma (1982a) reported 2n = 138 in *A.manihot sub sp tetraphyllus var pungens* for wild type; Hamon and Yapo (1986) observed 2n = 66-144 for *Abelmoschus esculentus* (cultivated type). Again, Pal *et al.* (1952) recorded 2n = 72-78 in *A. ficulneus* (wild), and 2n = 38 in *A. angulosus* (wild), 2n = 185-198 in *Abelmoschus caillei* (cultivated), and 2n = 58 in *Abelmoschus tuberculatus* (wild type*)* were recorded by (Singh *et al*., 1975), Joshi *et al*. (1974), and Kuwada (1957) respectively. From these, okra can be regarded as a polytypic complex (Singh *et al*., 1975) that exhibits both high polyploidy and hybridity of which the parental wild species is yet to be determined.

Aladele *et al. (*2008) collected 93 accessions of okra comprising 50 West African genotypes (*Abelmoschus caillei*) and 43 Asian genotypes (*A. esculentus*) and assessed for genetic distinctiveness and relationship using randomly amplified polymorphic DNA (RAPD), and concluded that all the thirteen primers used revealed clear distinction between the two genotypes. There was more diversity among the Asian genotypes; possibly due to the fact that they were originally collected from six different countries in the region. Six duplicate accessions were discovered while accession TOT7444 distinguished itself from the other two okra species, an indication that it might belong to a different species. This recent study at molecular level emphasises the need for a deeper study into the variable polymorphism at chromosomal level in the genus *Abelmoschus*.

Kumar *et al*. (2010) examined the possible outcome of a recombination of these species and their possible contrasting characters after a cross between Asian genotype and the West African genotype. The table below details their findings and observations.

Table 2.1: Potential of recombination breeding involving two *Abelmoschus spp*

Species		Contrasting traits
A. esculentus (common okra) 95% cultivated area	Amphidiploid (2n=130-140) *A. tuberculatus* or *A. ficulneus* (2n=58-60) x unknown?	Poor adaptation in humid zone, more susceptible to biotic stresses, less vigorous, short life cycle (suitable for short rainy season areas), usually neutral, cultivated in both rainy (rain fed) and dry (irrigated) seasons
A. caillei (West African okra) 5% cultivated area	Amphipolyploid (2n=196-200) *Å. esculentus* (2n=130-140 x *A. manihot* (2n = 60-68)	Better adaptation in humid zone, tolerant/ resistant to biotic stresses, more vigorous longer life cycle, mostly photoperiod sensitive and cultivated mainly in dry seasons

(Source: Kumer *et al*., 2010)

2.3 HEALTH BENEFITS AND NUTRITIONAL VALUE

The okra fruit is a reservoir of important and valuable nutrients (Candlish *et al*., 1987; Grubben *et al*., 1977), nearly half of which is soluble fibre in the form of gums and pectins. Soluble fibre helps to lower serum cholesterol and reducing the risk of heart disease (Brown *et al*., 1999).

The other half is insoluble fibre which helps to keep the intestinal tract healthy, decreasing the risk of some forms of cancer, especially colorectal cancer (Schneeman, 1998). About 10% of the recommended levels of vitamin B6 and folic acid are also present in a half cup of cooked okra (Hamon and Charrier. 1997). Okra has several health

benefits, as it is rich in vitamin A, thiamin, vitamin B6, vitamin C, folic acid, riboflavin, calcium, zinc and dietary fibre (Norman, 1992).

Okra is recommended for pregnant women, as it is rich in folic acid, which is essential in the neural tube formation of the foetus between the 4th and 12th weeks of pregnancy (Allen, 2007). It is rich in amino acids, with the likes of tryptophan, cystine and other sulphur amino acids. It is the ideal vegetable for weight loss and is a storehouse of health benefits, provided it is cooked over low flame to retain its properties (Hamon and Charrier., 1997).

Justo (2011) reported that, a 100 g edible portion of okra fruit contains 90 g water, 2 g protein, 1 g fibre and 7 g carbohydrates. Its energy value is 145 kJ/100 g and it is a good source of vitamins and minerals. It is also very rich in calcium (70-90 mg/100 g). Therefore, the consumption of okra plays an important role in human nutrition.

2.4 ECONOMIC IMPORTANCE OF OKRA

Okra (*Abelmoschus esculentus* (L) Moench) is an annual crop, which requires warm conditions for growth and is available in almost every market all over Africa (Schippers, 2000). It is grown purposely for its leaves and young pods which are frequently eaten green as vegetable. Okra leaves are considered good cattle feed, but this is seldom compatible with the primary use of the plant.

Okra mucilage is suitable for medicinal and industrial applications. In the medical field, the mucilage is used as a plasma replacement or blood volume expander (Purseglove,

1974). Industrially, okra mucilage is usually used to glace certain papers and is also useful in confectionery among other uses (Farinde *et al.*, 2007).

Worldwide production of okra as fruit vegetable was estimated at 6,000,000 tons per year. In West Africa, it was estimated at 500,000 to 600,000 tons per year (Burkill, 1997). Schippers (2000) observed a great diversification of okra with the most important production regions localized in Ghana, Burkina Faso and Nigeria. The West and Central Africa region accounts for more than 75% of okra produced in Africa, but the average productivity in the region is very low (2.5 t/ha) compared to East Africa (6.2 t/ha) and North Africa (8.8 t/ha) (FAOSTAT, 2006). Nigeria is the largest producer (1,039,000 t) followed by Cote d'Ivoire, Ghana and others (FAOSTAT, 2008).

The three most important vegetables grown by 28% of the rural poor in Ghana include pepper tomato and okra (Diao, 2010). According to Oppong-Sekyere *et al.* (2011), fresh okra is a vegetable that can be found in almost all markets in Ghana, during the rainy season and in a dehydrated form during the dry season, particularly in Northern Ghana due to its strong commercial value for poor women farmers and its importance as food in the diets of the inhabitants of the cities and villages.

2.5 SEED AS POTENTIAL EDIBLE OIL AND FLOUR SOURCE

Okra seed oil is rich (60 to 70%) in unsaturated fatty acids (Rao, 1985; Savello *et al.*, 1980). The seed protein is rich in tryptophan (94 mg/g N) and also contains adequate amounts of sulphur-containing amino acids (189 mg/g N). This rare combination makes okra seeds remarkably useful in reducing human malnutrition (NAP, 2006). Okra seed

protein with good protein efficiency ratio (PER) and net protein utilization (NPU) values is comparable to many cereals (except wheat) and its oil yield is comparable to most oil seed crops except oil palm and soybean (Rao, 1985). Moreover, okra seed oil has potential hypocholesterolemic effect (Rao *et al.,* 1991).

The potential for wide cultivation of okra for edible oil as well as for cake is very high (Rao, 1985). Okra seed flour could also be used to fortify cereal flour (Adelakun *et al.,* 2008). Okra seed flour has been used to supplement corn flour for a very long time in countries like Egypt to make better quality dough (Taha El-Katib, 1947).

2.6 STATUS OF OKRA IN THE LOCAL AND EXPORT MARKETS IN GHANA

The horticultural export industry in Ghana is one of Africa's success stories, growing at 20% annually (Sefa-Dedeh, 2006). Ghana ranks among the top six suppliers of horticultural produce to the European Union markets. Figures from Ghana Export Promotion Council suggest that vegetable exports have grown from 886 metric tonnes, valued at $439,000 in 1993 to 34,764 metric tonnes valued at $7,700,000 in 2003 (Sefa-Dedeh, 2006). The vegetables exported included chilli pepper, mini aubergine, tinda, okra, cluster beans, yard long beans, and green pepper and sponge gourds. Ghana contributes 2% to the total world production of 4.8 million tons of okra pods produced per annum (Gulsen *et al.,* 2007). Okra has a strong commercial value for poor women farmers particularly in Northern Ghana (Oppong-Sekyere *et al.,* 2011).

2.7 ENVIRONMENTAL REQUIREMENTS OF OKRA

Okra is a warm season crop, growing best between the minimum mean temperature of 18 °C and a maximum mean of 35°C (Grubben, 1997; Ezeakunne 1984). Okra is sensitive to low temperatures and develops poorly below 15^{o}C (Marsh, 1993).In recent years there has been interest in growing it in heated greenhouses in Northern Europe (Buchholz *et al.,* 2006). It can be grown in a wide range of soil types provided the drainage is good. It is intolerant of wet and poorly drained and acidic soils (Incalcaterra and Curatolo, 1997). Okra does not do well in tight, water logged soils, but will tolerate a soil pH range from 6.0 to 7.5 (Incalcaterra and Curatolo, 1997).The optimum soil temperature for seed germination is 24°C-32°C (Martin, 1983). Germination is poor at 20°C or below. Short day length stimulates flowering of most cultivars (Martin, 1983). Flowering starts at a very early stage of growth at day lengths of less than 11 hr. under long days, the flower buds tend to abort (Chauhan, 1972). Germination takes 5-14 days (Hamon *et al*., 1991).The vegetable is best eaten just after it has been picked but it can be stored for several days. Okra keeps for 7-10 days if stored at 45°C-50°C and a relative humidity of 90%-95% (Martin, 1983). Okra is very sensitive to ethylene gas, therefore it is not recommended to be stored with vegetables and fruits that give off ethylene gas such as apples and pears (Lutz and Hardenburg, 1966).

2.8 WEST AFRICAN OKRA

The West African okra is photoperiod sensitive (short day) and cultivated primarily for its fresh pods and leaves. Nutritionally, okra pods contain 88 ml water, 2.1 g protein, 0.2 g fat, 8 g carbohydrate and 332.72 mg vitamins in 100 g of edible portion (Berry *et al.,*

1988). Genetic studies in West African okra are limited. There is the existence of genetic diversity in West African okra accessions as reported by (Ariyo, 1993). Studies conducted by (Adeniji, 2003) and (Adewusi, 2011) indicated that West African okra are either pigmented or non-pigmented; pubescent or glabrous and arranged in vertical or horizontal direction. Heritability of metric characters has been identified as a genetic relationship between the parents and the offspring. These genetic components have been widely used to assess the degree to which a character is transmitted from parent to the offspring. Information on heritability could as well indicate the possibility and extent to which improvement in a character is possible. More often in biological research, broad and narrow sense heritability is used to evaluate the proportion of heredity and environment in the expression of a character. In West African okra, high narrow sense heritability has been reported for pod length, pod width, plant height and number of seeds per pod (Adeniji, 2003). West African okra pods are consumed fresh (a maximum of 7 days after anthesis) obviously a high number of pods per plant are a desirable characteristic for genetic improvement in West African okra.

2.9 WEST AFRICAN OKRA AS A POTENTIAL DONOR SPECIES

West African okra (*A. caillei*), accounts for about 5% of the total world production of the crop (Siemonsma and Kouame, 2004) and very important in tropical areas of Benin, Cote d'Ivoire, Ghana, Cameroon, Nigeria and Togo. It is also identified as an amphipolyploid species (Siemonsma, 1982) known for possessing a gene pool of variations that may be useful for okra improvement of both temperate and tropical types (Martin *et al.,* 1983). *A.*

caillei is gradually replacing common okra in the tropical-humid region because of its better adaptation under humid zone and tolerance to biotic stresses (Siemonsma, 1982). Indeed, under very limited and erratic rainfall in the Sudano-Sahel, earliness of *A. esculentus* (being amphidiploid) was compared with *A. caillei* (being amphipolyploid) and *A. caillei was* preferred during early domestication. In Asia, *A. caillei* has been utilized as a resistant source to breed yellow vein mosaic virus resistant common okra variety (Nerkar and Jambhale, 1985).The inter-specific cross between *A. caillei* and *A. esculentus* is successful with the possibility of gene transfer, although partial hybrid breakdown barrier must be overcome (Fatokun, 1987). The study on geographical distribution and extent of natural outcrossing in Benin and Togo suggests that genetic integrity of these two species is not threatened (Hamon and Hamon, 1991).

2.10 MECHANICS OF POLLINATION

Pollination is an essential stage in the reproduction of flowering plants. It is the transfer of pollen from male to the female part of a flower within the same species (Crane, (1991).This leads to sexual reproduction if fertilization (fusion of the male and female gametes) occurs. Sexual reproduction ensures mixing of genes, which do not occur when plants propagate themselves through asexual means, such as budding or division. Abiotic factors such as wind and water aid pollination, but insects and other animals provide the vast majority of terrestrial plant pollination (Adamson, 2011). Pollination occurs in two different ways, either cross-pollination or self-pollination. The reproductive system of

okra is a mixture of cross and self-pollination. Insects play a significant role in the fertilization of flowers but the indiscriminate use of pesticides reduces the number of various pollinators tremendously.

2.11 CROSS-POLLINATION

This is the transfer of pollen from the anther of a flower to the stigma of a flower on another plant of the same species. A plant is cross-compatible if it can normally be pollinated with pollen of another cultivar, but it is cross-incompatible if it is not receptive to pollen of certain cultivars (McGregor, 1976). According to (Shrestha, 2008) not only the self-sterile varieties require cross-pollination, but also the self-fertile forms need it by means of which they are able to produce more and better quality seeds and fruits if pollinated preferably by honeybees or by other insects. Kasina (2009) reported that beans and cowpeas usually have about 17% and 23% protein content when not pollinated by the carpenter bees but when pollinated, the protein content increases to 19% and 25% respectively. Sunflower oil content increases from 35% to 45% after pollination by honey bees while well pollinated melon and butternut is large, sweeter and juicy. According to Gustafson and Bergh (1966) cross-pollination is necessary for the best setting of fruit in avocados.

2.12 SELF-POLLINATION

This is the transfer of pollen from an anther to a stigma of the same flower or to the stigmas of flowers on the same plant. A plant is said to be self-fertile or self-compatible if it can produce fruits without the need for the transfer of pollen to it from another cultivar so that no inter-planting of cultivars is necessary (Snow *et al*., 1996). Such a plant may not necessarily be self- pollinating. An external agent, such as the wind or insects, may be necessary to transfer the pollen from the anthers to the stigma within the flower or between flowers on the same plant (McGregor, 1976). If the plant is not receptive to its own pollen, it is self-sterile. Even self-pollinating plants are frequently benefited by cross-pollination, the transfer of pollen from one flower to another. They may also benefit from having the pollen more thoroughly transferred and distributed over the stigma at the most receptive period (McGregor, 1976).

2.13 HERITABILITY AND TYPES OF GENETIC VARIABILITY

Generally the success of any crop improvement program largely depends on the magnitude of genetic variability, genetic advance, character association, direct and indirect effects on yield and yield attributes. Genetic variability arises as a result of mutation, inter variety or inter specific genetic recombination. Genetic diversity is important for selection of parents to recover transgressive segregants (Kiran Patro and Ravisankar, 2004). These variations tend to accumulate in the germplasm that has been under selection pressure by the target environment. Therefore, local germplasm that evolved under target environments would provide the desired variability for selection.

However, target traits are to be integrated in superior high yielding cultivars, since landraces and wild species have some undesirable feature. Introgression of target traits may result in deterioration of yield and quality due to linkage. The basic approach for development is to select locally adapted germplasm containing genetic variability for high yield potential and drought adaptive traits (Beck *et al*., 1990; Vasal *et al*., 1997). In okra variability among the cultivated genotype for different traits has been reported by (Gill *et al.,* 1997). Genetic variability and heritability studies have been done in a wide range of crops in other breeding programmes, *e.g.* okra (Bisht *et al.*, 2006), kenaf (Mostofa *et al.,* 2002), roselle (Ibrahim and Hussein, 2006), tomato (Foolad *et al.,* 2006), cowpea (Aremu *et al.,* 2007) or eggplant (Islam and Uddin, 2009). Determination of heritability estimates, using different methods (Wray and Visscher, 2008; Obilana and Fakorede, 1981) will provide information on the proportion of phenotypic variance that is due to genetic factors for different traits but heritability estimate alone is not a sufficient measure of the level of possible genetic progress that might arise not even when the most outstanding individuals are selected in a breeding programme.

The value of heritability estimates is enhanced when used together with the selection differential or genetic advance (Ibrahim and Hussein, 2006). Information on the amount and direction of association between yield and yield related characteristics is important for rapid progress in selection and genetic improvement of a crop (Asish *et al.,* 2008). This will indicate the inter-relationship between two or more plant characters and yield, providing suitable means for indirect selection for yield.

Estimation of type and amount of genetic variability associated with the target traits is very important. Determination of the type of genetic variability may help in the formulation of comprehensive breeding programme regarding the further improvement of the target trait. Additive or non-additive type of genetic variability has been found to be associated with many traits. Additive type of genetic variability arises as a result of cumulative effect of minor alleles while non-additive type of genetic variability arises as a result of dominance and epistasis. Dominance arises as a result of intragenic interaction while epistasis is due to intergenic interaction. In addition, these intergenic or intragenic interactions have also been found to be affected by the external stimuli such as drought, pests and diseases (Rauf and Sadaqat, 2008).

Specialized biometrical techniques are required to determine the type of genetic variability associated with the traits. These biometrical techniques are dependent on different mating designs such as diallel, line × tester, North Carolina design and generation mean analysis for the estimation of type of genetic variability. Among these mating designs generating mean analysis has been the most powerful biometrical analysis since it gives additional information about the epistatic interactions. Information derived from these analyses can be further utilized for the formulation of an effective breeding strategy. A population with preponderance of additive genetic variability can be easily managed and selected through progeny rows. The additive genetic variability has also been used for the estimation of narrow sense heritability (ratio of additive genetic

variability to the total genetic variability) and genetic advance. Heritability and genetic advance provide further information regarding the proportion of genetic variability which can actually be selected and how much improvement can be brought through selection. A population high in non-additive genetic variability is difficult to manage and further improvement in trait will be slow. Recurrent selection has been recommended for the population high in non-additive genetic variability in order to break the intergenic or intragenic interaction. Ahmad (2004) reported that additive gene action for fresh fruit yield per plant, days to first flower and seed yield per plant selection are effective in early segregating generations.

