

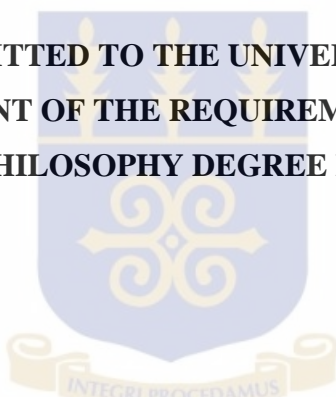
**GENETIC CONTROL AND BREEDING FOR SEED SIZE AND
COLOUR IN COWPEA [*Vigna unguiculata* (L.) Walp]**

By

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
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ABSTRACT

Consumer preference is very important in acceptance of an improved crop variety. Participatory rural appraisal (PRA) was therefore conducted on preferred traits of cowpea [*Vigna unguiculata* (L) Walp] among Ghanaian consumers in Ketu North, Ho and Akatsi districts. It was confirmed that the age long preference for large white cowpea among Ghanaian consumers still exists. Based on the results of the PRA, cowpea germplasm was assembled and used in genetic studies on seed coat colour and size. The total number of germplasm used for the various experiments was 126 from which 111 were from Ghana. Sixteen morphological traits were used in characterisation which clustered the improved varieties together indicating selection for similar traits. Molecular characterisation with 458 Single Nucleotide Polymorphic (SNP) markers clustered the germplasm based on seed coat colour and showed close relationship between improved varieties and materials from similar breeding programmes when analyzed with Darwin software. The software package structure was used to separate accessions into three groups, and the program correctly identified varieties that are known to have hybrid origins such as Zaayura and CB27. The SNP markers were also used in association mapping for cowpea seed size with Genstat statistical package. Eighteen of the SNP markers were observed to be highly associated with seed size and could be used in marker assisted selection for the trait. Eight of these 18 SNPs, one each located on Linkage Groups (LGs) 1, 5, 7, 10 and 11 and three on LG 4 were associated with both seed weight and thickness. Generation mean analysis (GMA) for seed size was conducted using CB27 and Gh3710 as the large and small seeded parents respectively. Results of the GMA analyzed with Genstat and SAS indicated that both fixable and non-fixable gene actions were important in seed size

inheritance. Eight genes were estimated to control seed size of cowpea from the experiment. The broad and narrow sense heritability estimates were 84% and 35% respectively. Duplicate gene action was observed to control seed size inheritance in cowpea. Small seed size was partially dominant over large seed size. Seven genotypes were used in hybridization and the segregating populations used in seed coat colour inheritance study in cowpea. It was realized that segregation for seed coat colour could be complex resembling quantitative inheritance depending on the parental genotypes. It was therefore suggested that the use of quantitative and molecular approaches could help in better understanding of seed coat colour inheritance in cowpea. Participatory selection with farmers was done from six different F_3 populations of cowpea from which four plants each were selected for further evaluation toward possible varietal development.



DEDICATION

EVERYONE WHO LOVES GOD



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LIST OF ABBREVIATIONS

AGRA:	Alliance for a green revolution in Africa
AFLP:	Amplified Fragment Length Polymorphism
BCP ₁	Backcross to parent 1 (Female parent)
BCP ₂	Backcross to parent 2 (Male parent)
cM:	Centi-Morgan
CSIR-CRI:	Council for Scientific and Industrial Research – Crop Research Institute
CSIR-PGRRI:	Council for Scientific and Industrial Research – Plant Genetic Resources Research Institute
CSIR-SARI:	Council for Scientific and Industrial Research – Savanna Agricultural Research Institute
CV%	Coefficient of variation expressed in percent
FAO:	Food and Agricultural Organization
F _{pr}	F probability
Grand \bar{X}	Grand mean
IITA:	International Institute of Tropical Agriculture
LD:	Linkage Disequilibrium

LG:	Linkage group
LSD	Least significant difference
MAS	Marker Assisted Selection
MT:	Metric ton
RAPD:	Random Amplified Polymorphic DNA
RFLP:	Restriction Fragment Length Polymorphism
S.E.	Standard error
SSR:	Simple Sequence Repeat
UCR:	University of California Riverside
WACCI:	West Africa Centre for Crop Improvement

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp] ($2n = 2x = 22$) is a major food crop for many people in Africa (Fatokun *et al.*, 2002) where the largest proportion of the world's production, trade and consumption take place. A total of 3,954,936.179 tonnes of dry cowpea grains was produced in Africa in 2012 representing 95% of world production (FAO, 2013). The crop is known for its high protein content which could be as high as 36.75% in the grains of some varieties (Olotuah and Fadare, 2012). Cowpea is consumed in several forms with over 50 different dishes known (Quaye *et al.*, 2009a; Boukar *et al.*, 2011). Improvement of cowpea is therefore, significant in tackling malnutrition and economic development in Africa.

Nearly all cowpea breeding programmes focus on higher grain yields and improved quality as primary breeding objectives (Timko and Singh, 2008). Seed size and number contribute directly to yield and together with seed coat colour, are among the determinants of consumer preferences of the crop (Hall *et al.*, 1997). In most West African communities white and cream colour types are preferred over other colours (Ogunkanmi *et al.*, 2006; Quaye *et al.*, 2009). In some parts of Ghana, seed size and coat colour are reported as the two most important grain quality traits of cowpea to farmers and consumers (Ansah, 2012).

Drabo *et al.* (1984), Lopes *et al.* (2003), Herridge *et al.* (2011) and Lucas *et al.* (2013a) have indicated that inheritance of seed size vary among genotypes. This makes it

important to study available genotypes for the trait of interest prior to embarking on breeding programmes. Similar to seed size, genetic control of seed coat colour in cowpea has been studied by a number of researchers with varying results (Oluwatosin, 2000; Asare *et al.* 2010).

Production of cowpea varieties with consumer preferred grain types could boost its cultivation and utilization in Ghana and other places where the crop is grown and used and thereby increase farmers' income. Ghana is a net importer of cowpea (FAOStat, 2011) although environmental conditions are favourable for the production of the crop in most of the agro-ecological zones of the country. This has a negative effect on food security and foreign exchange earnings of the nation. Most of the imported cowpea varieties have larger seeds than the common varieties in cultivation in the country. In addition, many of the Ghanaian varieties are not as white or creamy as the imported varieties. These imported cowpea varieties are not however adapted to the environmental conditions in Ghana due to genotype by environment interaction (Hall *et al.*, 1997). Consumer acceptability and preference are important considerations in a breeding programme. In this regard, cowpea farmers in Ghana who also double as consumers are important stakeholders to be considered in developing new varieties of the crop.

Studies that combine inheritance of these two traits are rare in Ghana. Considering the varieties released to date varieties that combine the preferred characteristics of large seed and white or cream seed coat colour are not available.

The objectives of the research were to:

- (a) ascertain production constraints of cowpea farmers and consumer preferences for seed size and colour,
- (b) assess genetic diversity of assembled cowpea germplasm with morphological markers,
- (c) assess genetic diversity of assembled cowpea germplasm with SNP markers,
- (d) determine association between SNP markers and seed size in cowpea,
- (e) determine genetic inheritance of seed colour and size in cowpea, and
- (f) identify promising cowpea lines for cultivation in the coastal savannah of Ghana.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and distribution of cowpea

Although the precise location of the origin of cowpea continues to be a debate (Kouam *et al.*, 2012), the crop is believed to have originated from Africa and it is currently cultivated throughout the tropics and to some extent in the subtropics. According to Tan *et al.* (2012), wild species of cowpea are found throughout Africa. Ogunkanmi *et al.* (2006) believed that cowpea originated from the savanna agro-ecological zone of Nigeria based on genetic diversity of the plant in that region. Based on wild species distribution and their hybridization with the cultivated *V. unguiculata*, Kanti (1975) also assert that West Africa is the centre of domestication of the crop.

Another school of thought suggested that cowpea originated from southern Africa and later spread to other parts of the continent including West Africa (Timko and Singh 2008). Ogunkanmi *et al.* (2006) and Kanti (1975) worked on cowpea plant materials from only West Africa, whilst the reports by Timko *et al.* (2007), Timko and Singh (2008) and earlier by Padulosi *et al.* (1997) were based on cowpea accessions from a wider geographic coverage. The assertion that cowpea originated from Southern Africa might be more accurate judged by the diversity in the plant materials studied. In that case one could conclude that although, *V. unguiculata* and its wild relatives are distributed across Africa; they might have originated from the southern part of the continent with West Africa as a secondary region of diversity.

Cowpea is cultivated all over the globe (Tan *et al.*, 2012) but more notably in Africa, South America and Asia (Lucas *et al.*, 2012). However, as much as 80% of the world production of cowpea comes from Africa with Nigeria and Niger as the largest producers (Timko *et al.*, 2008). In 2012 the proportion of cowpea produced in Africa rose to 95% (FAO, 2013) making the continent very important in cultivation, trade and utilization of the crop.

2.2 Importance and cultivation of cowpea

Cowpea is a major food crop for many people in Africa (Obembe, 2008). Singh (2007) believes that cowpea is the most nutritious source of food and fodder in West Africa. Langyintuo *et al.* (2003) also stated that the crop is the most economic legume in Africa. Protein content is usually used as the benchmark for nutrient level in food. Protein content of cowpea grain is between 20 to 32% (Philips *et al.*, 2003; Moalafi *et al.*, 2010; Tchiagam *et al.*, 2011a; Tan *et al.*, 2012), however, some varieties contain as high as 36.75% (Olotuah and Fadare, 2012). Cowpea is eaten as a whole grain or milled and used in preparing different meals across Africa and beyond.

In Ghana, consumption of cowpea is higher than production (Langyintuo *et al.*, 2003; Al-Hassan and Diao, 2007). Despite the importation of cowpea, Ghana is considered as one of the important countries where the crop is cultivated (Singh *et al.*, 2003; Majiwa *et al.*, 2007). There was importation of 3,380 million tonnes of cowpea grains to supplement the Ghana's production of 219,257 million tonnes in 2010/2011. This is however, a tremendous improvement over the deficit of 1990 which was 113,000 million tonnes (Al-

Hassan and Diao, 2007). Cowpea is imported into Ghana from neighboring countries such as Togo, Burkina Faso and Niger.

The inability of Ghana to produce enough cowpea to feed the citizenry is multi-faceted. First, commercial cowpea production is done mainly in the Northern and Upper West regions, although, production can be done over a wider geographic area in Ghana. For example, Upper West and Northern regions produced 75,969 and 105,841 million tonnes, respectively, in 2010 out of the reported national total of 219,300 million tonnes (MOFA, SRID, 2011). Production in the southern part of the country unlike the North and Upper West is mainly subsistence. Another major factor that might have been affecting production and consumption of cowpea in Ghana is varietal preference (Langyintuo *et al.*, 2003).

2.3 Challenges to cowpea cultivation

There are a number of challenges facing cowpea production. The importance of insect pests, diseases and grain quality has made them major goals in many cowpea improvement programmes (Timko and Singh, 2008). Viruses, nematodes, fungal and bacterial diseases are known to cause significant yield reductions depending on the type of disease, geographic location and cultivation practices (Adegbite and Amusa, 2008; Orawu *et al.*, 2012). Incidence and severity of diseases may vary depending on season, year or variety (Menendez *et al.*, 1997).

A myriad of insect pests are known throughout the cowpea world contributing to low yields, especially, in West Africa (Langyintuo *et al.*, 2003; Majiwa *et al.*, 2007; Makoi *et al.*, 2010). These pests affect practically every part of the cowpea plant and could result in total yield loss (Jackai and Daoust, 1986). It has been recognized that for cowpea to contribute significantly toward food security and poverty reduction in Africa, proper insect pest management strategies need to be developed (Majiwa *et al.*, 2007). Insect pest infestation like disease incidence depends on a number of factors including seasonal variation in weather conditions such as rainfall. Aphid infestation for instance is high when rainfall is erratic (Asafo-Adjei and Singh, 2005). In South Africa, sowing is recommended for late November to early December to reduce insect pests' incidence (www.daff.gov.za, 2011). Due to the significant yield reduction caused by insect pests, screening for resistance is a common activity in breeding programmes (Makoi *et al.*, 2010).

Erratic rainfall, inefficient nutrient uptake, soil salinity and weeds particularly *Striga gesneroides* are also considered major constraints to cowpea cultivation in Africa (Majiwa *et al.*, 2007; Gonne *et al.*, 2013). These constraints, however, are not limited to only cowpea but are known in other crops as well.

2.4 Inheritance of seed size

Seed size is a very important component of grain yield and is controlled by several genes (quantitative trait loci) (Xian – Jun *et al.*, 2007). Seed formation starts with floral induction which is controlled by a number of factors including the plant and its age,

environmental conditions, dry matter accumulation among others. The final seed size is believed to be controlled by genes (Li *et al.*, 2008). These genes restrict the period of cell proliferation thereby determining the maximum size the seed can reach. A number of genes controlling seed size have been reported by different authors and for different crops. A point to note is that, some authors use seed size and weight synonymously (Drabo *et al.*, 1984).

Arabidopsis is probably the plant in which the mechanism of seed size inheritance is best understood. However, even in the Arabidopsis where three genes (Auxin response factor2, Apetala2 and Da1) have been identified to control the trait, the subject is not completely understood (Adamski *et al.*, 2009). Two loci with two alleles each have been reported to control seed size in chickpea with additive and dominant epistasis (Upadhyaya *et al.*, 2011). Hossain *et al.* (2010) reported dominance of small seed over large in chickpea. Differences in seed size inheritance might exist for different crops.

In cowpea, Drabo *et al.* (1984) reported eight genes to control seed size while Lopez *et al.* (2003) reported five. However, there are reports of six, ten and other numbers of genes to control the trait by other authors (Aryeetey and Laing, 1973; Lopes *et al.*, 2003). Selection of parents may be a contributing factor to the varying reports. Also, variability in General and Specific Combining Ability (GCA and SCA) for seed size was reported by Tchiagram *et al.* (2011) in cowpea. Apart from the genotypes contributing to varying estimates of genetic parameters, the environment also has effect. The use of molecular

techniques will probably help in better understanding of the genetics of seed size as the use of molecular approach would limit the environmental influence on the estimates.

2.5 Inheritance of seed coat colour

Variability in the colour and pattern of the seed coat are basic phenotypic features used to discriminate genotypes of higher plants (McClellan *et al.*, 2002; Acquah, 2007). Pigmentation of the seed coat of plants is the result of accumulation of pigments such as anthocyanins within the epidermal layer (Todd and Vodkin, 1993). These anthocyanins are water-soluble pigments produced through the shikimic acid pathway (Chalker-Scott, 1999).

Two major and five minor types of anthocyanins have been extracted from cowpea (Hallie *et al.*, 2005; Ha *et al.*, 2010). The anthocyanin synthesis process can be divided into steps the first being the condensation of three molecules of malonyl CoA and one of p-coumaroyl CoA catalyzed by chalcone synthase (Dooner *et al.*, 1991). After a number of intermediary steps, anthocyanidin is produced and then converted to anthocyanin by the addition of glucose molecule catalyzed by “UDP glucose flavonoid 3-oxy-glucosyltransferase” (Holcroft and Kader, 1999).

In addition to a general colour factor which is either present or absent, it is believed that the expression of any pigment in the cowpea plant is the result of the interaction among a number of pigment genes (Mustapha, 2009). According to Arshad *et al.* (2005), the inheritance of seed coat colour and flower of some plants follows monogenic inheritance.

Monogenic control of both flower and seed coat colour is reported in *Asparagus* beans (Xu *et al.*, 2011) while three gene pairs have been reported in *Capsicum* (Zewdie and Bosland, 2003). However, as many as 35 genes have been identified to affect flower colour in petunia (Holton and Cornish, 1995).

Padi (2003) reported monogenic inheritance of seed coat colour from the cross of white and brown types of cowpea with the former recessive to the latter. Oluwatosin (2000) and Mustapha (2007) on the other hand stated that black and brown coat colours are digenically dominant to cream in cowpea. In contrast, black seed coat colour is recessive to cream in soybean (Hernandez-Garcia, 2011). Oluwatosin (2000) also reported that red coat colour is monogenically dominant to cream in cowpea. Monogenic control of seed coat colour was observed when Bambey 21 and CB27 were crossed, however, the result was different and complex with Montiero and Bambey21 (Fatokun *et al.*, 2002).

A number of regulatory genes as well as intensifiers have been identified in maize to control pigmentation of the seed, however, more are suspected to control the trait (Selinger and Chandler, 1999). Differences in number of genes reported in the control of seed coat colour in cowpea based on genotypic differences mean that the mechanism is not well understood. On eye pattern inheritance, Padi (2003) stated that different gene actions control seed coat colour inheritance in cowpea including monogenic but did not discuss eye pattern in relation to coat colour. Mustapha (2009) reported recessive epistasis to control seed coat colour in cowpea but did not elaborate on how this works and concluded that several genes might be involved in the control of seed coat colour in

cowpea. Egbadzor *et al.* (2010) reported two genes with recessive epistasis to control seed coat colour and eye pattern but the observation was made in only one population. Asante (2001) reported both recessive and dominant epistasis as influencing seed coat colour and eye pattern in cowpea. Asare *et al.* (2010) did not observe any relationship between different cowpea accessions with respect to seed coat colour when they were characterized with Simple Sequence Repeat markers (SSRs). A better understanding of the inheritance of pigmentation in cowpea may reveal more genes as over 20 have been reported in maize (Dooner *et al.*, 1991).

In *Arabidopsis thaliana*, pigmentation of the seed coat is not linked with either that of the leaves nor the flower buds (Sagasser *et al.*, 2002). Mutant plants lacking anthocyanin in the seed coat therefore accumulate anthocyanin in leaves and stems to the level of the wild type. However, the mutant plants had yellow seed coat which might mean the presence of a different pigment other than anthocyanin. Seed coat colour however, might be linked with a number of properties in the seed as the trait was reported in red clover to affect seed quality and seedling growth rate (Atis *et al.*, 2011).

Padi (2003) reported that depending on the choice of parents non-allelic genes may interact to produce novel flower and seed coat colour types. According to Cone *et al.* (1993), several genes are known to regulate anthocyanin pigmentation in maize. Some of these genes interact in determining the expression of anthocyanin pigmentation (Goodman *et al.*, 2004). This could complicate breeding for specific seed coat colour type. It is imperative, therefore, to study all contributing factors together.

2.6 Quantitative Trait Loci (QTL)

Quantitative Trait Loci (QTL) is a segment of the genome that is contributing to variation in the trait of interest (Falconer and Mackay, 1996; Tan *et al.*, 2012). This refers to associating the genotype and the phenotype in a population exhibiting genetic variation (Gupta *et al.*, 2005). QTL mapping represents the foundation of the development of markers for MAS as it is a step in gene discovery. Among other advantages, Marker Assisted Breeding (MAS) increases the precision in breeding programmes (Naidoo *et al.*, 2012) and it is effective in analyzing genetic control of complex traits (Young, 1996). The use of QTL in crop improvement is therefore, on the increase (Mace and Jordan, 2011).

QTL have been used in the study of different traits in crops including diseases (Young, 1996), seed size and shape (Maughan *et al.*, 1996; Park *et al.*, 2000; Song *et al.*, 2007), drought tolerance (Lanceras *et al.*, 2004). QTL have also been identified for drought, bacteria, nematode, fungal, seed weight, insects and Striga resistance in cowpea (Vallejos and Chase 1991; Yu *et al.*, 1998; Park *et al.*, 2001; Kelly *et al.*, 2003). Loci controlling resistance to thrips were identified by Omo-Ikerodah *et al.* (2008) in cowpea through QTL mapping. However, it is believed that identification of more QTL in cowpea is imperative to MAS in the crop (Tan *et al.*, 2012).

2.7 Association mapping

Khan (2013) defined association mapping as “a method used to identify marker alleles associated with phenotypic traits”. Association mapping is also known as Linkage

Disequilibrium (LD). It is a method used in identifying genes controlling quantitative traits and has been used in both plants and animals genetic studies (Myles *et al.*, 2009). The procedure is described as a “powerful gene tagging” tool (Abdurakhmonov and Abdugarimov, 2008). “Association mapping identifies quantitative trait loci (QTL) by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm” (Zhu *et al.*, 2008). Association mapping detects and locates QTL by the strength of the correlation between a trait and a marker (Mackay and Powell, 2006). Association mapping is said to “rely on the decay of LD, initially present in a population, at a rate determined by the genetic distance between loci and the number of generations since it arose” (Mackay and Powell, 2006).

Association mapping like QTL mapping helps to dissect the inheritance of complex traits in plants and has the advantage over the latter by reducing the research time (Zhu *et al.*, 2008). LD thus offers greater precision in QTL location than family-based linkage analysis. Natural populations of diverse germplasm used for association mapping increase the chance of high level of linkage disequilibrium (Kumar *et al.*, 2011; Abdurakhmonov and Abdugarimov, 2008). Association mapping focuses on associations of traits and markers within populations of unrelated individuals (Ersoz *et al.*, 2009)

The strength of association mapping depends on the LD (Myles *et al.*, 2009). LD measures the extent to which alleles at different loci are inherited together and therefore determines the extent to which association mapping can be performed. The trait and

marker are in LD if they are inherited together more or less often than it could have happened in equilibrium.

Association mapping is quite new so has been reported for only few crops and traits at the moment. However, the few results suggested that the procedure is promising so it has started gaining popularity. Wu *et al.* (2014) used the technique to find SNP markers associated with *Fusarium* wilt disease resistance in asparagus beans. Wang *et al.* (2008) also used it to mark iron deficiency chlorosis loci in soybean. Significant associations were established between some SNP markers and fruit weight, locule number, and soluble solid content of tomato (Ranc *et al.*, 2012).

2.8 Generation mean analysis

Phenotype can be qualitative or quantitative and is controlled by genes and environment. Quantitative traits are controlled by multiple genes and show continuous range from one extreme to the other (Zdravkovic *et al.*, 2011). Varieties of self-pollinated crops like cowpea are expected to have uniform phenotypes, however, environmental contribution accounts for their continuous variation in terms of quantitative traits (Chahal and Gosal, 2006). Plant breeders are interested in the genetic control of quantitative traits. To decipher these, one of the procedures used is generation mean analysis which was proposed by Mather and Jinks (Viana, 2000; Piepho and Mohring, 2010).

Generation mean analysis is used to obtain information on gene action and effects. It “provides information on the relative importance of additive and dominance effects in populations created from two inbreds” (Acquaah, 2007). Generation mean analysis has been used in the study of different traits in various crops. In lentil, Khodambashi *et al.* (2012) used the method to determine gene action for different traits such as pod length, number of seeds per pod and 100 seed weight. Tchiagam *et al.* (2011b) used it in studying inheritance of sucrose content in cowpea. Adeyanju *et al.* (2012) also used generation mean analysis to study dual purpose traits in cowpea.

2.9 Cowpea production and seed characteristics in Ghana

The major cowpea cultivation regions are found in the Guinea Savanna agro-ecological zone and to a lesser extent in the Sudan and Coastal Savanna zones. Although, cowpea production outside of the Savanna zones in Ghana is mostly on a subsistent manner, some varieties are known to perform well across the various agro-ecological zones (Asafo-Adjei *et al.*, 2005; Addo-Quaye *et al.*, 2011). Cowpea is the second most important economic leguminous crop in Ghana, second to groundnut in terms of area under cultivation, quantity produced and consumed annually. Available data show that the area under cultivation peaked in the year 2003 with 190,400 ha (MOFA, SRID, 2011). However, there have been slight reductions in the area under cowpea cultivation from 2003 to 163,700 ha in 2010. Reduction in farm size may be due to different factors including the level of farmers’ interest in the crop. Despite the decline in farm size, total cowpea grain production per annum has been increasing over the years. Production increased from 142,300 MT in 2004 to 201,300 MT in 2010. This is an indication of

higher yields which may be due to a combination of factors including better agronomic practices and higher yielding varieties.

Cowpea consumption is higher than production in Ghana resulting in importation. There was an importation of 3,380MT of cowpea grains to supplement the country's production of 219,257MT in 2010/2011. The inability of Ghana to produce enough cowpea to feed the citizenry is multi-faceted. First of all, cowpea is mainly produced in the Guinea Savanna zone in the country, where production can only be done within a short period in the year due to long drought period. For example, Upper West and Northern regions produced 75,969 and 105,841 MT respectively in 2010 out of the national total of 219,300 (MOFA, SRID, 2011). Another major factor that may be affecting the production and consumption of cowpea in Ghana could be varietal preference (Langyintuo *et al.*, 2003). Ghanaians are known to prefer cream seeded grains cowpea (Quaye *et al.*, 2009).

Production of cowpea with consumer preferred grain type could boost its cultivation in Ghana. The most popular cowpea variety in Ghana is "Asontem". Asontem was developed by the International Institute of Tropical Agriculture (IITA) and introduced in Ghana by the CSIR – Crop Research Institute (CRI) in 1984 (Asafo-Adjei *et al.*, 2005). Limitation of Asontem may be its red seed coat. "Nhyira" is another popular cowpea variety released by CRI. Although it has cream seed coat, its seeds are small and not desired by consumers. The most preferred, and consequently, imported grain types of cowpea are large, white or cream coloured. Varieties on the market with these two important traits are imported and expensive.

There are good indications for increased production of cowpea in the coastal savanna agro-ecological zone of Ghana. Cowpea is a short season crop with relatively high drought tolerant ability (Muchero *et al.*, 2009a). The crop is easily cultivated by the resource poor farmers who form the majority of the population. However, high proportion of the cowpea found in markets in the southern sector of Ghana comes mainly from the northern part or from different countries including Togo, Burkina Faso, Niger and Nigeria. Also most of the imported types; Nigerien Cowpea as an example (Langyintuo, 2004) is not normally grown in Ghana. Breeding of cowpea with acceptable consumer traits would boost its cultivation to feed the already existing market in Ghana especially along the coast.

2.10 Core collection

Plant genetic resources (germplasm) are important to meet the food security needs of the world. However, they are vulnerable in the wild. Germplasm are therefore, conserved in genebanks all over the world to protect them from extinction and also provide easy access for plant breeders, researchers and other users (van Hintum *et al.*, 2012). The number of accessions of a given plant species at a genebank could be very large making conservation activities difficult. In addition, as the number of accessions increases, often times, some genotypes become duplicated in the collection (Acquaah, 2007). The aim of establishment of a core collection is to reduce duplicates and the cost associated with the management of the germplasm (Krichen *et al.*, 2012). A core collection is a

representative sample of the germplasm collections. The core becomes the immediate source of accessions for genetic studies, breeding and other activities. A core collection is particularly useful to the breeder in that it minimizes the number of genotypes to consider in crosses or screening studies (Reeves *et al.*, 2012). A mini core collection is done when the core collections also become too large (Upadhyaya *et al.*, 2010).

An important point to consider in developing a core collection is that the total allelic and morphological diversity of the original collections must be captured in the core as much as possible (El Bakkali *et al.*, 2013). Genetic markers are thus used in assessing the diversity of the collection before selecting the core samples as the representation of the entire collection. Different molecular and morphological markers are therefore used in identifying the core collection (Cunff *et al.*, 2008). Krichen *et al.* (2012) stated that using a combination of morphological and molecular markers in developing a core collection gives a better result than using one marker type alone.

The number of markers and the level of polymorphism might be more important than marker type in core collection selection. Oliveira *et al.* (2010) used entirely morphological markers to select 10% core from 15,558 accessions of the USDA soybean germplasm. Simple Sequence Repeat (SSR) markers were used in establishing the core collection of sugar beet (Reeves *et al.*, 2012) and olives (El Bakali *et al.*, 2013). Oliveira *et al.* (2014) however, used Single Nucleotide Polymorphism (SNP) markers in establishing a core collection for cassava which captured all the alleles in the original collection. Zhang *et al.* (2009) laid emphasis on statistical method of identifying core

collection more than the marker type. It could be concluded that marker type, level of polymorphism of markers and statistical methods are all important in selection a core germplasm to represent the total collection.

According to Odong *et al.* (2013), a core collection may differ based on the purpose of their establishment. However, a core collection identifies a subset of the entire collection to be used for various purposes. The established core collections are being utilized in different crops for breeding and genetic studies. Lucas *et al.* (2013a) used cowpea core collections of the United States Department of Agriculture in studying seed size inheritance in the crop. Upadhyaya *et al.* (2007) identified chickpea genotypes of important agronomic traits from a core 1,956 accessions. Screening would have been extremely difficult if (Upadhyaya *et al.*, 2007) were to use the entire collection.

CHAPTER THREE

3.0 FARMERS' KEY PRODUCTION CONSTRAINTS AND PREFERRED TRAITS IN COWPEA: THE CASE OF THE AKATSI AND KETU NORTH DISTRICTS OF GHANA

3.1 INTRODUCTION

Cowpea is the second most important food legume in Ghana after groundnut. Ghanaians are known to prefer white or cream seeded cowpeas which are sweet and easy to cook (Quaye *et al.*, 2009). Production of cowpea with consumer preferred grain types could boost its cultivation in Ghana. The improved cowpea varieties under cultivation in the country were mostly released by the Crops Research Institute (CRI) and Savanna Agricultural Research Institute (SARI) both of the Council for Scientific and Industrial Research (CSIR). The CRI and SARI also did some of the varietal releases in collaboration with the International Institute of Tropical Agriculture (IITA). Some of these varieties have large seeds such as “Asontem” but lack white seed coat colour while those with white or cream seed coat colour are small seeded such as “Nhyira”. Deficiency in these two consumer traits (colour and size) makes these varieties less desirable by farmers and consumers of the commodity in Ghana. Many of these varieties were also not developed with farmers as partners in the breeding programme.

Currently, cowpea production in the coastal belt of Ghana is subsistent, with only pockets of commercial production. The major cowpea growing zone is the forest-savannah transition zone and to some extent the guinea savannah. An extensive cowpea growing zone on the coast is around Ohawu in the Ketu North District covering a land area of 16,687.34ha within which cowpea farming is the main economic activity for some

families. This is one of the pockets of cowpea growing areas on the coastal belt of Ghana. Despite the low production of cowpea on the coastal belt of Ghana, the demand for the crop is very high leading to importation from other parts of the country and abroad. The introduction of the school feeding programme in Ghana in 2005 has led to an increase in the demand of already low supply of cowpea grains on the coastal belt.

A number of constraints are known to militate against cowpea production. Some of these constraints are widespread while others are more or less localized. Cowpea aphid-borne mosaic virus disease for instance was reported to be a major virus disease for the crop worldwide (Orawu *et al.*, 2012). In general, a number of diseases are known to be a problem to cowpea production (Adegbite and Amusa, 2008). Insect pests are reported to be a major problem to cowpea cultivation in Africa attacking the plant at every stage of development (Makoi *et al.*, 2010). However, these insects may vary from locality to locality and even from season to season. *Striga gesnerioides* infestation could be devastating in some cowpea growing zones (Reiss and Bailey, 1998).

Knowledge on quality or consumer preferences of cowpea and constrains to its production would help in developing acceptable varieties of the crop for cultivation in a given locality. It is important to diagnose the consumer preferences and the reason(s) for the shortfall in production which warrants importation of cowpea to the coastal savannah zone of Ghana. The objectives of the study were to:

- (a) identify the production constraints of cowpea in the coastal savannah zone of Ghana, and
- (b) determine farmers' and consumers' preference for cowpea seeds.

3.2 MATERIALS AND METHODS

3.2.1 The study site

The study was done in the cowpea growing area of Ohawu. The area is about 16,700ha, including parts of Ketu North and Akatsi Districts and Keta Municipal (Figure 3.1) in the Volta Region of Ghana. Focus group discussions were conducted at Avenorpedo and Ohawu in the Akatsi and Ketu North Districts respectively. Akatsi District is located between latitude 6° S – 7° N and 0° W – 1° E and shares a boundary with Ketu North to the east which also shares a boundary with the Republic of Togo to its east. Questionnaires were administered in Ho Municipality in addition to Ketu North and Akatsi. Ho municipality is in the North West direction to Akatsi with Adaklu-Anyigbe District in-between them.

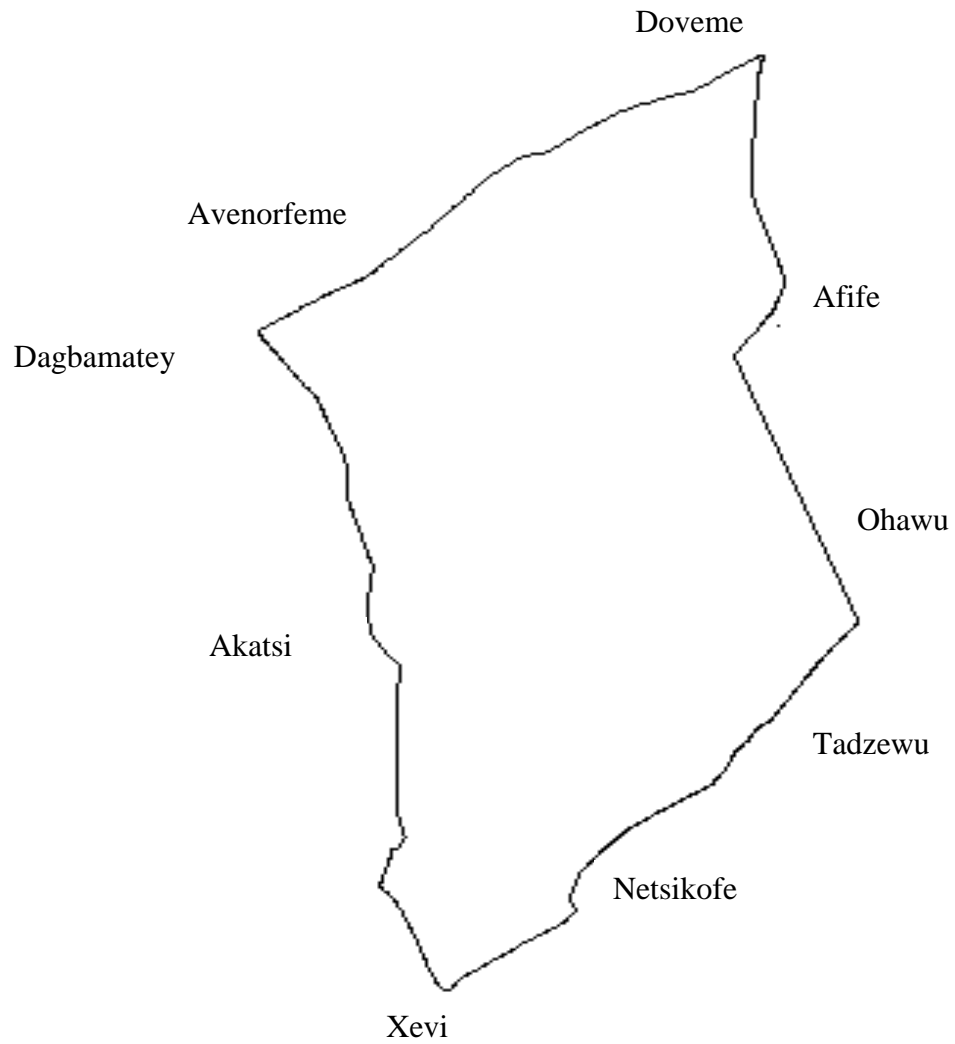


Figure 3.1: Map of Cowpea growing areas in the Ketu North and parts of Akatsi Districts and Keta Municipal

ATTRIBUTES:

TOTAL AREA (2D) = 16,687.34ha

PERIMETER (3D) = 60.32km

LATITUDE = 6°03'50.74812''N

LONGITUDE = 0°54'49.85784''E

ALTITUDE (HAE) = 52.010m



Scale 1:200,000

0

8.000



3.2.2 Respondents

Interaction was made with a total of 355 individuals. Interview guide was used for focus group discussion at the two sites (Avenorpedo and Ohawu). Participants for the focus group discussion were identified by the help of the Agricultural Extension Officers of the area. Questionnaires were administered to individual respondents at the various locations. Thirteen men and 11 women participated in the focus group discussion at Avenorpedo while that of Ohawu was 10 men and 6 women. Sixty farmers each were interviewed in Akatsi, Ketu North, and Ho. The farmers at Akatsi were made up of 36 men and 24 women. There were 28 men to 32 women and 40 men to 20 women at Ketu North and Ho respectively. Ten cowpea seed dealers and 15 food vendors from each of the 3 districts were also interviewed. In addition, a total of 60 cowpea traders, were interviewed from the 3 districts and Agbogbloshie market. Only one seed dealer from Akatsi and 2 each from Ho and Ketu North were females. However, all the food vendors and cowpea traders were females.

