INTROGRESSION OF COWPEA APHID RESISTANCE GENE INTO SUSCEPTIBLE COWPEA [*Vigna unguiculata* (L.) WALP] CULTIVARS THROUGH MARKER ASSISTED BACKCROSSES

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY CROP SCIENCE (GENETICS AND PLANT BREEDING) DEGREE

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DECLARATION OF ORIGINALITY

I declare that the content of this thesis has not been submitted for any degree. I also certify that to the best of my knowledge, all references and any help received in preparing this thesis have been duly acknowledged.

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ABSTRACT

Aphid infestation is recognized as one of the major constraints that limit cowpea production in Africa. Host-plant resistance has proven to be the most efficient and sustainable means to controlling aphid infestation and its associated challenges. The objectives of this project were to characterize cowpea varieties using 38 agro-morphological and 20 SSR markers, and also to deploy aphid resistant locus into three locally preferred aphid susceptible cowpea cultivars. A total of 22 cowpea varieties were evaluated in a Randomized Complete Block Design (3 blocks) on the experimental fields of CSIR-CRI, Fumesua in the minor rainy season of Ghana using the IBPGR cowpea descriptor. Characterization results showed a relatively high level of genetic diversity among the varieties which ranged from 1 - 0.007, 1 - 0.001, 0.652 - 0.109 for qualitative, quantitative and molecular markers respectively. Principal component analysis, dissimilarity matrices, principal component biplot, and clustering separated the accessions according to some qualitative and quantitative traits with the aid of Genstat version12.0 and Statistical Package for Social Sciences (SPSS) version 16.0 software. Among the 20 SSR markers screened, 19 primer pairs were polymorphic. One to six alleles per primer were detected with polymorphic information content (PIC) varying from 0.107 (SSR-6608) to 0.656 (SSR-6613) with mean of 0.293 and allele frequency ranging from 0.136 (SSR-6371) to 0.841 (SSR-6608) with mean of 0.445. The SSR marker CP171F/172R successfully distinguished between 5 aphid resistant varieties and 15 aphid susceptible varieties. The aphid resistant locus from a donor (SARC-1-57-2) was then deployed into three locally preferred cultivars (Asontem, Nhyira and Asetenapa) in a marker assisted backcross up to BC_2 generation. After genotyping the BC_1 individuals, 63 individuals who

picked up the gene as revealed by the marker were selected to develop 261 BC_2 seeds. The characters documented in this research will guide selection in subsequent backcrosses; diversity observed can also be exploited by breeders for forensic identification of cultivars and also used in cowpea improvement programmes. This research has also deployed the aphid resistant locus into three locally preferred cultivars. Successive backcrosses will lead to the complete improvement of the three cultivars.

DEDICATION

This work is dedicated to my elder brother Dr. Daniel Kwabena Danso of University of Education, Winneba – Kumasi campus.

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ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ALCA	Average Linkage Cluster Analysis
ANOVA	Analysis of Variance
APS	Ammonium persulphate
BC	Backcrossing
bp	Base pair
CABMV	Cowpea aphid-borne mosaic virus
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
DNA	Deoxyribonucleic acid
GDP	Gross Domestic Product
FADW	Filtered Autoclaved Distilled Water
FAO	Food and Agriculture Organization of the United Nations
IBPGR	International Board for Plant Genetic Resources
IFAD	International Fund for Agricultural Development
IITA	International Institute of Tropical Agriculture
MABC	Marker Assisted Backcrossing
MAS	Marker Assisted Selection

PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphism DNA
RFLP	Restriction Fragment Length Polymorphism
SARI	Savannah Agricultural Research Institute
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TEMED	Tetramethylethylenediamine
v-PAGE	Vertical-Polyacrylamide Gel Electrophoresis

CHAPTER ONE

1.0 INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp] is a very important leguminous crop in Ghana and other tropical and sub-tropical countries (Singh *et al.*, 1990). Cowpea is a major source of protein to human diet and also cultivated as fodder for livestock (Ofuya, 1993). Cowpea is an early maturing crop that tolerates poor soil fertility and water stress (Souza *et al.*, 2004). This makes it an important food crop in areas hard hit with climate change, famine, poor soil fertility, poverty and high population growth rate (Souza *et al.*, 2004; Singh and Ajeigbe, 2007).

Nigeria and Niger among other West African countries cover more than 80% of total cowpea cultivated area in the world (F.A.O, 2016). Ghana produces less than 1% of the total world production (F.A.O, 2016); however, cowpea is an economic crop in Northern Ghana (Boukar *et al.,* 2011; MOFA, 2010). The yield per hectare of cowpea in Africa is relatively low compared to other parts of the world (Boukar *et al*., 2011). In 2014, the estimated average best yield of cowpea in Africa was 432 kg/ha as compared to Europe's average best yield of 3,383 kg/ha (F.A.O, 2016). Poor soil fertility, low yielding traditional varieties, insect pest and disease attack and unfavourable weather are among the reasons for low yields in most African countries including Ghana (Diehl and Sipkins, 1985; Montimore *et al.,* 1997).

Aphid infestation is recognized as one of the major constraints that limit cowpea production in Africa (Singh, 1990; Annan *et al.*, 1996; Hordzi, 2011; Kusi *et al.*, 2010a). The cowpea aphid (*Aphis craccivora*) is a soft-bodied, pear-shaped insect pest that primarily infests the seedlings of cowpea and causes direct damage on the crop by sucking plant sap, resulting in stunted plants and distorted leaves (Singh and Jackai,

1985; Summers *et al.*, 2006). The more damaging effect of cowpea aphid is the transmission of aphid–borne cowpea mosaic viruses (CABMV) which can reduce yield by 13-87% under field conditions (Atiri, 1984; Thottappilly and Rossel, 1985).

In Ghana, several adapted cowpea cultivars developed for improved yield, preferred seed colour, early maturity, abiotic stresses and high food quality by research institutions are susceptible to aphid infestation (Asare *et al*., 2010; Kusi *et al*., 2010b). As a consequence, farmers do not attain the expected yield and quality of such cultivars.

Chemical, cultural, physiological and biological control strategies have been developed to overcome this problem, some of which present severe side effects on the soil, food quality, yield and environment (Singh and Jackai, 1985; Erbaugh *et al.*, 1995; Ofuya, 1997; Monfankye, 2014). So far, Host-Plant Resistance has proven to be an efficient and adopted method of aphid resistance (Babura and Mustapha, 2012). Crop plant may also avoid damage from a pest through the mechanism of escape where the sensitive phases of development do not coincide with the optimum conditions for the pest's development (Caurtera *et al.*, 1999; Acquaah, 2012).

The best way to manage pest infestation in crop production is to integrate pest management techniques that include the use of resistant cultivars (Babura and Mustapha, 2012; Kusi, 2014). Several aphid – resistant cowpea lines have been developed and tested against aphid populations from several locations in Africa and Asia by IITA and other research institutions (Bata *et al.*,1987; Ofuya, 1997; Singh, 2004; Obeng-Ofori, 2007). This is usually done by the introgression of aphid resistant gene obtained from a resistant variety into an adapted variety which is susceptible to aphid infestation through backcrosses (Acquaah, 2012).

For a quick and reliable introgression of new traits into adapted cultivars, the application of DNA markers coupled with phenotypic screening has proven to be an efficient way to achieve one's objectives (Miah *et al.*, 2015). Through DNA Marker Assisted Selection (MAS), aphid resistance QTLs have been successfully transferred to susceptible cultivars (Boukar, 2008; Kusi, 2014).

The characterization of crops is the first step in any crop improvement programme (De Vicente *et al.*, 2005). It involves recording characters which are highly heritable and which can easily be scored visually in most environmental conditions (IBPGR, 1993). Cowpea varieties tend to have narrow range of adaptation due to the fact that, cultivars are developed for one agro-ecological zone and are usually not very productive in other zones (Agyemang *et al*., 2014).

It is also important to note that genetic diversity in the available gene pool is the foundation of all crop improvement programmes (Magloire, 2005). Analysis of SSR markers coupled with morphological markers have proven to be efficient means of genotype identification, diversity studies and associating molecular markers to phenotypic traits (Senior *et al.*, 1998).

1.1 Justification

More recently, Kusi (2014) identified the SSR marker CP171F/172R to be tightly linked to the aphid resistance gene locus in the resistant line SARC1-57-2. The resistant line SARC1-57-2 was further used to improve the susceptible Zaayura cultivar. Further tests showed that the aphid resistance gene locus found during the improvement of Zaayura was stable across the major cowpea growing regions in Ghana – Upper East, Upper West, Northern, Brong-Ahafo, Central and Volta regions (Kusi, 2014). The stability of the gene locus across environment indicates that this gene locus can be

reliably deployed into other susceptible cultivars. Over the years, the CSIR-Crops Research Institute and CSIR-Savannah Agricultural Research Institute have released cowpea varieties which are being grown all over the country and beyond. Released varieties (cultivars) such as Asontem, Nhyira and Asetenapa are high yielding and adapted to the forest transition, coastal and Savannah areas but they are susceptible to *Aphis craccivora* (Egbadzor *et al*., 2013). There is, therefore, the need to further develop these cultivars to have resistance to cowpea aphids.

The success of this work will ensure that farmers obtain aphid resistant cultivars that possess the qualities they were bred for and will further reduce the dependence on pesticides.

1.2 Objectives

The general objective of the study was to deploy the aphid resistant gene into susceptible cowpea cultivars through marker assisted backcrosses and specifically to:

1. characterize 22 cowpea genotypes using SSRs and agro-morphological markers.
2. discriminate between aphid resistant and susceptible cowpea varieties using the recommended marker CP 171F/172R.
3. deploy aphid resistant gene into three susceptible locally preferred cultivars by marker assisted backcrosses up to BC_2.
4. check gene introgression by subjecting improved cultivars to molecular screening.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Domestication of Cowpea

Cowpea [*Vigna unguiculata* (L.) Walp] is one of the most ancient crops known to man. Cowpea (2n = 2x = 22), belongs to the family Fabaceae and subfamily Faboideae (Pasquet, 2000). The history of cowpea dates back to ancient West African cereal farming, 5000 to 6000 years ago, where it was closely associated with the cultivation of sorghum and pearl millet (Davis *et al.*, 1991). West Africa is noted by most literature as the centre of diversity and origin of cowpea (Ba *et al.*, 2004; Gomez, 2004). The African origin of cowpea was suggested as early as 1847 (Richard, 1847), and since Piper (1913), no one is contesting it, since wild cowpea plants are found only in tropical Africa and Madagascar (Steele 1976). Cowpea has evolved from native wild types and its genetic diversity is greater than that of any other crop in the dry African savannah (IFAD, 2000). However, where the crop was first domesticated is still uncertain and different centres of diversity have been proposed, i.e. Northeast Africa (Vavilov, 1926; Steele, 1976; Pasquet, 2000), West Africa (Faris, 1965; Marèchal *et al.*, 1978; Ng, 1990; Vaillancourt and Weeden, 1992), Eastern and Southern Africa (Baudoin and Marechal, 1985) and India (Pant *et al.*, 1982). Evidence from amplified fragment length polymorphism (AFLP) analysis has also suggested North Eastern Africa to be the origin of domestication (Coulibaly *et al.*, 2002).

The wild cowpea *Vigna unguiculata* var. *spontanea* is the likely progenitor of cultivated cowpea (Pasquet, 1999). *Vigna unguiculata* ssp. Dekindtiana is thought to be the immediate progenitor of cultivated cowpea as members of this group can be hybridized with cultivated cowpea (Ehlers and Hall, 1996). Natural hybrids between cultivated

and wild (ssp dekindtiana) cowpeas occur and form "weedy" population in some parts of West Africa. In several studies assessing genetic variability based on isozymes (Panella and Gepts, 1992; Vaillancourt *et al.*, 1993), seed storage protein diversity (Panella *et al.*, 1993) and chloroplast DNA (Vaillancourt and Weeden, 1992), the cultivated cowpea has been shown to have a narrow genetic base suggesting the crop went through a 'genetic bottleneck' during domestication.

Cowpea has become an essential crop in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, Central and South America (Singh and Tarawali, 1997).

2.2 Cultivation and Production Level

Cowpea is well adapted to environmental stresses that affect crop production such as drought, high temperatures and other biotic stresses relative to other crops (Martin *et al.*, 1991).

The crop is of major importance to the livelihoods of millions of people in the tropics. For resource-poor small-holder farmers, the crop serves as food, animal feed, cash and manure (DAFF, 2011). Going beyond its importance for food and feed, cowpea can be regarded as a pivot of sustainable farming in regions characterized by systems of farming that make limited use of purchased inputs like inorganic fertilizer (DAFF, 2011). The crop can fix about 240 kg/ha of atmospheric nitrogen and make available about 60-70 kg/ha nitrogen for succeeding crops grown in rotation with it (Aikins and Afiukwa *et al.*, 2011).

Cowpea is now grown throughout the tropics and subtropics and has become a part of diet of about 110 million people (DAFF, 2011).

About 90% of the production and more than 95% of the area under production is spread over the vast Sudan Savanna and Sahelian zones of sub-Saharan Africa (FAO, 2016). This ranges from Senegal going east through Nigeria and Niger to the Sudan, in Kenya and Tanzania, and from Angola across Botswana to Mozambique (Timko *et al.*, 2007). Substantial quantities of cowpea are also produced in South America (largely in the semi-arid north eastern Brazil). As at 2014, the total area grown to cowpea globally was 12.6 million hectares and about 10.8 million hectares of these was in West Africa (FAO, 2016). The global annual production of cowpea in the year 2014 was 5.6 million tons, of which West Africa accounted for 81% (FAO, 2016). It has been reported that Nigeria, being the largest producer of cowpea in the world accounted for more than 2.1 million tons which represents about 38% of the total world cowpea production in the year 2014 (FAO, 2016). The average yield per hectare of cowpea in Nigeria was only 578 kg/ha in the year 2014 (FAO, 2016). In the year 2014, an average of 1.6 million tons of cowpea was produced by Niger; Burkina Faso producedan amount of 573,048 tons; Mali produced 149,248 tons (FAO, 2016). Ghana produces an average of 143,000 metric tons annually on about 156,000 hectares of land making Ghana the fifth highest producer of cowpea in Africa (MOFA, 2010; Boukar *et al.,* 2011). Cowpea is an important crop in Ghana due to its contribution to national GDP, farmers' incomes, food and nutrition security (CORAF/WECARD cowpea report, 2011). The per capita consumption of cowpea in Ghana is about 9 kg each year (Coulibaley *et al*., 2009). Commercial production is restricted to some parts of the Volta, Northern, Upper East, Upper West and Brong Ahafo regions (Tweneboah, 2000). However, Ghana still

imports about 10,000 tons of cowpea annually from Burkina Faso and Niger (Coulibaly *et al*., 2009).

2.3 Morpho-physiological Characteristics of Cowpea

Cowpea is an annual herb reaching heights of up to 80 cm with a strong taproot and many spreading lateral roots in the surface soil (Kay, 1979; Fox and Young, 1982). Growth forms vary and many are erect, trailing, climbing, or bushy, usually indeterminate growers under favourable conditions (Singh *et al*., 2002).

Leaves are alternate and trifoliate. The first pair of leaves is simple and opposite. Leaves exhibit considerable variation in size (5 – 16 cm x 3 -11 cm) and shape (linear, hastate, lanceolate, globose to ovate) and they are usually dark green (Timko *et al*., 2007). The leaf petiole is 5-25 cm long; the stems are straight, smooth or slightly hairy and sometimes tinged with purple (Ehlers *et al*., 1997).

The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are borne in alternate pairs, with usually only two to three flowers per inflorescence (Doumbia, 2012). Flowers are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, yellow, pink, pale blue or purple in colour (IBPGR, 1983; DAFF, 2011). Flowers open in the early day and close at approximately midday. After blooming (opening once) they wilt and collapse (DAFF, 2011).

Fruits are pods that vary in size, shape, colour and texture. They may be erect, crescent-shaped or coiled. They are usually yellow when ripe, but may also be brown or purple in colour (DAFF, 2011).

There are usually 8 - 20 seeds per pod (Ehlers and Hall, 1997). Seeds vary considerably in size, shape and colour. They are relatively large (2 -12 mm long) and weigh 5 -30 g/100 seeds. Seed shape is correlated with that of the pod; where individual seeds are

separate from adjacent ones during development, they become reniform (Ehlers and Hall, 1997), but as crowding within the pod increases, the seeds become globular. The testa may be smooth or wrinkled, white, green, buff, red, brown, black, speckled, blotched, eyed (hilum white surrounded by a dark ring) or mottled in colour.

Cowpea can thrive in dry environments and produce the dry grain yield of about 1000 kg/ha in a Sahelian environment with only 181 mm of rainfall and high evaporation rate (Hall and Patel, 1985). The optimum temperature for growth and development is around 30 °C. Varieties differ in their response to day length, some being insensitive and flowering within 30 days after sowing when grown at a temperature around 30 °C (Singh *et al*., 2002).

Cowpeas are grown on a wide range of soils but the crop shows a preference for sandy soils, which tend to be less restrictive on root growth (Singh *et al*., 2002). It is more tolerant to infertile and acid soils than many other crops (Ehlers and Hall, 1996). This adaptation to lighter soils is coupled with drought tolerance through reduced leaf growth, less water loss through stomata, and leaf movement to reduce light and heat load under stress (Wein and Summerfield, 1980).

2.4 Uses and Nutritional Value of Cowpea

Cowpea is usually utilized as grain crop, vegetable and fodder for livestock (Hall *et al.*, 2003). The primary use of cowpea is for the dry pu1se, green pods, green seeds, seedlings, and tender young leaves are often used as pot herbs (Singh *et al*., 2002). The vegetation also makes excellent hay, and the surplus culled and broken seeds can be used as a protein concentrate for domestic animals.

Cowpea is usually cooked and consumed whole or processed into flour for culinary preparations that include frying and steaming (Allen *et al*., 1998). Some important local names for cowpea include "Beng"in Dagari, "Ayi" in Ewe, "waakye", and "ewa" in much of West Africa and "caupi" in Brazil (Osseo-Asare, 2015). Malnutrition among the children in developing countries is mainly due to the consumption of cereal based meals which are bulky in carbohydrate with little or no proteins (Geissler *et al*., 1998). Cowpea provides protein constituent of the daily diet of the economically depressed rural class, due to its potential to reduce malnutrition; it is sometimes being referred to as "poor man's meat" (Geissler *et al*., 1998).

As a grain legume, cowpea is an important source of human dietary protein and calories. The grains contain about 25% proteins and 64% carbohydrate, while young leaves, pods and peas contain vitamins and minerals (Nielson *et al.*, 1993). The high protein and lysine content makes cowpea natural supplement for high carbohydrates, tubers and cereals, which are common staple foods among the sub-Saharan people (IITA, 1982). Other nutrients contained in cowpea seeds are 11% water, 5.9-7.3% crude fibre, 3.4-3.9% Ash, 1.3-1.5% Fat, 0.146% Phosphorus, 0.14% Calcium and 0.005% Iron (Kay, 1979; Gomez, 2004).

2.5 Insect Pest of Cowpea

Despite cowpea being a popular and nutritionally important crop in many parts of the tropical world, it is very susceptible to insect infestation both in the field and in storage (Fatokun, 2002; Adam and Baidoo, 2008). The activities of a spectrum of insect pest which ravage the crop in the field at different stages can result in low yields (Olatunde *et al*., 1991) and sometimes total yield losses and crop failure can occur (Singh and

Jackai, 1985). Studies have shown that in unprotected monocrops, yield losses due to the major serious field pests may range from 20-100% (Youdeowei, 1989). The pest problem is more serious in Africa than in Asia or Latin America (Singh and Jackai, 1985). Every stage in the life cycle of cowpea has at least one major insect pest (Tables 2.1) that can cause serious damage and impact yield negatively (Fatokun *et al*., 2002). The major insect pests which severely damage cowpea during all growth stages are the cowpea aphid (*Aphis craccivora* Koch), foliage beetles (*Ootheca* sp, *Medythia* spp), the flower bud thrips (*Megalurothrips sjostedti Trybom*) the legume pod borer (*Maruca vitrata* Fabricius) and the sucking bug complex, of which *Clavigralla* spp, *Anoplocnemis* spp, *Riptortus* spp, *Mirperus* spp, *Nezara viridula* Fab and *Aspavia armigera* L are most important and are prevalent (Jackai and Adalla, 1997). Without their control, reasonable grain yield cannot be obtained (Jackai and Daoust, 1986).

Table 2.1 Cowpea growth stages and pest incidence

Growth stages	Days after planting 20 30 40 50 60 70	Insect pests
Foliage	_____	Aphids, Leafhoppers, Foliage Beetle
Flower Budding	_____	Flower Thrips
Flowering	_____	Flower Thrips, pod borers
Podding	_____	Pod sucking bugs, pod borers
Late Podding	_____	Pod sucking bugs, pod borers (population decline due to crop senescence)
Spraying by growth stage	* * * * * *	

Source: Jackai and Adalla (1997)

2.5.1 Aphids

Aphids are small soft-bodied plant-sucking insects which occur throughout the world (Jackai and Adalla, 1997). They belong to the order Hemiptera and sub-order Homoptera (Summers *et al*., 2006). Aphids prefer plants in the Fabaceae (beans, peas and groundnuts) family, but they are highly polyphagous and have been found on many plant species (Dixon, 1985). They differ from other plant sucking bugs of the sub-family Aphodoidea by the fact that the females of at least a few generations are parthenogenetic and viviparous (Dixon, 1985). Although many species are small and inconspicuous, they become abundant in a short period. As many as 200 million aphids per acre may live on the above-ground parts of plants (Dixon, 1985).

2.5.1.1 The Cowpea Aphid, *Aphis craccivora* Koch.

The cowpea aphid is cosmopolitan in distribution, occurs in the temperate, subtropical and tropical regions of the world (Jackai and Adalla, 1997). This aphid is a soft-bodied, pear-shaped insect; has antennae which are shorter than the body length (about two third as long as the body) and a pair of cornicles (tailpipe-like appendages) (Summers *et al*., 2006).

Generally, apterae (wingless) are more common. Alate (winged) are produced under stressful conditions such as over-crowding and a decline in food quality. *Aphis craccivora* alatae have the dorsal shield broken up into segmental bands with large marginal and postsiphuncular sclerites. Cowpea aphid nymphs are pale green to grey with powdery coating (Summers *et al*., 2006).

Aphid colonies are often attended by ants in mutualistic symbiosis where the ants provide protection against some predators while the aphids supply honey dew

(Whitworth and Ahmad, 2009). Young and adult cowpea aphids feed by sucking plant juices. They are found primarily on the growing points of the host plant, including tips, flowers and developing bean pods (Singh *et al*., 1990).

2.5.1.2 Damage and economic importance of *Aphis craccivora* Koch

Aphis craccivora Koch (Homoptera: Aphididae) is phloem feeding insect, and a major insect pest of cowpea in Africa, Asia, and the Americas (Obeng-Ofori, 2007). In West Africa, during the last decade, aphid populations have continuously increased, consequently causing major losses (Singh *et al*., 1990).

Cowpea aphids usually feed on the young succulent terminal growth parts by piercing the plant tissue and penetrating the phloem with their needle-like mouthparts (Ross, 2000). They tend to feed in clusters on newly expanding leaves, blooms, and stems. They inject toxins into the plant while feeding which results in reduced plant vigour and yields (Whitworth and Ahmad, 2009). While feeding, aphids produce considerable amount of honeydew upon which sooty mold grows. The black sooty mold reduces photosynthesis and may make leaves unpalatable to livestock. The honeydew also makes the leaves of the legumes sticky, which causes problems with harvest for fodder (Ross, 2000).

