

EVALUATION OF GENETIC DIVERSITY IN GARDEN EGG (*Solanum aethiopicum*) GERMPLASM IN GHANA

BY

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JULY, 2013**DECLARATION**

I, Leander Dede Kubie, thereby declare that except for the reference to other people's work, which have been duly cited, this thesis is the result of my original findings and this thesis has neither in whole, nor part, been presented for a degree in Ghana or elsewhere.

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ABSTRACT

Analysis of genetic relationships among accessions is a prerequisite to any viable genetic improvement. The main objective of the study was to determine the genetic variation among 20 garden egg accessions in Ghana based on morphological traits as well as calcium and iron content of fruits. Twenty garden eggs accessions were studied in a Randomized Complete Block Design with three replications. There was large variability between and among accessions. The fruit length: diameter ratio, number of fruits per plant, weight per fruit, calcium and iron content recorded a higher genetic and phenotypic coefficient of variation and genetic advance. However, high heritability was recorded for days to first flowering, days to 50% flowering, fruit length, yield, calcium and iron content. Phenotypic correlation coefficients between yield and other traits showed significant estimates only for days to flowering and canopy span, the former being negative and the latter positive. The calcium and iron content of fruit was negatively correlated. The first PCA accounted for 35.6% of the total variation and was dominated by leaves per plant and fruits per plant. The second PCA contained 20.7% of the total variation and was also dominated by yield and fruit size (weight per fruit and fruit diameter). The third PCA was dominated by days to flowering and plant height and contained 14% of the total variation. For all the traits evaluated, A8 and A10 were high yielding, short, had broad canopy span, matured early, had high number of leaves, branches and fruits though quite dissimilar to each other in the cluster diagram.

DEDICATIONS

I dedicate this work to my husband Mr Edward Mawusi Melomey, for his encouragement and support both financially and emotionally and my lovely daughter Seyram Sefakor McMelomey.



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LIST OF ABBREVIATIONS

ACC	Administrative Committee on Coordination
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
Ca	Calcium
CLCA	Complete Linkage
cm	Centimetre
cpDNA	Chloroplast DNA
CSRI	Council for Scientific and Industrial Research
EGGNET	Eggplant Genetic Resource Network
FAO	Food and Agriculture Organization
Fe	Iron
GA	Genetic advance
GCV	Genetic Coefficient of Variation
h^2	Broad sense heritability
HCl	Hydrochloric Acid
HNO ₃	Nitric Acid
IFPRI	International Food Policy Research Institute
IRRI	International Rice Research Institute
ISRIC	International Soil Research and Information Center
ISSS	International Society of Soil Science
LSD	Least Significant Difference
m	Mean
MDS	Multidimensional Scaling
ml	Millilitre
m/L	Millilitre/Litres
MLCA	Meta – Cluster Algorithm

mm	Millimetre
N.P.K	Nitrogen Phosphorus Potassium
PCA	Principal Component Analysis
PCOA	Principal Coordinate Analysis
PCV	Phenotypic Coefficient of Variation
r	Correlation Coefficient
RAPD	Random Amplification of Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
r_p	Phenotypic Correlation Coefficient
SCN	Subcommittee on Nutrition
SDS-PAGE	Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis
SLCA	Single Linkages Cluster Analysis
SSR	Single Sequence Repeat
t/ha	Tonnes/hectare
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UPGMC	Unweighted Pair-Group Method using Centroids
UPOV	International Union for the Protection of New Varieties of Plants
\bar{x}	Population mean
σ_e^2	Error variance
$\sigma_{G(AB)}$	Genetic covariance between traits A and B
σ_p	Square root of the phenotypic variance
σ_g^2	Genotypic variance
σ_p^2	Phenotypic variance

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CHAPTER ONE

1.0 INTRODUCTION

Garden egg or scarlet eggplant (*Solanum aethiopicum* L., gilo group) is one of the most important vegetable crops in tropical Africa. The plant is believed to have originated from Africa (Norman, 1974). According to Lester (1998), *S. aethiopicum* was domesticated in Africa from the wild relative *S. anguivi* Lam. Other cultivated eggplants that are related to the scarlet eggplant are *S. melongena* and *S. macrocarpon* which are also mainly cultivated in Africa. The plant is essentially self pollinated and most landraces are virtually purebred lines (Lester and Niakan, 1986).

Four cultivar-groups have been recognized in *S. aethiopicum*, and include Gilo, Shum, Kumba and Aculeatum (Lester and Daunay, 2003). The Gilo group represents what is largely known in Ghana as garden eggs. The Gilo group is the most important cultivar-group, which includes cultivars with smooth fruits that are popular in West and East Africa, and cultivars with more or less strongly ribbed fruits. *Solanum aethiopicum* vary in fruit shape, fruit size, length, diameter, weight, seed content, colour and taste. The garden egg cultivars also vary in petiole length, leaf blade, branching habit, time of flowering, time of fruit set and maturity (Blay, 1991; Ofori, 1999). Throughout Africa, local garden eggs are very popular and play an important part in many diets. The plants are easy to raise, relatively free of disease and pests, and provide a steady supply of both food and income. Garden egg consumption has a lot of health benefits including effectiveness in blood cholesterol reduction, positive help in heart problems and weight loss (Muanya, 2009). Garden egg is very rich in iron and low in vitamin C as compared to tomato (Okon *et al.*, 2010) and also contains some appreciable amount of calcium (Horna *et al.*, 2007).

It has proven to be beneficial to patients suffering from anaemia because of its rich iron content. A rich meal of garden eggs is used to induce lactation in freshly delivered women (Blay, 1991). There is increasing awareness and advocacy for the fortification of foods with iron since one in three people worldwide is at the risk of iron deficiency (Hill, 1998). The need to focus on producing crops that contain iron or fortified with iron has become very urgent. Screening accessions for iron rich materials is therefore a vital breeding objective.

Nonetheless very little research works on the diversity and characterization of genetic resources of *S. aethiopicum* have been published (Stedje and Bukenya-Ziraba, 2003; Sunseri *et al.*, 2010). Similarly, very few works have been devoted to the genetic improvement of the garden egg plant (Lester, 1986; Lester and Niakan, 1986). According to Spooner *et al.* (2003), establishment of cultivar groups is a key step that can enhance the utilization and conservation of genetic resources. However, there are no clear rules for classifying garden egg accessions into established cultivar groups or for creating new groups.

Analysis of genetic relationships in crop species is an important component of crop improvement programs as it serves to provide information about genetic diversity and is a platform for stratified sampling of breeding populations (Mohammadi and Prasanna, 2003). The assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications. These include the analysis of genetic variability in cultivars (Smith, 1984), identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998) and introgressing desirable genes from diverse germplasm into available varieties (Thompson *et al.*, 1998).

Diverse data sets have been used by researchers to analyze genetic diversity in crop plants; most important among such data sets are pedigree, passport or morphological data, biochemical and molecular data (Smith and Smith, 1992; Bernardo, 1993; Godt and Hamrick, 1997; Sunseri *et al.*, 2010). It is desirable that primary characterization descriptors have high heritability values (Nyquist, 1991), which allow greater selection efficiency and genetic advance (Wricke and Weber, 1986). Additionally, the genotypic coefficient of variation is important statistics as it provides reliable indication of the expected amount of improvement or genetic advance when selection is based on these traits

Estimates of genetic variation in the available accessions of a crop are an essential pre-requisite for genetic improvement. Compared with other tropical vegetable crops, little published work exist on the extent of genetic variation in the garden egg plant (Ofori *et al.*, 2008). The evaluation of morphological and agronomic characters as well as the nutritional composition can provide relevant information on yield and quality traits, as well as other information of great interest to horticulturists and breeders.

The objectives of the study were to

- a) determine genotypic variation among garden egg accessions
- b) estimate genetic relationship among genotypes
- c) assess variability in iron and calcium content among the garden egg accessions
- d) estimate heritability and genetic advance based on selection among accessions

CHAPTER TWO

2.0 Literature Review

2.1 Origin and Distribution

Garden egg (*Solanum aethiopicum* L.) belongs to the family Solanaceae, subfamily Solanoideae, subgenus *Leptostemonum* section *Oilganthes*, Dunal Bitter. *Solanum aethiopicum* comprises of four groups namely the gilo, shum, aculeatum and kumba. The aculeatum is less important and is mostly found growing in the wild in Africa (Lester, 1986). The Solanaceae is an important family in the plant kingdom (Bremer *et al.*, 2003). It includes 91 genera and an estimated number of 2450 species with great variation in habit, morphology and ecology (Mabberley, 2008). The family is ranked as third in economic importance and is regarded as a source of many morphologically different domesticated crop species beneficial to human health, diet, beauty and ornamental use (Mueller *et al.*, 2005; Sekara *et al.*, 2007). Tomato, potato, pepper, petunia, datura, tobacco and eggplant are some of the valuable family members (Doganlar *et al.*, 2002; Knapp *et al.*, 2004). The Solanaceae family members are well adapted to different agro ecological environments, which are widely spread across the globe (Knapp *et al.*, 2004).

Garden egg is mostly found in tropical Africa, South America and Asia. It is grown throughout tropical Africa and South America (mainly Brazil) and occasionally grown in Southernmost France and Italy. It can be grown for various parts of the plants and for food. In savanna areas especially in Uganda, it is grown for both the leaves and immature fruits while in the humid zone of West Africa it is mostly grown for its immature fruits (Norman, 1992).

2.2 Botany Of Garden Egg

The term African eggplant describes both the Scarlet eggplant (*Solanum aethiopicum*) and the gboma eggplant (*S. macrocarpon*) (Lester *et al.*, 1990; Schippers, 1997).

According to Lester *et al.* (1986) *S. anguivi* and the groups of *S. aethiopicum* could be separated from each other on pubescence of the leaves, prickliness of the plants, number of flowers and fruit characters. *S. anguivi* is the most likely wild ancestor of *S. aethiopicum* based on morphological data.

The shum and gilo groups have previously been regarded as different species, but are now more often treated as ‘groups of cultivars’ (Lester & Niakan, 1986). Based on cytological data Anaso (1991) proposed that both gilo- and shum-groups are of hybrid origin and that the Gilo has evolved from the shum group by further hybridisation and selection.

The garden egg particularly the gilo group looks like the common eggplant (*Solanum melongena*) during its vegetative stage of growth. Garden egg is grown as annual under temperate conditions in countries like Southern Europe and South of the United States of America but grown as a perennial herb in both the tropical and subtropical climate. (Dupriez and Deleaner, 1989).

2.2.1 Leaves

Bukenya and Carasco (1999) describes the leaves to be alternate, simple; stipules absent; petiole up to 11 cm long; blade broadly ovate, obtuse or cordate at base, acute to obtuse at apex, slightly to deeply lobed at margin, pinnately veined; upper leaves are smaller, narrower, less lobed and

often sub opposite. The peduncle is often short or even absent and the rachis may be short to long.

2.2.2 Flowers

The flowers are bisexual, regular, white and sometimes pale purple. The stamens are inserted near the base of the corolla tube and alternate with corolla lobes. The filaments are short and thick, the anthers are connivent, yellow, opening by terminal pores and the ovary superiors are 2–6-celled. The style is as long as or slightly longer than stamens and the stigma is small and obtuse. Cultivars of eggplant (*Solanum melongena* L.) bear hermaphrodite flowers in clusters as well as singly (Som and Maity, 1986). According to Hepper (1987), flowers are either solitary or in few-flowered inflorescences and latter is supra-axillary with lower flower hermaphrodite and upper ones male. Kanahama *et al.* (1989) reported that the fundamental inflorescence type in eggplant is a scorpioid cyme. A study on flower types in 29 cultivars showed that they all bear flower cluster along with a solitary flower and in most of the cultivars, the flowers in cluster are - either short- styled and medium or all medium styled (Chadha and Saimbhi, 1977).