3.2.3 Data Collection and Analysis

Different strategies were employed in data collection including telephone calls, informal contacts as well as planned interviews. The respective district agricultural officers were very instrumental in identifying qualified respondents using convenience sampling. Results recorded in excel were imported into SAS 9.2 and summarized into averages, frequencies or percentages.

3.3 RESULTS

3.3.1 Priority Traits of Cowpea

Pair wise ranking of grain coat colour, wholeness, size, sweetness and ease of cooking (Table 3.1) was done to identify which of these traits farmers considered most important on cowpea grain. Sweetness and ease of cooking were the two most important traits mentioned during the focus group discussion comparing the results of the two sites.

3.3.2 Preferred Coat Colour of Cowpea

The five most common seed coat colour types of cowpea in Ghana were ranked by the participants of the focus group discussion. The results are presented in Table 3.2. White or cream seed coat coloured cowpea was ranked highest and black the least.

Table 3.1: Pair-wise ranking of some quality seed traits of cowpea during focus group discussions

Traits	Coat colour	Wholeness	Seed size	Sweetness	Ease of cooking	Score	Rank
<i>Avenorpedo</i>							
Coat Colour	-----	Wholeness	Seed size	Sweetness	Ease of cooking	0	5th
Wholeness		-----	Seed size	Wholeness	Ease of cooking	2	2nd
Seed size			-----	Sweetness	Ease of cooking	2	2nd
Sweetness				-----	Ease of cooking	2	2nd
Ease of cooking					-----	5	1st
<i>Ohawu</i>							
Coat Colour	-----	Wholeness	Seed size	Sweetness	Ease of cooking	0	5th
Wholeness		-----	Wholeness	Sweetness	Ease of cooking	2	3rd
Seed size			-----	Sweetness	Ease of cooking	1	4th
Sweetness				-----	Sweetness	4	1st
Ease of cooking					-----	3	2nd

Table 3.2: Pair-wise ranking of preferred coat colour of cowpea

Colour	Black	Brown	Mottle	Cream	Red	Score	Rank
Black	-----	Brown	Mottle	Cream	Red	0	5 th
Brown		-----	Mottle	Cream	Red	1	4 th
Mottle			-----	Cream	Red	2	3 rd
Cream				-----	Cream	4	1 st
Red					-----	3	2 nd

NB: Only one Table was used because results from both Avenorpedo and Ohawu were the same

3.3.3 Constraints to Cowpea Cultivation

Five constraints at Avenorpedo and four at Ohawu were identified and ranked. Three top constraints namely, drought, insect pests attack and weed infestation were identified at the two locations of the PRA. Mould and problem of storage were additionally mentioned at Avenorpedo while only harvesting problem was added to the three at Ohawu (Table 3.3).

Table 3.3: Constraints identified during PRA

Constraint Number	Constraint	Community	Rank
1	Drought	Avenorpedo / Ohawu	1st
2	Insect Pest	Avenorpedo / Ohawu	2nd
3	<i>Cyperus spp</i>	Avenorpedo / Ohawu	3rd
4	Mould	Avenorpedo	4th
5	storage	Avenorpedo	5th
6	Harvesting	Ohawu	4th

3.3.4 Farmers' Perceived Limitations to Cowpea Production

The major limitations to cowpea production perceived by farmers were insect pests, drought and diseases (Table 3.4). Insect pest attack was the most reported limitation to cowpea production across the survey communities following the PRA. The three districts perceived these three constraints in the same order of importance.

Table 3.4: Farmers' Perceived Limitations to Cowpea Production

Limitation	District			Total (n = 180)
	Akatsi (n = 60)	Ho (n = 60)	Ketu North (n = 60)	
Diseases	3 (5.1)	15 (25)	3 (5)	21 (11.7)
Mould	0	0	2 (3.3)	2 (1.1)
Insect pests	28 (47.5)	38 (63.3)	50 (83.3)	116 (64.4)
Taste	0	0	1 (1.7)	1(0.6)
Beetle	1 (1.7)	0	0	1(0.6)
Difficult to cook	0	0	1 (1.7)	1(0.6)
Drought	24 (40.7)	0	2 (3.3)	26 (14.4)
Excess vegetation	0	0	1 (1.7)	1(0.6)
Labour	1 (1.7)	0	0	1(0.6)
Processing	1 (1.7)	7 (11.7)	0	8 (4.4)
Storage	1 (1.7)	1 (11.7)	0	2 (1.1)
Total	59	60	60	180 (100)

Note: Percentage of respondents from a district in bracket

3.3.5 Cowpea Grain Problems Reported by Food Vendors

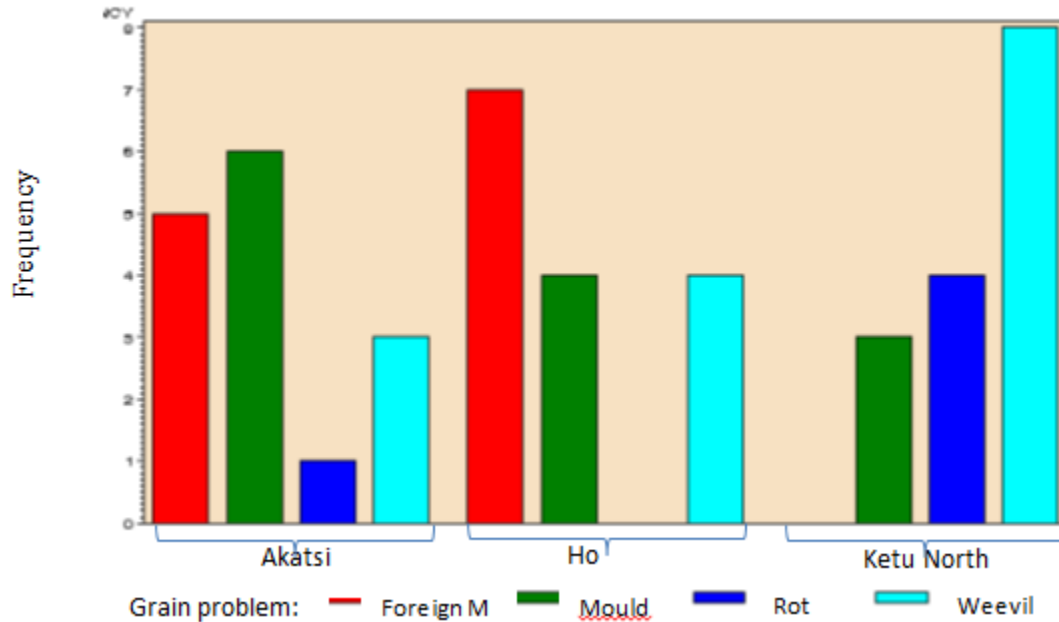


Figure 3.2: Grain Problems Reported by Food Vendors from Different Districts

Foreign materials, mould, rot and weevils were reported by food vendors as problems, however, the significance of each differed from district to district (Figure 3.2). Mould was the most reported grain problem at Akatsi as against foreign materials and weevils at Ho and Ketu North respectively

3.3.6 Preferred varieties of cowpea

Most farmers, grain sellers and food vendors preferred white coat cowpea and large seeds to other types (Table 3.5). Seventy-six percent of the 180 farmers preferred large seeded cowpea to the small and medium. Seventy-three percent of 60 cowpea grain sellers preferred large seeds. Seven percent, 11% and 82% food vendors respectively prefer small, medium and large seeded cowpea. One hundred and fifty six farmers prefer white

coat coloured cowpea. Majority of grain sellers and food vendors also preferred white coat cowpea to red and other types.

Table 3.5: Preferred varieties of cowpea by farmers, grain sellers and food vendors

Group of people	Seed size				Seed colour			
	Small	Medium	Large	Total	Red	White	Others	Total
Farmers	13	20	137	180	16	156	8	180
Grain sellers	6	10	44	60	4	47	9	60
Food vendors	3	5	37	45	2	42	1	45

3.3.7 Source of Cowpea Grains at Various Markets

Cowpea grains found in the market at Ho, Akatsi and Dzodze were from the farms of the traders themselves, Akatsi market or from Togo (Table 3.6). All the traders in Ho imported their cowpea from Togo. Akatsi traders obtain 10% of their grains from the locality and 90% from Togo. Dzodze traders obtained 25%, 20% and 55% respectively from Akatsi, Own farm and Togo.

Table 3.6: Sources of Cowpea Grains at Various Markets

Source	Market			Total
	Akatsi	Ho	Dzodze	
Akatsi	2 (10)	0	5 (25)	7
Own farm	0	0	4 (20)	4
Togo	18 (90)	20 (100)	11 (55)	49
Total	20	20	20	60

Note: percentage of respondents from market in brackets

3.4 DISCUSSION

Farmers' perceived most limiting factors to cowpea cultivation differed from one district to the other as well as for individual farmers. A number of limitations were mentioned, the most of which were insect pests attack in all the districts. In Ho in particular, the respondents believed that insect pests and diseases were the most important constraints to cowpea cultivation. Insect pests are known to be major constraints to cowpea production in Africa (Makoi *et al.*, 2010) and other regions where the crop is cultivated (Gonne *et al.*, 2013). Farmers in the study area confirmed that insect pests attack were big problems to them. There were few farmers who thought that although insect pests were problems, they could deal with them better than other problems such as drought and *Cyperus spp.*

Quite a number of farmers at Akatsi believed that drought was one of the most limiting factor to cowpea cultivation, although, cowpea is a short season crop with relatively high drought tolerant ability (Muchero *et al.*, 2009a). Drought may be a serious problem as the coastal savannah of Ghana is generally known to receive just a little rainfall. Drought as stated by the farmers causes a delayed planting of cowpea and other crops in the area.

Although, the number of people who mentioned limitations other than insect pests were not many, they were very passionate about their perceived constraints. These other constraints may also complicate insect pest problems, example being drought reported by Abdel-Galil *et al.* (2007). Most of the other limitations related to grain quality. Storability as mentioned by some farmers could be a great limitation to cowpea cultivation. There are different dimensions to the problem of storability. First of all, because of lack of

appropriate storage methods and facilities, farmers were compelled to sell their produce early after harvest although the prices are generally low at that time. Another dimension of storability problem was reported especially for red type beans. According to farmers, red type beans do not cook well when stored for long periods. Storability is also a problem because of storage insect pests especially for cream seeded beans. Closely related to storability is mould problem identified by some farmers. Mould is also related to delay harvesting particularly when it keeps raining after pod ripening. Farmers who stated labour problem as a big limitation explained that obtaining enough labour especially during harvesting time was a problem. Processing difficulty can also be linked with that of harvesting.

A keenly debated issue was the *Cyperus spp* problem. Although, all the farmers identified it as a problem, some believed that good management by spraying before sowing of the cowpea seeds and early weeding controlled the weed significantly. It could be concluded that insect pests were the most important constraints to cowpea cultivation in the study area followed by drought.

Ease of cooking was the most important trait to consider in choosing a cowpea variety for cultivation and consumption according to the focus group at Avenorpedo in the Akatsi District. Almost every one of the group agreed that once a variety takes a long time to cook, it may not be accepted in the area. Colour did not seem to matter in choosing cowpea variety, however, it was discovered that apart from one local variety called Awakli, all the leading varieties in the area were cream coated.

Unlike at Avenorpedo, sweetness was the most important trait to consider in cowpea according to the focus group at Ohawu in the Ketu North District. The second most important trait was easy to cook. The finding for colour was similar to that of Avenorpedo. In prioritizing traits, colour was not ranked high during the focus group discussions. However, most of the varieties grown as well as marketed throughout the surveyed areas had cream seed coat. This agrees with Langyintuo *et al.* (2004) that nearly all cowpeas sold in Ghana and Cameroon markets are white. The colour and seed size preference in cowpea identified by Dovlo (1975) and Drabo *et al.* (1984) have not changed. A similar report was also made by Ansah (2012). All the farmers interviewed in Ho stated that they preferred cream seeded cowpea grains to any other colour. Although, the majority of respondents in both Akatsi and Ketu North also preferred cream seeds some had divergent views on cowpea seed coat colour. One third of the respondents in Ketu North preferred mottle type of cowpea to any other colour including cream and white. The preference was explained that their local variety, Awakli which is brown mottle is very nutritious and stores better after harvest. This Awakli variety is the only non-cream coated variety grown on large scale in the area.

Farmers' notion on high nutrient usually meant high protein. A research conducted by Alayande *et al.* (2012) revealed that a brown type variety had more crude protein than a cream and white types. It may not be appropriate to use that result for all cream/white and brown varieties: more samples should be compared before the generalization. However, the coincidence of then claim by the farmers the findings of Alayande *et al.* (2012) warrant further investigation.

The majority of traders interviewed in Agbogbloshie, Akatsi, Ho and Dzodze markets responded that they preferred cream/white grain. Although, all the traders in Accra and Ho preferred cream/white grains, two respondents each from Akatsi and Dzodze did not have any preferences and one in Dzodze preferred the mottled seed. Traders' preferences were in line with those of food vendors and farmers. Most of the cowpeas observed during the survey were cream/white as well.

Responses from vendors of four different food types of cowpea indicated that cream/white grains were more suitable for their meals with the exception of "ayikple". ayikple is a traditional food for the "Ewe tribe". This meal is no longer common and according to the vendor, she prepares this food during the harvesting season of cowpea. This was the only vendor of "ayikple" interviewed. The opinion of just one respondent may not be adequate to make conclusion. The earlier revelation that the farmers of Akatsi and Ketu North preferred mottle cowpeas for their own consumption may be strengthened by the attestation of the "ayikple" vendor for her preference of mottle grains for her trade. This is partly due to the fact that the people believe that the variety Awakli is more nutritious than the white grains and partly due to the traditional nature of "ayikple". One vendor of "waakye" also believed that all types of cowpea were good for her trade.

All "borbor" sellers believed that the white grain cowpeas were better for their trade than other colours. Further investigations revealed that they preferred the white grain because they were mostly softer and cooked faster. "Borbor" in Ewe language means soft.

“Borbor” is cooked beans usually eaten with “gari”. The full name is “ayiborbor” which means soft beans. Some of the borbor dealers were asked to explain the reasons for choosing white over red as the “borbor” which is called red-red by some people in Ghana is eaten with red palm oil therefore the name. The response in all cases was that the red types were not as soft as the white types. Akla (Kose) dealers also preferred white grains to other types but for with different reasons. “Akla” sellers use white grains because they are easier to process.

Response to the question on preferred seed size of cowpea was one sided. Traders in cowpea grains whether for food or seed preferred larger grains to smaller ones supporting Langyintuo *et al.* (2004). Farmers and food vendors also preferred larger grains to smaller types. Cowpea consumers in Ghana are ready to pay a premium for larger grains just like many other West African countries as reported by Mishili *et al.* (2007). Improving the size of a given variety would therefore enhance its adoption provided other traits remain same.

Seed dealers mentioned a number of traits that they thought should be improved upon in cowpea. Three of the most important traits according to the interviewees were insect pest resistance, yield and drought tolerance. This agrees with what was discovered with farmers.

No trader obtained her goods from a single source. In all the markets, it was evident that most of the cowpeas at the time were imported from different countries such as Togo,

Nigeria, Burkina Faso and Niger. It has been reported that Ghana imports cowpea from Mali and Cote d'Ivoire also (Langyintuo *et al.*, 2003). At the time of the survey none of the traders at Ho had supplies from Ghana. Few traders stated having bought their supplies from Akatsi (Ghana) in Akatsi and Ketu North.

Although a lot of cowpea is produced at Akatsi and Ketu North, they are sold at the farm gate just after harvest. This situation can be attributed to the demand and supply scenario. Traders have to buy from other markets off season to Akatsi like other locations. Most of the supplies were from Togo in all of the markets. However, they might not be from Togo farmers but bought from different countries. Most traders were also able to identify varieties from these countries as they called them by the country's name. Because of variation in the planting season and demand and supply issues, cowpea like any other commodity, can be moved from one country to the other in the West African sub-region.

3.5 CONCLUSION

Interaction with farmers in Ho, Ketu North and Akatsi districts of the Volta region of Ghana revealed that a lot of cowpea is produced in these areas. These grains are bought at the harvesting period leaving no trace of them few months thereafter. Almost all cowpea grains found in the markets were imported from Togo, Nigeria, Niger and Burkina Faso. Cowpea production in Ghana is evidently lower than consumption, especially in the southern zone where the survey was conducted. The imported large grains with white seed coat are the most preferred type by consumers in Ghana. Also, cowpea consumers desired a product that is sweet and easy to cook. A number of farmers believed that their traditional variety of cowpea which is brown mottled in colour is more nutritious than the

cream types which were most widely cultivated. Further investigation into this perception is important. Insect pests attack, diseases, drought, *cyperus spp* and harvesting difficulty were the most important constraints narrated by farmers.

CHAPTER FOUR

4.0 DIVERSITY ASSESSMENT OF 118 COWPEA ACCESSIONS USING MORPHOLOGICAL TRAITS

4.1 INTRODUCTION

Diversity in a given germplasm forms the raw material for the improvement of that crop. Cowpea is one of the crops with several hundreds of accessions conserved in genebanks all over the world (Fatokun *et al.*, 2002). The high number of cowpea accessions may be due to the presence of high morphological diversity within the species and the importance of the crop among other reasons. There are instances that even closely related genotypes of cowpea have conspicuous differences (Omoigui *et al.*, 2006) and this could lead to assigning them different accession numbers.

There are about three hundred accessions of cowpea collected mainly from different parts of Ghana in conservation at the Plant Genetic Resources Research Institute (PGRRI) of the Council for Scientific and Industrial Research (CSIR), Bunso. Bennett-Lartey and Ofori (1999) studied the variability of some qualitative traits of some of the accessions. Cobbinah *et al.* (2011) have also reported morphological characterization of some of the Bunso cowpea collection. Opong-Konadu *et al.* (2005) characterized the cowpea germplasm based on seed protein while Asare *et al.* (2010) used Simple Sequence Repeat (SSRs) in their characterization. All of these authors used only samples of cowpea accessions being conserved at CSIR – PGRRI. Cowpea germplasm have very wide diversity depicting the geographic location and the planting system from which they were collected (Menendez *et al.*, 1997). Therefore, in order to increase coverage of the

germplasm conserved, elite genotypes from different sources were included in the current diversity study.

Despite the effectiveness of the use of molecular markers in diversity studies (Tan *et al.*, 2012; Tanhuanpaa and Manninen, 2012), morphological and agronomic traits remain imperative to plant breeders (Krichen *et al.*, 2012). Morphological characterization is used routinely by plant breeders for the initial description and classification of germplasm in order to select genotypes for cultivation by farmers or in breeding programmes (Krichen *et al.*, 2012). In addition the desired traits must be expressed in the target environment and this makes morphological characterization crucial in plant breeding.

In characterizing cowpea germplasm from Cameroon, Gonne *et al.* (2013) were able to identify genotypes more tolerance to thrips attack. Genotypes tolerant to thrips attack could be selected for cultivation or used in developing varieties tolerant to the pest. Oladejo *et al.* (2011) observed morphological traits that correlated with seed yield in their collection of cowpea so could use them as indirect selection for yield. Some cowpea genotypes were observed to have protein almost double that of some genotypes when a collection was evaluated for protein and mineral concentration (Boukar *et al.*, 2011). Thus morphological characterization helps in initial description and classification of germplasm in order to select genotypes with valuable traits for direct use by farmers or in breeding programmes (Krichen *et al.*, 2012)

Knowledge on the key traits of the germplasm is essential in making decisions on parent selection for breeding purposes and reduces the number of germplasm that a researcher has to screen. The objective of this study was to characterize cowpea germplasm and select genotypes to be used as parents for genetic studies and breeding programmes.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials and cultural practices

The bulk of the germplasm was assembled from CSIR-PGRRI, Bunso, Ghana. The genotypes consisted primarily of landraces; and a few of them were commercial varieties in cultivation in Ghana. The rest of the germplasm came from different sources but were all obtained through the University of California Riverside, California, USA.

4.2.2 Experimental design and trial management

Planting was done in the West Africa Centre for Crop Improvement (WACCI) farm, University of Ghana using augmented design with three commercial varieties as checks in April, 2011. Plants were grown under rain-fed conditions with no commercial fertilizer applied. An insecticide (cymethoate) was applied at 1.5L/ha at 5 and 7 weeks after planting to reduce insect damage. Harvesting was done as soon as pods turned colour completely and were sun dried in pods. The experiment was repeated in the experimental farm of PGRRI - CSIR, Bunso and the averages of the two sites used for analysis.

4.2.3 Data collection and Analysis

Scoring for the traits was done based on “Key access and utilization descriptors for cowpea genetic resources (Mahalakshmi *et al.*, 2007)”. Data was collected on plant vigour, growth habit, flower colour, plant pigmentation, mature and immature pod pigmentation, seed coat and eye colour, pod attachment, terminal leaflet shape and seed shape.

The quantitative traits measured were number of days to flowering, number of pods per peduncle, pod length, seeds per pod and 100 seed weight. Both the qualitative as well as the quantitative data were scored as modalities and Sokal and Sneath dissimilarity calculated used for factorial display (Perrier *et al.*, 2003).

$$\left\{ dij = \frac{u}{2m+u} \right\} \text{ (Perrier } et al., 2003)$$

Notations

dij = dissimilarity between two genotypes i and j

u = number of unmatching variables

m = number of matching variables

4.3 RESULTS

4.3.1 Distribution of qualitative traits studied

Distributions of the various qualitative traits studied varied in the cowpea collection studied (Table 4.1). Sixty four percent of the accessions were not vigorous while 34% and 2% were intermediate and vigorous respectively.

Diverse growth habits were exhibited by the various cowpea lines in the collection. Prostrate types were the most observed (31 %). Climbing, prostrate and erect cowpea types were all observed (Figure 4.1).



Figure 4.1: CB27, Gh4524 and Gh3710 representing erect (A), climbing (B) and prostrate (C) types of cowpea respectively.

Accessions with violet and white petals accounted for 74.7% and 25.3% respectively.

Varying degree of plant pigmentation (Figure 4.2) was observed in the germplasm

collection; however, very slightly pigmented types were the most observed with 48.7% of the total observation. Only 6.2% of the accessions did not have any plant pigmentation.

Table 4.1: Frequency distribution of qualitative variable descriptors of cowpea accessions studied

Descriptor and class	Frequency of class (%)							
	0	1	2	3	4	5	6	7
Plant vigour: 1 = None; 2 = Intermediate; 3 = Vigorous		64	34	2				
Growth habit: 1 = acute erect; 2 = Erect; 3 = Semi erect; 4 = Intermediate; 5 = Semi prostrate; 6 = Prostrate; 7 = Climbing		1	16	23	19	1	31	9
Flower colour: 1 = White; 2 = Violet		25.3	74.7					
Plant pigmentation: 0 = None; 1 = Very slight; 2 = Moderate; 3 = Intermediate	6.2	48.7	35.4	9.7				
Immature pod pigment: 0 = None; 1 = pigmented tip; 2 = Uniform pigment; 3 = Pigmented sutures	75.8	17	1.8	5.4				
Mature pod pigment: 1 = Black; 2 = Straw; 3 = Dark brown		5.3	84.1	10.6				
Seed coat colour: 1 = White; 2 = Cream; 3 = Brown; 4 = Red; 5 = Black; 6 = Other		11.6	12.5	16.1	24.1	22.3	13.4	
Eye colour: 0 = Absent; 1 = Brown; 2 = Black; 3 = Mottled	81.3	1.8	16	0.9				
Pod attachment: 1 = Pendant; 2 = 30 – 90°; 3 = Erect		58.7	34.9	6.4				
Terminal leaflet: 1 = Globose; 2 = Sub globose; 3 = Sub hastate; 4 = Hastate		3.6	61.3	23.4	11.7			
Seed shape: 1 = Rhomboid; 2 = Ovoid; 3 = Kidney; 4 = Globose		44.1	39.4	12.8	3.7			

There were genotypes with no anthocyanin pigmentation of immature pods (75.8%) through to solid anthocyanin pigmentation which was 17%. Mature and dried pods pigmentation also varied in the collection.

The variability in seed coat colour and eye was very high in the collection (Figure 4.3). There were some accessions with two or more different seed coat colours. This type was designated others.

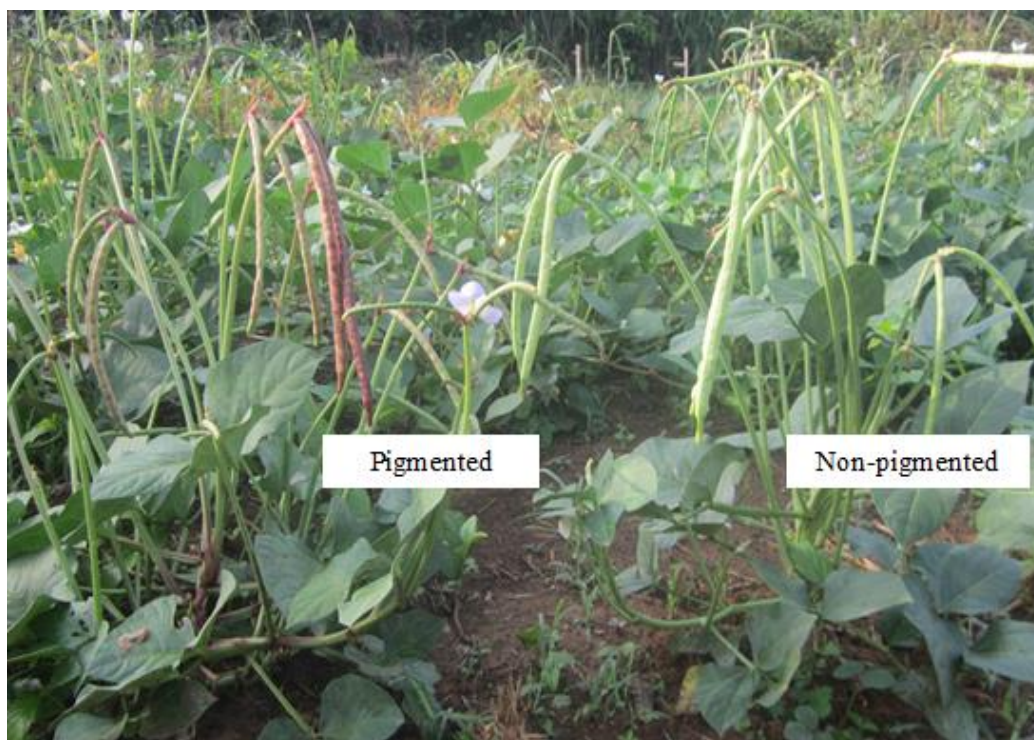


Figure 4.2: Cowpea genotypes UCR779 (left) and Bambey21 (right) showing pigmented and non-pigmented pods respectively.



Figure 4.3: A mixture of cowpea seeds from different accessions showing coat colour variability.

All three types of pod attachment namely; pendant, 30 – 90° and erect were exhibited by genotypes in the collection (Figure 4.4). The most observed pod attachment type was pedant with 58.7%.

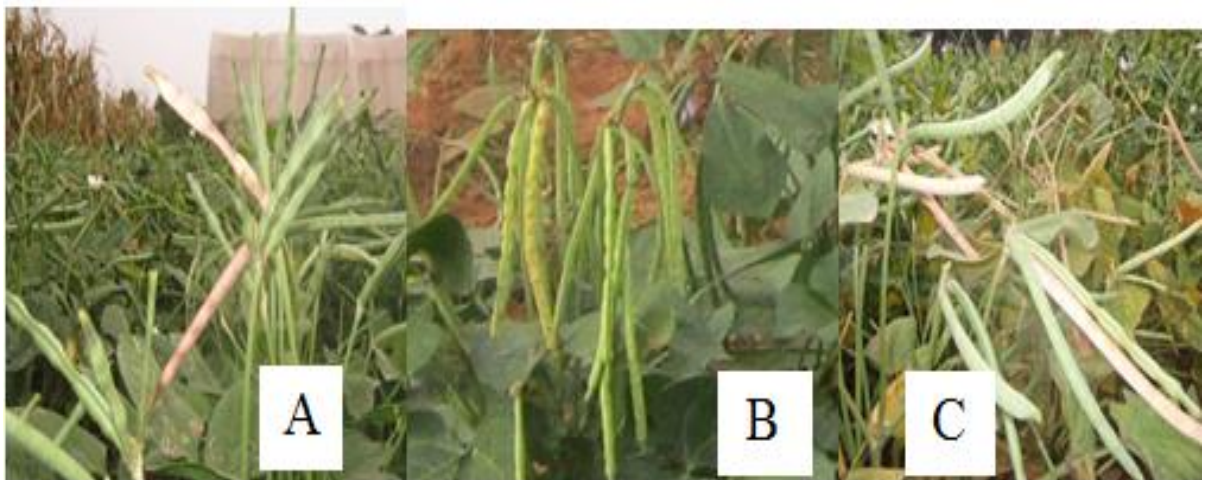


Figure 4.4: Cowpea genotypes Nhyira (A), CB27 (B) and Gh4537 (C) showing erect, pendant and 30° - 90° types of pod attachment respectively.

Sub-globose leaf shape was the most common terminal leaflet types with 61.3% occurrence. Hastate and globose terminal leaflets were conspicuous; however, they were less frequent than sub-globose and sub-hastate types (Figure 4.5).

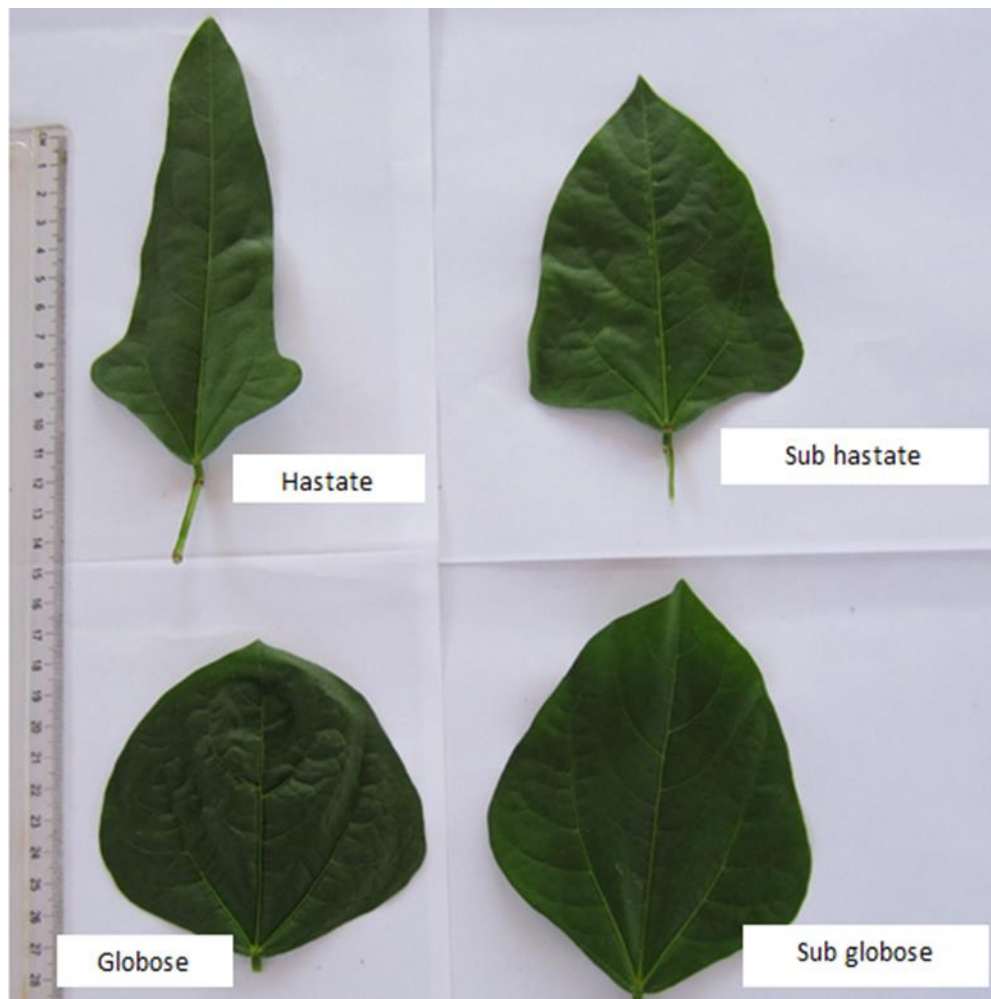


Figure 4.5: Variability in cowpea terminal leaflet shapes observed.

Seed shapes observed were kidney (12.8%), ovoid (39.4%), globose (3.7%) and rhomboid (44.1%).

4.3.2 Diversity in quantitative traits studied

Flowering was recorded for first appearance of opened flowers on each cowpea accession. The earliest flowering was 31 days after sowing (Figure 4.6). The latest accession flowered 75 days after planting.