The cowpea aphid also serves as a vector which transmits aphid-borne mosaic viruses such as cowpea mosaic virus (CABMV), cucumber mosaic virus and groundnut rosette virus to plant hosts such as cowpea (Bock and Conti, 1974).

The presence of sucking insects acts as a sink for the phloem, redirecting a large part of photosynthates away from the tissue for which it was intended and into the insect gut. In this way, an infestation of phloem feeding insects may interfere with the normal

partition of photosynthates between plant organs (Bardner and Fletcher, 1974). The intensity of pest attack can be described as the product of three effects: the numbers of the pest present, their development stage and the duration of the pest attack. It is the combination of these three factors in relation to the crop that influences crop yield (Dent, 1991). Singh and Allen (1980) estimated yield losses of 20% to 40% in cowpea due to *A. craccivora* infestation in Asia and up to 35% in Africa.

2.5.1.3 Reproduction and Life Cycle

In most places reproduction is entirely parthenogenetic with no sexual stage in the life cycle; however, sexual morphs have been recorded from Germany and India (Mackean, 2006).

Apterae and alate forms are always females that reproduce asexually and give birth to live young (Dixon, 1985; Schreiner, 2000). Within a few days, nymphs molt, shedding their skin four times from first instar to reproductive adult (Dagg, 2002). This rapid asexual reproduction is the key to the dramatic population growth achieved by the cowpea aphid in a short time (Dagg, 2002).

The reproduction system of the parthenogenetic aphid consists of ovarioles (the number of which is determined prior to birth) that contain the developing embryos (Wellings *et al*., 1980). Each ovariole usually contains several embryos at different stages of development (Dixon, 1985). There are many factors, both intrinsic and extrinsic, that can affect an aphid's reproductive capacity (Dixon, 1985). The reproductive capacity correlates positively with adult weight (Wellings *et al*., 1980). It may either increase or

decline with nutritional quality (Wellings *et al*., 1980), and may be correlated positively or negatively with ovariole number (Dixon, 1985).

2.5.1.4 Management of *Aphis craccivora* Koch

The control of *A. craccivora* involves a number of strategies including the use of insecticides, biological control, cultural practices and host plant-resistance (Jackai and Adalla, 1997).

Majority of African farmers still rely on indigenous pest control approaches to manage pest problems, although many government extension programmes encourage the use of pesticides (Kusi, 2014). Sometimes, however, farmers spray their farms as many as eight to ten times during the growing season (Omongo *et al*., 1998). Insecticides that have been reported to be effective against *A. Craccivora* include: carbofuran granules which when applied to the soil gives good control of A. *craccivora* infestation at the cowpea seedling stage (Jackai and Dacoust, 1986). Foliar application of phosphamidon, dimethoate, thiometon and pirimicarb are effective against the pest (Jackai and Dacoust, 1986). Lambda cyhalothrin, a synthetic pyrethroid, at the rate of 20 g active ingredient ha^{-1} has been one of the most common insecticides used in Ghana for the control of cowpea aphid (Kusi *et al*., 2010b).

Increased applications of insecticides, however, have many associated hazards such as toxicity to humans and other animals, development of resistance by insects, and pollution of the environment as most of them are non-biodegradable (Jackai and Adalla, 1997).

Baidoo *et al.* (2013) conducted a trial in Ghana to compare two neem-based products with lambda cyahalothrin for control of *Aphis craccivora* on cowpea. Significantly more cowpea aphids were collected on the control plots than on the treated plots. The numbers of aphids on the two types of neem-treated plots did not differ significantly. The two neem products were effective in reducing the cowpea aphid population.

Current pest management research activities carried out by national and international agricultural research programmes in Africa focus on biological control and breeding of resistant host plants (Kusi *et al.*, 2010b). The potential of using natural enemies to control insect pest is much higher in the tropics than in the temperate regions due to the high arthropod diversity and year-round activity of natural enemies (Gullan and Cranston, 1994).

Cowpea aphids are usually eaten by predators such as lady beetles, lacewings, big eyed bugs, damsel bugs, and syrphid flies. The presence of ladybeetle is an indication that aphids are present (Whitworth and Ahmad, 2009).

Munyuli (2009) conducted experiments in the Democratic Republic of Congo to investigate the effect of insect predators on *Aphis craccivora* and the yield of groundnut and cowpea. The assays were conducted using split-plot field-cage experiments and three treatments involved insecticide sprays, release of a mixture of indigenous insect predators (ladybeetles, syrphid larvae and earwigs) and a control. The results indicated that the release of predators significantly reduced the aphid population in the cowpea and groundnut fields. Overall yields were lower in the control treatments, and no significant differences were observed in field cages treated either with predators or with insecticides. In comparison to control treatments, biological control with predators increased yield by up to 58% and 66% for groundnut and cowpea respectively. This

approach was recommended to small-scale farmers in order to obtain better yield and marginal economic return at low cost of production.

Some cultural practices can also help reduce *A. Craccivora* populations. For instance, the use of border-strip cutting during harvest helps maintain populations of parasites and predators in balance. Heavy rainfall can reduce populations significantly (Singh *et al.*, 1985).

The uses of biological and cultural controls are acceptable on organically certified crops (Whitworth and Ahmad, 2009). Organically certified insecticides such as azadirachtin (Neemix), neem oil (Trilogy), and pyrethrin (PyGanic) are registered for use on legumes to control aphids. Studies conducted in California, however, have shown that at best they provide some suppression of populations but do not control them (Whitworth and Ahmad, 2009).

2.5.2 Host-Plant Resistance

Snelling (1941) defined resistance as "including those mechanisms which enable a plant to avoid, tolerate or recover from attacks of insects under conditions that will cause great injury to other plants of the same species". Kumar (1984) also defined resistance broadly as the inherent ability of a crop plant to restrict, retard or overcome pest infestation and thereby improve the yield and/or the quality of the harvestable crop product. Some crops also avoid damage from pest by an escape mechanism where the sensitive phases of development do not coincide with the optimum conditions for pest's development (Cuatera *et al*., 1999). A plant is said to be resistant when a pest cannot

establish a compatible relationship under any condition with a certain plant genotype (Painter, 1951).

From the point of view of the farmer, horticulturalist and other stakeholders of agriculture, the use of resistant cultivars represents one of the simplest and the most convenient methods of insect pest control provided that the cultivar does not require expensive input of fertilizer in order to guarantee high yields (Egbadzor *et al*., 2013).

Using host-plant resistance as a pest control method comes along with its own merits and demerits. Some of the merits include: (i) it is relatively inexpensive. Usually, seed of resistant cultivars is not more expensive than seed of no-resistant cultivars; (ii) the technique is easy to apply because the grower only has to buy seeds of resistant cultivars; (iii) completely resistant cultivars need much no chemicals for pest control and even partially resistant cultivars need much less to control pests; (iv) resistant cultivars can be incorporated into integrated pest management programmes and when combined with biological control they have cumulative effect; (v) adverse environmental effects are minimal or nil, pollution is much reduced; and (vi) resistant cultivars, except transgenic cultivars, are acceptable to public (Kumar, 1984).

Some of the disadvantages of resistant cultivars are: (i) it takes a long time to develop a resistant cultivar; (ii) resistant cultivars may control only one pest, while pesticides are often effective against several pests; (iii) resistance must be introduced in each new cultivar; and (iv) the pest may adapt to the resistance and this limits the durability of resistant cultivars (Kumar, 1984). The disadvantages of resistant cultivars are, however, much less than their advantages.

Public concerns about the effects of pesticides have compelled governments to make laws to reduce the use of pesticides (Baidoo *et al*., 2006). The best way to avoid or

reduce the use of pesticides in crop production is to introduce integrated pest management techniques that include the use of resistant cultivars (Jackai and Adalla, 1997). Consequently the prospects for the future development of many more resistant cultivars appear promising.

Host plant resistance can be broken into three categories: non-preference (interference with insect behaviour), antibiosis (interference with insect biology), and tolerance (Painter, 1951). The non-preference has since been replaced by antixenosis (Kogan and Omar, 1978).

2.5.2.1 Antixenosis

Antixenosis is the inability of a plant to serve as host to an insect herbivore (Eickhoff *et al.*, 2006). The basis of this resistance mechanism can be morphological (e.g. leaf hairs, surface waxes, tissue thickness) or chemical (e.g. repellents or antifeedants). These plants would have reduced initial infestation and/or higher migration rate of the insect than susceptible plants. Some plant morphological characteristics that can interfere with or modify the behaviour of the insect are colour, shape, type of cuticle wax, hairiness of plant stalks and leaves (Kogan and Omar, 1978).

2.5.2.2 Antibiosis

Plants produce a wide variety of defensive compounds (allelochemicals) that protect them from herbivores (Eickhoff *et al*., 2008). These compounds may reduce growth, inhibit reproduction, alter physiology, delay maturation, or induce various physical or behavioural abnormalities in herbivores. By purposely selecting for plants with high levels of allelochemicals, or by breeding such plants with less resistant ones, it is often

possible to develop new cultivars that resist pest injury yet still retain desirable horticultural characteristics (Eickhoff *et al*., 2008).

Often, both a resistant and susceptible variety will have the same basic response to a pest, but the resistant variety will respond more quickly or more dramatically than the susceptible variety, reducing the amount of damage the pest causes. Plants that express antibiosis affect the biology of pests.

Dahms (1972) illustrated the antibiotic effectof resistant plant on differential rate of aphid development. Nymphs matured in five days (susceptible variety), 10 days (intermediate antibiosis) and 20 days (high antibiosis). Mortality of immature arthropods was one of the most important factors limiting the increase of arthropod population, which was also illustrated by Dahms (1972).

2.5.2.3 Tolerance

Plant tolerance is the degree to which a plant can support an insect population that under similar conditions would severely damage a susceptible plant (Cuartera *et al*., 1999). When two cultivars are equally infested, the less tolerant one produces low yield. A tolerant plant may be colonized by a pest to the same extent as susceptible plants, but there is no reduction in yield both in quantity and quality (Eickhoff *et al*., 2008). Plants with an ability to tolerate insect damage at times may produce more yield than the plants of a non-tolerant susceptible cultivar at the same level of insect infestation. Tolerance often occurs in combination with antixenosis and antibiosis components of resistance (Sharma, 2009).

2.5.2.4 Resistance of Cowpea to *Aphis craccivora* Koch

Antibiosis has been identified as the main mechanism responsible for aphid resistance in cowpea (Singh, 1977; Ansari, 1984; Ofuya, 1988) and is controlled by a single dominant gene (Singh and Ntare, 1985; Bata *et al*., 1987; Ombakho *et al*., 1987; Pathak, 1988).

A large number of aphid resistant lines have been developed and evaluated in international yield trials. These lines, which need no insecticide protection against aphids include, TVu36, TVu300, TVu310, TVu410, TVu2996, TVu3000, IT84S-2246, IT87S-1459, IT84S-2049 and IT93K-503-1 (Bata*et al*., 1987; Ofuya, 1997). Others include IT8S-728-5, IT83S-728-13, IT83S-742-2, IT84E-1-108 (Obeng-Ofori, 2007). Other aphid resistant varieties include: IT90K-59, IT90K-76, IT97K-499-35 and IT00K-1251 (Singh, 2004). The resistance in genotype IT84S-2246 is the source of resistance in varieties IT90K-59, IT90K-76, IT97K-499-35 and IT00K-1251 (Singh, 2004).

Screening for aphid resistance has been conducted in IITA and several resistant lines have been identified (Singh and Jackai, 1985) and used in the breeding programmes to develop aphid resistant cultivars (Singh and Ntare, 1985). However, the resistance to *A. craccivora* of all the identified cowpea cultivars at IITA has recently broken down (Sharma, 2009).

In response to breakdown of resistance in several aphid resistant varieties, new resistant cultivars have been developed in recent studies. Souleymane *et al*. (2013) and Kusi *et al.* (2010a) conducted separate experiments to search for new sources of resistance in African cowpea. Souleymane *et al.* (2013) screened 109 cowpea cultivars and 92 wild cowpea accessions in Nigeria to search for new source of resistance to A. *craccivora*.

IT97K-556-6 showed the highest level of tolerance and was recommended for breeding programmes aimed at introgressing aphid resistance gene into susceptible cultivars.

SARC-1-57-2 was identified as resistant to A. *craccivora* among 22 cowpea varieties evaluated with seedling screening method (Kusi *et al*., 2010a). The stability of SARC-1-57-2 was further tested in all major cowpea growing zones in Ghana. The study confirmed that the aphid resistance gene in SARC-1-57-2 is stable against A. *craccivora* in all major cowpea growing belts of Ghana. SARC-1-57-2 is an advanced line (F_6) developed from a cross between Apabgaala and UCR 01-11-52 (Kusi *et al*., 2010a). Inheritance of aphid resistance gene follows the mendallian inheritance pattern.

2.6 Role of Characterization in crop improvement programmes

Characterization of crops is the first step in any crop improvement programme (De Vicente *et al.*, 2005). Characterization helps in diversity studies, identification, and in the selection of suitably diverse parents to obtain heterotic hybrids. It is also needed for germplasm conservation purposes. Various morphological, biochemical and molecular markers are used for the characterization of cowpea germplasm.

2.6.1 Morphological characterization

Morphological Characterization involves recording characters which are highly heritable and can easily be scored visually in most environmental conditions (IBPGR, 1993).

The main advantages of conducting morphological characterization are that: (i) published descriptor lists are readily available for most major crop species including *Vigna unguiculata* (L.) *Walp*, (ii) it can be carried out *in situ* (on-farm) and (iii) it is

relatively inexpensive and relatively easy to carry out (Hoogendijk and Williams, 2001).

Morphological markers are analysed at the phenotype level and comes along with the following disadvantages; they are influenced by environmental conditions, they are labour intensive and require large populations of plants in performing breeding experiments. Moreover, they need large plots of land and/or greenhouse space in which to be grown; but they have remained useful till now as a highly recommended first step that should be undertaken before more in-depth biochemical or molecular studies (e.g. DNA fingerprinting) are attempted (Smith & Smith, 1992).

The agro-morphological variability in several cowpea varieties have been studied across the world (Ghalmi *et al.,* 2010; Aminasaun e*t al.*, 2015; Bennett-Lartey and Ofori, 2000). However, there is little information on the agro-morphological characteristics on newly improved cultivars. Recent agro-morphological characterization studies on cowpea varieties focus on parameters that benefits breeding for early maturity, pest and disease resistance and resistance to parasitic weeds (Singh *et al*., 1997). On the other hand, other characteristics such as number of pods per plant, number of days to flowering, growth habit, flower colour, pod shape etc have been less researched (Cobbinah *et al.*, 2011).

2.6.2 Molecular marker-based characterization

The limitations in morphological characterization led to the development of molecular makers. Molecular marker-based characterization also known as DNA fingerprinting is a useful complement to morphological and physiological characterization of varieties. Molecular maker techniques are based on naturally occurring polymorphisms in DNA sequences.

Fingerprinting with molecular markers allows precise, objective and rapid variety identification of plant varieties (Beleke and Beleke, 2014). Molecular makers are stable and detectable in all tissues regardless of growth differentiation, development, pleitropic effect, epistatic effects and not confounded to environment where they grow (Bhat, 1995; Milee *et al*, 2008). There are several types of genetic markers and the choice of a particular marker depends on the level of diversity information needed. Some of the molecular markers used for molecular characterization are Restriction fragment length polymorphism (RFLP), Random amplified polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple sequence repeats (SSRs) or short tandem repeats and Single Nucleotide Polymorphism (SNPs). One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis (Mohan *et al*., 1997).

2.6.2.1 Simple Sequence Repeats (SSR) markers

Simple Sequence Repeats (SSRs), also known as Microsatellites, are co-dominant markers that are routinely used to study genetic diversity in different crop species. These markers occur at high frequency and appear to be distributed throughout the genome of higher plants. These are DNA sequences that consist of two to five nucleotide core units such as (AT)n, (CTT)n and (ATGT)n, which are tandem repeats. The regions flanking the microsatellites are generally conserved among varieties of the same species, allowing the selection of PCR primers that will amplify the intervening SSR in all varieties. Variation in the number of tandem repeats, n, results in different

PCR product lengths. Due to mutations these repeats are highly polymorphic even among closely related cultivars, causing variations in the number of repeating units. They can detect a large number of alleles; level of heterozygosity is high and follows Mendelian inheritance (Wu and Tanksley 1993). Microsatellites have become the molecular markers of choice for a wide range of applications in genetic mapping and genome analysis (Li *et al.,* 2001), genotype identification and variety protection (Senior *et al*. 1998), seed purity evaluation and germplasm conservation (Brown *et al*., 1996), diversity studies (Xiao *et al*., 1996), paternity determination and pedigree analysis (Ayres *et al*. 1997), gene and quantitative trait locus analysis (Blair and McCouch 1997) and marker-assisted breeding (Weising *et al*., 2005). For identification of molecular markers linked to agronomically important genes, SSR is one of the best choices as compared to RAPD and AFLP for high polymorphic information and less costly (Young, 1999, Appiah-Kubi *et al*., 2014). Doumbia *et al.* (2014) used 20 SSR markers to characterise 94 accessions of cowpea from Ghana and Mali and established the genetic relationship that exist among the accessions.

2.6.2.2 Marker-assisted selection (MAS)

A major breakthrough to plant breeding is the use of molecular markers to select varieties not only for qualitative traits but also for quantitative traitsloci (QTLs). Many agriculturaly important variations like productivity, quality, tolerance to environmental stresses, and some types of disease and pest resistance are controlled by QTLs and greatly depend on genetic x environmental interactions (Abdurakhmonov and Adukarimov, 2008).

A marker can either be located within the gene of interest or be linked to a gene determining a gene of interest (Brown *et al*., 1996). Such markers can be detected early in the selection procedure thus the breeder can significantly reduce the number of seedlings grown and screened. This helps to reduce expenses and to increase efficiency of breeding (Kurt *et al.,* 2005). MAS can aid selecting for all target alleles that are difficult to assay phenotypically especially in the early generations. MAS also helps distinguish between heterozygote and homozygote varieties based on molecular data (Acquaah, 2012).

2.6.2.3 Marker assisted backcrossing (MABC)

Marker assisted backcrossing is a method in plant breeding to transfer favourable traits from a donor plant into an elite genotype (Acquaah, 2012). Markers can be used in MABC to either control the target gene or to accelerate the reconstruction of the recurrent parent genotype. Traditional backcross breeding requires 6 -8 backcrosses to fully reconstruct recurrent parent genotype, while MABC may reduce this to 3 – 5 generations. The theoretical proportion of the recurrent parent genome after n generations of backcrossing is given by $(2n+1-1)/2n+1$ (where n = number of backcrosses; assuming an infinite population size) (Acquaah, 2012). The percentages of recurrent parent recovery after each backcross generation are presented in Table 2.2.

Table 2.2 Percentage recurrent parent genome after backcrossing

Generation	Recurrent parent genome (%)
BC1	75.0
BC2	87.5
BC3	93.8
BC4	96.9
BC5	98.4
BC6	99.2

Source: Acquaah, 2012

Although the initial cost of marker-assisted backcrossing would be more expensive compared to conventional breeding in the short term, the time savings could lead to economic benefits. This is an important consideration for plant breeders because the accelerated release of an improved variety may translate into more rapid profits by the release of new cultivars in the medium term to long-term (Morris *et al*., 2003).

2.6.2.4 The Aphid resistance marker CP 171F/172R

Kusi (2014) screened 50 DNA markers (SSR primers) and identified the marker CP 171F/172R (left sequence: 5' – GTAGGGAGTTGGCCACGATA – 3'; Right sequence: 3'-CAACCGATGTAAAAAGTGGACA-5') to be tightly linked to the aphid resistance locus. The marker displayed a segregation pattern at band size of 176bp, consistent with the phenotypic scores obtained following aphid infestation of 128 lines in an earlier experiment conducted by the same author. CP 171F/172R was co-dominant and segregated in the expected 1:2:1 fashion following chi square analysis. The marker CP

171F/172R is about 50 cM away from the aphid resitance locus on linkage group 2 (Andargie *et al*., 2011; Menendez *et al.*, 1997).

2.6.3 Description of some locally preferred cowpea varieties

2.6.3.1 Nhyira (IT87D-611-3)

Nhyira, which was released by CSIR-CRI in 2005, is one of the farmer preferred cowpea varieties in Ghana (Egbadzor *et al*., 2013). The name Nhyira is the Akan word for blessing. This early maturing variety (65-68 days) is high yielding with average annual yield of 2,460 Kg/ha (CSIR-CRI, 2006). It has rough globose seeds, and grows to a height of 57cm without any pubescence on either stem or leaf. The leaf is broad, green in colour and obcuneate in shape. The flower is purple in colour and mid vein is light green. The immature pods are green and the mature pods are brown in colour (Agyemang *et al*., 2014). Nhyira has an erect growth habit and also drought tolerant. Nhyira is rich in energy, iron, phosphorus and protein (Agyemang *et al*., 2014). It is also resistant to Anthracnose and Cercospora leaf spot disease. Nhyira is moderately resistant to many viruses and tolerant to leaf hoppers (CSIR-CRI, 2006; Kusi, 2014). The variety is cultivated in all the six cowpea growing regions in Ghana. Farmers prefer it for its resistance to pest, disease and drought while consumers also prefer it due to its early cooking and sweetness (CSIR-CRI, 2010).

2.6.3.2 Asontem (IT82D-32)

The name Asontem is an Akan word meaning early maturing (65 – 70days). This variety was developed by the International Institute of Tropical Agriculture (IITA) and

introduced in Ghana by CSIR-CRI in 2005 (Asafo-Adjei *et al.*, 2005). It produces red and medium sized smooth seeds weighing about 15 g/100 seeds (CSIR-CRI, 2006). Asontem has a semi-erect growth pattern. Though some consumers reject it for its red colour (Egbadzor *et al*., 2013), others prefer the colour especially for the preparation of certain foods such as waakye in Ghana. Public perception ascribe red seed colour to high nutrients. This makes Asontem one of the consumer preferred varieties. Farmers also ascribe red seed colour to pest and disease resistance thus making Asontem one of the farmer preferred varieties.

2.6.3.3 Asetenapa (IT32D-1951)

The name Asetenapa is an Akan word which means good living. It is an early maturing cowpea variety released by CSIR-CRI in 1991. It bears smooth, ovoid seeds and grows to a height of 53 cm without any pubescence on either the stem or the leaf. The leaves are broad and are borne on long peduncles (CSIR-CRI, 2010). The leaf is green in colour and obcuneate in shape. The flower is whitish cream and mid-vein colour is light green (Agyemang *et al*., 2014). The immature pods are bright green and almost straight. Asetenapa seeds contain 11.2% moisture, 22.6% protein, 0.9% fat, 3.5% ash, 61.7% carbohydrate, 345.6% energy, 164.5 mg/100g calcium, 6.7 mg/100g iron and 828.0 mg/100g phosphorus (Agyemang *et al*., 2014). Asetenapa is highly preferred in the local market for its seed colour, taste and early cooking. Farmers also prefer it for its high yield (2,500Kg/ha) (Egbadzor *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

Following the identification of SARC-1-57-2 as a credible source of resistance to the cowpea aphid, it is now possible to use this gene to improve upon the field resistance of existing cowpea cultivars (Kusi, 2014). While doing this, it is also important to maintain the identity of the cultivars.

3.1 Collection of Germplasm

The cowpea germplasm for the project was made up of 19 released varieties, 1 advanced line, 1 exotic line and 1 landrace collected from CSIR-CRI and CSIR-SARI (Table 3.1).