2.14 GENETIC IMPROVEMENT

In countries such as India and USA a number of okra varieties have been developed through breeding efforts. A greater majority of these were introduced in West and Central Africa (WCA) countries and are still popular. There are a number of very good reports on genetic studies in okra, especially from Nigeria by Ariyo and associates. Multivariate analysis of characters such as pod yield, branch per plant, leaves per plant, days to flowering, plant height at flowering and maturity, pods per plant, edible pod length and width, mature pod length, duration of lowering, life span, seeds per pod and 100 seed weight of 30 genotypes collected from different geographical areas revealed no relationship between clustering pattern and geographical distribution of okra genotypes (Ariyo, 1987). Pod yield and several yield-contributing characters lack stability due to strong environmental influence, suggesting the need for breeding for specific

environments (Ariyo, 1990). Diversity in pod shape/size and flowering behavior account for most of the variation between the genotypes of WCA origin (Duzyaman, 1997) and scope for further gain in pod yield per plant is limited because of low phenotypic and genotypic variability (Ariyo, 1990).

To break the yield barrier in existing genotypes of common okra (*A. esculentus*) and breed for different market types, a hybridization-based breeding strategy is desirable. Despite okra's recognized potential and consumption in the developing world in general and West Africa in particular, it has been considered an economically minor crop (Duzyaman, 1997).

Commercial okra cultivation in the region faces many challenges including photoperiod sensitivity and cold temperatures that limit year-round availability of fresh pods; shelf-life, fiber/mucilage content, and pest resistance, especially root-knot nematodes, tomato fruit worm and begomoviruses. To overcome these challenges, a long term breeding project was warranted. Since, 2003, AVRDC-The World Vegetable Center and its partners, have been introducing, testing and promoting new cultivars. Efforts are sustained through pure line selection for high yielding cultivars with high mucilage content. In 2007, okra improvement activities were initiated at the center's outreach office that execute AVRDC/ICRISAT joint vegetable breeding project at Sadore, Niger. Okra has large acreage under rain-fed conditions; breeding goal should be focused on developing okra lines for both rain-fed and irrigated production systems. Efforts should be made to screen germplasm against root knot nematode. Considering the potential of

West African okra (*A. caillei*), there is the need to also develop inter-specific crosses and to efforts to overcome hybrid breakdown barriers, to facilitate pre-breeding and broadening of genetic base.

2.15 GENETIC BASES OF HETEROSIS

The ultimate goals of an okra breeder is to develop high yielding varieties (pod and seed yield), through selection and breeding, utilizing available genetic resources. The final product of okra plant i.e. pod and seed yield are the outcome of inter play between genetic and non-genetic components and due to complex nature of the interaction selection. Phenotypically diverse genotypes, presumably of diverse origins, are regarded to be more effective in obtaining promising crosses (Duzyaman and Vural, 2003). It was found that dominance gene effect was also found indicating the presence of both additive and dominance genetic variation in fruit length. Singh (1975) reported that pod length in okra is affected by both additive and dominance genes indicating the presence of both additive and dominance genes. The inheritance of fruit length in okra was obtained as partial dominance (Partap *et al.*, 1980; Stino *et al.*, 1970), additive gene effect and dominance gene action (Singh, 1975). Over dominance was also observed in fruit length suggesting that hybrid vigor can be exploited in okra for increasing yield (Sharma and Mahajan, 1978). The yield was influenced directly and/or indirectly by pod weight and pod length suggesting that these traits would be most useful as selection criteria in breeding for yield improvement (Duzyaman and Vural, 2003; Patel and Dalal, 1994).

2.16 INSECTS AND PESTS

Okra is affected by several species of insect pests and a number of diseases from seedling stage to harvesting. Economic losses depend on the degree of damage, pest density, environmental conditions, stage of growth and the parts of the plant destroyed by pests (Valeriana, 2011). Insect pests reported to infest okra in Ghana include flea beetles (*Podagrica sp*.), cotton stainer (*Dysdercus superstitus*), white fly (*Bemisia tabaci*), and green stink bug (*Nezera viridula*) among others (Bi-Kusi, 2013; Senjobi *et al*., 2013; Obeng-Ofori and Sackey 2003). Among these pests, flea beetles (*Podagrica sp*.) are the most important in Ghana (Asare-Bediako *et al*., 2014; Bi- Kusi, 2013; Obeng-Ofori and Sackey, 2003). The feeding activity of *Podagrica* sp. causes damage comprising characteristic perforations of leaves, and irregular holes reducing the photosynthetic surface area of the leaves leading to a great reduction of yield in okra (Echezona and Offordile, 2011). However, effective management of pest infestation and diseases is very important in order to improve yield of okra.

2.17 DISEASES

Damping off at the seedling stage can cause heavy losses and this can be controlled by treating sown seeds with recommended fungicides. Okra plants are subject to attack by mosaic and leaf curl viruses (early protection against the vector, the White fly, is necessary). Infected plants produce poor quality pods. Okra is subject to infection by some fungal diseases such as powdery mildew and leaf spot at the late vegetative state to

the reproductive stage. These two fungal infections spread rapidly on overcrowded fields via wind or workers passing through the field during harvesting.

2.18 MOLECULAR MARKERS

Reports on marker development in okra are very scanty and have been limited to characterization of cultivars. An agreement between clustering patterns obtained from morphological traits and molecular markers in *Abelmoschus* spp. has been demonstrated (Martinello *et al.,* 2001). Ninety-three accessions of common (*A. esculentus*) and West African (*A. caillei*) okra could be distinguished using random amplified polymorphic DNA (RAPD) markers (Aladele *et al.,* 2008). Use of sequence related amplified polymorphism (SRAP) in marker aided selection (MAS) for various traits in Turkish germplasm has been done (Gulsen *et al.,* 2007). Recently, 20 okra accessions from Burkina Faso were analyzed using16 primers designed to amplify SSR regions of *Medicago truncatula*. Two accessions were found distinct from the other 18, based on the presence of an unique 440 bp fragment generated by primer MT-27 and also based on presence of hairs on fruits and delayed maturity of these two accessions (Sawadogo *et al.,* 2009).

2.19 WEED COMPETITION

Weeds on the okra field compete for nutrients, sunlight and water that are needed for photosynthesis as well as space. They also serve as alternate host for pest and diseases in

the production of okra. Weeds have very high adaptive features such as high dormancy rates production of many seeds as well as several methods of propagation making their control very difficult. The most devastating group of weeds to okra are stargrass (*Cynodon dactylon*), crows foot grass (*Eleusine indica*) the common okra sedge, purple nut edge (*Cyperus rotundus*) (Norman 1992). The broad leaf weeds of okra are the giant pig weed (*Trianthema portulacastrum*); spiny amaranthus (*Amaranthus spinosus*); morning glory (*Ipomoea triloba*); *Trianthema* for instance is known to harbor small larvae that feed on okra (Tindall, 1983).

Herbicide application is considered the easiest and least expensive method of weed control (Lamont, 1999). Application of broad-spectrum herbicides is known to cause death to okra seedlings (from herbicide drift). Phytotoxicity always happens in the okra field due to over reliance on herbicides for weeds control.

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CHAPTER THREE

HYBRIDISATION STUDIES OF OKRA (*Abelmoschus spp.* (L.) Moench)

3.1 INTRODUCTION

Artificial sexual hybridisation is a conventional breeding approach which involves crossing of selected genotypes in order to introgress genes, to introduce new genetic variability for generating new or novel varieties (Sharma, 1994). This is usually aimed at incorporating genes of desirable traits such as disease resistance and high yield in one genotype into the genetic background of the other genotype to produce superior hybrids. It is a very useful approach in quantitative genetic analysis such as studies of combining ability and expression of heterosis towards improvement of targeted traits.

In cross-pollinated species such as sweet potato, the phenomenon of self-incompatibility and high levels of cross-compatibility promotes high rates of cross-fertilisation after hand pollination (Jousselin *et al*., 2004; Ahn *et al*., 2002).

However, due to genetic and reproductive barriers such as homogamy in which synchronisation of anther dehiscence, stigma receptivity or close proximity of stigmas with anthers and self-pollinated crops generally exhibit very low cross-fertilisation rates after artificial pollination (Bhojwani and Razdan, 1983; Yeung and Thorpe, 1981). Differences in genetic and floral morphology such as the effects of heterostyly- different style length among genotypes utilised as parents have been identified to contribute to the

low success rates after artificial pollination (Valdiani *et al.,* 2012; Lattoo *et al*., 2006; De-Block and Igersheim, 2001).

For instance in cassava, the genotype of the female plays prominent role in determining success of the crosses than the pollen source (Hershey, 1981). Apart from floral and genetic factors, environmental conditions particularly low temperature and high humidity are also known to play very important roles in determining hybridisation success after hand pollination (Ahn *et al*., 2002; Ma *et al*., 1996).

As a result, assessment of crossability relationships among genotypes of self-pollinating crops such as okra is a first step for systematic and effective planning of crop improvement programmes through artificial hybridisation in order to efficiently utilise genetic diversity (Valdiani *et al.,* 2012).

The major problem underlying okra productivity is low yielding potential of current varieties and reduction in yield due to frequent infestation of pests and diseases, especially the fruit and shoot borer and Yellow Vein and Mosaic Viruse (YVMV) (Reddy *et al*., 2012), as well as the lack of improvement on the well adapted local landraces. YVMV transmitted by white fly (*Bemisia tabaci*) is the main limiting factor in cultivation affecting fruit yield, causing a loss of 50 to 95 per cent depending on the stage of the crop growth at which infection occurs (Sastry and Singh, 1974) and fruit quality of okra. Since the disease cannot be controlled by chemical means, the only practical solution to this problem is to develop tolerant and resistant varieties (Mogili *et al*., 2013). Interspecific hybridization is considered a possible mechanism of plant diversification.

Interspecific hybridization followed by backcrossing and selection in the segregating generations is an effective method for developing high yielding disease resistant varieties. Hybridization including wild and cultivated species has long been used for transfer of genetic material in crops. A promising breeding method for creation of new variability is wild hybridization that became a more common practice after the advancement of hybridization techniques (Mujeeb-Kazi and Rajaram, 2002). Wild relatives of crops have been recognised as an important source of useful traits for breeding programmes.

Also, yield levels have been improved substantially through intensive and concerted breeding efforts, and as further yield advances seem to be more difficult necessitating the application of newer breeding approaches. The required goal of increasing productivity in the quickest possible time can be achieved only through heterosis breeding.

It is therefore important to enhance productivity of the local landrace varieties of okra through breeding to boost vegetable farmers' interest in large-scale cultivation of the crop and for adoption by Ghanaian and export market. Intra-specific and inter-specific hybridisation among locally adapted cultivars may therefore play a very important role in producing a broad-based segregating population from which recurrent selection could be carried out to obtain hybrids which combine high yield and disease resistance with other desirable traits such as high nutritional and anthocyanin contents, as well as fruit characteristics required for export.

This study seeks to investigate crossability through inter-specific and intra-specific hybridization of okra from cultivars assembled from different parts of the country.

3.1.1 Objectives of the study

The main objective of this study was to investigate crossability relationships among ten local landraces and exotic cultivars of okra for key yield-determining traits in order to obtain relevant information on their breeding behaviour for use in future breeding programmes towards improvement of landraces in Ghana.

The specific objectives were;

1. To investigate cross compatibility among seven accessions of *Abelmoschus esculentus* and five of *Abelmoschus caillei.*

2. To assess the possibility of inter-specific and intra-specific hybridisation among accessions of *Abelmoschus esculentus* and *Abelmoschus caillei.*

3. To estimate crossability index and fruit set percentage for both inter-specific and intra-specific hybridisation.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The experiment was carried out at the research farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of Ghana Atomic Energy Commission (GAEC) at Kwabenya, near Accra. The experimental site is located at 05º 40' N and longitude 0º 13' W at an elevation of 76 m above sea level within the coastal savanna agro-ecological zone. The soil at site belongs to the Nyigbenya-Haatso series, which is typically well-drained savanna Ochrosol (Ferric Acrisol), derived from quartzite and Schist (FAO/UNESCO, 1994).

3.2.2 Germplasm Assembly

Twelve (12) accessions of okra were collected from six geographic regions of Ghana (East, Volta, Ashanti, Greater-Accra, Upper East and Brong-Ahafo regions) (Table 3.1) and used as parents to produce F_1 hybrids.

Table: 3.1. Okra accessions used in the study

Accession code	Origin	Accession name	Accession owner
T1*(A. esculentus)*	Upper East region	Yire marna1	Amitaaba
T2 *(A. esculentus)*	Upper East region	Yire marna 2	Amitaaba
T3*(A. esculentus)*	Upper East region	Yire marna 3	Amitaaba
T4*(A.caillei)*	Upper East region	Yire marna 4	Amitaaba
AM*(A.caillei)*	East region	Amanfrom	Ahiakpa
VT*(A. esculentus)*	Volta region	Volta	Ahiakpa
ID*(A. esculentus)*	GreaterAccra region	Indiana	Ahiakpa
AS*(A. esculentus)*	Ashanti region	Agric Short	Ahiakpa
YL *(A.caillei)*	Brong-Ahafo region	Yeji Local	Ahiakpa
KG*(A.caillei)*	Ashanti region	Kortebotor green	Ahiakpa
KP*(A.caillei)*	Ashanti region	Kortebotor pink	Ahiakpa
CSL(*A. esculentus)*	Greater Accra region	Clemsin spineless	Agrimat Ghana

3.2.3 Land Preparation

A total land area of 45 m x 30 m was acquired and cleared; all stumps were removed and ploughed to a fine tilth for planting.

3.2.4 Experimental Design

Single row planting was used with four replications, each replicate measuring 17.5 m x 12 m, and separated by a distance of 2 m from the other. All rows within the various replications were randomly assigned by drawing lots to avoid bias and each plot was labelled.

3.2.5 Seed Sowing.

Seeds of the various accessions were sown on 9th April, 2014 after a heavy rainfall that facilitated uptake of water by the seeds for germination. The seeds were sown at a depth of 2 cm, at a spacing of 1 m x 0.70 m between and within rows to allow free movement during emasculation, with three to four seeds per hill and thinned to two per hill after germination.

3.2.6 Field Management Practices

No fertiliser was applied, but weeds were controlled and other agronomic and management practices were carried out. Weeding was done fortnightly. The rainfall pattern during this period was not quite consistent and regular hence there was the need for supplementary water for irrigation to support plant growth and development.

3.2.7 Emasculation and crossing operation

Fig.3.1 shows the procedure for emasculation at flowering. Five healthy plants of each accession were selected to serve as parents for hybridisation. Emasculation was carried

out prior to pollination by first identifying a matured bud as shown in step one. In step two, the calyx of the matured bud is cut open to expose the corolla. Step three shows the use of a sterilized surgical blade to remove the corolla by cutting it close to the receptacle to expose both the androecium and gynoecium without damaging the fruit bud. The anthers are then removed to feminize the hermaphroditic flowers. Finally, in step four the calyx is folded back and sealed in paper envelops to prevent insect and self-pollination.

Step1

Step2

Step3

Step4

Fig. 3.1: Procedure for emasculation Source: experimental Field 2014.

Pollination was performed the next morning from 6:00 am to 10:00 am by dusting the receptive stigma of the emasculated flower with pollen from selected male parents. For each cross, twenty (20) flowers were pollinated and reciprocal crosses were also made. Immediately after emasculation and pollination, the flower buds were covered with paper bags and clipped to prevent contamination from undesirable pollen and properly tagged in order to identify the pollen donor and recipient as well as the time that each particular cross was made. Between successive emasculation of flowers belonging to different accessions, the pair of forceps was sterilized with alcohol to prevent contamination with pollen from earlier sources. Three days after pollination, the paper bags were removed to allow the fruits to develop properly.

3.2.8 Tools for emasculation

Tools used for the emasculation process were, a pair of forceps, 70 % alcohol, cotton wool, a stapler, and paper envelops.

3.2.9 Evaluation of hybridisation success.

Success of hybridization was assessed through observation of each flower three days after pollination. Fertilised flowers developed fruit capsules with white seeds between 3 to 4 days after pollination depending on the accessions crossed and where fertilization failed, the flowers dropped 2 to 3 days after pollination without developing any fruit capsules.

3.2.10: Estimation of hybridization success.

Fruit set percentage of each cross was calculated according the formula of Nunekpeku *et al.,* (2012).

$$FS\ (\%) = \frac{NFF \times 100}{NFP}$$

Fruit set percentage = (FS)

Number of fruit formed = (NFF)

Number of flowers pollinated = (NFP)

3.2.11 Estimation of crossability index

$$CI = \frac{(\Sigma\ FS\ F_1)}{(\Sigma\ FSP_1 + FS\ P_2)/2} \times 100$$

Where, CI (%) = Crossability index,

$FS\ F_1$ = fruit set for F_1 hybrid,

FSP1 = fruit set for parent one,

$FS\ P_2$ = fruit set for parent two and

Σ = summation.

3.3 RESULTS

3.3.1 Crossability studies among accessions of *Abelmoschus esculentus* and *caillei.*

Table 3.2 shows crossability success among 12 accessions okra, five *A. caillei* T4, KG, KP, YL and AM and seven accessions of *Abelmoschus esculentus* T1, T2, T3, CSL, VT, ID and AG in inter-specific and intra-specific hybridization using reciprocal crosses.

Successful hybridization was obtained only in one direction of direct crosses and not reciprocal crosses between *A. esculentus* cultivars and *A. caillei* in this study. Successful hybridisation between *A. esculentus* when used as female (♀) and *A. caillei as male* (♂) was achieved, all attempted reciprocal crosses failed to develop fruit capsules and dropped off two or three days after pollination.

This was partly due to poor synchronization of flowering, and inability of some of the accessions to produce enough flowers to complete reciprocal crosses among the accessions of *Abelmoschus caillei*

However, hybridisation attempts among accessions belonging to *Abelmoschus esculentus* mating group, was successful though to varying levels in different cross combinations.

Accessions T1, T2, T3, ID, VT and AG emerged as most compatible female and male parents, yielding 30 successful hybrids in reciprocal crosses.