Murtasow *et al.* (1971) and Prasad and Prakash (1968) found that the number of flowers per inflorescence varied from 2 to 4 and 3 to 5, respectively. Furthermore, they observed that the flower developed first is larger than others, while the latter has short styles. Popova (1961) stated that the style length or the position of stigma in relation to anthers varied with cultivars and can also vary in different flowers of the same cultivar. She found that stigmas are either above, on the same level or below anthers. Krishnamurthi and Subramanium (1954) reported 4 types of flowers in eggplant depending on the length of style, *viz.*, long-styled with big size ovary,

medium-styled with medium size ovary, pseudo short-styled with rudimentary ovary and true short-styled with very rudimentary ovary. A study of large collection of eggplant from many countries showed that, in 21.5% of varieties, the stigma protruded above anthers, 28.0% below anthers and 50.5% at the same level with anthers (Murtasow *et al.*, 1971). Such study of flowers is absent in garden egg.

2.2.3 Fruits

According to Tindall (1976), the gilo fruit ranges from subglobose to ellipsoid 2.5 – 12 cm in length and 3 – 6 in diameter. A wide variation has been observed in fruit shape, immature fruit colour, fruit size, diameter of corolla and taste. The fruit ranges from smooth to more or less strongly ribbed. The colour of the fruit can be pure white, creamy white, dark green, pale green, purple or brown. Some fruits can be stripped with two or more colours (Chinedu *et al.*, 2011). According to Som and Maity (1986) in the eggplant, the fruit is borne either singly or in clusters. Several studies in eggplant have shown relationship between fruit set and stigma position. Some of them showed that fruit-set is high where the stigma is above or on the same level while short styled stigmas do not set fruits at all (Krishnamurthi and Subramaniam, 1954; Passam and Bolmatis, 1997 and Prasad and Prakash, 1968). Popova (1961) and Mohideen *et al.* (1977) also found the percentage of fruit-set is high in long- styled flowers. Chadha and Saimbhi (1977) pointed out that fruiting habit in a cultivar is directly related to the length of style.

Under worse environmental conditions, the eggplant (*Solanum melongena* L.) is characterized by great variability of fruit setting and yielding. Amoako and Yeboah- Gyan (1991) reported that the plant is partially self-inconsistent and requires cross - pollination for better fruit setting.

Appropriate flower pollination is one of the principal conditions for achieving good quality yields and seeds in eggplants (Mc Gregor, 1976; Polverente *et al.*, 2005).

2.3 Importance Of Garden Eggs

Scarlet eggplant, African eggplant or garden egg (*Solanum aethiopicum* L.) is an important solanaceous crop in West Africa, grown as a commercial crop for domestic consumption and also for export (Anon., 1997; Daunay *et al.*, 2001). The African garden egg (*Solanum aethiopicum*) is one of the most important vegetable crops in Ghana and probably the third most consumed vegetable in the country. Medicinal properties are attributed to its roots and fruit (IFPRI, 2007).

The Scarlet Eggplant, *Solanum aethiopicum* L. is grown in Uganda for its edible leaves (the Shum group) and edible fruits (the Gilo group). The fruits of the weedy *S. anguivi* Lam. are collected and eaten and it is believed that the soup from the cooked green fruits offers treatment of hypertension (Bukonya and Carasco, 1999).

According to Chinedu *et al.* (2011), garden egg is very rich in nutrients. It helps the body to boost the immune system, enhance free circulation of blood as well as strengthen the body tissues. Garden egg is low in sodium, low in calories and very rich in high dietary fibre. It is also high in potassium, a necessary salt that helps in maintaining the function of the heart and regulate blood pressure as well as protect the heart against cholesterol effects. According to Lawande and Chavan (1998), eggplant is a fairly good source of iron, calcium, phosphorus, potassium and vitamin B group.

Fortifying food with iron has become a major concern because more than 2 billion people worldwide are iron-deficient but iron is essential to most life forms and to normal human physiology (ACC/SCN, 2000; Stoltzfus, 2001) A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, brain development and decreased immunity (Haas and Brownlie, 2001 and Bhaskaram, 2011).

Similarly, calcium builds and maintains strong bones, teeth, and connective tissue. Calcium deficiency leads to slow growth in children (IRRI, 2004).

The Igbo people in Nigeria community can hardly do without eating garden egg because it is good for the sight. In a study to assess the “Effects of garden egg on some visual functions of visually active Igbos of Nigeria”, the experts found that the consumption of garden egg fruits may be of great benefits to glaucoma patients (White, 2010).

2.4 Challenges Facing Garden Egg Production

According to Horna *et al.* (2007), although garden egg is highly demanded locally and also has great export potential, no official variety has been bred and released for commercial cultivation. In order to get this vegetable onto the export market, a formal variety must be released which will serve as the basis for setting quality standards which is a critical factor for success on the international market.

As with many indigenous crops of African origin, the physiological activity of the African eggplant or garden egg has not been studied at all. Such studies are however important to ascertain the physiological behavior of existing material in the plant genetic pool.

Garden egg remains largely unsupported by research, as such it is far short of its potential. At the moment, only a handful of researchers are championing its cause, because this small group is enthusiastic about the crop's promise. If given attention, Africa's own eggplant could attain a very big future (Nkansah, 2001).

In addition, there is also limited information presently on garden eggs. The information on garden egg germplasm is limited if not unavailable making it difficult for researchers to develop improvement programmes (Nkansah, 2001).

2.5 Potential for Garden Egg Production

According to Lester and Seck (2004), In the Caribbean, and Southeast Asia, a rough estimate for a few countries indicates an annual fruit production of 8000 t in Senegal, 60,000 t in Côte d'Ivoire and 4500 t in Burkina Faso. Commercial production is increasing in countries like Uganda, Côte d'Ivoire and Senegal. These countries export the fruit to European countries to earn foreign currency. Small-scale growers account for at least 80% of the total production.

In Ghana, garden egg is also produced on a large scale for both the local market and for export. (Daunay *et al.*, 2001)

In order to realize the full potential of garden egg production, there is the need to establish a garden egg database, into which research information could be incorporated, and subsequently disseminated to farmers, researchers, and interested amateur gardeners. Accurate and illustrated botanical descriptions of the local 'cultivars' used as vegetables could also be useful.

There are excellent chances for genetically enhancing African garden eggs for increased yield and other features through selection hybridization. There is a need to identify varieties better adapted to specific growing conditions. In addition, there is potential to create varieties with fruits of even more shapes, colors and tastes.

In order to increase production, research institutions, universities and farming programs across Africa should initiate localized garden egg support and improvement projects. Also, the public should be educated on both the nutritional and health benefit of garden egg so that their interest in garden egg consumption can be rekindled. Garden egg could quickly fit into development-support programs across the continent. These programs can include urban agriculture, soil protection, traditional foods, home gardens, sustainable development, women's welfare, and rural development.

There is also the need to foster optimal cultivation practices without necessarily long-term research operations; instead, making the most use of whatever knowledge is already available while further research goes on. In this regard, indigenous knowledge on the plant types used in the various countries should be gathered and socioeconomic surveys on the production and use of garden eggs in traditional settings across Africa are warranted as well. Programs to provide bulk samples of quality seed might also be helpful in increasing garden egg production (Fox and Young, 1982).

2.6 Improvement of Garden Egg

Solanum aethiopicum and *S. macrocarpon* are of interest for genetic improvement considering

the presence of several traits of agronomic interest in its germplasm (Alba *et al.*, 2005). Daunay *et al.* (1993) reported the possibility of production and characterization of fertile somatic hybrids of *S. melongena* and *S. aethiopicum* Aculeatum group. *S. anguivi*, *S. aethiopicum* Gilo group and Shum group obviously are easily separated on morphological characters but it is not verified if these differences are based in genetic differences.

Attempts at crossing the cultivated eggplant species with their wild relatives have had limited success due to sexual incompatibility (Collonier *et al.*, 2001). Lester and Thitai (1989) reported that a cross between *S. anguivi* and *S. aethiopicum* were interfertile but Omidiji (1979) also made crosses between *S. aethiopicum* Shum and Gilo- groups, which yielded fully fertile hybrids. Based on cytological data Anaso (1991) proposed that both Gilo- and Shum-groups are of hybrid origin and that the Gilo has evolved from the Shum group by further hybridisation and selection.

Pessarakli and Dris (2004) reported that conventional breeding methods involve prolonged breeding and hybridization techniques with low success rate. Subsequently, Rizza *et al.* (2002) obtained dihaploid plants through anther culture of somatic hybrids between *S. melongena* and *S. aethiopicum* Gilo group which were characterized by complete resistance to fungal wilt caused by *Fusarium oxysporum f. sp. melongenae*.

Sakata and Lester (1997) studied cpDNA variation in *S. melongena* and related species and found that all species, except *S. anguivi* and *S. aethiopicum*, were clearly separated in the cpDNA analysis.

Kashyap *et al.* (2003) reviewed the application of plant tissue culture techniques including somaclonal variation, somatic hybridization, somatic embryogenesis and genetic transformation for successful introgression of genes from wild species into cultivated varieties. Novel variable

traits against biotic and abiotic stresses can now be created and genetically engineered into cultivated species (Kantharajah and Golegaonkar, 2004). Seedless eggplant fruits engineered with the chimeric *Def Ha-iaaM* parthenocarpic gene (Donzella *et al.*, 2000) and transgenic *Bacillus thuringiensis* (Bt) eggplant hybrids expressing a natural insecticidal resistance against fruit and shoot borers have been developed (Science Daily, 2007).

Fruit shape, size, colour and glossiness and quality (skin thickness, flesh texture, colour and flavour, seediness, transportability and early maturity) are desirable fruit traits that can be targeted in garden egg breeding programme.

2.7 Genetic Diversity Studies In Eggplant and Garden Egg

Genetic diversity arises primarily as variants in the linear sequence of nucleotides in DNA. Mutations can happen in the coding region of genes, or the spacer regions within and between genes, in the number of copies of genes, the linkage relation between several genes or indeed in whole chromosomes. A small portion of these changes translates into protein variation, into marker polymorphisms, characters, physiological and morphological variation in agronomic characters and ultimately into varieties given different names by farmers Brown (2008).

Mostafa and Mohammed (2011), postulated that genetic diversity studies provides the understanding of genetic relationships among populations and hence directs assigning lines to specific heterogenous groups useable in identification of parents and hence choice selection for hybridization.

Precise information on evaluation of genetic diversity is important to know the source of genes for a particular trait within the available germplasm. Multivariate analysis acts as a useful tool to quantify the degree of divergence between the biological populations at genotypic level and to assess the relative contribution of different components to the total divergence both inter and intra cluster levels (Murty and Arunachalam, 1966).

According to Rao (2004), plant genetic resources for food and agriculture are the basis of global food security and increased self-sufficiency in vegetable crop production is a major goal in developing breeding program. Osei *et al.* (2010), garden egg varies in several agronomic traits. These agronomic traits include branching habit, time of flowering, time of fruit maturity and fruit yield. Wide morphological diversity exists in eggplant, their related species and wild types observed for plant morphology, physiology and biochemical properties (Daunay *et al.*, 1991; Collonier *et al.* 2001). Fruit shape, size, colour and taste are the most noticeable characters that vary among individuals (Frery *et al.* 2007).

The ability to characterize morphological diversity is indispensable for effective management and sustainable use of eggplant genetic resources in breeding programmes. Primary characterization involves measuring plant characters that can be easily recorded through visual observations at different plant growth stages. Such traits include leaf size, fruit shape, size and colour, plant prickliness and hairiness. Secondary characterization deals with more complicated morphological traits of agronomic importance such as pest and disease resistance, fruit set, yield potential and biochemical properties (Ayad *et al.* 1995).

Morphological crop descriptors allow a quick and easy discrimination between phenotypes. They are generally highly heritable traits that can be easily recorded through visual observations and

that are equally expressed in all environments. Morphological descriptors for *S. melongena*, *S. aethiopicum* and *S. macrocarpon* have been developed by IPGRI/FAO, EGGNET and UPOV which provide internationally accepted definitions for these descriptors and include a complete description of important quantitative and qualitative traits illustrated by figures and measured either in metric or arbitrary scale.

To carry out an effective breeding program, information concerning the extent and nature of genetic diversity within a crop species is essential. It is very useful for characterizing individual accessions and cultivars and as a general guide in the selection of the parents for hybridization (Furini and Wunder, 2004). Better knowledge on genetic diversity or genetic similarity could also help to sustain long term selection gain (Chowdhury *et al.*, 2002).