Table 3.1 Cowpea varieties evaluated for genetic diversity and aphid resistance

No.	Local Name	Accession Name	Source of material	Status	Year of Release
1	Hewale	IT93K-192-4	CSIR-CRI	Released variety	2012
2	Asomdwoe	IT94K-410-2	CSIR-CRI	Released variety	2012
3	Videza	IT95K-142-20	CSIR-CRI	Released variety	2012
4	Nhyira	IT87D-611-3	CSIR-CRI	Released variety	2005
5	Tona	IT87D-2075	CSIR-CRI	Released variety	2005
6	Asetenapa	IT32D-1951	CSIR-CRI	Released variety	1999
7	Adom	CR-06-07	CSIR-CRI	Released variety	1999
8	Ayiyi	IT83S-728-13	CSIR-CRI	Released variety	1992
9	Bengpla	IT83S-818	CSIR-CRI	Released variety	1992

Table 3.1 Continued

No.	Local Name	Accession Name	Source of material	Status	Year of Release
10	Asontem	IT82D-32	CSIR-CRI	Released variety	1999
11	Soronko	TVX2724-OIF	CSIR-CRI	Released variety	1999
12	Agyenkwa	11(8)-1	CSIR-CRI	Released variety	2015
13	Zamzam	11(9)-5	CSIR-CRI	Released variety	2015
14	Hansadua	11(9)-2	CSIR-CRI	Released variety	2015
15	Nketewade	11(9)-3	CSIR-CRI	Released variety	2015
16	Zaayura	SARC4-75	CSIR-SARI	Released variety	2008
17	Songotra	IT97K-499-35	CSIR-SARI	Released variety	2008
18	Padi-Tuya	SARC3-122-2	CSIR-SARI	Released variety	2008
19	Apabgaala	ITXP48-2	CSIR-SARI	Released variety	2003
20		SARC-1-57-2	CSIR-SARI	Advanced line	
21	Sanzi		Northern Ghana	Landrace	
22	Bra-01		Brazil	Exotic	

3.2 Agro-Morphological and Molecular Characterization of Cowpea Varieties

DNA fingerprinting in addition to agro-morphological characterization of plant varieties is an important stage in any breeding programme which involves artificial selection of materials. This project involved the selection of backcrossed materials which look exactly as the parent material except for the inclusion of the cowpea aphid resistant

gene. It was therefore important to take data on the molecular and agro-morphological traits of the parent materials before the start of the marker assisted backcross.

3.2.1 Agro-morphological Characterization of Cowpea Varieties

This study was conducted in the minor rainy season of Ghana on the experimental fields of CSIR-CRI, Fumesua. The varieties were planted on 4th August, 2015 and harvested from 3rd to 7th November, 2015. The weather condition within the period of the study is presented in Appendix 1. The experimental field was ploughed and harrowed with a tractor. The field was lined and pegged and divided into 3 blocks with the slope of the land being the source of variation. Ten seedlings of each variety (with 20 cm spacing) were sowed on a 100 cm x 60 cm ridge. In all, there were 22 ridges in each of the 3blocks. The few rains were supplemented with manual irrigation when necessary. Two manual weedings were done on the experimental site. The first weeding was done in the third week after planting and the second weeding in the sixth week after planting. No fertilizer was applied since the objective was to characterise the varieties under farmer's field conditions. However, insects were controlled with the Lambda power® insecticide.

A total of 38 agro-morphological traits (Table 3.2) made up of 14 quantitative traits and 24 qualitative traits were measured using the IBPGR cowpea descriptors with slight modifications.

Table 3.2 List of Agro-morphological traits measured

	Qualitative Traits	Quantitative Traits
1	Pod pigmentation	Pod length (PL)
2	Leaf base pigmentation	Number of locules (NL)
3	Node pigmentation	Number of Seeds per pod (NSP)
4	Petiole pigmentation	Seed length (SL)
5	Branches pigmentation	Seed width (SW)
6	Stem pigmentation	Number of pods per peduncle (NPP)
7	Peduncle pigmentation	Number of peduncles (NP)
8	Sepal pigmentation	Leaf length (LL)
9	Twinning tendency	Leaf width (LW)
10	Raceme position	100 seed weight (SWg)
11	Petiole hairs	Number of pods per plant (NPPP)
12	Stem hairs	Plant height (PH)
13	Leaf texture	Leaf Area Index (LL/LW)
14	Leaf shape	Number of Days to 50% flowering (ND50F)
15	Leaf apex shape	
16	Leaf base shape	
17	Growth habit	
18	Seed colour	
19	Seed eye colour	
20	Flower colour	
21	Pod shape	
22	Pod colour at 40 days	
23	Pod tip shape	
24	Seed shape	

3.2.1.1 Qualitative traits

3.2.1.1.1 Growth habit

Growth habit was evaluated in the 6th week after planting. The varieties were classified as;

0 Erect (branching angle less acute than above)

1 Semi-erect (branches perpendicular to main stem, but not touch ground)

2 Intermediate (most lower branches touch ground)

3 Semi-prostate (main stem reaches 20 or more centimetres)

4 Prostrate (plants flat on the ground; branches spread several metres)

5 Climbing

3.2.1.1.2 Plant pigmentation

Pigmentation status was recorded for stem, branches, petioles, peduncles and sepals in the 6th week after sowing. The level of pigmentation was classified as;

0 Not pigmented

1 Very slight

2 Moderate at the base and tips of petioles

3 Intermediate

4 Extensive

5 Solid

Node and Leaf base pigmentations were simply classified as;

0 Not pigmented

1 Pigmented

Pod pigmentation was also classified at three levels;

0 Not pigmented

1 Pod tip pigmented

2 Entire pod pigmented

3.2.1.1.3 Terminal leaflet shape

The leaf shape for the terminal leaflet of a young, mature leaf in the 6th week after sowing was recorded for each variety as follows;

1 Sub-globose

2 Globose

3 Sub-hastate

4 Hastate

The terminal leaf apex shape was also recorded for each variety as;

1 Acute

2 Oval

The shape of the leaf base of the terminal leaf was also recorded for each variety as;

1 Cuneate

2 Semi-hastate

3 Hastate

3.2.1.1.4 Twinning tendency

The twining tendency for each variety was classified as;

0 None

1 Slight

2 Intermediate

3 Pronounced

3.2.1.1.5 Plant hairiness

Varieties were classified based on the plant hairiness on the stems, leaves and petioles in the 6th week as;

0 Smooth

1 Hairy

3.2.1.1.6 Raceme position

The raceme position for the varieties were recorded in the 6th week after sowing and classified as;

1 Mostly above canopy

2 In upper canopy

3 Throughout canopy

3.2.1.1.7 Immature pod colour

The colour for the immature pod of each varietywas classified as;

1 Green

2 Green with purple tips

3 Purple-green

4 Purple

3.2.1.1.8 Pod shape

The pod shape for each variety was classified as;

1 Straight

2 Bent

3 S-Shaped

4 Sickle

The pod tip shape was also classified as;

1 Blunt

2 Pointed

3.2.1.1.9 Flower colour

The flower colour of each plant was recorded after comparison with a colour chart.

3.2.1.1.10 Seed shape

Seed shape was classified as;

1 Kidney

2 Ovoid

3 Crowder

4 Globose

5 Rhomboid

3.2.1.1.11 Seed colour

The seed colour of each plant was recorded after comparison with a colour chart.

3.2.1.1.12 Seed eye colour

The seed eye colour of each plant was recorded after comparison with a colour chart.

3.2.1.2 Quantitative traits

3.2.1.2.1 Plant height

The plant height was scored as the linear distance between the apex of the plant and the soil surface along the main axis of the plant with a thread and later stretched on a meter rule to ascertain the real numerical value. Values obtained were recorded to the nearest centimetre.

3.2.1.2.2 Leaf length

The leaf length of an individual cowpea plant was measured from the leaf base along the midrib to the leaf apex with a 15 cm rule. Leaf length was measured as the mean of three randomly selected terminal leaflets to the nearest centimetre.

3.2.1.2.3 Leaf width

Leaf width was scored by using a 15 cm rule to measure the widest width of the terminal leaflets used in sub section 3.2.1.2.2. The mean width was calculated and recorded to the nearest centimetre.

3.2.1.2.4 Leaf Area Index

The leaf area index was calculated as the ratio of leaf length to leaf width of each terminal leaflet measured in subsections 3.2.1.2.2 and 3.2.1.2.3.

3.2.1.2.5 Days to 50% flowering

This involved recording the number of days that elapsed from the day of sowing to the stage when 50% of plants had begun to flower for all varieties.

3.2.1.2.6 Pod length

The pod length was recorded as mean of the 10 longest pods measured from 5 randomly selected plants. The measurement was done with a 30 cm thread and later stretched on a 30 cm rule to ascertain the real numerical value.

3.2.1.2.7 Number of locules per pod

The mean number of locules per pod was counted for the 10 pods used in subsection 3.2.1.2.6.

3.2.1.2.8 Number of seeds per pod

The mean number of seeds per pod was counted for the 10 pods used in subsection 3.2.1.2.6 for consistency.

3.2.1.2.9 Number of pods per peduncle

This was recorded as the mean number of pods of 10 randomly selected peduncles for each variety.

3.2.1.2.10 Number of peduncles per plant

The mean number of peduncle per plant for 5 randomly selected plants was recorded for each variety.

3.2.1.2.11 Number of pods per plant

The mean number of pods per plant for 5 randomly selected plants was recorded for each variety.

3.2.1.2.12 Seed length and Seed width

The mean length and width of 10 randomly selected seeds of each variety were recorded with the aid of a micrometre screw gauge to the nearest centimetre.

3.2.1.2.13 Hundred Seed Weight (g)

After threshing, 100 seeds of each variety were weighted on an electronic balance to ascertain the mass to the nearest gramme.

3.2.2 Molecular characterization of cowpea varieties

The molecular work of this project was conducted in the Biotechnology laboratory of the CSIR-Crops Research Institute, Kumasi – Ghana.

3.2.2.1 DNA extraction

Leaf explants used for this study were harvested from two week old seedlings and kept in liquid nitrogen. The DNA was extracted using DNeasy™ Plant Mini extraction kit (Qiagen, Germany) following the methodology below:

For each cowpea variety, 100 mg of leaf tissue was weighed and ground in 2 ml eppendorf tubes with the aid of liquid nitrogen. Buffer AP1 (400 µl) and RNase A (100 mg/ml) were added and vortexed vigorously. The mixture was then incubated at 65ºC for 10 minutes, mixed 2-3 times by inversion during incubation. A hundred and thirty microlitres of Buffer AP 2 was added to lysate, mixed and incubated on ice for 5 minutes. This was followed by centrifugation for 14000 rpm for 5 minutes. The lysate was pipetted into the QIA shredder min spin column and centrifuged at 14000 rpm for 2 minutes. The flow-through fraction was transferred into a new eppendorf tube without disturbing the debris pellet. 1.5 volumes of Buffer AP 3/E was added to lysate followed by mixing. A volume of 650 µl of the mixture including any precipitate was pipetted into the DNeasy mini spin column and then centrifuged at 8000 rpm for 1 minute. This step was repeated again after the flow-through was discarded. The DNeasy mini spin column was placed into a new 2 ml collection tube and 500 µl Buffer AW added. The mixture was centrifuged at 8000 rpm for 1 minute. The flow through was discarded and the collection tube reused. Five hundred microlitres of Buffer AW was added to the DNeasy min spin column and centrifuged at 14000 rpm for 2 minutes and then empty spanned for 2 minutes. The DNeasy min spin column was transferred to a new 1.5 ml ependorf tube and 50 µl Buffer AE added. The mixture was incubated at room temperature for 10 minutes, centrifuged at 8000 rpm and 50 µl Buffer AE added again. This step was to elute the DNA finally from membrane.

3.2.2.2 DNA Quality check

Samples were assessed by electrophoresis through a 0.8% (w/v) agarose gel to check the quality of DNA extracted.

3.2.2.3 DNA concentration check and dilution

The concentration of the DNA was determined on a Nanodrop (spectrophotometer 2000C). The genomic DNA samples were diluted to a final concentration of 20 ng/µL with 1×TE buffer (10 mM Tris-HC1, pH 8.0; 1 mM EDTA) and stored at -20 °C for further use.

3.2.2.4 Polymerase Chain Reaction using SSR markers

A total of 20 SSR primers were used to fingerprint the 22 cowpea varieties (Table 3.3). Information about the primers was obtained from the SSR panel in (Timko, 2009). The primers were synthesized by Inqaba Biotechnical Industries Ltd., Pretoria, South Africa. Polymerase chain reaction (PCR) amplification was conducted in 20 µl volume tubes. Each PCR reaction contained 6 µl "One Taq Quick-Load 2x Master Mix" (composed of 20mM Tris-HCl, 1.8mM $MgCl_2$, 22mM NH_4Cl, 22 mM KCl, 0.2 mM dNTPS and 25 units/ml One Taq DNA Polymerase), 2.0 µl Molecular Grade Distilled Water (MGDW), 0.5 µl of each primer pair and 1µl of genomic DNA sample to make a total volume of 10µl. The PCR amplifications were performed in a thermal cycler C1000 (Seegene, Korea). The thermal cycler was set to initial denaturation at 94^{o}C for 1 minute followed by 35 cycles of denaturation for 30 seconds at 94^{o}C, annealing at 55^{o}C for 30 seconds, extension at 72^{o}C for 1 minute and ended with final extension at 72^{o}C for 10 minutes.

Table 3.3 SSR primers used for molecular characterization and their sequences

NO.	SSR NAME	ORIGINAL NAME	LEFT SEQUENCE	RIGHT SEQUENCE	ANNEALING TEMPERATURE (°C)	PRODUCT SIZE (bp)
1	SSR-6265	CP215/CP216	CAGAAGCGGTGAAAATTCAAC	GCATGTTGCTTTGACAATGG	55	239
2	SSR-6258	CP201/CP202	GGTTTCCTAGTTGGGAAGGAA	ATTATGCCATGGAGGGTTCA	55	260
3	SSR-6243	CP171/CP172	GTAGGGAGTTGGCCACGATA	CAACCGATGTAAAAAGTGGACA	55	176
4	SSR-6218	CP117/CP118	GTGGAAGGAATGGGTCCAG	AGGAAATTTGCATTCCCTTGT	55	287
5	SSR-6217	CP115/CP116	GGGAGTGCTCCGGAAAGT	TTCCCTATGAACTGGGAGATCTAT	55	294
6	SSR-6353	CP397/CP398	TCATGGGTTAAATTTGCTTCAA	AAACCATGTGGTTGTTGCAC	50.9	109
7	SSR-6352	CP395/CP396	GTTGTGAGCTTCCCCAGATG	AATTTTGAACCCACCACCAG	55	127
8	SSR-6336	CP359/CP360	TGAAAAACAACGATATGCAGAAG	TCAGTCTTAGAATTGAGTTTTCTTCG	55	247
9	SSR-6323	CP333/CP334	CAAAGGGTCATCAGGATTGG	TTTAAGCAGCCAAGCAGTTGT	55	218
10	SSR-6277	CP239/CP240	CACCCCCGTACACACACAC	CACTTAAATTTTCACCAGGCATT	50.9	157
11	SSR-6436	CP573/CP574	GCAGAATCCTTGTGAACCTG	TTTCGCAATATGCCCTTTTC	50.9	280
12	SSR-6375	CP443/CP444	GCTCGGATATGGTCCTGAAA	TCAGTGTCAGCACCATACCC	55	293
13	SSR-6371	CP435/CP436	TGCTCATCGTGCTTTGTCTT	CACTTCAGACTTAGAGCGAAGAAA	55	189
14	SSR-6370	CP433/CP434	CAACTTCACAGCCCTCACAA	TTGAAGGTATGGCCTTTTGTTT	55	253

Table 3.3 continued

NO.	SSR NAME	ORIGINAL NAME	LEFT SEQUENCE	RIGHT SEQUENCE	ANNEALING TEMPERATURE (°C)	PRODUCT SIZE
15	SSR-6356	CP403/CP404	TGCAATATGGACCAGAAGAAA	ATGCCCCAACAACAACATTT	55	158
16	SSR-6613	Y31	CTATTGGAATCTTGCCGTTG	CTTTACCTTTATGCAAACCAATTC	55	333
17	SSR-6608	Y26	CTAAATTATAATATTCGTCGGTC	GGTTAAGGAAAAGAGGGTAGG	55	299
18	SSR-6603	Y21	GAGAACTTCACGCACAATAG	CGCGGTAGCATGATTGAATTTTG	55	330
19	SSR-6587	Y1	GATATAGAATAGCATATTTAACATATTAG	GTTGAAAGTTTGATAGTAAAGTGG	55	319
20	SSR-6451	CP605/CP606	AAAGAGATACACATGCCTAACA	GACCAACAGCGACTTTGAGC	55	142
14	SSR-6370	CP433/CP434	CAACTTCACAGCCCTCACAA	TTGAAGGTATGGCCTTTTGTTT	55	253
15	SSR-6356	CP403/CP404	TGCAATATGGACCAGAAGAAA	ATGCCCCAACAACAACATTT	55	158
16	SSR-6613	Y31	CTATTGGAATCTTGCCGTTG	CTTTACCTTTATGCAAACCAATTC	55	333
17	SSR-6608	Y26	CTAAATTATAATATTCGTCGGTC	GGTTAAGGAAAAGAGGGTAGG	55	299
18	SSR-6603	Y21	GAGAACTTCACGCACAATAG	CGCGGTAGCATGATTGAATTTTG	55	330
19	SSR-6587	Y1	GATATAGAATAGCATATTTAACATATTAG	GTTGAAAGTTTGATAGTAAAGTGG	55	319
20	SSR-6451	CP605/CP606	AAAGAGATACACATGCCTAACA	GACCAACAGCGACTTTGAGC	55	142

3.2.2.5 Gel electrophoresis

The PCR products were ran on vertical Polyacrylamide gel electrophoresis (v-PAGE) (Criterion cell model, vertical centrion tank) to separate and resolve the bands. The 6% polyacrylamide gel was prepared as shown in Table 3.4.

Table 3.4 Preparation of 15ml of 6% Polyacrylamide gel

Reagent	Volume
Filtered Autoclaved Distilled Water (FADW)	11.335 ml
10x TBE	1.25 ml
40% acrylamide solution	2.25 ml
40% Ammonium per sulphate (APS)	150 µl
TEMED	15 µl
Total Volume	15 ml

The liquid polyacrylamide gel was quickly dispensed into a 10 ml cassette using a transfer pipette after preparation. A twenty-six tipped comb was used to create wells and allowed to polymerise. The tape covering the base of the cassettes was then removed before submerging the cassette into 1x TBE buffer contained in the vertical electrophoresis tank. The wells were then loaded with the PCR products after the comb was removed. A 100bp ladder (Invitrogen®) was loaded in the first well to determine band sizes of the PCR products. The gel was run to at least half way to the end of the tank and a spatula was used to prise off the cassette to release the gel gently. The gel

was stained with 0.5% Ethidium bromide and shook on an electric shaker for 1 hour. The gel was photographed under Ultraviolet light for further analysis.

3.2.2.6 Scoring of bands from v-PAGE

Scoring of bands was done with the alpha imager® scoring software version 3.4.0 along with a 100-bp DNA ladder (Invitrogen®) to identify the molecular-weight of the DNA samples.

3.3 Molecular Screening of Cowpea Varietiesfor Aphid resistance locus

A polymorphic test was conducted on the 22 cowpea varieties using the marker CP 171F/172R (left sequence: 5' – GTAGGGAGTTGGCCACGATA – 3'; Right sequence: 3' - CAACCGATGTAAAAAGTGGACA-5') as recommended by Kusi (2014) to distinguish resistant varieties from susceptible varieties at the genomic level. This included the SARC-1-57-2 and Apabgaala which were the resistant and susceptible checks respectively.

DNA samples were taken from the leaf primordial of the 22 varieties two weeks after planting. DNA was extracted using the Qiagen extraction kit as detailed in sub section 3.2.2.1.

PCR was run using the SSR marker CP 171F/172R according to the steps documented in sub section 3.2.2.4. The PCR products were run on a non-denaturing v-PAGE following the steps in subsection 3.2.2.5. The photographed bands of the samples along with the checks were analysed to determine the polymorphic state of the varieties at the aphid resistance loci.

3.4 Development of F_1 progenies

The F_1 generation was developed from a cross between the resistant line SARC-1-57-2 (Male) and three susceptible cultivars – Asontem, Nhyira and Asetenapa (Females) on the experimental field of CSIR-CRI, Kumasi. The parent materials were planted on 18th September, 2015. Each crossing block was made up of onerow (2 m long) of the male variety interspersed in two rows (2 m long) of the female variety. Each row was made of 10 plants with 20 cm spacing. Artificial pollination was done between the hours of 06:00 hrs and 08:00 hrs when most petals are open. Artificially pollinated floral buds were well tagged with labels (Plate 3.1). The F_1seeds were harvested on 13th November, 2015.

Plate 3.1: Artificial cross pollination (For each cross, the immature anthers in the floral bud of the female parent were emasculated with the aid of sterilized forceps and then manually pollinated with the pollen from the male variety)

3.5 Development of BC_1 Progenies

The F_1 generation developed from crosses between three susceptible cultivars (Asontem, Nhyira and Asetenapa) and the resistant line SARC-1-57-2 were grown again on the fields on CSIR-CRI along with recurrent parent varietieson 13th January, 2016 to develop BC_1 progenies. Crosses between the F_1 (donor) and the susceptible lines (recurrent parents) followed the procedure outlined in sub-section 3.4. Recurrent parents were used as females. BC_1 seeds were harvested on 12th April, 2016.

3.6 Molecular Screening of BC_1

The BC_1 seeds were planted along with recurrent parents (Asontem, Nhyira and Asetenapa) on 20th April, 2016 at CSIR-CRI. All the individuals from the backcross were genotyped to select individuals with successful introgression using the SSR marker CP 171F/172R. DNA extraction, PCR amplification and Scoring followed the procedure outlined in sub-section 3.2.2.

3.7 Development of BC_2 seeds

Pollen grains from selected BC_1 plants were used as males to pollinate recurrent parent varieties (females) following the steps in sub section 3.5. BC_2 seeds (offsprings) were harvested for future backcrosses on 27th June, 2016.

CHAPTER FOUR

4.0 RESULTS

4.1 Agro-Morphological Characterization of Cowpea Varieties

4.1.1 Qualitative traits

4.1.1.2 Plant Pigmentation

4.1.1.2.1 Leaf base pigmentation

The study revealed two patterns of leaf base pigmentation (Table 4.1). Most of the varieties had pigmented leaf base (77.00%) while 23.00% were not pigmented. Varieties whose leaf bases were not pigmented are Bengpla, Apabgaala, Ayiyi, Asontem, and Adom(Table 4.2).

4.1.1.2.2 Node pigmentation

Majority of the varieties had pigmented leaf nodes (95.45%) while 4.55% were not pigmented (Table 4.1). Out of 22 varieties, only the nodes of Asontem were not pigmented (Table 4.2).

4.1.1.2.3 Petiole pigmentation

The study revealed that the pattern of petiole pigmentation varied. The petiole of most varieties had moderate pigmentation at the base and tips of the petiole (31.82%). This was followed by intermediate pigmentation (27.27%), very slight (22.73%) and not pigmented (13.63%) respectively. Varieties with solid petiole pigmentation were the least represented (Table 4.1). Sanzi was the only variety with solid leaf petiole pigmentation in this study (Table 4.2).