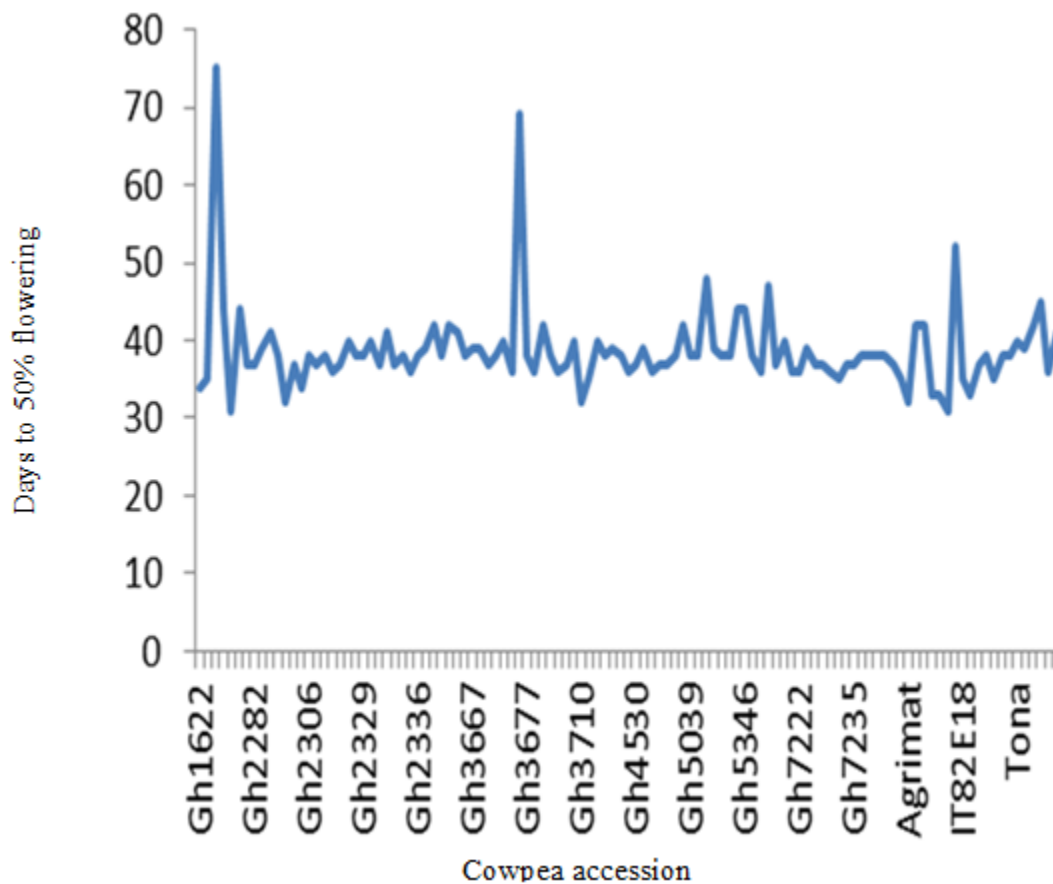


Figure 4.6: Variability in number of days to flowering

The mean number of pods per peduncle varied from 1 to 4 (Figure 4.7). The mean number of pods per peduncle for 71.19% of the accessions was two. Only 3.39% has four pods per peduncle.

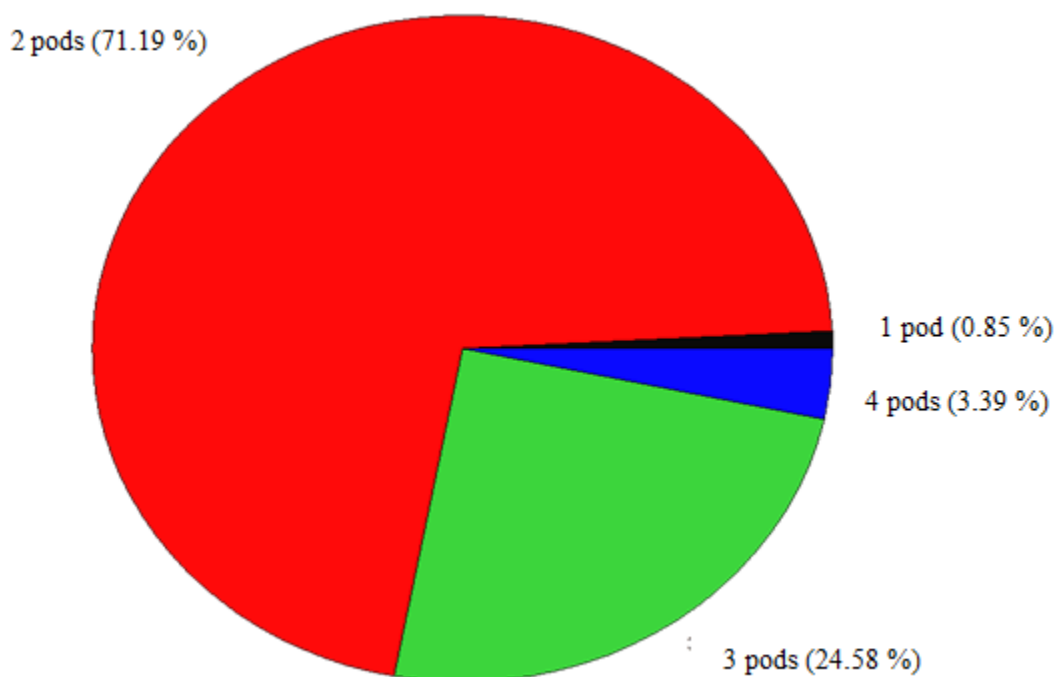


Figure 4.7: Mean number of pods per peduncle and their proportions in the collection

Number of seeds per pod ranged from 11.33 to 21.67 with a mean of 16.92 (Figure 4.8). Twenty five percent of the accessions had more than 18 seeds per pod. About 25% had less than 16 seeds per pod.

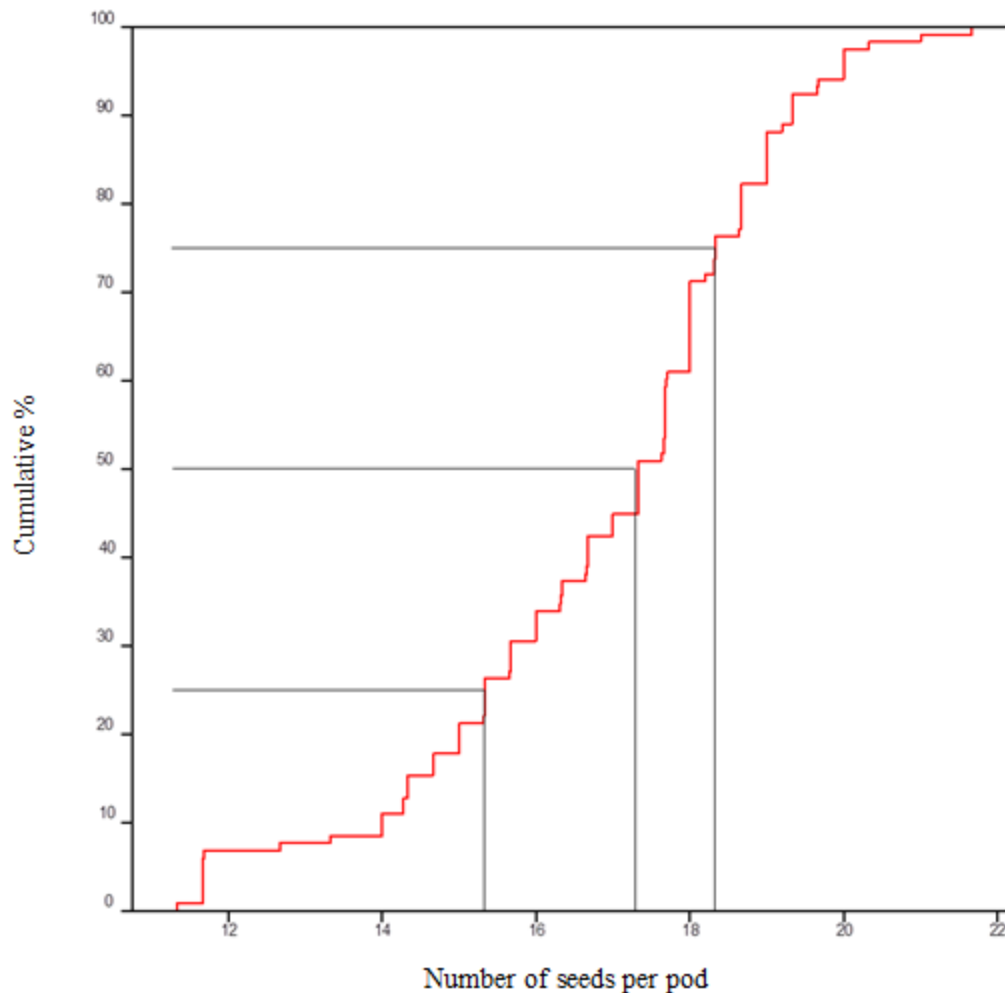


Figure 4.8: Distribution of mean number of seeds per pod

One hundred seed weight ranged from 5.54g to 20.84g and had a mean of 10.73g (Figure 4.9). Twenty five percent of the collection had seed weight heavier than 12g. The smallest 25% had seed weight less than 10g.

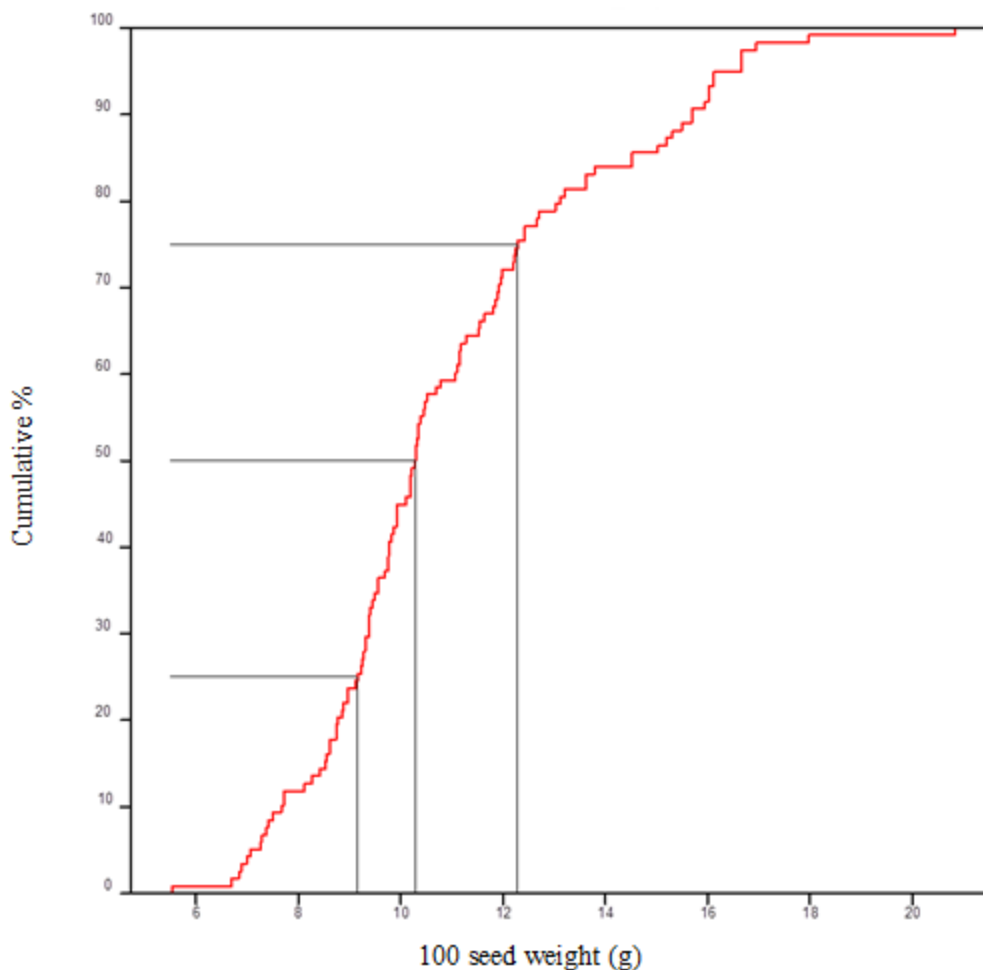


Figure 4.9: Mean seed weight (g) for cowpea accessions

4.3.3 Factorial display of the cowpea accessions

Factorial plot (Figure 4.10) was drawn based on dissimilarities calculated with the traits scored with modalities. Some genotypes did not share any of the 16 traits with any other genotype resulting in 100% dissimilarity among those genotypes. Some genotypes on the other hand shared most of the traits which resulted in their dissimilarity indices less than 10%. The factorial plot generally divided the genotypes into two: genebank materials and foreign or improved varieties.

4.4 DISCUSSION

Although, cowpea is reported to have narrow genetic base (Li *et al.*, 2001; Asare *et al.*, 2010), variability was observed in all of the 16 morphological traits studied confirming the report of Omoigui *et al.* (2006) that even closely related genotypes may have morphological differences. Variability in vigour was observed in the seedlings of different genotypes. Plant vigour as determined by growth in height and width showed that vigorous plants had advantage in early establishment and dominance over weeds. Therefore vigorous plants are preferred over less vigorous ones. Early establishment may also be important in drought resistance. Most vigorous plants observed were the spreading types such as Gh7218 and Gh7174. These lines would be good candidates in developing varieties for cover cropping. Accessions Gh7178 and Gh2336 were vigorous and erect, but have red and black seed coats respectively, which are less attractive to consumers in Ghana (Quaye *et al.*, 2011).

Diverse growth habits were exhibited by the cowpea accessions in the collection. This is also important in deciding on the planting system to choose for the variety. The climbers, prostrate and erect cowpea types would definitely be used in different planting systems such as sole or intercropping (Hall *et al.*, 1997). Plant architecture determines how much sunlight the plant can capture. Although a closed canopy as in erect types is likely to be conducive for microbial infection, they are also likely to have higher harvest index.

Large variation in flowering date may be related to differences in response to photoperiod among the accessions (Ishiyaku *et al.*, 2005; Timko and Singh, 2008). There were some

local accessions such as Gh3710 and Gh2293 which also flowered within 32 days of sowing. Gh3675 flowered 69 days after sowing and some did not flower until 75 days. It could be concluded with certainty that very early maturing cowpea genotypes are available in the collection.

According to Othman *et al.* (2006) and Egbadzor *et al.* (2012), there is pleiotropic control of flower, pod and seed coat pigmentation in cowpea. Makoi *et al.* (2010) reported relationship between seed coat pigmentation and insect pest resistance. Linkage of flower colour to other traits could facilitate indirect selection for important economic traits.

Varying degree of plant pigmentation was shown in the 118 cowpea genotypes. It was observed that the local accessions generally showed more intense anthocyanin pigmentation than the exotic ones. Yacine and TVU14676 for instance did not show any anthocyanin pigmentation. It was observed that a given immature pod pigment may result in different mature pod pigment while different types of immature pod pigments may also mature to similar pigments. Genotypes with darker mature pods were observed to shatter easily at maturity.

Seed colour influences consumer preference in cowpea (Mustapha, 2008). It is known that higher grain yields and improved grain quality are the primary breeding objectives of nearly all cowpea breeding programmes (Timko and Singh, 2008). Grain colour is one of the qualities that consumers prefer in cowpea and this preference has cultural dimension. The variability in seed coat colour and eye was very high in the collection (from black to

white). Mixture of different coat colours is a sign of within accession variability in some of the genotypes, and was observed only in the local genotypes.

Erect type pods were mostly above the canopy which are said to be easier to harvest (Bennett-Lartey and Ofori 1999; Cobbinah *et al.*, 2011) especially when borne on long peduncles. Pendant pods with short peduncles were within the canopy. However, pendant pods were generally long and had more seeds than erect types which would contribute to higher seed yields. Varieties with pendant pods borne on relatively long peduncles are desirable.

Seed shapes observed were kidney, ovoid, globose and rhomboid. A critical observation revealed that genotypes with some seed shapes had fewer seeds per pod. For instance, kidney shaped seeds were normally fewer per pod compared to rhomboid types of seeds which are usually many per given pod. Seed weight was measured at about 14% moisture content. Seed weight is reduced as the number of seeds per pod increases. Number of seeds per pod ranged from 9 to 21. Pod length ranged from 10.33 to 22.00 cm while 100 seed weight was from 6.69g to 20.84g.

The factorial display showed general diversity in the cowpea collection based on the 16 morphological traits. Two main clusters are recognizable in the display demarcated with a diagonal green line. The genotypes on the left side of the green line are mainly improved varieties in cultivation in Ghana with names written in blue and foreign genotypes written in red. Clustering of the improved genotypes together is an indication

of having been selected for similar traits. Most of the improved or foreign varieties share similar traits such as erect growth habit, early maturity and they bear multiple pods per peduncle among other traits. Consequently, this leads to the narrowing of the genetic base of crops. The clustering of genebank materials away from the improved varieties means that there are available genotypes for possible improvement in cowpea. This also shows the importance of conservation in safeguarding genetic erosion. Few genebank materials clustering with improved types may also help in using local materials for cowpea improvement; nevertheless, the use of foreign materials has its own advantage.

4.5 CONCLUSION

Variability was observed in all the 16 morphological traits used in characterizing the 118 cowpea genotypes. Despite the variability, some of the genotypes were similar for most of the traits resulting in low dissimilarity values. Some of the genotypes also did not have any trait in common resulting in 100% dissimilarity. Improved cowpea varieties clustered together away from genebank materials in a factorial plot. The experiments strengthened the proposition for conservation of plant genetic resources and in this case cowpea, to safeguard genetic erosion. In addition, it is clear that introduction of genotypes from other regions and breeding programmes increases the genetic diversity for breeders' use. Cowpea genotypes including CB27, Nhyira, Gh3710, Bambey21, UCR779, Tona and PadiTuya were selected for the study of seed coat colour and seed size inheritance.

CHAPTER FIVE

5.0 DIVERSITY ASSESSEMENT OF 113 COWPEA ACCESSIONS USING 458 SNP MARKERS

5.1 INTRODUCTION

Cowpea germplasm are being conserved at the genebank of the Plant Genetic Resources Research Institute (PGRRI) of the Council for Scientific and Industrial Research (CSIR). Most of these germplasm were collected in the 1980s and 1990s from different parts of Ghana (Bennett-Lartey, 1992; Asare *et al.*, 2010). Cowpea accessions of CSIR – PGRRI have been characterized based on morphological (Bennett-Lartey, 1992) seed protein (Oppong-Konadu *et al.*, 2005) and Simple Sequence Repeat (SSR) markers (Asare *et al.*, 2010). In addition, morphological traits of the collections are monitored during the regular regeneration activities.

Using morphological markers, Cobbinah *et al.* (2011) reported multiple duplicates of cowpea genotypes within the studied germplasm from Bunso. The reason for the high number of duplicates might be the limited number of morphological markers and the low amount of variability each revealed. Asare *et al.* (2010), using SSRs, could also not discriminate among some of the Bunso cowpea germplasm. Although, SSRs could be effective in diversity study in crops, only 20 polymorphic markers were used. The markers were not polymorphic enough to separate all of the accessions from each other.

Single Nucleotide Polymorphism (SNPs) markers were reported to be powerful tools in genetic diversity studies of living organisms including plants (Deulvot *et al.*, 2010). They detect variability between organisms at the nucleotide level. SNPs are more effective in

characterizing compared with other markers such as Amplified Fragment Length Polymorphisms (AFLPs) and SSRs (Varshney *et al.*, 2007). This is because SNPs are the most abundant type of markers ever discovered (Gupta *et al.*, 2001).

SNPs are numerous in the genome of plants and other living organisms (Galeano *et al.*, 2009; Deulvot *et al.*, 2010) and they serve as good tools for diversity studies (Varshney *et al.*, 2007). The more common a marker type is, the better it is in diversity studies (Acquaah, 2007), and for that matter, SNPs may be the best choice for diversity studies at the moment. However, SNP technology is quite new and has not yet been used in diversity studies of cowpea (Tan *et al.*, 2012). The objective of this study, therefore, was to use SNP markers to:

- (a) assess genetic diversity within cowpea germplasm in Ghana, and
- (b) develop a core collection of cowpea germplasm for Ghana.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials

A total of 113 cowpea accessions collected from Ghana and abroad were used for the experiment. These included 102 accessions collected from different parts of Ghana. One hundred and one (101) accessions of the 102 are being conserved at CSIR – PGRRI genebank at Bunso, Ghana, while one accession (WACCI01) was obtained from West Africa Centre for Crop Improvement (WACCI), University of Ghana. Four accessions were lines selected from GH4524 based on seed coat colour differences (Figure 5.1). Six of the accessions were improved varieties being cultivated in Ghana namely: ‘Asontem’,

‘Nhyira’, ‘Zaayura’, ‘Tona’, ‘Paddy Twua’ and ‘Bawuta’. In addition there were two lines each from University of California Riverside (UCR779 and CB27) and from International Institute of Tropical Agriculture (IITA) in Nigeria (IT97K-556-6 and IT82E-18). The accession labeled “market” is one of the popular cowpea varieties imported to Ghana from Togo and was, therefore, included in the imported accessions.

Seeds were germinated in sterilized top soil contained in nursery boxes at the Crop Science Department Garden, University of Ghana. Leaf discs of one week old plants were sampled from one plant per accession and shipped to the laboratory of KBiosciences in the United Kingdom where genomic DNA was extracted. The DNAs were genotyped using 500 SNPs from the cowpea panel (Muchero *et al.*, 2009b; Lucas *et al.*, 2011). Twenty-three of the markers did not give good result to be included in the results.

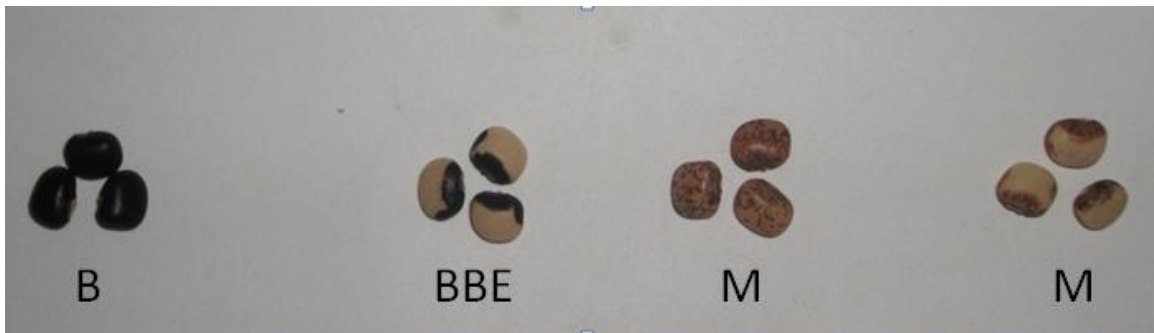


Figure 5.1: Gh4524 Lines.

Note: Seed coat colour is a distinguishing trait of Gh4524 lines. B, BBE, M and ME for Black, Big black eye, Mottle and Mottle eye respectively.

5.2.2 The SNP markers used

The SNP markers used were distributed across the cowpea genome. Figure 5.2 shows a map of the eleven linkage groups of cowpea, indicating the positions of the SNP markers on the genome. The length of each linkage group and the number of markers on it is inserted. Twelve of the 477 markers were unmapped and this is the reason for the sum of markers in Figure 5.2 to be 465.

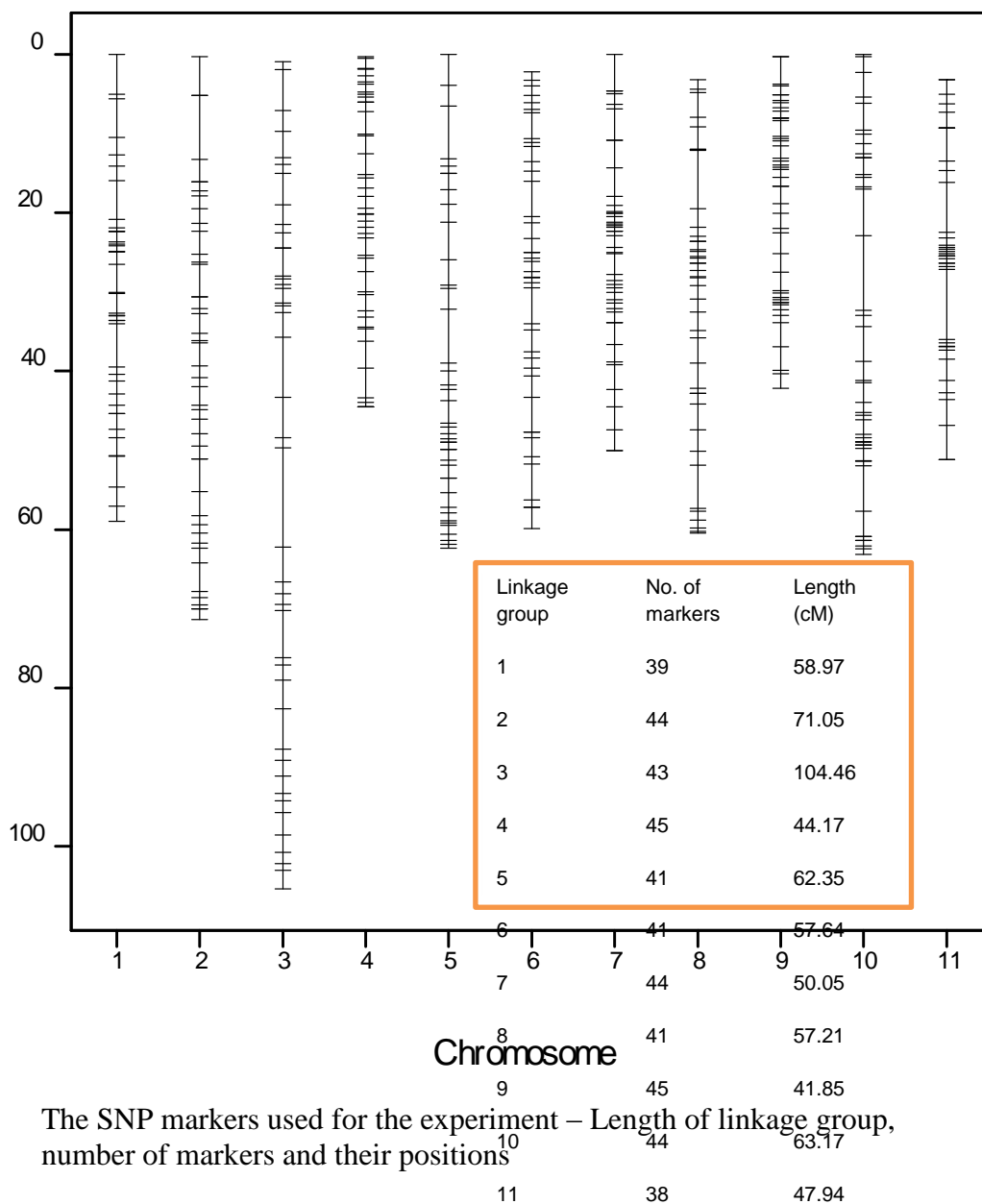


Figure 5.2: The SNP markers used for the experiment – Length of linkage group, number of markers and their positions

5.2.3 Data analysis

The software Darwin (Perrier and Jacquemoud-Collet, 2006) was used to analyze the data. Dissimilarity was calculated using simple matching formula:

$$\{d_{ij} = 1 - \frac{1}{L} \sum_{l=1}^L \frac{m_l}{\pi}\} \text{ (Perrier } et al., 2003)$$

Notations

d_{ij} = dissimilarity between units i and j

L = number of loci

π = ploidy

m_l = number of matching alleles for locus l .

The dissimilarity calculated was used for tree construction using the hierarchical clustering of Weighted Paired Group Method with Arithmetic Mean (WPGMA). The dissimilarity was also used for factorial analysis and plotted with number of axes equal to five. A Maximum Length sub-tree was constructed to select a representative core of the accessions.

The software Structure (Pritchard *et al.*, 2000) was used to detect the underlying populations structure among the cowpea accessions studied.

5.3 RESULTS

5.3.1 Allelic diversity

Out of the 477 SNPs, 458 were polymorphic. SNP data revealed that some of the markers although polymorphic, only few, sometimes just one genotype had the alternative allele in the collection. The percentage of the cowpea accessions that had the same allele at a

particular locus thus varied greatly; from 0% versus 100% to 50% versus 50%. For the purpose of discussion, alleles that were shared by less than 10% of the accessions in the collection were referred to as rare.

The cowpea accessions, GH7888 (a genebank material), ‘Zaayura’ and IT97K-556-6 shared a rare allele. IT97K-556-6 is an IITA line while ‘Zaayura’ is a commercial variety released by CSIR - Savanna Agricultural Research Institute. Another rare allele was shared by UCR779 and ‘Zaayura’. UCR 779, a Botswana landrace, resistant to aphid (Muchero *et al.*, 2009a) was one of the most unique accessions in the collection. It was the only line with “A” against “T” for one marker. “Asontem” (IT82E-16) which is one of the improved varieties in Ghana developed by IITA in collaboration with CSIR – Crops Research Institute, also had a rare allele at a locus. The last example of a rare allele observed in the collection was “T” for GH7167, GH2288 and CB 27 where all other accessions had “C”. The allelic diversity thus varied greatly for the cowpea accessions.

5.3.2 Heterozygosity

Some of the cowpea accessions were heterozygous at some of the marker loci. Heterozygosity at a locus may indicate accessions undergoing segregation. Many of the accessions in the collection had at least one heterozygous site.

5.3.3 A bi-plot of the cowpea accessions

The SNP markers discriminated all of the cowpea accessions with dissimilarity ranging from 0.6% to 63.4% with a mean of 34.3% (Fig. 5.3). General diversity of the germplasm

is displayed in a bi-plot in Figure 5.4. Lines and a circle were drawn in Figure 5.4 to aid in explanation. Three major clusters were identified in the Figure demarcated by the “y” shaped two green lines. Members of each cluster mostly had similar seed coat colour.

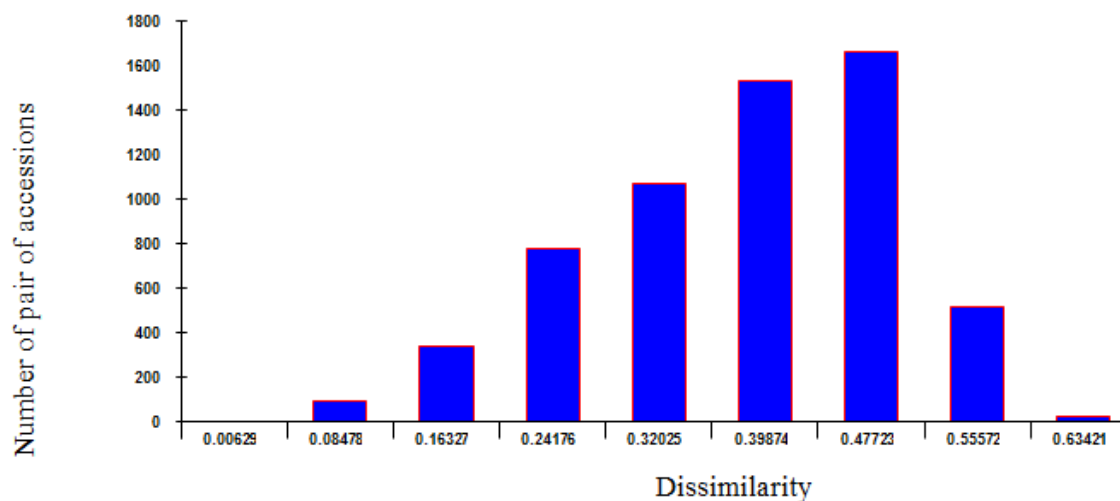


Figure 5.3: Bar-graph showing the number cowpea accessions with corresponding dissimilarity indices.

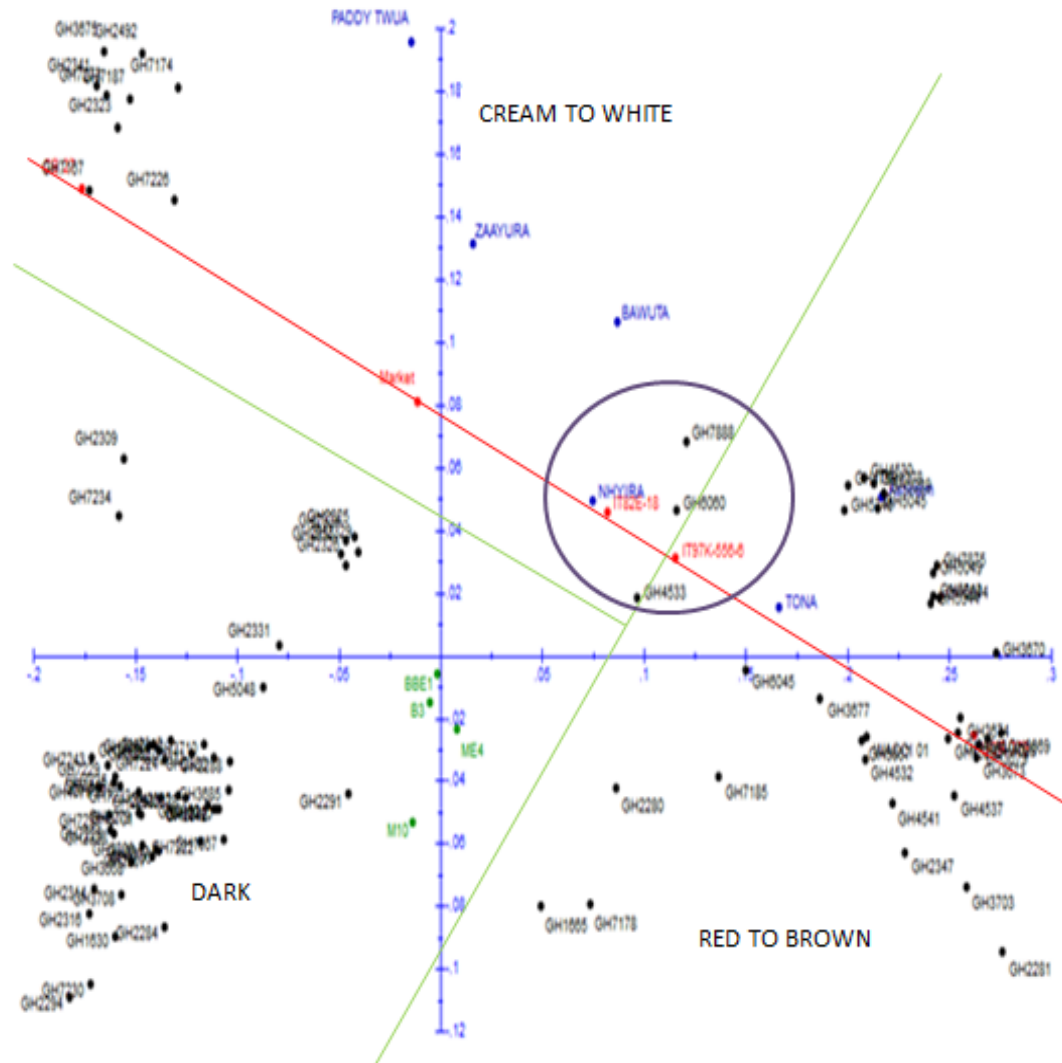


Figure 5.4: A bi-plot showing cowpea accessions using 458 polymorphic SNP markers

5.3.4 Dendrogram of the cowpea accessions

Figure 5.5 is a dendrogram of the cowpea accessions drawn with the calculated values of dissimilarities using hierarchical clustering with WPGMA method. For legibility of the accessions, different portions of Figure 5.5 were shown in Figures 5.6, 5.7 and 5.8.