According to Sharma and Jana (2002), assessment of genetic variation in a species is a prerequisite for initiating an efficient breeding program, as it provides a basis for tailoring desirable genotypes.

Further more, a previous knowledge of the structure of the genetic diversity within a large collection of germplasm may be of great help to make decisions on management procedures, as well as on breeding strategies to use in current and future breeding programs (Kumar *et al.*, 2007).

Improvement in yield and quality is normally achieved by selecting genotypes with desirable character combinations existing in the nature or by hybridization. Selection of parents identified on the basis of divergence analysis would be more promising for a hybridization program. (Singh and Gopalakrishman, 1999).

Genetically diverse parents are likely to segregate and or to produce high heterotic crosses. The more diverse the parents are the greater the chances of obtaining high heterotic F1s and broad spectrum of variability in segregating generations. Understanding genetic relationship within and among cultivars could increase hybrid vigour and reduce or avoid re-selection within existing germplasm (Arunachalam, 1981). Genetic diversity study also permits to select the genetically divergent parents to obtain the desirable recombinant in the segregating generations of eggplants.

Studying genetic diversity is critical to success in plant breeding. It provides information about the quantum of genetic divergence and serves a platform for specific breeding objectives. It identifies parental combinations exploitable to create segregating progenies with maximum genetic potential for further selection, as proven by (Aremu *et al.*, 2007).

It exposes the genetic variability in diverse populations and provides justification for introgression and ideotype breeding programmes to enhance crop performance.

Choice of parents has been identified to be the first basic step in meaningful breeding programme (Rahim *et al.*, 2010). Furthermore, the choice of parent selection in diversity studies is valuable because it is a means of creating useful variations in subsequent progenies. Diversity studies on garden eggs at their respective primitive levels will led to the development of widely distributed cultivars and varieties with proven characteristics based on stability and adaptability of performance with consistent tolerance to adverse weather conditions and resistant to diseases. The report of Mohammadi *et al.* (2010), revealed that appropriate parent selection for hybridization in flax using a definite diversity study technique, increased flax yield tremendously.

2.8 Evaluation of Germplasm

Genetic variation is indispensable for effective management and use of genetic resources. Diversity in plant species is assessed on the basis of morphological or phenotypical traits, biochemical techniques based on isozyme patterns and protein profile like SDS-PAGE, and by using DNA based molecular techniques like RFLP, AFLP, RAPD and SSR markers (UPOV, 1991).

2.8.1 Morphological Characterization

Morphological characterization is essential to describe the distinctive characteristics of cultivars and landraces (UPOV, 1991) and therefore considered as the starting point in eggplant plant diversity structure and gene-to-phenotype relationship analysis. It is also useful for characterizing individual accessions and cultivars as a genetic guide in selection of parents for hybridization (Singh *et al.*, 2006)

Polygenic morphological and pomological characteristics serve as genetic markers for germplasm characterizations. The evaluation of morphological characters alone to assess genetic diversity may not be very effective. The diversity based on phenological and morphological characters usually varies with environment. Evaluation of these traits requires comprehensive characterization of genotypes prior to identification. Morphological and phenological characters help to collect the basic information for improvement through breeding programme and further evaluation. Systematic characterization and evaluation of plant genetic resources are prerequisites for effective use of the materials through conventional methods or modern

techniques.

Morphological characterization provides a base and raw picture of germplasm of specific plant species about variability and genetic diversity. These characters can be used as a base for further molecular level studies for confirmation of these morphological characters

2.8.2 Agronomic Evaluation

According to Islam and Uddin, (2009) there were higher genotypic values for number of seeds, height at flowering and days to flowering in sweet potato. Similar reports were made by Hitomi *et al.* (1998) when they carried out a laboratory experiment to investigate the influence of auxin type on the array of somaclonal variants generated from somatic embryogenesis of egg plant. In addition, (Vadivel and Bapu, 1990) confirmed this finding in their attempt to find out the genetic diversity in eggplants.

Abrefa and Ofori (2012), cited Desmukh *et al.* (1986) categorization of PCV and GCV values into following classes; as high (>20%), medium (10-20%) and low (<10%). Degwione *et al.* (2011) reported PCV values ranged from 6.8% in fruit diameter to 27.8% in number of seeds per fruit and GCV ranged from 4.0% in fruit diameter to 22.7% in fruit length. The development of effective breeding programme depends existence of genetic variability.

Singh *et al.* (2011) conducted a research in *Pisum sativum* and reported that the values of the genotypic correlation were little higher and in most cases almost the same as phenotypic correlation coefficient values. Fruit weight was significantly and positively associated with fruit diameter and fruit length at phenotypic level. In addition, at genotypic level fruit weight showed

positively correlation with fruit length. There was significant positive phenotypic and genotypic correlation between days to flowering and height at flowering as well as fruit length.

Abrefa and Ofori (2012) stated that height at flowering revealed significant and highly positive association with fruit length at both phenotypic and genotypic levels in groundnut. Swamy *et al.* (2003) also affirmed a positive association between height at flowering and fruit length. However, Denton and Nwangburuka (2011) reported significant negative association at phenotypic level and positive correlation at genotypic level between plant height and fruit length in *Solanum anguivi*.

Abrefa and Ofori (2012) reported that fruit diameter showed significant positive association with number of seeds per fruit. This collaborates with findings of Tumbilen *et al.* (2011) in their study of the diversity in garden eggs. However, in related studies Ofusu-Budu (1984) and Badea *et al.* (1996) reported that seed content of fruit of garden egg varies with fruit size and it is higher in large fruits. In addition, number of seeds per fruit showed significant negative correlation with fruit length.

Rana and Kalloo (1989) stated that the number of fruits per plant has a close bearing with total fruit yield in tomato. Therefore, the varieties showing high number of fruits per plant might be high yielding. However, along with number and size, weight of fruit also contributes directly to total yield.

Saeed *et al.* (2007) found out that variation between the accessions, on the basis of coefficient of variability was greater in traits like number of fruits per plant and number of flowers per plant. Therefore the material was rich in variability for those traits (Singh, 2004).

Kaushik *et al.* (2011) reported that the magnitude of GCV and PCV were almost equal for number of leaves, days to 50% flowering, fruit length, fruit diameter and fruit yield except for plant height and fruit shelf life indicating that least influence of environment in expression of these traits whereas in case of plant height and fruit shelf life, the magnitude of PCV was higher than their respective GCV denoting environmental factor influencing their expression. The magnitude of genotypic and phenotypic coefficient of variation was higher for number of leaves at 30 days after transplanting, fruit length (cm) and fruit yield. The number of leaves at 30 days after transplanting, fruit length (cm) and fruit yield described greater genotypic as well as phenotypic variability among the accession and sensitiveness of the attributes for mating further modifications by selection. Similar observations were also made in tomato by Singh and Narayan (2004), Mehta and Asati (2008) and Sharma *et al.* (2009).

Yildirim *et al.* (1997) observed that both yield components (tuber number and tuber weight) were associated with tuber yield, but they indicated that tuber numbers were important than average tuber weight. Also the number of tubers in a plant and the percent of dry matter percent had a positive and significant correlation with tuber yield. On the contrary, Hosseinzadeh (2002) did not observe a significant correlation between the number of tubers in each plant and tuber yield either Siyadat *et al.* (2000). Also, negative and significant correlation between the tuber weight and the number of tubers in plant has been reported by Islam *et al.* (2002) and Tsegaye *et al.* (2006) according to the correlation analysis showed that sweet-potato yield is related to many traits.

2.9 Estimation of Genetic Diversity

The appropriate statistical measures of diversity to use have long been a matter of discussion (Magurran, 2003). Several researchers have employed different data source and type from diverse crops to study genetic diversity. Such data source includes morphological and agronomic, pedigree, proximate or biochemical and molecular data (Aremu and Ibrinde, 2011).

According to Aremu *et al.* (2008), the choice of statistical method to be used is dependent on the achievable objectives laid out in the studies

Franco *et al.* (2001) stressed the need for careful considerations to be made when measuring genetic diversity within and between crop populations in research. Such considerations include:

1. The use of multivariate data collected from morphological or agronomic traits. Such data may effectively display discrete, continuous, binomial or ordinal variables.
2. The use of multiple sets arising from morphological, biochemical and DNA-based data. Use of such multiple data sets in diversity studies help to reveal the adequacy in terms of strength and constraints in the choice of each of the data sets. Studied phylogenetic relationships among triticeae species using individual and combined analysis of data sets consisting of morphological and DNA-based traits and discovered divergent results in the analyzed individual and combined data.
3. Expected objective to be achieved. This dictates choice of statistical tool in measuring genetic distance and the level of clustering of the intragenic factors in use. Variation recorded in the measurement of genetic diversity in genotype relationships are based on genetic distances and class groupings.

Genetic relationship among and with breeding materials can be identified and classified using multivariate grouping methods. The use of established multivariate statistical algorithm is important in classifying breeding materials from germplasm, accessions, lines, and other races into distinct and variable groups depending on genotype performance. The frequently used techniques irrespective of the data source (morphological, biochemical and molecular marker data) are cluster analysis, Principal Component Analysis (PCA), Principal Coordinate Analysis (PCOA), Canonical Correlation and Multidimensional Scaling (MDS).

According to Amenu (2011), Cluster analysis presents patterns of relationships between genotypes and hierarchical mutually exclusive grouping such that similar descriptions are mathematically gathered into same cluster. Cluster analysis have five methods namely unweighted paired group method using centroids (UPGMA and UPGMC), Single Linkages (SLCA), Complete Linkage (CLCA) and Median Linkage (MLCA). UPGMA and UPAMC provide more accurate grouping information on breeding materials used than the other clusters.

Principal components, canonical and multidimensional analyses are also used to derive a 2-or 3-dimensional scatter plot of individuals such that the geometrical distances among individual genotypes reflect the genetic distances among them.

Cluster and principal component analysis can be jointly used to explain the variations in breeding materials in genetic diversity studies.

2.10 Heritability of Agronomic Traits

Broad-sense heritability estimates the total genetic effects influencing a trait and includes additive, dominance, and epistatic effects (Nyquist, 1991). In crosspollinated species, narrow-

sense heritability is typically more useful to plant breeders because it measures the additive gene effects, which are passed on to the progeny more predictably than dominant or epistatic gene effects. However, when working with apomictic or asexually propagated crops, in which hybrid vigor and both additive and non-additive gene action are fixed, estimates of broad-sense heritability are more appropriate (Poehlman and Sleper, 1995).

Assessment of genetic variation is the most appropriate statistical tool to find out the magnitude of heritability, genetic coefficient of variation and response to selection using appropriate selection intensity for traits of interest.

Heritability helps the plant breeder to plan and executes effective breeding strategy. The occurrence of high heritability and genetic advance for number of fruit/plant and average fruit weight in tomato had been earlier found (Mittal *et al.*, 1996). Some other researcher reported moderate to high genotypic coefficient of variation and phenotypic variability, heritability and genetic advance for number of flowers/cluster, number of fruits per plant and fruit yield; and concluded that yield was positively associated with number of fruits per plants (Srivastava *et al.*, 1998). Mohanty (2003) also stated high genetic variability, coefficient of variation and heritability for number of branches per plant, number of fruits per plant, fruit weight, plant height and number of days to harvest in tomato and further reported that number of fruits per plant and average fruit weight had positive direct effects on tomato yield.

Makeen *et al.* (2007) evaluated twenty mungbean genotypes to estimate genetic variability, heritability and genetic advance for quantitative characters and reported highly significant differences for all traits with greater magnitude of heritability for plant height and test weight.

Characters with high heritability are less affected by the environment but on the other hand characters with low heritability are typically highly affected by the environment.

Abrefa and Ofori (2012) reported that fruit diameter had the lowest heritability value (34.5%) followed by number of fruiting branches (54.7%). Fruit length had the highest heritability estimate (94.7%) followed by days to flowering (83.5%). The heritabilities for fruit weight, height at flowering and number of seeds per fruit were 76.0%, 74.1% and 66.3% respectively. Denton and Nwangburuka (2011) reported almost similar result in *Solanum anguivi*. In a related study, Islam and Uddin (2009) reported high heritability estimates of 91.5% for fruit length and 62.7% for days to first flowering in *Solanum melongena*. They emphasized that the high heritability values recorded for fruit length, days to flowering, fruit weight and height at flowering suggest that selection could be practiced for these agronomic traits.