4.1.1.2.4 Branch Pigmentation

As shown in Table 4.1, four levels of branch pigmentation were observed. The branches of 31.82% of the varieties studied were not pigmented, 27.27% had very slight pigmentation. Another 27.27% had moderate pigmentation at the base and tips of petioles while only 3.00% had

intermediate pigmentation. Bengpla, Videza, Asetenapa, Zamzam, Asomdwoe, Agyenkwa and Nhyira are among the varieties whose branches are not pigmented (Table 4.2).

4.1.1.2.5 Stem pigmentation

In this study, three kinds of stem pigmentation were observed (Table 4.1). The stem of most of the varieties was not pigmented (86.36%). About 9.09% of the varieties had very slight stem pigmentation while 4.55% had intermediate pigmentation (Table 4.1). Sanzi and Adom had very slight pigmentation on the stem while Padi-Tuya had intermediate stem pigmentation. The stems of all the other varieties were not pigmented (Table 4.2).

4.1.1.2.7 Peduncle pigmentation

Three levels of peduncle pigmentation were recorded in this study (Table 4.1). Most of the varieties were not pigmented (77.27%). Varieties with intermediate (18.18%) and very slight (4.55%) peduncle pigmentation were also recorded. Videza had very slight pigmentation while Apabgaala, Asontem, Sanzi and Songotra also had intermediate pigmentation of peduncle (Table 4.2).

4.1.1.2.8 Pod pigmentation

The varieties exhibited three different kinds of pod pigmentation as shown in Table 4.1. The pods of most of the varieties studied were not pigmented (68.20%); 13.60% had only the pod tips pigmented while 18.20% had the entire pod pigmented. Varieties with entire pod pigmentation include Sanzi, Songotra and Padi-Tuya (Table 4.2).

4.1.1.2.9 Sepal pigmentation

The sepals of the varieties showed three kinds of sepal pigmentation (Table 4.1).

The sepals of most varieties studied were not pigmented (68.18%) while 13.64% and 18.18% had intermediate and solid sepal pigmentation respectively.

4.1.1.3 Terminal leaflet description

4.1.1.3.1 Terminal leaflet apex shape

All the 22 varieties studied in this project had acute terminal leaf apex (Table 4.1). In other words, no other leaflet apex shape was encountered aside the acute shape.

4.1.1.3.2 Terminal leaflet base shape

In this study, three kinds of leaf base shapes were observed (Table 4.1). These were Cuneate (59.09%), Semi-hastate (27.27%) and Hastate (13.64%).

4.1.1.3.3 Terminal leaflet shape

This study recorded three terminal leaflet shapes (Table 4.1 and Plate 4.1). The terminal leaflet shape of most of the varieties studied was Sub-globose (54.55%). About 36.36% were Hastate while 9.09% were Globose. Varieties with globose terminal leaflet shape were Bengpla and Asomdwoe (Table 4.2).

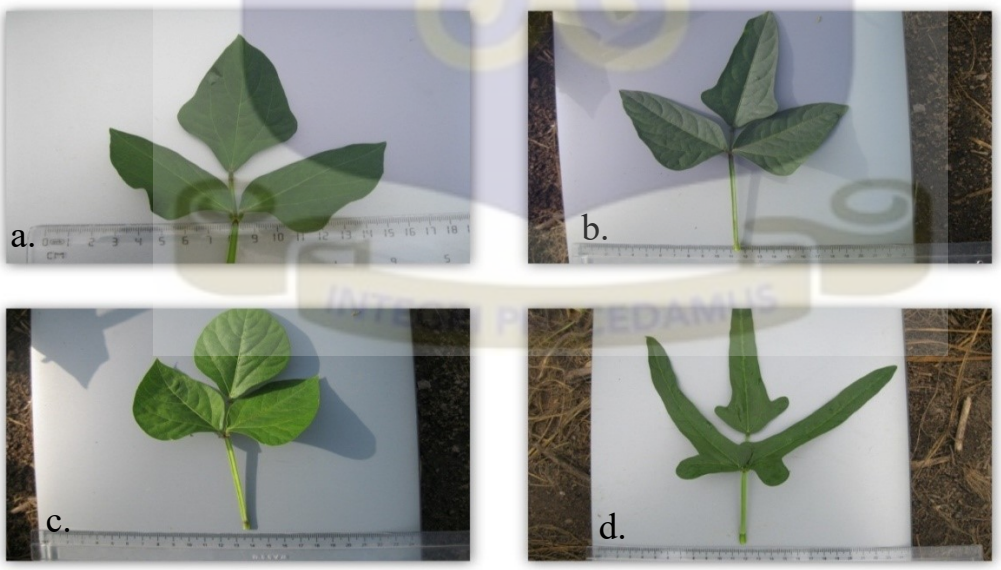

Plate 4.1: Variation observed in terminal leaf shape - a. Sub-globose; b. Semi-hastate; c. Globose; d. Hastate

4.1.1.4 Growth Habit

There was great variation in the growth habit exhibited by the cowpea varieties studied (Table 4.1). Varieties with Erect and Semi-erect growth habits formed 36.36% each of the population. Semi-prostrate and Climbing varieties followed with 9.09% each, while Intermediate and Prostrate varieties scored the least (4.55% each). Adom and Asontem were the two protrate varieities encountered in this study (Table 4.2).

4.1.1.5 Twining tendency

Varieties with no twining (none) and slight twining were represented by 36.36% each of the total number of varieties studied. Varieties with Intermediate and Pronounced twining tendencies also followed with 13.64% each (Table 4.1).

4.1.1.6 Plant hairiness

4.1.1.6.1 Petiole hairs

The petioles of the varieties were described as either smooth or hairy based on the presence or absence of glandular hairs. In all, 63.64% had no hairs (smooth) on the petiole while 36.36% had hairs (hairy) (Table 4.1).

4.1.1.6.2 Stem hairs

The stem of the varieties were described as either smooth or hairy based on the presence or absence of glandular hairs. About 90.91% had no hairs (smooth) on the petiole while 9.09% had hairs (hairy) on the stem (Table 4.1). The varieties with hairy stem were SARC-1-57-2 and Adom (Table 4.2).

4.1.1.6.3 Leaf hairs

In this study, 59.09% of the varieties had smooth leaf texture while the other 40.91% had Hairy leaf texture (Table 4.1).

4.1.1.7 Raceme position

About 40.91% of the varieties studied had their raceme position in the upper canopy while 31.82% had the raceme positions occurring mostly above the canopy (Table 4.1). Some of the varieties (27.27%) also had their raceme occurring throughout the canopy.

4.1.1.8 Flower and Pod description

4.1.1.8.1 Flower colour

The observed flower colours in this study were purple (22.73%) and white (77.27%) as shown in Table 4.1 and Plate 4.2.

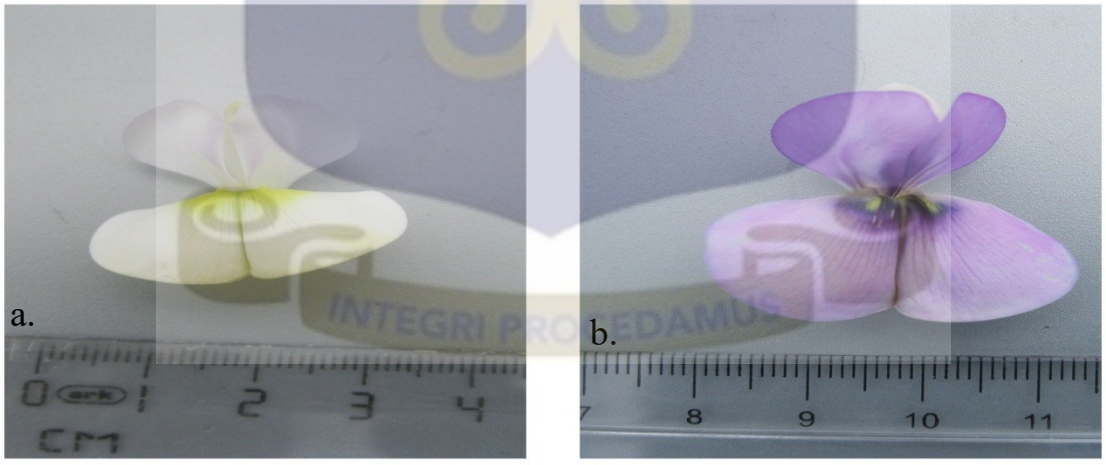

Plate 4.2: Variation observed in flower colour a. White flower b. Purple flower

4.1.1.8.2 Pod Shape

In this study, four kinds of pod shapes were identified (Table 4.1). Pods of most varieties were Straight and Bent (40.91% each). The other pod shapes encountered were Sickle – shaped pods and S-shaped pod represented by 9.09% each (Plate 4.3).

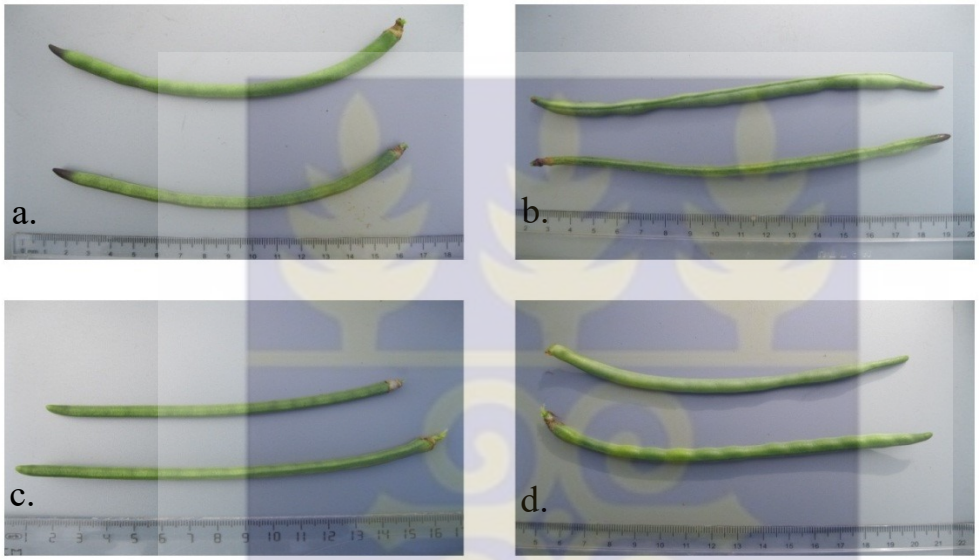

Plate 4.3: Variation observed in pod morphology a. Bent shaped pod with purple tips; b. S – shaped pods with purple tips; c. Straight shaped pods; d. Sickle shaped pods

4.1.1.8.3 Pod Tip Shape

The observed pod-tip shapes encountered in this study were Blunt (9.09%) and Pointed (90.91%) (Table 4.1). Varieties with blunt pod tips were Soronko and Videza (Table 4.2).

4.1.1.9 Seed description

4.1.1.9.1 Seed Colour

Seed colour in this study showed much variation (Table 4.1 and Plate 4.4). Most varieties had white coloured seeds (72.73%), followed by Brown coloured seeds (13.64%) and Red coloured seeds (9.09%). Only 4.55% of the varieties had Dark – brown coloured seeds.

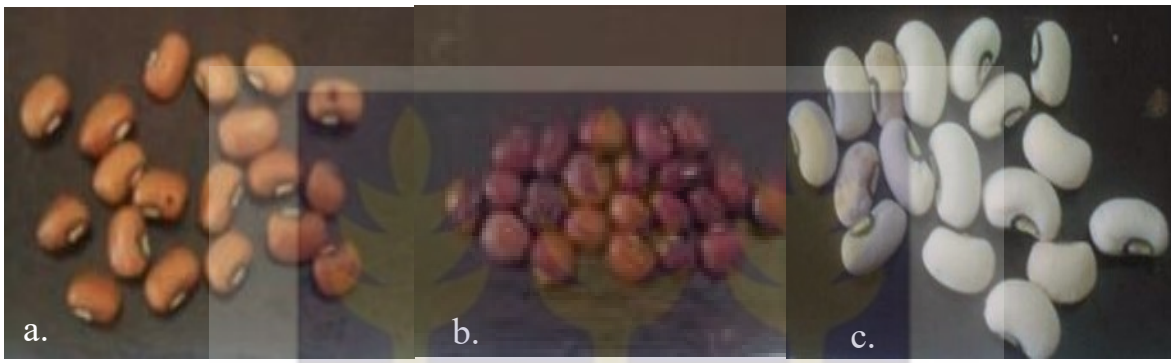

Plate 4.4 Variation observed in seed colour a. Brown seeds b. Red seeds c. White seeds

4.1.1.9.2 Seed eye colour

Four kinds of seed eye colour were encountered in this study (Table 4.1). Half of the varieties had black seed eye colour (50.00%). Other seed eye colours encountered were brown, Dark-brown and Greenish brown represented by 36.36%, 9.09% and 4.55% respectively.

4.1.1.9.3 Seed shape

There was variation in the seed shape of the cowpea varieties studied (Table 4.1). Varieties with Kidney shaped seeds and ovoid shaped seeds dominated with 31.82% each. This was followed by Rhomboid seed shape (22.73%). The least occurring seed shape was Globose seed shape represented by 13.64%.

The qualitative data summeries and description of the individual 22 cowpea varieties are presented in Tables 4.1 and 4.2 below.

Table 4.1: Summary Statistics of Qualitative Characters

Qualitative Characters	0	1	2	3	4	5
Leaf base- Pigmentation 0 – Not Pigmented; 1 – pigmented	5 (22.0%)	17 (77.0%)				
Node Pigmentation 0 – Not pigmented; 1 - pigmented	1 (4.6%)	21 (95.4%)				
Petiole Pigmentation 0 – Not pigmentation; 1 – Very slight; 2 – Moderate at the base and tips of petioles; 3 – Intermediate ; 4 – Extensive; 5 – Solid	3 (13.6%)	5 (22.7%)	7 (31.8%)	6 (27.3%)		1 (4.6%)
Branch Pigmentation 0 – Not pigmentation; 1 – Very slight; 2 – Moderate at the base and tips of petioles; 3 – Intermediate ; 4 – Extensive; 5 – Solid	7 (31.8%)	6 (27.3%)	6 (27.3%)	3 (13.6%)		
Stem Pigmentation 0 – Not pigmentation; 1 – Very slight; 2 – Moderate at the base and tips of petioles; 3 – Intermediate ; 4 – Extensive; 5 – Solid	19 (86.4%)	2 (9.1%)		1 (4.6%)		
Peduncle Pigmentation 0 – Not pigmentation; 1 – Very slight; 2 – Moderate at the base and tips of petioles; 3 – Intermediate ; 4 – Extensive; 5 – Solid	17 (77.3%)	1 (4.6%)		4 (18.2%)		
Pod Pigmentation 0 – Not Pigmented; 1 – Pod tip pigmented; 2 – Entire pod pigmentation	15 (68.2%)	4 (18.2%)	3 (13.6%)			
Sepal Pigmentation 0 – Not pigmentation; 1 – Very slight; 2 – Moderate at the base and tips of petioles; 3 – Intermediate ; 4 – Extensive; 5 – Solid	15 (68.2%)			3 (13.6%)		4 (18.2%)
Terminal Leaflet Shape 1 – Sub-globose; 2 – Globose; 3 – Sub-hastate; 4 – Hastate		12 (54.5%)	2 (9.1%)	6 (27.3%)	2 (9.1%)	
Terminal Leaf Apex Shape 1 – Acute; 2 – Oval		22 (100%)				
Terminal Leaf Base Shape 1 – Cuneate; 2 – Semi-hastate; 3 – Hastate		13 (59.1%)	6 (27.3%)	3 (13.6%)		

Table 4.1 continued

Qualitative Characters	0	1	2	3	4	5
Twining Tendency 0 – None; 1 – Slight; 2 – Intermediate; 3 – Pronounced	8 (36.4%)	8 (36.4%)	3 (13.6%)	3 (13.6%)		
Petiole Hairs 0 – Smooth; 1 – Hairy	14 (63.6%)	8 (36.4%)				
Stem Hairs 0 – Smooth; 1 – Hairy	20 (90.9%)	2 (9.1%)				
Leaf Hairs 0 – Smooth; 1 – Hairy	13 (59.1%)	9 (40.9%)				
Raceme Position 1 – Mostly above canopy; 2 – In upper canopy; 3 – throughout canopy		7 (31.8%)	9 (40.91%)	6 (27.3%)		
Immature Pod Colour 1 – Green; 2 - Green with purple tips; 3 – Purple-greeen; 4 – Purple		19 (86.4%)	1 (4.55%)	1 (4.55%)	1 (4.55%)	
Pod Shape 1 – Straight; 2 – Bent; 3 – S-Shaped; 4 – Sickle		9 (40.9%)	9 (40.9%)	2 (9.1%)	2 (9.1%)	
Pod Tip Shape 1- Blunt; 2 – Pointed		2 (9.1%)	20 (90.9%)			
Seed Shape 1 – Kidney; 2 – Ovoid; 3 – Crowder; 4 – Globose; 5 – Rhomboid		7 (31.8%)	7 (31.8%)		3 (13.6%)	5 (22.7%)
Seed Colour 1-Brown; 2 – White; 3 - Red; 4 – Dark brown		3 (13.6%)	16 (72.7%)	2 (9.1%)	1 (4.6%)	
Seed Eye Colour 1-Brown; 2 – Black; 3 – Dark brown 4 – Greenish brown		8 (36.4%)	11 (50.0%)	2 (9.1%)	1 (4.6%)	
Growth Habit 0 – Erect; 1 - Semi-erect; 2 – Intermediate; 3 – Semi-prostate; 4 – Prostate; 5 - Climbing	8 (36.4%)	8 (36.4%)	1 (4.5%)	2 (9.1%)	1 (4.5%)	2 (9.1%)

Table 4.2: Qualitative traits of 22 cowpea varieties

Variety	PodP	LBP	NP	PetP	BP	StP	PedP	SepP	TT	RP	PetH	StH	LH	TLSh	LBSh	GH	SSh	SC	SEC	FC	PSh	PTSh
Soronko	0	1	1	2	1	0	0	0	0	3	0	0	1	1	2	0	1	1	1	1	1	1
Nketewa	0	1	1	2	1	0	0	0	0	1	0	0	0	3	2	1	2	2	2	2	2	2
Hansadua	0	1	1	2	2	0	0	0	0	1	1	0	1	3	2	1	1	2	1	2	2	2
Bengpla	1	0	1	1	0	0	0	3	2	2	1	0	1	2	1	0	3	2	2	2	2	2
Tona	0	1	1	1	1	0	0	0	1	3	0	0	0	1	1	0	4	1	1	1	1	2
Apabgaal	0	0	1	1	1	0	2	0	0	1	0	0	0	3	2	1	4	1	1	2	1	2
Bra 01	0	1	1	3	3	0	0	0	0	1	0	0	0	1	1	0	4	2	1	2	1	2
Videza	0	1	1	2	0	0	1	0	1	3	0	0	0	1	1	3	2	2	2	2	1	1
Zaayura	0	1	1	3	3	0	0	0	1	1	1	0	1	3	3	3	1	2	1	2	1	2
Asetenap	1	1	1	3	0	0	0	3	1	2	0	0	0	1	1	2	1	2	2	2	4	2
Asontem	0	0	0	0	1	0	0	0	3	2	0	0	0	4	3	5	1	2	3	2	3	2
Zamzam	0	1	1	0	0	0	0	0	1	2	0	0	0	1	1	1	3	2	2	2	2	2
SARC-1-5	1	1	1	3	2	0	2	0	2	2	1	1	1	1	1	1	4	3	1	1	2	2
Ayiyi	0	0	1	1	1	0	0	0	3	2	0	0	1	3	2	0	2	2	3	2	2	2
Asomdwoe	0	1	1	2	0	0	0	2	1	3	1	0	0	2	1	0	2	2	2	2	1	2
Sanzi	2	1	1	5	2	1	2	2	1	2	0	0	0	1	1	4	1	4	2	1	1	2
Agyenkwa	0	1	1	3	0	0	0	0	0	2	1	0	0	1	1	1	2	2	2	2	2	2
Songotra	2	1	1	2	2	0	2	2	0	1	0	0	0	1	1	1	1	2	2	2	1	2
Padi-Tuya	2	1	1	3	2	2	0	3	0	1	0	0	0	3	2	1	4	2	2	2	2	2
Nhyira	0	1	1	1	0	0	0	0	1	3	1	0	1	1	1	0	4	2	4	2	4	2
Adom	1	0	1	0	3	1	0	3	3	2	1	1	1	4	3	5	4	3	2	1	3	2
Helewa	0	1	1	2	2	0	0	0	2	3	0	0	1	1	1	0	3	2	1	2	2	2

PodP = Pod pigmentation; LBP = Leaf Base pigmentation; Node pigmentation; PetP = Petiole pigmentation; BP = branches pigmentation; StP = Stem pigmentation; PedP = Peduncle pigmentation; TT = Twining tendency; RP = Raceme position; PetH = Petiole hairs; StH = Stem hairs; LH = Leaf hairs; TLSh = Terminal leaf shape; LBSh = Leaf base shape; GH = Growth habit; SS = Seed Shape; SC = Seed colour; SEC = Seed eye colour; FC = Flower colour; PSh = Pod shape; PTSh = Pod tip shape.

The codes have been explained in Chapter 3, subsection 3 and Table 4.1.

4.1.1.10 Dissimilarity matrix and cluster analysis based on qualitative traits

The qualitative data obtained from the 22 varieties was used to generate a dissimilarity matrix (Appendix 5) with the aid of the Statistical Package for Social Sciences (SPSS) sotftware version 16.0. The level of dissimilarity was measured at rescaled squared Euclidean distance. Genetic distance ranged between 0.007 and 1.00. From the dissimilarity matrix presented in Appendix 5, there was no distance between Hansadua and Nketewade suggesting that the two varieties are very similar. The results also established a genetic distance of 0.007 between Zamzam and Hansadua. The highest genetic distance of 1.00 was recorded between Agyenkwa and Adom and was closely followed by Soronko and Adom which scored 0.957.

A dendrogram was constructed with the aid of Statistical Package for Social Sciences (SPSS) version 16.0 using the qualitative data collected on the 22 varieties (Figure 4.1). A summary of the composition of the clusters is also presented in Appendix 2. The varieties were grouped into two major clusters at rescaled distance of 9 (Cluster A and Cluster B). Cluster A centered a total number of 8 varieties. Cluster A had two sub clusters – subcluster I and subcluster II. Three out of the four newly released cultivars of CSIR-CRI clustered within subcluster I. Cultivars released by CSIR-SARI also clustered within sub-cluster I. Sub-cluster II consisted of two varieties grown in northern Ghana (i.e. Zaayura and Sanzi). Cluster B was also made up two sub-clusters with 10 varieties. Nine out of the ten varieties that occurred in cluster B were released by CSIR-CRI.