5.3.5 Result of structure analysis

The result of analysis made with Structure is presented in Figure 5.9. Three populations were assumed and indicated with blue, green and red colours. The number of accessions belonging to the blue, green and red discretely was 8, 22 and 38 respectively. Thus the total number of accessions without admixed genomes was 68.

5.3.6 Core 48 cowpea accessions

Maximum length sub tree method (Perrier *et al.*, 2003) was used to identify forty-eight core accessions for breeding purposes (Figure. 5.10). These 48 accessions were found to be very diverse morphologically.

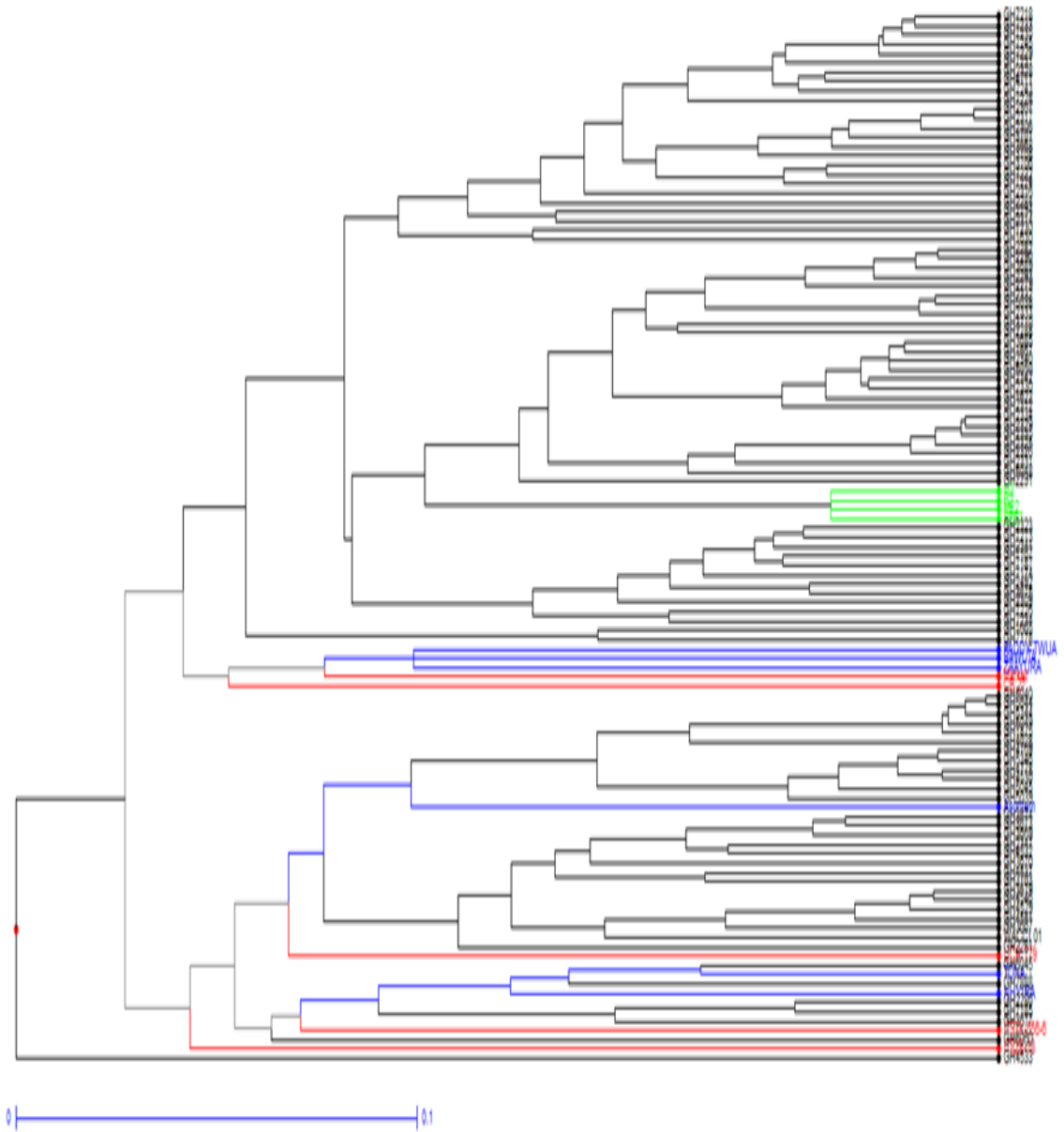


Figure 5.5: Dendrogram of 113 cowpea accessions constructed from dissimilarity using 458 polymorphic SNP markers.

Note: Accessions written in black represent genebank materials obtained from Bunso and WACCI01 while those in blue and green are improved varieties from Ghana and Gh4524 lines respectively. Accessions in red are from IITA, UCR and the one named "market".

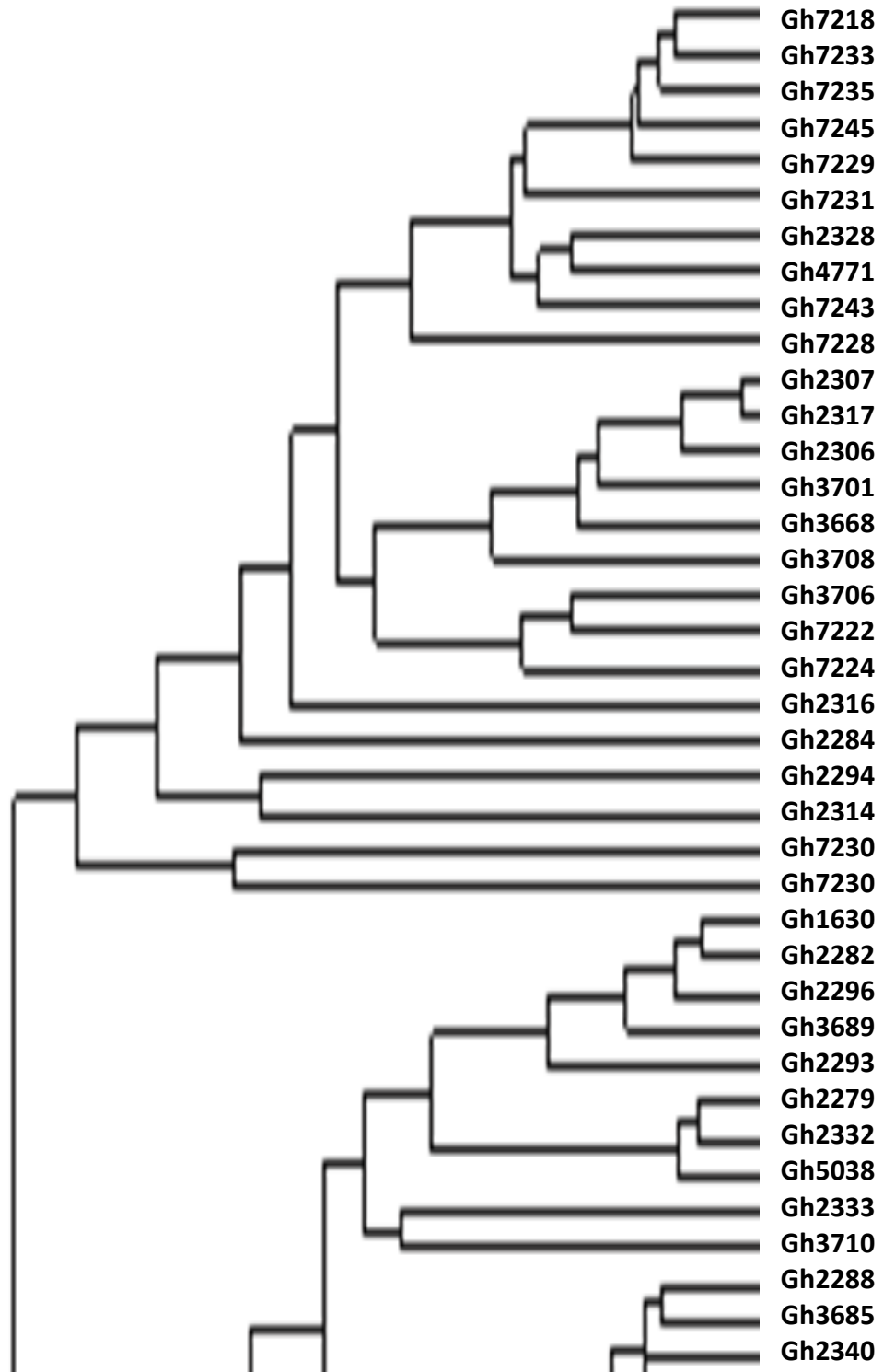


Figure 5.6 First 38 cowpea accessions in Figure 5.5

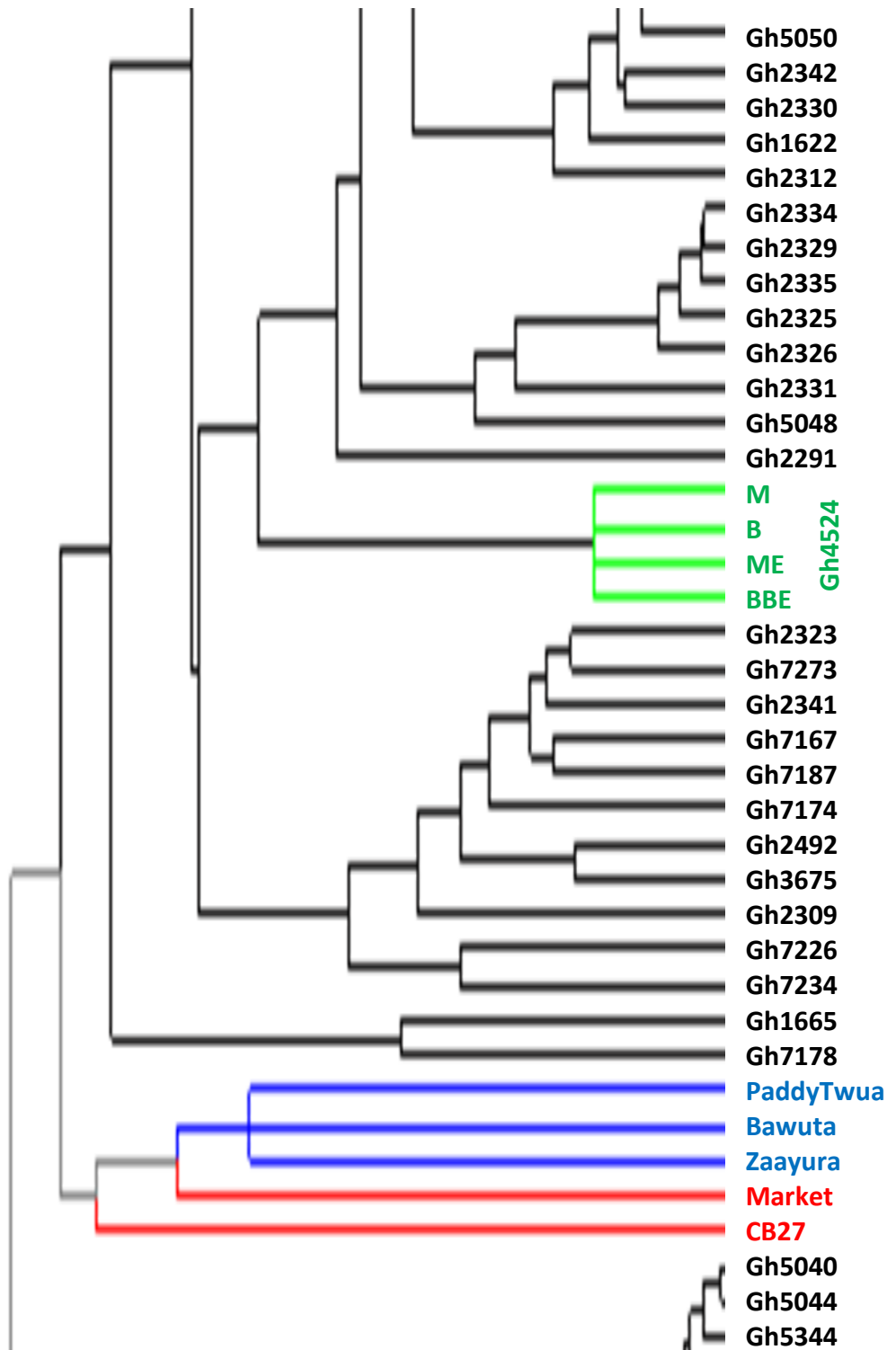


Figure 5.7: Middle 38 cowpea accessions in Figure 5.5

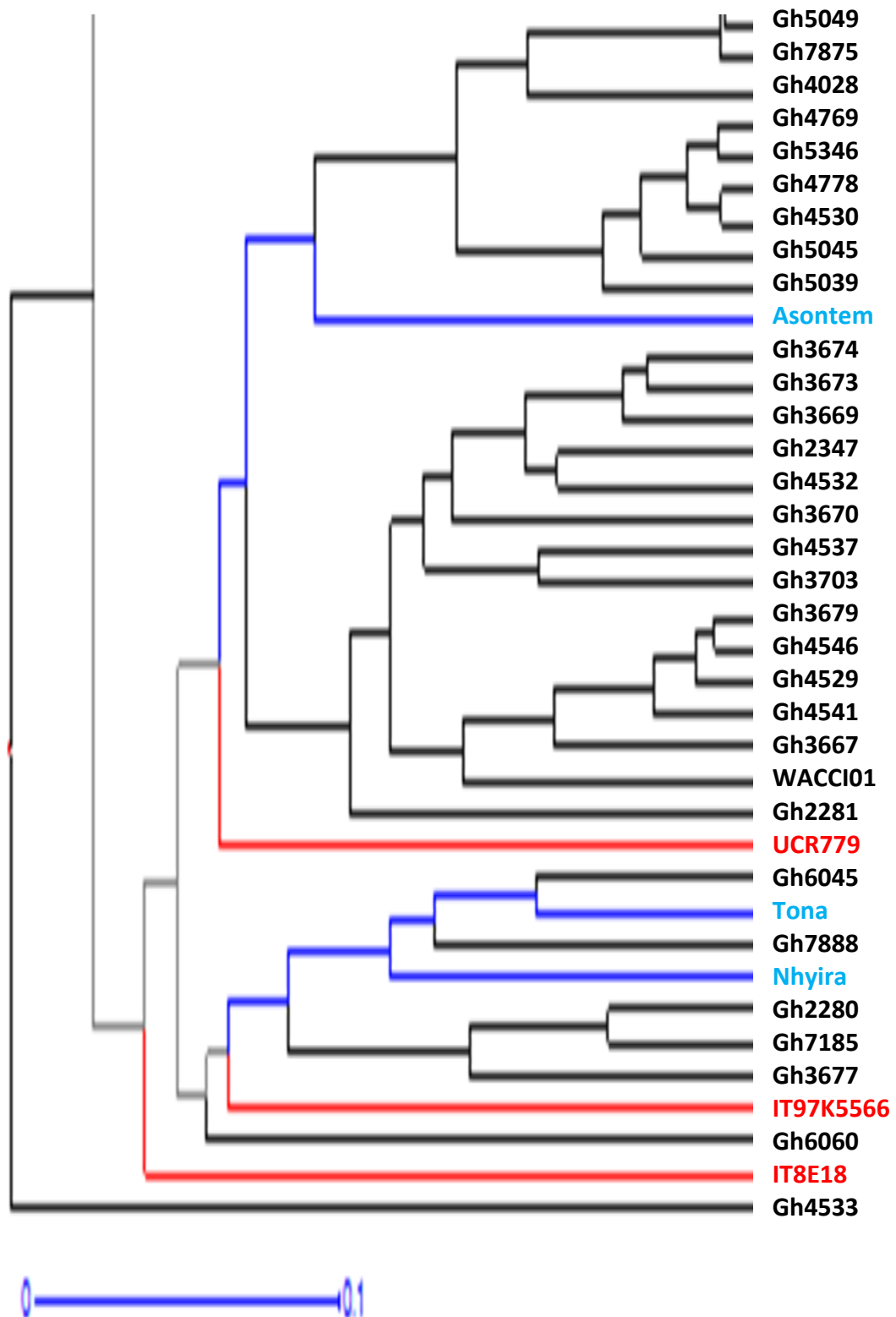


Figure 5.8: Last 37 cowpea accessions in Figure 5.5

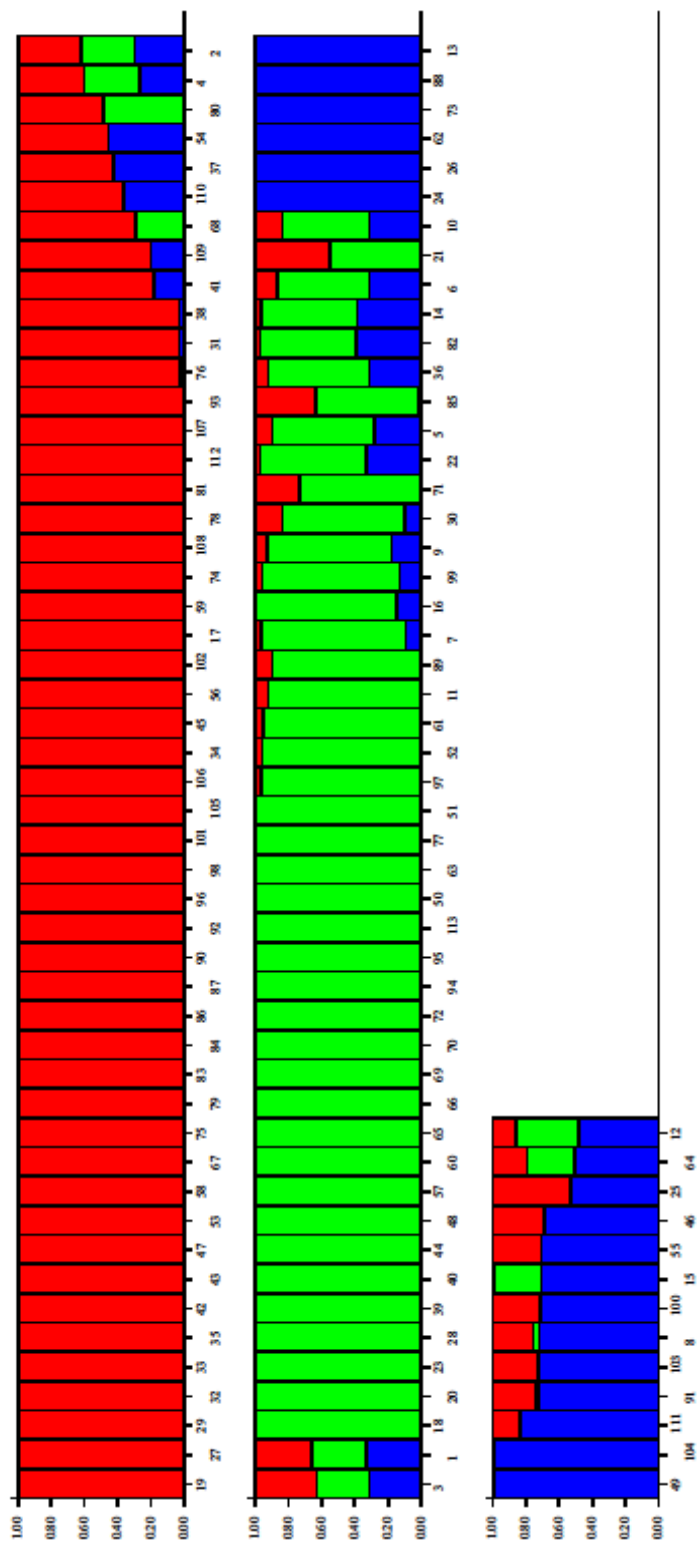


Figure 5.9: Estimated population structure for the cowpea accessions studied.

Note: Each accession is represented by a vertical line, which is partitioned into coloured segments indicating the estimated membership fractions for it. Estimated Ln Prob of Data = -32014.2: Mean value of Ln likelihood = -31663.4: Variance of Ln likelihood = 701.5: Mean value of alpha = 0.071: Mean value of Fst 1 = 0.7: Mean value of Fst 2 = 0.56: Mean value of Fst 3 = 0.49: Overall proportion of membership of the sample in each of the 3 clusters (Inferred Clusters 1 (red) = 0.46; 2 (green) = 0.35; 3 (blue) = 0.19)

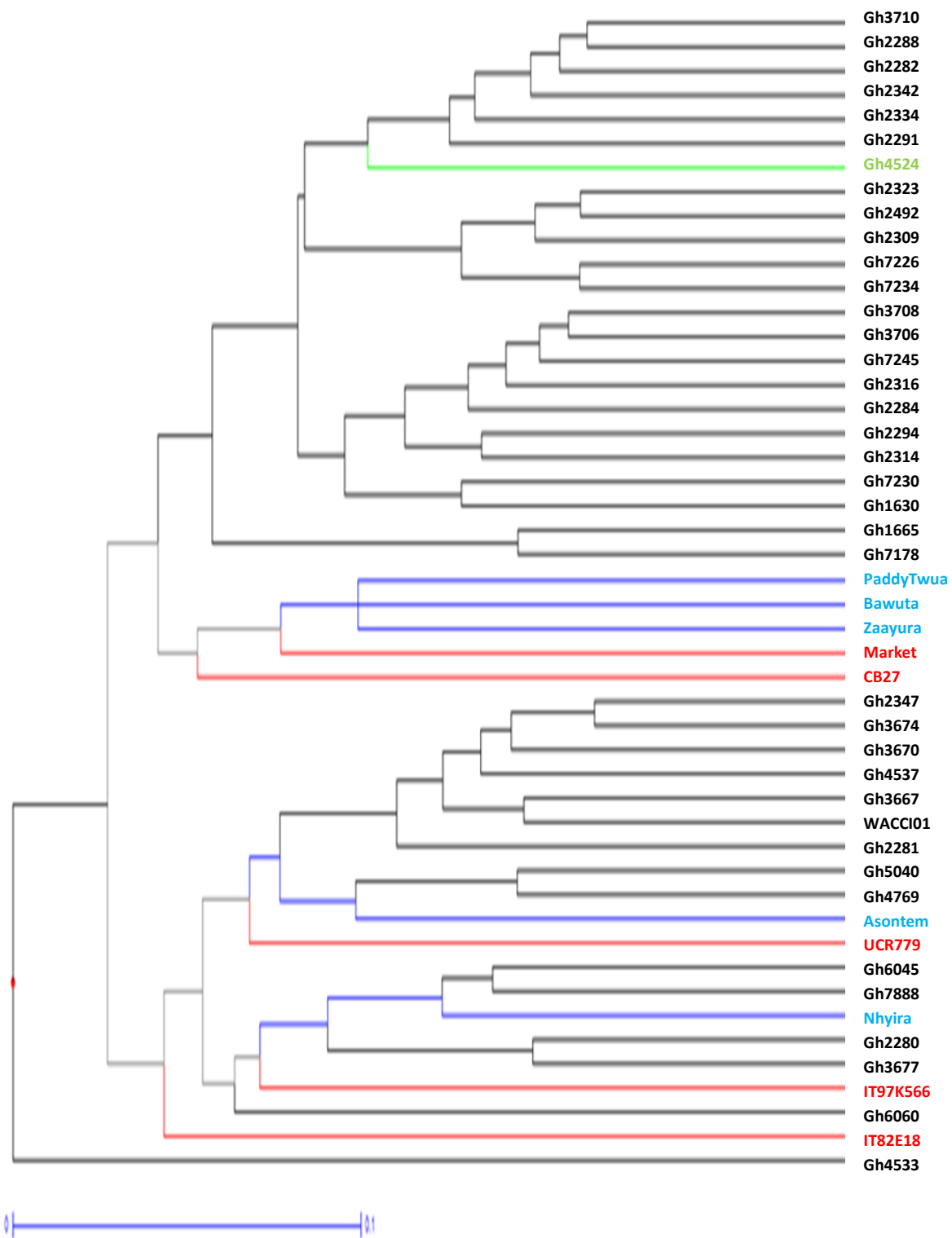


Figure 5.10: Dendrogram of 48 core cowpea accessions.

5.4 DISCUSSION

The 458 SNP markers were able to discriminate between all the cowpea accessions studied. Previous studies (Bennett-Lartey 1992; Oppong-Konadu *et al.*, 2005; Asare *et al.*, 2010) could not discriminate all accessions, but the increased number of markers used here and their high levels of polymorphism allowed the discrimination of even closely related accessions such as BBE, M, B and ME (segregated lines of Gh4524). This confirms the robustness of SNP markers in diversity studies reported (Varshney *et al.*, 2007; Lucas *et al.*, 2013b).

Definite patterns were identified in the cowpea collection. Both Ghanaian and foreign elite accessions clustered together (Figures 5.4 and 5.5). Patterns could also be seen in Figure 5.4 based on the seed coat colour similarities of the cowpea accessions. However, accessions collected from different regions of Ghana did not cluster together in most cases. Asare *et al.* (2010) also did not observe strong geographic relationship in the PGRRI cowpea collections when they used SSR markers in diversity studies. Tanhuanpaa and Manninen (2012) in their studies on *Phleum pretense* diversity with SSRs markers also did not observe significant correlation between the various accessions and their geographic origins. Geography does not always reflect underlying genetic structure (Rosenberg *et al.*, 2002).

Only 150 markers did not have any cowpea showing heterozygosity. Some markers generally revealed higher levels of heterozygosity. There were 23 accessions which were heterozygous for a particular marker. Most of these accessions clustered together (Figures

5.4 and 5.5). Gh7234 for instance had as many as 90 heterozygous sites. This suggests that some of the genebank cowpea accessions are not pure. Phenotypic analysis strengthened the assertion that seeds of Gh7234 were different in terms of seed coat colours with the dominant being dark mottling. Similar observation was made for Gh7231 which had 13 heterozygous sites. However, some of the improved varieties including the foreign ones (IT97K-556 and CB27) also had one or more heterozygous sites. High heterozygosity which has been detected in such crops as plantains (Tenkouano *et al.*, 1999), Scot pines (Gupta *et al.*, 2001) and cassava (Dyer *et al.*, 2011) was unanticipated in this study. The high heterozygosity observed in some of the cowpea accessions might be due to outcrossing (Lucas *et al.*, 2011; Kouam *et al.*, 2012) during regeneration at the genebank and to the fact that some of them have hybrid origin. There were only five accessions (Gh2282, Gh2340, Gh2347, Gh3706 and Gh7218) that were homozygous at all the loci.

Three major clusters were identified on the factorial display of the accessions indicated by two green lines which formed a “y” shape (Figure 5.4). Accessions of the same cluster generally had similar seed coat colours with only a few exceptions. Some of these exceptions were Gh2281 and Gh7185 with dark seed coat colours clustering in the red to brown seed coat colour group while Gh2284 and Gh5048 with red seeds clustered with dark colours. Seed coat colour is frequently used as a morphological trait in classifying crop varieties (Adesoye and Ojobo, 2012) and may also be linked with other important traits such as insect resistance (Atis *et al.*, 2011). The clusters according to the seed coat colours were; Dark, Cream to White and Brown to Red (Figure 5.4). The boundaries

between the dark seed coat colour cluster and the other two were very conspicuous. However, the boundary between the white and red seed coat colour clusters was not very clear. Six accessions in the purple outlined circle formed a sub-cluster between the white and red seed coat colour clusters. Even though, the six accessions formed a sub-cluster, each individual was closely linked to its respective major cluster, with the exception of IT82E-18 (Figure 5.4). In contrast, Asare *et al.* (2010) did not observe clustering pattern based on seed coat colour when they characterized cowpea collections with SSRs markers. However, in this study clear patterns based on seed coat colour were observed. Similarly in maize, SNP markers were used to identify kernel colour gene (Sharma *et al.*, 2011).

All the foreign accessions fell on a straight line (red). They also fell in their appropriate seed coat colour clusters. These are elite germplasm (improved varieties) and have been selected for similar traits over a long period of time. The improved varieties from both Ghana and abroad are found on or above the red line (Figure 5.4). Local accessions that clustered with these elite accessions could be very useful materials for cowpea breeding programmes, especially in Ghana, for being genetically close to the elite varieties and being adapted to the local climate. For instance, the dissimilarity between GH7888 (a genebank material), and ‘Zaayura’ was as small as 0.026. GH7167, GH2288 and CB 27 clustered together. CB27 was released in California in 1999 and is resistant to Fusarium wilt race 3 and moderately susceptible to aphid (Muchero *et al.*, 2009). Phenotypically CB27 did not share much similarity with Gh2288. Seed mass of CB27 was twice that of Gh2288. The kidney shaped seed of CB27 had white seed coat with black eye. This type

of cowpea has a high preference in Ghanaian markets (Langyintuo *et al.*, 2003). Gh2288 on the other hand had dark mottling seed coat colour. Accession CB27 is erect while Gh2288 is prostrate. Few traits shared by CB27 and Gh2288 are, pigmented immature pod tip which dry up to straw, pendant pods that were slightly curved and sub-hastate terminal leaflets.

No elite genotype fell in the dark coat coloured cluster (Figure 5.4). Commercial varieties of cowpea are mainly white or brown to red coat coloured in Ghana as they are the types preferred by consumers (Quaye *et al.*, 2011; Langyintuo *et al.*, 2003). Separation of many Ghanaian accessions away from elite and commercial varieties may mean availability of diversity that could be exploited for cowpea improvement. Despite claims of limited genetic variation in cowpea (Asare *et al.*, 2010; Kumar *et al.* 2011 Tan *et al.*, 2012), there is substantial morphological and genetic evidence that cowpea is a very diverse taxon (Huynh *et al.*, 2013). This experiment has shown that the studied germplasm has some amount of diversity that can be used for cowpea improvement. Special interest would be to use the landraces in broadening the genetic base of the improved cowpea varieties similar to what was suggested for asparagus bean in China (Tan *et al.*, 2012).

Clustering of materials such as CB27, Paddy Twua (Padi Tuya), ‘Bawuta’ and ‘Zaayura’ is very significant. This is because Padi ‘Tuya’ and a number of varieties released by CSIR – SARI are known to have parentage from California Black eye (Padi *et al.*, 2004a). Close relationship between “Market” and CB27 (Figures 5.4 and 5.5) was also not surprising. “Market” is an imported cowpea picked from a market and is suspected to

originate from California Black-eye because of its seed features. Clustering of CB27 and Market had confirmed their relatedness. The dendrograms in Figures 5.5 to 5.8 support pedigree knowledge as seen in the clustering of Gh4524 lines and UCR779 with IT82E-18 which are both from South/East Africa, Botswana and Mozambique, respectively. The SNP markers generated reliable information in this diversity study. An exception was that IT82E-18 did not cluster with Asontem (IT82E-18 in Ghana). It could probably be that the Asontem collected was not the IT82E-18 as it has been in the hands of farmers for a long time. Farmers might be calling a morphologically similar variety Asontem. Another possibility resulting in the non-clustering of IT82E-18 and the supposed Asontem is that the plant genotyped as Asontem could be a rogue as described by Lucas *et al.* (2013b).

Members in the blue population in the cluster plot, some of which are Gh2323, Gh7167 and Gh7174 clustered at the top left corner in Figure 5.4. With the exception of Gh7178 (13 in Figure 5.9), all the accessions within the blue cluster have white or cream seed coat colour. Gh2323 and Gh7273 which are both members of the blue population in Figure 5.9 were the closest relatives in Figure 5.5. The cowpeas in the green population in Figure 5.9 are mostly red seed coated and also showed close relationship in the dendrogram in Figure 5.5. Accessions such as Gh5039, Gh5040 and Gh5049 in the green population clustered together in Figure 5.4. Similar patterns were also observed for the red population in Figure 5.9. However, accessions in this group were more diverse in terms of seed coat colour. The clustering pattern in the dendrogram and factorial plot with “Darwin” thus had some similarities with that of “Structure”. In this study the result of

the structure analysis made biological sense especially when it is compared to the phenotype of the cowpea accessions and the analysis made with Darwin.

Different combinations of admixtures of genomes for different cowpea accessions were observed. Some of the accessions had genomes from two different populations while others were from all the three. All of the improved varieties had genome from different populations (Figure 5.9). “Zaayura”, “CB27” and “Market” had similar patterns with the exception of having slightly different proportions for the various segments. These three varieties are believed to have been bred from materials with common parentage (Padi *et al.*, 2004b). The four accessions obtained from Gh4524 (numbers 1, 2, 3 and 4 in Figure 5.9) showed very similar patterns and had portions of their genomes from different sources. Some other accessions from the genebank as well showed inheritance of segments of genomes from different populations. Cowpea is predominantly inbreeding and it is shown by the mean F_{ST} value of 0.07 indicating that most of the accessions are essentially from one population. However, mean value of 3.4% outcrossing has been reported (Kouam *et al.*, 2012) which might be the reason for some of the genebank materials to be admixed. The observation in this study thus confirms this phenomenon.

The establishment of a core germplasm collection helps in easy management and identification of variations for breeding purposes (van Hintum *et al.*, 2000). Where the germplasm collection is very large, management goes beyond core to mini core collection (Upadhyaya *et al.*, 2010). Forty-eight core accessions were consequently identified from the fingerprinting for conservation and crop improvement. The core 48 accessions include UCR779, CB27, IT97K-556-6 and IT82E-18 which are internationally known

cowpea lines. These materials had unique alleles that are not likely to be available in the genebank in Ghana. Five of the improved varieties from Ghana were included in the 48 core accessions. These 48 accessions include all of the 11 improved varieties in the study. Bringing these improved accessions which were hitherto not in the collection into the activities of the genebank might mean expansion of the gene pool of the cowpea which is considered to be narrow (Tan *et al.*, 2012). Expansion of a gene pool is important for crop improvement (Varshney *et al.*, 2007).