Saeed *et al.* (2007) stated that the value of heritability for number of fruits per plant was 0.96 which showed that about 96% of estimate of variation was under genetic control and the coefficient of variability which was 13.92%, suggested that 96% of 13.92% variation could be transferred to the progeny. Singh and Mandhar (2004) also stated that, the relatively low value of coefficient of variability of 7.69% and heritability of 0.36 indicated that the character was partially under the control of genes. The value of high broad sense heritability (0.9715) of yield per plant that 97% of the variation observed was genetically determined. The results are in agreement with the findings of Mittal *et al.* (1996) and Mohanty (2003).

The highest value of broad sense heritability (0.97) for yield per plant showed that about 97% of the variation observed for this trait was genetically determined and will transfer to the progeny (Singh, 2004). Similar results have been reported by Srivastava *et al.* (1998), and Mohanty

(2003).

Kaushik *et al.* (2011) also indicated that the heritability in broad sense ranged from 58.2 for fruit shelf life to 99.9 for fruit yield. The high heritability was recorded for all the number of leaves, days to 50% flowering fruit length, fruit diameter and fruit yield except for plant height except fruit shelf life (58%). Their findings were in collaboration with Pradeepkumar *et al.* (2001), Haydar *et al.* (2007) and Hidayatullah *et al.* (2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Location

The experiment was carried out at the Sinna gardens of the Department of Crop Science and the University of Ghana Farms, Legon, Accra. According to Cobbina (1987), the experimental site is located between latitude of $5^{\circ} 38' 45''$ N and longitude $00^{\circ} 11' 13''$ E with a mean annual temperature of 31° C. The soils are mostly poor in nutrient but well trained, easy to work with and light textured (Brammer, 1962). According to the world Reference Base and FAO systems, the soils fall within Lexisols/Luvisols/Plinthosls (ISSS/ISRIC/ FAO, 1998; FAO, 1988). However, according to Ghana Soil Classification Systems, the soils are generally called savannah Ochrosols (Brammer, 1962).

3.2 Genetic Materials

The genetic material comprised of 20 garden egg accessions, which were obtained from the existing collections at University of Ghana, Plant Genetic Resource Institute of Ghana and Council for Scientific and Industrial Research (CSIR) of Ghana, Bunsu. The 20 garden egg accessions used were A1, A1 (2F), SA1, A2, A2 (2F), A3, A4, A5, A6, A6B, A6C, A6F, A7, SA8, A8, A9, A10, A11C, A12B, and Legon 1.

3.3 Nursery, Field Preparation and Planting

3.4 Seeds of each of the accessions were nursed in soil contained in nursing trays on the 21st of July 2012 at the Sinna gardens at the Department of Crop Science, University of Ghana, Legon, Accra. Five seeds were sown per cell in the nursing tray and later pricked out to one per tray two weeks after planting. Ridomil was applied at the rate of 3 g/L of water to the seedlings a day after pricking out to prevent damping off disease. N.P.K. 19-19-19 at a rate of 70-90 g/L was applied in solution to the seedlings about three weeks after nursing. This was repeated at weekly interval to provide nutrients to the seedlings. Cydim Super at the rate of 2 ml/L was sprayed onto the leaves of the seedling to prevent leaf miners. Daily watering was carried out in the absence of rain. The experimental area was ploughed and then harrowed. Lining and pegging was done a day before transplanting. One-month-old seedlings were then transferred to the University of Ghana Farm.

3.4 Experimental Design and Field Layout

A Randomized Complete Block Design (RCBD) with three replications was used on the field. The seedlings were planted at 80 cm x 80 cm between rows and 80 cm x 80 cm within rows. Each accession was planted in two rows with five plants per row providing a total of 10 plants per accession. Data were collected from six tagged plants (three from each of the middle rows) throughout the experiment expect for fruit length, fruit diameter and single fruit weight for which plants two, four and six were used.

3.5 Agronomic Practices

N.P.K. (15-15-15) was applied to the seedlings three days after transplanting as a starter solution. Cydim Super at the rate of 35ml/15L of water and Levo at 5ml/15L of water were sprayed alternatively every two weeks to ward off leaf miners, white flies and mice.

N.P.K. (15-15-15) at the rate of 250 kg/ha was applied to each plant by side placement two weeks after transplanting and Sulphate of Ammonia at the rate of 125 kg/ha was also applied in split doses at flowering and fruiting.

Weeding was done as and when necessary and watering was done daily since the soil was very dry through out the growing season. The physiologically matured fruits were harvested using a knife.

Table 3.1 Mean monthly temperature ($^{\circ}\text{C}$), rainfall (mm) and relative humidity (%) during the growing period

Month	Temperature ($^{\circ}\text{C}$)		Rainfall (mm)	Relative humidity (%)	
	Minimum	Maximum		Minimum	Maximum
July	23.1	28.8	20.9	68	86
August	22.4	28.2	11.5	67	86
September	23.2	30.0	42.5	67	86
October	23.8	31.2	88.3	67	89
November	24.6	32.4	14.0	63	88

Source: Ghana Meteorological Agency, Memepehuasem, Legon, 2012.

3.6 Data Collection

3.6.1 Morphological Characterization

3.6.1.1 Vegetative Characteristics

Number of leaves: The number of leaves of the six record plants was counted when the plants were five weeks old after transplanting.

Plant height: A meter rule was used to measure the height of the six record plants from the soil level to the tip when the plants were five weeks old after transplanting.

Number of branches: The number of primary branches of the six record plants was counted at when the plants were five weeks old after transplanting.

Girth at flowering: Vernier caliper was used to measure the girth of the stem at 10cm from the soil surface of the six record plants at flowering.

Span of canopy at flowering: Steel tape was used to measure the two broad diagonals of the canopy of the six record plants were flowering.

Span of canopy at maturity: A Steel tape was used to measure the two broad diagonals of the canopy of the six record plants when the fruits have reached physiological maturity.

3.6.1.2 Reproductive Characteristics

Days to first flowering: The number of days from transplanting to the appearance of the first flower among the six record plants.

Days to 50% flowering: The number of days from transplanting to the day three of the six record plants has flowered.

Position of stigma in relation to anther tips: Visual observation of the level of the stigma in relation to the anther tips.

Number of fruits per plant: The total number of fruits for each of the six plants was counted.

Fruit length: Vernier caliper was used to measure the fruit from the apex of the fruit to the base of the fruit stalk.

Fruit diameter: Vernier caliper was used to measure the broadest part of the fruit diameter.

Yield: The total weight of each of the six record plants was measured using a measuring scale.

3.6.2 Variation In Iron and Calcium Content Among 17 Garden Egg Accessions

The experiment was carried out in the laboratory at Food Research Institute of Ghana in Accra. Samples of the garden egg accessions were transported to the food Research Institute in clean containers.

At the laboratory, samples were thoroughly washed with ordinary water and then with doubly distilled water. The stalks were removed and the edible portion of the fruits was analyzed. From each of the garden egg accessions sub-samples were selected and sliced into pieces using a carbon steel knife on a plastic slicing board. The sliced sub-samples were homogenized by thorough mixing. Each sub-sample was further homogenized in a home-styled blender with stainless steel blades. From the homogenized samples, three (3 g) was taken for the analysis of each mineral.

3.6.2.1 Analysis of Ash Content

Three grams (3 g) of the sample was dry-ashed in an electric furnace at 550 °C for 24 hours. The resulting ash was cooled in a desiccator and weighed. The ash was then dissolved with 2 ml of concentrated Hydrochloric acid (HCl) and few drops of concentrated Nitric acid (HNO₃) were added.

The solution was placed in a boiling water bath and evaporated almost to dryness. The content was then transferred to 100 ml volumetric flask and diluted to volume with deionized water. For each of the garden egg accessions two ash solutions were prepared for a duplicate analysis.

The ash content is expressed as

$$\% \text{ ash (dry basis)} = \frac{\text{Weight after ashing}}{\text{Weight before ashing}} \times 100$$

3.6.2.2 Analysis of Iron content

Twenty (20) ml of each of the digested garden egg accession ash solution was pipetted into 50 ml volumetric flask and 30 mg of crystalline ascorbic acid was added with a small spatula and a small volume of deionized water was used to rinse the neck of the flask. The solution was allowed to stand for 10 minutes for complete reduction of the iron to ferrous state. 10 ml of ammonium acetate solution was added and the pH of the solution was adjusted to 4.5 using ammonia and hydrochloric acid. 2 ml of dipyriddy solution was also added and deionized water also added to increase the volume to 50 ml. The solution was then left for 60 minutes for the full

development of the red color. The optimal density of the solution was measured near 500 nm using a calorimeter. The above procedure was repeated for 0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml, 15.0 ml and 20.0 ml of iron standard solution. The resulting calibration curve was used to determine the iron in the sample.

3.6.2.3 Analysis of Calcium Content

Twenty (20) ml of the ash solution was pipetted into a 150 ml beaker. 10ml of hydrochloric acid solution was added to the ash solution. Distilled water was also added to produce 60ml. 3 drops of methyl red indicator was added and then later heated to boiling point. 15ml of saturated ammonium oxalate solution and 5 g of urea were added to the hot solution and then allowed to boil to a temperature of 70 - 80° C for 10 minutes. Ammonia solution was added drop wise while the solution was stirred until it was neutral. The color of the solution changed from red to yellow. The solution was covered with a water glass and allowed to stand for 4 hours. The mixture was filtered through a coarse filter paper and care was taken so that the precipitate was not disturbed. The precipitate was washed with a small volume of cold water until it was free from chloride. The filter paper together with the precipitate was transferred into a beaker. The precipitate was then dissolved with hot dilute sulphuric acid. The solution was then titrated immediately with N/50 potassium permanganate maintaining the temperature at 60 °C. A blank titration was also carried out at same time.

Amount of calcium content was calculated as follows:

$$\text{Calcium content} = \frac{\text{Titre value} - \text{Blank value}}{\text{Weight of sample}} \times 100$$

3.7 Data Analysis

Genstat statistical software was used for data analysis. Means, ranges and standard errors were determined for all quantitative traits. Quantitative morphological data were subjected to Analysis of Variance (ANOVA) to assess significance of the differences among accessions. The least significant difference (LSD) was used to separate the mean performance of genotypes that were significantly different. To estimate the extent of variability, genotypic and phenotypic coefficients of variability (GCV and PCV) were estimated by adopting the formula of Johnson and Robinson (1955). Heritability and genetic advance were estimated according to the methods suggested by (Hanson *et al.*, 1956) and (Johnson Robinson, 1955) respectively.

Table 3.2 Format of analysis of variance

Source of variation	df	Mean square	Expected mean square
Replication (r)	r-1	<i>M1</i>	-
Accessions (a-1)	a-1	<i>M2</i>	$r\sigma_g^2 + \sigma_e^2$
Residual	(r-1)(a-1)	<i>M3</i>	σ_e^2
Total	(ra-1)		

Where σ_g^2 is the genetic variance and σ_e^2 is the error variance.

The genotypic Coefficient of variation (GCV) was calculated as follows:

$$\text{GCV} = \frac{\sigma_g}{\bar{x}} \times 100$$

Where σ_g is the genotypic standard deviation

The phenotypic Coefficient of variation (PCV) was calculated as follows:

$$\text{PCV} = \frac{\sigma_p}{\bar{x}} \times 100$$

Where σ_p is the phenotypic standard deviation

In determining the GCV and PCV, the population mean was estimated by the grand mean (\bar{x}) from the analysis of variance.

The genetic variance was calculated from the ANOVA table as follows:

$$\sigma_g^2 = \frac{M2 - M3}{r}$$

$$M2 = r\sigma_g^2 + \sigma_e^2$$

$$M3 = \sigma_e^2$$

The phenotypic variance was estimated as:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2/r$$

Broad Sense heritability (H) was estimated as:

$$H = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/r}$$

According to Johnson *et al.* (1955), response to selection or expected genetic advance (as a percentage of the mean) after one generation of selection was calculated at 10% selection intensity using the following formula:

$$GA = \frac{i\sigma_p h^2}{m} \times 100$$

$$GA(\bar{x}\%) = \frac{i\sigma_g \sqrt{h^2}}{m} \times 100$$

$$GA(\bar{x}\%) = iGCVh$$

Where GA = genetic advance; σ_p is the phenotypic standard deviation, h^2 is the broad sense heritability and m is the mean of the unselected population and i is the selection differential.