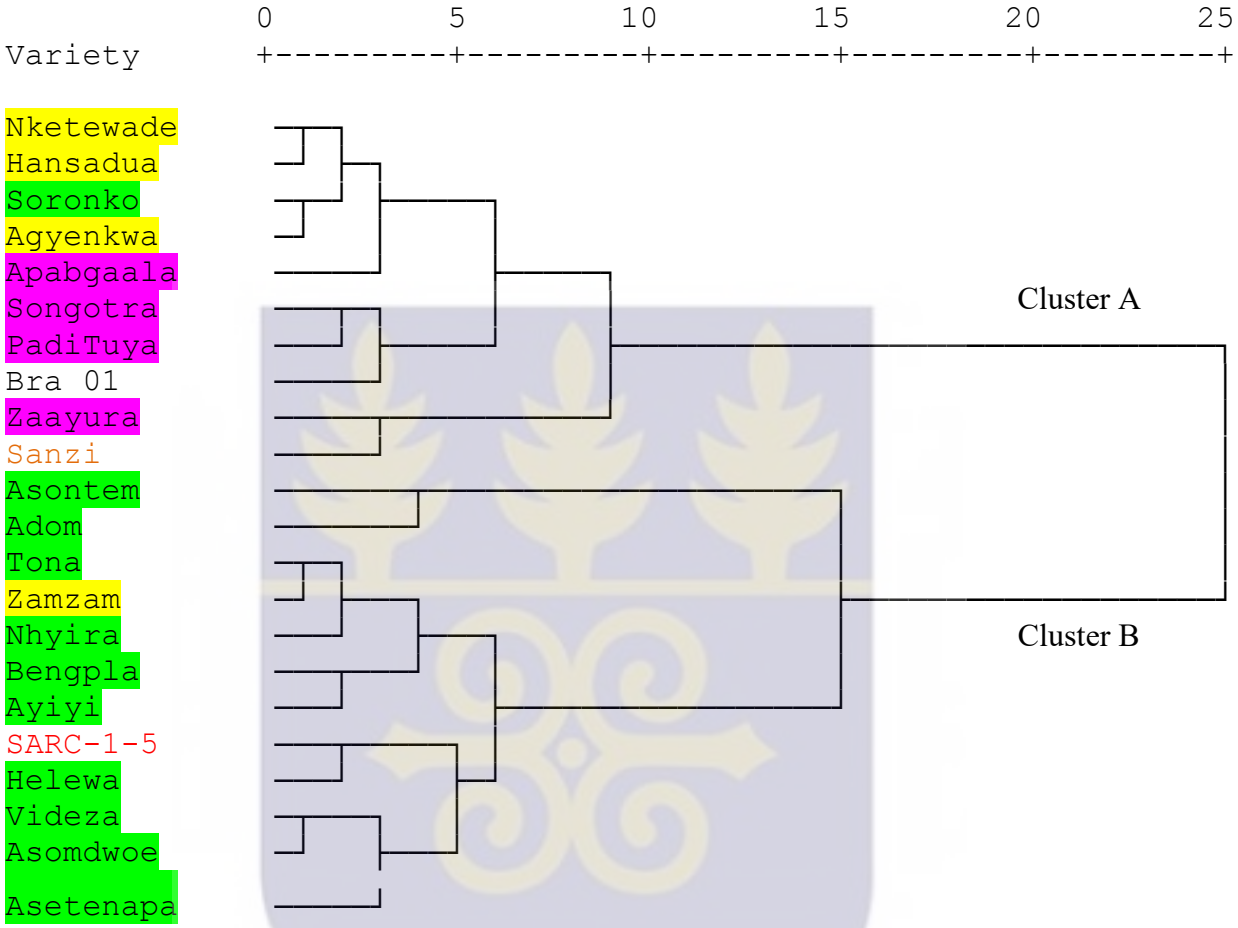

Figure 4.1 Dendrogram of 22 cowpea varieties based on qualitative traits (average linkage between groups) generated with the aid of SPSS version 16.0

Colour legend

Green outline = Old cultivars released by CSIR-CRI; Yellow outline = New cultivars released by CSIR-CRI; Mauve outline = Cultivars released by CSIR-SARI; Brown font = Landrace from northern Ghana; Black font = Exotic variety from Brazil; Red font = Advanced line developed by CSIR-SARI

4.1.2 Quantitative traits

The mean, standard error and coefficient of variation for the individual varieties are presented in Table 4.3. Analysis of variance (ANOVA) tables have also been presented in Appendix 9a to 9l.

4.1.2.1 Plant height

The mean plant height in this study was 38.52 ± 1.48 cm (Table 4.3). Soronko had the highest plant height of 63.33 ± 0.88 cm while Apabgaala had the least plant height of 13.67 ± 1.45 cm. From the analysis of variance, there was significant difference ($P < 0.001$) in the plant height among the varieties which implies that the plant heights of the varieties are not the same.

4.1.2.2 Terminal Leaf length

The terminal leaf length varied between 15.17 ± 0.42 cm (Asontem) and 7.17 ± 0.19 cm (Sanzi) (Table 4.4). The mean terminal leaf length in this study was 10.45 ± 0.75 cm (Table 4.3). From the analysis of variance, there was significant difference ($P < 0.001$) in the terminal leaf length among the varieties which implies that the terminal leaf lengths of the 22 varieties are not the same.

4.1.2.3 Terminal Leaf width

The mean terminal leaf width was 6.46 ± 0.51 cm (Table 4.3). Soronko had the highest terminal leaf width of 9.50 ± 0.23 cm while Sanzi recorded the least terminal leaf width of 4.47 ± 0.21 cm (Table 4.4). From the analysis of variance, there was significant difference ($P < 0.001$) in the terminal leaf width among the varieties. This implies that the terminal leaf widths of the varieties are not the same.

4.1.2.4 Terminal Leaf Area Index

The terminal leaf area index ranged between 3.45 ± 0.41 (Asontem) and 1.10 ± 0.02 (Bengpla) (Table 4.4). The mean terminal leaf area index was 1.69 ± 0.11 (Table 4.3). From the analysis of variance, there was significant difference in terminal leaf index among the varieties which indicates that the terminal leaf area indeces of the 22 varieties are not the same.

4.1.2.5 Hundred Seed Weight (g)

The mean 100 seed weight was 15.08 ± 0.78 g (Table 4.3). Hundred seed weight for the varieties in this study ranged between 25.33 ± 0.17 g (Bra-01) and 8.17 ± 0.60 g (Sanzi). From the analysis of variance, there was significant difference ($P < 0.001$) in the 100 seed weight among the varieties.

4.1.2.6 Seed length (cm)

The highest seed length was scored by Padi-Tuya (0.97 ± 0.09 cm), followed by Songotra, Bra-01 and Soronko with seed length of 8.33 cm each. Sanzi, Bengpla and Hewale jointly scored the least seed length (Table 4.4). The mean seed length for the varieties in this study was 0.73 ± 0.03 cm (Table 4.3). From the analysis of variance, there was significant difference ($P < 0.001$) in the seed length of the varieties which implies that there is variation in the seed length of the 22 varieties.

4.1.2.7 Seed width (cm)

The mean seed width for the varieties in this study was 0.53 ± 0.02 cm (Table 4.3). Bra-0 recorded the highest seed width of 0.80 ± 0.00 cm while Videza had the least seed length of 0.30 ± 0.00 cm (Table 4.4). From the ANOVA, there was significant difference ($P < 0.001$) in seed width of the varieties studied which indicates that the seed widths of the 22 varieties are not the same.

4.1.2.8 Pod length (cm)

Adom recorded the highest pod length of 19.77 ± 0.63 cm while Sanzi recorded the least pod length of 13.67 ± 0.44 cm (Table 4.4). The mean pod length in this study was 16.22 ± 0.63 cm (Table 4.3). From the analysis of variance, there was significant difference among the varieties studied which indicates that the pod lengths of the 22 varieties are not the same.

4.1.2.9 Number of Days to 50% Flowering

Bengpla recorded the least number of days to 50% flowering (52 days) while Asomdwee recorded the highest number of days to 50% flowering (66 ± 0.00 days) as presented in Table 4.5. The mean number of days to 50% flowering was calculated as 60 ± 0.38 days (Table 4.3). From the analysis of variance, there was significant difference ($P < 0.001$) among the varieties studied which implies that there is variation in the number of days each variety reaches 50% flowering.

4.1.2.10 Number of peduncles per plant

Bra-01 and Tona jointly recorded the highest number of peduncles per pod (19 pods) as shown in Table 4.5. The least number of peduncles per plant (10 ± 0.57 pods) was scored by Asontem. The mean number of peduncles per plant was 14.39 ± 1.32 pods (Table 4.3). Analysis of variance showed that there was significant difference ($P = 0.008$) in number of peduncles per plant among the varieties studied. This implies that the number of peduncles per plant for the 22 varieties is not the same.

4.1.2.11 Number of pods per peduncle

The mean number of pods per peduncle in this study was 2.44 ± 0.24 pods (Table 4.3). The highest number of pods per peduncle of 3.33 was scored by jointly scored by Soronko, Apabgaala and Asomdwee (Table 4.5). From the ANOVA, there was significant difference ($P < 0.001$) in the

number of pods per peduncle among the varieties which implies that number of pods per peduncle of the 22 varieties are not the same.

4.1.2.12 Number of locules per pod

Number of locules per pod ranged between 11 locules (Zaayura) and 17 locules (Ayiyi and Soronko) as shown in Table (Table 4.5). The mean number of locules per pod in this study was 14.23 ± 0.81 locules (Table 4.3). Analysis of variance showed that there was significant difference in number of locules per pod among the varieties which indicate variation in the number of locules per pod among the 22 varieties.

4.1.2.13 Number of seeds per pod

The highest number of seeds per pod of 16.33 ± 0.88 was scored by Ayiyi while the least number of seeds per pod of 10 ± 0.58 was also scored by Asomdwee (Table 4.5). The mean number of seeds per pod was 13.33 ± 0.83 (Table 4.3). From the Analysis of variance, there was significant difference in number of seeds per pod among the varieties which implies that there is variation in the number of seeds per pod among the 22 varieties.

Table 4.3: Summary statistics of quantitative traits of 22 cowpea varieties

Parameter	Mean	SE	SD	CV	Min	Max
Plant height (cm)	38.52	1.48	12.01	31.18	13.67	63.33
100 seed weight (g)	15.08	0.78	3.64	24.16	8.17	25.33
Leaf length (cm)	10.45	0.29	2.36	22.63	7.17	15.17
Leaf width (cm)	6.46	0.20	1.61	24.92	4.47	9.50
Leaf area index	1.69	0.11	0.95	30.06	1.10	3.45
Number of days to 50% flowering	60	0.45	3.69	6.13	52	66
Number of pods per peduncle	2	0.10	0.79	32.26	1	3
Number of peduncles per plant	14	0.42	3.42	23.75	10	19
Number of pods per plant	21	0.73	5.93	28.75	9	37
Number of locules	14	0.25	2.07	14.53	9	19
Number of seeds per pod	13	0.27	2.21	16.61	9	18
Pod length (cm)	16.22	0.24	1.95	11.99	13.67	19.77
Seed length (cm)	0.73	0.02	0.13	18.13	0.60	0.97
Seed width (cm)	0.53	0.01	0.11	19.84	0.30	0.80

Table 4.4: Quantitative statistics on 22 cowpea varieties

VARIETY	PL		SL		SW		LL		LW		SWg		PH		LL/LW	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Soronko	17.8 ± 0.70^{a}	6.81	0.83 ± 0.03^{fg}	6.93	0.50 ± 0.00^{cde}	0.00	13.50 ± 0.50^{abc}	6.42	9.50 ± 0.23g	4.21	14.33 ± 0.33de	4.03	63.33 ± 0.88k	2.41	3.17 ± 0.37defghi	20.23
Nketewa	15.73 ± 0.94^{cdef}	10.35	0.67 ± 0.03^{bcd}	8.66	0.53 ± 0.07^{cde}	21.65	8.17 ± 0.95^{ab}	20.21	4.83 ± 0.38a	13.78	15.00 ± 0.00ef	0	35.00 ± 3.51defg	17.38	2.88 ± 0.33abcdefg	19.55
Hansadua	16.46 ± 0.69^{efg}	7.30	0.73 ± 0.03^{cdef}	7.87	0.56 ± 0.03^{de}	10.18	9.10 ± 0.93^{abc}	17.69	5.67 ± 0.62abc	19.03	16.17 ± 0.60g	6.44	26.33 ± 1.86bc	12.21	2.03 ± 0.05ab	4.31
Bengpla	16.57 ± 0.37^{efg}	3.88	0.6 ± 0.00^{ab}	0.00	0.50 ± 0.00^{cd}	0.00	9.07 ± 0.70^{abc}	13.30	8.20 ± 0.55fg	11.63	13.00 ± 0.00bc	0	46.67 ± 2.73ij	10.13	3.73 ± 0.73fghi	34.02
Tona	16.67 ± 0.15^{efg}	1.51	0.67 ± 0.03^{bcd}	8.66	0.46 ± 0.03^{bc}	12.37	11.83 ± 0.60^{def}	8.80	8.23 ± 0.55fg	11.67	13.00 ± 0.58bc	7.69	38.33 ± 3.48defgh	15.72	3.71 ± 0.61fghi	28.23
Apabgaala	15.20 ± 0.96^{abcde}	10.99	0.80 ± 0.00^{efg}	0.00	0.53 ± 0.03^{cde}	10.82	9.10 ± 0.81^{abc}	15.38	5.07 ± 0.30abc	10.13	14.50 ± 0.29de	3.45	13.67 ± 1.45a	18.41	2.74 ± 0.33abcdef	20.68
Bra 01	16.43 ± 1.29^{efg}	13.61	0.83 ± 0.03^{fg}	6.93	0.80 ± 0.00^{g}	0.00	9.70 ± 0.93^{bcde}	16.59	6.83 ± 0.62def	15.78	25.33 ± 0.17j	1.14	33.67 ± 2.40cde	12.37	2.77 ± 0.38abcdef	23.45
Videza	13.93 ± 0.66^{abc}	8.16	0.53 ± 0.03^{a}	10.83	0.30 ± 0.00^{a}	0.00	10.27 ± 0.73^{bcde}	12.26	7.00 ± 0.65def	16.10	13.00 ± 0.58bc	7.69	41.33 ± 0.88fghi	3.7	1.92 ± 0.16a	14.17
Ayiyi	17.46 ± 0.52^{fgh}	5.19	0.70 ± 0.00^{bcde}	0.00	0.60 ± 0.00^{ef}	0.00	9.33 ± 0.44^{abc}	8.18	4.93 ± 0.32ab	11.16	12.00 ± 0.58b	8.33	32.33 ± 3.28cd	17.59	3.89 ± 0.21ghi	9.45
SARC-1-57-2	19.56 ± 0.70^{ij}	6.18	0.90 ± 0.00^{gh}	0.00	0.53 ± 0.03^{cde}	10.82	8.07 ± 0.23^{ab}	5.01	5.87 ± 0.32abcd	9.39	18.00 ± 0.00h	0	23.00 ± 3.00b	22.59	2.06 ± 0.29abc	24.68
Zaayura	16.17 ± 0.76^{defg}	8.17	0.90 ± 0.00^{gh}	0.00	0.57 ± 0.03^{de}	10.19	11.03 ± 0.77^{cdef}	12.07	6.70 ± 0.87cdef	22.54	21.00 ± 0.58i	4.76	41.00 ± 3.21efghi	13.58	3.37 ± 0.56efghi	28.92
Asetenapa	16.00 ± 0.29^{defg}	3.13	0.63 ± 0.09^{abc}	24.12	0.47 ± 0.03^{bc}	12.37	11.97 ± 0.65^{ef}	9.39	8.30 ± 0.26fg	5.52	13.00 ± 0.00bc	0	44.67 ± 2.96hij	11.49	3.42 ± 0.21efghi	10.85
Asontem	18.70 ± 0.59^{hij}	5.43	0.53 ± 0.03^{a}	10.83	0.50 ± 0.00^{cd}	0.00	15.17 ± 0.43^{g}	4.86	4.57 ± 0.73a	27.73	13.50 ± 0.29cd	3.7	47.00 ± 0.58ij	2.13	2.71 ± 0.20abcdef	12.80
Zamzam	14.43 ± 0.52^{abcd}	6.25	0.80 ± 0.06^{efg}	12.5	0.50 ± 0.00^{cd}	0.00	8.83 ± 0.18^{abc}	3.46	5.73 ± 0.20abcd	6.13	17.00 ± 0.00gh	0	34.00 ± 2.65def	13.48	2.22 ± 0.06abcd	4.82
Asomdwoe	13.73 ± 0.13^{ab}	1.68	0.73 ± 0.03^{cdef}	7.87	0.47 ± 0.03^{bc}	12.37	10.30 ± 0.49^{bcde}	8.30	7.23 ± 0.54def	12.85	14.00 ± 0.00cde	0	45.00 ± 2.52hij	9.69	2.60 ± 0.17abcde	11.54
Sanzi	13.67 ± 0.44^{a}	5.54	0.6 ± 0.00^{ab}	0.00	0.40 ± 0.00^{b}	0.00	7.17 ± 0.19^{a}	4.49	4.47 ± 0.12a	4.66	8.17 ± 0.60a	12.74	15.33 ± 0.67a	7.53	3.48 ± 0.26efghi	12.74

Table 4.4 continued

VARIETY	PL		SL		SW		LL		LW		SWg		PH		LL/LW	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Agyenkwa	15.57 ± 0.66^{bcde}	7.39	0.80 ± 0.00^{efg}	0.00	0.56 ± 0.07^{de}	20.38	9.37 ± 0.49^{abcd}	9.08	6.03 ± 0.50abcd	14.29	16.00 ± 0.00fg	0	46.67 ± 1.86ij	6.89	3.11 ± 0.15cdefgh	8.13
Songotra	16.07 ± 0.96^{defg}	10.37	0.83 ± 0.03^{fg}	6.93	0.6 ± 0.06^{ef}	16.67	9.33 ± 0.93^{abc}	17.32	6.50 ± 0.58bcde	15.38	15.00 ± 0.58ef	6.67	41.00 ± 4.04efghi	17.07	4.07 ± 0.23hi	9.70
Padi-Tuya	16.80 ± 0.65^{efg}	6.71	0.97 ± 0.09^{gh}	15.8	0.67 ± 0.03^{f}	8.66	13.33 ± 1.57^{fg}	20.37	7.87 ± 0.89efg	19.54	20.83 ± 0.17i	1.39	42.33 ± 2.33ghi	9.55	5.30 ± 0.69j	22.40
Nhyira	15.63 ± 0.26^{cdef}	2.88	0.63 ± 0.03^{abc}	9.12	0.57 ± 0.03^{de}	10.19	9.97 ± 0.75^{bcde}	13.09	6.97 ± 0.26def	6.47	14.00 ± 0.58cde	7.14	38.00 ± 5.51defgh	25.1	3.20 ± 0.42	22.87
Adom	19.77 ± 0.64^{j}	5.57	0.77 ± 0.03^{def}	7.53	0.53 ± 0.03^{cde}	10.82	13.13 ± 2.07^{fg}	27.26	4.57 ± 0.98a	37.05	13.00 ± 0.00	0.00	48.00 ± 0.00ij	0	4.22 ± 0.25i	10.37
Helewa	14.47 ± 0.95^{abcd}	11.41	0.60 ± 0.00^{ab}	0.00	0.50 ± 0.00^{cd}	0.00	12.07 ± 1.08^{ef}	15.56	7.07 ± 0.74def	18.20	12.00 ± 0.00b	0.00	50.67 ± 1.86j	6.34	2.99 ± 0.26bcdefg	14.80

PL =Pod length; SL = Seed length; SW = Seed width; LL = Leaf length; LW = Leaf width; SWg =100 seed weight; PH = Plant height; LL/LW = Leaf Area index

Means followed by the same letter are not significantly different at significance level of 0.05

Table 4.5 Numeric data on 22 cowpea varieties

VARIETY	NPP		NP		ND50F		NPPP		NL		NSP	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Soronko	3 ± 0.33^{e}	17.32	12 ± 0.58^{abcd}	8.33	61 ± 0.58^{gh}	1.64	20 ± 2.03^{bcdef}	17.86	17 ± 1.15^{h}	11.76	16 ± 1.33^{hij}	14.74
Nketewa	2 ± 0.00^{b}	0	17 ± 1.53^{ef}	15.56	56 ± 0.00^{bc}	0	20 ± 1.56^{bcdefg}	21.79	15 ± 1.00^{efgh}	11.55	14 ± 0.88^{defghi}	11.18
Hansadua	2 ± 0.33^{bc}	24.74	16 ± 1.53^{cdef}	16.54	58 ± 0.58^{de}	1.72	29 ± 0.67^{ij}	4.03	15 ± 0.88^{efgh}	9.96	15 ± 1.00^{ghij}	11.55
Bengpla	1 ± 0.33^{a}	43.3	17 ± 1.15^{ef}	11.76	52 ± 0.33^{a}	1.1	15 ± 2.52^{abc}	29.06	14 ± 0.67^{defgh}	7.87	15 ± 0.67^{fgij}	7.87
Tona	2 ± 0.33^{bc}	24.74	19 ± 4.16^{f}	37.95	58 ± 0.58^{de}	1.72	16 ± 2.19^{abcd}	23.18	14 ± 0.67^{cdefg}	8.06	14 ± 0.67^{efghij}	8.06
Apabgaala	3 ± 0.33^{e}	17.32	17 ± 2.60^{def}	27.06	62 ± 0.00^{hi}	0	23 ± 2.96^{efghi}	21.99	12 ± 1.15^{abc}	16.67	12 ± 1.15^{abcde}	16.67
Bra 01	1 ± 0.33^{a}	43.3	19 ± 1.15^{f}	10.53	57 ± 0.58^{cd}	1.75	21 ± 2.96^{cdefg}	24.05	12 ± 0.33^{abc}	4.68	12 ± 0.33^{abcdef}	4.68
Videza	4 ± 0.00^{f}	0	15 ± 2.89^{bcdef}	33.33	60 ± 0.58^{fg}	1.67	32 ± 2.60^{j}	14.24	13 ± 0.88^{bc}	11.46	13 ± 0.88^{cdefgh}	11.46
Ayiyi	2 ± 0.00^{b}	0	12 ± 0.58^{abcd}	8.33	55 ± 0.33^{b}	1.04	14 ± 0.88^{ab}	10.66	17 ± 0.58^{h}	5.88	16 ± 0.88^{j}	9.35
SARC-1-57-2	2 ± 0.00^{b}	0	13 ± 1.67^{abcde}	22.79	55 ± 0.58^{b}	1.82	28 ± 4.58^{hij}	28.35	14 ± 1.00^{bcdefg}	12.37	13 ± 1.20^{cdefgh}	15.61
Zaayura	2 ± 0.33^{bc}	24.74	14 ± 0.88^{abcde}	11.18	60 ± 0.58^{fg}	1.67	19 ± 3.61^{bcde}	32.87	11 ± 1.15^{a}	18.18	11 ± 0.88^{ab}	14.32
Asetenapa	2 ± 0.33^{bc}	24.74	15 ± 1.20^{def}	12.49	60 ± 0.58^{fg}	1.67	18 ± 1.20^{abcde}	11.78	15 ± 1.20^{fgh}	13.29	12 ± 0.67^{abcdef}	9.36
Asontem	3 ± 0.33^{cd}	21.65	10 ± 0.58^{a}	10	59 ± 0.00^{ef}	0	22 ± 1.53^{defgh}	12.03	16 ± 0.33^{gh}	3.53	16 ± 0.00^{ij}	0
Zamzam	2 ± 0.00^{b}	0	14 ± 0.88^{abcde}	11.18	58 ± 0.33^{de}	0.99	26 ± 0.88^{ghij}	5.8	13 ± 0.67^{abcdef}	8.66	11 ± 1.20^{ab}	19.52
Asomdwoe	3 ± 0.00^{e}	17.32	14 ± 0.33^{abcde}	4.22	66 ± 0.00^{l}	0	26 ± 1.76^{fghij}	11.9	11 ± 0.33^{ab}	4.95	10 ± 0.58^{a}	10
Sanzi	2 ± 0.33^{bc}	24.74	10 ± 0.88^{ab}	14.78	63 ± 0.58^{ij}	1.59	18 ± 1.45^{bcde}	13.73	13 ± 0.67^{bcdef}	8.45	14 ± 0.58^{defghij}	7.14
Agyenkwa	3 ± 0.00^{de}	0	14 ± 1.20^{abcdef}	14.52	64 ± 0.58^{jk}	1.56	21 ± 0.88^{cdefg}	7.39	13 ± 1.33^{bcdef}	16.9	13 ± 0.88^{bcdefg}	12.06
Songotra	2 ± 0.33^{bc}	24.74	15 ± 0.88^{abcdef}	10.41	62 ± 0.00^{hi}	0	15 ± 0.88^{abc}	9.96	15 ± 1.00^{efgh}	11.55	14 ± 0.58^{defghij}	7.14
Padi-Tuya	2 ± 0.00^{b}	0	13 ± 0.88^{abcde}	11.46	60 ± 0.58^{fg}	1.67	12 ± 1.33^{a}	19.79	13 ± 0.00^{abcde}	0	12 ± 0.88^{abcd}	13.09
Nhyira	3 ± 0.67^{cd}	43.3	12 ± 0.33^{abcde}	4.68	64 ± 0.00^{jk}	0	21 ± 2.60^{cdefg}	21.82	13 ± 0.67^{bcdef}	8.45	11 ± 0.58^{abc}	9.09
Adom	2 ± 0.00^{b}	0	11 ± 0.88^{abc}	13.48	65. ± 0.33^{l}	0.88	16 ± 0.88^{abcd}	9.75	16 ± 0.88	9.35	16 ±1.20^{hij}	13.29
Helewa	3 ± 0.67^{cd}	43.3	16 ± 2.33^{def}	24.74	65 ± 0.58kl	1.54	22 ± 1.73^{defgh}	13.64	14 ± 1.20^{defgh}	14.19	14 ± 1.15^{defghij}	14.29

NPP = No. of pods per plant; NP = No. of peduncles per plant; ND50F = No. of days to 50% flowering; NPPP = No. of pods per peduncle; NL = No. of locules per pod; NSP = No. of seeds per pod.