Table 3.2 Cross compatibility success among 12 accessions of *Abelmoschus esculentus* and *caillei*

Male parent (♂)

Female parent (♀)	T1	T2	T3	VT	ID	AG	CL	T4	AM	YL	KB	KG
T1	X	√	√	√	√	√	*	√	√	√	√	N
T2	√	X	√	√	√	√	*	√	√	√	√	N
T3	√	√	X	√	√	√	*	√	√	√	√	N
VT	√	√	√	X	√	√	*	√	√	√	√	N
ID	√	√	√	√	X	√	*	√	√	√	√	N
AG	√	√	√	√	√	X	*	√	√	√	√	N
CL	*	*	*	*	*	*	X	*	*	*	*	*
T4	N	N	N	N	N	N	*	X	N	N	N	N
AM	N	N	N	N	N	N	*	N	X	N	N	N
YL (♀	N	N	N	N	N	N	*	N	N	X	N	N
KB(♀)	N	N	N	N	N	N	*	N	N	N	X	N
KG(♀)	N	N	N	N	N	N	*	N	N	N	N	X

√ = Successful cross X = Self N = Not successful * = Cross not carried out

Contrary, Table 3.3 shows direct and reciprocal crosses (whether used as male or female) among all six accessions of *Abelmoschus esculentus* that were all successful.

Table 3.3 Hybridisation success (%) among six accessions of *Abelmoschus esculentus* in pairwise crosses.

Male parent (♂)

Female parent (♀)	T1	T2	T3	VT	ID	AG
T1	x	√	√	√	√	√
T2	√	x	√	√	√	√
T3	√	√	x	√	√	√
VT	√	√	√	x	√	√
ID	√	√	√	√	x	√
AG	√	√	√	√	√	x

√= Successful cross

Table 3.4 displays parental accessions, the number of flowers selfed, the average number of seeds per pod and percentage seed germination. Twenty flowers were selfed for all the accessions and all were successful.

T2 and VT gave the highest values (95%) and YL the least value (60%) for percentage fruit set percentage.

Accessions VT and T3 (69 and 25) gave the highest and least value respectively for average number of seeds per pod.

Accessions VT and YL recorded the highest and lowest values for germination percentage respectively.

Table 3.4 Percent fruit set, average number of seeds per fruit and percent germination among parental accessions okra (*Abelmoschus esculentus*).

Paretal accession	NFC	FS (%)	ANSP	CI (%)
T1	20	80	48	90
T2	20	95	39	80
T3	20	75	25	85
T4	20	70	54	70
VT	20	**95**	**69**	**95**
ID	20	75	32	90
AM	20	**95**	65	90
KB	20	70	68	70
YL	20	60	38	65
AG	20	75	59	80

Bolded and underlined values represent highest and lowest percentages fruit set (FS %), number of flowers crossed (NFC), average number of seeds per pod (ANSP) and percentage crossability index (CI %) respectively.

Table 3.5 displays F1 accessions, the number of flowers crossed the average number of seeds per pod, percentage seed germination and crossability index obtained from reciprocal crosses of intra-specific hybridization. Twenty flowers were crossed for all accessions.

VT x T1 gave the highest values (75%), while T3 x ID, ID x VT and T1 x AG scored the least value (40%) for percentage fruit set.

For average number of seeds per pod, VT x T2 (48) and T3 x ID (16) gave the highest and least value respectively. In all, VT x T3 recorded the highest value (90.00%) and ID x AG the least (30%) for percentage germination, while crossability index values ranged between 90.32% and 45.71% for the crosses between T3 x T1 and T1 x AG respectively

Table 3.5 Percentage fruit set; average number of seeds per pod, percentage germination and crossability index of 29 F_1s obtained from intra-specific hybridisation.

OFFSPRING	NFC	FS (%)	ANSP	G (%)	CI (%)
T2 x T1	20	65	35	70	74.29
T3 x T1	20	70	44	60	**90.32**
VT x T1	20	**75**	47	65	85.71
AG x T1	20	65	45	75	74.29
T1 x T2	20	55	43	85	62.86
T3 x T2	20	70	40	80	82.35
ID x T2	20	45	28	75	52.94
VT x T2	20	70	**48**	60	73.68
AG x T2	20	60	46	75	63.16

Bolded and underlined values represent highest and lowest fruit set percentages FS (%), average number of seeds pod (ANSP), percentage seed germination G (%) and crossability index CI (%) respectively.

Table 3.5 (cont'd)

OFFSPRING	NFC	FS (%)	ANSP	G (%)	CI (%)
T1 x T3	20	55	37	80	70.97
T2 x T3	20	65	43	75	76.47
ID x T3	20	50	20	60	66.67
VT x T3	20	65	22	**90**	76.45
AG x T3	20	55	20	75	64.71
T1 x ID	20	45	20	55	58.06
T2 x ID	20	60	18	55	70.59
T3 x ID	20	40	16	63	53.33
VT x ID	20	60	34	60	70.59
AG x ID	20	50	26	70	58.82
T1 x VT	20	60	25	65	77.42
T2 x VT	20	55	31	75	58.89
T3 x VT	20	50	23	75	58.82
ID x VT	20	40	27	52	47.06
AG x VT	20	60	45	70	63.16
T1 x AG	20	40	41	60	45.71
T2 x AG	20	45	40	70	47.35
T3 x AG	20	60	30	50	70.59
ID x AG	20	45	29	30	52.94
VT x AG	20	70	36	75	73.68

Bolded and underlined values represent highest and lowest fruit set percentages FS (%), average number of seeds pod (ANSP), percentage seed germination G (%) and crossability index CI (%) respectively.

Table 3.6 displays F_1 accessions, the number of flowers crossed, the average number of seeds per pod, percentage seed germination and crossability index obtained from direct crosses of inter-specific hybridization. Twenty flowers were crossed for accessions.

The cross VT x AM gave the highest value of (50%), while the crosses T3 x KB and ID x KB scored the least value of (25%) for fruit set percentages.

While for average number of seeds per pod, the crosses VT x AM and ID x YL gave the highest and least value of 43 and 6 seeds respectively.

For germination percentage, the cross ID x KB recorded the highest value of (85%) while, the cross AG x YL gave the least value (10%).

With respect to crossability index, the cross T3 x AM exhibited the highest crossability of (60.00%) while the crosses T3 x KB and ID x KB gave the least crossability index of (34.48%)

Table 3.6 Percentage fruit set; average number of seeds per pod, percentage germination and crossability index of 23 F_1s obtained from intra-specific hybridisation.

OFFSPRING	NFC	FS (%)	ANSP	G (%)	CI (%)
T1 X AM	20	35	30	45	45.16
T2 X AM	20	30	41	43	35.29
T3 X AM	20	45	29	65	**60.00**
ID X AM	20	40	15	43	53.33
VT X AM	20	**50**	**43**	40	58.82
AG X AM	20	45	31	55	52.94
T1 X T4	20	40	19	40	53.33
T2 X T4	20	35	24	50	42.43
T3 X T4	20	35	27	30	48.28
ID X T4	20	30	14	41	41.38
VT X T4	20	45	40	40	54.55
AG X T4	20	40	29	20	48.49
T1 X KB	20	30	27	50	40.00
T2 X KB	20	40	30	55	48.49
T3 X KB	20	<u>25</u>	20	60	<u>34.45</u>
ID X KB	20	<u>25</u>	12	**85**	34.48
VT X KB	20	45	31	60	54.55

Bolded and underlined values represent highest and lowest fruit set percentages FS (%), average number of seeds fruit (ANSP), percentage seed germination G (%) and crossability index CI (%) respectively.

Table 3.6 (Continued)

OFFSPRING	NFC	FS (%)	ANSP	G (%)	CI (%)
AG X KB	20	35	22	40	42.43
T1 X YL	20	40	9	35	57.14
T2 X YL	20	40	21	45	51.61
T3 X YL	20	35	25	30	51.85
ID X YL	20	30	6	40	44.44
VT X YL	20	35	23	35	45.16
AG X YL	20	35	25	10	45.16

Bolded and underlined values represent highest and lowest fruit set percentages FS (%), average number of seeds fruit (ANSP), percentage seed germination G (%) and crossability index CI (%) respectively.

3.4 Discussions

3.4.1 Cross compatibility studies among accessions of *A. caillei* and *A. esculentus.*

Results of the investigation revealed that all six accessions of *A. esculentus* utilised in this study are compatible with one another in both direct and reciprocal crosses, a strong indication of no major crossability barriers among genotypes of this sub-species of okra. Highest fruit set, average number of seeds per crossed fruits and highest crossability index were observed.

However, crosses between *A. caillei* and *A. esculentus* were successful only when *A. esculentus* was used as female and *A. caillei* as male. Sheela (1994) stated that reciprocal differences in compatibility of the two species exist.

In contrast, successful crossing between these two species was also reported by Hamon and Hamon (1991), where highest fruit set, average number of seeds per crossed fruits and highest crossability index were observed when *A. caillei* was used as female parent.

Outcomes of crossability studies help breeders to determine appropriate strategies or breeding designs to adopt to transfer genes of desirable traits present in one genotype into the genetic background of other genotypes to produce novel varieties (Ahn *et al*., 2002). Where genotypes are compatible with one another in both direct and reciprocal crosses as was achieved for accessions of *A. esculentus* in this study, a full diallele analysis can be carried out.

However, where complete pairwise crosses are not achieved as it came out for accessions of *A. caillei* and *A. esculentus*, partial diallele or other breeding designs whereby genotypes can be used as either male or female parents only such as North Carolina design would be suitable (Todd, 2013; Griffing, 1956).

Accessions T3 and T1 were the most compatible female and male parents with the greatest CI (90.32%) value. This shows their inherent potential as pollen donor and recipient respectively, since all accessions were crossed under the same conditions. Hence, T3 and T1 would be suitable maternal and paternal parents respectively to cross with the other accessions in future breeding of okra in Ghana through intra-specific hybridisation. T4, AM, KB and YL also failed to register any success as female parents, thus could be utilised only as paternal parents (pollen donors) in breeding programmes. Similar results were reported by Nunekpeku *et al.,* (2012) who studied crossability in cassava and apple respectively.

3.4.2 Hybridisation success among accessions of *A. caillei* and *A. esculentus* in pairwise crosses.

Results of the investigation reveal that hybridisation success of all accessions of *A. esculentus* utilised in the study were high with percentage fruit set ranging from (40% to75%), which indicates high fertility rates of the genotypes of this sub-species of okra. In line with observations of Valdiani *et al.,* (2012) as well as De-Block and Igersheim (2001), On the other hand, hybridisation success of crosses among accessions of *A. caillei*

and A. esculentus was generally in one direction only - when *A. caillei* was used as male parent. Accessions involving ID as female and T1 as male produced shriveled seeds which failed to germinate – a sign of hybrid inviability.

Major causes of hybrid inviability might be due to non-compatibility of the parental chromosomes, cytoplasmic genic interactions and non-compatibility between embryo and the surrounding tissue called somaplastic sterility Stebbins (1958). Crosses which produced shriveled and non-viable seeds indicate involvement of post-zygotic sterility during inter-specific hybridization. Similar results were obtained by Sheela, (1986). In this incompatibility, zygote formation might be prevented by failure or ineffectiveness of pollen growth or failure of fertilization Allard (1990). However, viable hybrids were achieved through embryo culture technique, by Gadwal *et al* (1968)

Genetic relationships among genotypes are known to play an important role in determining hybridisation success after artificial pollination (Koelling *et al.*, 2011; Lattoo *et al.*, 2006; Ogburia and Okele, 2001).

Consequently, genetic difference among the accessions of *A. caillei* and *A. esculentus* in this study could have also contributed to the observed variations in reciprocal crosses.

From the present findings, it was observed that highest percentage fruit set, number of seeds per crossed fruit, percentage germination were achieved when VT was used as female and highest crossability index obtained when T3 was used as female parent while crossing with T1, T2, T3 and T1 respectively. This is in line with findings of Jambhale (1980). However, Sheela (1994) observed that reciprocal crosses registered higher

compatibility than the direct crosses, while Cheriyan (1986) observed no differences between direct and reciprocal crosses involving *A. esculentus* and *A. manihot*. Lowest fruit set was observed in the inter-specific hybridisation betweenT3 x KB and ID x KB. Also, ID x YL registered the least value for average number of seeds per fruit and least crossability index was observed in the cross between AG x YL. Low seed set and recovery of shriveled seeds may be due to partial or complete failure of the endosperm owing to genetic imbalance.

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CHAPTER FOUR

ESTIMATES OF HETEROSIS IN F_1 HYBRID PROGENY OF OKRA

(*Abelmoschus spp.* (L.) Moench)

4.0 Introduction

Heterosis (hybrid vigour) is the superior performance of a hybrid compared with the performance of its parents. Expression of heterosis depends on the differences in the gene frequency of the parental materials that are used for hybridisation.

Mid-parent heterosis (MPH) refers to the superiority of a hybrid over the mean of its parents, while better-parent heterosis refers to superior performance of a hybrid line over the mean performance of the better-parent for a given trait (Bernado, 2002).

The phenomenon of heterosis has been a powerful force in the evolution of crop plants and has been exploited extensively in crop production (Birchler *et al*., 2003). Heterosis for increased fruit size, fruit weight and fruits per plant in okra was first reported by Vijayaraghvan and Warriar (1946). Heterosis is expressed as relative heterosis, heterobeltiosis and standard heterosis, depending on the criteria used to compare the performance of a hybrid. However, from the practical point of view, standard heterosis is the most important of the three types of heterosis because it is aimed at developing desirable hybrids superior to the existing high yielding commercial varieties (Chaudhary, 1984).

Heterosis is thought to result from the combined action and interaction of allelic and non-allelic factors and is usually closely and positively correlated with heterozygosity (Falconer, 1989).

Further, exploitation of hybrid vigour depends on the direction and magnitude of heterosis as well as biological feasibility.

An understanding of heterosis would be helpful in improving the yield ability as well as yield contributing characters.

Heterosis works like a basic tool for the improvement of crops in F_1 generation. Through heterosis, seed cotton along with quality traits had been improved significantly (Naquibullah *et. al*., 2000). Ahmad (2002) reported that substantial heterosis of 26% and 19.2% in pod length and yield per plant could be exploited by producing F_2. Similarly, Wammanda (2010) reported heterosis of 10.6%, 15.4% and 17.2% in pod length, number of pods per plant and plant height respectively in okra.

The ultimate goal of an okra breeder is to develop high yielding varieties (pod and seed yield), through selection and breeding, utilizing available genetic resources. Increased pod and seed yield are the outcome of inter play between genetic and non-genetic component and also, due to complex nature of gene interaction and selection.

Phenotypically diverse genotypes, presumably of diverse origins, are regarded to be more effective in obtaining promising crosses (Duzyaman and Vural, 2002).

In crop breeding programme, to achieve high degree of heterotic response, it is essential to have knowledge about performance of desirable parents.

There has been considerable improvement in the yield of tropical okra by pedigree selection and more recently, by the development of commercial hybrids, based on hand emasculation and pollination by Sood and Sharma (2001). It is therefore possible to enhance productivity of the local landrace varieties of okra through breeding to boost vegetable farmers' interest in large-scale cultivation of the crop and for adoption by Ghanaian and export market. Intra-specific and intra-specific hybridisation among locally adapted cultivars may therefore play a very important role in producing a broad-based segregating population from which recurrent selection could be carried out to obtain hybrids which combine high yield and disease resistance with other desirable traits such as high nutritional and anthocyanin contents, as well as fruit characteristics required for export.

In all, a great deal of variation has been observed in okra (*Abelmoschus spp.* L.) particularly the West Africa type, *Abelmoschus caillei* but, no serious breeding effort have been made to harness its genetic richness to advance the improvement of the crop. Previous international efforts have been limited to intensive cultivation based on resistance to pests and diseases. There is still enormous scope for cultivar improvement in Africa particularly Ghana as we have the potential yet have not improved our local landraces to increase production of the crop to meet standards of the export market.

Heterosis breeding based on the identification of parents with desirable characteristics and their cross compartibilities is capable of producing F_1 hybrids and transgressive segregants with superior yield. This study was, therefore, undertaken to elicit information about the nature and magnitude of heterosis for yield and its components in okra so as to formulate suitable breeding strategy and isolate potential parents and promising offspring for further exploitation.

4.1.1 Objectives of the study

The main objective of this study was to investigate expression of heterosis by F_1 offspring obtained from intra-specific and inter-specific hybridisation among accessions of okra for seven key quantitative agro-morphological traits.

The specific objectives were to;

1. Estimate expression of heterosis among 27 F_1 offspring obtained from intra-specific hybridisation for six quantitative agro-morphological traits.
2. Estimate expression of heterosis by 24 F_1 offspring obtained from inter-specific hybridisation for seven quantitative agro-morphological traits.

4.2 Materials and Methods

4.2.1 Experimental site

The experiment was conducted at the Research Farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Kwabenya, in the Greater Accra Region of Ghana. The BNARI Research Farm is located at latitude 05° 40′ N and longitude 0° 13′ W, and elevated at 76 m above sea level within the coastal savanna agro-ecological zone. Kwabenya has an annual average temperature of 28 ℃ and receives an annual rainfall less than 1000 mm (Ghana Meteorological Authority, 2005). The soil at site is the Nyigbenya-Haatso series, which is a typically well-drained savanna ochrosol (Ferric Acrisol) derived from quartzite and schist (FAO/UNESCO, 1994).

4.2.2 Planting material

Ten accessions of okra (*A. esculentus* and *A. caillei*) (refer to chapter three) and 51 F_1 hybrids obtained from inter-specific and intra-specific hybridization were used for the study. Total entries were 61 including 10 parental lines.

4. 2.3 Experimental design and planting

An experimental plot size of 85.4 m x 25 m was pegged and marked out, within the prepared land leaving the rest of it as a periphery. Approximately 2.0 m was created

around replications to serve as a control for pests and to ease movement around the field. The Randomised Complete Block Design was used with four replications, each replicate measuring 42.7 m x 12.5 m, and separated by a distance of 2.0 m from the other.

Seeds of the various accessions were sown on 26th August, 2014 after a heavy downpour of rainfall that facilitated uptake of water by the seeds and boosted germination. The seeds were sown at a depth of 2.0 cm, at a spacing of 0.70 m x 0.50 m between and within rows with three to four seeds per hole later thinned to two per hill after germination.

4.2.4 Data collection

Using ten randomly selected plants per entry, data were collected on eight quantitative agro-morphological traits namely;

(i) Days to 50% germination	(DG)
(ii) Days to flowering	(DF)
(iii) Plant height at first fruiting	(PHAFF)
(iv) Length of petiole	(LOPE)
(v) Length of pod	(LOF)
(vi) Fresh fruit weight	(FW)
(vii) Number of branches	(NOB)
(viii) Number of seeds per fruits	(NSPP)

4.2.5 Data analysis

Means were subjected to Analysis of variance (ANOVA) to determine the level significance of variability among accessions for each of the parameters. Genstats Statistical Software Package (12th edition), Statsgraphics Centurion software (version 16.1) and Microsoft Excel Software (2010) edition were used for the data analyses.