The genetic correlation between yield (A) and other traits (B) ($r_{G(AB)}$) were calculated from the genetic variance and covariance components from the ANOVA as follows:

$$r_{G(AB)} = \frac{\sigma_{G(AB)}}{\sqrt{(\sigma^2_{G(A)} \sigma^2_{G(B)})}}$$

Where $\sigma_{G(AB)}$ is the genetic covariance between traits A and B. This was estimated by summing up the two traits and estimating the variance of the resulting data using the mean squares from the ANOVA. The $\sigma^2_{G(A)}$ and $\sigma^2_{G(B)}$ are the genetic variances of traits A and B respectively.

Principal Component Analysis was carried out using 11 vegetative and reproductive traits subjected to Principal Component Analysis and Cluster analysis was done to determine the relationship between the garden egg accessions.

CHAPTER

4.0 RESULTS

4.1 Variability In Quantitative Traits

Large differences were observed between accessions for the various vegetative and reproductive traits scored (Table 4.1). For each trait, the minimum and maximum values vary largely from their respective means based on the standard error. Number of branches per plant and stem diameter recorded the lowest standard error with respect to their means. At flowering the number of leaves ranged from 13-103 and the plant height ranged from 12.2 – 57.5. Flowering started at 21 days after transplanting in some accessions whereas in others flowering started at 41 days after transplanting (Table 4.1). On the average, three days after the first opened flowers were seen, 50 % of the plants for the particular accession produced opened flowers.

4.2 Variability In Qualitative Traits

In terms of shape of the fruits, both length and diameter varied more than three-fold between plants. The fruit length: diameter ratio which is an approximation to the shape of the fruits varied from 0.38 to 1.74 indicating the large variation available between the plants evaluated. The number of fruits produced per plant was similarly very variable with a range of 4 – 78. The size of fruits produced, estimated by the weight of fruits varied six-fold. Fruit yield varied between plants from 1.9 to 30 t/ha.

The floral buds per inflorescence vary from 1 to greater than 5 among accessions (Figure 4.3). Also in terms of stigma position to anther tips, 19 of the accessions had long style flower and only 1 accession had short style flower (Table 4.2 and Figure 4.2)

Table 4.1 Means, standard errors and ranges of quantitative traits evaluated in 20 garden egg accessions

Trait	Mean± s.e	Range
Number of leaves per plant at flowering	54±3.71	13 – 103
Plant height (cm) at flowering	33.9±1.90	12.2 – 57.5
Canopy span at flowering (cm)	67.1±2.21	41.2 – 88.7
Canopy span at maturity (cm)	89.5±2.31	62.6 – 113.0
Number of branches per plant	8±0.38	3 – 12
Stem diameter at flowering (mm)	11.8±0.38	6.5 – 16.0
Days to first flowering	33±1.14	22 – 41
Days to 50% flowering	36±1.01	25 – 45
Fruit length (cm)	4.1±0.20	2.1 – 6.8
Fruit diameter (cm)	4.3±0.18	2.31 – 7.00
Fruit length: diameter ratio	0.97±0.06	0.38 – 1.74
Number of fruits per plant	27.3±3.05	4 – 78
Weight per fruit (g)	34.8±3.06	13.4 – 78.7
Yield (t/ha)	13.4±1.17	1.9 – 30.0
Ca concentration (mg/100g)	30.1±2.83	12.4 – 50.0
Fe concentration (mg/100g)	3.0±0.40	0.7 – 5.9

¹ s.e = Standard error

Table 4.2. Floral bud per inflorescence and stigma position in relation to anther tips in 20 garden egg accessions

Accessions	No. of Floral bud/Inflorescence	Stigma position in relation to anther tips
A1	1 and 2	Short styled flower
A1 (2F)	1 and 2	Long style flower
SA1	1,2 and 3	Long style flower
A2	1 and 2	Long style flower
A2 (2F)	1 and 2	Long style flower
A3	1 and 2	Long style flower
A4	1	Long style flower
A5	1 and 2	Long style flower
A6	1 and 2	Long style flower
A6B	>5	Long style flower
A6C	1, 2 and 3	Long style flower
A6F	2 and 3	Long style flower
A7	2	Long style flower
SA8	1 and 2	Long style flower
A8	1 and 2	Long style flower
A9	1 and 2	Long style flower
A10	1 and 2	Long style flower
A11C	1, 2, 3 and 4	Long style flower
A12B	1 and 2	Long style flower
Legon 1	1	Long style flower

Table 4.3 Leaf production, plant height and number of branches for 20 accessions of garden eggs under field conditions

Accessions	Leaves at flowering	Height at flowering (cm)	Branches per plant
A8	76.9	20.9	8.2
A10	70.7	23.0	7.6
A9	68.8	36.2	9.3
SA8	67.4	36.6	8.9
A2	57.8	41.2	8.1
A3	57.7	34.3	8.3
A6B	56.6	26.0	7.6
A1	53.6	32.8	8.5
A11C	53.2	39.0	8.6
SA1	53.2	33.1	7.7
A6C	52.8	37.1	7.8
A6	50.3	38.4	8.1
A5	50.0	37.4	8.9
A12B	49.6	39.1	8.8
A4	47.9	35.7	7.5
L1	47.1	33.9	7.5
A6F	47.0	27.9	7.4
A7	45.1	43.7	6.5
A1(2F)	42.6	27.5	7.5
A2(2F)	35.4	33.7	5.7
LSD _{0.05}	7.97	3.47	0.99

There were significant differences ($P < 0.01$) among accessions for vegetative traits evaluated, including number of leaves, plant height, canopy span, stem diameter and number of branches developed. At flowering, differences between accessions for number of leaves became more distinct with accession A8 having the highest number of leaves, and only accession A10 having leaf numbers not significantly smaller than A8 (Table 4.3). Accessions A8 and A10 that had the highest numbers of leaves were the shortest plants at flowering. The tallest plants at flowering were A7, A2 and A12B (Table 4.3).

At flowering, the span of the canopy was largest in the shorter plants. Accessions A5 had the largest span, with eight other accessions having canopy spans not significantly different from that of A5. A11C had the smallest span but was not significantly different from the span of five other accessions including A6F, A1 (2F), A6B, A4 and SA1 (Table 4.4)

Ranking of accessions for canopy span at maturity showed that the span at flowering generally reflects the final span of the accession, except for accession A4. A4 though among the accessions with the least span at flowering, increased its canopy span to 96.5 cm which was not significantly different from that of accession A3 that had the largest span at maturity.

Stem diameter at flowering ranged from 10.0 mm in A4 to 13.2 mm in A12B. Mean comparisons show that the stem diameter of five other accessions including Legon 1, A2, A3, A1 and A5 were not significantly different from that of A12B (Table 4.4).

The branches per plant ranged from 6 to 9 in A2 (2F) and A9, respectively. Only in accession A7 is the number of branches not significantly different from that in accession A2 (2F).

The number of days to first opened flowers, and days to 50 % flowering were both significantly different among accessions. Of all the traits evaluated, the days to flowering traits had the least

Table 4.4 Canopy span at flowering and maturity and girth at flowering for 20 accessions of garden eggs under field conditions

Accessions	Canopy span (cm)		Girth at flowering (mm)
	At flowering	At maturity	
A8	72.8	93.2	12.3
A10	70.0	94.1	11.8
A9	73.2	92.6	11.4
SA8	69.6	88.8	12.2
A2	69.4	89.8	13.0
A3	69.3	98.1	12.6
A6B	64.2	80.7	11.6
A1	68.8	89.2	12.6
A11C	59.6	81.2	11.3
SA1	64.4	87.3	12.3
A6C	65.4	90.2	11.3
A6	65.8	91.6	11.4
A5	73.7	94.1	12.4
A12B	69.2	93.1	13.2
A4	64.3	96.5	10.0
L1	65.7	82.3	13.1
A6F	61.4	84.6	11.1
A7	66.0	84.7	11.0
A1(2F)	62.7	87.6	11.4
A2(2F)	65.6	86.6	10.9
LSD _{0.05}	5.4	5.7	0.9

coefficients of variation. Significant differences ($P < 0.01$) were found between accessions for the fruit length, fruit diameter and the fruit length: diameter ratio, the number of fruits per plant, weight per fruit and fruit yield, as well as the calcium and iron contents. Fruit yield and the yield related traits had the largest coefficients of variation among all the traits evaluated.

Accessions A10 and A8 were the earliest to flower, each initiating flowering at 22 DAT with 50% of the plants developing opened flowers by 25 DAT (Table 4.5). Accession A2 (2F) was the last to flower, and took 41 days to produce the first opened flowers. Whereas accessions A11C, A1 and A7 had on average a day difference between the time to first and 50 % flowering, accessions A6B and SA8 had on average six days difference between time to first and 50% flowering.

Accession A2 (2F) developed the longest fruits whereas the shortest fruits were produced by accession SA8. Fruits with the longest diameter were produced in accession SA1, and accession A6B produced fruits with the shortest diameter (Table 4.5). Differences in the length and diameter of the fruits produced by individual accessions gave rise to fruits with varying shapes, approximated by the length: diameter ratio. Notably accessions A4, A6B, A6C, and A2 (2F) that had ratios of 1.2 and above appeared more slender in shape. Accessions with fruit length: diameter ratios between 0.7 to 0.8 appeared more roundish. The shape of the fruits was related to the weight such that those with longer diameter (4.8 to 5.1 cm) such as A1 (2F), A1, SA1 and A2 were significantly heavier than those accessions that produced fruits with diameter in the range of 3.5 to 3.7 cm. Accessions that produced fruits of small size (weights ranging from 20 to 24 g) produced significantly a large number of fruits per plant compared with accessions such as A6F and A1 (2F) that produced fruits with weights of 42.3 and 52.6 g, respectively (Table 4.5).

Table 4.5 Flowering date, fruit length and diameter of 20 garden egg accessions

Accessions	Days to first flowering	Days to 50% flowering	Fruit length (cm)	Fruit diameter (cm)	Fruit length/diameter ratio
A10	22	25	4.7	4.4	1.1
A8	22	25	3.0	3.7	0.8
A6B	28	34	4.3	3.5	1.2
SA8	29	35	2.7	3.7	0.7
A9	20	34	3.8	3.6	1.1
L1	31	35	4.5	4.2	1.1
A2	31	36	4.7	4.9	1.0
A5	31	36	3.2	4.7	0.7
A3	33	37	4.7	4.2	1.1
A4	34	35	4.7	3.7	1.3
A6	34	35	4.7	4.2	1.1
A12B	35	37	3.9	3.7	1.1
A6F	35	37	4.3	4.7	0.9
SA1	35	37	3.4	5.1	0.7
A6C	35	38	4.9	4.3	1.2
A11C	36	37	3.2	3.9	0.8
A1	36	37	3.9	4.8	0.8
A1(2F)	37	39	3.3	5.0	0.7
A7	37	38	3.6	4.5	0.8
A2(2F)	41	45	5.2	4.2	1.2
LSD _{0.05}	2	1	0.2	0.2	0.1

There were exceptions though, such as accessions A2 (2F) and A6B that produced relatively few fruits that were of small sizes. The highest yield of 17.9 t/ha was produced by accession A10, though yields produced by six other accessions were not significantly different from that produced by A10. Accession A6B produced the lowest yield of 7.3 t/ha (Table 4.6).

Among the 17 accessions evaluated for fruit mineral content, accession A6 had the highest calcium content which was four times higher than that in accession A10 that produced the lowest fruit content of calcium. A10 however, produced the highest fruit content of iron (5.8 mg/100g of fruit) whereas the lowest fruit content of iron was 0.8 mg/100g of fruit in accession A6F (Table 4.6). Only accession A6 combined high levels of iron and calcium in its fruit. On the other hand, accessions A6F and A11C had low contents for both nutrients.