Means followed by the same letter are not significantly different at significance level of 0.05

4.1.3 Correlation analysis

A correlation matrix of 14 quantitative trait variables was computed using Pearson correlation coefficient (Table 4.6) with the aid of Statistical Package for Social Science (SPSS) v.16 software.

There was a strong correlation between 100 seed weight and seed length (r = 0.788), as well as seed width (r = 0.604). This implies that for most cowpea varieties, 100 seed weight increases as the seed size increases.

The strongest positive correlation in this study occurred between number of locules and number of seeds per pod (r = 0.837). This value implies that number of seeds per pod increases as number of locules increases for most cowpea varieties characterised in this study.

Number of seeds per pod correlated positively with pod length (r = 0.561) which implies that, most varieties with longer pods had more seeds.

A strong positive correlation also existed between seed length and seed width (r = 0.627). This implies that most varieties with long seeds also have broad seeds.

Table 4.6 Correlation coefficient values among 14 quantitative trait of cowpea

SWg	1													
LL/LW	-0.203	1												
LL	-0.126	0.238	1											
LW	0.01	-0.59	0.432	1										
ND50F	-0.068	0.034	0.221	0.228	1									
NL	-0.464*	0.245	0.179	-0.179	0.475	1								
NP	0.148	-0.298	-0.197	0.396	-0.072	-0.231	1							
NPP	-0.224	0.076	0.334	0.176	0.021	-0.16	-0.141	1						
NPPP	0.244	-0.099	-0.201	-0.153	0.169	-0.316	0.079	0.431*	1					
NSP	-0.483	0.199	0.132	-0.19	0.196	0.837**	-0.199	-0.091	-0.277	1				
PL	0.142	0.069	0.336	-0.01	0.154	0.55*	-0.242	-0.429	-0.379	0.561**	1			
PH	-0.238	-0.007	0.747**	0.476**	0.277	0.236	-0.128	0.297	-0.183	0.174	0.139	1		
SL	0.788**	-0.154	-0.021	0.074	-0.148	-0.33	-0.051	-0.242	-0.107	-0.311	0.304	-0.157	1	
SW	0.604**	0.129	-0.062	-0.19	-0.143	-0.036	-0.009	-0.402*	-0.26	-0.08	0.368	-0.252	0.627**	1
	SWg	LL/LW	LL	LW	ND50F	NL	NP	NPP	NPPP	NSP	PL	PH	SL	SW

** = Correlation is significant at the 0.01 level (2-tailed). * = Correlation is significant at the 0.05 level (2-tailed).

4.1.4 Quantitative Principal Component analysis

The 14 quantitative trait variables were subjected to stepwise discriminant analysis. The analysis revealed that the first three principal components accounted for approximately 85.91% of the total variation among the 22varieties (Table 4.7).

Most of the genetic variation observed was explained by the first principal components (48.99%), followed by the second (26.91%). Hundred seed weight had high positive loadings, while number of days to 50% flowering had high negative loadings on the first principal components (PC1) (Table 4.6). This implies that PC1 is primarily a measure of increasing 100 seed weight and decreasing days to 50% flowering. In this case, varieties with high PC1 values tend to have heavy seeds and also required less number of days to bear 50% of their flowers.

The second principal component (PC2) also correlated positively with 100 seed weight and number of days to 50% flowering (Table 4.7). This principal component can be viewed as a measure of increasing 100 seed weight and increasing number of days to 50% flowering. In this instance, varieties with high PC2 values have heavy seeds and also require several days to produce 50% of their flowers.

The third principal component (PC3) with total variation of 10.01% correlated positively with number of days to 50% flowering and plant height. It however, correlated negatively with leaf area index which implies that varieties with high PC3 values have high number of days to 50% flowering, high plant height and low leaf area index values.

Plant height and leaf area index had strong positive loadings with respect to principal component four (PC4) while number of days to 50% flowering and 100 seed weight had high negative loadings with respect to PC4. This implies that cowpea varieties with high

PC4 values have high plant height, high leaf area index but low number of days to flowering and low 100 seed weight.

The principal component analysis reduced the fourteen quantitative traits to four most important traits which contributed greatly to the variation observed in the 22 varieties. These were 100 seed weight, Plant height, Number of days to 50% flowering and Leaf Area Index (Table 4.7).

Table 4.7: Five Principal components for 14 quantitative traits of cowpea

	PC1	PC2	PC3	PC4	PC5
Latent roots	98.34	54.02	20.08	12.91	6.41
Percentage variation	48.99	26.91	10.01	6.49	3.29
Cumulative percent variation	48.99	75.9	85.91	92.4	95.69
Latent vectors (loadings)					
100 Seed Weight (g)	0.5964	0.3712	0.2302	-0.405	0.2058
Leaf length	-0.0183	0.1341	0.0326	0.2869	0.1565
Leaf width	0.0359	-0.0401	0.2988	0.1375	0.1568
Leaf Area Index	-0.0845	0.2471	-0.4073	0.3069	0.0617
Days to 50% flowering	-0.38	0.4439	0.471	-0.3836	-0.3482
Number of locules	-0.03	-0.0328	-0.0677	0.275	-0.0912
Number of peduncles per plant	0.0443	-0.0488	0.0772	-0.0386	0.0021
Number of pods per peduncle	-0.0829	0.0347	0.0623	-0.0937	0.2207
Number of pods per plant	-0.0138	-0.0105	-0.0296	-0.2352	0.4233
Number of seeds per pod	-0.0237	-0.0307	-0.1446	0.2703	-0.1111
Pod length	0.0552	0.0608	-0.1525	0.2742	-0.0245
Plant height	-0.0253	0.1145	0.3761	0.6014	0.2596
Seed length	0.138	0.1211	0.0759	-0.145	-0.2546
Seed width	0.1458	0.0953	0.0247	0.0012	-0.4076

PC = Principal Component

4.1.5 Principal components biplot

Agraphical representation of the accessions on principal components 1 and 2 was constructed with the aid of Genstat 12th edition (Figure 4.2). The varieties widely separated along the axis of the two principal components. Adom, Asontem, Hewale and Asomdwoe had high PC1 values which indicate that such varieties have heavy seeds and also require less number of days to bear 50% of their flowers. Meanwhile, Bra-01, Soronko, Padi-Tuya and Zaayura also had high PC2 values meaning such varieties have heavy seeds but require several days to bear 50% of their flowers. Bra-01 (exotic variety) had the lowest PC1 value while Sanzi (landrace) had the lowest PC 2 value.

The Landrace (Sanzi) diverged into the lower right quadrat while the exotic variety (Bra-01) diverged into the upper left quadrat of the graph. Most of the earlier varieties released by CSIR-CRI mostly grouped in the upper right quadrat of the graph, while most of the newly released varieties also grouped around the origin. Varieties developed by CSIR-SARI concentrated in the upper half of the graph except for SARC-1-57-2 and Apabgaala which diverged into the lower half of the graph.

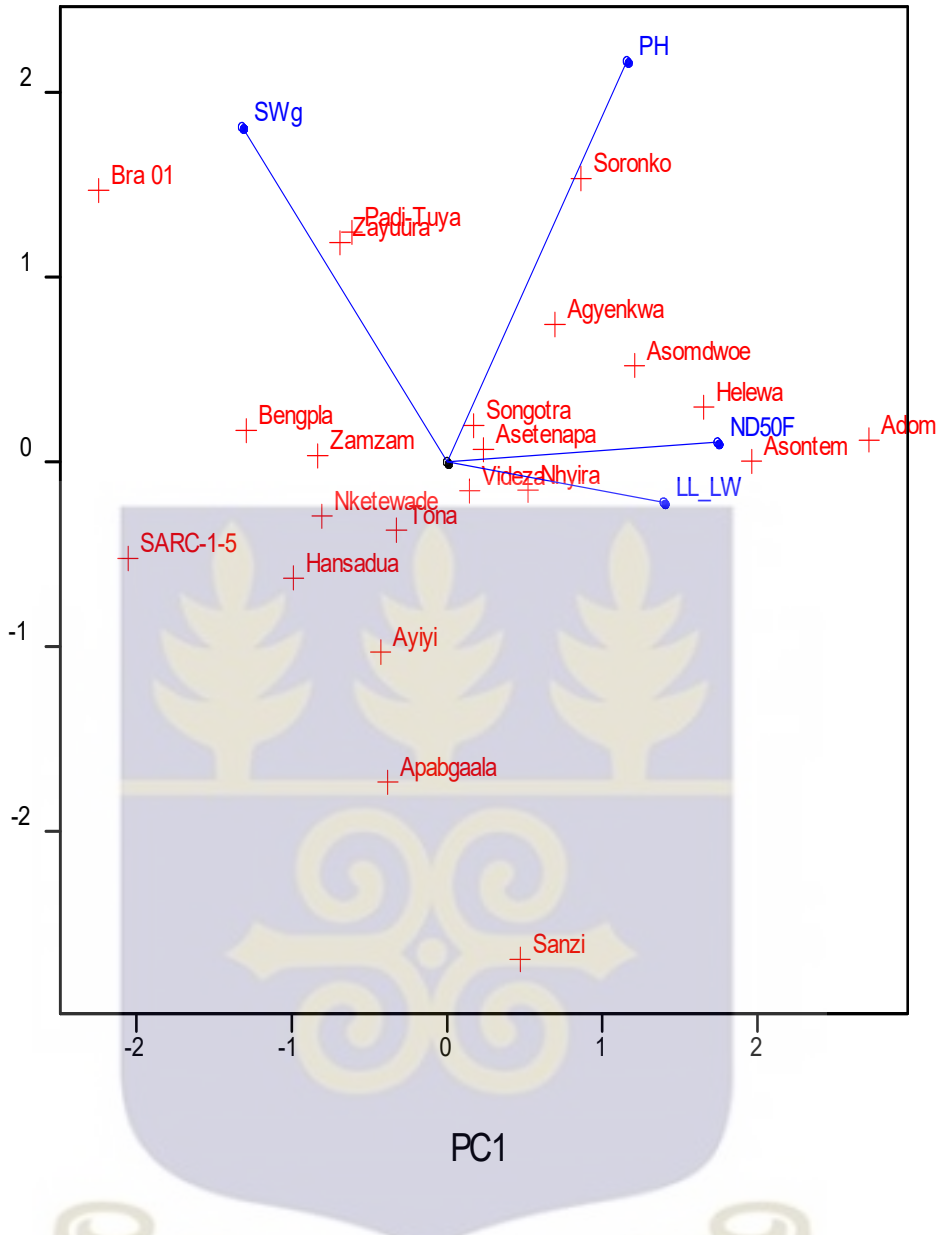

Figure 4.2: PC 1 and PC 2 biplot using quantitative trait scores of 22 cowpea varieties generated with the aid of Gentat 12th edition.

4.1.6 Dissimilarty matrix and Cluster analysis based on quantitative traits

The quantitative data obtained from the agro-morphological characterization, was fed into the Statistical Package for Social Sciences (SPSS) software to generate a dissimilarity matrix based on rescaled squared Euclidean distance as shown in Appendix 6. Dissimilarity ranged between 0.001 and 1.00. The results showed that there was no distance between Asetenapa and Songotra indicating that these two varieties are very similar. The minimal distance of 0.001 was found between SARC-1-57-2 and Hansadua while the highest distance of 1.000 (most divergent) was found between Soronko and Apabgaala.

A dendrogram was generated with the aid of the Genstat 12th edition using all the agro-morphological data collected (Figure 4.3). The 22 varieties were clustered into four major clusters at similarity coefficient 0.85. The Average Linkage Cluster Analysis (ALCA) cluster analysis separated the 22 varieties with Euclidean similarity distance. Cluster A consisted of only Soronko (released by CSIR-CRI). Cluster B was made up of two CSIR-CRI varieties (Asontem and Adom). The largest group was cluster C which was composed of 11 varieties with three sub-clusters diverging at similarity coefficient 0.86. The composition of the sub-clusters have been summarised in Appendix 3. There were three sub-groups under cluster C. All the varieties in sub-group I and II except SARC-1-57-2 were released by CSIR-CRI. On the other hand, subgroup III consisted of two varieties released by CSIR-SARI (i.e. Zaayura and Padi-Tuya) and the exotic Bra-01. Cluster D was made up of 8 varieties with two sub-clusters at similarity coefficient 0.875. Sub-group I consisted of five CSIR-CRI released varieties and two CSIR-SARI varieties.

Sub-cluster II on the other hand consisted of only Sanzi (landrace).

The nature of clustering suggests that the source of the varieties contributes to the differences observed among the varieties.

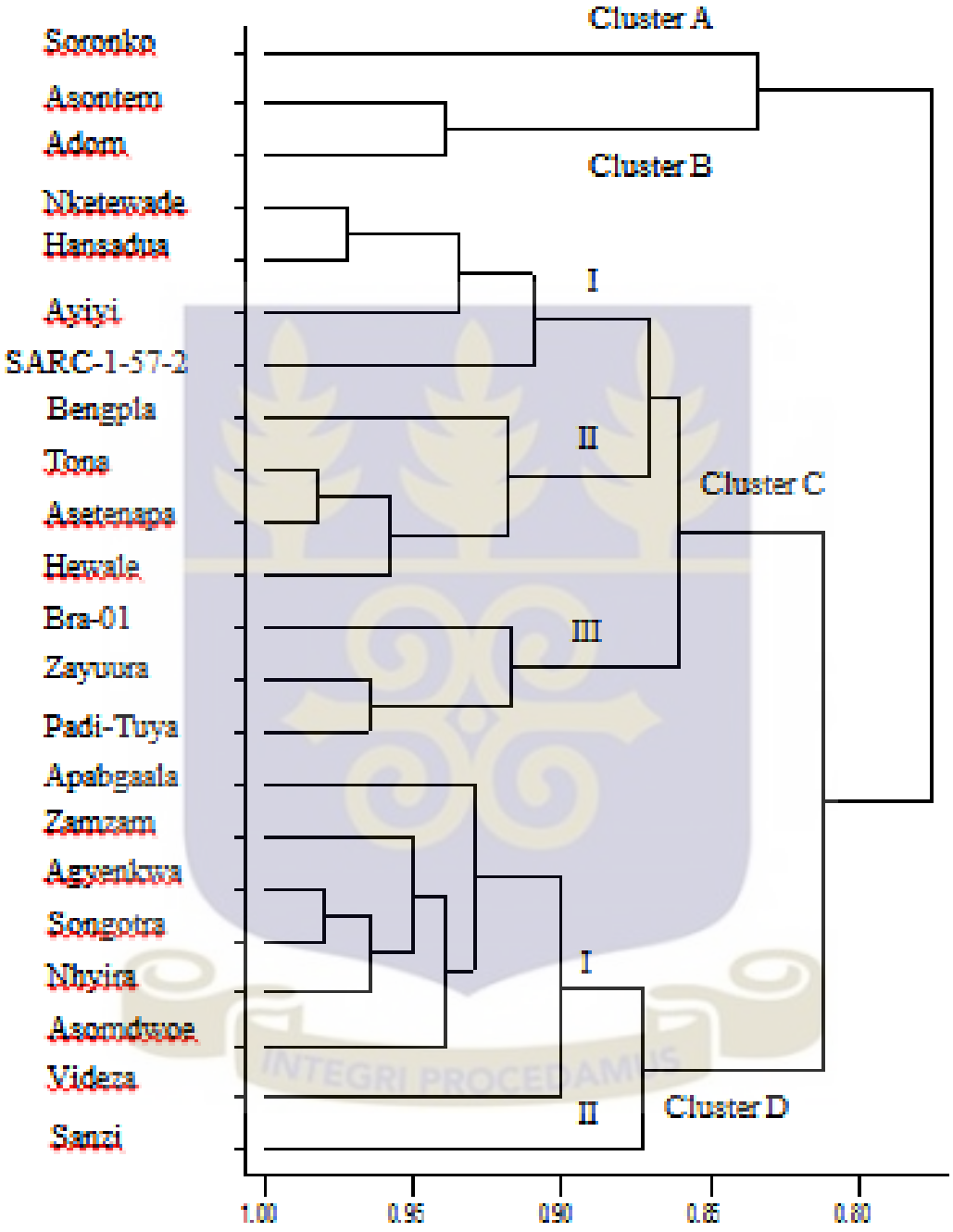

Figure 4.3: Dendrogramof 22 cowpea varieties generatedfrom 14 quantitative parameters using Euclidean similarity distance generated with the aid of Genstat 12th edition

4.2 Molecular Characterization of Cowpea varieties

Twenty pairs of SSR primers (forward and reverse) were used to analyse genetic diversity of 22 cowpea varieties. The bands observed on the gels after v-PAGE were scored on the basis of presence/absence (1/0) with the aid of the AlphaImager version 3.41 software. The primers generated a total of 481 bands across the selected varieties out of which 428 (88.98%) were polymorphic. Only 1 (SSR 6336) out of the 20 primers did not show polymorphism among the varieties, and therefore was excluded from the analysis. Scores from the informative bands were used to generate a matrix which was subjected to resolving power analysis and cluster analysis using Power marker® software.

The size of amplified alleles ranged from 90bp to 391bp (Table 4.8). The primers SSR-6613 and SSR-6608 recorded the highest and lowest number of polymorphic bands of 79 and 5 respectively. The number of alleles varied from 1 to 6 (Table 4.8). The allele frequency ranged from 0.136 (SSR-6371 to 0.841 (SSR-6608) with mean of 0.445 among the varieties. The polymorphic information content (PIC) representing the allele diversity for a specific locus ranged from 0.107 (SSR-6608) to 0.656 (SSR-6613) with a mean of 0.293.

Table 4.8 Resolving power analysis of SSR primers

Primer	Allele size range (bp)	No. of alleles	Allele frequency	Number of bands	Number of Polymorphic bands	Polymorphic Information Content (PIC)
SSR-6265	217 – 282	5	0.445	49	49	0.631
SSR-6258	195 – 269	2	0.523	23	21	0.242
SSR-6243	165 – 187	2	0.5	22	20	0.202
SSR-6218	149 – 287	2	0.409	18	16	0.266
SSR-6217	230 – 294	2	0.341	15	15	0.242
SSR-6353	90 – 115	2	0.523	23	22	0.199
SSR-6352	113 – 141	3	0.257	17	17	0.246
SSR-6323	220 – 285	3	0.348	23	22	0.331
SSR-6277	114	1	0.318	7	7	0.340
SSR-6436	266 – 370	3	0.454	30	29	0.405
SSR-6375	296 – 333	2	0.522	23	22	0.405
SSR-6371	164 – 195	2	0.136	6	6	0.185
SSR-6370	254 – 275	2	0.386	17	14	0.091
SSR-6356	127 – 147	2	0.5	22	22	0.223
SSR-6613	250 – 391	6	0.598	79	79	0.656
SSR-6608	233 – 300	2	0.841	37	5	0.107
SSR-6603	358 – 386	2	0.477	21	20	0.261
SSR-6587	336 - 352	2	0.409	18	14	0.204
SSR-6451	110 – 155	3	0.469	31	28	0.325
Mean		**2.526**	**0.445**	**25.32**	**22.53**	**0.293**

4.3 Dissimilarity matrix and Cluster analysis based on SSR markers

Following the gel scoring, the molecular data was fed into DARwn 6.0.010 sofware (Perrier and Jacquemoud-Collet, 2006) to generate a dissimilarity matrix and a dendrogram. From the dissimilarity matrix (Appendix 7), there was no distance between Videza and Asomdwoe which implies that the two varieties are very similar. A short distance of 0.109 was found between Zamzam and Agyenkwa while the highest distance (most divergent) was found among three pairs of varieties: Agyenkwa and Adom; Hewale and Ayiyi; Zamzam and Helwale at genetic distance of 0.652.

The dendrogram grouped the 22 varieties into four major clusters (A, B, C and D) at genetic distance of 0.20 (Figure 4.4). Cluster A was made up of only Hewale (CRI-released variety) (Appendix 4). Cluster B had two sub-clusters diverging at genetic distance of 0.125. Sub-cluster I consisted of only Zaayura while subcluster II consisted of Asomdwoe and Videza. The third major cluster was cluster C which consisted of 9 varieties grouped into two subclusters diverging at genetic distance of 0.15. Cluster D also consisted of two sub-clusters. The first sub-cluster comprised of 5 varieties (Adom, Asontem, Tona, Nhyira and Soronko) while the second sub-cluster consisted of 3varieties (Hasnsadua, Bengpla and Songotra). Unlike the results observed in the qualitative and quantitative cluster analysis, the dendrogram generated from the molecular data did not group the varieties based on geographical homogeneity.