The sphericity index as explained by Perrier *et al.* (2003) was considered in choosing the 48 core cowpea accessions. The sphericity index for all the 113 accessions was 0.69. This figure meant that there was high redundancy in the collection, compared to the final three accessions which had the highest sphericity index of 1. The core 48 accessions selected had sphericity index of 0.79 which was quite low indicating high redundancy which could permit further reduction in the number of accessions included in the core collection. However, as many as 10 improved varieties were included in the core collection when the number of accessions was reduced to 20 with sphericity index of 0.88. This meant that with 20 core accessions, only 50% of the accessions would be from the genebank. To avoid further narrowing of the genetic base of the cowpea germplasm for breeding purposes (Sharawy and El-Fiky 2002; Fang *et al.*, 2005; Asare *et al.*, 2010; Tan *et al.*, 2012), the 48 core accessions were, therefore, accepted to increase the genetic base of the core collection.

The core accessions varied in morphological traits such as growth habit where there were a wide range spanning from erect to spreading types. Plant pigmentation, leaf shape and flower colour also varied among the core accessions. Seeds with different coat colours, sizes and shapes were found within the core accessions. Some of the accessions in the core collection had been reported to have resistance to biotic stresses. Examples include CB27 and UCR779 which are resistant to Fusarium wilt and aphid respectively (Muchero *et al.*, 2009a). These accessions could be used as parents to develop varieties resistant to biotic stresses such as aphid borne mosaic virus which is a serious constraint to cowpea cultivation in many parts of Africa (Orawu *et al.*, 2012). Further evaluation of the core 48 accessions may reveal other traits that might be of interest to cowpea breeders.

5.5 CONCLUSION

The markers were efficient in discriminating among all the accessions used in the study including closely related materials such as Gh4524 lines. Accessions known to be related by ancestry such as CB27 and Paddy Twua, clustered together, demonstrating the reliability of the markers. The information provided in this diversity study could be useful in cowpea improvement in Ghana and elsewhere. A total of 48 accessions were identified as a core collection for breeding purposes. These core accessions were morphologically very diverse and included UCR779, CB27, IT97K-556-6 and IT82E-18. These are elite materials obtained from different countries. Improved varieties from Ghana such as 'Bawuta' and 'Nhyira' are also in the 48 core accessions. The genetic diversity of the selected core could be of importance for future plant breeding for the development of superior varieties of cowpea.

CHAPTER SIX

6.0 IDENTIFICATION OF SNP MARKERS ASSOCIATED WITH SEED SIZE IN COWPEA

6.1 INTRODUCTION

One of the important traits desired in cowpea is large seed size in West Africa (Drabo *et al.*, 1984; Langyintuo *et al.*, 2003; Tchiagam *et al.*, 2011a; Egbadzor *et al.*, 2013). However, many breeding objectives have not been directly focused on seed size compared with other traits such as biotic and abiotic stresses tolerance (Hall *et al.*, 1997; Orawu *et al.*, 2012). This is not to say that seed size has never been studied. Conflicting gene actions have been reported to control seed size inheritance in cowpea through classical studies (Drabo *et al.*, 1984). This is an indication of the complexity of the trait. Association mapping or linkage disequilibrium which is an alternative to traditional QTL mapping is a powerful method in complex traits studies (Abdurakhmonov and Abdukarimov, 2008) and therefore can be used to better understand inheritance of seed size in cowpea. Association mapping has advantage over QTL mapping in terms of time, allele number and quality of resolution (Zhu *et al.*, 2008).

SNPs present in the coding sequences may determine the mutant phenotype and will show 100% association with the trait and could therefore be useful for MAS and gene isolation (Gupta *et al.*, 2001). The objective of the research, therefore, was to map seed size in the cowpea genome using association population of mainly Ghanaian genotypes with SNP markers. Identification of significant association between SNP markers and seed size could help in marker assisted selection of the trait.

6.2 MATERIALS AND METHODS

6.2.1 Plant Materials

A total of 78 cowpea accessions were used in the association mapping. Sixty-nine of the accessions were random samples of gene-bank materials from the Plant Genetic Resources Research Institute of the Council for Scientific and Industrial Research (CSIR - PGRRI), Bunso, collected from across Ghana. There were 5 improved varieties from Ghana and 4 from outside Ghana. Four of the five improved varieties were Tona, Nhyira, Asontem and Zaayura, all in cultivation in Ghana. The fifth variety, labeled “Market” was taken from the market. The four genotypes obtained from outside Ghana were UCR779, CB27, IT82E-18 and IT97K-556-6.

The plant materials were grown in single rows without replication at WACCI farms, University of Ghana in April 2011 under rainfall condition. Dry pods from five plants out of ten per row were bulk harvested and their seeds removed for data collection. Leaf tissues of one week old plants from each genotype were sampled and sent to the KBioscience Laboratory in the United Kingdom for genotyping with SNP markers. Genotypic (SNP) and phenotypic (seed weight and seed thickness) data were used for association mapping.

6.2.2 Data collection and analysis

Seeds were dried to approximately 12% moisture content in the sun after harvest and measurement taken on their sizes (mass, length, width and thickness). Seed length, width

and thickness were measured with electronic digital calipers and mass of 100 seeds with balance. The representation of the seed length, width and thickness is shown in Plate 1.

Phenotypic and genotypic data were recorded on excel sheet. The former was imported directly into GenStat for analysis, however, the latter was transported into notepad before importation into GenStat. Preliminary single environment analysis was done on the phenotypic data: vital information including F Probability and heritability estimates reported.

The procedure for single trait single environment association mapping (VSN International, 2012) was followed to identify SNP markers linked with seed mass and thickness.

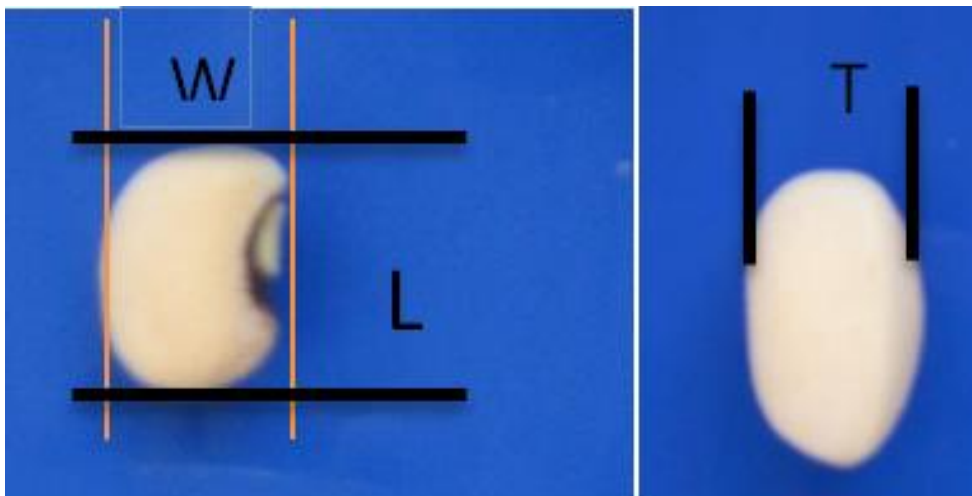


Figure 6.1: Cowpea Seed Width (W), Length (L) and Thickness (T)

6.3 RESULTS

Preliminary single environment analysis was run twice: first with genotypes as random effects in order to estimate heritability and variance parameters, the second time with Genotypes fixed to get unshrunk means for QTL analysis. Vital information from this analysis is presented in Table 6.1.

Table 6.1: Results from Preliminary Single Environment Analysis on Seed Size

Seed Trait	Minimum	Maximum	Mean	F. Probability	Heritability
100 seed Mass (g)	6.58	20.27	10.48	0.006	0.96
Width (mm)	4.94	7.53	5.89	0.081	0.82
Length (mm)	5.65	9.9	6.94	0.06	0.85
Thickness (mm)	2.3	5.82	3.56	0.016	0.94

Note: Number of observations (n) is 78 for all traits.

Correlations between the various attributes of seed size namely seed mass, seed length, seed width and seed thickness were estimated (Fig 6.2).

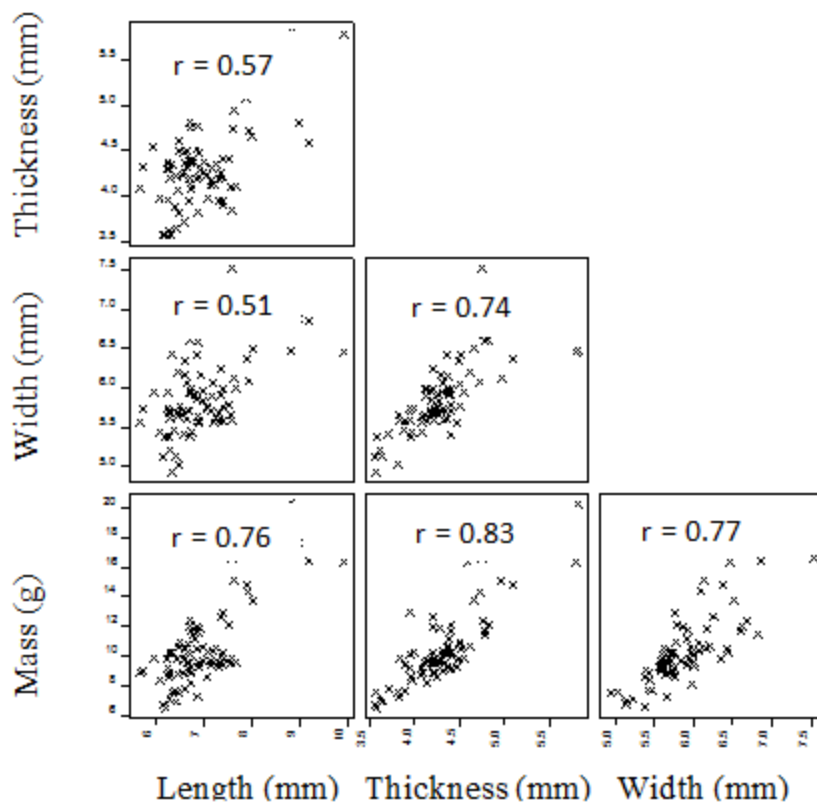


Figure 6.2: Correlation between seed mass, length, thickness and width.

The markers used for the mapping were fairly distributed across the cowpea genome as shown in Figure 6.3. Figure 6.4 shows strength of association between various markers and seed mass while Figure 6.5 shows their positions in the cowpea genome. Figure 6.6 and 6.7 are similar to Figure 6.4 and 6.5 but for seed thickness. Some of the markers having significant association with the traits are shown in Table 6.2.

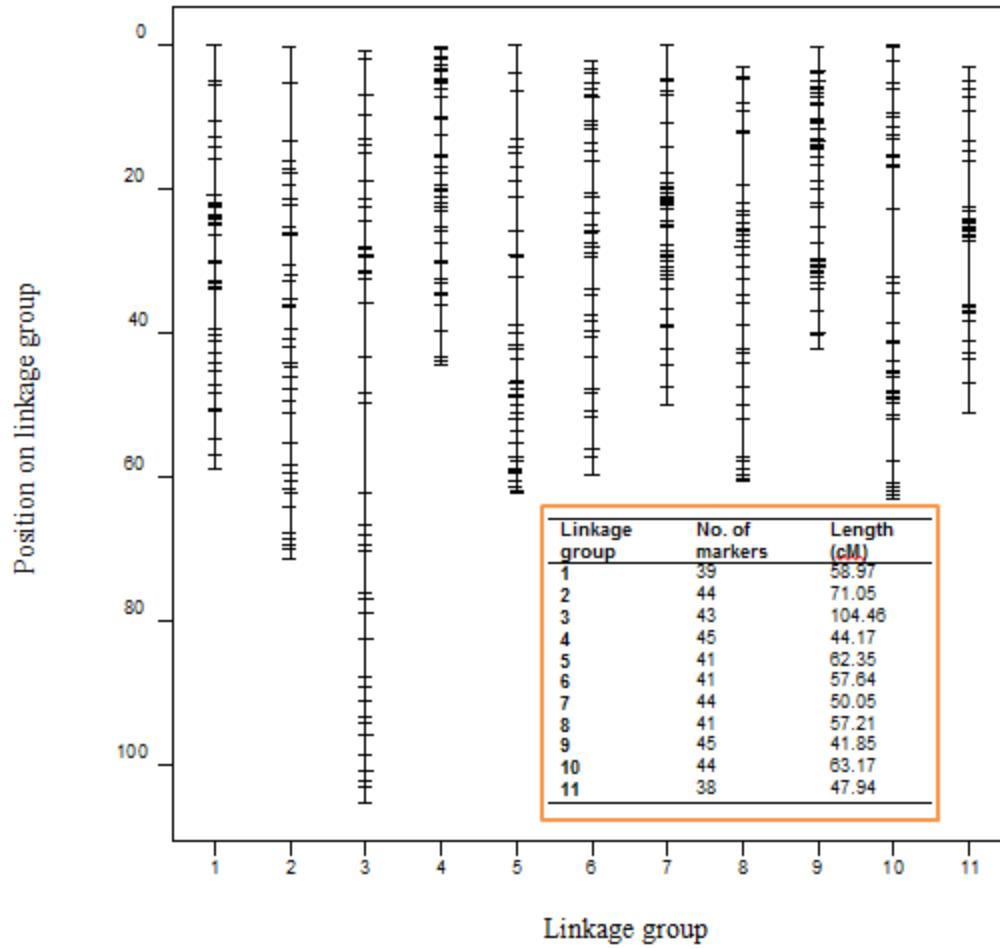


Figure 6.3: Markers mapped onto linkage groups

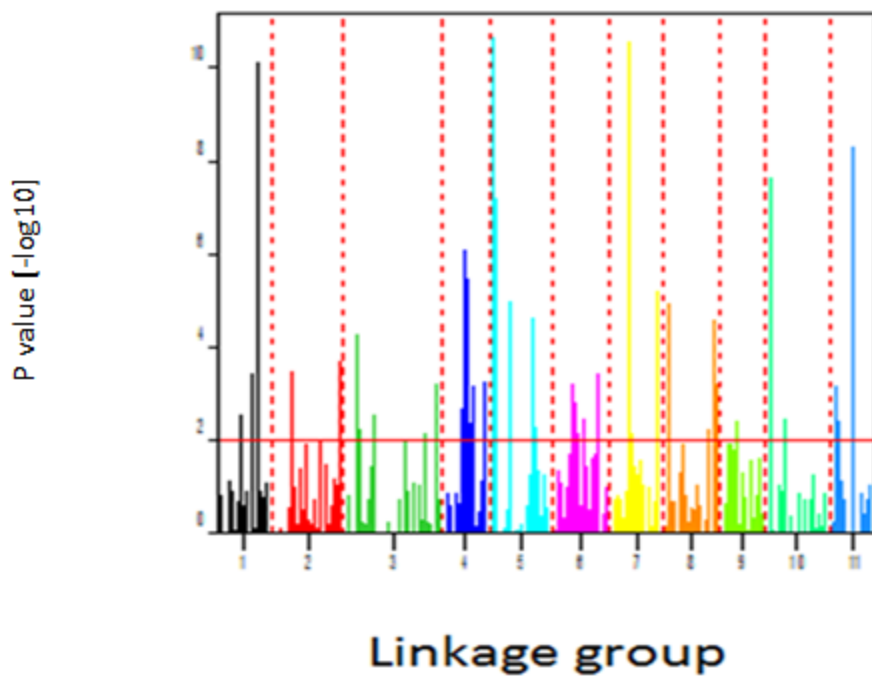


Figure 6.4: Markers' association with seed mass at different p levels

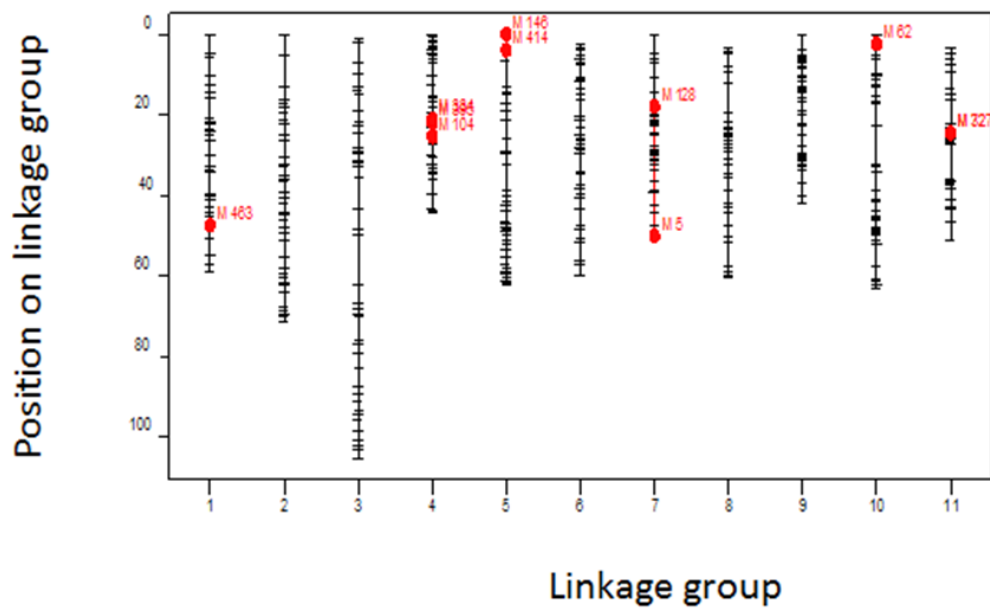


Figure 6.5: Markers' association with seed mass at different p levels

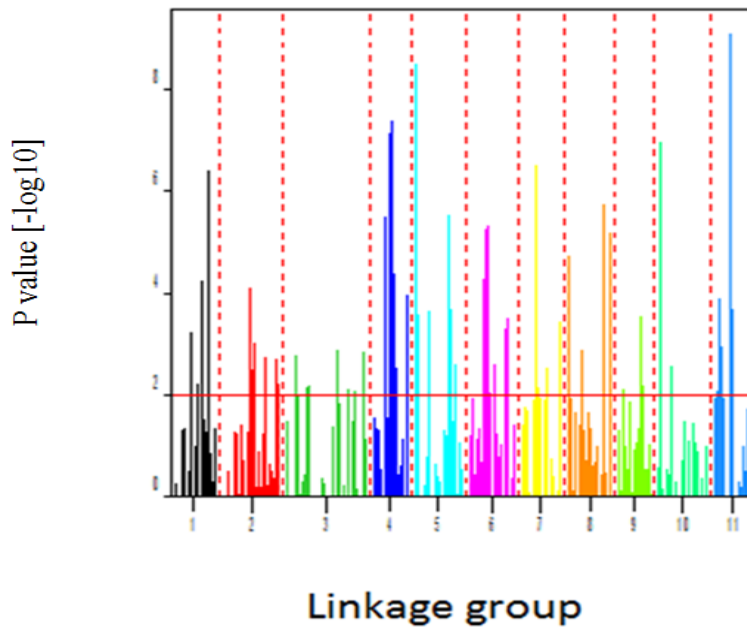


Figure 6.6: Markers' association with seed thickness at different p levels

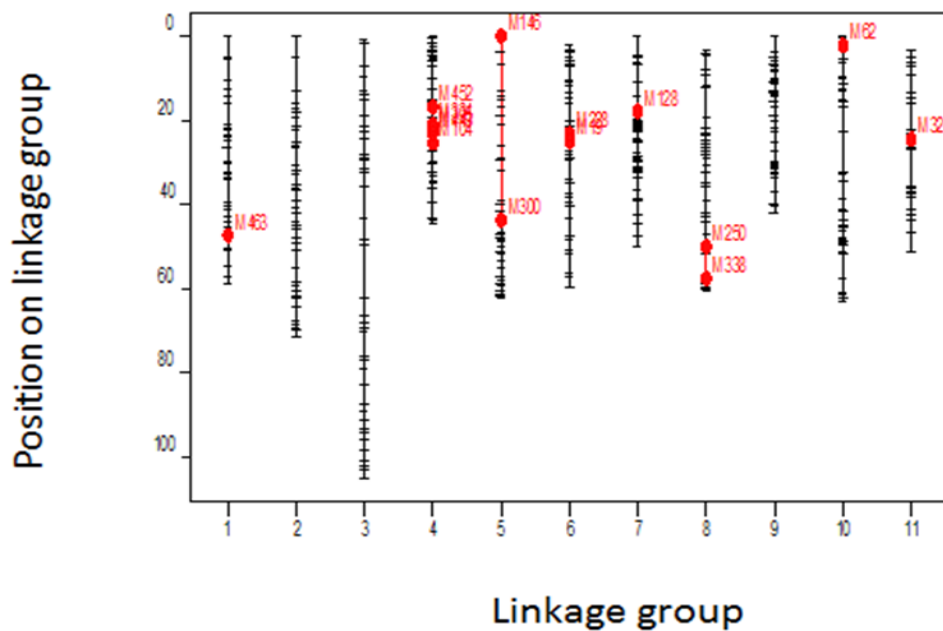


Figure 6.7: Positions of markers with significant association with seed thickness [$-\log_{10}(p) \geq 5.0$]

Table 6.2: Markers with significant association with traits [$-\log_{10}(p) \geq 5.0$]

Marker	Linkage group	Position	Trait
9175_1561	1	47.4	Mass and Thickness
8899_1022	4	16.8	Thickness
5061_428	4	21.1	Mass and Thickness
7102_965	4	21.9	Mass and Thickness
8625_1231	4	23.2	Thickness
13269_270	4	25.4	Mass and Thickness
1441_128	5	0.0	Mass and Thickness
7967_1210	5	3.9	Mass
4098_1148	5	43.7	Thickness
17588_963	6	23.3	Thickness
10279_316	6	25.0	Thickness
13872_1420	7	18.0	Mass, and Thickness
16822_160	7	50.0	Mass
2395_247	8	50.1	Thickness
5135_477	8	57.7	Thickness
11851_914	10	2.3	Mass and Thickness
12183_298	11	24.4	Mass
4882_531	11	24.6	Mass and Thickness

6.4 DISCUSSION

The insignificance of variability in seed length and width in the collection of cowpea studied might mean that these traits might not be important in seed size as compared with thickness and mass. Seed length and width have more to do with seed shape than size. The length and width of cowpea seed are related to shape such as globose, rhomboid and kidney shapes. These shapes are recognized no matter the size of the seed and among other things, it is the length and width of the seed that determine the shape. In this case, there could be small or large kidney shaped cowpea seed. Disproportionate change in length and width may alter the shape of the seed. For instance, increasing the width of a 'globose' seed without a change in the length may change the seed shape to 'crowder'. Increasing the length of globose seed may lead to ovoid if the width remains the same.

Variability in 100 seed mass and seed thickness of the genotypes studied were significant (0.006 and 0.016 F. probability respectively). Although, differences between genotypes with respect to seed width and length were not significant, their F probabilities were quite small (0.08 and 0.06 respectively). Because the differences in the seed width and length were not significant, only 100 seed mass and seed thickness were used for association mapping with the SNP markers. Notwithstanding, heritability estimate for all four seed size traits were very high: between 82% and 96%. It is however, important to note that the heritability estimate for seed width and length (82% and 85%) respectively were lower than that of 100 seed mass (96%) and seed thickness (94%). High heritability for

seed mass obtained in this study (96 %) is close to 98.9 % reported by Omoigui *et al.* (2006).

There were positive correlations between all the traits studied. The highest correlation (0.83) was between 100 seed mass and seed thickness: the traits with significant variability. The smallest correlation value of 0.51 was between seed length and width. The strong positive correlation between seed mass and the other three traits suggests that seed mass can be used to represent seed size. The thicker the seed, the heavier it is expected to be, hence the high correlation between thickness and mass.

In addition to wide variability observed in seed mass between the genotypes, seed mass was easier to measure compared with the other three. This also suggests that there could be substantial error in measuring seed width, length and thickness than weighing to obtain the 100 seed mass. Significant associated markers were observed for seed mass on all the eleven Linkage Groups (LGs) at the default level of $p < 0.05$. There was just one associated marker on LG 9. LG 2 and 7 had two associated markers each with seed mass. There were 3 significant markers each for LGs 1 and 11. Marker trait significance level was raised from the default of 2 to 5. The number of significant SNPs associated with 100 seed mass also reduced drastically. No marker on LGs 2, 3, 6, 8 and 9 were significantly associated with seed mass. LGs 1, 10 and 11 had one significant associated marker each. LG 4 had 3 significant markers while 5 and 7 had two markers each. This result does not agree with Fatokun *et al.* (1992) where cowpea seed weight genes were mapped on LGs 2 and 6. However, Fatokun *et al.* (1992) used RFLPs and not SNPs. The

current studies, however, had some agreement with Kelly *et al.* (2003) who used a combination of AFLP, RAPD and RFLP and mapped seed weight genes of cowpea on chromosomes 1, 3, 4, 6 and 7.

The trend in significance of association between markers and trait for seed thickness was similar to that of 100 seed mass. The two traits shared a number of significant markers. This could be an indicative that seed thickness is responsible for seed mass and consequently seed size in cowpea. At $[-\log_{10}(p) \geq 5.0]$, there were 18 significant SNPs for the two traits. Seed mass had only 3 significant markers that were not significantly associated with seed thickness. Seed thickness on the other hand had 7 significant markers that were not significant with seed mass. The significant SNPs shared with seed mass and seed thickness could be studied further as they might be the most important seed size markers in cowpea. In this regard, the single markers with significant association on chromosome 1, 10 and 11 are strongly suggested. Park *et al.* (2000) also observed significant association between some seed thickness and mass QTLs in common bean.

A key issue in the study of heredity and variations at the molecular level is the detection of associations between DNA sequence variation and the heritable phenotypes (Gupta *et al.*, 2001). “SNPs are often linked to genes” (Acquaah, 2007), suggesting the association of these ones with seed size genes. The association between SNP markers discovered in this experiment could be used in marker assisted breeding of large seeded cowpeas which is of economic value in West Africa.

6.5 CONCLUSION

Results of the experiment showed seed mass and seed thickness as the major determinants of seed size in cowpea. Seed mass and thickness were significantly different within the population studied. It was also observed that these two traits were highly correlated (0.83). Seed width and length could be more of shape determinants than size. There were significant marker – trait association for both seed mass and thickness for 8 SNPs used in the experiment at $[-\log_{10}(p) \geq 5.0]$. The markers with significant association with seed size are distributed throughout the cowpea genome from LG 1 to 11 with the exception of 2, 3 and 9. It would be important to estimate the contribution of each of these loci to seed size differences. The wide distribution of the significant loci among other things confirmed quantitative nature of seed size.

CHAPTER SEVEN

7.0 GENETIC CONTROL OF SEED SIZE IN COWPEA

7.1 INTRODUCTION

Cowpea was harvested on 1,126,786.1 hectares of land worldwide in 2013 out of which 98% was from Africa. The total grain from Africa in the year (2013) was about 6 million tonnes corresponding to 95% of the world production (FAO, 2014). Nigeria, Niger and Burkina Faso in West Africa produce more than 70% of the world cowpea. This makes cowpea a West African crop in terms of cultivation, trade and consumption.

Variability in seed size and seed yield per plant exist for cowpea. The average grain yield of cowpea in farmers' field in West Africa was reported as 492kg/ha (Bourkar *et al.*, 2013). Grain yield between 200kg/ha and 2,183kg/ha for different genotypes of cowpea were reported by Kiari *et al.* (2011). Actual grain yield of over 2,500kg/ha had however, been reported from research fields in the region (Singh *et al.*, 2003) with estimated potential over 3,000kg/ha (Agyeman *et al.*, 2014). The gap between the actual and potential yield can be narrowed by practicing good agronomic practices on the farmers' field. The seed yield of cowpea can be improved by breeding for increased seed size and the number of seeds per plant (Lopes *et al.*, 2003) which would also contribute towards the narrowing of the yield gap.

Cowpea seed size which is an important component of seed yield and is also an essential market trait as consumers in Ghana tend to prefer larger seeds to smaller ones

(Langyintuo *et al.*, 2003). A survey in some markets in Ghana has revealed that the varieties in cultivation in the country are generally smaller seeded than imported ones. It will be imperative to breed larger seeded cowpea; however, the mode of inheritance of the trait must first be understood before setting it as a breeding goal.

Generation mean analysis is used to estimate genetic effect of quantitative traits on crops. The method is used to estimate the contribution of additive and dominance genes and their interactions to the inheritance of the trait in question (Zdravkovic *et al.*, 2011). The method had been used to obtain reliable estimates for different traits on different crops such as days to maturity in sesame (Sharmila *et al.*, 2007), yield and its related traits in lentil (Khodambash *et al.*, 2012) and drought resistance in common bean (Hinkossa *et al.*, 2013). The procedure of generation mean analysis had been used to report genetic control of a number of traits on cowpea. Lopes *et al.* (2003) reported the genetic control of seed size in cowpea. While Tchiagam *et al.* (2011b) used the method for the control of sucrose in cowpea; Ishiyaku *et al.* (2005) used it for time to flowering in the crop.

Inheritance of seed size of cowpea has received research attention by some plant breeders in the past. Drabo *et al.* (1984) stated in their literature review that contradictory reports had been given on inheritance of seed size in cowpea since 1950. In their conclusion, Drabo *et al.* (1984) stated that cowpea seed weight (size) is governed by eight genes acting mainly additively with small size partially dominant over large. Lopes *et al.* (2003) reported that five genes control the inheritance of seed size in cowpea with high narrow sense heritability. Additive gene effect is desirable as progress can be made through

selection. Yilwa (2012) on the other hand reported large seed to be dominant over small seed, continuing the contradiction and therefore warranting the current study. The objective of this study was to determine the genetic control of seed size in cowpea.

7.2 MATERIALS AND METHODS

7.2.1 Plant materials

The cowpea genotypes, CB27 and Gh3710 were used for the experiment. CB27 which was obtained from the University of California Riverside was used as the large seeded parent while Gh3710 obtained from the Plant Genetic Resources Research Institute of the Council for Scientific and Industrial Research (CSIR-PGRI), Bunso, Ghana, was the small seeded parent. Both CB27 and Gh3710 flowered within the same number of days after planting (32days). The parents were crossed and subsequently the generations, F₁, F₂, BC₁, BC₂ developed and then together with the parents used for generation mean analysis for seed size.

7.2.2 Study site

The experiment was conducted at Ohawu [Latitude = 6°03'50.74812''N; Longitude = 0°54'49.85784''E (Fig. 3.1)] in the coastal savanna zone of Ghana from June to August, 2013. The soil of the area is sandy loam. The area is characterized with low annual rainfall and had only 47.6mm of rainfall from June to August, 2013, during which the experiment was conducted.

7.2.3 Experimental design and field establishment

The experiment was conducted in the field under rain-fed condition. The layout was randomized complete block design with 10 replications for the parents and F₁ populations while the backcrosses and F₂ were replicated 30 and 60 times respectively. Each experimental unit consisted of three plants from which the middle plant was used for data collection. The spacing between rows was 100 cm and the within the row, 50 cm, while between block spacing was 200 cm. One hundred seed weight was weighed with digital scale in grams per plant. Seed thickness of ten seeds per plant was measured with a pair of calipers.

7.2.3 Data analysis

Data on seed thickness (mm) and 100 seed weight (g) was analyzed with GenStat edition 15 (VSN international, 2012). Means of the various generations were used to draw a box-plot. The means and variances obtained were used to estimate genetic parameter such as effective factors and narrow sense heritability. SAS version 9.2 was used to estimate genetic control of seed thickness and 100 seed weight.

7.2.3.1 Heritability of 100 seed weight in the broad sense

Broad sense heritability (H_b^2), of 100 seed weight of cowpea was estimated by the formula of Ito and Brewbaker (Zewdie and Bosland, 2003). $H_b^2 = (VF_2 - VE) / VF_2$:

Where;

- H_b^2 = Broad sense heritability
- VE = Error variance = $1/4 (VP_1 + VP_2 + 2VF_1)$
- VF_2 = Variance of F_2 family
- VP_1 = Variance of parent 1
- VP_2 = Variance of parent 2
- VF_1 Variance of F_1 family

7.2.3.2 Narrow sense heritability

The method of Warner (1952) was used to estimate narrow-sense heritability (h^2_{ns}): $h^2_{ns} = [2VF_2 - (VBCP_1 + VBCP_2)] / VF_2$, where: VF_2 , $VBCP_1$, and $VBCP_2$ are the variances of the F_2 , CB27/ F_1 and Gh3710/ F_1 respectively.

7.2.3.3 Number of effective factors (genes)

The number of effective factors controlling seed weight was estimated using the method of Burton (1951) as:

- $k = [0.25(0.75 - h + h^2) D^2] / (VF_2 - VF_1)$

Where:

- $D = P_1 - P_2$
- $h = (F_1 - P_2) / P_1 - P_2$
- $k =$ minimum number of effective factors
- $VF_2 =$ Variance of F_2 population
- $VF_1 =$ Variance of F_1 population
- $P_1 =$ mean of parent 1
- $P_2 =$ mean of parent 2

7.2.3.4 The degree of dominance

The degree of dominance (d) was calculated as the deviation from the mid-parent value towards one of them (Figure 7.1).

$$d = F_1 - \frac{1}{2}(P_1 + P_2)$$

Where:

$F_1 =$ mean of F_1 ,

$P_1 =$ mean of P_1 and

$P_2 =$ mean of P_2

Degree of dominance = d/a

7.2.3.5 Gene action controlling 100 seed weight

Generation mean analysis was used to estimate genetic control of seed weight in cowpea

The GMA model is stated below:

$$Y = m + \alpha a + \beta d + \alpha^2 aa + 2\alpha\beta ad + \beta^2 dd$$

- α and β are the coefficients for a and d, respectively (Table 7.2)
- Y = the observed mean
- m = mean = mean of the F₂
- a = pooled additive effects
- d = pooled dominance effects
- aa = additive x additive gene interaction effects
- ad = additive x dominance gene interaction effects
- dd = dominance x dominance gene interaction effects

The mode of inheritance of 100 seed weight in cowpea was estimated by generation mean analysis with six generations (P₁, P₂, F₁, F₂, BCP₁, and BCP₂) of CB27 and Gh3710.

Following significant differences in 100 seed weight of the various generations, their means and variances were used to perform generation mean.

7.3 RESULTS

7.3.1 Seed weight and thickness of various generations

Analysis of variance showed significant differences between 100 seed weight and seed thickness of the various generations of CB27 and Gh3710 used for the experiment.

Boxplot of the mean seed weight is shown in Figure 7.1 and that of seed thickness in Figure 7.2.