4.3 Variance Components and Heritability

The genotypic variance component of each trait estimated following the analysis of variance were all significant based on the associated standard errors (Table 4.7). Each genetic variance component was more than two times the value of its associated standard error. The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) in all the traits evaluated; both were moderately low, ranging from 7.28 to 57.66 and 5.02 to 57.56, respectively (Table 4.7).

Table 4.6 Fruit yield, calcium and iron content of 20 garden egg accessions

Accessions	Weight per fruit (g)	Fruits per plant	Yield (t/ha)	Ca content (mg/100g)	Fe content (mg/100g)
A10	44.0	27	17.9	12.4	5.8
A8	24.3	42	15.9	37.7	4.8
A6B	22.0	21	7.3	47.7	1.5
SA8	20.7	42	12.6	31.3	1.1
A9	20.0	48	14.9	15.6	4.0
L1	35.0	23	11.7	-	-
A2	45.0	24	16.2	35.3	1.3
A5	39.2	23	13.2	48.3	1.6
A3	36.6	26	14.4	-	-
A4	27.3	37	15.9	28.2	4.5
A6	38.5	19	11.8	48.6	4.7
A12B	22.6	40	13.7	19.1	4.2
A6F	42.3	16	10.6	19.2	0.8
SA1	47.8	21	15.7	17.6	5.1
A6C	36.8	25	13.7	35.2	1.5
A11C	22.8	35	12.2	19.3	1.7
A1	51.4	20	15.4	36.5	1.9
A1(2F)	52.6	14	10.9	-	-
A7	38.1	26	15.3	38.9	2.0
A2(2F)	30.3	18	8.1	21.3	4.9
LSD _{0.05}	5.4	6	2.8	5.3	0.4

- Not determined

The heritability estimates were low for canopy span, moderate for stem diameter, number of leaves, plant height and fruit diameter, and high for the other traits evaluated. The heritability estimates for the calcium and iron contents were close to unity (Table 4.7).

The genetic advance was low for the canopy span and girth at flowering, moderate for plant height, number of leaves and days to flowering, and high for yield and yield component traits.

The genetic advance for the contents of iron and calcium were very high.

4.4 Trait Associations

Pearson correlation coefficients were obtained between 13 vegetative, reproductive and fruit mineral content traits (Table 4.8). Tall plants had fewer leaves ($r = -0.487$), flowered later ($r = 0.623$) and had higher content of ash in their fruits ($r = 0.677$) than the short plants. The number of leaves per plant was positively correlated with the number of branches and number of fruits developed, and negatively correlated with number of days to 50% flowering. The plant height was negatively correlated with leaves per plant but positively correlated with 50% flowering.

The stem diameter at flowering showed a positive correlation only with the number of branches per plant. Plants that developed larger fruits had lower content of total minerals in the fruits ($r = -0.480$), and developed fewer number of fruits ($r = -0.726$). The weight of the fruits was highly positively and significantly correlated with the fruit diameter, but not with the length of the fruits. As such, the number of fruits developed was negatively correlated with the fruit diameter.

Table 4.7 Variance components (\pm standard error), genotypic and phenotypic coefficients of variance, genetic advance and heritability estimates for various traits assessed on 20 garden egg accessions

Trait	Genotypic variance	GCV (%) ¹	PCV (%) ²	Broad Sense Heritability	Genetic Advance (%)
Number of leaves per plant, 37 DAT	96.0 \pm 33.80	18.07	22.22	0.66	25.86
Plant height, 37 DAT	34.6 \pm 11.72	17.37	19.56	0.79	27.15
Canopy span at flowering	11.4 \pm 4.91	5.02	8.32	0.36	5.34
Canopy span at maturity	20.5 \pm 8.01	5.06	7.28	0.48	6.20
Number of branches per plant	0.6 \pm 0.23	9.65	11.30	0.73	14.50
Girth at flowering	0.6 \pm 0.23	6.67	9.20	0.52	8.50
Days to first flowering	23.2 \pm 7.66	14.79	14.91	0.98	25.82
Days to 50% flowering	18.6 \pm 6.11	12.12	12.19	0.99	21.21
Fruit length	0.5 \pm 0.17	17.58	18.95	0.86	28.69
Fruit diameter	0.2 \pm 0.08	11.43	13.82	0.68	16.63
Fruit length: diameter ratio	0.04 \pm 0.01	20.95	22.98	0.83	33.61
Number of fruits per plant	92.2 \pm 31.34	35.16	39.90	0.78	54.54
Weight per fruit	108.0 \pm 36.25	29.88	32.79	0.83	47.93
Yield	6.6 \pm 2.48	19.26	20.43	0.89	31.96
Ca concentration	142.4 \pm 51.46	39.61	39.89	0.99	69.21
Fe concentration	3.0 \pm 1.07	57.56	57.66	0.99	101.14

¹GCV = Genotypic coefficient of variation; ²PCV = Phenotypic coefficient of variation; ³ Days after transplanting

Also the weight of the fruits was negatively and significantly correlated with fruits per plant. Neither the fruit content of iron nor calcium had a significant correlation with the total mineral content (ash content). The relationship between the fruit content of iron and calcium of the 17 accessions are shown in Figure 4.1. In terms of the iron content, the accessions fall into two discrete groups, whereas for the calcium content, there was a more normal distribution. The phenotypic correlation between the iron and calcium contents was not significant (Table 4.8).

Phenotypic and genetic correlation coefficients were obtained between fruit yield and the 13 other traits studied (Table 4.9). The only significant phenotypic correlations were with canopy span and days to 50% flowering. Except for the weight per fruit, stem diameter, fruits per plant and fruit diameter, the genotypic correlations were higher than their corresponding phenotypic correlations. Based on the associated standard errors, the genotypic correlations were significant only with the number of fruits per plant, branches per plant, leaves per plant, canopy span and days to 50% flowering.

4.5 Principal Component and Cluster Analysis

The multivariate analyses included 11 vegetative and reproductive traits. The first component accounted for 35.6% of the total variation, the second contains 20.7% and the third contains 14%, amounting in total to 70.3% of the variation explained in the data. The first component was accounted for by the number of leaves per plant and the number of fruits per plant (Table 4.10). Both traits had negative scores, indicating they are positively correlated, with accessions A8, A9, SA8, A10 and A12B having the best expression for the index of these traits that dominated the first principal component axis (Figure 4.9).

Table 4.8 Pearson correlation coefficients between various traits of 20 garden egg accessions

Ash content	-									
Branches per plant	0.122	-								
Ca content	-0.150	0.044	-							
Canopy span	-0.082	0.323	-0.031	-						
Days to 50% flowering	0.510	-0.354	-0.007	-0.417	-					
Fe content	0.028	-0.191	-0.377	0.501*	-0.315	-				
Fruit diameter	-0.268	-0.224	0.012	-0.067	0.231	-0.148	-			
Fruit length	-0.178	-0.544*	-0.061	0.097	0.236	0.221	0.121	-		
Fruits per plant	0.378	0.549*	-0.288	0.350	-0.384	0.188	-0.694**	-0.475	-	
Stem diameter	-0.028	0.527*	0.091	0.170	-0.202	-0.100	0.251	-0.373	0.078	-
Leaves plant	-0.315	0.558*	-0.081	0.302	-0.854**	0.144	-0.310	-0.417	0.581*	0.349
Plant height	0.677*	0.105	0.136	-0.039	0.623*	-0.309	0.148	-0.018	0.054	-0.003
Weight per fruit	-0.480*	-0.238	0.074	0.050	0.071	-0.039	0.940**	0.272	-0.726**	0.196
	Ash content	Branches per plant	Ca content	Canopy span	Days to 50% flowering	Fe content	Fruit diameter	Fruit length	Fruits per plant	Stem diameter

* P < 0.05; **P < 0.01

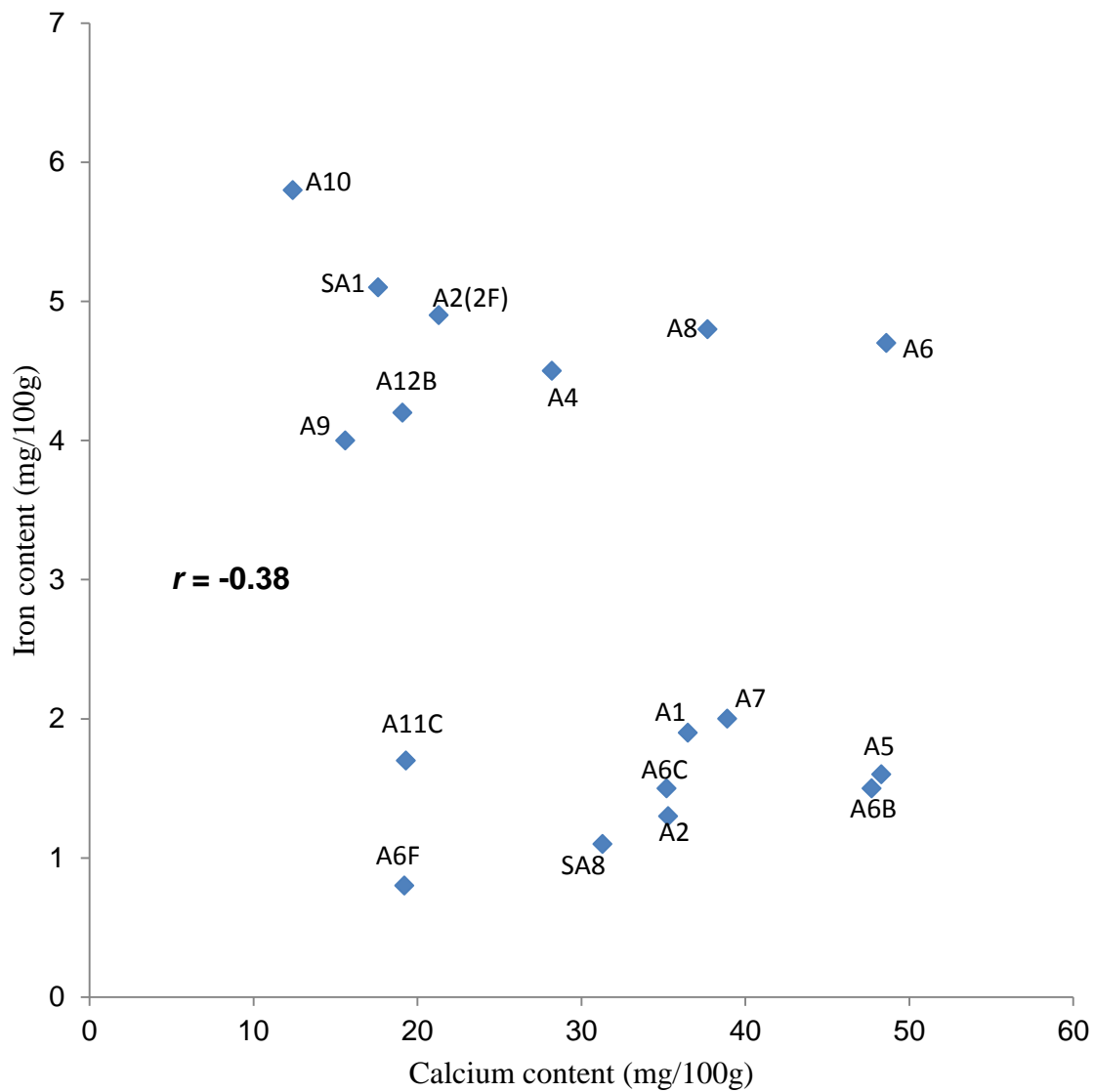


Figure. 4.1 Relationship between the fruit contents of Fe and Ca among 17 garden egg accessions evaluated under field conditions.

Table 4.9 Phenotypic and genetic correlations between yield and mineral among 20 garden egg accessions

Trait	Phenotypic correlation (r_p)	Genetic correlation (r_g)
Ash content of fruit	-0.199	-
Branches per plant	0.286	0.368 \pm 0.141
Ca content of fruit	-0.224	-
Canopy span	0.606*	0.897 \pm 0.023
Days to 50% flowering	-0.516*	-0.780 \pm 0.049
Fe content of fruit	0.299	-
Fruit diameter	0.281	0.185 \pm 0.182
Fruit length	-0.176	-0.190 \pm 0.181
Fruits per plant	0.355	0.315 \pm 0.153
Stem diameter	0.257	0.185 \pm 0.182
Leaves per plant	0.464	0.646 \pm 0.079
Plant height	0.044	0.082 \pm 0.205
Weight per fruit	0.353	0.167 \pm 0.186

* $P < 0.05$

- Not determined because of incompatible number of replicates between trait and yield.