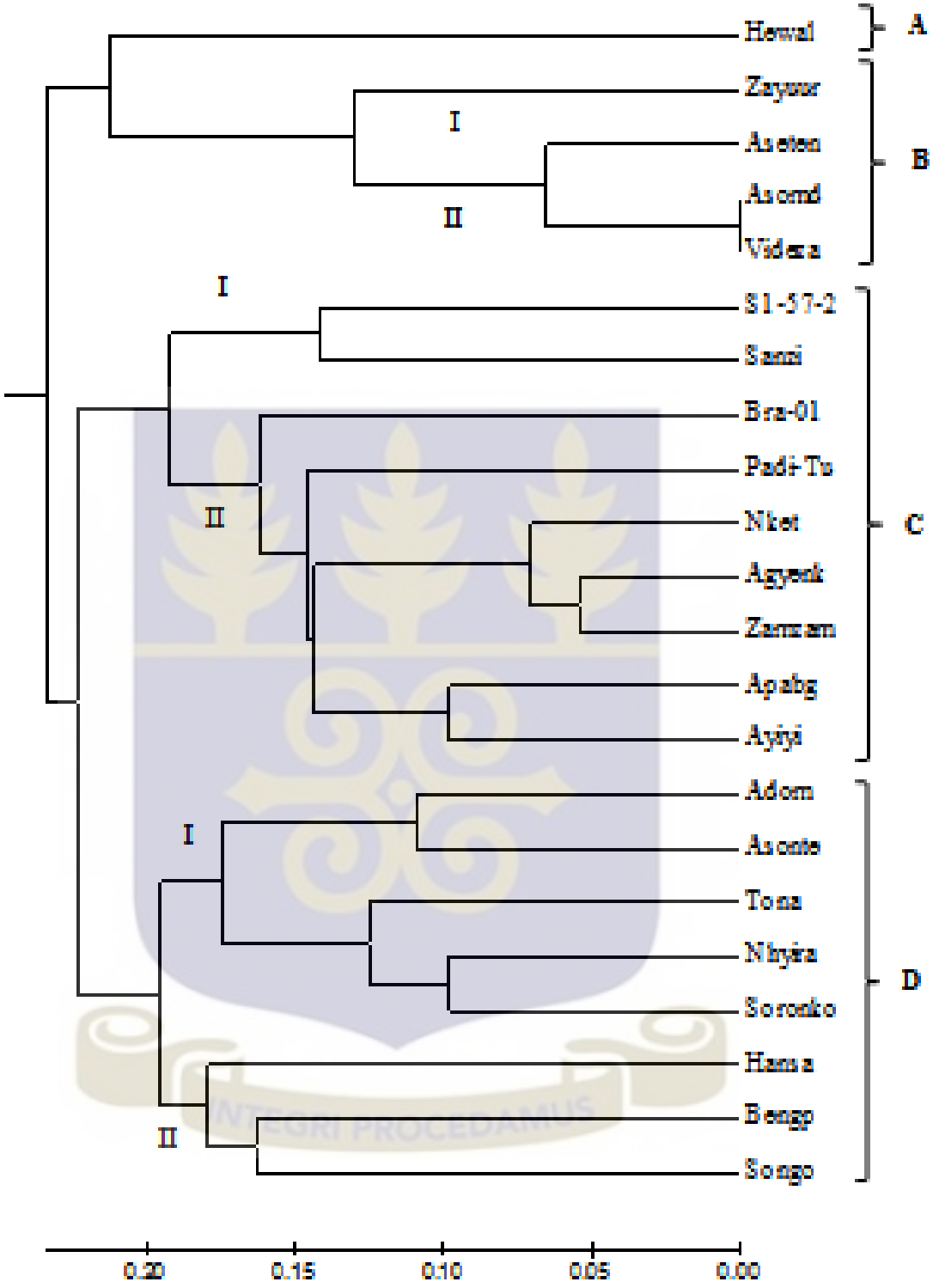

Figure 4.4: Dendrogram of 22 cowpea varieties using 19 SSR primers based on Nei (1983) distance generated with the aid of DARwn 6.0.010 sofware

4.4 Molecular Screening of Cowpea Varieties against *A. craccivora*

The SSR marker CP171F/CP172R was used to identify aphid resistance status of 20 cowpea varieties in addition to two checks (Plate 4.5).

Plate 4.5.CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%) for 22 cowpea varieties. M = 100bp ladder; R = Resistant Check (SARC-1-57-2); S = Susceptible Check (Apagbaala); 1 = Nhyira; 2 = Hewale; 3 = Asomdwee; 4 = Adom; 5 = Soronko; 6 = Bengpla; 7 = Hansadua; 8 = Agyenkwa; 9 =Padi-Tuya; 10 = Zaayura; 11 = Nketewade;12 = Zamzam;13 = Ayiyi; 14 = Songotra; 15 = Videza; 16 = Asontem; 17 = Tona; 18 = Asetenapa; 19 = Sanzi; 20 = Bra 01

The aphid resistance status of the 22 varieties screened with the SSR marker CP171F/172R is presented in Table 4.9. Susceptible band size was 168bp while that of the resistant was 180bp. They all fall within the margin of error for the expected band size. Five out of the twenty varieties were identified as resistant varieties. The other fifteen varieties were susceptible to cowpea aphid infestation.

Table 4.9: Aphid resistance status of 22 cowpea varieties

Resistant varieties	Susceptible varieties
SARC-1-57-2 (Check)	Apagbaala (Check)
Adom	Hewale
Sanzi	Nhyira
Tona	Agyenkwa
Soronko	Asontem
Songotra	Zamzam
	Videza
	Asetenapa
	Bra-01
	Bengpla
	Nketewade
	Padi-Tuya
	Ayiyi
	Asondwee
	Zaayura
	Hansadua

4.5 Hybridization and Development of F1 progenies

Successful hybridization of SARC-1-57-2 (resistant line) with three farmer preferred aphid susceptible cowpea varieties (Asontem, Nhyira and Asetenapa) resulted in the development of a total of 34 hybrid (F_1) pods and 233 hybrid seeds (Table 4.10).

Table 4.10: Number of pods and seeds obtained from Pollination (F1)

Entries	No. of Pods	No. of Seeds
SARC-1-57-2 x Asontem	13	85
SARC-1-57-2 x Nhyira	11	76
SARC-1-57-2 x Asetenapa	10	72
Total	34	233

4.6 Development of BC_1 progenies

Following the methodology in sub-section 3.2.1.3, BC_1 seeds were developed from crosses between the hybrids (F_1) and recurrent parents (Asontem, Asetenapa and Nhyira) as shown in Table 4.11.

Table 4.11: Number of pods and seeds obtained from Pollination (BC_1)

Entries	No. of Pods	No. of Seeds
(SARC-1-57-2 x Asontem) x Asontem	11	52
(SARC-1-57-2 x Nhyira) x Nhyira	9	49
(SARC-1-57-2 x Asetenapa) x Asetenapa	5	38
Total	25	139

4.7 Molecular Screening of BC_1

4.7.1 Asontem

A total of 47 seedlings developed from the cross (SARC-1-57-2 x Asontem) x Asontem were subjected to genotypic screening using the SSR marker CP 171F/172R (Plate 4.6a and 4.6b). Out of the 47 BC_1 individuals screened, the segregation at the marker locus was 25:22 (homozygous susceptible: heterozygous) (Table 4.12). The segregation fit the expected 1:1 ratio for heterozygous and homozygous susceptible individuals based on Chi-squared tests for goodness of fit ($\chi 2 = 0.191$; $P < 0.75$) (Appendix 10a). Individuals showing heterozygosis at the aphid resistance locus were selected for development of BC_2 generation.

Plate 4.6a.CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%) for BC_1progeny developed from Asontem. Marker amplify once for homozygotes and twice for heterozygotes. Arrow pointing to the expected band size. M= 100bp ladder, The 1 and 2 represent susceptible homozygous and heterozygous individuals respectively of the BC_1 progeny.

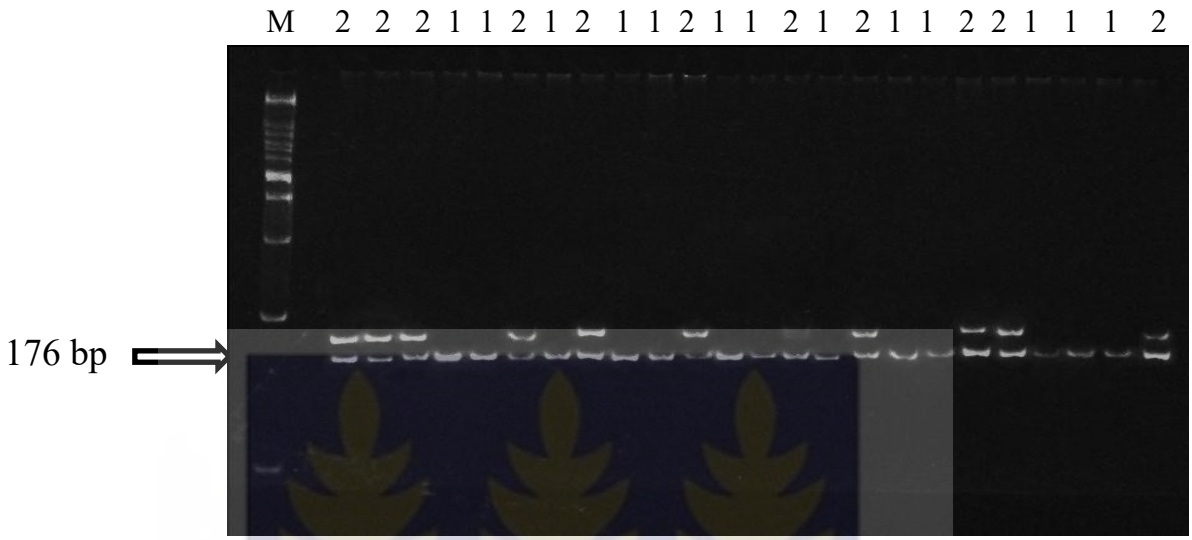

Plate 4.6b.CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%) for BC_1progeny developed from Asontem. Marker amplify once for homozygotes and twice for heterozygotes. Arrow points to the expected band size. M= 100bp ladder; The 1 and 2 represent susceptible homozygous and heterozygous individuals respectively of the BC_1 progeny.

4.7.2 Nhyira

Forty-seven BC_1 seedlings developed from (SARC-1-57-2 x Nhyira) x Nhyira were screened with the SSR-marker CP 171F/172R (Plates 4.7a and 4.7b). The marker segregated in the ratio 22:25 (homozygous susceptible: heterozygous) ($\chi 2 = 0.191$; $P < 0.75$) (Table 4.12, Appendix 10b). Individuals showing heterozygosis at the aphid resistance locus were selected for development of BC_2 generation.

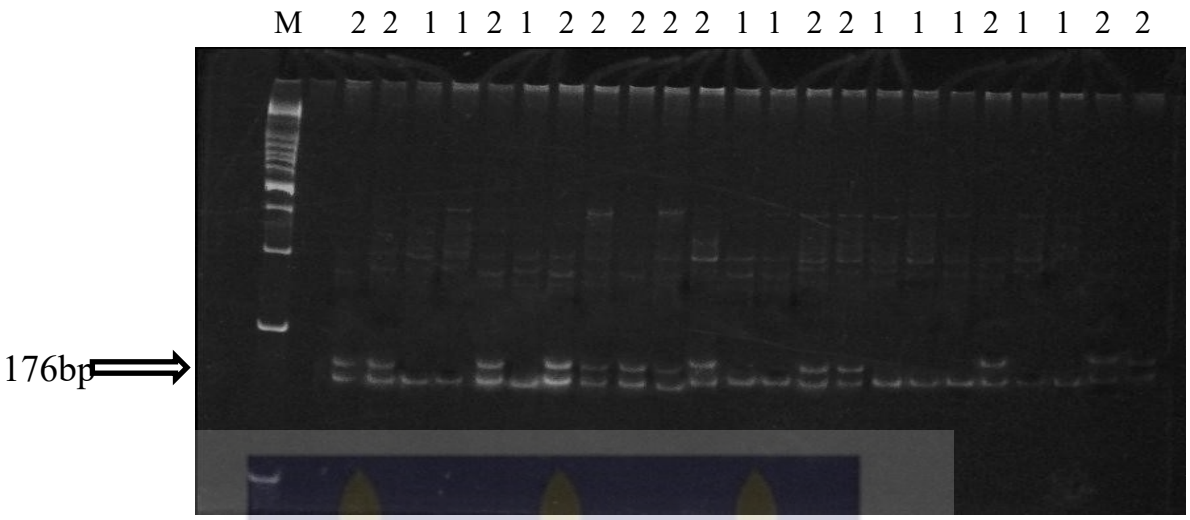

Plate 4.7a. CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%) for BC_1progeny developed from Nhyira. Marker amplify once for homozygotes and twice for heterozygotes. Arrow pointing to band of interest. M= 100bp ladder, The 1 and 2 represent susceptible homozygous and heterozygous individuals respectively of the BC_1 progeny.

Plate 4.7b. CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%)for BC_1 progeny developed from Nhyira. Marker amplify once for homozygotes and twice for heterozygotes. Arrow pointing to band of interest. M= 100bp ladder, The 1 and 2 represent susceptible homozygous and heterozygous individuals respectively of the BC_1 progeny.

4.7.3 Asetenapa

Thirty-four seedlings developed from the cross (SARC-1-57-2 x Asetenapa) x Asetenapa were subjected to genotypic screening using the SSR marker CP 171F/172R (Plates 4.8a and 4.8b). Out of the 34 BC_1 individuals screened for Asetenapa, the segregation at the marker locus was 18:16 (homozygous susceptible: heterozygous) (Table 4.12). The segregation fit the expected 1:1 ratio for heterozygous and homozygous susceptible individuals based on Chi-squared tests for goodness of fit ($\chi 2 = 0.191$; $P < 0.75$) (Appendix 10c). The 34 individuals that showed heterozygosis at the aphid resistance locus were selected for development of BC_2 generation.

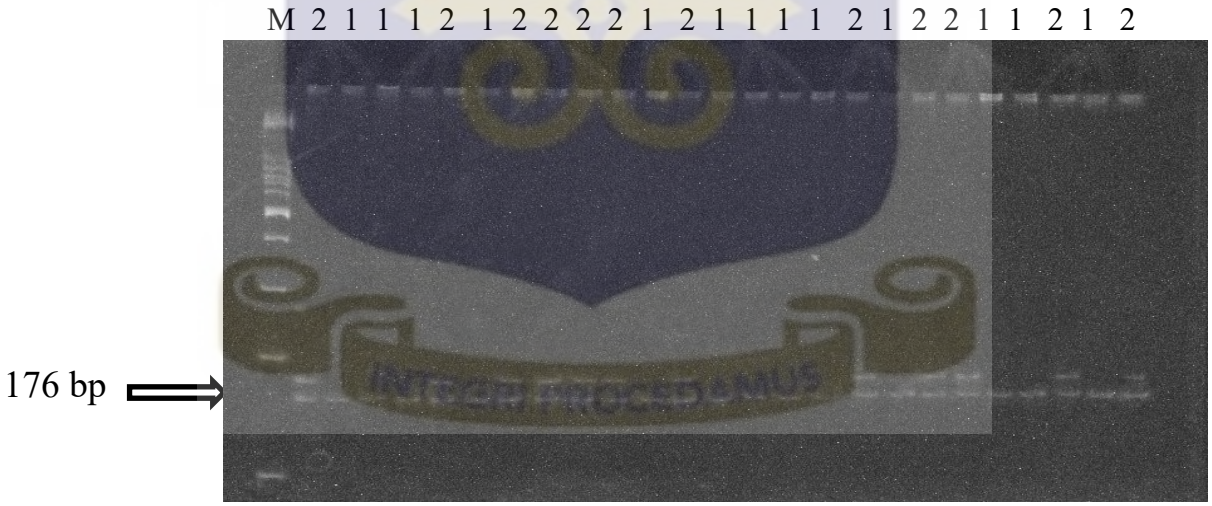

Plate 4.8a. CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%)for BC_1 progeny developed from Asetenapa. Marker amplify once for homozygotes and twice for heterozygotes. Arrow pointing to band of interest. M= 100bp ladder, The 1 and 2 represent susceptible homozygous and heterozygous individuals respectively of the BC_1 progeny.

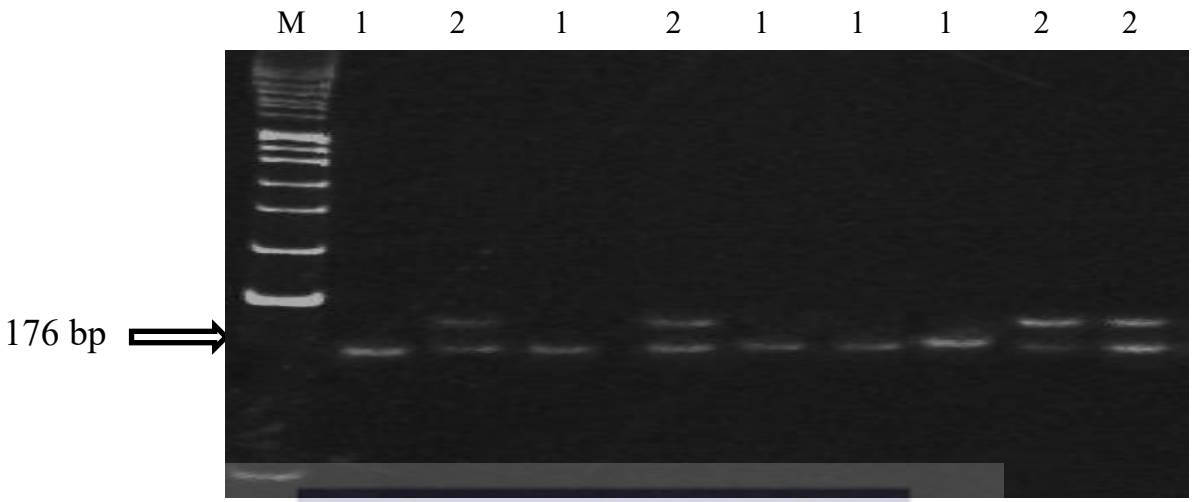

Plate 4.8b. CP171F/172R marker scored on ethidium bromide stained PAGE gel (6%)for BC_1 progeny developed from Asetenapa. Marker amplify once for homozygotes and twice for heterozygotes. Arrow pointing to band of interest. M= 100bp ladder, The 1 and 2 represent susceptible homozygous and heterozygous individuals respectively of the BC_1 progeny.

Table 4.12: Marker-assisted selection of BC_1 progenies

Entries	No. of varieties screened	No. of selected varieties
(SARC-1-57-2 x Asontem) x Asontem	47	22
(SARC-1-57-2 x Nhyira) x Nhyira	47	25
(SARC-1-57-2 x Asetenapa) x Asetenapa	34	16
Total	128	63

4.8 Development of BC_2 progenies

Following the methodology in sub section 3.2.1.3, 261 BC_2 seeds were developed from crosses between selected heterozygous BC_1 individuals and recurrent parents (Asontem, Asetenapa and Nhyira) as shown in Table 4.13.

Table 4.13 Number of pods and seeds obtained from Pollination (BC_2)

Entries	No. of Pods	No. of Seeds
BC_1 x Asontem	15	84
BC_1 x Nhyira	19	103
BC_1 x Asetenapa	12	74
Total	46	261

CHAPTER FIVE

5.0 DISCUSSION

5.1 Agro-morphological characterization and diversity studies of 22 cowpea varieties

In this study, unique identity of varieties as well as variation were observed among the cowpea varieties with respect to the qualitative and quantitative agro-morphological traits that were measured except for leaf apex shape. This was expected since most of the varieties used in this study are released varieties that have met requirements of the National Variety Release and Registration Committee (NVRRC) and have been tagged as unique and stable as at the time of their release. It is however likely that some of the characters at the time of release may have been modified by the environment. Moreover, it is necessary to occasionally check the stability of the characteristics that were found on the cultivars at the time of varietal release.

5.1.1 Qualitative characters

Pigmentation in cowpea is as a result of the presence of anthocyanin in some varieties (Singh and Rachie, 1985). It appears as purple colouration on different parts of the plant in different patterns (Fery, 1985). The intensity and location of the purple pigment is however controlled largely by genetic factors (Javanmardi, 2002). It was observed in this study that, the nodes of all the varieties except Asontem were pigmented. This gives Asontem a unique identity among the other varieties. The cowpea production manual of CSIR-CRI (2006) was silent on the variation in vegetative pigmentation found on the cowpea varieties released by the institute. This

study has identified variation in plant pigmentation and will add on to the information known about the varieties released by CSIR-CRI. In this study, varying degrees of purple pigmentation were observed at the petiole, branches, stem, sepal, immature pods and flowers as documented by IBPGR (1983). Majority of the varieties had pigmented petiole (86.37%) and branches (68.18%) (Table 4.1). Such varieties possess anthocyanin in their branches and petioles. However, most of the varieties had no pigmentation on their stem (86.36%), sepal (68.18%) and immature pods (68.20%) as shown in Table 4.1. Such varieties do not possess anthocyanin in their stem, sepal and immature pods. Cobbinah *et al*. (2011) also reported that most of the accessions used in their work had no pigmentation on the immature pods.

All the levels of twining tendency documented in IBPGR (1983) cowpea descriptor were observed in this study. However, majority of the varieties had either no twining tendency or very slight twining tendency (Table 4.1). This result suggests that most of the accessions need no staking since only a few showed trailing tendencies. A similar trend was observed by Cobbinah e*t al.* (2011), where the percentage of varieties with twining tendency was only 3.7%. This also implies that the cost and labour associated with the provision of stakes aimed at preventing rotten would not be necessary and this can reduce the cost of production (Cobbinah *et al*., 2011).

It was again observed that, all the varieties had acute terminal leaf apex shape (Table 4.1). Such monomorphic traits are not useful in diversity studies (Acquuah, 2012).

Nualsri *et al*. (2010) reported seven different kinds of terminal leaflet shapes in cowpea and yardlong bean, some of which were identified in this current study as shown in Plate 4.1. Bengpla and Asomdwoe were the only varieties with globose terminal leaflet shape (Table 4.2).

In this study, three kinds of terminal leaflet base were reported (Table 4.1). All the three classes of terminal leaflet base were also captured by Nualsri *et al*. (2010).

The International Board for Plant Genetic Resources, IBPGR (1983) descriptor described seven growth habits. Six out of the seven growth habits were identified in this study. The difference may be due to the fact that the IBPGR used large number of cowpea germplasm collected from major cowpea growing around the world as compared to the relatively smaller sample size collected from only Ghana.

The growth habit of cowpea varieties plays an important role in the choice of cropping system by peasant farmers and also during harvesting of pods. Cowpea accessions with prostrate growth habit are used by peasant farmers in mixed cropping (Doku, 1970; Rachie and Rawal, 1976), while the erect types have good returns for high intercrop adaptability.Cobbinah *et al* (2011), also observed that the prostrate types had protracted periods of pod maturation resulting in an uneven periods of harvesting and also required bending very low to pick the pods. Moreover, it was observed in this study that some of the pods of the prostrate varieties touched the soil and were quick to rot on the field. Majority of the varieties used in this work had semi-erect or erect growth habit (72.72%) (Table 4.1). This is contrary to the findings of Doumbia (2012) where majority of the Ghanaian cowpea accessions studied had semi-prostrate growth habit. The difference may be attributed to the fact that most of the varieties used in the current study are released varieties that were selected against prostrate and climbing growth habits in other to enable easy harvesting and also prevent rotting of pods on the field. On the other hand, the cowpea accessions used by Doumbia (2012) involved many landraces that are well known for their prostrate growth habit.

The presence of plant hairs is one of the antixenotic mechanisms by which plants resist the damage by insect pests. Plant hairs can also make harvesting and crosses quite difficult due to the itchy nature. In this study, 63.64% of the varieties had no hairs on the petiole (Table 4.1), 90.91% had no hairs on the stem and 59.09% had no hairs on the leaf. This confirms that insect tolerant varieties without plant hairs (e.g. SARC-1-57-2, Sanzi and Tona) do not employ antixenosis as mechanism of insect tolerance but rather antibiosis and tolerance (Kusi, 2014). However, the insect resistance ability of hairy varieties such as Bengpla and Adom may be attributed to the combination of antixenosis and antibiosis. It was found out in this work that Tona did not have any form of plant hairs just as was reported by CSIR-CRI (2006).