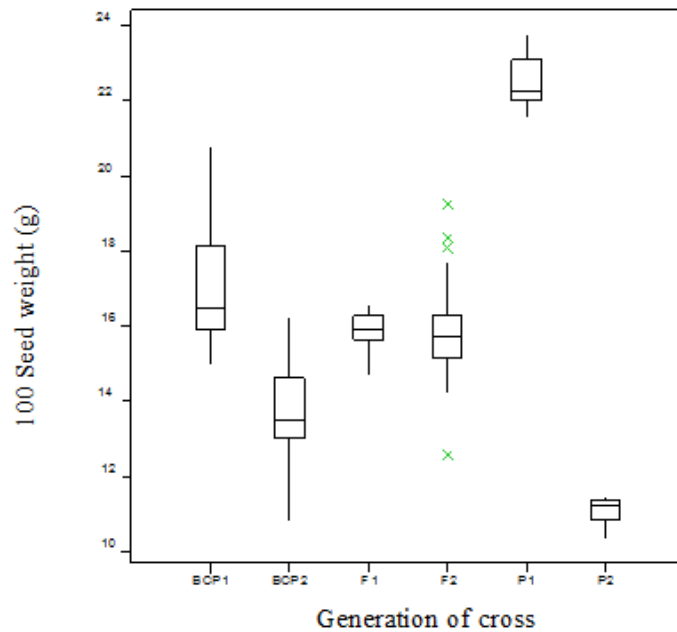


Figure 7.1: Boxplot of 100 seed weight (g) of 6 generations of cowpea developed from CB27/Gh3710

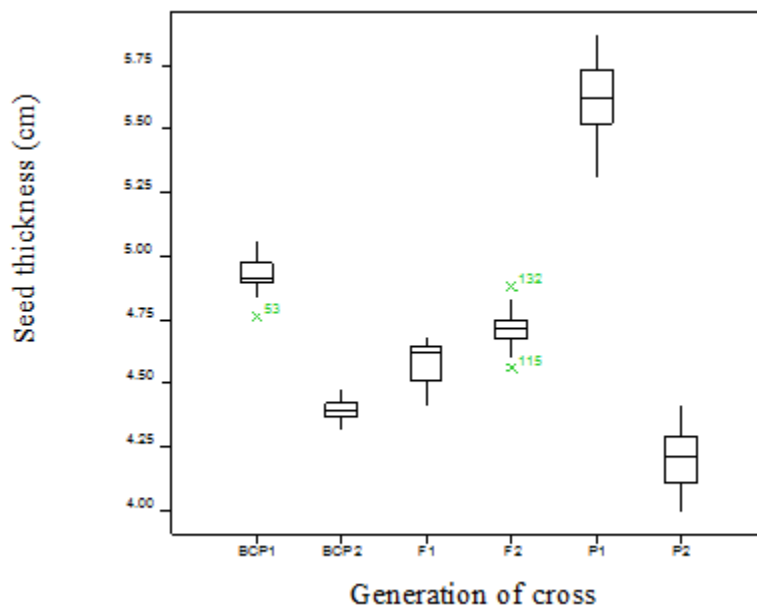


Figure 7.2: Boxplot of seed thickness (cm) of 6 generations of cowpea developed from CB27/ Gh3710

The means and standard errors (SE) of 100 seed weight of the various generations are shown in Table 7.1. Matrix pairing of the generations is shown in Table 7.1 and the generations without significant differences in 100 seed weight indicated with “ α ”. There were only two non-significant pairs of generations; F_1 against F_2 and BCP_1 .

Table 7.1: Average of 100 seed weight (g) and standard errors for various cowpea generations with a matrix showing LSDs (5%)

Generation	Mean \pm SE	LSD					
1 (BCP_1)	16.79 \pm 0.23	-					
2 (BCP_2)	13.52 \pm 0.23	0.63	-				
3 (F_1)	15.92 \pm 0.42	0.97 α	0.97	-			
4 (F_2)	15.98 \pm 0.19	0.63	0.63	0.97 α	-		
5 [CB27 (P_1)]	22.58 \pm 0.42	0.97	0.97	1.1	0.97	-	
6 [Gh3710 (P_2)]	11.20 \pm 0.42	0.97	0.97	1.1	0.97	1.1	-
	Generation	1	2	3	4	5	6

Note: the number of observations for P_1 , P_2 and $F_1 = 10$; BCP_1 and $BCP_2 = 30$ and $F_2 = 60$. Non-significant pairs indicated with asterisks (α)

7.3.2 Vital genetic estimates of 100 seed weight of cowpea

Table 7.2 contains estimates of broad and narrow sense heritability, number of effective factors and the degree of dominance in the control of 100 seed mass of cowpea from the experiment.

Table 7.2: Vital genetic estimates of 100 seed weight of cowpea

Estimate	Result
Broad sense heritability	84%.
Narrow sense heritability	35%.
Number of effective factors (genes)	8
The degree of dominance	16%

The degree of dominance which is the deviation of the F_1 from the mid parent towards one of the parents is represented in Figure 3 based on Falconer and Mackay (1996)

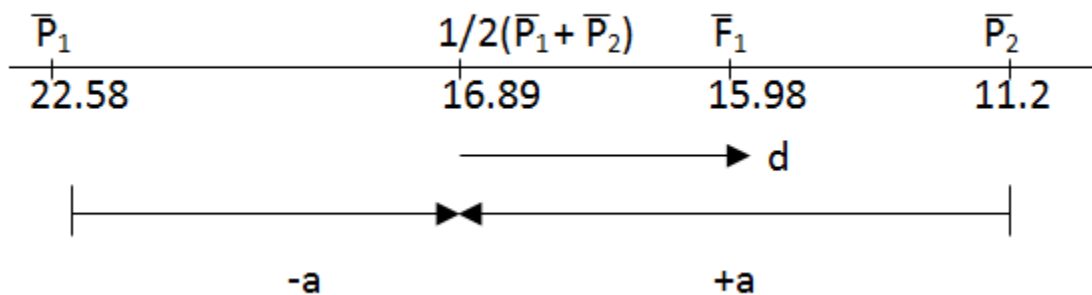


Figure 7.3: Diagrammatic representation of the genotypic values based on Falconer and Mackay (1996)

7.3.3 Gene action controlling 100 seed weight

Following significant differences in 100 seed weight of the various generations of CB27 and Gh3710, their means and variances were used to perform generation means analysis. Vital results from regression analysis from SAS are presented in Table 7.3. The generation mean analysis indicated that there were additive and dominance gene effects and their interactions in the inheritance of 100 seed weight of cowpea.

Table 7.3: Analysis of variance and parameter estimates for genetic control of 100 seed weight of cowpea.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	18.747	1.722	10.89	<.0001
Rep	1	0.008	0.012	0.65	0.5151
a	1	-5.678	0.217	-26.21	<.0001
d	1	-9.706	3.469	-2.80	0.0058
aa	1	-1.99	1.682	-1.18	0.2387
ad	1	4.775	0.674	7.09	<.0001
dd	1	6.61	1.917	3.45	0.0007

Note: Generations of CB27 and Gh3710 were used. [a] additive; [d] dominance; [aa] additive-additive; [ad] additive-dominance; [dd] dominance-dominance gene effects.

Table 7.4 shows the results of generation mean analysis for seed thickness of cowpea. There were significant additive and dominance gene effects and their interactions in the inheritance of seed thickness in cowpea.

Table 7.4: Analysis of variance and parameter estimates for genetic control of seed thickness of cowpea.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	5.09313	0.08190	62.19	<.0001
Rep	1	0.00079576	0.00064608	1.23	0.2201
a	1	-0.68200	0.01539	-44.30	<.0001
d	1	-1.13094	0.18774	-6.02	<.0001
aa	1	-0.16527	0.07871	-2.10	0.0375
ad	1	0.30374	0.05576	5.45	<.0001
dd	1	0.63661	0.11994	5.31	<.0001

Note: Generations of CB27 and Gh3710 were used. [a] additive; [d] dominance; [aa] additive-additive; [ad] additive-dominance; [dd] dominance-dominance gene effects

7.4 DISCUSSION

Significant differences were observed between the various generations of plants from the cross of CB27 and Gh3710 in terms of 100 seed weight and seed thickness. The results of both 100 seed weight and seed thickness followed the same trend (Figure 7.1 and 7.2). Therefore, only 100 seed weight was presented and used for the discussion to avoid repetitions. The means 100 seed weight of F₁, F₂ and the backcrosses fell between the two parental means of 11.2g and 22.58g. This result is similar to that of Lopez *et al.*

(2003), although, comparatively each of their generations had smaller weight with their small and large seeds having 100 seed weight of 4.82g and 15.14g respectively. Lucas *et al.* (2013a) on the other hand used slightly heavier genotypes (11.6g and 26.41g) as the parents in QTL studies for seed size compared with the current experiment.

The broad sense heritability estimated for 100 seed weight in this research was 84%. This value is very close to 83.3% reported by Damarany (1994), in Fatokun *et al.* (2002). Drabo *et al.* (1984) also reported a close figure of 85% while Manggoel *et al.* (2012) reported 86.84%. Omoigui *et al.* (2006) and Tchiagam *et al.* (2011a), however, reported broad sense heritability as high as 98.9% and 90% respectively for the trait. The association mapping with SNP markers (Chapter 6) estimated broad sense heritability for 100 seed weight as 96%. Environment can therefore, be seen as a major influence on heritability estimate for seed size in cowpea.

The narrow sense heritability estimated was 35%. The low narrow sense heritability observed may have been caused by the significant additive dominance and dominance dominance epistatic effects. However, such low narrow sense heritability has been reported. Adeyanju *et al.* (2012) for example reported 15 and 25% from two different crosses. Relatively higher narrow sense heritability for 100 seed weight of 67% was reported by Tchiagam *et al.* (2011b). These reports suggest that breeding methodology to improve seed size in cowpea may be influenced by the genotype and environment in which the breeding is to take place.

The results from this experiment are consistent with that of Drabo *et al.* (1984) that small seed size is partially dominant over larger seed. Partial dominance genetic variance was evident in that the F_1 of CB27/Gh3710 was more similar to Gh3710 than CB27 in terms of seed size. This means that small seed size is partially dominant over large seed. Dominance gene effect was observed to be important in seed size inheritance in lentil as well (Khodambashi *et al.*, 2012). However, the finding in this experiment was contrary to the observation of Lopez *et al.* (2003) and Yilwa (2012) who stated that large seed in cowpea is rather partially dominant over the small. These contradictory findings may be due to the genotypes used in the various studies. It was also observed that seeds from none of the P_2 , F_1 , F_2 , BCP_1 or BCP_2 generations was equal to the weight of seeds from P_1 (CB27). Similarly, Fatokun *et al.* (1992) had no F_2 seeds with a similar seed weight as the heavy parent used in their experiment.

The number of gene pairs estimated to control seed weight was eight, similar to observations made by Drabo *et al.* (1984) in spite of the different genotypes used in the separate trials. Lopez *et al.* (2003), however, estimated five factors.

Additive and non-additive gene effects and their interactions were shown to significantly control seed size with cowpea in the exception of additive – additive ([aa]) in this current experiment (Table 7.2). However, the magnitude of the significant fixable gene action [a] (Hakizimana *et al.*, 2004) has smaller magnitude compared with the non-fixable [d], [ad] and [dd]. Gene interaction observed was “duplicate” because [d] was negative while [dd] was positive (Khodambashi *et al.*, 2012). The negative [d] also shows that small seed is dominant over large seed. The presence of two or more of the eight factors

estimated to control seed size in this experiment would act in duplicate manner (Suzuki *et al.*, 1989) to affect the trait. Although, [aa] gene effect was not significant, the associated negative sign indicates the dispersion of seed weight genes in both parents (CB27 and Gh3710).

Significant [a] gene effect is an assurance that gains can be made with selection in the long term (Acquah, 2007) and for seed size in cowpea. Seeds of the backcross generations in this experiment are indication of the practicality of this principle by being significantly larger or smaller than the F_1 s. The backcross to the larger seeded parent (CB27) resulted in larger seeds while the F_1 crossed to the small seeded parent (Gh3710) were also smaller than the F_1 .

Both fixable and non-fixable gene effects are important and therefore make breeding for cowpea seed size complex. The presence of significant [a] gene effect would make selection from early generation effective. The implication is that selection for seed size for cowpea should commence from the segregation population through advanced levels where varieties are at evaluation stage.

7.5 CONCLUSION

It was observed in the experiment that eight genes control seed weight in cowpea using the genotypes CB27 and Gh3710 as parents in generation mean analysis. Small seed size appeared to be partially dominant over large seed. Additive, dominant, additive – dominant and dominant – dominant gene actions were found to be significant. The

inheritance of seed weight is complex and thus suggests that a combination of different approaches should be used to improve the trait against the use of a single approach. Selection from early generation to improve seed size in cowpea is feasible because of the significant additive gene effect. However, the low narrow sense heritability could make selection less effective. Selection and backcrossing to the larger seeded parent is important to fix alleles for large seed size.

CHAPTER EIGHT

8.0 INHERITANCE OF SEED COAT COLOUR IN COWPEA

8.1 INTRODUCTION

The colour of the seed of cowpea and that of other plants is important to the plant and human beings who use the seed for various purposes. Seed viability and seedling vigor have been found to vary based on seed coat colour as reported in clover (Atis *et al.*, 2011). In clover, red seed coat is preferred for good seed quality and rapid growth of seedlings. In cowpea electrical conductivity and water absorption are reported to be higher in white seed coat types than coloured types (Peksen *et al.*, 2004) leading to differences in response to germination stimuli. Nutrient content which is of interest to man has also been reported to vary according to the seed coat colour of some legumes. The brown seed coat cowpea was found to have higher protein than the white type (Alayande *et al.*, 2012; Petchiammal and Hopper, 2014). In horse gram and common bean, the black seed coat types were reported to have higher protein (Petchiammal and Hopper, 2014).

Seed coat pigmentation is mainly due to the presence of pigments such as anthocyanins in the plant. Some anthocyanin genes are known to control the pigmentation of multiple parts of plants (Chandler *et al.*, 1989; Chalker-Scott, 1999) while some such as *pac1* gene is known to control colour of only the seed coat in maize (Selinger and Chandler, 1999). Anthocyanins are known to be important to plants in response to abiotic stresses such as drought, cold temperatures and excessive radiation (Chalker-Scott, 1999). Anthocyanins

have also been implicated in salt tolerance in *Arabidopsis thaliana* (Oh *et al.*, 2011). High levels of anthocyanins have also been reported to help the plant in biotic stress tolerance (Makoi *et al.*, 2010; Sharma *et al.*, 2011). Cowpea seedlings raised from coloured seeds were observed to have less incidence of insect pests' attack compared to those from white seeds (Makoi *et al.*, 2010). It was proposed by Nakabayashi *et al.* (2014) to breed crops for higher levels of flavonoids to enhance their tolerance to biotic and abiotic stresses. Besides the roles of anthocyanin to the plant, it controls pigmentation of the seed coat which consequently has influence on consumer preference.

The preferred seed coat colour types differ for different crops and for different consumers. Seed coat colour is reported to be important to the consumers of chickpea in India (Agbola *et al.*, 2002). This was confirmed by Shiyani *et al.* (2005). In Nigeria groundnut with red seed coat is preferred (Ndjeunga *et al.*, 2010). The seed coat colour of choice for cowpea consumers in Ghana and some other West African countries is the white type (Langyintuo *et al.*, 2004).

Seed coat colour has mainly been reported as a qualitative trait in cowpea (Yilwa 2012), although a few other reports have reported contrary observations (Oluwatosin 2000). The objective of the research was to estimate the number of genes that control seed coat colour in cowpea. This would help in designing breeding programmes to produce consumer preferred seed coat colour type cowpea.

8.2 MATERIALS AND METHODS

8.2.1 Study site

Crosses were made at the West Africa Centre for Crop Improvement (WACCI) farm, University of Ghana in 2012. F₁ and the F₂ were planted at the experimental field of the Plant Genetic Resources Research Institute of the Council for Scientific and Industrial Research (CSIR-PGRRI), Bunso, Ghana.

8.2.2 Plant Materials

Six bi-parental crosses were made between seven different true breeding cowpea genotypes namely; UCR779, CB27, Nhyira, Gbode, Bambey21, Tona and Gh3710 (Table 8.1 and Figure 8.1). The F₁s were grown in one of the research fields of CSIR – Plant Genetic Resources Research Institute, Bunso, Ghana, in December, 2012. The F₁s harvested were grown in February, 2013 in Bunso and allowed to self. Dry pods of F₂ plants were harvested on a plant by plant basis and the seed coat colour assessed.

Table 8.1: Cowpea genotypes used and parents for seed coat colour studies

Genotype	Source	Seed coat colour	Eye colour
UCR779	University of California, Riverside	Brown	Absent
Nhyira	Crop Research Insittute, Ghana	Cream	Brown
Gbode	Seed grower	White	Black
Bambey21	University California, Riverside	White	Absent
Tona	Crop Research Insittute, Ghana	Brown	Absent
Gh3710	Plant Genetic Resources Research Institute, Ghana	Dark mottle	Absent
CB27	University of California, Riverside	White	Black



Figure 8.1: Seeds of the cowpea genotypes used in hybridization

8.2.2 Cross formation

Emasculation was done on matured but closed flowers in the evening. Pollination was done the following morning and labeled. Appropriate markers were used to identify true hybrids depending on the traits in which the parents were polymorphic. For example for the cross of Bambey21 and UCR779, the former was made the female parent and the latter the male. The F_1 plant of this cross had pigmented parts including stem and peduncle signifying success. The seed coat colour of the F_1 also was black seeded. Unsuccessful cross would have resulted in non-pigmented plant with seeds with white seed coat.

8.2.3 Data collection and analysis

Ripe pods from each F_2 plant from each population were harvested separately. Pods were sun dried and seeds removed after drying. Seeds were grouped according to seed coat colour differences. Chi-square goodness of fit test was used to test various genetic ratios. It was not possible to group some of the seeds from most populations because their seed coat colour variations were almost continuous. Photographs of the parent phenotypes, their F_1 s and F_2 s for such populations were taken for discussion.

8.3 RESULTS

Results of the seed coat colour segregations for the various crosses are shown in Tables 8.2 to 8.4 and Figures 8.2 to 8.7.

Pigmentation of F_1 seeds of the cross of Bambey21 and Gbode resembles that of Gbode indicating the dominance of the latter over the former (Table 8.2). The F_2 progenies although, had some variability within groups, can be separated into the two parental types in terms of coat colour. The number of plants exhibiting Gbode seed coat colour type were 138 to 39 Bambey21 type fitting 3: 1 ratio, suggesting monogenic control of the trait (Table 8.2).

Table 8.2: Expected and observed seed coat colour of cowpea hybrids

Cross	F ₁ Seed Testa Pigmentation	
	Expected	Observed
Bambey21 x Gbode	Black eye (Gbode)	Black eye
CB27 x UCR779	Solid black	Solid black
Bambey21 x Tona	Solid brown	Solid black
Bambey21 x UCR779	Solid brown	Solid black
CB27 x Gh3710	Solid black	Solid black
Bambey21 x Nhyira	Solid brown	Cream with black eye

Table 8.3: Segregation pattern (3:1 ratio) of F₂ plants for seed coat colour for the cross of Bambey21/Gbode

Class	df	Observed (O)	Expected (E)	$(O - E)^2$	$(O - E)^2 / E$	P value (0.05)
Gbode type		138	133	25	0.188	
Bambey21 type		39	44	25	0.568	
X^2	1				0.756	3.84

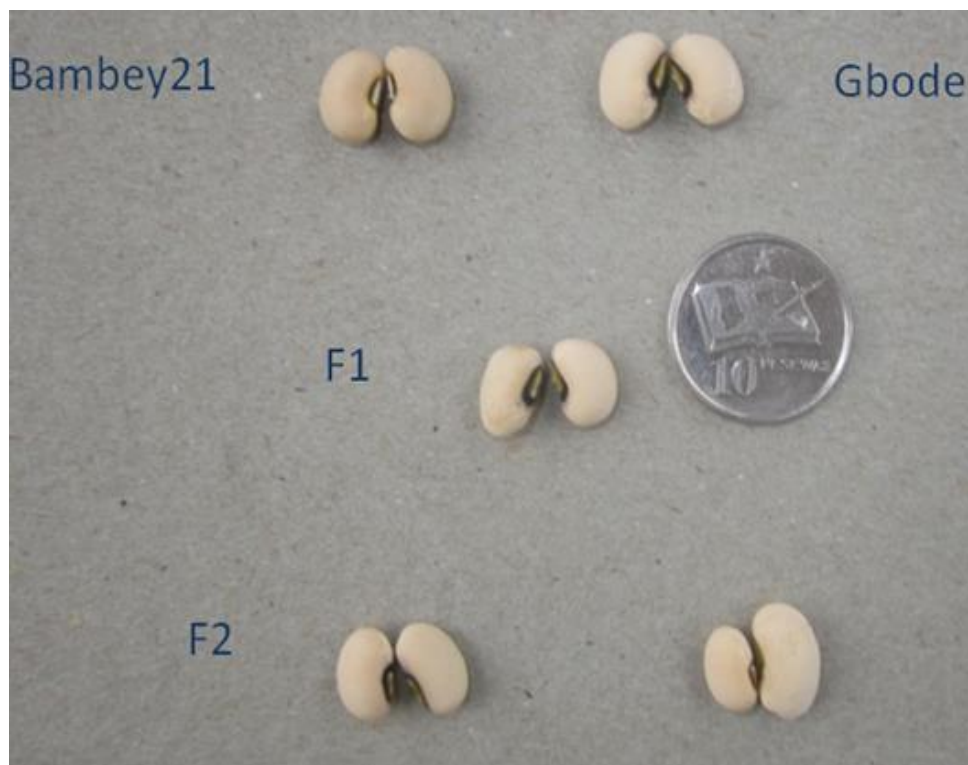


Figure 8.2: Seeds of Bambey21 and Gbode with their F₁ and F₂s



Figure 8.3: Seeds of Bambey21 and Nhyira with their F₁ and F₂s

Nhyira has a small brown eye while Bambey21 has none. The F_1 of the cross between these genotypes had white seed coat with small black eye. The F_2 had eye and eyeless seeds. The eyes were either black or brown. Some of the black eyes were larger than others, however, there was no clear distinction between the small and the larger types in some cases. There were 16 brown eye, 45 black eyes and 18 eyeless seeded plants fitting 1:2:1 ratio. The chi-square test for this is presented in Table 8.4.

Table 8.4: Segregation pattern (1:2:1 ratio) of F_2 plants for seed coat colour for the cross of Bambey21/Nhyira

Class	df	Observed (O)	Expected (E)	$(O - E)^2$	$(O - E)^2 / E$	P value (0.05)
No eye	18	19.75	3.06	0.155		
Black eye	45	39.5	30.25	0.766		
Brown eye	16	19.75	14.063	0.712		
X^2	2			1.633	5.99	



Figure 8.4: Seeds of UCR779 and CB27 with their F₁ and F₂s

The seed coat of the F₁ between brown seeded UCR779 and CB27 (white with black eye) was black. The F₂ seeds had varied coat pigment from black to white. Some of the seeds had mottle coat. There were some solid seeds in that they did not have distinct eye colour. The eye types varied from normal eye to holstein type. Ten seed coat colour groups were identified.



Figure 8.5: Seeds of Bambey21 and UCR779 with their F₁ and F₂s

Bambey21 and UCR779 produced black seeded F₁ plants which segregated into seeds with black to white. There were both eye and eyeless seeds. A number of plants produced seeds with seed coat colour in-between the three main colours: black, brown and white. It was difficult to classify some of the seeds into the black, brown or white as they were not clearly distinct from each other. At least nine groups were identified in an attempt to group the plants based on seed coat colour.



Figure 8.6: Seeds of Bambey21 and Tona with their F₁ and F₂s

The seed produced by F₁ plants of the cross of Bambey21 (solid white) and Tona (solid brown) were solid black. F₂ plants produced black, brown and white seeds with a lot of intermediates. Some of the seeds were solid while some had eyes. The seeds could be classified into ten different groups according to seed coat colour.

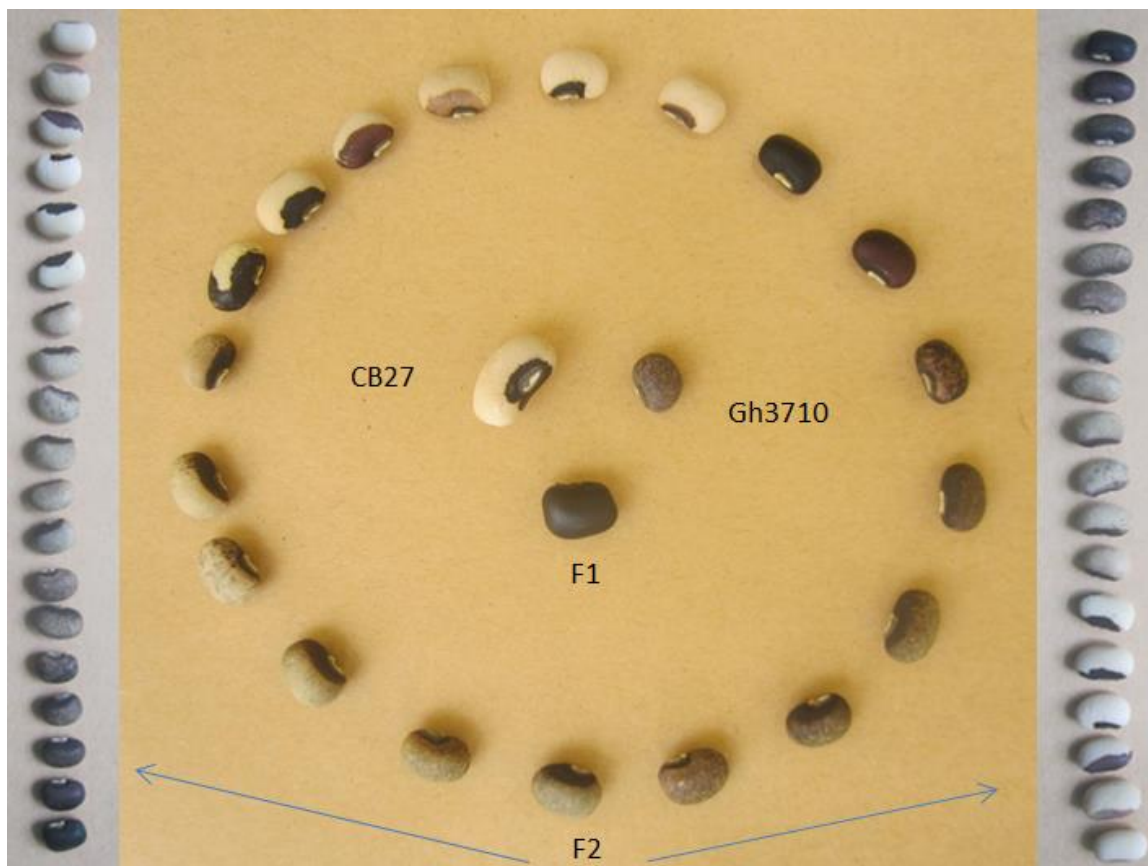


Figure 8.7: Seeds of CB27 and Gh3710 with their F₁ and F₂s

The hybrid of black eye CB27 and dark mottle GH3710 bore black seeds. The F₂ seed coat colours were much varied from black to white and very difficult to classify. Most of the F₂ seeds had eye, however, eye could not be recognized on some.

8.4 DISCUSSION

Whilst individuals of two of the segregating populations could be grouped into definite seed coat colour groups, it could not be done for the other four. This suggests that no single cross may be able to give full insight into seed coat colour inheritance in cowpea, which also indicates the possibility of many genes controlling the trait.

The F₂ of Bambey21 (all white) and Gbode (white with black eye) from F₁ that resembles Gbode could be grouped into two based on seed coat colour. This segregation agrees with the observation made from the cross of Bambey21 and CB27 (Fatokun *et al.*, 2002). The Chi-square calculated was 0.76 for the 3:1 ratio indicating monogenic control. This result also agrees with Arshad *et al.* (2005) and Mustapha (2009) for blackgram and cowpea seed coat colour respectively. Similar results were reported by Yilwa (2012).

The F₂ progenies of Bambey21 and Nhyira had three groups of seeds based on coat colour; black eye, brown eye and solid white. It was interesting to notice black eye seeds at the F₁ even though neither the male nor female parent had black seed coat. Production of black seed coat by white and brown parents suggests heterosis of seed coat colour and will be discussed later. This cross could also be likened to Bambey21/Gbode, however in this case the heterozygous produced a distinct coat colour resulting in three different classes of coat colour at the F₂.

It became more difficulty and ambiguous in grouping plants according to seed coat colour as one moved progressively from Figure 8.1 to 8.6. The cross between UCR779

(solid brown seed coat) and CB27 (white with black eye) produced F₁ with solid black seed coat. Black is reported to be dominant over brown (Mustapha, 2008). The black at the eye region of CB27 is dominant over brown of UCR779 and because the latter lacks eye gene, solid black F₁ was produced. Different seed coat colours and patterns were observed in the F₂. At least ten seed coat colour groups were identified in the F₂, indicating that many genes might be involved similar to observations made in maize (Chandler *et al.*, 1989).

Result of the cross and the segregating population of Bambey21/Tona were unexpectedly very complex. Bambey21 and Tona are all white and all brown respectively. However, the F₁ of this cross was solid black seeded. If dominance of black seed coat of cowpea over brown and other colours were universal, then white genotype crossed with brown could not produce black F₁. It is suggested that the result of this cross was due to epistasis or that the colours behaved in quantitative manner in which heterosis was observed from the cross with respect to seed coat colour. The cross of Bambey21/Nhyira suggested that Bambey21 has eye gene but not *expressed because of lack of colour. The F₂ of Bambey21/Tona therefore had some eyed genotypes because of the eye gene from Bambey21. There was gradual fading of colour from solid black to white in the segregating population. The varying seed coat colour might correlate with anthocyanin types and quantities.

Bambey21/UCR779 came next to Bambey21/Tona in terms of seed coat colour inheritance as their F₁s were black and the segregating population had a number of

groups with seed coat colour varying from black to white. Here also the expression of black at F_1 did not suggest its dominance over white and brown because if that were true, black could not have hidden under the white or brown of the parents. Epistasis may explain the expression of black at this F_1 phenotype better than simple dominance and recessive inheritance. Yilwa (2012) reported four different crosses of white and brown in which she observed brown at the F_1 and white and brown at the F_2 , with ratios suggesting two genes controlling the trait. Our observation is contrary to this as more than two coat colour types were observed in our crosses of brown to white. In *Capsicum annum*, it is known that environmental factors greatly influence anthocyanin synthesis and therefore, pigmentation of various plant parts including the seed (Lightbourn *et al.*, 2007).

The most complex segregation for seed coat colour was observed from the cross of CB27 X Gh3710. The Gh3710 is dark mottling. F_1 of CB27/Gh3710 was black and could be attributed to the black eye of the CB27. The F_2 showed gradation from black to white with much difficulty in grouping them. This clearly showed quantitative nature of seed coat colour inheritance in cowpea.

Observations indicate continuous variation in the seed coat colour rather than classes. Attempt to identify nine classes in the segregation population of the cross of Bambey21/UCR779 means that at least four gene loci were involved in the seed coat colour inheritance. However, even the classification of seeds into coat colour groups in this research was arbitrary. The F_2 of the crosses of Bambey21/Tona and CB27/Gh3710 on the other hand suggest more than four loci in each case. In this case, study of seed coat

colour as a quantitative trait may be appropriate. The segregation observed in the crosses of this experiment may not represent all the possible allele combination due to segregation distortion (Lambrides *et al.*, 2004). The use of molecular tools in the study of seed coat colour segregation may give more accurate result.

Quantitative traits are known to be affected by environmental factors. Anthocyanin which is known to determine seed coat colour and pigmentation of other plant parts has the primary role of stress tolerance in plants especially when expressed in vegetative parts. Higher anthocyanin pigmentation of vegetative parts is caused by harsh environmental conditions (Chalker-Scott, 1999). Seed coat colour pigmentation may also be influenced by environmental factors such as solar radiation.

Overlap within genotypes and phenotype which is a phenomenon of quantitative traits is very likely in the attempt to classify seed coat colour in some of the crosses being discussed. The groupings are therefore, arbitrary. This is another proof of quantitative nature of seed coat pigmentation in cowpea. If white is taken as the lowest value for pigmentation and black as the highest, the cross of Bambey21 (white) and Tona (brown) produced black offspring; an indication of heterosis and F_2 that spanned the whole spectrum of seed coat colour variability. Breeding for specific coat colour would thus be difficult as the F_2 showed much variability. Regarding seed coat colour of cowpea as qualitative trait will be misleading in breeding programmes as quantitative traits are more difficult to breed (Acquah, 2007).

8.5 CONCLUSION

Segregation with respect to seed coat colour in cowpea depends on the genotypes used. Some populations satisfy one or two genes segregation ratios while others resemble more than two genes. Use of simple Mendelian ratios may not be able to fully explain the inheritance of seed coat colour in cowpea as some seeds in segregating populations could not be assigned into groups. The use of quantitative and molecular approaches may hold the key to better explain the results.

CHAPTER NINE

9.0 SELECTION OF PROMISING COWPEA GENOTYPES THROUGH FARMER PARTICIPATION

9.1 INTRODUCTION

The ultimate goal in a participatory plant breeding is to harness farmers' knowledge and researchers' scientific approach in developing desirable plant varieties (Nkongolo *et al.*, 2008). Farmers in most cases have their own desired traits that the scientists identify through their participation (Singh *et al.*, 2003; Mukanga *et al.*, 2011). The desired traits of a particular crop may vary from one community to the other. Apart from being a sound procedure whereby both the plant breeders and farmers learn from each other participatory plant breeding leads to early adoption of a newly developed variety because of the awareness created before its release (Jumaboev *et al.*, 2013). In addition, the farmers feel as owners of the variety developed as the method is a bottom-up approach.

White coat coloured cowpea with large seed size are preferred in most parts of Ghana (Quaye *et al.*, 2011; Ansah 2012; Egbadzor *et al.*, 2013). The preference for large seed sizes is not limited to cowpea but known in other crops with rice as an example (Song *et al.*, 2007). In cowpea, many breeding objectives have not directly focused on seed size compared with such traits as biotic and abiotic stress tolerance (Hall *et al.*, 1997; Orawu *et al.*, 2012). Breeding of new cowpea varieties needs to consider other traits such as acceptable seed size and coat colour.

Participatory selection of large cream cowpea for varietal development would boost the production and utilization of the crop in Ghana and reduce its importation. Cowpea selection with farmers at Ohawu which is a noted cowpea production area will help in timely address of gene environment interaction issues and also lead to early adoption (Hall *et al.*, 1997; Acquah 2007). The plant breeders involved would bring their scientific knowledge in the selection process while farmers would also bring their expertise and consider their desired traits apart from those set up in the breeding objectives making the process a holistic one.