4.2.6 Estimation of heterosis.

Percent mid parent heterosis (MPH %) and better parent heterosis (BPH %) were calculated based on the formulae of Falconer and Mackay, (1989) ;

$$(1)\quad \text{MPH \%} = \frac{(F_1 - [P_i + P_j] / 2)}{[P_i + P_j] / 2} \times 100$$

$$(2)\quad \text{BPH \%} = \frac{(F_1 - BP)}{B} \times 100$$

Where, MPH % = mid parent heterosis of F_1 hybrid,

F_1 = mean value of F1 hybrid,

P_i = mean value of first parent,

P_j = mean value of second parent,

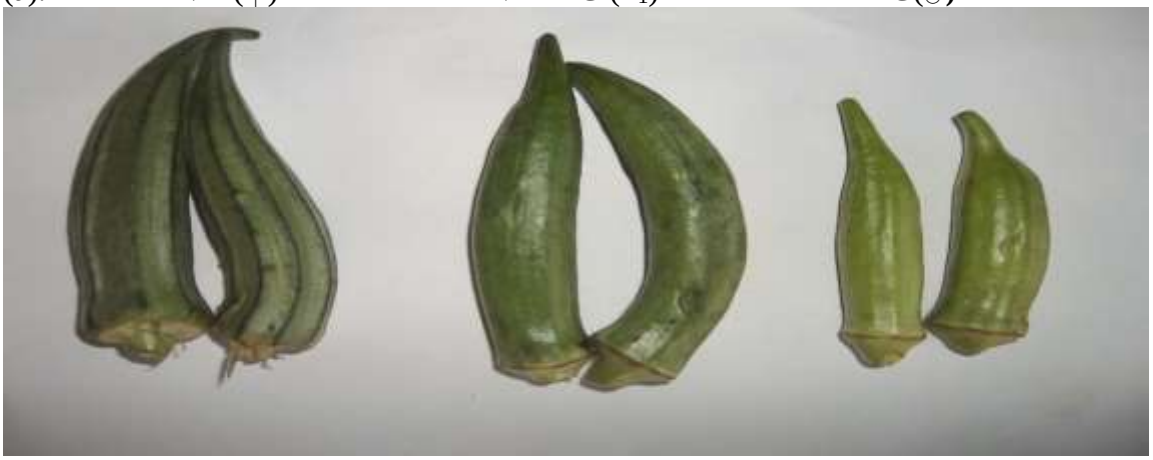
BPH % = heterobeltiosis of F_1 Hybrid and BP = mean value of better parent.

4.3 RESULTS

4.3.1 Morphological Traits of fresh pods of Accessions

Figure 4.1 shows the resultant F_1 for a cross between VT (♀) and Ag (♂**).** It shows improved fruit size, fruit length and fruit weight with reference to both parents indicating better-parent heterosis. In the reciprocal cross, the F_1 exhibits improved fruit size compared to both parents.

(a). VT (♀) VT×AG (F_1) AG(♂**)**



(b). VT (♂) AG ×VT (F_1) AG (♀**)**

Figure 4.1: Photographs of fresh okra obtained from field showing different levels of heterosis in;
a. VT was used as female and AG as male. b. AG was used as female and VT as male.

4.3.2 Estimates of heterosis.

Table 4.1 shows estimates of mid parent heterosis (MPH) and better parent heterosis (BPH) expressed by 24 F_1 hybrids obtained from inter-specific hybridisation among ten local landraces of okra for seven agro-morphological quantitative traits. Generally, extensive variation was recorded with respect to both MPH and BPH ranging from -68.87 to 152.76 and -566.67 to 51.45 respectively. Most crosses produced positive MPH for length of pod (LOF) and number of seeds per plant (NSPP) and 0.00 for number of branches (NOB), relatively few of the F_1 offspring gave positive values for days to flowering (DF) and plant height at first fruiting (PH). The crosses T2 x T4 and AG x YL produced highest MPH (152.76) and BPH (51.45) values for NOB and LOP respectively. While the cross ID x KB and VT x KB (-53.59 and -566.67) recorded least values in PHAFF and NOB for MPH and BPH respectively.

Values recorded by the crosses T3 x KB and AG x T4 (10.78 and 1.77) as well as VT x KB and ID x KB (-30.93 and -121.03) emerged highest and least for MPH and BPH respectively for DF.

Again, the cross T1 x AM recorded highest MPH (24.22) and the cross T3 x T4 recorded the highest BPH (-24.85) for PH, while ID x KB (-53.59 and 468.13) gave least values.

Similarly, AG x YL registered highest MPH (51.60) and BPH (51.45) for LOF, while ID x T4 and T2 x YL gave the least values (-17.49and -76.57).

With respect to DG, values of the crosses T1 x YL and AG x AM (-9.98and -24.01) as well as ID x AM and T2 x KB (-48.77and -200) gave highest and least MPH and BPH respectively.

Also VT x YL and AG x YL (17.17and 12.54) and ID x T4 (-22.52 and -49.59) scored highest and least values for FFW, while VT x YL and T1 x YL (36.87 and 18.42) and T3 x KB (-35.39 and -102.68) emerged highest and least for NSPP.

Table 4.1: Estimates of percent (%), mid parent heterosis (MPH) and better parent heterosis (BPH) expressed by 24 F1 offspring of okra for seven quantitative traits.

Offsping	DF		PH		LOF		DG		FFW		NOB		NSPP	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
T1XAM	-15.25	-43.18	**24.22**	-68.44	-2.20	-12.07	-18.00	-77.14	2.22	1.16	-15.91	0.00	-2.80	-15.29
T2XAM	5.65	-35.23	-21.63	-196.34	34.71	-24.38	-38.83	-150.15	-7.08	-10.73	-10.17	0.00	-28.63	-60.89
T3XAM	-19.49	-59.82	-1.13	-153.85	0.80	-15.04	-44.50	-160.36	2.87	1.03	39.13	0.00	-19.68	-102.68
IDXAM	-3.77	-58.92	-33.12	-245.01	-13.83	-21.16	-48.77	-123.33	-17.89	-39.68	-33.30	0.00	-14.91	-64.59
VTXAM	-21.07	-47.78	-33.51	-169.28	8.10	11.64	-33.29	-75.05	-0.52	-2.50	0.00	-333.33	2.34	-5.03
AGXAM	-24.02	-70.53	-21.41	-140.91	1.37	-42.62	-21.12	-24.01	-3.70	-18.37	-38.07	-286.60	11.50	7.27
T1XT4	-4.03	-9.86	0.21	-36.57	2.49	1.38	-38.83	-92.37	-2.30	-3.86	121.17	0.00	3.47	-6.25
T2XT4	-2.92	-17.14	-6.02	-51.17	-15.03	-19.52	-15.09	-90.09	-5.53	-12.10	**152.76**	0.00	6.54	-15.26
T3XT4	-11.56	-19.67	12.21	**-24.85**	3.11	-3.24	-45.45	-140.24	-2.17	-2.90	47.55	0.00	13.71	-42.68
IDXT4	-3.93	-25.26	-11.63	-66.01	-17.49	-19.46	-44.50	-100.23	-22.52	-49.59	-47.24	0.00	-23.43	-72.94
VTXT4	-3.71	-25.02	-14.52	-48.80	5.29	-5.69	-28.15	-56.29	-5.56	-6.24	35.69	0.00	2.90	-6.36
AGXT4	10.56	**1.77**	-26.36	-60.50	3.46	-54.79	-33.36	-39.11	-7.66	-26.53	35.39	**26.80**	-5.69	-12.71
T1XKB	-20.67	-63.64	-50.94	-361.56	10.90	-2.00	-40.93	-138.57	3.29	-0.05	-20.00	0.00	-28.97	-57.63
T2XKB	-5.74	-38.09	-49.11	-407.42	7.11	-9.57	-46.27	-200	-0.20	-1.24	-15.53	0.00	0.63	-37.27
T3XKB	**10.78**	-30.77	-47.53	-439.01	1.08	-20.00	-41.53	-190.09	0.75	-3.53	-26.67	0.00	-35.39	-157.32
IDXKB	-30.84	-121.03	-53.59	-468.13	5.52	-4.87	-49.93	-153.81	-2.52	-18.34	-68.87	0.00	-25.00	-97.91
VTXKB	-30.93	-74.26	-29.07	-249.19	9.41	8.35	-40.36	-106.19	2.79	-1.48	-21.21	-566.67	1.45	0.98
AGXKB	-17.75	-77.69	-48.07	-288.20	11.01	-22.37	-32.15	-48.02	-6.87	-18.93	-48.57	-420.00	0.51	-2.54
T1XYL	1.37	-35.61	6.08	-135.82	3.93	-57.12	**-9.98**	-69.28	-12.77	-37.90	21.18	0.00	27.91	**18.42**
T2XYL	8.34	-46.66	0.63	-176.47	-4.06	-76.57	-13.54	-110.21	8.96	2.04	30.27	0.00	-0.87	-2.63
T3XYL	0.00	-48.72	-20.67	-261.52	16.88	-45.94	-35.12	-150.15	-9.39	-35.02	-26.67	0.00	0.94	-14.68
IDXYL	-14.73	-96.84	-32.39	-299.14	11.58	-39.20	-39.98	-115.47	6.65	5.10	-18.18	0.00	5.98	-8.34
VTXYL	-12.25	-51.47	-23.49	-175.37	26.01	-3.63	-25.61	-68.86	**17.17**	-2.33	-36	-466.67	**36.87**	11.39
AGXYL	4.38	-46.42	-29.89	-187.62	**51.60**	**51.45**	-26.95	**-32.05**	16.83	**12.54**	-8.67	-193.40	-7.73	-46.47

Bolded and underlined values represent highest and lowest heterosis combiners respectively for each trait. PH = Plant height at first fruiting; LOF = Length of pod; DG = Days to 50% germination; FFW =Fresh fruit weight; NOB= Number of branches per plant; NSPP = number of seeds per pod; DF=days to 50% fruiting.

Table 4.2 shows estimates of mid-parent heterosis (MPH) and better-parent heterosis (BPH) expressed by 27 F_1 hybrids obtained from intra-specific hybridisation among four cultivars of *A. caillei* and six *A. esculentus* for six agro-morphological quantitative traits. Generally, extensive variation was recorded with respect to both MPH and BPH ranging from -55.75 to 102.12 and -107.81 to 102.78 respectively. Most crosses produced positive MPH for length of pod (LOF), length of petiole (LOPE), plant height (PH) and days to 50% germination (DG), but relatively few of the F_1 offspring gave positive values for BPH. The cross T3 x T1 produced the highest MPH (102.78) and BPH (102.12) values for DG, while the cross T3 x AG (-107.81) recorded the least values for NSPP and the cross ID x VT (-55.75) for BPH and MPH respectively. Values recorded by the crosses ID x AG and T2 x AG (1.33and 18.65) as well as the cross T3 x T2 (-46.54 and-22.99) gave the highest and least values for BPH and MPH respectively for LOPE.

Again, the crosses T3 x VT and VT x AG recorded the highest MPH (30.25) and the cross T3 x T4 the highest BPH (7.32) for LOP, while ID x VT and AG x T3 (-55.75and -75.26) gave the least MPH and BPH values respectively.

Similarly, the cross T3 x T1 registered the highest MPH (102.78) and BPH (102.12) for DG, while the crosses ID x VT and AG x ID gave the least values (-30.37 and-86.67).

With respect to NSPP values, the cross T2 x T3 (13.24 and 32.73) gave the highest and the least MPH and BPH values respectively.

Also, the cross T2 x T3 (44.92 and 47.30) scored highest and least values for PH, while the cross T3 x AG (18.75 and 5.05) gave the highest MPH and BPH values and the crosses VT x AG and ID x AG (-27.40 and -45.27) least for stem FFW.

Table 4.2: Estimates of percent (%) Mid Parent Heterosis (MPH) and Better Parent Heterosis (BPH) expressed by 27 F1 offspring of okra for six quantitative traits.

Offspring	LOPE		LOP		DG		NSPP		PH		FFW	
	MPH	BPH	MPH	BPH	MPH	BMP	MPH	BPH	MPH	BPH	MPH	BPH
T2 X T1	2.19	-15.03	10.02	4.48	30.95	19.44	27.90	3.52	8.58	2.25	8.69	4.47
T3 X T1	-4.72	-21.91	-0.41	-5.75	**102.78**	**102.12**	19.68	-17.35	-4.49	-16.65	-8.73	-10.71
VT X T1	0.35	-17.02	6.78	-9.24	3.70	-23.33	-18.35	-41.16	11.48	4.29	-0.02	-1.53
AG X T1	-6.99	-38.24	4.55	-60.08	5.56	-38.33	-10.31	-24.27	15.73	7.10	-11.29	-28.93
T1 X T2	-20.45	-41.49	-10.78	-17.64	46.43	30.55	-5.69	-13.64	37.29	32.94	3.60	-0.79
T3 X T2	-22.99	-46.54	-17.34	-21.06	30.16	22.22	24.57	2.93	14.02	11.57	-2.13	-7.29
ID X T2	18.62	-9.36	-33.94	-41.12	-1.58	-16.67	-21.63	-35.50	0.51	-4.62	0.95	-13.07
VT X T2	-13.31	-37.67	-11.97	-31.69	8.33	-22.22	-9.14	-46.97	-8.18	-25.66	5.12	-0.12
AG X T2	-18.63	-79.92	-3.03	-75.26	-2.26	-80.56	-4.83	-28.23	15.66	0.00	-11.29	-22.83
T1 X T3	-13.24	-16.59	-27.81	-34.83	13.23	0.00	11.87	-28.71	-3.03	-15.03	-7.20	-9.20
T2 X T3	6.94	-11.07	6.61	3.75	101.59	100.0	**32.73**	**13.24**	**47.30**	**44.92**	-20.39	-27.94
VT X T3	1.053	-6.38	0.97	-19.19	-7.41	-19.45	-8.20	-103.84	24.62	8.09	-25.00	-27.04
AG X T3	9.66	-13.60	-1.66	-79.97	-3.03	-83.33	-12.81	-84.56	27.42	9.79	-13.81	-34.05
T1 X ID	1.54	-8.63	-9.73	-14.06	-23.15	0.00	-1.35	-26.71	10.01	-1.97	1.48	-18.22
T2 X ID	11.42	-18.28	10.15	4.08	49.21	41.67	-11.6	-24.79	12.45	8.12	-21.26	-38.48
VT X ID	1.71	-7.14	2.08	-7.35	-23.70	-40.00	-31.41	-102.25	4.52	-17.23	-19.74	-36.32
AG X ID	12.68	-3.87	6.73	-47.17	-26.50	-86.67	-8.99	-51.63	11.54	-11.19	-10.71	-14.56
T1 X VT	-8.54	-14.09	2.02	-14.39	-3.70	-16.67	-29.47	-54.44	5.38	-2.20	-27.40	-12.76
T2 X VT	14.11	-4.47	-7.93	-10.63	77.78	72.22	-15.42	-54.90	17.37	3.967	-14.40	-20.72
T3 X VT	1.01	-6.35	22.54	**7.32**	0.00	-33.33	-6.65	-94.19	8.54	-8.50	-1.07	1.91
ID X VT	-4.7	-14.24	-55.75	-12.02	-30.37	-46.67	-6.45	-50.01	14.34	-5.07	-19.81	-45.27
VT X AG	12.24	-13.19	**30.25**	-0.69	-5.56	-43.33	-34.66	-47.58	-18.42	-20.54	14.75	0.22

Bolded and underlined values represent highest and lowest heterosis combiners respectively for each trait. PH = Plant height at first fruiting; LOP = Length of pod; DG = Days to 50% germination; FFW = Fresh fruit weight; NOB= Number of branches per plant; NSPP = number of seeds per pod; DF=days to fruiting.

Offspring	**LOPE**		**LOP**		**DG**		**NSPP**		**PH**		**FFW**	
	MPH	BPH	MPH	BPH	MPH	BMP	MPH	BPH	MPH	BPH	MPH	BPH
AG X VT	2.52	-25.19	12.48	2.68	-2.23	-31.11	-26.90	-41.88	-2.95	-5.87	-18.48	-38.45
T1 X AG	5.00	-20.82	5.64	-57.7	20.94	-16.67	2.15	-7.91	0.58	-9.56	18.04	5.05
T2 X AG	**18.65**	-26.92	26.92	-23.06	8.59	-63.89	-4.18	-7.74	-10.43	-30.67	-17.32	-29.51
T3 X AG	18.43	0.22	45.32	-18.26	-19.70	-77.78	-26.61	-107.81	3.01	-20.53	**18.72**	**5.64**
ID X AG	18.02	**1.33**	7.68	-2.36	0.00	-46.67	-34.59	-86.64	-15.33	-44.39	1.18	-1.97

Bolded and underlined values represent highest and lowest heterosis combiners respectively for each trait. PH = Plant height at first fruiting; LOP = Length of pod; DF = Days to 50% germination; FFW = Fresh fruit weight; NOB= Number of branches per plant; NSPP = number of seeds per pod; DF=days to fruiting.

4.4 Discussion

4.4.1 Expression of Heterosis among F_1 offspring

Analysis of variance (ANOVA) (appendix 6a and 6b) exhibited significant differences among treatments for all the characters in all the crosses under study. These results are in agreement with observations of Louis *et al*., (2013) and Mostofa *et al*., (2011) who also recorded significantly high heterosis over mid and better parent(s) for all studied traits. This indicated the presence of appreciable genetic diversity for the characters showing significant variances. Wide genetic variability among the parents utilised for the hybridisation could have accounted for greater hybrid vigour of the F_1 offspring (Hallauer and Miranda, 1988). Higher yield is the basic objective of all crop improvement programmes and unless a new hybrid has a potential equal to or exceeding that of current cultivar or hybrid, it will fetch no success even if it has excellent quality.

The cross, T3 x T1 came out with the highest BPH for days to 50% germination while the crosses AG x T4, AG x YL and T2 x T3, recorded highest BPH for (days to 50% flowering and number of branches per plant), (length of pod and Fresh fruit weight) and (Plant height and number of seeds per pod) respectively.