The second principal component axis was dominated by yield and fruit size (weight per fruit and fruit diameter), each of which was positively correlated with the other. Each of these three traits had negative latent score, placing emphasis on only accessions A8 and A10 among the first set of five accessions separated by the first principal component 1 axis. The third component was

dominated by plant height and days to 50% flowering, which places accent on accessions A10 and A8 (among the first set of five identified by (PC1) as being short and early to flower.

Table 4. 10 Latent vector scores of principal component analysis

Traits	PC 1	PC 2	PC 3
Branches per plant	-0.361	-0.094	-0.342
Canopy span	-0.235	-0.247	-0.083
Days to 50% flowering	0.368	0.209	-0.405
Fruit diameter	0.295	-0.497	-0.117
Fruit length	0.207	0.020	0.199
Fruits per plant	-0.434	0.223	-0.160
Stem diameter	-0.137	-0.298	-0.258
Leaves per plant	-0.440	-0.140	0.204
Plant height	0.078	0.129	-0.711
Weight per fruit	0.276	-0.540	0.016
Yield	-0.258	-0.415	-0.139
% Variation	35.6	20.7	14

The hierarchical cluster analyses revealed three distinct clusters (Figure 4.10). At similarity index of 85% the first cluster includes 11 accessions. The second includes accessions A11C, SA8, 12B, A9 and A4. The third include A10 and A8. Accessions A2 (2F) and A6B appear unique based on the traits for which the 20 accessions were assessed.



LONG SYLE FLOWER



SHORT SYLE FLOWER

Figure 4.2 Variation in the stigma position in relation to anther tips among accessions



1



2



3



4



>5

Figure 4.3. Variability in number of floral buds per inflorescence among accessions.



A1



SA1



A1 (2F)



A2

Figure 4.4 Variability in fruit shape at various stages of development in accessions A1, SA1, A1 (2F) and A2.



A2 (2F)



A3



A4



A5

Figure 4.5 Variability in fruit shape at various stages of development in accessions A2 (2F), A3, A4 and A5.



A6



A6B



A6C



A6F

Figure 4.6 Variability in fruit shape at various stages of development in accessions A6, A6B, A6C and A6F



A7



A8



SA8



A9

Fig 4.7 Variability in fruit shape at various stages of development in accessions A7, A8, SA8 and A9.



A10



A11



A12B



Legon 1

Fig 4.8 Variability in fruit shape at various stages of development in accessions A10 A11C, A12B and Legon 1

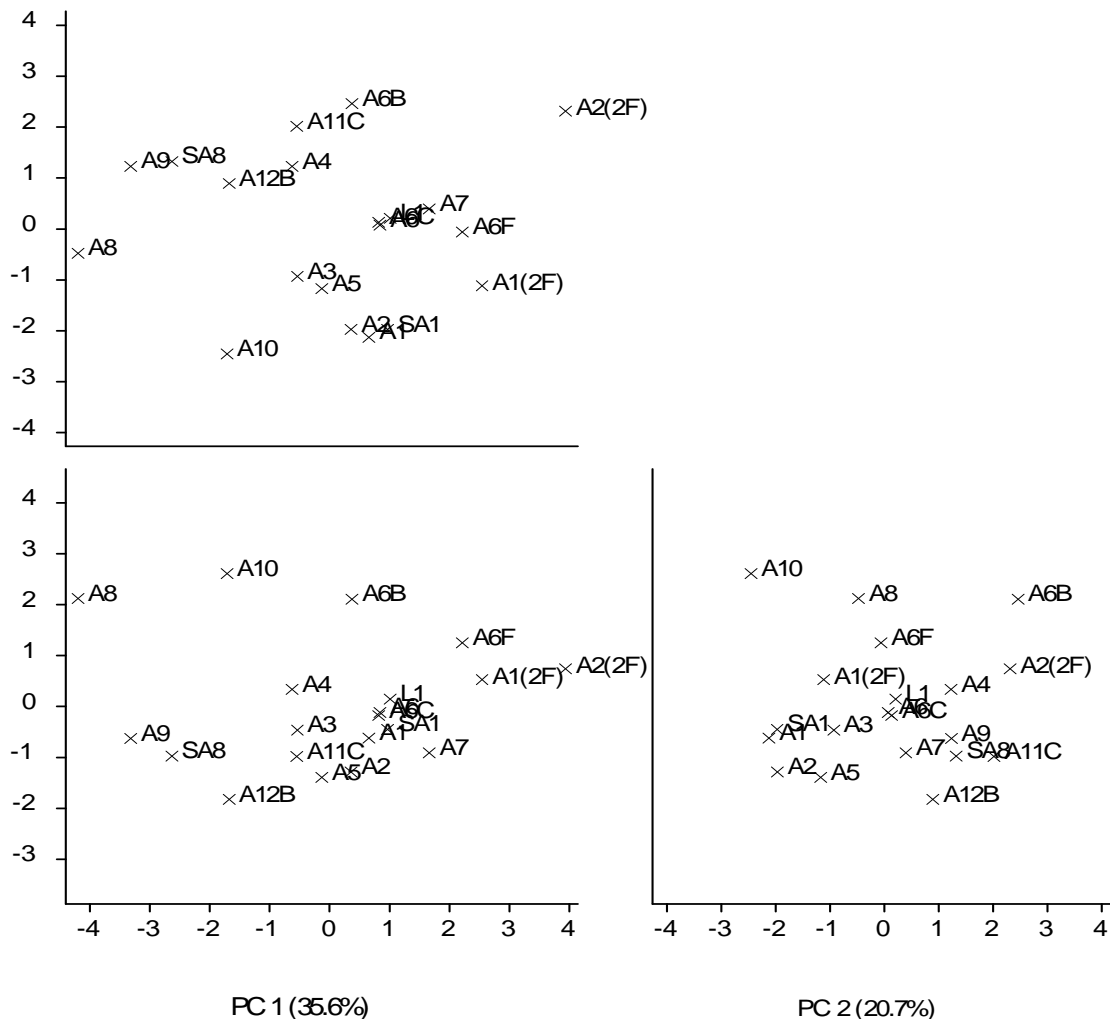


Fig 4.9 Ordination in the space of the first three principal component axes of vegetative and reproductive traits of twenty garden egg accessions evaluated under field conditions.

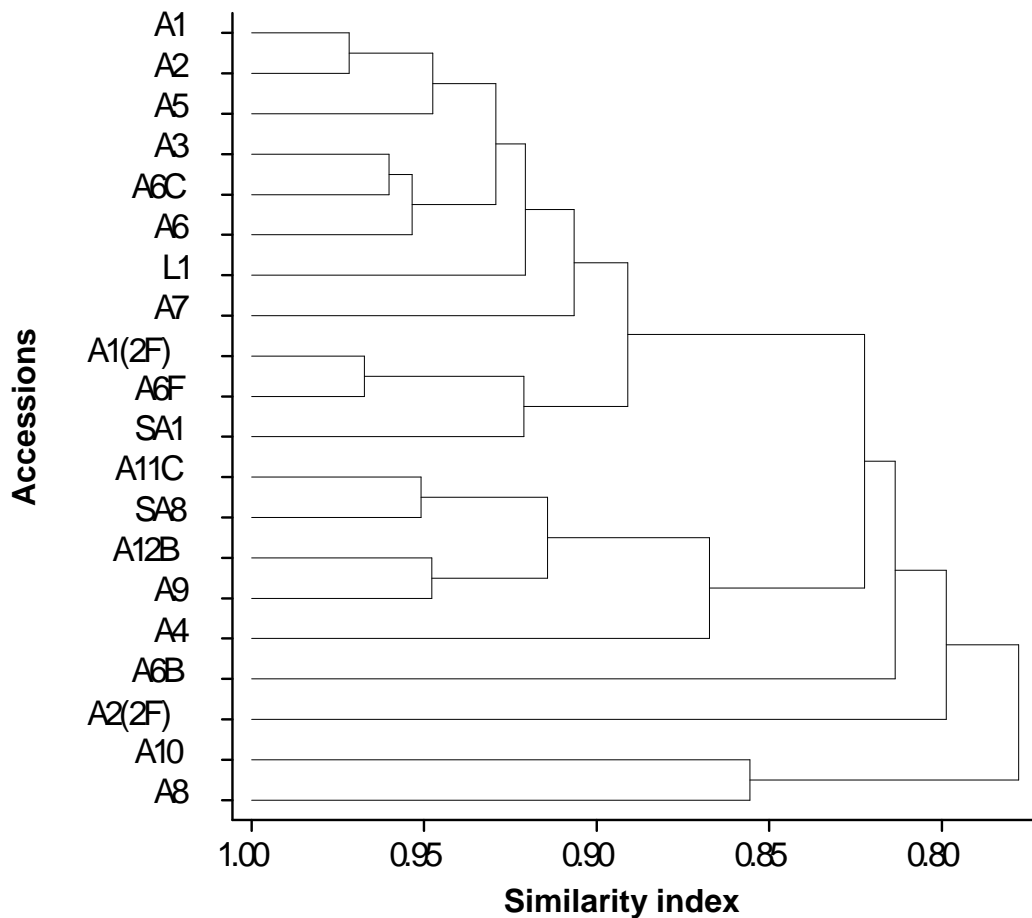


Fig 4.10 Hierarchical cluster analyses of 20 garden egg accessions based on the group average method using environmental correlation matrix of various reproductive and vegetative traits.

Chapter 5

5.0 Discussion

Large ex situ germplasm collections have been established for the major seed crops at world-based or regional gene banks (Plucknett *et al.*, 1987; Adham and Van Sloten, 1990). A thorough characterization of these genetic resources is critical to efforts aimed at genetic improvement of the crops. Large-scale evaluations of morpho-physiological traits desirable for crop improvement have also been undertaken in some instances with the aim of providing breeders with information of practical interest. The lack of such information has frequently been advocated as one of the main constraints to a more extensive and more effective utilization of germplasm collections in breeding programs (Frankel, 1970; Duvick, 1984; Williams, 1991).

5.1 Within-Accession Variations

For an inbred species such as the garden egg, its genetic evaluation or characterization is often based on the assumption that an accession or variety is a homogeneous sample of genetically pure seed. Large variations between plants within accessions are an indication of plausible variation within the accessions that could arise from seed admixtures or to a highly variable soil in terms of fertility. The large standard errors associated with the means for morphological traits observed in this study may therefore arise from genetic variation among plants within accessions, or a highly variable soil in terms of fertility. Evidence of within-cultivar heterogeneity has been documented experimentally in cultivars of many inbred crop species including rice, soybean and red clover with the use of morphological, isozyme and molecular markers (Wang and Tanksley, 1989; Kresovich *et al.*, 1992; Kongkiatngam *et al.*, 1995; Virk *et al.*, 1996; Haun *et al.*, 2011).

For accessions that have not been purified genetically after collection, a high degree of heterogeneity would be expected within accessions. This can constrain efforts at agronomic trait evaluations, making phenotypic or molecular characterization of germplasm an imperative first step to purify the accessions. Conventional soil analyses are typically too expensive to capture soil heterogeneity at a scale of plant to plant positions in the field. The confounding influences of the environment on the use of plant morphological features in assessing diversity of accessions have been elaborated by Gilbert *et al.*, 1999. Thus, whether the observed large variations in the current study are the result of genetic differences between plants within accessions or due to a variable soil environment will require further investigation such as the use of molecular markers to ascertain genetic purity.

The position of stigma in relation to anthers varies with cultivars and can also vary in different flowers of the same cultivar. Some accessions had single flower while others had cluster flowers per inflorescence. A particular accession could also contain 1, 2, 3 and 4 flowers per inflorescence on the same plant. This agrees with work done by Som and Maity (1986), Chadha and Saimbhi (1977) in eggplant, Murtasow *et al.* (1971) and Prasad and Prakash (1968). The stigma was mostly above the anther tips except in one accession that the stigma was below the anther tips. Popova (1961) found that stigmas are either above, on the same level or below the anthers.