The objective of this study was to use researcher-farmer participatory approach to select cowpea lines toward variety development with a focus on seed size and colour. Seven cowpea genotypes with unique traits of interest were used in developing the populations from which selections were made.

9.2 MATERIALS AND METHODS

9.2.1 Plant materials, crosses and population development

Seven cowpea genotypes from different sources were chosen for the varietal development. The genotypes are listed in Table 9.1 and shown in Figure 8.1.

Bambey21 was hybridized with Tona, Nhyira, UCR779 and Gbode in a screen house at WACCI farm, University of Ghana in September, 2012. CB27 was also crossed with Gh3710 and UCR779. The F_1 s of all the crosses were advanced to F_2 at Bunso, in

December, 2012. In March, 2013, F₂ plants were grown and selection was made towards farmer participatory selection at Ohawu.

Table 9.1: Cowpea genotypes selected for varietal development

Genotype	Source	Distinctive Trait of interest
CB27	UCR	Large white seed
UCR779	UCR	Large seed, aphid resistance
Gh3710	CSIR – PGRRI	Local adapted, early maturing
Bambey21	Senegal, through UCR	White, erect
Tona	CSIR – CRI	Local variety
Nhyira	CSIR – CRI	Local variety
Gbode	Farmer, Ho	Farmer/consumer preferred

Note: CSIR – CRI; Council for Scientific and Industrial Research – Crop Research Institute; CSIR – PGRRI; Council for Scientific and Industrial Research – Plant Genetic Resources Research Institute; UCR - University of California Riverside

9.2.2 Geographic Location and Selection of farmers

Fifty cowpea farmers around Ohawu in the Ketu North District of the Volta Region were selected with the assistance of the Agricultural Extension Officers in the area. Some of the communities covered fell under two Districts namely Keta Municipal and Akatsi (Figure 3.1). The farmers who were drawn from 25 cowpea growing communities from Doveme, Dagbamatey, Xevi and Tadzewu were made up of 25 males and females each. Seven scientists and 15 other persons with different backgrounds were also involved in the selection exercise.

9.2.3 Field planting

The field for planting of the cowpea populations was at Ohawu in the Ketu North District of the Volta Region. Clearing of vegetation was done in the first week of June followed by ploughing. Planting of seeds was done on the 17th of June with a spacing of 60cm by 80cm. Hoe Weeding which was done in the third and fifth week of planting and the plants sprayed with cymethoate (insecticide) applied at 1.5L/ha. Harvesting was done when pods were dry.

9.2.4 Cowpea Lines Selection with farmers

Selections were made from six different populations. Line selections were made from F₃ population of CB27/Gh3710. Selection from the other five F₃ populations, four of which had Bambey21 as the female parent and the males as, Gbode, Nhyira, Tona and UCR779 were based on single plants. The fifth population was UCR779/CB27. Individual farmers indicated their three best lines (in the case of CB27/Gh3710) or the three best individual plants (from the five other populations) 58 days after planting by placing cards under them. After harvesting plants with preferred seed coat colour were included in the second stage of selection which was based on seed size. Seed weights of the selected lines were measured.

9.2.5 Statistical analysis

Apart from analysis of variance which was performed on 100 seed weight, no other statistical analysis was performed.

9.3 RESULTS

9.3.1 Participatory field selection

There were three rounds of selection, each of the first two serving as culling level for the subsequent. At the first stage, farmers and other participants in the selection indicated the preferred plants based on their own assessment. All plants with at least one farmer's endorsement qualified for the next stage of the selection. The result of the selection is presented in Table 9.2. The number of plants selected per population varied from the least of 13 for Bambey21/Tona to the maximum of 26 for Bambey21/UCR779. Some of the plants were selected by a single participant while some were selected by many of them with the highest value of 36 for plant number 5 in the UCR779/CB27 population.

Seeds of all plants selected by the group (Table 9.2) were harvested at seed maturity and included in the next level of selection. Harvesting started 58 days after planting. The second stage of selection targeted seed coat colour. Table 9.3 shows the number of plants selected per population. This resulted in vast difference between the number of selected and accepted plants as shown in Table 9.3.

Table 9.2: Plants Selected from various Populations and the number of Participants that selected them

Plant No.	Number of participants who selected a given plant					
	CB27/ Gh3710	Bambey21/ UCR779	Bambey21/ Gbode	Bambey21/ Nhyira	UCR779/ CB27	Bambey21/ Tona
1	5	18	2	5	1	27
2	16	3	15	23	7	17
3	11	4	12	3	17	1
4	2	4	7	2	2	2
5	2	4	1	3	36	10
6	8	1	14	25	7	1
7	21	2	15	6	20	10
8	21	3	9	5	28	32
9	11	1	5	2	1	1
10	1	1	7	13	7	15
11	1	7	10	3	2	4
12	17	9	1	9	1	10
13	2	1	9	17	1	17
14	2	1	30	14	4	
15	2	4	8	5		
16	2	21				
17	17	1				
18	5	1				
19	5	22				
20	10	16				
21		1				
22		2				
23		9				
24		1				
25		5				
26		3				

9.3.2 Selection based on seed coat colour

Table 9.3: Number of plants accepted per population based on seed coat colour from population

Population	Number of field selected plants	Number of plants accepted
CB27/Gh3710	20	4
Bambey21/UCR779	26	7
Bambey21/Gbode	14	7
Bambey21/Nhyira	15	6
UCR779/CB27	15	7
Bambey21/Tona	13	5

9.3.3 Selection based on seed size

The number of plants selected for analysis of variance per population is shown in Table 9.4 in the “number of plants accepted” column. The least number of plants selected was 4 for CB27/Gh3710 whilst the most was 7 for three populations namely, Bambey21/UCR779, Bambey21/Gbode and UCR779/CB27. The numbers selected from Bambey21/Nhyira and Bambey21/Tona was 6 and 5 respectively. Analysis of variance for 100 seed weight was performed on the seeds of accepted plants including the parents as checks for each population and the results are shown in Table 9.4. The four heaviest seeds were accepted as the selected plants from each of the populations (Figure 9.1).



Figure 9.1: Seeds of plants selected from different cowpea populations.

Note: Female and male parents on left and right respectively and progenies in-between.

Table 9.4: One hundred seed weight (g) of accepted plants as compared with their parents

UCR779/CB27		Bamby21/UCR779		Bamby21/Gbode		Bamby21/Tona		CB27/Gh3710		Bamby21/Nhyira	
Genotype	Weight	Genotype	Weight	Genotype	Weight	Genotype	Weight	Genotype	Weight	Genotype	Weight
UCR779	19.20	Bamby21	16.46	Bamby21	16.46	Bamby21	16.46	CB27	20.28	Bamby21	16.46
CB27	20.28	UCR779	19.20	Gbode	17.98	Tona	16.7	Gh3710	10.54	Nhyira	13.02
*Plant 1	18.98	*Plant 1	22.78	Plant 1	16.76	Plant 1	14.84	*Plant 1	14.72	Plant 1	13.48
Plant 2	18.84	*Plant 2	21.18	Plant 2	17.88	*Plant 2	21.82	*Plant 2	15.48	*Plant 2	25.08
Plant 3	20.46	Plant 3	16.94	*Plant 3	28.28	*Plant 3	14.88	*Plant 3	18.50	*Plant 3	15.08
*Plant 4	27.54	Plant 4	18.20	*Plant 4	22.52	*Plant 4	18.84	*Plant 4	20.88	*Plant 4	17.20
Plant 5	21.66	*Plant 5	25.44	*Plant 5	20.46	*Plant 5	16.54			Plant 5	14.84
*Plant 6	22.92	*Plant 6	18.38	Plant 6	18.24					*Plant 6	17.76
*Plant 7	29.00	Plant 7	16.88	*Plant 7	18.62						
Grand \bar{X}	22.10		19.5		19.69		17.15		16.73		16.62
<i>F.pr</i>	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001
<i>S.E</i>	0.82		0.75		0.62		0.39		0.59		0.49
<i>CV%</i>	3.7		3.9		3.1		2.2		3.5		2.9
<i>LSD (5%)</i>	1.06		0.97		0.8		0.5		0.78		0.63

Note: *: Accepted Plants

9.4 DISCUSSION

At the time of selection (58 days after planting) farmers were mainly interested in yield and early maturity. They therefore chose plants with matured pods that were long and many per peduncle. Some farmers opened pods to be sure on what the seeds looked like before selecting them, although, they had been advised not to do so. Although some of the farmers were independent and chose what they decided on, others tended to be influenced by friends leading to many of them selecting the same plants. Farmers were in general enthused with the selection activities and this will contribute in developing varieties with farmers' desired traits (Quaye *et al.*, 2009).

After harvesting some of the plants selected were rejected because of having less desirable consumer quality seed coat. Accepted seeds were mainly cream / white seed coated (Langyintuo, *et al.*, 2003; Quaye *et al.*, 2011). However, very few accepted plants had mottle or brown seed colour because of the extremely large number of farmers' endorsement. The plants with unattractive seed coat pigmentation but with high endorsement by farmers may also be used in further breeding programmes.

A number of selected plants had similar morphology at the vegetative stage and also the same seed features. It would not be possible to develop and release such plants as separate varieties through single seed descent; therefore, the one with highest number of farmers' selection was used to represent them. In so doing the use of bulking was also avoided as it was less effective than the single seed descent (Tchiagam *et al.*, 2011a).

This led to a drastic decrease in the number of plants selected to the number accepted for seed size evaluation.

All of the four plants selected from CB27/Gh3710 had average 100 seed weight significantly higher than that of the smaller seeded parent (Gh3710). The heavier parent, CB27 was heavier than all the selected plants except “Plant number four”. However, the difference between CB27 and “Plant 4” was not significant. Smaller seeds were not expected as selection was against them at the F₂ stage. All of the plants had cream seeded coat with three having black eye and the fourth mottling. The three plants with black eyes were different in the size of the eye as well as the seed weight. All of the selected lines had dry pods before 60 days after planting which are earlier than 65 to 70 days considered by Singh *et al.* (2003) as early maturing. Early maturity is a very important objective in breeding cowpea and other crops as it is known to be an adaptive feature (Ishiyaku *et al.*, 2005), especially in these times of shortened rainfall periods (Muchero *et al.*, 2009b). In this respect, the selected plants of CB27/Gh3710 are very promising.

Apart from “Plant 6”, all of the selected ones from the population of Bambey21/UCR779 were significantly heavier than both parents. This shows effectiveness of selection for seed size in early generations of cowpea reported by Lopes *et al.* (2003). Effectiveness in selection in early generations for seed size in Chickpea has also been reported (Upadhyaya *et al.*, 2011). The idea from chickpea may be applicable in cowpea as synteny has been reported between cowpea and some of its legume relatives for a number of traits (Lucas *et al.*, 2013a). One of the plants had brown seed coat but was included in

the selection because of the high number of farmers that endorsed it during the selection. The brown seeded plant may not be released with that coat colour as a variety but rather subsequently used in hybridization. The three other plants had cream seeds with one having black eye and two with brown eyes but clearly different in seed sizes.

All the seeds of Bambey21/Gbode selected had cream seed coat with black eye but different shapes and sizes. Among the selected plants of Bambey21/Nhyira and Bambey21/Tona were plants with seeds similar to Bambey21. They however, had different growth habits. Bambey21/Tona had some plants with seeds not having attractive pigmentation but they were included in the selection for their high yields. High yield is one of the most important traits used by farmers in selection. Two plants from Bambey21/Tona were very similar so one is likely to be dropped.

Plants of UCR779/CB27 population generally had large parts with heavy seeds. Apart from “plant1” all of the selected plants were transgressive segregants for large seeds. This is an indication of partial or complete dominance of large seed (Acquaah, 2007). Transgressive segregation was however, not observed in CB27/Gh3710 used for generation mean analysis reported in chapter 6 of this Thesis. This is an indication that genotypic difference is important in estimating gene action. Plant number 7 of UCR779/CB27 had 100 seed weight of 29.00g which was the largest across all populations from which selections were made. All the other plants selected across the various populations had seed size larger than at least that of the smaller seeded parent.

9.5 CONCLUSION

Crop varieties developed through farmers' participation have higher adoption rate compared with those developed by researchers alone. This was the reason for involving farmers from the cowpea growing communities around Ohawu in the Volta Region of Ghana in selecting cowpea plants toward the development of varieties. Farmers' participation and researchers' knowledge were used to select 24 plants from 6 different bi-parental populations. These selected plants would be advanced toward varietal development focusing on seed size and colour among other traits. The expected varieties to come out of this breeding programme have a very high chance of being adopted because of the level of commitment shown from the onset by farmers.

CHAPTER TEN

10.0 GENERAL DISCUSSION

10.1 GENERAL DISCUSSION

The acceptability of a newly developed crop variety depends largely on its end user traits. Seed size and coat colour are known important end user traits of cowpea for a long time in Ghana and other places. Recently, Ansah (2012) reported that these two traits continue to be the two most important end user traits in the acceptability of cowpea in some localities in Ghana. There is also cultural influence on end user traits notably seed coat colour of cowpea in Ghana as one type of colour may be acceptable among the people of one cultural or ethnic group but not by the other. A series of studies that focused on seed size and coat colour inheritance of cowpea were undertaken along the south eastern coast of Ghana.

Farmers identified insect pests, diseases, drought, *Cyperus spp* (weed) and harvesting difficulty as the major constraints to cowpea farming. This is a confirmation of what is known elsewhere (Adipala *et al.*, 2000; Asante *et al.*, 2001; Makoi *et al.*, 2010). On consumer traits, ease of cooking was the most important trait to consider in choosing cowpea for cultivation and consumption according to the focus group at Avenorpedo. Ease of cooking, however, was ranked second at Ohawu after sweetness. Other traits of importance mentioned included, seed coat colour, wholeness of grain and seed size.

Preference for cowpea with cream large seeds which is sweet and easy to cook among Ghanaian consumers has not changed according to the survey. It would therefore, be important to ensure that newly developed cowpea varieties for Ghana have large seeds with cream coat colour.

It is paramount to understand the inheritance of seed coat colour and size in cowpea to be able to develop cowpea varieties with cream seed coat pigment and large seeds. The first step in any breeding programme is to assemble plant genetic resources which are the raw materials for breeding (Van Hintum *et al.*, 2000). One hundred and eighteen cowpea genotypes comprising gene-bank materials, landraces and improved varieties from Ghana, Nigeria, Senegal, Botswana and United States of America were assembled and characterized using 16 morphological traits. Information on the 16 traits was used to calculate dissimilarity between the genotypes with the method of Sokal and Sneath (Perrier *et al.*, 2003). The dissimilarity ranged from 7% to 100% with a mean of 43%. Factorial plot clustered the improved varieties together indicating the importance of conservation in safeguarding against genetic erosion (El Bakkali *et al.*, 2013).

SNP markers were used in characterization of 113 cowpea accessions comprising of 108 from Ghana and 5 from abroad. The SNP markers discriminated among all of the cowpea accessions and the dissimilarity values which ranged from 0.006 to 0.63 were used for factorial plot of the accessions. Unexpected high levels of heterozygosity were observed in some of the cowpea accessions (Dyer *et al.*, 2011). The high heterozygosity observed in some of the cowpea accessions might be due to outcrossing (Lucas *et al.*, 2011:

Kouam *et al.*, 2012) during regeneration at the genebank and to the fact that some of them have hybrid origin. Accessions known to be closely related clustered together in a dendrogram drawn using hierarchical clustering with WPGMA method. The software package “Structure” was used to separate accessions into three groups, and this programme also correctly identified varieties that are known to have hybrid origins. The structure plot also showed closely related accessions having similar genome patterns. The SNP markers were more efficient in discriminating among the cowpea germplasm than morphological (Bennett-Lartey, 1992), seed protein polymorphism (Oppong-Konadu *et al.*, 2005) or SSR (Asare *et al.*, 2010) reported earlier on samples from the same source. This confirms the strength of SNP markers in diversity studies as reported by Varshney *et al.* (2007).

Two different approaches namely association mapping and generation mean analysis were used in the study of seed size inheritance. Genome wide Association between the marker scores and phenotypes were analyzed using single trait single environment association mapping algorithm in GenStat. Significant association was established between eighteen SNP markers and cowpea seed mass and thickness at $-\log_{10}(p) \geq 5.00$. However, eight of these markers had significant association with both seed mass and thickness and therefore could be the most important markers in seed size studies in cowpea. These markers could be exploited for marker assisted breeding of larger seeded cowpea. Linkage groups (LG) 1, 10 and 11 had one significant associated marker each. LG 4 had 3 significant markers while 5 and 7 had two markers each. This result does not agree with Fatokun *et al.* (1992) where cowpea seed weight genes were mapped on LG 2

and 6. However, Fatokun *et al.* (1992) used RFLP markers and not SNPs. On the other hand, the current studies had some agreement with that of Kelly *et al.* (2003) who mapped seed weight genes of cowpea on LG 1, 3, 4, 6 and 7 with a combination of AFLP, RAPD and RFLP markers. This study had high agreement with Lucas *et al.* (2013b) who also used SNP markers in their studies.

Generation mean analysis revealed a high broad sense heritability (84%) and relatively low narrow sense heritability (35%) for seed size in cowpea using the genotypes CB27 and Gh3710. Varying figures have been reported for narrow and broad sense heritability of seed size in cowpea in the past (Drabo *et al.*, 1984; Fatokun *et al.*, 1992; Omoigui *et al.*, 2006; Tchiagam *et al.*, 2011a; Manggoel *et al.*, 2012). Varietal differences thus seem to greatly influence seed size heritability. Eight genes were estimated to control seed size inheritance in cowpea in this study which is in agreement with the observation of Drabo *et al.* (1984). This is however, contrary to a more recent work by Lopes *et al.* (2003) who reported five genes. Small seed size was found to be partially dominant over large seed. Duplicate gene action was observed to control seed size inheritance in the study (Suzuki *et al.*, 1989). Results of the generation mean analysis suggest complexity in the inheritance of seed size in cowpea. The implication for breeding is that a combination of methodologies such as single seed descent and backcrosses with selection from early generation through to advanced levels may be used to make progress.

Experiment was set up to estimate the number of genes that might be controlling seed coat colour inheritance in cowpea with different genetic ratios. Six different crosses were

made and their segregating populations studied. Most of the hybrids had black seed coat suggesting that simple dominant-recessive might not fully explain seed coat colour inheritance in cowpea reported by some authors (Arshad *et al.*, 2005; Mustapha 2009; Yilwa, 2012) especially from parents that were not black. The number of seed coat colour phenotypes produced varied from 2 to 10 for different populations suggesting that the mode of inheritance varies from genotype to genotype. Individuals of some of the populations could not be classified as they exhibited continuous variation. Chi-square goodness of fit test could not be conducted on some of the crosses. It was suspected that many genes might be involved in seed coat colour inheritance in cowpea and as such the use of molecular and quantitative principles may be helpful in understanding the genetic control of the trait.

Participatory plant breeding leads to early adoption of a newly released variety as farmers would be aware of whatever advantage it might have over prior existing ones as early as possible. Cowpea farmers around Ohawu in the Volta Region of Ghana where participatory rural appraisal was carried out previously were involved in participatory selection of promising cowpea plants. The farmers were chosen from 25 communities within the cowpea growing area by the help of agricultural extension officers. Fifty farmers including 25 males and females each, 7 scientists and 15 other persons with different backgrounds were involved in the selection exercise. Selections were made from six F₃ populations with parents of various unique traits obtained from Botswana, Ghana, Nigeria, Senegal, or United States of America. Four most liked cowpea plants from each

of the six populations were selected to be advanced and tried in multi-locations towards probable release.

10.2 CONCLUSION

The results of participatory rural appraisal and survey revealed that the major constraints to cowpea production along the coastal savanna of Ghana are disease and insect pests' incidence, weed problems, drought and harvesting and processing difficulties. Traits of interest to consumers of cowpea are ease of cooking, sweetness, large seed size and cream coat colour and wholeness of grains.

Morphological and molecular characterization revealed wide diversity among the cowpea germplasm assembled from different places. These diverse genetic resources have useful materials that could be used in cowpea improvement programme. Some cowpea accessions have therefore been selected as core for future breeding programmes.

Seed size in cowpea was seen to be influenced mainly by seed weight and thickness. Eight SNP markers were found to significantly associate with seed size in cowpea. These identified markers could be exploited in marker assisted selection.

Generation mean analysis revealed that at least 8 genes control seed size inheritance in cowpea. Although, additive gene effect was significant, non-additive genes were, however, observed to have larger control on the inheritance of the trait. Small seed was observed to be partially dominant over large seed.

Several genes with their interactive effects were suspected to control seed coat colour inheritance in cowpea. Variability in the F₂ generation of some crosses resembling quantitative inheritance suggesting that the best way to understand the inheritance of the trait is to use quantitative and molecular approaches.

Variety selection with farmers resulted in 24 promising lines which would be developed into varieties.

10.3 RECOMMENDATIONS

- Seed size and coat colour in cowpea are very important consumer traits and should therefore be considered in the development and release of new varieties.
- Introduction of germplasm from different sources offer diversity for breeding. Breeders should therefore consider materials from other breeding programmes in selecting parents for cowpea improvement.
- For fast gain in seed size, marker assisted selection should be used in cowpea improvement.
- Quantitative approach such as the use of colorimeters and QTL should be used to better understand the inheritance of seed coat colour in cowpea.
- Selection should be made from early generation through advanced level for seed size in cowpea because of the importance of additive and non-additive gene effects in the inheritance of the trait.

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APPENDICES:

Appendix 1: Passport data of the cowpea accessions

GH number	Local name	Collection date	Region	Location	Lat	Long	Seed colour	% Heterozygous
GH1622	Ayi-dze	21-12-82	Volta	Djolo	06°35'N	00°27'E	Black	0.43
GH1630	Asedua	01-12-83	Volta	Anum	06°29'N	00°10'E	Black	0.71
GH1665	Asedua	26-11-82	Eastern	Nkawkaw			Red	31.45
GH1667	AduaNsawa	30-11-82	Eastern	Kan No. 2	06°40'N	01°20'W	Brown	0.21
GH2272	Banga	13-10-87	BrongAhafo	Tuobodom	07°43'N	01°69'W		-
GH2279	Sanji	14-10-87	North	Tianjeni	09°15'N	00°37'W	Red	0.67
GH2280	Sanji	14-10-87	North	Labaraga	09°15'N	02°10'W	Brown	0.43
GH2281	Sanji	14-10-87	North	Boterly			Dark	0.24
GH2282	Sanji	14-10-87	North	Tua	09°55'N	00°07'W	Black	0.00
GH2284	Sanji	14-10-87	North	sang	09°18'N	02°25'W	Red	6.99
GH2288	Isagi	14-10-87	North	Tuwuwa	09°26'N	02°00'W	Black	0.85
GH2291	Sanji	15-10-87	North	Ziong	09°13'N	01°02'W	Red	3.81
GH2293	Sanji	15-10-87	North	Limoh	09°29'N	01°13'W	Red seeds	0.22
GH2294		15-10-87	North	Limoh	09°29'N	01°13'W	Mottled	0.51
GH2296	Sanji	15-10-87	North	Duobunantor	10°21'N	00°35'W	Red	0.21
GH2306	Bonda	16-10-87	Upper West	Tumu	10°40'N	02°01'W	Black	0.21
GH2307	Bondawa	16-10-87	Upper West	Buoti	10°53'N	02°07'W	Black	0.21
GH2309	Bibitakone	17-10-87	Upper West	Nandom	10°50'N	03°15'W	Black	7.86
GH2312	Dapiala	17-10-87	Upper West	Sombo	10°15'N	02°27'W	Red	0.45
GH2314	Bengah	17-10-87	Upper West	Kampala	10°0'N	02°24'W	Mottled	2.50
GH2316	Bengah	17-10-87	Upper West	Ga	09°26'N	02°30'W	Mottled	0.23
GH2317	Achibe	17-10-87	North	Tuna	09°32'N	02°36'W	Mottled	0.21
GH2323	Bengah	19-10-87	North	Malume	08°25'N	02°17'W	White	1.49

Appendix 1: Passport data of the cowpea accessions (Continued)

GH2325	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Mixed	0.43
GH2326	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	White	0.21
GH2328	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Red	0.22
GH2329	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	White	0.21
GH2330	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Red	0.64
GH2331	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Mottled	0.42
GH2332	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	White	0.21
GH2333	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Mottled	0.85
GH2334	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Cream	0.21
GH2335	Asedua	30-11-87	Eastern	Akoradarko	06°22'N	00°24'W	Red	0.21
GH2336	Asedua	30-11-87	Eastern	Ahomahomaso	06°27'N	00°29'W	Mottled	-
GH2338	AduaNsawa	12-1-87	Eastern	Bepongkwahu	06°42'N	00°47'W	Red	-
GH2340	AduaNsawa	12-1-87	Eastern	Asakraka	06°34'N	00°35'W	Black	0.00
GH2341	AduaNsawa	12-1-87	Eastern	Abene	06°38'N	00°34'W	White	0.21
GH2342	AduaNsawa	12-1-87	Eastern	Abene	06°38'N	00°34'W	Red	0.63
GH2347	Yor	18-12-87	Eastern	Nkurakan			Black	0.00
GH2492	-	20-10-87	Eastern	Ohwimase				1.35
GH3666	Ayi	23-10-93	Volta	Juapong	06°18'E	0°11'N	Red	-
GH3667	Ayi	23-10-93	Volta	Juapong	06°18'E	0°11'N	Dark	0.21
GH3668	Ayi	23-10-93	Volta	Juapong	06°18'E	0°11'N	Black	0.43
GH3669	Ayi	23-10-93	Volta	Juapong	06°18'E	0°11'N	Black	0.21
GH3679	Yor	26-10-93	Eastern	Takunya	06°06'N	0°11'W	Other	-
GH3684	Asedua	29-10-93	Eastern	Tanoso	06°54'N	0°43'W	Brown	-
GH3685	Asedua	30-10-93	Eastern	Fukuokrom	07°16'N	02°19'W	Black	0.43
GH3689	Sanji	1-11-93	Eastern	Defaa	08°54'N	0°39'N	Brown	0.43
GH3701	Sanji	11-2-93	Eastern	Kpong	06°10'w	0°03'N	Brown	0.42

Appendix 1: Passport data of the cowpea accessions (Continued)

GH3703	Tuya	11-2-93	Northern	Loagri			Red	0.47
GH3706	Benga	4-11-93	Northern	Tanina	09 ⁰ 50N	0 ⁰ 29W	Brown	0.00
GH3708	Sega	11-4-93	Northern	Grupe	09°20'W	02°20'N	Mottled	0.45
GH3710	Tuya	11-5-93	Northern	Nabori			Dark	0.21
GH4028	Aduansawa	19-5-96	Northern	Owusukrom			Dark	0.23
GH4524	Yor	29-7-96	Accra	Prampram			Black	0.24
GH4529	Tolonye	30-7-96	Accra	Kasseh			Mottled	0.43
GH4530	Yor	30-7-96	Accra	Kasseh			Brown	0.43
GH4532	Ayiyibor	31-7-96	Volta	Dabala			Red	0.65
GH4533	Ayi	31-7-96	Volta	Dabala			Cream	1.32
GH4537	Ayi	08-1-96	Volta	Ziope			Brown	0.22
GH4541	Ayi	08-1-96	Volta	Ziope			Mottled	0.63
GH4546	Ayi	08-3-96	Volta	Ziope			Dark	0.21
GH4769	Bianga	22-10-96	Upper West	Wa			Red	0.42
GH4771	Bianga	22-10-96	Upper West	Wa			Black	0.21
GH4778	Gonja	27-10-96	North	Yipala			Red	0.21
GH5038	Vakli	11-4-96	Volta	Akuni No. 2			Mottled	0.21
GH5039	ekye	11-4-96	Eastern	Abonse			Red	0.44
GH5040	Yor	11-5-96	Eastern	AhabasoGyaes			Red	0.21
GH5043	Asedua	11-6-96	Eastern	New Tafo			Brown	-
GH5044	Yor	11-6-96	Eastern	Sutapong			Red	0.21
GH5045	Yor	11-6-96	Eastern	Sutapong			Red	0.66
GH5048	AduaNsadua	11-6-96	Eastern	Suminakese			Red	0.21
GH5049	Asedua	11-6-96	Eastern	Okumaning			Red	0.21
GH5050	Asedua	11-8-96	Ashanti	Kokoben			Red	0.42

Appendix 1: Passport data of the cowpea accessions (Continued)

GH5344	Asedua	11-6-96	Ashanti	Juaben	Red	0.42
GH6045	Soronko		Ashanti	Fumesua	Brown	0.63
GH6046	Ayiyi	08-3-98	Ashanti	Fumesua	White	-
GH6060	Ayiyi	28-8-98	Eastern	Nsawam	Cream	0.21
GH7161	Sanji	17-2-03	Upper East	Kukobila	Black	-
GH7166	Tue	17-2-03	Upper East	Sandema	White	-
GH7167	Tue	18-2-03	Upper East	Wiaga	White	0.42
GH7172	Sena	18-2-03	Upper East	Papa Zoye	White	-
GH7174	Tse	18-2-03	Upper East	Bolga	Cream	0.42
GH7178	Benga	19-2-03	Upper East	Zebila	Red	36.49
GH7187	Goatana	20-2-03	Upper East	Navrongo	Dark	0.21
GH7218	Sona	04-1-03	Upper West	Babison	Dark	0.00
GH7220	Bonda	04-1-03	Upper West	Naveriwie	Brown	
GH7222	Bondabene	04-1-03	Upper West	Kong	Brown	0.66
GH7223	Sombene	04-1-03	Upper West	Kong	Dark	-
GH7224	Sompla	04-1-03	Upper West	Kong	Dark	0.21
GH7225	Sompla	04-1-03	Upper West	Kong	White	-
GH7226	Ben	04-1-03	Upper West	Kong	White	0.43
GH7228	Bene	04-2-03	Upper West	Kalsegra	Dark	3.24
GH7229	Bene	04-2-03	Upper West	Kalsegra	Dark	0.21
GH7230	Bene	04-2-03	Upper West	Kalsegra	Mottle	0.24
GH7231	Bene	04-2-03	Upper West	Kalsegra	Dark	2.44
GH7233	Bene	04-2-03	Upper West	Kalsegra	Brown	0.42
GH7234	Bene	04-2-03	Upper West	Kalsegra	Dark	20.09
GH7235	Bene	04-2-03	Upper West	Kalsegra	Dark	0.21
GH7243		04-2-03	Upper West	Kaleo	Dark	0.42

Appendix 1: Passport data of the cowpea accessions (Continued)

GH7245	Sonorni	1-4-03	Upper West	Kunchogu	Other	0.43
GH7875	Asedua	15-3-06	Eastern	Anyinam	Red	0.21
GH7888	Asetenapa	16-8-06	Ashanti	C.R.I Fumesua	Cream	0.63
IT97K556				IITA	Brown	0.43
IT82E18				IITA	Brown	0.88
IT845				IITA	Red	-
IT93K631				IITA	Brown	-
Songotra				CSIR-SARI	White	-
Tona				CSIR-CRI	Brown	0.42
Nhyira				CSIR-CRI	Cream	0.42
Zaayura				CSIR-SARI	White	1.07
PaddyT				CSIR-SARI	White	0.21
Asontem				CSIR-CRI	Red	2.77
Market				Ho Market	White	2.70
UCR 779				UCR	Brown	0.21
Bambey2				UCR	White	-
CB 27				UCR	White	0.64
2425B				UCR		-
2425B-1				UCR	Cream	-
524B				UCR		-
Danila				UCR	Cream	-
Tvu7778				UCR	Dark	-
Tvu14676				UCR	Black	-
Yacine				UCR	Brown	-
Mourid				UCR	Cream	-
Wacci01				WACCI	Dark	0.66

Appendix 2: PARTICIPATORY RURAL APPRAISAL: FOCUS GROUP DISCUSSION QUESTIONS

General questions

1. Geographic boundaries of the district
2. Major towns and villages
3. Major crops grown
4. Minor crops grown
5. The ranking of cowpea among other crops in the area
6. Other economic activities of the area

30 min

Questions on cowpea

1. Varieties of cowpea
2. Major cowpea producing communities
3. System of cowpea cultivation (mix, mono etc cropping)
4. Cowpea farm size
5. Apart from food for human consumption, what are the other uses of cowpea?
6. Meals made from cowpea
7. Any special use of cowpea? Medicine?
8. Markets where you sell your cowpea
9. Desirable traits
10. Production constraints

30 min

Activity 1: The most challenging constraints to cowpea cultivation in the your area

Constraint	Insect pests	<i>Cyperus spp</i>	Mould	Preservation	Drought	Score	Rank
Insect pests							
<i>Cyperus spp</i>							
Mould							
Preservation							
Drought							

30 min

Activity 2: Preferred traits in choosing cowpea varieties

Trait	Coat Colour	Wholeness	Seed Size	Sweetness	Easy to cook	Score	Rank
Coat Colour							
Wholeness							
Seed Size							
Sweetness							
Easy to cook							

30 min

Activity 3: Preferred grain coat colour of cowpea

Colour	Black	Brown	Mottle	Red	White	Score	Rank
Black							
Brown							
Mottle							
Red							
White							

30 min

Appendix 3: Interview guide: Survey on Cowpea Farming in Ho, Ketu-North and Akatsi
Districts: End Use Traits and Challenges with Cultivation

Section 1. Tracking information

Name of interviewer Questionnaire no.

Location Date of interview.....

Section 2. Respondent and household information

(1) Name

(2) Gender: Male [] Female []

(3) Relationship of respondent to head (main bread winner) of household

Head [] Daughter/Son [] Parent [] Not related []

Wife/Husband [] Sister/Brother [] Other relatives []

(4) How many persons belong to your household?

Males under 15

Females under 15

Male over 15

Females over 15

(5) What are the main sources of livelihood of your household?