From the mean performance of the genotypes, it is evident that, in general, the mean values of crosses were desirably higher than those of some parents for days to 50% germination, fruit length, average fresh fruit weight and harvest duration.

Therefore, it can be inferred that heterosis breeding would be advantageous in okra improvement compared to the open pollinated cultivars as hybrids will have the advantage of higher yields together with uniform maturity, size and color of the fruits. The results suggest that heterosis for fruit yield is obtained through component heterosis. Even the slight hybrid vigor for individual yield components may have additive or synergistic effects on yield. The study further demonstrates the presence of heterosis for important quantitative traits in okra. In the present study, the significance of the heterotic performance was highly affected by the genetic background of parental genotypes. The high heterosis among these germplasm for most of the characteristics studied indicates that considerable potential exist in these materials for developing hybrids. The results suggest that yield of okra can be substantially increased through heterosis breeding. It also suggests that hybrid vigor is available for commercial production of hybrid okra and that isolation of pure lines from the progenies of heterotic F_1s is a possible way to enhance the fruit yield of okra.

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CHAPTER FIVE

MORPHOLOGICAL CHARACTERISATION OF ACCESSIONS OF OKRA (*Abelmoschus Spp* L.).

5.1 INTRODUCTION

Characterisation of genetic resources refers to the process by which accessions are identified, differentiated or distinguished according to their characteristics (Oseikita and Akinyele, 2008). Characterisation and identification of genetic variability within germplasm collections are a preliminary requirement for the exploitation of useful traits in plant breeding (Oka, 1991).

The key objectives of okra germplasm characterisation have generally been to identify high yielding genotypes with resistance to yellow vein mosaic virus (YVMV), fruit borer (*Spodoptera spp*.), jassid (*Cicadellidae*) and higher vitamin C content in the species that can be utilised for the improvement of a crop (Nwangburuka *et al*., 2011; Bisht and Bhat, 2006). According to Beeching *et al*., (1993) one important aspect of crop improvement is assessment of genetic diversity of desirable characteristics such as increased yield, wide adaptability, pests and diseases resistance among other traits which exist within populations of crop species.

Among other purposes, characterisation plays a key role in investigations of genetic diversity patterns and identification of duplicates within crop germplasm collections. It also facilitates studies of correlation among characteristics of agronomic importance

(CIAT, 2007). The efficacy of the method deployed for the characterisation process largely determines the potential genetic value of a particular germplasm (De vicente *et al,* 2005).

Generally, characterisation and genetic assessment is centered on use of genetic markers; any measurable character (phenotypic or genotypic whose inheritance can be traced through different generations) which is capable of detecting a variation in either a protein or DNA sequence to identify the characteristics of a genetic material (De vicente *et al.*, 2005).

Morphological markers are the traditionally accepted and proven tools as a first step, among a host of other techniques, for characterisation of plant germplasm. Despite the challenge of their ambiguity due to contribution of multiple genes and modifications or interactions with the environment, they still remain very useful primary methods for germplasm characterisation (Staub *et al*., 1996). They constitute the most readily available technique, thus published descriptor lists are readily available for most major crop species including okra. Characterization based on phenotypic traits is not easily reproducible, particularly, since they are affected by environmental variations (Staub *et al*., 1996). In addition, it requires a large tract of land or greenhouse space to grow large populations of plants; it is labour intensive and difficult to manage (Ahiakpa, 2009; Vogel *et al*., 1996). However, the techniques require little skills and are relatively inexpensive to carry out (Hoogendijk and William, 2001).

Morphological characterisation of crops is facilitated by the use of standard descriptors, which provide an international format for producing a universally understood language for plant genetic resources (De vicente *et al*., 2005). Notwithstanding, the technique remains useful as a highly recommended first step to be undertaken prior to more in-depth biochemical or molecular studies in okra germplasm (Oppong-Sekyere, 2011; Smith and Smith, 1992).

A number of morphological, biochemical and molecular (DNA) markers have been developed and widely used to investigate diversity in plant genetic resources. However, resolution of diversity using biochemical analyses has received little attention due to their reliance on proteins/enzymes which are usually limited for most traits in plant germplasm (De vicente *et al.,* 2005).

On the contrary, molecular techniques which comprise a large variety of DNA-based markers are very efficient for analysis of variation in germplasm collections due to their ability to detect or amplify anonymous loci (expressed or non-expressed sequences). However, due to requirement of high expertise and sophisticated facilities, molecular techniques are very expensive to carry out (Soni *et al*., 2010). This study seeks to use morphological characterization to ascertain the differences in characteristics of the accessions utilised and their F_1s.

5.1.2 Objective of the study

The main objective of this study was to morphologically characterise ten local landraces of okra (*Abelmoschus esculentus* and *caillei)* and their 51 F_1offspring obtained from both intra-specific and inter-specific hybridisation in order to identify promising genotypes for selection. The specific objectives were to:

1. Assess the level of genetic variability among the accessions for fifteen qualitative and eight quantitative traits in order to identify and recommend lines with superior agro-morphological characteristics for selection.

2. Determine genetic relatedness among parental accessions and the F_1 offspring in order detect possible duplicates.

3. Study the degree of association between the quantitative traits to determine appropriate strategies to adopt in future hybridisation programmes towards improvement of useful traits.

4. Identify agro-morphological parameters which contribute most to total variability among the accessions to be utilised in future breeding work

5.2 MATERIALS AND METHODS

5.2.1 Experimental site

The research was carried out at the research farm of Biotechnology and Nuclear Agriculture Research Institute (BNARI) of Ghana Atomic Energy Commission (GAEC), Kwabenya, near Accra. The experimental site is located at 05º 40' N and longitude 0º13'W at an elevation of 76 m above sea level within the coastal savannah agro-ecological zone. The soil at the Nyigbenya-Haatso series, which is typically well-drained savanna Ochrosol (Ferric Acrisol), derived from quartzite and Schist (FAO/UNESCO, 1994).

5.2.2 Weather Conditions

The maximum and minimum average temperatures for the period (April, 2014-January, 2015) of the experiment were 30.10ºC and 23.20ºC, respectively. The mean annual rainfall was 220 mm. Average relative humidity, sunshine and wind speed for the same period were 42.00, 195.35 w/m^2 and 742.72 m/s, respectively (Local Weather Station Data, 2015).

5.2.3 Experimental Design

The Randomised Complete Block Design was used with four replications.

5.2.4 Field Management Practices

Ten (10) parents and fifty one (51) F1s of okra accessions were planted on an 85.4 m x 25 m size of land.

No fertiliser was applied, but weeds were controlled and other agronomic and management practices were carried out. Weeding was done fortnightly. The rainfall pattern during this period was quite consistent and regular hence there was no need for supplementary water to support plant growth and development.

5.2.5 Seed Sowing

Seeds of the various accessions were sown on 21st August 2014 for parents and F_1s after a heavy downpour of rainfall that facilitated uptake of water by the seeds and boosted germination. The seeds were sown at a depth of 2 cm, at a spacing of 0.70 m x 0.50 m between and within rows with three to four seeds per hill and thinned to two per hill after germination.

5.2.6 Data Collection

Data were collected using the International Plant Genetic Resources Institute (IPGRI, 1991) Descriptor List for okra. Data were taken on 23 characters, which include the following parameters and were grouped into four growth stages of the plant;

(a) Vegetative characters: general aspect of the plant, branching type (BRT), stem pubescence, stem colour, leaf shape and leaf colour. Data were taken on these characters prior to first fruiting of all accessions ensuring that all accessions within each block receive uniform treatment.

(b) Inflorescence characters: number of epicalyx segments (NES), shape of epicalyx segments (SES), persistence of epicalyx segments (PES), petal colour, and colouration of petal base.

(c) Reproductive characters: days to 50% germination (DG), maximum plant height (cm) (PHAFF). A tape measure was employed in taking plant height at the fruiting stage.

(d) Fruit characters: position of fruit on main stem (PFMS), fruit colour, fruit length at maturity, length of peduncle, fruit shape, number of ridges per fruit (NRPP), fruit pubescence and number of days to 50% fruiting (DG). Position of fruit on main stem was determined on five data plants prior to harvesting of fruits. Measuring rule was used for measuring length of pods and peduncle of pods after harvest. Number of ridges was recorded by counting the quantity of ridgeline or natural striations through the fruit and then coded accordingly. This was done on five pods of each accession. Data on fruit pubescence was taken by visual assessment of hairiness or smoothness of pods and a practical hand'feel' on harvested fresh fruits.

5.2.7 Data analysis

Qualitative data were evaluated based on the morphological descriptors to identify the extent of variation within the parents and F_1 populations for the selected qualitative traits. The quantitative data were subjected to Analysis of variance (ANOVA) to determine the level of significance of variability for the various parameters. A p-value of 0.05 or less was considered as statistically significant. Duncan's multiple range test was deployed to determine differences among means. Cluster analysis based on similarity matrices was performed to generate a dendrogram in order to determine genetic relationships among

the genotypes. Correlation analysis was also carried out to determine degree of association between the quantitative agro-morphological traits. Contribution of each trait to total genetic diversity within the populations studied was determined through principal component analysis based on correlation matrix of agro-morphological variables. Statsgraphics Centurion software (version 16.1) and Microsoft Excel Software (2010 edition) were used for the Data analyses.

5.3 RESULTS

5.3.1 Morphological traits of dry pods of accessions

The photographs below show phenotypic characteristics of dried fruits of F_1s and some parents at maturity.

AG × KB AG × AM

VT × AG VT × T4

Figure 5.1: Photographs of dried fruits of okra accessions.

Figure 5.1: (cont'd)

T2 × YL

AG × T4

AG

KB

Figure 5.1: Photographs of dried fruits of okra accessions.

5.3.2 Variations in morphological characteristics of some of the parental accessions in the field.

The photographs below show phenotypic characteristics of parental accessions growing on the field

VT

T1

Figure 5.2 Photographs of accessions with fruits on the field

Figure 5.2(cont'd)

T3

T4

ID

AG

Figure 5.2 Photographs of accessions with fruits on the field

5.4 Variation in eight quantitative traits among 33 accessions of okra *(Abelmoschus esculentus)* including F1 offspring.

Table: 5.1.shows data on comparative performance of 33 okra genotypes comprising 6 parents and 27 F_1s. The analysis of variance revealed that the differences among the selected genotypes were very highly significant (appendix-6a and 6b) for all the characters investigated, indicating the presence of variability among them.

There were very highly significant differences (p<0.001) in the mean number of days taken for seedlings of the 33 accessions of okra to emerge. Genotype AG took the longest mean number of 8.33 days to emerge while the crosses ID x VT, T3 x T3 and T2 x T2 recorded the shortest mean number of 3.33 days.

Growth parameters differed significantly among the okra accessions. The tallest plants at 50% flowering were recorded for the cross AG x T1 with a mean value of 44.57. This was closely followed by those of T1 x T2 and AG x T3 with mean heights of 44.30 cm and 41.83 cm respectively. The least value was recorded for ID a parental generation with a mean value of 27.77 cm. Mean square estimates for plant height at flowering showed very highly significant (p<0.001) differences amongst the 33 parents and their offspring.

Number of days to 50% flowering differed very highly significantly (p<0.001) among the parents and F_1 varieties of okra evaluated. The cross T1 x AG recorded the highest mean number of days to flower with a value of 46.33 days and accession ID had the least mean value of 31.70 days.

With respect to pod length, it differed very highly significantly (p<0.001) among the 33 okra varieties. Pods of VT x T2 gave the heighest mean value of 18.33 cm, while AG had the least mean value of 6.57 cm. There were also very highly significant (p<0.001) differences in the average number of seeds per pod among the 33 accessions of okra evaluated. The crosses VT x VT and ID x AG the highest and least mean values of 66.33 and 29.00 respectively.

Table 5.1: Variability in eight Quantitative Traits among 6 accessions and 25 inta-specific hybrids of okra

Accession	LOF	*LOPE*	NSPP	FFW	DF	DOP	PHAFF	DG
T1	14.83±1.61^{hijk}	3.53±0.06^{g}	48.00±1.73^{jklm}	39.67±0.90^{mn}	41.33±0.58^{jk}	3.13±0.15^{jklm}	34.73±1.12^{defgh}	4.33±0.58^{jk}
T2 x T1	16.70±0.96^{imnop}	4.23±0.26^{hi}	49.33±6.03^{jklm}	41.30±0.92^{no}	35.33±0.58^{cde}	2.63±0.78^{defgh}	35.47±1.11^{efghi}	4.67±0.58^{cde}
T3 x T1	15.47±1.72^{ijklm}	3.33±0.15^{dfg}	43.67±2.08^{ghijk}	36.78±1.35^{jk}	39.33±2.52^{ghij}	2.47±0.21^{bcdef}	30.03±1.00^{abcd}	7.67±0.58^{ghij}
VT x T1	14.13±1.10^{ghij}	3.43±0.31^{efg}	46.67±1.53^{ijkl}	40.00±1.67^{mno}	38.33±2.08^{fghi}	2.80±0.27^{fghijk}	41.83±0.76^{klmno}	4.33±0.58^{fghi}
AG x T1	11.07±1.69^{bcd}	2.80±0.10^{b}	45.33±0.58^{hijk}	31.19±0.28^{fg}	41.33±1.53^{jk}	2.10±0.10^{ab}	44.30±1.35^{mno}	6.67±0.58^{jk}
T1 x T2	13.53±1.56^{gh}	3.30±0.26^{defg}	42.67±1.52^{fghij}	39.36±1.40^{lmn}	37.33±2.08^{efg}	3.00±0.10^{hijklm}	44.83±3.75^{o}	5.33±0.58^{efg}
T2	15.63±1.03^{jklmn}	4.77±0.25^{j}	39.33±2.08^{efgh}	36.35±0.99^{ijk}	37.67±1.15^{efgh}	3.07±0.31^{ijklm}	30.60±1.04^{acbd}	3.33±0.58^{efgh}
T3 x T2	13.23±0.85^{fgh}	3.17±0.06^{bcdefg}	40.00±2.00^{efghi}	38.25±0.82^{klm}	38.33±1.15^{fghi}	2.67±0.31^{defgh}	33.90±1.75^{cdef}	4.33±0.58^{fghi}
ID x T2	10.03±0.55^{b}	4.50±0.17^{hij}	28.00±3.00^{ab}	32.66±0.78^{gh}	32.67±1.15^{ab}	2.67±0.15^{defgh}	29.30±0.61^{abc}	3.67±0.58^{ab}
VT x T2	18.33±2.52^{op}	3.50±0.27^{fg}	48.00±2.00^{jklm}	40.29±1.58^{mno}	37.00±1.73^{defg}	2.50±0.10^{cdef}	32.57±1.50^{abcde}	4.67±0.58^{defg}
AG x T2	10.67±0.61^{bc}	2.83±0.10^{bc}	45.67±1.53^{hijk}	29.47±1.55^{cdef}	40.67±1.15^{ij}	3.07±0.12^{ijklm}	41.87±2.01^{klmno}	5.67±0.58^{ij}
T1 x T3	11.23±0.97^{bcd}	3.03±0.06^{bcde}	37.33±0.58^{defg}	37.37±2.23^{jkl}	37.67±0.58^{efgh}	2.77±0.31^{efghij}	30.47±1.50^{abcd}	4.33±0.58^{efgh}
T2 x T3	17.07±0.21^{mnop}	4.40±0.20^{hij}	42.67±1.53^{fghij}	30.75±0.76^{efg}	35.67±0.58^{cbe}	3.33±0.22^{m}	43.27±1.62^{lmno}	6.67±0.58^{cde}
T3	16.40±0.66^{klmno}	3.47±0.31^{fg}	25.00±1.00^{a}	40.90±0.86^{no}	43.67±2.31^{kl}	3.23±0.25^{lm}	28.17±0.76^{ab}	3.33±0.58^{kl}
VT x T3	16.40±0.66^{ghij}	3.43±0.06^{efg}	40.33±4.51^{efhi}	30.44±0.97^{def}	40.67±0.58^{ij}	3.10±0.20^{ijklm}	42.67±2.39^{klmno}	4.00±0.00^{ij}
AG x T3	11.30±0.80^{bcde}	3.10±0.27^{bcdf}	35.67±5.03^{cdef}	27.33±0.47^{bc}	43.67±1.53^{kl}	2.13±0.15^{abc}	44.57±2.38^{no}	5.67±0.58^{kl}
T1 x ID	12.93±1.01^{efg}	3.23±0.15^{cdefg}	39.33±4.51^{efgh}	34.51±0.80^{hi}	38.67±1.15^{fghi}	2.03±0.06^{a}	34.50±9.26^{defg}	3.33±0.58^{fghi}
T2 x ID	16.33±0.65^{klmno}	4.23±0.49^{hi}	31.33±3.21^{abcd}	24.81±1.44^{a}	34.00±1.73^{bc}	3.17±0.21^{klm}	32.83±3.82^{bcde}	5.67±0.58^{bc}
ID	14.03±0.71^{ghij}	2.83±0.06^{bc}	32.00±1.00^{bcd}	28.35±1.20^{bcd}	31.70±0.58^{a}	2.97±0.15^{hijklm}	27.77±1.45^{a}	4.33±0.58^{a}
VT x ID	13.10±0.79^{fg}	3.13±0.06^{bcdefg}	33.67±0.58^{bcde}	27.57±1.06^{bc}	36.33±1.53c^{def}	2.67±0.21^{defgh}	35.47±5.50^{efghi}	3.67±0.58^{cdef}
AG x ID	10.97±0.85^{bcd}	2.83±0.12^{bc}	40.33±4.51^{efghi}	26.68±0.73^{ab}	39.00±1.00^{ghij}	2.33±0.21^{abcd}	38.83±1.56^{ghijkl}	4.67±0.58^{ghij}
T1 x VT	13.50±0.50^{gh}	3.13±0.12^{bcdefg}	40.33±3.51^{efghi}	35.61±0.75^{ij}	37.00±1.00^{defg}	2.63±0.12^{defgh}	39.57±3.17^{hijklm}	4.67±0.58^{defg}
T2 x VT	12.60±1.06^{defg}	4.60±0.26^{ij}	44.67±4.51^{hijk}	32.85±2.55^{gh}	35.33±0.58^{cde}	2.90±0.20^{ghijkl}	41.60±3.50^{klmno}	7.67±0.58^{cde}
T3 x VT	17.20±0.53^{nop}	3.43±0.06^{efg}	42.67±6.66^{fghij}	42.06±1.60^{o}	37.00±1.00^{defg}	3.13±0.21^{jklm}	38.00±2.00^{fghijk}	4.33±0.58^{defg}
ID x VT	12.60±0.46^{befg}	2.93±0.06^{bcd}	50.33±5.51^{klm}	27.56±0.71^{bc}	34.67±0.58^{bcd}	2.90±0.10^{ghijkl}	38.97±3.41^{ghijkl}	3.33±0.58^{bcd}