5.2 Genetic Variance and Heritability of Traits

The success of breeding programs is principally dependent on the genetic variation available in the breeding materials. Evaluating and understanding the extent of genetic variability existing in the garden egg collections in Ghana is therefore important for the effective utilization of the

germplasm to improve the crop. The significant estimates of the genetic variance for all traits evaluated provide an indication that the assembled accessions as a group have an appreciable level of genetic variability. This observed variability among the accessions plays an important role in any crop-breeding program and will determine the limit of selection for yield improvement in the garden egg in Ghana. The relative amount of genotypic variation is best expressed as the genotypic coefficient of variation (GCV), since this variable takes into account the mean value as well as the unit of measurement into consideration, and provides a measure for comparing genetic variability in various metrical characters. Among the traits evaluated, the flowering traits had the highest proportion of GCV: PCV estimates with correspondingly high estimates of broad sense heritability, supporting findings in many other crops that time to flowering is typically under strong genetic control (Sachs and Coulman, 1983; Rahman and Bahl, 1986; Thurling and Ratinam, 1989). In general, the reproductive traits were under a stronger genetic control compared with the morphological traits based on the proportion of GCV to PCV estimates. Burton (1952) suggested that the genetic coefficient of variation together with heritability estimates gave a better picture of the extent of heritable variation. In breeding improved garden egg varieties, traits related to the appearance of the fruit such as size and shape are important since they influence consumer preferences. In general, consumers prefer large size fruits. For the morphological traits, particularly the canopy span and girth, the lower estimates of broad sense heritability are indicative of the higher influence of the environment in expression of these traits. These indicators of the extent of genetic control on metric characters in the accessions evaluated have implications for planning an efficient selection program. Selection for traits that are under strong environmental influences will need to be conducted in higher number of replicates in both time and space to improve upon their precision (Hallauer, 2007). Breeding

methodologies that control environmental variation well, such as the use of family selection or progeny testing, will improve the rate of gain for these traits because of the relatively large environmental variance associated with their assessment (Amand and Wehner, 2001). Conversely, for traits under high genetic control such as days to flowering (an indication of earliness to maturity) and fruit size, simple selection methods in the early generations after hybridization can be effective in selecting for these traits. It is worth noting, however, that since quantitative traits are affected largely by environmental factors and the heritability estimates were obtained from a single year and single site study, they are biased upwards. Haussmann *et al.* (2002) noted that such upward bias in the estimation of genetic variances and heritability estimates leads to discrepancies between expected and realized responses to selection.

Heritability estimates are indicative of the relative importance of genetic variation to the total variation in a population and hence they depend on the absolute size of genetic and environmental variations. The genetic component varies with the type of population (e.g. individuals, as in this study, or progenies of particular mating designs) while the environmental component is dependent on the experimental conditions and number of replications in which the traits are measured (Holland *et al.*, 2003; Hallauer, 2007). In this study, the broad-sense heritability estimates were very high for the Fe and Ca contents of the fruit. This is probably arising from parameters that were determined in the laboratory with low environmental variation compared with other traits that were assessed in the field with larger components of environmental variation.

The relative magnitude of additive (fixable) and non-additive (non-fixable) genetic variation is confounded in the estimates of broad-sense heritability (Roy, 2000 and Chahal and Gosal, 2002). Since garden egg is predominantly a self pollinated crop, the germplasm represents a mixture of

pure-lines (Falconer and Mackay, 1996). The genetic component of variability among the garden egg accessions is therefore attributable to additive and additive-based epistatic interaction of genes controlling the different traits assessed. Progress in breeding for particular desirable phenotypes should be effective given the large size of the heritable components of the variation.

The value of heritability estimates is enhanced when used together with the selection differential or genetic advance (Falconer and Mackay, 1996). Combined high heritability estimate and genetic advance is an indication that variation is attributable to high degree of additive effect and selection would prove effective (Panse, 1957). In addition to the contents of Fe and Ca in the fruit, high response to selection is expected to result from selection for the weight per fruit and number of fruits per plant, both of which are important components of final yield. In *Solanum melongena*, Patel *et al.* (2004) similarly found high response to selection for yield and yield components.

5.3 Correlation Between Traits

In crop breeding particularly for complex traits such as yield, correlations between traits are of value both from a practical point of view in determining secondary traits for selection and from a physiological perspective, in order to understand possible trade-offs between improvement for yield and component traits. If a negative correlation exists, then selection pressure for either one of these traits will result in positive gains in that trait but negative gains in the other. The phenotypic correlation coefficients between yield and other traits showed significant estimates only for days to flowering and canopy span, the former being negative and the latter positive.

This implies that early flowering garden egg accessions were of higher yield than latter flowering types. Also, those accessions with larger span of the canopy were higher yielding than those with narrow span.

Phenotypic correlations arise from both genetic and environmental causes. Lynch (1999) noted that genetic correlations depend on the general pleiotropic effects of genes and, or on the pattern of gametic-phase disequilibrium between genes affecting different traits. A set of closely linked genes present on one chromosome tend to be inherited together and may be the cause of genetic correlations. For both days to flowering and canopy span, the genetic correlation coefficients were larger than their corresponding phenotypic correlation coefficients indicating a stronger influence of genetic causes for the correlations. Though the phenotypic correlations between yield with number of leaves, and with number of branches were not significant at the 5% probability level, their corresponding genetic correlation coefficients were associated with standard errors that were less than twice the estimates, indicating significance. A strong environmental influence probably reduced the significance of the phenotypic correlation coefficients.

The branches per plant were significant and positively correlated with number of fruits per plant and this concurs with the work done by Cramer and Wehner (2000) in cucumber.

Also, the fruit weight was significantly and positively correlated with fruit diameter which implies that the diameter of the fruit rather than the length, is a better indicator of the fruit weight. This is in agreement with the work done by Tatis *et al.* (2009) in eggplant and Ben-Chaim and Poran (2000) in chilli. Again fruit weight was highly significant and negatively correlated to fruit per plant. This result agrees with the findings of Wessel-Beauver (1992) in

tomato, Pimentel *et al.* (2008) in passionfruit, and Monpara and Kamani (2007) and Tatis *et al.* (2009) in eggplant. Universal compensation between yield related characters is a common observation in crops, making it difficult to simultaneously increase yield through positive selection for all yield related traits concurrently (Kambal, 1969; Osman *et al.*, 2013). These results indicate that the improvement of garden egg through a higher number of fruits per plant will lead to progressive reduction in the fruit weight, compromising yield and quality when large fruits are required, as emphasized by Bertin *et al.* (1998), Antonini *et al.* (2002). There must be a balance between fruit per plant and fruit weight for progress in breeding for yield. Because of this universal compensation among the yield characters, a selection index giving weights to the various characters that determine yield have been advocated, rather than selecting on single yield related traits (Hardwick and Andrews, 1980).

The correlation between calcium and iron contents of the fruit though negative, was low and not statistically significant, implying that it should be possible to simultaneously select for high fruit contents for the two important nutrients. Fruit weight was positively correlated with calcium content. This finding concurs with the findings of Izhaki (2004) and Izhaki *et al.* (2002) that larger fruits tend to be rich in calcium and small fruits tend to be poor in calcium.

5.4 Relatedness of genotypes and Implications for improvement

Multivariate statistical tools have found extensive use in summarizing and describing the inherent variation among crop genotypes. In particular, principal component analysis (PCA) and hierarchical cluster analyses techniques identify plant traits that characterize the distinctness among selected genotypes. These are often extended to the classification of a population into

groups of distinct orders based on similarities in one or more characters, and thus guide in the choice of parents for hybridization and development of crop ideotypes (Donald, 1968). Leaves per plant and fruits per plant dominated the first component of the PCA that accounted for 35.6% of the variation. Yield and fruit size (weight per fruit and fruit diameter) dominated most for the 20.7% of the variation accounted for by the second principal component. The third principal component was dominated by days to flowering and plant height. For all these traits, two accessions A8 and A10 had the best expression, evident by their positions on the PCA plots. These two accessions though quite dissimilar to each other in the cluster diagram, were more closely related than to the other accessions evaluated. It is well documented that crosses between unrelated and consequently genetically distant parents show greater hybrid vigor than that between closely related parents (Mehta *et al.*, 2004). Information about genetic diversity also permits the classification of parent lines into heterotic groups, which is particularly important for hybrid breeding. Thus, the prominent characters coming together in different principal components and contributing towards explaining the variability have the tendency to remain together which may be kept into consideration during utilization of these characters in breeding program.

In the cluster analysis, A8 and A10 are clustered together. In all the traits evaluated, these two accessions were short, early maturing, had high number of leaves, high number of branches, broad canopy span and larger number of fruits. Accessions A2 (2F) and A6B had low yield and small fruits. However, A2 (2F) flowered late but A6B flowered early.

CHAPTER SIX

6.0 CONCLUSION

The study revealed that substantial variation exists within the 20 accessions of garden egg evaluated. The accessions showed wide variability for fruit contents of Fe and Ca. Fruits contents of Fe and Ca were, however, negatively correlated. Most of the traits, particularly those associated with fruit yield had high volumes for both broad sense heritability and genetic advance which provide indications that recurrent selection procedures may be used to fix these traits after hybridization and selection. The general compensation between yield related traits might, however, constrain progress to developing high yielding varieties with large fruit size. The high yielding accessions were A8 and A10. Both accessions were high yielding, short, had broad canopy span, matured early, had high number of leaves, branches and fruits.

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Appendix

Mean squares of leaf production, plant height and branches

Sources of variation	df	Mean square ¹		
		Leaves at flowering	Height at flowering	Branches per plant
Replication	2	1514.5	297.9	6.2
Accession	19	1867.2*	645.1*	12.7*
Residual	338	147.5	27.8	2.3
Total	359	247.0	62.2	2.9
CV (%)		22.4	15.6	19.1

¹ ANOVA was performed on individual plant data* Significant at $P < 0.01$

Mean squares of canopy span and stem diameter

Sources of variation	df	Mean square ¹		
		Canopy span at flowering	Canopy span at maturity	Stem diameter at flowering
Replication	2	2172.6	1827.9	63.0
Accession	19	240.8*	395.3*	12.9*
Residual	338	59.5	65.6	1.7
Total	359	83.6	96.4	2.6
CV (%)		26.6	9.1	11.0

¹ ANOVA was performed on individual plant data
Significant at $P < 0.01$

Mean squares of days to first flowering and days to 50% flowering

Sources of variation	df	Mean square	
		Days to first flowering	Days to 50% flowering
Replication	2	0.35	0.02
Accession	19	70.86*	56.52*
Residual	38	1.14	0.65
Total	59	23.57	18.62
CV (%)		3.27	2.26

* Significant at $P < 0.01$

Mean squares fruit length, fruit diameter and fruit length : diameter ratio

Sources of variation	df	Mean square ¹		
		Fruit length	Fruit diameter	Fruit length: diameter ratio
Replication	2	1.67	4.14	0.23
Accession	19	22.96*	11.07*	1.74*
Residual	858	0.25	0.33	0.03
Total	879	0.75	0.58	0.06
CV (%)		12.43	13.57	16.3

¹ ANOVA was performed on individual plant data

* Significant at $P < 0.01$

Mean squares from the analysis of variance of yield component traits and yield for 20 garden egg accessions evaluated under field conditions

Sources of variation	df	Mean square ¹		
		Number of fruits per plant	Weight per fruit	Yield
Replication	2	45.28	60.92	11.57
Accession	19	1729.45*	1992.81*	137.03*
Residual	335	79.52	66.19	18.28
Total	356	167.14	168.99	24.56
CV (%)		32.66	23.39	32.00

¹ ANOVA was performed on individual plant data

* Significant at $P < 0.01$

Mean squares from the analysis of variance of calcium and iron contents for 17 garden egg accessions

Sources of variation	df	Mean square	
		Ca concentration	Fe concentration
Replication	1	6.58	0.09
Accession	16	291.01*	6.07*
Residual	16	6.17	0.03
Total	33	144.29	2.96
CV (%)		8.25	5.78

* Significant at $P < 0.01$