	Most income income	Some income	Very little	No
Cash crop	[]	[]	[]	[]
Root and tuber crops	[]	[]	[]	[]
Cereals	[]	[]	[]	[]
Vegetables	[]	[]	[]	[]
Cowpea	[]	[]	[]	[]
Livestock	[]	[]	[]	[]
Buying and selling	[]	[]	[]	[]
Government service	[]	[]	[]	[]
Pension	[]	[]	[]	[]
Other	[]	[]	[]	[]

Section 3. Cowpea varieties

- (6) What is the importance of cowpea in your household consumption?
 Very important [] Quite important [] Not important [] Not eaten []
- (7) What is the importance of cowpea in your household income?
 Very important [] Quite important [] Not important []
- (8) Which of these varieties of cowpea do you know?
 Asontem [] Awakli [] Dapango [] Cynthia [] Borta []
 Tsedebanu [] Obama [] Wenchi [] Butter [] Gbovena []
- (9) Which of these cowpea varieties do you grow most?
 Asontem [] Awakli [] Dapango [] Cynthia [] Borta []
 Tsedebanu [] Obama [] Wenchi [] Vuvana [] Other [],
 Name it (them)
- (10) What is the reason for your choice in (9) above?
 High yield [] Easy to cook [] Taste [] Pests resistance []
 Drought tolerance [] High market price [] Other []
- (11) Which of these is the greatest limitation to your preferred variety?
 Drought [] Difficult to cook [] Low yield [] Tasteless [] Pest attack []
 Disease susceptibility [] Other []
- (12) What is your main reason for growing cowpea?
 Household consumption [] Cash [] Both consumption and cash []
- (13) Which cowpea varieties do you consume most?
 Awakli [] Obama [] Dapango [] Wenchi [] Borta [] Asontem []
 Gbovena [] Tsedebanu []
- (14) Why do you prefer this variety for consumption?
 No other [] Good taste [] High nutrient [] Medicine [] Easy to cook []
 Other []
- (15) Do you plan to continue growing these cowpea varieties? Yes [] No []
 If no why?

Section 4. Sources of seed

- (16) How did you obtain seed you used last growing season?
 Retained own seed [] Exchange with other farmers [] Gift from relatives []
 Provided by Gov't or NGO [] Purchased [] Other []

(17) If you purchase seed, from where?

From another farmer [] Local market [] Local Agro dealer []
Seed company [] Other []

(18) Which sources of seed do you prefer?

Own seed [] From other farmers [] Gift from relatives []
Provided by Gov't or NGO [] Other []

Section 5. Cowpea diseases

(19) Which diseases are problem for your cowpea?

Dies in seedling stage [] Blight [] Leaf spot [] Leaf curl []
Rot at base [] Pod rot [] Other []

(20) Which pest are problem for your cowpea?

White flies [] Aphids [] Thrips [] Leaf eaters [] Leaf miner []
Maruca [] Other []

(21) How do you protect your cowpea from these pests and diseases?
.....

Section 6. Seed traits

(22) Which cowpea seed colour type do you grow most?

White [] Black [] Red [] Brown [] Mottle [] Other []

(23) Which colour type will you be most reluctant to grow?

White [] Black [] Red [] Brown [] Mottle [] Other []

(24) Which size of seed do you like best?

Small [] Medium [] Large []

Section 7. Production level and marketing

(25) What is your normal cowpea farm size?

Less than 1 acre [] 1 – 2 acres [] 2 – 3 acres [] 3 – 5 acres [] More than 5 acres []

(26) During which season(s) do you cultivate cowpea?

Rainy [] Dry [] Throughout []

(27) Where do you sell your cowpea?

Farm gate [] House [] Local market [] District market [] Other []

(28) To whom do you sell your crop?

Locally to others [] Market traders [] Processors []
 Shop/hotel/super market [] Other []

Appendix 4: Diagnostic Questions for Cowpea Traders in Ketu North, Akatsi, Ho and Agbogbloshie markets on End Use Traits

Section 1. Tracking information

Name of interviewer ----- Questionnaire no -----

Location ----- Date of interview -----

Section 2. Respondent and household information

(1) Name

(2) Gender: Male [] Female []

(3) Relationship of respondent to head (main bread winner) of household

 Head [] Daughter/Son [] Parent [] Not related []
] Wife/Husband [] Sister/Brother [] Other relatives []

(4) How many persons belong to your household?

 Males under 15

 Females under 15

 Male over 15

 Females over 15

(5) How long have you been dealing in cowpea?

 Less than 1 year [] 1 –2 years [] 2 - 5 years[]
]

 More than 5 years[]

(6) What is the importance of cowpea trading in your household income?

 Very important [] Quite important [] Not important []

(7) Which are the main varieties of cowpea you know?

 Awakli [] Obama [] Dapango [] Wenchi [] Borta []

 Asontem [] Gbovena [] Tsedebanu []

 Others, list

(8) Which of the cowpea varieties do you deal in most?

Awakli [] Obama [] Dapango [] Wenchi [] Borta []

Asontem [] Gbovena [] Tsedebanu []

. Others, list

(9) What is your main reason for the choice in 8 above?

High market demand [] Availability []

Others [], state

(10) Which cowpea varieties do you consume most?

.....

(11) Why do you prefer this variety for consumption?

No other [] Good taste [] High nutrient [] Medicine [] Other []

]

(12) Do you plan to continue dealing in these cowpea varieties? Yes []

No []

(13) Where do you obtain cowpea from for selling?

Own farm [] Other farmers [] From relatives []

Market [] Specify Other []

(14) Where do you sell the cowpea?

House [] Local market [], name..... Other []

(15) What are the problems for your cowpea?

Weevils [] Rot [] Mould [] Low price []

High cost [] Difficult to cook [] Other []

(16) Which colour type of cowpea sells best?

Black [] White [] Brown [] Red []

Mottle [] Other [] specify

(17) Which colour type are customers most reluctant to buy?

White [] Black [] Red [] Brown [] Mottle [] Other []

(18) Which size of seed do you like best?

Small [] Medium [] Large []

(19) Whom do you sell to? You may tick more than one

People from the same community [] To nearby villagers []
 Market traders [] Processors [] Shop/hotel/super
 market [] Other []

Appendix 5: Diagnostic Questions on desirable end use traits of cowpea for vendors in food made from cowpea in Ho, Ketu North and Akatsi Districts

Section 1. Tracking information

Name of interviewer ----- Questionnaire no. -----

 Location ----- Date of interview -----
 --

Section 2. Respondent and household information

(20) Name

(21) Gender: Male [] Female []

(22) Relationship of respondent to head (main bread winner) of household

Head [] Daughter/Son [] Parent [] Not related []
] Wife/Husband [] Sister/Brother [] Other relatives []

(23) How many persons belong to your household?

Males under 15

Females under 15

Male over 15

Females over 15

(24) What is the importance of cowpea in your trade?

Very important [] Quite important [] Not important [] Not
 eaten []

(25) What is the importance of cowpea in your household income?

Very important [] Quite important [] Not important [] Not
 grown []

Section 3. Cowpea and its products

(26) Which are the main varieties of cowpea you know?

Asontem [] Dapango [] Avakli [] Obama [] Wenchi []
Butter [] Vuvana [] Tinabawu [] Other [] name

.....

(27) Which food do you trade in?

Ayikple [] Waakye [] Borbor [] Akla (kose) [] Aklankui []
Ayidzenkple [] Other []

(28) What type of cowpea is most suitable for your meal?

White [] Red [] Mottle [] Brown [] Black [] Any of above []
]

(29) How regular do you prepare and sell this food?

< 3 months a year [] 3 – 6 months [] Year round []

(30) How long have you been in the business?

< 1 year [] 1 – 2 years [] 2 – 5 years [] Over 5 years []

(31) Which cowpea varieties do you use in preparing the food?

Awakli [] Obama [] Dapango [] Wenchi [] Borta []
Asontem [] Gbovena [] Tsedebanu [] Other []

(32) What is the reason for your answer in (12) above?

No other [] Good taste [] High nutrient [] Less expensive [] Colour []
] Grain quality [] explain Other []

(33) Where do you buy cowpea grains for your food preparation?

Own farm [] Farmers in the community [] Local market []
Other []

(34) What are the problems for your grains?

Weevils [] Mould [] Rot [] Other []

(35) Which cowpea seed colour type do you use?

White [] Black [] Red [] Brown [] Mottle [] Other []

(36) Which colour type will you be most reluctant to use?

White [] Black [] Red [] Brown [] Mottle [] Other []

(37) Which size of seed do you like best?

Small [] Medium [] Large [] Any []

Appendix 6: Survey on cowpea seed system in Ho, Ketu North and Akatsi Districts
Section 1. Tracking information

Name of interviewer ----- Questionnaire no. -----

Location ----- Date of interview -----

Section 2. Respondent and household information

(38) Name -----

(39) Gender: Male [] Female []

(40) Relationship of respondent to head (main bread winner) of household

Head [] Daughter/Son [] Parent [] Not related []
] Wife/Husband [] Sister/Brother [] Other relatives []

(41) How many persons belong to your household?

Males under 15

Females under 15

Male over 15

Females over 15

(42) What is the importance of cowpea in your trade?

Very important [] Quite important [] Not important []

Section 3. Main survey questions

(43) Which are the main varieties of cowpea you know? Tick all that you know.

Avakli [] Obama [] Dapango [] Wenchi [] Butter []
 Asontem [] Vuvena [] Tinabawu [] Other [] List

.....

(44) Which of the cowpea varieties do you trade in?

Avakli [] Obama [] Dapango [] Wenchi [] Borta []
 Asontem [] Gbovena [] Tsedebanu [] Other [] List

.....

...

(45) What is your reason for the choice in (7) above?

No alternative [] High yields [] Taste [] Disease resistance []
 Pest resistance [] Quality grain [] Easy to cook [] Large grain []
 Colour of grain [] Other [] State

.....

(46) Where do you buy your seeds from?

Own farm [] Farmers in the community [] Other seed dealers [] Local
 market [] Other [] State

.....

(47) Where do you sell the seeds?

House [] Local market [] District market []
 Other [] State

(48) What quantity do you sell in a year?

Less than 50kg [] 50 – 100kg [] 100 – 200 kg [] 200 – 500kg [] above
 500kg []

(49) Which cowpea seed colour type do you trade in most?

White [] Black [] Red [] Brown [] Mottle [] Other []

(50) Which colour type will you be most reluctant to trade in?

White [] Black [] Red [] Brown [] Mottle [] Other []

(51) Which size of seed do you like best?

Small [] Medium [] Large []

(52) During which season(s) do you sell cowpea seeds most?

Major [] Minor [] Both major and minor []

(53) What will you like to see improved on the varieties you trade in?

Yield [] Disease resistance [] Pest resistance [] Drought tolerance [] Taste []
 Other [] State

Appendix 7: Rainfall Figures at Ohawu (Ketu North District) from June to August 2013

June		July		August	
Day	Quantity	Day	Quantity	Day	Quantity
1 st	3.6mm	4 th	30.0mm	Nil	
2 nd	3.6mm	15 th	3.6mm		
5 th	14.4mm	24 th	14.0mm		
9 th	21.6mm				
11 th	9.6mm				

Appendix 8: Description of cowpea accessions based on traits used in characterisation (Mahalakshmi *et al.*, 2007).

Trait	Cowpea accession					
	GH1622	GH1630	GH1665	GH1667	GH2279	GH2280
Vigour	Intermediate	Intermediate	None	None	Intermediate	None
Growth habit	Prostrate	Prostrate	Erect	Prostrate	Prostrate	Semi-erect
Days to flower	34	35		44	31	44
Flower colour	Violet	Violet		Violet	Violet	Violet
Plant pigment	Very slight	Moderate	Moderate	Moderate	Moderate	Moderate
Pods/peduncle	2	3	3	2	2	2
Immature pod	Sutures	Non	None	None	Sutures	
Mature pod	Black	Straw	Straw	Straw	Dark brown	Straw
Eye colour	Eye absent	Eye absent	Black	Eye absent		Eye absent
Seed coat colour	Black	Other	Cream	brown	Red	Brown
Pod attachment	30 - 90°	30 - 90°	Pendant	Pendant	30 - 90°	30 - 90°
Terminal leaflet	Sub-globose	Sub-globose	Sub-globose	Sub-globose	Sub-globose	Sub-hastate
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Rhomboid	Ovoid	Ovoid	Rhomboid		Rhomboid
Pod length (cm)	13.33	14.33	14.67	17.33	15.67	14.00
Seeds per pod	15.67	15.67	16.67	18.67	17.33	15.33
100 seed (g)	8.27	11.10	15.49	8.43	8.88	10.20

Continuation of appendix 8

Trait	Cowpea accession					
	GH2281	GH2282	GH2284	GH2288	GH2291	GH2293
Vigour	Intermediate	None	None	None	None	Intermediate
Growth habit	Intermediate	Prostrate	Prostrate	Prostrate	Semi-erect	Prostrate
Days to flower	37	37	39	41	38	32
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Very slight	Intermediate	Moderate	Moderate	Very slight	Intermediate
Pods/peduncle	2	2	2	2	1	3
Immature pod	Tip pigment	Sutures		Tip pigment	Tip pigment	Sutures
Mature pod	Straw	Dark brown	Dark brown	Straw	Dark brown	Dark brown
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Other	Black	Red	Black	Red	Red
Pod attachment	Pendant	Pendant	Pendant	Pendant	30 - 90°	30 - 90°
Terminal leaflet	Sub-globose	Sub-hastate	Sub-hastate	Sub-hastate	Sub-hastate	Sub-globose
Pod curvature	Straight	Slightly	Straight	Slightly	Slightly	Straight
Seed shape	Rhomboid	Ovoid		Ovoid	Ovoid	Kidney
Pod length (cm)	17.33	13.00	18.00	17.67	14.67	15.67
Seeds per pod	20.00	14.33	20.00	19.00	18.00	18.00
100 seed (g)	9.76	9.70	10.22	8.96	9.74	9.38

Continuation of appendix 8

Trait	Cowpea accession					
	GH2294	GH2296	GH2306	GH2307	GH2312	GH2314
Vigour	None	Intermediate	None	None	None	Intermediate
Growth habit	Prostrate	Prostrate	Semi-erect	Semi-erect	Semi-erect	Semi-erect
Days to flower	37	34	38	37	38	36
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Moderate	Intermediate	Very slight	Very slight	Moderate	Very slight
Pods/peduncle	2	2	2	2	3	3
Immature pod	None	Sutures	None	None	None	Tip pigment
Mature pod	Straw	Black	Straw	Straw	Straw	Dark brown
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Black	Red	Black	Black	Red	Black
Pod attachment	30 - 90°	Pendant	30 - 90°	Pendant	Pendant	Pendant
Terminal leaflet	Sub-globose	Sub-hastate	Sub-globose	Sub-globose	Sub-globose	Sub-globose
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Ovoid	Kidney	Ovoid	Ovoid	Ovoid	Ovoid
Pod length (cm)	15.67	14.67	15.33	13.67	17.67	17.67
Seeds per pod	17.33	15.00	16.00	15.67	19.67	17.67
100 seed (g)	9.92	10.18	7.38	8.74	7.41	8.61

Continuation of appendix 8

Trait	Cowpea accession					
	GH2317	GH2325	GH2326	GH2329	GH2330	GH2331
Vigour	Intermediate	Intermediate	None	Intermediate	Intermediate	Intermediate
Growth habit	Intermediate	Intermediate	Intermediate	Intermediate	Erect	Prostrate
Days to flower	37	40	38	38	40	37
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Very slight	Very slight	Very slight	Very slight	Very slight	Very slight
Pods/peduncle	3	2	2	2	2	2
Immature pod	None	None	None	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Brown	Eye absent	Black	Eye absent	Eye absent
Seed coat colour	Other	Red	Black	Brown	Red	Other
Pod attachment	30 - 90°	Pendant	Pendant	Pendant	Pendant	Pendant
Terminal leaflet	Sub-globose	Sub-globose	Hastate	Sub-hastate	Sub-globose	Sub-hastate
Pod curvature	Straight	Slightly	Straight	Slightly	Slightly	Slightly
Seed shape	Rhomboid	Kidney	Rhomboid		Kidney	Rhomboid
Pod length (cm)	16.33	19.67	16.33	19.33	16.67	17.00
Seeds per pod	17.67	21.67	15.33	21.00	17.00	15.67
100 seed (g)	9.25	9.54	11.98	9.92	6.69	10.31

Continuation of appendix 8

Trait	Cowpea accession					
	GH2332	GH2333	GH2334	GH2335	GH2336	GH2338
Vigour	None	None	Intermediate	None	Intermediate	None
Growth habit	Prostrate	Prostrate	Climbing	Semi-erect	Erect	Intermediate
Days to flower	41	37	38	36	38	39
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Intermediate	Moderate	Very slight	Moderate	Moderate	Very slight
Pods/peduncle	2	2	2	2	3	3
Immature pod	None	None	None	Tip pigment	None	Tip pigment
Mature pod	Straw	Straw	Straw	Dark brown	Straw	Black
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Other	Other	Brown	Black	Black	Red
Pod attachment	30 - 90°	Pendant	Pendant	30 - 90°	30 - 90°	Pendant
Terminal leaflet	Sub-hastate	Sub-globose	Sub-hastate	Sub-globose	Sub-globose	Sub-hastate
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Ovoid	Ovoid	Kidney	Rhomboid	Kidney	Rhomboid
Pod length (cm)	11.00	15.33	22.00	15.67	17.33	16.00
Seeds per pod	16.33	15.00	20.00	17.67	17.33	19.33
100 seed (g)	9.17	10.30	9.86	6.99	6.89	8.62

Continuation of appendix 8

Trait	Cowpea accession					
	GH2340	GH2341	GH2342	GH2347	GH3666	GH3667
Vigour	None	None	None	None	Intermediate	None
Growth habit	Erect	Prostrate	Prostrate	Erect	Intermediate	Semi-erect
Days to flower	42	38	42	41	38	39
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Moderate	Moderate	Intermediate	Very slight	Very slight	Very slight
Pods/peduncle	2	2	3	2	3	2
Immature pod	None	Tip pigment	None	None	None	None
Mature pod	Straw	Other	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Black	Brown	Red	Black	Red	Other
Pod attachment	Pendant	Pendant	Pendant	Pendant	Pendant	
Terminal leaflet	Sub-hastate	Sub-globose	Sub-globose	Sub-hastate	Sub-globose	Sub-globose
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Ovoid	Globose	Ovoid	Rhomboid	Ovoid	Rhomboid
Pod length (cm)	17.67	17.00	13.33	20.67	17.33	17.67
Seeds per pod	17.67	17.33	14.67	20.33	18.33	19.00
100 seed (g)	7.71	13.79	6.84	9.95	10.47	9.56

Continuation of appendix 8

Trait	Cowpea accession					
	GH3668	GH3669	GH3670	GH3673	GH3674	GH3675
Vigour	None	Intermediate	Intermediate	None	Intermediate	Intermediate
Growth habit	Prostrate	Intermediate	Erect	Semi-erect	Semi-erect	Prostrate
Days to flower	39	37	38	40	36	69
Flower colour	White	Violet	Violet	Violet	White	White
Plant pigment	None	Very slight	Very slight	None	Very slight	Moderate
Pods/peduncle	2	3	2	2	3	2
Immature pod	None	Non	None	Pigmented tip	Pigmented tip	Pigmented tip
Mature pod	Dark brown	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Black	Black	Brown	Black	Black	Black
Pod attachment	Pendant	Pendant	Pendant	Pendant	30 - 90°	
Terminal leaflet	Sub-globose	Sub-globose	Sub-globose	Sub-globose	Sub-globose	Sub-globose
Pod curvature	Slightly	Slightly	Straight	Straight	Slightly	Slightly
Seed shape	Ovoid	Rhomboid	Rhomboid	Rhomboid	Rhomboid	Rhomboid
Pod length (cm)	16.67	17.33	16.67	19.00	16.67	21.33
Seeds per pod	16.67	18.67	17.33	19.00	17.67	18.00
100 seed (g)	13.20	9.32	9.76	9.31	8.53	11.86

Continuation of appendix 8

Trait	Cowpea accession					
	GH3677	GH3679	GH3684	GH3685	GH3689	GH3701
Vigour	Intermediate	None	None	Intermediate	None	None
Growth habit	Erect	Semi-erect	Semi-erect	Erect	Prostrate	Intermediate
Days to flower	38	36	42	38	36	37
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Very slight	Moderate	Intermediate	Very slight	Moderate	Very slight
Pods/peduncle	2	3	2	3	2	2
Immature pod	None	None	Tip pigment	Non	Uniformly	None
Mature pod	Straw	Straw	Black	Straw	Black	Dark brown
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Brown	Other	Red	Red	Brown	Brown
Pod attachment	Pendant		Pendant	Pendant	30 - 90°	Pendant
Terminal leaflet	Sub-globose	Sub-globose	Sub-globose	Sub-globose	Sub-hastate	Sub-globose
Pod curvature	Slightly	Slightly	Straight	Slightly	Slightly	Straight
Seed shape	Rhomboid	Rhomboid	Rhomboid	Rhomboid	Ovoid	Kidney
Pod length (cm)	18.00	17.00	18.33	16.67	16.33	19.00
Seeds per pod	18.00	18.00	18.67	17.33	17.00	18.00
100 seed (g)	9.77	10.29	11.27	7.50	11.18	10.10

Continuation of appendix 8

Trait	Cowpea accession					
	GH3703	GH3706	GH3710	GH4028	GH4524M	GH4524BBE
Vigour	None	None	Vigorous	Intermediate	None	Intermediate
Growth habit	Semi-erect	Semi-erect	Prostrate	Erect	Climbing	Climbing
Days to flower	40	37	32	35	40	38
Flower colour	Violet	Violet	Violet	Violet	Violet	White
Plant pigment	Very slight	Very slight	Intermediate	Very slight	Moderate	Moderate
Pods/peduncle	2	2	2	3	2	2
Immature pod	None	None	Sutures	None	None	Pigmented tip
Mature pod	Straw	Dark brown	Black	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Black
Seed coat colour	Red	Brown	Black	Red	Other	Cream
Pod attachment	Pendant	30 - 90°	30 - 90°	Pendant	Pendant	Pendant
Terminal leaflet	Sub-globose	Sub-hastate	Sub-globose	Sub-globose	Hastate	Hastate
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Rhomboid	Ovoid	Kidney	Rhomboid	Rhomboid	Rhomboid
Pod length (cm)	15.33	16.33	14.00	18.67	15.67	16.00
Seeds per pod	16.67	17.00	14.67	20.00	16.33	16.00
100 seed (g)	7.27	8.76	7.71	10.67	12.56	12.43

Continuation of appendix 8

Trait	Cowpea accession					
	GH4524ME	GH4524B	GH4529	GH4530	GH4532	GH4533
Vigour	Intermediate	None	Intermediate	Intermediate	None	Intermediate
Growth habit	Climbing	Climbing	Intermediate	Climbing	Semi-erect	Climbing
Days to flower	38	39	36	37	39	36
Flower colour	White	Violet	Violet	Violet	Violet	White
Plant pigment	Moderate	Moderate	Very slight	Moderate	Moderate	Very slight
Pods/peduncle	2	2	2	2	2	2
Immature pod	None	Tip pigment	None	None	Tip Pigment	Tip pigment
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Mottled	Eye absent	Eye absent	Eye absent	eye absent	Black
Seed coat colour	Cream	Black	Other	Red	Black	Cream
Pod attachment	Pendant	Pendant	Pendant	Pendant	Pendant	Pendant
Terminal leaflet	Hastate	Hastate	Sub-globose	Hastate	Sub-hastate	Hastate
Pod curvature	Slightly	Slightly	Slightly	Slightly	Straight	Slightly
Seed shape	Rhomboid	Rhomboid	Rhomboid	Rhomboid	Rhomboid	Ovoid
Pod length (cm)	14.67	16.00	17.67	17.00	20.00	17.67
Seeds per pod	15.33	15.67	18.67	18.67	18.00	15.33
100 seed (g)	11.96	13.02	8.86	11.54	10.35	15.19

Continuation of appendix 8

Trait	Cowpea accession					
	GH4537	GH4546	GH4771	GH4778	GH5039	GH5040
Vigour	None	None	None	None	None	None
Growth habit	Intermediate	Intermediate	Prostrate	Prostrate	Climbing	Semi-erect
Days to flower	37	37	38	42	38	38
Flower colour	Violet	Violet	Violet	Violet	Violet	Violet
Plant pigment	Very slight	Very slight	Moderate	Moderate	Very slight	Very slight
Pods/peduncle	2	2	2	2	3	2
Immature pod	None	None	None	None	None	None
Mature pod colour	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Brown	Other	Black	Red	Red	Red
Pod attachment	30 - 90°	Pendant	30 - 90°	Pendant	Pendant	30 - 90°
Terminal leaflet	Sub-globose	Sub-hastate	Sub-globose	Sub-hastate	Hastate	Sub-globose
Pod curvature	Slightly	Slightly	Slightly	Straight	Slightly	Slightly
Seed shape	Rhomboid	Rhomboid	Ovoid	Rhomboid	Rhomboid	Rhomboid
Pod length (cm)	16.33	16.67	18.33	15.67	17.33	17.00
Seeds per pod	17.33	18.33	17.67	18.00	17.67	18.33
100 seed (g)	10.28	9.83	8.74	13.12	12.67	10.51

Continuation of appendix 8

Trait	Cowpea accession					
	GH5043	GH5044	GH5045	GH5048	GH5050	GH5344
Vigour	Intermediate	None	None	Intermediate	None	None
Growth habit		Semi-erect	Climbing	Climbing	Erect	Semi-erect
Days to flower	48	39	38	38	44	39
Flower colour	Violet	Violet	White	Violet	Violet	Violet
Plant pigment	Very slight	Very slight	Very slight	Moderate	Moderate	Very slight
Pods/peduncle	2	2	2	2	3	2
Immature pod		None	None	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Brown	Red	Red	Red	Red	Red
Pod attachment	Pendant	Pendant	Pendant	Pendant	Pendant	30 - 90°
Terminal leaflet	Hastate	Globose	Hastate	Sub-hastate	Sub-globose	Sub-globose
Pod curvature	Straight	Straight	Slightly	Slightly	Slightly	Slightly
Seed shape	Ovoid	Rhomboid	Rhomboid	Rhomboid	Ovoid	Rhomboid
Pod length (cm)	19.00	17.33	18.33	17.33	15.67	14.33
Seeds per pod	17.67	18.67	18.67	17.67	17.67	18.33
100 seed (g)	12.20	11.89	12.25	10.38	7.67	10.84

Continuation of appendix 8

Trait	GH5346	GH6045	GH6060	GH7174	GH7178	GH7185
Vigour	None	None	None	Vigorous	Intermediate	None
Growth habit	Semi-erect	Semi-erect	Semi-erect	Prostrate	Erect	Prostrate
Days to flower	44	38	36	47	37	40
Flower colour	Violet	Violet	White	White	Violet	Violet
Plant pigment	Very slight	Very slight	Very slight	Very slight	Very slight	Moderate
Pods/peduncle	2	2	2		4	2
Immature pod	None	None	None	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Dark brown
Eye colour	Eye absent	Eye absent	Brown	Black	Eye absent	Eye absent
Seed coat colour	Brown	Brown	Cream	Cream	Red	Black
Pod attachment	Pendant	Pendant	Pendant	30 - 90°	30 - 90°	Pendant
Terminal leaflet		Sub-globose	Sub-hastate	Sub-globose		Sub-globose
Pod curvature	Slightly	Straight	Slightly	Straight	Slightly	Slightly
Seed shape	Rhomboid	Rhomboid	Ovoid	Kidney	Ovoid	Rhomboid
Pod length (cm)	17.67	15.33	17.67	11.67	16.67	17.67
Seeds per pod	19.67	14.33	19.33	13.33	19.00	19.00
100 seed (g)	11.81	10.44	11.16	17.94	7.07	10.35

Continuation of appendix 8

Trait	GH7218	GH7222	GH7223	GH7224	GH7228	GH7229
Vigour	Intermediate	Non	None	None	None	Intermediate
Growth habit	Prostrate	Semi-erect	Prostrate	Prostrate	Prostrate	Prostrate
Days to flower	36	36	39	37	37	36
Flower colour	Violet	Violet	Violet	Violet	White	Violet
Plant pigment	Very slight	Moderate	Very slight	Very slight	Very slight	Moderate
Pods/peduncle	2	2	3	2	2	2
Immature pod	None	None	None	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Other	Brown	Other	Other	Other	Other
Pod attachment	30 - 90°	Pendant	30 - 90°	Pendant	Pendant	Pendant
Terminal leaflet	Globose	Sub-globose	Sub-globose	Globose	Sub-globose	Sub-globose
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Ovoid	Ovoid	Ovoid	Globose	Ovoid	Ovoid
Pod length (cm)	14.67	17.33	13.67	13.33	15.67	13.67
Seeds per pod	15.00	18.00	14.00	16.33	16.33	16.00
100 seed (g)	9.49	9.23	8.12	10.19	8.77	9.78

Continuation of appendix 8

Trait	GH7231	GH7234	GH7235	GH7243	GH7245	GH7875
Vigour	Intermediate	None	None	None	None	Intermediate
Growth habit	Prostrate	Prostrate	Prostrate	Prostrate	Prostrate	Intermediate
Days to flower	35	37	37	38	38	38
Flower colour	Violet	Violet	Violet	Violet	Violet	White
Plant pigment	Moderate	Very slight	Very slight	Moderate	Moderate	Moderate
Pods/peduncle	2	2	2	2	2	2
Immature pod	None	None	None	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent
Seed coat colour	Other	Other	Other	Other	Other	Red
Pod attachment	Pendant	30 - 90°	Pendant	30 - 90°	30 - 90°	Pendant
Terminal leaflet	Globose	Sub-globose	Sub-globose	Sub-globose	Sub-globose	Sub-globose
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Globose	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid
Pod length (cm)	13.67	13.33	15.33	17.00	13.33	20.67
Seeds per pod	14.00	15.00	16.67	18.00	15.33	19.00
100 seed (g)	9.16	9.39	9.12	9.41	8.98	9.93

Continuation of appendix 8

Trait	Cowpea accession					
	GH7888	2425B-1	524B	Agrimat	Asontem	Bawuta
Vigour	None	Intermediate	Intermediate	None	None	None
Growth habit	Semi-erect	Prostrate	Erect	Intermediate	Semiprostrate	Semi-erect
Days to flower	38	37	35	32	42	42
Flower colour	White	White	White	White	Violet	White
Plant pigment	Moderate	None	Moderate	Very slight	Very slight	Very slight
Pods/peduncle	3	2	2	3	2	2
Immature pod	None	None	Tip pigment	Tip pigment	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Black	Black	Black	Black	Eye absent	Black
Seed coat colour	Cream	Cream	Cream	Cream	Brown	Cream
Pod attachment	30 - 90°	30 - 90°	30 - 90°	Pendant	Pendant	30 - 90°
Terminal leaflet	Sub-hastate	Sub-globose	Sub-hastate	Sub-globose	Hastate	Sub-hastate
Pod curvature	Straight	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Ovoid	Ovoid	Kidney	Kidney	Rhomboid	Ovoid
Pod length (cm)	17.67	16.00	16.00	16.67	18.00	15.00
Seeds per pod	17.67	16.00	11.67	9.00	18.00	17.67
100 seed (g)	11.07	12.70	16.12	16.02	10.78	12.29

Continuation of appendix 8

Trait	Cowpea accession					
	Bombay 21	C- black eye	CB 27	Danila	IT82E-18	IT845
Vigour	Intermediate	None	Intermediate	None	Non	None
Growth habit	Erect	Erect	Acute erect	Prostrate	Erect	Erect
Days to flower	33	33	31	52	35	34
Flower colour	White	White	White	White	Violet	White
Plant pigment	None	Very slight	Very slight	Moderate	Moderate	None
Pods/peduncle	3	3	2		2	2
Immature pod	None	Pigmented tip	Pigmented tip	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Black	Black	Black		Eye absent
Seed coat colour	Cream	Cream	Cream	Cream		Red
Pod attachment	30 - 90°	30 - 90°	Pendant		Pendant	Pendant
Terminal leaflet	Sub-globose	Sub-globose	Sub-hastate	Sub-globose	Sub-globose	Sub-hastate
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Slightly
Seed shape	Kidney	Kidney	Kidney	Rhomboid		Kidney
Pod length (cm)	17.67	19.67	14.33	13.67	20.33	10.33
Seeds per pod	11.67	12.67	11.67	12.67	18.33	14.67
100 seed (g)	15.95	15.03	16.66	11.19	13.61	

Continuation of appendix 8

Trait	Cowpea accession					
	IT845	IT93K-63-1	IT97K556-6	Market	Mourid	Nhyira
Vigour	None	None	Intermediate	Non	None	None
Growth habit	Erect	Intermediate	Intermediate	Intermediate	Intermediate	Semi-erect
Days to flower	33	37	38	35	38	38
Flower colour	White	Violet	Violet	White	White	White
Plant pigment	Intermediate	Very slight	Moderate	Moderate	Moderate	Very slight
Pods/peduncle	3	2	2	3	4	3
Immature pod	None	None	None	Uniformly	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Black	Eye absent	Eye absent	Black	Black	Black
Seed coat colour	Cream	Black	Brown	Cream	Cream	Cream
Pod attachment	30 - 90°	30 - 90°	Pendant	30 - 90°	30 - 90°	30 - 90°
Terminal leaflet	Sub-globose	Sub-globose	Sub-hastate	Sub-globose	Sub-hastate	Sub-globose
Pod curvature	Slightly	Slightly	Slightly	Slightly	Slightly	Straight
Seed shape	Ovoid	Rhomboid	Kidney	Rhomboid	Rhomboid	Globose
Pod length (cm)	11.67	15.00	17.33	15.33	13.67	14.33
Seeds per pod	11.33	18.00	14.33	16.67	14.67	14.00
100 seed (g)	11.92	8.56	15.69	14.52	11.51	9.28

Continuation of appendix 8

Trait	Cowpea accession					
	Tona	TVU 7778	TVU14676	UCR 779	Yacine	Zaayura
Vigour	Non	None	None	None	None	None
Growth habit	Intermediate	Intermediate	Erect	Intermediate	Semi-erect	Intermediate
Days to flower	40	39		45	36	
Flower colour	Violet	Violet		White	White	White
Plant pigment	Moderate	Moderate	None	Very slight	None	Very slight
Pods/peduncle	3	2	2	2	2	2
Immature pod	Non	None	None	None	None	None
Mature pod	Straw	Straw	Straw	Straw	Straw	Straw
Eye colour	Eye absent	Eye absent	Eye absent	Eye absent	Eye absent	Black
Seed coat colour	Brown	Other	Black	Black	Brown	Cream
Pod attachment	Pendant	Pendant		30 - 90°	30 - 90°	30 - 90°
Terminal leaflet	Sub-globose	Sub-globose	Sub-hastate	Sub-globose	Sub-globose	Sub-hastate
Pod curvature	Slightly	Slightly	Straight	Straight	Slightly	Slightly
Seed shape	Kidney	Ovoid	Rhomboid	Rhomboid	Kidney	Rhomboid
Pod length (cm)	19.00	15.67	14.33	17.67	16.33	11.33
Seeds per pod	19.33	17.00	15.33	18.00	14.33	11.67
100 seed (g)	12.21	7.23	15.31	11.64	16.95	20.84