LOF = Length of pod LOPE = Length of petiole NSPP = Number of seeds per plant FFW = Fresh Fruit weight DF = Days to 50% flowering DOP = Diameter of pod PHAFF = Plant height at 50% flowering DG = Days to 50% germination

Table 5.1 continued

Accession	LOF	*LOPE*	NSPP	FFW	DF	DOF	PHAFF	DG
VT	11.77±1.44^{cdef}	3.33±0.32^{defg}	66.33±2.08^{o}	40.36±1.14^{mno}	38.67±1.53^{fghi}	2.77±0.25^{efghij}	40.37±1.42^{jklmno}	5.33±0.58^{fghi}
AG x VT	10.33±1.53^{bc}	2.83±0.22^{bc}	45.00±1.00^{hijk}	28.74±0.42^{bcde}	38.33±0.58^{fghi}	2.13±0.12^{abc}	39.90±4.55^{ijklmn}	6.67±0.58^{fghi}
T1 x AG	11.27±0.87^{bcd}	3.03±0.06^{bcde}	53.00±8.00^{lmn}	41.18±1.60^{no}	46.33±0.58^{m}	2.73±0.25^{efghi}	38.50±2.50f^{ghij}k	7.67±0.58^{m}
T2 x AG	13.97±1.11^{ghi}	4.13±0.32^{h}	53.67±4.16^{mn}	27.46±1.09^{bc}	36.33±2.08^{cdef}	2.83±0.06^{fghijk}	32.47±3.44^{abcde}	6.33±0.58^{cdef}
T3 x AG	15.33±0.72^{ijkl}	3.40±0.30^{efg}	30.00±2.00^{abc}	41.52±1.50^{no}	41.33±1.53^{ij}	2.80±0.20^{fghijk}	36.03±2.51^{efghij}	4.67±0.58^{jk}
ID x AG	11.07±0.86^{bcd}	2.97±0.21^{bcd}	29.00±1.00^{abc}	29.59±0.80^{cdef}	37.33±1.15^{efg}	2.53±0.31^{defg}	29.60±2.25^{abc}	6.33±0.58^{efg}
VT x AG	11.93±0.95^{p}	3.10±0.10^{bcdef}	43.67±3.06^{ghijk}	40.44±1.34^{mno}	40.00±1.00^{hij}	2.47±0.06^{bcdef}	34.50±4.27^{defg}	6.00±0.00^{hij}
AG	6.57±1.01^{a}	2.20±0.30^{a}	57.00±7.21^{n}	30.25±0.39^{def}	45.33±0.58^{lm}	2.40±0.20^{abcde}	41.87±1.03^{klmno}	8.33±0.58^{lm}
Mean	13.12±2.52	3.40±0.63	42.12±9.25	34.29±5.60	38.42±3.50	2.73±0.39	36.65±5.70	5.20±1.48
CV%	19.18%	18.32%	21.97%	16.32%	9.12%	14.41%	15.56%	28.40%

LOF = Length of pod LOPE = Length of petiole NSPP = Number of seeds per plant FFW = Fresh Fruit weight DF = Days to 50% flowering DOF = Diameter of fruits PHAFF = Plant height at 50% flowering DG = Days to 50% germination

5.4.1 Variation in seven quantitative traits among 28 accessions of okra *(Abelmoschus caillei)* including F_1 offspring.

Table 5.2 shows variation in seven quantitative agro-morphological traits of 4 accessions of okra and 24 F_1 offspring obtained from inter-specific hybridisation among the accessions. Very highly significant variations ($p \leq 0.001$) were observed among the accessions for all 7 quantitative traits.

Generally, values for days to first flowering (DF), number of branches per plant (NBPP), plant height at 50% flowering (PHAFF) and Days to 50% germination (DG), recorded for parental accessions were higher than those recorded for the F_1 offspring. However, for fresh fruit weight (FFW), length of pod (LOF) and number of seeds per plant (NSPP) values obtained for the F_1 offspring were comparatively higher than those of parental accessions. Overall, T4, VT x YL, YL, T3xT4, recorded highest values for (FFW, NSPP, DF, and LOF) respectively, while KB gave the highest values for (PHAFF, NOB and DG) respectively. On the contrary, AG x T4, ID x T4, T3 x KB, T3 x T4 and YL recorded lowest values for PHAFF, FFW, and NSPP, (DG and LOF) respectively.

Similarly, ID x T4 registered the lowest values for (NOB and DF).

Table 5.2: Variability in Quantitative Traits among 28 accessions of Okra obtained from inter-specific hybridization

Accession	LOF	DG	DF	NOB	NSPP	FFW	PHAFF
AM	12.43±0.45^{fgh}	8.67±0.58^{h}	65.33±2.52^{j}	23.00±2.00^{de}	59.67±1.53^{ij}	38.83±1.23^{jkl}	119.67±4.51 ^{h}
T1xAM	13.33±0.42^{hijk}	5.33±0.58^{de}	46.33±4.04^{de}	13.33±2.52^{abcd}	52.33±2.08^{gh}	40.12±1.24^{jkl}	95.90±13.67^{g}
T2xAM	12.60±.36^{gh}	3.67±0.58^{abc}	53.00±5.29^{abc}	10.33±2.52^{ab}	35.33±2.52^{abc}	34.93±2.30^{fghi}	60.50±6.38^{bcde}
T3xAM	14.53±0.35^{lmn}	3.33±0.58^{ab}	42.00±3.00^{abcde}	16.00±4.00^{bcd}	34.00±3.61^{ab}	40.70±1.40^{kl}	76.33±25.11^{efg}
IDxAM	11.40±0.40^{def}	3.33±0.58^{ab}	46.67±6.66^{de}	7.67±2.52^{ab}	39.00±3.61^{bcd}	27.58±0.58^{ab}	51.63±2.97^{abcd}
VTxAM	13.80±0.26^{ijklm}	4.67±0.58^{cd}	43.67±3.51^{bcde}	13.00±2.65^{abcd}	65.67±5.13^{jk}	39.39±1.11^{jkl}	51.33±9.02^{abcd}
AGxAM	9.63±0.47^{b}	6.67±0.58^{f}	39.00±2.00^{ab}	8.67±1.53^{ab}	69.67±1.53^{kl}	33.27±3.01^{defg}	60.67±13.80^{bcde}
T4	14.50±0.50^{lmn}	7.67±0.58^{g}	44.67±2.08^{cde}	6.33±1.53^{ab}	47.67±2.52^{efg}	40.80±2.17^{l}	60.33±5.51^{bcde}
T1xT4	15.03±0.61^{no}	3.67±0.58^{abc}	40.33±1.53^{abc}	7.00±2.00^{ab}	54.67±1.53^{ghi}	39.35±1.19^{jkl}	47.63±4.25^{abcd}
T2xT4	12.80±0.20^{ghij}	4.67±0.58^{cd}	38.67±1.53^{ab}	8.00±2.00^{ab}	51.67±4.51^{fgh}	36.48±0.58^{ghij}	44.67±3.51^{abc}
T3xT4	15.93±1.22^{o}	3.00±0.00^{a}	37.00±2.00^{a}	4.67±1.53^{ab}	45.00±5.00^{def}	39.71±1.07^{jkl}	53.33±6.11^{abcd}
IDxT4	11.77±0.25^{efg}	3.33±0.58^{ab}	36.67±1.15^{a}	1.67±1.53^{a}	37.33±4.51^{bc}	26.82±0.85^{a}	42.00±2.65^{abc}
VTxT4	13.83±0.21^{jklm}	4.67±0.58^{cd}	43.33±1.53^{bcde}	6.33±27.78^{de}	65.00±3.61^{jk}	38.36±1.58^{ijkl}	40.63±1.18^{abc}
AGxT4	10.90±0.60^{cde}	5.33±0.58^{de}	45.33±1.15^{cde}	7.67±1.15^{ab}	58.00±6.00^{hi}	32.85±1.00^{def}	35.00±19.47^{a}
KB	11.50±0.50^{def}	10.33±0.58^{i}	75.33±3.51^{k}	30.00±2.00^{e}	69.33±2.08^{kl}	37.10±2.78^{hijk}	177.67±3.21^{i}
T1xKB	14.60±0.36^{mn}	4.30±0.58^{bcd}	47.33±2.08^{e}	12.00±3.61^{abcd}	41.67±3.79^{cde}	39.65±1.13^{jkl}	52.10±16.23^{abcd}

LOF = Length of pod DG = Days to 50% germination DF = Days to 50% flowering NOB = Number of branches NSPP = Number of seeds per pod FFW = Fresh Fruit weight PHAFF = Plant height at 50% flowering.

Table 5.2 continued

Accession	LOF	DG	DF	NOB	NSPP	FFW	PHAFF
T2XKB	14.53±0.57^{lmn}	3.67±0.58^{abc}	52.00±2.65^{f}	12.67±1.53^{abcd}	54.67±5.03^{ghi}	36.65±2.90^{ghij}	53.00±4.00^{abcd}
T3XKB	14.10±0.66^{klmn}	4.00±0.00^{abc}	63.33±2.52^{ij}	11.00±2.65^{abc}	30.00±3.61^{a}	38.99±1.34^{ijk}	54.00±5.57^{abcd}
IDXKB	13.47±0.76^{hijk}	3.67±0.58^{abc}	37.00±2.00^{a}	4.67±2.52^{ab}	38.33±6.51^{bcd}	31.90±2.94^{cdef}	47.67±2.52^{abcd}
VTXKB	12.73±0.85^{ghi}	4.67±0.58^{cd}	41.67±2.08^{abcd}	13.00±4.58^{abcd}	70.00±2.00^{kl}	39.81±0.78^{ijk}	77.07±16.49^{efg}
AGXKB	10.03±0.15^{bc}	6.33±0.58^{ef}	46.33±1.53^{de}	9.00±2.02^{ab}	67.67±2.08^{kl}	31.37±1.69^{cde}	57.00±6.56^{bcde}
YL	6.53±0.50^{a}	9.00±1.00^{h}	77.00±2.00^{k}	22.00±1.73^{cde}	38.00±3.00^{bcd}	27.44±1.44^{ab}	134.67±5.51^{h}
T1XYL	11.10±0.80^{de}	6.00±0.00^{ef}	61.33±2.08^{hij}	13.33±4.16^{abcd}	55.00±5.00^{ghi}	29.24±1.55^{abc}	92.50±7.37^{g}
T2XYL	10.63±0.51^{bcd}	5.33±0.58^{de}	60.67±2.52^{hij}	14.33±2.08^{bcd}	38.33±2.53^{bcd}	36.91±1.44^{hij}	85.67±11.68^{fg}
T3XYL	13.40±0.80^{hijk}	4.00±1.00^{abc}	58.00±2.65^{gh}	11.00±2.65^{abc}	34.33±4.51^{abc}	30.69±1.01^{bcde}	66.57±14.65^{def}
IDXYL	11.47±0.93^{def}	4.00±0.00^{abc}	46.33±3.06^{de}	9.00±2.65^{ab}	35.33±4.51^{abc}	29.75±1.18^{abcd}	56.60±11.57^{bcde}
VTXYL	11.53±0.45^{def}	5.33±0.58^{de}	53.67±3.51^{fg}	8.00±2.65^{ab}	73.00±6.25^{l}	39.72±1.73^{jkl}	68.87±21.84^{def}
AGXYL	9.93±0.67^{bc}	6.33±0.58^{ef}	59.67±1.53^{hi}	12.33±2.52^{abcd}	47.67±4.16^{efg}	33.71±5.10^{efgh}	63.63±4.41^{cde}
Mean	12.43±2.07	5.18±1.91	50.01±11.32	11.88±7.84	50.30±13.62	35.44±4.77	68.81±32.35
CV%	16.678%	36.9758%	22.6119%	65.9567%	27.0706%	13.452%	47.0216%

LOF = Length of pod, NSPP = Number of seeds per plant, DF = Days to first flowering, FW = Fresh Fruit weight, NOB = number of branches, PHAFF = Plant height at first fruit, DG = Days to 50% germination and NSPP = number of seed per plant.

5.4.2 Variation in qualitative traits among 10 accessions of okra (A. *esculentus* and *caillei*) and their F_1 offspring.

Table 5.3 shows variability of qualitative traits among 10 local accessions of okra (A. *esculentus* and *caillei*) and 51 F_1 offsprings obtained from different cross combinations. The accessions showed greatest variability with respect to fruit colour. In general, five groups of colour namely; red, green, deep green, green with red patches and yellowish were recorded across both parents and hybrids. Petal colour colour gave the least variation with two categories either yellow or cream but majority (66.67 %) expressed cream. Regarding leaf colour, a greater number of the parents and F_1s were green with 21% showing green with red patches.

Expression of pubescence on leaf and stem varied from glabrous, to conspicuous pubescence. However, most of the accessions produced slight pubescence. For stem pubescence, (50%) produced slight pubescence with the least (13.33%) being downy and rest prickly.

Pod length was categorised into very long, long, medium and small. (50 %) of the F_1 offspring produced very long or long, followed by medium with (30%) and (20%) recording small.

Table 5.3 Variation in qualitative agro-morphological traits among 10 local accessions of okra (*A. esculentus* and *A. caillei*) and their F_1 offspring.

Accessions	PES	SES	NES	RCPB	PC	FP	FC	PFMS	Stem pubescence	NRPPD	FS	Pod length	Leaf shape	Fruit peduncle	Leaf colour
T1	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Downy	Green	Erect	Slight	8 to 10	1	Very long	2	1 to 3cm	Green
T2 X T1	Persistent	Lanceolate	More than 10	Inside only	Cream	Downy	Green with red patches	Erect	Slight	8 to 10	1	Very long	3	1 to 3cm	Green with red veins
T3 X T1	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Slightly rough	Green	Erect	Glabrous	8 to 10	3	Very long	2	1 to 3cm	Green
VT XT1	Persistent	Triangular	More than 10	Inside only	Yellow	Prickly	Deep green	Erect	Slight	8 to 10	3	Medium	3	1 to 3cm	Green
AG X T1	Persistent	Triangular	More than 10	Both sides	Cream	Slightly rough	Green	Erect	Conspicuous	Non(smooth fruit)	12	Medium	2	1 to 3cm	Green
T1 X T2	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Downy	Green	Erect	Slight	8 to 10	1	Very long	2	1 to 3cm	Green
T2	Partially Persistent	Lanceolate	From 5 to 7	Inside only	Cream	Prickly	Red	Erect	Conspicuous	5 to 7	1	Very long	3	More than 3cm	Green with red veins
T3 X T2	Partially Persistent	Lanceolate	More than 10	Both sides	Yellow	Slightly rough	Yellowish green	Erect	Glabrous	8 to 10	1	Very long	2	1 to 3cm	Green
ID X T2	Persistent	Lanceolate	From 5 to 7	Both sides	Cream	Slightly rough	Green	Erect	Glabrous	5 to 7	3	Small	9	More than 3cm	Green
VT X T2	Partially Persistent	Lanceolate	More than 10	Inside only	Cream	Prickly	Deep green	Erect	Slight	8 to 10	3	Long	2	1 to 3cm	Green
AG X T2	Persistent	Triangular	More than 10	Both sides	Cream	Prickly	Green	Erect	Conspicuous	Non(smooth fruit)	12	Small	2	1 to 3cm	Green with red vein
T1 X T3	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Downy	Yellowish green	Erect	Glabrous	8 to 10	3	Medium	2	1 to 3cm	Green
T2 X T3	Partially Persistent	Lanceolate	More than 10	Inside only	Cream	Prickly	Red	Erect	Conspicuous	8 to 10	1	Medium	3	More than 3cm	Green with red vein
T3	Partially Persistent	Lanceolate	More than 10	Inside only	Yellow	Slightly rough	Yellowish green	Erect	Glabrous	8 to 10	3	Very long	2	1 to 3cm	Green
VT X T3	Persistent	Triangular	More than 10	Inside only	Cream	Prickly	Yellowish green	Erect	Slight	8 to 10	3	Very long	2	1 to 3cm	Green
AG X T3	Persistent	Triangular	More than 10	Both sides	Yellow	Slightly rough	Green	Erect	Conspicuous	Non(smooth fruit)	12	Medium	2	1 to 3cm	Green

NES= Number of Epicalyx Segment; RCPB = Red Colouration of Petal Base; PES = Persistence of Epicalyx Segment; SES = Shape of Epicalyx Segment; PFMS = Position of Fruit on Main Stem; NRPPD = Number of Ridges per Pod; FS =Fruit shape FP=Fruit Pubescence PC=Petal Colour.

Table 5.3 (continued)

Accessions	PES	SES	NES	RCPB	PC	FP	FC	PFMS	Stem pubescence	NRPPD	FS	Pod length	Leaf shape	Fruit peduncle	Leaf colour
T1 X ID	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Slightly rough	Green	Erect	Glabrous	8 to 10	1	Medium	9	1 to 3cm	Green
T2 X ID	Partially Persistent	Lanceolate	From 5 to 7	Inside only	Cream	Prickly	Red	Erect	Conspicuous	5 to 7	3	Very long	3	More than 3cm	Green
ID	Persistent	Lanceolate	From 5 to 7	Both sides	Cream	Slightly rough	Yellowish green	Erect	Glabrous	5 to 7	3	Very long	9	1 to 3cm	Green
VT X ID	Persistent	Triangular	More than 10	Inside only	Cream	Slightly rough	Green	Erect	Slight	8 to 10	3	Long	2	1 to 3cm	Green
AG X ID	Persistent	Lanceolate	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Erect	Conspicuous	Non(smooth fruit)	3	Small	2	1 to 3cm	Green
T1 X VT	Non-persistent	Lanceolate	More than 10	Inside only	Yellow	Prickly	Yellowish green	Erect	Slight	8 to 10	3	Very long	2	1 to 3cm	Green
T2 X VT	Persistent	Lanceolate	More than 10	Inside only	Cream	Prickly	Red	Erect	Conspicuous	5 to 7	1	Long	3	More than 3cm	Green with red vein
T3 X VT	Partially Persistent	Lanceolate	More than 10	Inside only	Cream	Slightly rough	Green	Erect	Glabrous	8 to 10	3	Very long	2	1 to 3cm	Green
ID X VT	Persistent	Triangular	From 5 to 7	Both sides	Cream	Slightly rough	Yellowish green	Erect	Slight	5 to 7	3	long	2	1 to 3cm	Green
VT	Persistent	Triangular	More than 10	Inside only	Cream	Prickly	Deep green	Erect	Slight	8 to 10	3	Small	2	1 to 3cm	green
AG X VT	Persistent	Triangular	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Erect	Slight	Non(smooth fruit)	12	Small	2	1 to 3cm	Green
T1 X AG	Persistent	Lanceolate	More than 10	Both sides	Cream	Prickly	Green	Erect	Slight	8 to 10	12	Medium	2	1 to 3cm	Green
T2 X AG	Persistent	Lanceolate	More than 10	Inside only	Cream	Prickly	Red	Erect	Conspicuous	5 to 7	1	Very long	3	1 to 3cm	Green with red vein
T3 X AG	Partially Persistent	Triangular	More than 10	Inside only	Yellow	Slightly rough	Yellowish green	Erect	Conspicuous	8 to 10	3	Small	2	1 to 3cm	Green
ID X AG	Persistent	Lanceolate	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Erect	Glabrous	5 to 7	3	Medium	9	1 to 3cm	Green
VT X AG	Persistent	Triangular	More than 10	Inside only	Cream	Slightly rough	Deep green	Erect	Slight	8 to 10	3	Medium	2	1 to 3cm	Green

NES = Number of Epicalyx Segment; RCPB= Red Colouration of Petal Base; PES= Persistence of Epicalyx Segment; SES= Shape of Epicalyx Segment; PFMS= Position of Fruit on Main Stem; NRPPD= Number of Ridges per Pod; FS = Fruit shape FP = Fruit Pubescence PC=Petal Colour

Table 5.3 continued

Accessions	PES	SES	NES	RCPB	PC	FP	FC	PFMS	Stem pubescence	NRPPD	FS	Pod length	Leaf shape	Fruit peduncle	Leaf colour
AG	Persistent	Triangular	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Erect	Conspicuous	Non(smooth fruit)	12	Small	2	1 to 3cm	Green
AM	Persistent	Lanceolate	More than 10	Both sides	Yellow	Downy	Yellowish green	Pendulous	Slight	None (smooth)	1	Long	8	More than 3cm	Green
T1 X AM	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Downy	Green with red patches	erect	Slight	8 to 10	3	Very long	2	1 to 3cm	Green
T2 X AM	Partially Persistent	Lanceolate	More than 10	Both sides	Cream	Downy	Red	Pendulous	Conspicuous	5 to 7	1	Medium	3	1 to 3cm	Green with red vein
T3 X AM	Persistent	Lanceolate	More than 10	Inside only	Yellow	Slightly rough	Yellowish green	Erect	Slight	8 to 10	1	Very long	2	1 to 3cm	Green
ID X AM	Persistent	Lanceolate	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Pendulous	Glabrous	5 to 7	3	Medium	9	1 to 3cm	Green
VT X AM	Persistent	Triangular	More than10	Inside only	Cream	Prickly	Deep green	Erect	Slight	8 to 10	3	Very long	8	1 to 3cm	Green
AG X AM	Persistent	Triangular	More than 10	Both sides	Yellow	Slightly rough	Green	Erect	Conspicuous	Non(smooth fruit)	12	Small	2	1 to 3cm	Green
T4	Non-persistent	Lanceolate	More than 10	Inside only	Cream	Prickly	Yellowish green	Erect	Slight	5 to 7	3	Very long	10	1 to 3cm	Mixed
T1 X T4	Non-persistent	Lanceolate	More than 10	Both sides	Yellow	Prickly	Yellowish green	Erect	Slight	8 to 10	3	Very long	7	1 to 3cm	Green
T2 X T4	Partially Persistent	Lanceolate	More than 10	Inside only	Cream	Prickly	Red	Erect	Slight	5 to 7	3	Medium	3	1 to 3cm	Mixed
T3 X T4	Partially Persistent	Lanceolate	More than 10	Inside only	Yellow	Slightly rough	Yellowish green	Erect	Slight	8 to 10	3	Very long	9	1 to 3cm	Green
VT XT4	Persistent	Triangular	More than 10	Inside only	Cream	Prickly	Deep green	Erect	Slight	8 to 10	3	Very long	3	1 to 3cm	Green

NES = Number of Epicalyx Segment RCPB = Red Colouration of Petal Base PES = Persistence of Epicalyx Segment SES = Shape of Epicalyx Segment PFMS = Position of Fruit on Main Stem NRPPD = Number of Ridges per Pod FS = Fruit shape FP = Fruit Pubescence PC = Petal Colour.

Table 5.3 (continued)

Accessions	PES	SES	NES	RCPB	PC	FP	FC	PFMS	Stem pubescence	NRPPD	FS	Pod length	Leaf shape	Fruit peduncle	Leaf colour
AG X T4	Non-persistent	Triangular	More than 10	Both sides	Cream	Slightly rough	Green	Erect	Conspicuous	Non(smooth fruit)	12	Small	2	1 to 3cm	Green with red vein
KB	Persistent	Lanceolate	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Pendulous	Conspicuous	5 to 7	3	Medium	6	More than 3cm	Mixed
T1 X KB	Persistent	Lanceolate	More than 10	Both sides	Yellow	Downy	Green	erect	Slight	8 to 10	3	Very long	2	1 to 3cm	Green
T2 X KB	Partially Persistent	Lanceolate	5 to 7	Inside only	Cream	Slightly rough	Green with red patches	Erect	Conspicuous	5 to 7	1	Very long	3	1 to 3cm	Mixed
T3 X KB	Persistent	Lanceolate	More than 10	Inside only	Yellow	Slightly rough	Yellowish green	Erect	Slight	8 to 10	3	Very long	2	1 to 3cm	Green
ID X KB	Persistent	Lanceolate	From 5 to 7	Both sides	Cream	Slightly rough	Yellowish green	Erect	Glabrous	5 to 7	3	Very long	9	1 to 3cm	Green with red vein
VTX KB	Persistent	Lanceolate	More than 10	Both sides	Cream	Slightly rough	Yellowish green	Erect	Conspicuous	5 to 7	3	Medium	2	1 to 3cm	Green
AG X KB	Persistent	Triangular	From 5 to 7	Inside only	Cream	Prickly	Green with red patches	erect	Slight	8 to 10	8	Small	2	1 to 3cm	Green with red vein
YL	Persistent	Triangular	From 5 to 7	Inside only	Cream	Prickly	Green with red patches	Pendulous	Slight	None (smooth)	2	Small l	2	More than 3cm	Green with red vein
T1 X YL	Non-persistent	Triangular	From 5 to 7	Inside only	Cream	Prickly	Green with red patches	erect	Slight	8 to 10	3	Medium	2	1 to 3cm	Green
T2 X YL	Partially Persistent	Lanceolate	From 5 to 7	Inside only	Cream	Prickly	Green with red patches	Erect	Conspicuous	5 to 7	1	Small	3	1 to 3cm	Mixed
T3 X YL	Persistent	Lanceolate	More than 10	Inside only	Yellow	Slightly rough	Yellowish green	Erect	Slight	8 to 10	3	Very long	2	1 to 3cm	Green
ID X YL	Persistent	Triangular	From 5 to 7	Inside only	Cream	Slightly rough	Yellowish green	Erect	Glabrous	5 to 7	3	Medium	9	1 to 3cm	Green with red vein
VT X YL	Partially Persistent	Triangular	From 5 to 7	Inside only	Cream	Prickly	Green	erect	Slight	8 to 10	3	Medium	2	1 to 3cm	Green
AG X YL	Persistent	Triangular	More than 10	Both sides	Cream	Slightly rough	Green with red patches	Erect	Conspicuous	Non(smooth fruit)	12	Small	2	1 to 3cm	Green with red vein

PES = Persistence of Epicalyx Segment SES = Shape of Epicalyx Segment NES = Number of Epicalyx Segment RCPB = Red Colouration of Petal Base PC = Petal Colour PFMS = Position of Fruit on Main Stem NRPPD = Number of Ridges per Pod FP = Fruit Pubescence FS = Fruit shape

Figure 5.3 shows the genetic relationship among 10 local accessions and 24 F_1 offspring obtained from their inter-specific crosses, based on furthest neighbour method (Euclidean). The accessions were separated into two clusters (A and B) at a genetic distance of 12.33%. Cluster A contains three accessions, made up of parental accessions AM, KB and YL.

Cluster B on the other hand subdivided repeatedly into two sub-clusters of B1 and B2 with B2 containing a single parental accessions, AG which is most distantly related to AM. However, B1 again separated into sub-sub-clusters (B1.1, B1.2, B1.3 and B1.4). The analysis revealed no duplicates and clustering pattern especially among parental accession, appears to reflect relationship based upon speciation as parental accessions (AM, KB, YL and T4) and their hybrids belonging to *A. caillei* are clustered towards one end of the dendrogram while members belonging to *A. esculentus* and their hybrids also clustered towards the opposite end.

5.4.3 Clustering pattern of 10 local accessions of okra (24) and their intra-specific F_1 offspring.

Fig 5.3 displays a furthest neighbour dendrogram showing genetic relatedness of 10 local accessions of okra and 24 F_1 offspring obtained from inter-specific hybridisation among the accessions

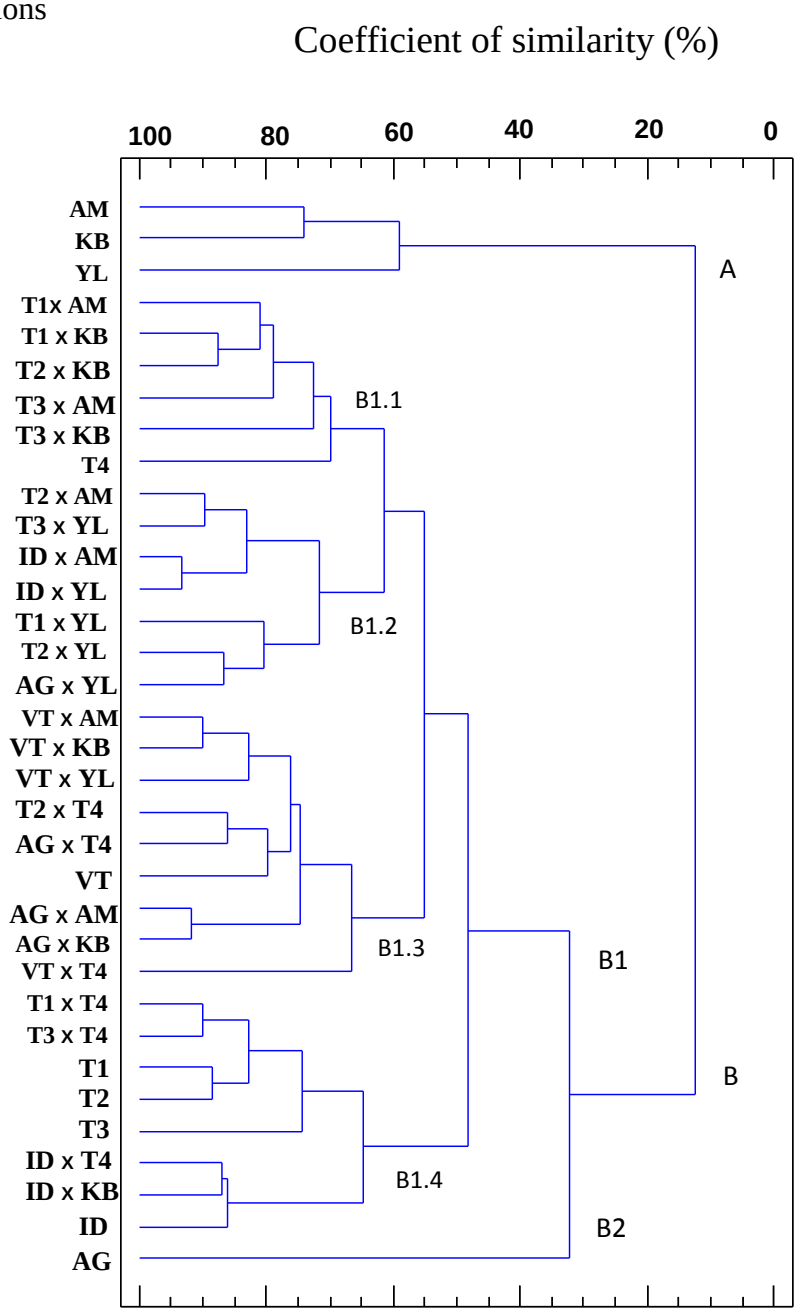

Figure 5.3: Dendrogram showing relatedness of 10 accessions of okra and 24 F_1 offsprings based on 7 quantitative.

5.4.4 Principal Components Analysis (PCA).

Table 5.4 displays factor scores of seven quantitative traits for three principal components accounting for variability among 10 local accessions of okra (*A. caillei*) and 24 F_1 offspring obtained from inter-specific hybridisation among the accessions as well as contributions and eigen values of each principal component.

Table 5.4: Association of seven quantitative traits with three principal components accounting for total variability among 10 accessions of okra and their F_1 offspring.

	Component		
Traits	1	2	3
LOF	**0.342138**	**0.481271**	-0.353075
DG	-0.491386	0.0818066	**0.200812**
DF	-0.462612	-0.104235	-0.369037
NOB	-0.396005	**0.275532**	-0.234668
PHAFF	-0.488485	0.0830871	-0.19164
NSPP	-0.16779	**0.40132**	**0.76343**
FFW	0.0622948	**0.711999**	-0.155574
Eigenvalue	3.22*	1.63	1.01
% variance	45.98*	23.31	14.45
Cumulative % variance	45.98	69.29	**83.75***

Bolded values represent variables which made significant contribution to total variance in respective axes. Maximum eigen value and percent variance are asterisked (*); minimum eigen value and percent variance are underlined. Maximum cumulative percent variance of the 3 principal components is bolded and asterisked.

Contributions of the three principal components were 45.98 %, 23.31 %, and 14.46% for the first (PC1), second (PC2) and third (PC3) respectively, with corresponding eigen values of 3.21837, 1.63171 and 1.01212 respectively, cumulating into maximum of 83.75 % of total variance.

From Table 5.4. The first component is far more important than the other two, since it accounts for 45.98% of the variation in the data. There appears to be a contrast between LOF and FFW on one hand and DG, DF, NOB, PHAFF and NSPP on the other, as the latter set all have negative values.

Factor scores of the variables indicate that length of fruit and fresh fruit weight exhibited significant positive association with PC1 indicating that breeding in this component will lead to increased fruit length and fresh fruit weight. The same effect will on the other hand, lead to reduction in days to 50% germination, days to 50% flowering, number of branches per plant, number of seeds per pod and plant height at first fruiting. Regarding PC2, the significant variables were length of pod, fresh fruit weight, number of seeds per fruit and number of branches per plant. This indicates increased values when breeding in component two while earliness could be achieved, since the value for earliness is negative in this component. Again, number of days to 50% germination and number of seeds per pod made significant positive contribution to the genetic variance in PC3.

Fig. 5.4 displays component weight of the seven quantitative agro-morphological characters.

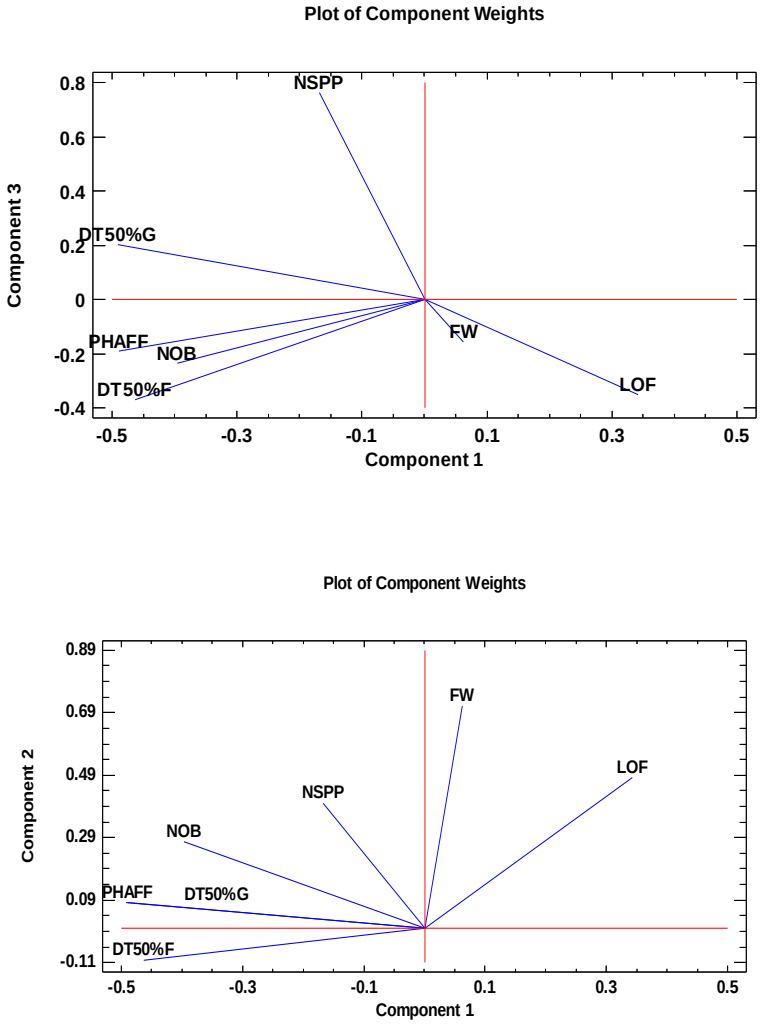

Fig. 5.4: Plot of component weight of 7 quantitative agro-morphological traits of okra (*A. esculentus* and *A. caillei*) and their F_1s.

5.4.5 Correlation studies among seven quantitative traits of okra.

Table 5.5 displays levels of association among 7 quantitative traits of 10 accessions of Okra and their inter-specific F_1 hybrids. Length of pod was very highly correlated with fruit weight with a correlation coefficient r = 0.5504. Again, fresh fruit weight was also significant and positively correlated with number of seeds per pod.

However, fresh fruit weight and length of pod are significant but negatively correlated with days to 50% flowering. This indicates that, selection for pod length in this study will also lead to increased fresh fruit weight. From the results, accession with low plant height matured early and produced large fruits, this is reflective of the values obtained from the correlation matrixes as pod length is significant but negatively correlated with days to 50% germination and days to 50% flowering with r = 0.5286 and – 0.3163 respectively.

From the studies plants that were tall had a lot of branches and produced many fruits which were smaller. This is shown in the significant but positive correlation between plant height and number of branches per plant with a correlation coefficient r = 0.6066.

Table 5.5: Correlation matrix for seven quantitative traits of okra (*A. esculentus* and *A. caillei*).

	LOF	DG	DF	NOB	PHAFF	NSPP	FFW
LOF							
DG	-0.5286***						
	0.0000						
DF	-0.3163**	0.4630***					
	0.0040	**0.0000**					
NOB	-0.1248^{ns}	0.4178***	0.6332***				
	0.2555	**0.0001**	**0.0000**				
PHAFF	-0.2947**	0.5061***	0.5995***	0.6066***			
	0.0073	**0.0000**	**0.0000**	**0.0000**			
NSPP	-0.1651^{ns}	0.4798***	-0.0608^{ns}	0.1256^{ns}	0.0861^{ns}		
	0.1326	**0.0000**	0.5798	0.2524	0.4330		
FFW	0.5804***	0.0154^{ns}	-0.0915^{ns}	0.1610^{ns}	0.0749^{ns}	0.2452*	
	0.0000	0.8886	0.4047	0.1424	0.4948	**0.0255**	

FFW = Fresh fruit weight; NSPP = Number of seeds per plant; LOF = Length of pod, PHAFF = Plant height at first fruiting, FFW = Fresh Fruit weight; NOB = Number of branches per plant; DG= Days to 50% germination, DF= days to 50% flowering.
Below each correlation coefficient (**bolded)** is P-value (underlined). *, **, *** = significant at $P \leq 0.05, 0.01, 0.001$ respectively
Ns = not significant at $P \leq 0.05$.

5.5 DISCUSSION

5.5.1 Variations in qualitative and quantitative agro-morphological traits of 10 accessions of okra (*A. esculentus* and *A. caillei*) and their intra-specific and inter-specific F_1 offspring.

The results of this study are indicative of the fact that all the 61 accessions of okra exhibited significant variation in morphological traits, but minimal variation in qualitative traits, among the accessions investigated. This corresponds with the results of Omalsaad *et al*., (2014), implying that the latter traits are not useful for studying genetic diversity of okra germplasm. Observation of significant differences among the quantitative traits is, however, an indication that genetic diversity exists among the accessions as well as hybrids, thereby providing a basis for selection. This is in consonance with what has been reported earlier by Ahiakpa *et al*., (2013); and Aladele *et al*., (2008), where it was demonstrated that such genetic variability existed amongst okra varieties evaluated in the respective studies.

Earliness in okra is determined by the number of days from sowing to 50% full-bloom. In this study, the accessions differed significantly in the number of days to 50% flowering. It has been demonstrated that on a general basis, early flowering is detrimental for overall productivity in okra as the source to sink ratio will be potentially limited for effective photosynthesis Aboagye *et al*., (1994).

Differences in flowering periods among the varieties in the current study imply that their maturity periods vary. Depending on the desire of the breeder or farmer, appropriate

selection can thus be made for either early or late maturing plants. Early maturing plant types for instance could be selected for areas with short rainy seasons in rain fed ecologies. Such genotypes will also be suitable for areas where farmers grow a second crop to take advantage of residual moisture after harvesting the early crop. This is supported by results obtained by Oppong-Sekyere *et al*., (2012) and Nwangburuka *et al*., (2011).

Plant height of the accessions evaluated was also significantly different. The height of the plant can potentially affect yield as those that are taller are usually more prone to wind damage in the event of heavy seasonal rains. Height at flowering and fruiting are of particular interest for breeding programmes because tall, thin stems increase rate of lodging near harvesting time (Doku, 2011) and this could culminate in loss of dry matter and a subsequent decrease in fruit yield. This is in consonance with reports by Doku (2011), Akinyele and Oseikita (2006) and Myanmar (1995) who worked on rice and okra respectively. Number of days to 50% flowering and plant height at maturity, among other agronomic characters, are some of the most variable traits that are necessary for selection programmes aimed at improving desirable traits in okra Akinyele and Oseikita (2006). It is suggestive from this that number of days to 50% flowering and plant height at flowering are controlled by the same genetic variables Choudhary *et al*., (2006) and Hussain *et al*., (2006). Consequently, selection for dwarf stature plants may thus be made on the F_1 hybrids as they were shorter than most parental varieties evaluated in the current study.

From this study, okra accessions exhibited varying degrees of fruit pubescence spanning from prickly, slightly rough (smooth), downy to little hairs on fruits but with the majority having slight pubescence. This is in contrast with the findings of Bisht *et al*. (1995), who found downy type of fruit pubescence to be the most pronounced followed by slightly rough while prickly fruits was the least in the okra accessions they studied. This could be an indication of preference of Ghanaian farmers' for smooth fruit types and thus, dis-selecting the hairy types (Oppong-Sekyere, 2011).

Petal colour was either cream or yellow. This is contrary to observation made by Myanmar (1995) who found petal colour to be 100% yellow for all 40 okra accessions examined. Akinyele and Akinlosotu (1991) also found similar results in their okra research. Variations observed in this study were conspicuous for position of fruit on main stem that was erect, horizontal or pendulous. Most of the accessions had their fruits in the erect position on main stem as against pendulous and horizontal positioning of fruits on main stem of the accessions. This is because different genotypes have the tendency of exhibiting different growth habits, whether as a result of selection or a natural adaption mechanism. This is similar to an observation made by Hanson (1991) working on tomato. Yellowish green was the most predominant fruit colour while green and green with red patches were least observed among the accessions. These results are similar to those found by Oppong-Sekyere (2011) and Myanmar (1995) in okra.

Ariyo (1993) indicated that the pattern of genetic variation observed in characters studied in West African okra suggests a lot of outcrossing among the taxon. The considerable

morphological variation observed in the characters of the accessions studied could perhaps, be attributed to the preponderance of out-crossing among these different accessions (Oppong-Sekyere, 2011and Adeniji, 2003). There were also intense variation in number of branches per plant, number of seeds per pod (fruit), stem pubescence, fruit shape, type of pod axis, branching type, fruit peduncle and fruit length. These agree with results found in okra morphological diversity studies by Amoatey *et al.*, (2015) and Khanorkar and Kathiria (2010).

5.5.2 Genetic relationship among 10 local accessions of okra and their intra-specific F_1 hybrids based on cluster analysis.

Variations among genotypes do not only indicate their genetic constitution but also their interactions with the environment. Hence combining qualitative and quantitative traits gives more desirable results in cluster analysis (Dixon and Nukenine, 2000).

The pattern of clustering from the cluster analysis based on both quantitative and qualitative traits, generally reflected variability in terms of genus characteristics, as all entries of cluster A are accessions of *A. caillei* and take a long time to mature and also are taller compared to those of cluster B. Separation of the entries into sub-clusters also reflected similarity based on parents and their offspring, since members of sub-sub-clusters were composed of parents and their offsping. Any pair of genotypes which share genetic similarity of above 95 % may be considered identical (Anderson *et al*., (2007). By applying this criterion to the results of the correlation analysis, no pair of entries is possible duplicates.

5.5.3 Contribution of 7 quantitative traits to total variability via principal components analysis (PCA).

The main aim for undertaking principal component analysis in genetic diversity studies is to identify variables which contribute most to genetic variability to be selected for characterising genotypes (Johnson and Wichern, 1992). Results of the principal component analysis showed that 83.75 % of the total variability among the okra accessions evaluated in this study was accounted for by the first three principal components thus, greater percentage of the total variance was explained by these components. This compares with 82.97 % and 75.77 % reported by earlier researchers (Ahiakpa *et al*., 2013; Torres-Morán *et al*., 2011) who also evaluated 30 accessions of okra (*Abelmoschus* spp L.) in Ghana and 12 local cultivars of roselle cultivated in Mexico respectively. They however, differ from findings of Doku, (2011) who observed 91.87% contribution of first four principal axes to total variance among 17 accessions of African rice (*Oryza glaberrima* Steud) in Ghana.

Fruit length and fresh fruit weight shared significant positive association with the first principal axes (PC1) which contributed most (45.98%) to the total genetic variance. This implies that genes controlling the inheritance of these traits accounted for most of the genetic divergence as pointed out by Adeniji and Aremu, (2007). Therefore, it provides good basis for their selection for future investigations pertaining to genetic diversity of okra germplasm.

5.5.4 Correlation analysis of 7 quantitative agro-morphological traits of okra.

Fruit traits are perhaps the most important traits in okra and their improvement is of particular interest in okra breeding programmes. Results of the correlation analysis reveal strong positive association between length of pod and days to 50% germination compared with fresh fruit weight. This implies that component breeding would lead to significant increase in fresh fruit weight if these traits are considered, since they are positively correlated as pointed out by Hazra and Basu (2000). Improvement of fresh fruit weight could also be accomplished indirectly through selection for number of seeds per pods and length of pod since both traits shared strong positive association with fresh fruit weight. However, in breeding for fresh fruit weight; plant height, number of branches per plant, days to 50% germination and days to flowering would have negative effect since there is negative association with these traits. Days to first flowering was positively correlated with plant height at 50% flowering, number of branches, length of fruit and days to 50% germination. Hence, breeding for earliness would have significant effect on these traits of importance.

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CHAPTER SIX

6.1. GENERAL CONCLUSIONS AND RECOMMENDATION

6.1.1 CONCLUSIONS

In general, genetic variation has been observed in Okra (*Abelmoschus spp L*.) particularly the West African type, *Abelmoschus caillei,* but ironically, no serious breeding effort have been made to harness the genetic richness to advance improvement of the crop. Previous international efforts have been limited to intensive cultivation with increased production based on early maturing and resistance to insect pests and diseases. There is still enormous scope for cultivar improvement in Africa particularly Ghana as we have the potential for okra production, yet have not improved our locally adapted landraces to increase production of the crop to meet standards of the export market. Hybridization studies on ten cultivars of *A. caillei* and *A. esculentus* was done to augment efforts towards the improvement of the crop in Ghana.

On bases of results obtained from the study the following conclusion may be drawn.

1a.The two species of Okra, *A. esculentus* and *A. caillei* were cross-compatible in one direction only, when accessions of *A. esculentus* were used as female parents. The reciprocal crosses were unsuccessful.

1b. Among the *A. esculentus* mating group made up of T1,T2,T3, ID, VT and AG hybridization among accessions was generally successful though to varying levels in different cross-combinations producing 30 hybrids.

1c. Intra-specific hybridization among *A. caillei* mating group made up of T4, AM, KB, and YL was generally unsuccessful as a result of non-synchronization of flowering periods.

1d. Crossability index ranged from 45.71% to 90.32% in crosses among *A. esculentus* while in *A. caillei* the range was 34.48% to 60%.

1e. percent fruit set ranged from 40.00 % to 75 % in crosses among *A. esculentus* mating group and 25% to 50% in *A caillei* mating group.

2a. Estimates of percentage (%) heterosis among inter-specific hybrids revealed that for earliness the highest value for mid-parent heterosis (MPH) was 10.78% and 1.77% for better parent heterosis (BPH) while the least values for (MPH) was – 30.84% and - 96.84% for (BPH.

2b. with reference to fresh fruit weight, mid-parent heterosis values ranged from -22.52% to 17.17%, better-parent heterosis ranged from -49.59% to 12.54%, indicating an increase in fruit size by 17.17% (MPH) and 12.54% (BPH) for offspring VT X YL and AG X YL. These accessions are very wide apart in terms of genetic relatedness showing that heterosis is very high in accessions that are distantly related as indicated in the dendrogram.

2c. The (MPH) values for pod length ranged from – 17% to 51.60% while (BPH) was from -76.57% to 51.45%. Accession AG x YL recorded the highest (MPH) and (BPH) values.

2d. Number of seeds per pod also revealed (MPH) values ranging from – 35.39% to 36.87 % while (BPH) ranged from – 157.32% to 18.42. The F1s VT x YL and AGxKB had the highest values.

3a. Extensive significant variation was observed among the 10 parental accessions and their 51 offspring obtained from both inter-specific and intra-specific hybridization investigated for (15) qualitative and (8) quantitative traits studied, as shown in appendix (a) and (b)

3b. Qualitative traits such as fruit colour, position of fruit on main stem, fruit pubescence and petal colour, the results indicates that yellowish green, erect, slightly rough and cream dominated respectively.

3c. Qualititative traits such as fresh fruit weight, pod length, number of seeds per pod and days to 50% germination were all very highly significant see appendix (a) and (b).

3d. Parental accessions belonging to the species A. esculentus were distantly related to those belonging to *A. caillei* and were positioned at opposite ends of the dendrogram. Their intra- and inter-specific hybrids occupied middle positions on

the dendrogram, a reflection of their mixed genetic origins. No two hybrids or parents were identical (duplicates).

3e. the first three components accounted for 83.75% total variance. Pod length and fruit weight were positive in principal component one (PC1) and two while pod length, number of branches, number of seeds per pod and fresh fruit weight are also positives in PC2. Days to 50% germination and number of seeds per pod were positive in (PC3). In all, number of days to 50% flowering was negative in all three components, indication earliness when component breeding is carried out in all the three components.

3f. Pod length was highly significant and positively correlated with fresh fruit weight and significant but negatively correlated with days to 50% germination. Fresh fruit weight was also significant and positively correlated with number of seeds per pod. This indicates that in breeding, selecting for high pod length will lead to increased fresh fruit weight. However, reduction in plant height, days to 50% flowering and days to 50% germination will reduce since, they were significant but negatively correlated and this is good for breeding for earliness.

6.1.2 RECOMMENDATIONS FOR FUTURE RESEARCH

On basis of results obtained in these studies and previous achievements of breeding of okra, the following recommendations are to be considered for future breeding work;

1. International and national institutions concerned with promoting and maximising the potentials of unexploited and under utilised crops like okra should collaborate to invest in a project towards sequencing of the genome of this versatile vegetable since this cannot be undertaken by a single entity.

2. Genes linked to agronomically important traits in okra should be genetically mapped through Quantitative Traits Loci (QTLs) to serve as a baseline data platform for researchers and breeders.

3. Further studies on inheritance of qualitative traits in okra should be carried out, preferably using molecular markers to fully understand the pattern of segregation with appropriate ratios. Use of molecular markers would also conclusively confirm results of morphological characterisation of the accessions.

4. Fruit length and fruit weight are parameters that made significant positive contribution to the overall genetic divergence observed among accessions, which

shows that, they would be potentially effective in differentiating genotypes of okra. Hence high priority should be given to these traits in future breeding work.

5. Cross-fertilization among genotypes that are cross-compatible in both direct and reciprocal crosses such as result obtained from *A. esculentus*, mating group with crossability index ranging from 40% (T1 X AM) to 90.32% (T3 X T1), is a strong indication of high fertility rates among genotypes belonging to this sub-species. Hence, a full diallel design could be utilized for gene transfer among the accessions of this group.

6. From the results obtained in the study, negative values for traits such as days to 50% germination and days to 50% flowering would be ideal for breeders who want to breed for earliness.

CHAPTER SEVEN

7.1 Appendix-(a) anova intra-specific hybrids

ANOVA Table for LOP

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	668.464	32	20.8895	20.39	0.0000
Within groups	67.6267	66	1.02465		
Total (Corr.)	736.091	98			

ANOVA Table for LOPE

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	34.9867	32	1.09333	23.63	0.0000
Within groups	3.05333	66	0.0462626		
Total (Corr.)	38.04	98			

ANOVA Table for DF

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	11.4851	32	0.358908	6.44	0.0000
Within groups	3.68	66	0.0557576		
Total (Corr.)	15.1651	98			

ANOVA Table for PHAFF

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2603.48	32	81.3587	9.19	0.0000
Within groups	584.587	66	8.85737		
Total (Corr.)	3188.07	98			

ANOVA Table for DG

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	193.293	32	6.0404	19.29	0.0000
Within groups	20.6667	66	0.313131		
Total (Corr.)	213.96	98			

ANOVA Table for NSPP

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	7523.88	32	235.121	17.86	0.0000
Within groups	868.667	66	13.1616		
Total (Corr.)	8392.55	98			

ANOVA Table for FFW

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2974.21	32	92.9442	62.75	0.0000
Within groups	97.7645	66	1.48128		
Total (Corr.)	3071.98	98			

ANOVA Table for DF

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1086.18	32	33.9432	19.31	0.0000
Within groups	116.0	66	1.75758		
Total (Corr.)	1202.18	98			

7.2 Appendix-(b) anova inter-specific hybrids

ANOVA Table for LOPANOVA Table for DG

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	337.866	27	12.5136	37.09	0.0000
Within groups	18.8933	56	0.337381		
Total (Corr.)	356.76	83			

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	285.655	27	10.5798	31.74	0.0000
Within groups	18.6667	56	0.333333		
Total (Corr.)	304.321	83			

ANOVA Table for DF ANOVA Table for NOB

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	10185.4	27	377.236	47.01	0.0000
Within groups	449.333	56	8.02381		
Total (Corr.)	10634.7	83			

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	3204.14	27	118.672	3.51	0.0000
Within groups	1892.67	56	33.7976		
Total (Corr.)	5096.81	83			

ANOVA Table for NSPP **ANOVA Table for PHAFF**

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	80182.9	27	2969.74	24.81	0.0000
Within groups	6704.3	56	119.72		
Total (Corr.)	86887.2	83			

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	14515.6	27	537.613	34.53	0.0000
Within groups	872.0	56	15.5714		
Total (Corr.)	15387.6	83			

ANOVA Table for FFW

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1681.68	27	62.2844	17.07	0.0000
Within groups	204.321	56	3.6486		
Total (Corr.)	1886.0	83			