Raceme position plays a very important role in harvesting since it enhances visibility. Racemes, held at the same level or within the canopy bears hidden pods within the canopy making harvesting and crosses more difficult and strenuous (Cobinnah *et al*., 2011). Majority of the varieties studied in this current work had the raceme either in the upper canopy (40.91%) or mostly above the canopy (31.82%) totaling 72.73% as shown in Table 4.1. This result is consistent with that of Cobbinah *et al*. (2011) who also recorded many accessions (89.50%) possessing racemes that occur either in the upper canopy or mostly above the canopy.

The IBPGR (1983) cowpea descriptor documented three flower colours (white, purple and mauve-pink). However, two out of the three colours were encountered in this study (Plate 4.2). Similar studies done by Doumbia (2012) and Cobbinah *et al*. (2011) presented all three flower colours as proposed by the IBPGR (1983) cowpea descriptor. The inability of the present work to realize the mouve-pink colour may be due to the relatively smaller sample size used as compared to those of the two authors. Studies done by Gibbon and Pain (1985) also reported additional flower colours such

as pale blue, yellow and pink, which were not observed in this study. Majority of the varieties in this study had white flower colour (77.27%). This was similar to what waspresented by Ezueh and Nwoffiah (1984) and Bennet-Lartey and Ofori (1999) in similar studies yet contrary to the results of Doumbia (2012) and Cobbinah *et al.* (2011) who reported dominance of the purple flowered varieties. Varieties with purple flower colour encountered in this study were Adom, Sanzi, Asontem, Tona, and Soronko (Table 4.2).

Doumbia (2012) mentioned four types of pod shapes but identified only one type in the 94 Ghanaian accessions observed. However, this current study identified all the four kinds of pod shapes (Plate 4.3). The observed difference may be attributed to genotypic differences that occur among the varieties.

Variability was also observed in the pod tip shape of the varieties (Table 4.1). Majority of the varieties had pointed pod tips (90.91%). Soronko was the only variety with blunt pod tip (Table 4.2). This can be a useful identification tool for Soronko.

Seed coat colour greatly influences the preference of consumers for cowpea varieties (Egbadzor *et al*., 2013). Variation in seed coat colour has been reported (Ehlers and Hall, 1997). Variation was also observed in seed coat colour by Doumbia (2012) when he studied 94 Ghanaian cowpea accessions. Most of the varieties in this present study were white coloured (72.73%) (Plate 4.4 and Table 4.1). This is similar to the result of Aremu *et al*. (2007) and Ehlers and Hall (1997). Asontem and Adom are among the varieties with red seed coat colour (Table 4.2) as reported by Egbadzor *et al.* (2013) and Agyemang *et al.* (2014) making them preferred varieties for the preparation of waakye and apapransa in Ghana (Osseo-Asare, 2015).

The IBPGR (1983) cowpea descriptor documented nine seed eye colours. However, this recent study identified four out of the nine seed eye colours (Table 4.1). The difference may be attributed to fact that the IBPGR report was based on the assessment of large number of cowpea accessions collected from cowpea growing area around the world as compared to this study where the assessment was based on a smaller number of cowpea accessions collected from only Ghana. The dominant seed eye colour was black (50.00%) (Table 4.1). This is contrary to the observation of Ghalmi *et al*. (2010) who concluded that most of the varieties assessed in their work which were obtained from Algeria had not seed eye colour. The difference may be due to the genotypic differences that occur in the germplasm of the Ghana and Algeria.

Kidney and Ovoid seed shapes were the most dominant seed shapes in this study with each represented by 31.82% of the population (Table 4.1). A similar report was given by Magloire (2005) and Ghalmi *et al.* (2010) in a morphological study of African cowpea varieties and a morphological study of Algerian cowpea landraces respectively. According to Ehlers and Hall (1997), where individual seeds are separate from adjacent ones during development, they become reniform (Kidney). But as crowding within the pod increases, the seeds become globular.

It was observed that some of the characteristics documented for Hewale by Agyemang *et al*. (2014) were different from what was observed in this study. For instance, flower colour and seed shape have been reported as being violet and rhomboid respectively. Meanwhile, the present study observed white flower colour and globose shaped seeds. The difference may be explained by mixing of genes or environmental changes that have affected those traits. It may also be due to mischaracterization of the variety.

5.1.1.1 Dissimilarity matrix and Cluster analysis based on qualitative traits

Results from dissimilarity matrix (Appendix 5) showed that there was no distance between Hansadua and Nketewade implying that the two varieties very similar. These two varieties are among the newly released cultivars of CSIR-CRI. They share many qualitative characteristics in common. Dissimilarity matrices based on quantitative data also showed short distance of 0.045 between the pair (Appendix 6). However, SSR-marker based dissimilarity matrix showed that the two varieties are different with moderate distance of 0.326 (Appendix 7) and can therefore not be same genotype. The highest distance of 1.00 was found between Agyenkwa and Adom. These two differ in many qualitative characteristics such as leaf shape, flower colour, plant pigmentation, plant hairs etc. (details presented in Table 4.2).

Cowpea cultivars are usually developed to adapt to particular agro-ecological zones (Agyemang *et al*., 2014). It is important to note that CSIR-CRI develops cowpea cultivars that adapt well to the environmental conditions of the transition zone while CSIR-SARI also develop cultivars that well adapt to the savannah agro-ecological environment. Cluster analysis based on qualitative analysis grouped the varieties according to the two agro-ecological zones where the two research stations are located in Ghana (Figure 4.1). Cowpea varieties released by CSIR-SARI (located in the Savannah zone) clustered in Cluster A (Appendix 3). Cluster B composed of mostly cowpea varieties released by CSIR-CRI (located in the Transition zone of Ghana). The nature of clustering in this study suggests possible geographical homogeneity within the clusters. However, the newly released varieties of CSIR-CRI clustered within cluster A together with the CSIR-SARI released varieties. This suggests that the newly released varieties (i.e. Nketewade, Hansadua and Agyenkwa) possess qualities that can help them adapt to the environmental conditions of the Guinea

Savannah. The presence of Soronko (CSIR-CRI cultivar) in cluster A also suggests that geographical homogeneity alone cannot explain the nature of clustering. The nature of cowpea germplasm used to develop these varieties should be investigated to ascertain the source of homogeneity.

5.1.2 Quantitative characters

The mean plant height of varieties in this study was 38.52 ± 1.48 cm with CV of 31.18 (Table 4.3). The mean plant height is relatively higher than what was reported by Doumbia (2010) and Magloire (2005) yet lower than the mean plant height reported by Peksen and Peksen (2013) and Animasaun *et al*. (2015). The high CV for plant height also suggests high variability in the plant height of the varieties accessed in this study. Variability in plant height of cowpea have also been reported by several authors (Bennet-Lartey and Ofori, 2000; Magloire, 2005; Doumbia, 2013; Animasaun *et al*. 2015). Generally, cowpea responds to high humid conditions by promoting vegetative growth rather than reproductive growth and vice versa for low humid conditions. The relatively high plant height in this study may be attributed to the fact that the current study was conducted in the minor rainy season of Ghana. The high humidity recorded during the experiment might have accounted for the relative increase in plant height (Appendix 1).

Comparing the current study to that of Doumbia (2012), it was observed that Asontem recorded the longest terminal leaflet length in both studies. The mean terminal leaflet length was 10.45 ± 0.75cm in this study (Table 4.3). This result is not far from the means reported by Animasaun *et al.* (2015); Magloire (2005), Doumbia (2012) and Cobbinah *et al*. (2011). Nkouannessi (2005) however reported shorter terminal leaflet length ranging between 3.41 cm and 8.93 cm. From Table 4.3, coefficient of variation

of 22.63 indicates high genetic variation in plant height among the varieties studied in this current work. Cobbinah *et al.* (2011) reported CV of 18.56 and 16.47 in similar studies conducted at Bunso and Pokuase respectively. Animasaun *et al.* (2015) and Doumbia (2012) also reported variability in terminal leaflet length of cowpea. There was strong positive correlation between leaf length and plant height (r = 0.747) (Table 4.6). It was physically observed that varieties with twining tendencies (e.g. Adom and Asontem) scored high values for both leaf length and plant height (Table 4.4).

The terminal leaflet width of the varieties in this study ranged between 4.47 cm and 9.50 cm with a CV of 24.92 (Table 4.3). Such high coefficient of variation indicates high genetic variability among the varieties. Cobbinah *et al.* (2011) also recorded a similar CV (23.07) for terminal leaflet width in a similar study conducted at Bunso in Ghana. Doumbia (2010) measured cowpea terminal leaflet width between 3 cm and 10 cm. Nkouannessi (2005) also recorded terminal leaflet width between 0.72 cm and 5.96 cm. The mean terminal leaflet width of 6.46 ± 0.51 cm recorded in this study is however not far from the mean terminal leaflet length reported by Cobbinah *et al.* (2011) at Pokuase (6.42 cm).

According to Ehlers and Hall (1997) cowpea weighs between 5 to 30 g/100 seeds. The mean 100 seed weight in this study was 15.08 ± 0.78 g with a CV of 24.16 (Table 4.3). Cobbinah *et al.* (2011) reported mean 100 seed weight of 11.44g and 14.32 g at Pokuase and Bunso respectively. Bernnet-Lartey and Ofori (2000) also reported mean 100 seed weight of 11.0 g and CV of 16.3. The variability measured in this study was higher than what was reported by Bennet-Lartey and Ofori (2000) (i.e. 16.3 g) and Cobbinah *et al.* (2011) (i.e. 20.19 g). This current study recorded 100 seed weight in the range of 8.17 g to 25.33 g. Doumbia (2012) also recorded a similar range between 8.0 g and 26.0 g. A strong and significant ($P < 0.05$) positive correlation was found

between 100 seed weight and seed length (r = 0.788), as well as seed width (r = 0.604) (Table 4.6). This implies that varieties with big seeds are likely to be heavier.

According to Egbadzor *et al*. (2013), consumers prefer big sized cowpea grains. This makes seed length and seed width important parameters considered by cowpea breeders in developing new varieties. Seed length range reported in this study (0.60 to 0.97 cm) was within the seed length range reported by Ehlers and Hall (1997) (i.e. 0.2 to 1.2 cm). Doumbia (2012) reported variability in the seed length and seed width of 94 Ghanaian cowpea varieties. This study recorded similar results and reports of CV of 18.13 and 19.84 for seed length and seed width respectively (Table 4.3). This shows that there is some level of variability in the size of cowpea varieties studied in this work for breeders to select preferred sizes. In addition to this, a strong and significant ($P < 0.05$) positive correlation (r = 0.627) was recorded between seed length and seed width (Table 4.6). Variability in seed size and pod dimensions has been indicated as cardinal to possibilities for the improvement of the cultivars through selection (Selvam *et al.,* 2000; Lesly, 2005).

It is much easier to harvest pods from varieties that bear longer pods. This is because it is quiet easy to find longer pods especially on erect varieties (Cobbinah *et al*., 2011). Also, positive correlation has been observed between pod length and number of seeds per pod (Amoatey, 1987). The variability in pod length (i.e. CV = 12.0) in the current study is not far from the variability reported by Cobbinah *et al*. (2011) (i.e. 12.57). Bennet-Lartey and Ofori (1999) also recorded CV of 13.4 in pod length of cowpea accessions collected from Ghana, but however concluded that though pod length was genetically controlled to a large extent, potential genetic variability for selection was low and hence may lead to very little change.

Early maturity is a relatively important agronomic character which is measured by criteria such as days to first flowering, days to 50% flowering and days to maturity (Singh and Rachie, 1985). All the varieties attained 50% flowering between 52 to 66 days (Table 4.5). Previous studies recorded days to 50% flowering ranging between 31 to 52 days (Cobbinah *et al*., 2011), 33 to 49 days (Bennet-Lartey and Ofori, 2000), 41 to 49 (Agyemang *et al*., 2014) and 50 to 84 days (Doumbia, 2012). Cowpea responds to high humidity and rainfall by prolonging the vegetative phase of growth. Variation in time of flowering could also be due to photoperiod during period of growth (Bennet-Lartey and Ofori, 2000). These phenomena coupled with genotypic differences in germplasm may have accounted for the differences observed in the number of days to 50% flowering in the various cowpea diversity studies. Variability in days to 50% flowering recorded in the present study is low (CV = 6.13%). Cobbinah *et al*. (2011) also reported low variability in days to 50% flowering with CV of 1.21%. According to Bennet-Lartey and Ofori (2000), low values of genotypic coefficient of variation indicate that breeders have very low variability to make their selection.

The number of peduncles per plant affects the yield of cowpea production. Breeders are therefore interested in selecting accessions with high number of peduncles. The number of peduncles per plant for this study ranged between 10 and 19 pods per plant with CV of 23.75% (Table 4.3). This result show the presence of variability among the varieties studied in this work.

From Table 4.3, the number of pods per peduncle ranged between 1 and 3 pods with majority of the varieties (68%) bearing an average of 3 pods per peduncle. Doumbia (2012) also reported the same range (1 to 3 pods) per peduncle in a variability study of 94 Ghanaian cowpea accessions. Doumbia (2012) and Goenaga *et al.* (2008)

however observed that majority of the lines used in the research had 2 pods per peduncle. This study also recorded CV of 32.26% for number of pods per peduncle indicating variability among the varieties with respect to number of pods per peduncle.

Number of pods per plant ranged between 9 and 32 pods with CV of 29% (Table 4.3). Cobbinah *et al*. (2011) observed a ranged of 3 to 57 pods/plant at Pokuase and 5 to 63 at Bunso in similar studies with CV of 50.07% and 34.30% respectively. The high CV value suggests that there is variability in the number of pods per plant among cowpea varieties. A high variability up to 88% was observed by Bennet-Lartey and Ofori (2000) when they studied the variability in number of pods per plat of 45 accessions of cowpea collected from four cowpea growing regions of Ghana. Bennet-Lartey and Ofori (2000) reported that a large proportion of the observed variability in this character was due to genetic differences. Doumbia (2013) also observed variability in the number of pods per plant of 94cowpea Ghanaian accessions.

It was observed in this study that the number of locules was not always equal to the number of seeds produced by a variety (Table 4.5). Insect pests such as the pod borers, grass hoppers and aphids suck the sap of immature pods preventing them from forming seeds. In this study, insect pests were controlled just as would happen on a farmer's field to ascertain the variability in the number of locules and number of seeds produced by each variety. Ayiyi scored the highest in both number of locules per pod (17) and number of seeds per pod (16) (Table 4.5). The mean for number of locules per pods and number of seeds per pod were 14.23 and 13.33 respectively in this study (Table 4.3). Although Doumbia (2012) did not observe any correlation between the two characters, the strongest positive correlation in this study occurred between number of locules and number of seeds per pod (r = 0.837). Another strong and

significant ($P < 0.05$) positive correlation occurred in pod length with respect to number of locules ($r = 0.561$) and number of seeds per pod ($r = 0.550$) (Table 4.6) which is in agreement with Amoatey (1987), who also found a positive and significant correlation ($P < 0.05$) between pod length and number of seeds per pod among local cowpea collection. Thus selection of long pods can result in a high number of locules and high number of seeds.

5.1.2.1 Principal component analysis of Quantitative traits

The principal component analysis reduced the fourteen quantitative traits to four most important traits which contributed greatly to the variation observed in the 22 varieties. These were 100 seed weight, Plant height, number of days to 50% flowering and Leaf Area Index (Table 4.7). Doumbia (2012) also observed a similar situation where 100 seed weight and number of days to 50% flowering contributed to variation found in the first two principal components. Sulnathi *et al.* (2007) also indicated in their study that days to maturity, 100 seeds weight and days to flowering contributed very much to the divergence between the accessions. From the principal component biplot shown in Figure 4.2, most of the varieties released by CSIR-CRI clustered around the origin except for Soronko which diverged into the right upper quadrat while the widely distant varieties (i.e. Bra-01, SARC-1-57-2, Sanzi) separated towards the peripheries.

5.1.2.2 Dissimilarity matrix and Cluster analysis based on quantitative traits

Principal Component Analysis (PCA) alone may not give an adequate character representation in terms of their relative importance and hence the need to be

complemented with statistics such as Average Linkage Cluster Analysis (ALCA) cluster analysis and dissimilarity matrix (Tatineni *et al*., 1996). Results from dissimilarity matrix (Appendix 6) showed that there was no distance between Asetenapa and Songotra suggesting similarity between the two varieties. However, qualitative and SSR-marker based dissimilarity matrices separated the two varieties at distances of 0.370 and 0.205 respectively implying that the two varieties differ in some characters. On the hand, the highest distance of 1.0 occurred between Soronko and Apabgaala (Appendix 6) implying that the two varieties are the most different in terms of quantitative characters. Soronko grows to about 63 cm with broad sub-globose leaves and bears brown seeds with blunt pod tip and adapted to the agro-economic conditions of the Transition zone while Apabgaala on the other hand is a short variety having small hastate leaves and bears white seeds with pointed tipped pods (Table 4.2).

The 22 varieties were clustered into four major clusters at similarity coefficient 0.85 using all the quantitative agro-morphological data collected (Figure 4.3). Doumbia (2012) reported two cluster groups in variability studies of cowpea accessions at similarity coefficient of 0.84. Soronko in Akan language means 'Unique' due how different its morphology compares to other varieties. Soronko has broad leaves, bears big brown seeds enclosed in long pods and grows to a height of about 60 cm (Tables 4.2 and 4.4). It is therefore of no surprise that cluster A of this study consisted of only Soronko (Figure 4.3). Adom and Asontem also formed cluster B as was expected since the two varieties were developed from a common yardlong bean ancestor (CSIR-CRI, 2006) but however diverged at similarity coefficient of 0.97. This implies that though the two varieties have a common ancestor, they differ in many morphological characteristics.

5.1.3 Genetic diversity based on SSR markers

In the present study, the 19 informative SSR primer pairs used to analyse the 22 cowpea varieties from Ghana and Brazil resulted in 1 to 6 alleles per primer pair with an average of 2.53. Asare *et al*. (2010) also reported 1 to 6 alleles per primer when they assessed the genetic diversity in cowpea germplasm from Ghana using SSR primers including some of the primers used in this study. However, there has been reports of number of allele per locus ranging from 1 to 9 (Diouf and Hilu, 2005), 5 to 12 (Sawadogo *et al*., 2010), 1 – 16 (Badiane *et al*., 2012), 2 to 5 (Adetiloye *et al*., 2013), 5 to 12 (Doumbia *et al*., 2014) and 2 to 17 (Ali *et al*., 2015) in previous cowpea variability studies. According to Ali *et al*. (2015), such variations in numbers of alleles can be attributed to the types of primers used in each study and/or the rate of polymorphism of each primer pairs.

In this study, the polymorphic information content (PIC) ranged from 0.107 to 0.631 with an average of 0.293. Asare *et al*. (2010) also reported PIC range from 0.07 to 0.66 in a variability study of cowpea germplasm from Ghana. However, other researchers have reported PIC values in the range of 0.02 to 0.73 (Li et al., 2001), 0.08 to 0.33 (Badiane *et al*., 2012), 0.61 to 0.92 (Doumbia *et al.*, 2014) and 0.33 to 0.83 (Ali *et al.,* 2015).

According to the Botstein *et al*. (1980) scale of informativeness, PIC value ≥ 0.5 is highly informative, 0.25 – 0.5 reasonably informative and ≤ 0.25 is slightly informative, and marker loci with many alleles and a PIC value near 1 are most desirable. Based on this, the most desirable markers used in this study were SSR-6265 and SSR-6613 (Table 4.8).

Three of the primers used in this study (i.e. SSR-6258, SSR-6243 and SSR-6323) were also used by Badiane *et al*. (2012) to study the genetic relationship among cowpea varieties from Senegal. The three primers showed polymorphism just as was reported by Badiane *et al.* (2012). Badiane *et al,* (2012) further observed thatthe primer that gave the highest allele frequency also recorded the lowest genetic diversity as well as lowest polymorphic information content (PIC). A similar situation was observed in this current study where SSR primer 6608 scored the highest allele frequency but the lowest PIC value. Previous studies conducted by Badiane *et al*. (2012) and Doumbia *et al.* (2014) reported low levels of polymorphism among SSR primers. This present work recorded high level of polymorphism in support of studies conducted by Asare *et al.* (2010), Afiukwa *et al*. (2011) and Choumane *et al*. (2000).

5.1.3.1 Dissimilarity matrix and Cluster analysis based on SSR-markers

The delineation of cowpea germplasm into groups of genetic relatedness will be valuable for guiding introgression efforts in breeding programmes and for improving the efficiency of germplasm management (Bao-Lam *et al*., 2013).

Molecular data obtained from the DNA fingerprinting was used to construct a dissimilarity matrix as shown in Appendix 7. The results showed that there was no distance between Videza and Asomdwoe implying that the two varieties are nearly the same. However, dissimilarity matrices generated from qualitative and quantitative data (Appendix 5 and Appendix 6) showed that there was distance of 0.055 and 0.033 respectively between the two varieties. Though the distances are small, they are significant enough to show some level of dissimilarity. Differences were observed in the quantitative traits as well as qualitative traits such as branch pigmentation, stem

pigmentation, petiole hairs, seed shape, and growth habit (Table 4.2). However, more informative primers would be required to distinguish the two varieties.

The dendrogram generated from the molecular data grouped the varieties into four clusters (Figure 4.4). Adom and Asontem were found in the same subgroup. This was replicated in the dendrograms generated from the qualitative and quantitative data (Appendix 2, Appendix 3, and Appendix 4). The trend re-enforces the closeness of the two varieties. They share several characteristics in common and this may be due to the fact that they were developed from a common ancestor. Unlike the results observed in the qualitative and quantitative cluster analysis, the dendrogram generated from the molecular data did not group the varieties based on geographical homogeneity. This may be explained by the fact that agro-morphological characterization is subject to environmental modifications, the molecular analysis showed clearly the differences that existed within the genome of the varieties.

5.2 Molecular Screening of Cowpea Varieties against *A. craccivora*

The SSR marker CP171F/171R successfully distinguished between aphid resistant varieties and aphid susceptible variety confirming the claim of Kusi (2014). Though Kusi (2014) compared molecular with phenotypic data, there is still the need for more confirmatory phenotypic experiments to support the diagnostic property of the marker CP171F/171R.

Addo-Quaye *et al*. (2011) reported that Ayiyi was aphid resistant; however, the molecular screening in this present work showed that Ayiyi doesn't possess the aphid resistant gene. Ayiyi is one of the oldest varieties introduced in Ghana by CSIR-CRI

(CSIR-CRI, 2006). The resistance of Ayiyi to aphid infestation is therefore likely to be due to antexenosis or an escape mechanism. The present study has identified Adom, Tona, Sanzi, Songotra and Soronko as potential sources of aphid resistant locus in addition to the known SARC-1-57-2 (Plate 4.5). However, aphid resistance status of these varieties should be confirmed using phenotyping approaches. Apart from Sanzi (black seed bean) the other three varieties are popular farmer preferred varieties that are already adapted to the Transition and Savannah agro-ecological zones of Ghana and are therefore recommended for breeding programmes aimed at deploying aphid resistant locus through crosses.

The study also identified 15 susceptible varieties in addition to Apabgaala (Table 4.9). Most of these susceptible varieties are farmer and consumer preferred varieties and therefore need to be improved against aphid infestation.

Zamzam, Agyenkwa, Hansadua and Nketwade have been reported as moderately tolerant to aphid infestation through phenotypic experiments (CSIR-CRI, 2015; GNA, 2015). However, the current study showed that all four varieties did not possess the aphid resistance locus. The aphid resistance that was observed in the previous study may therefore be as a result of the presence of a different QTL other than the one linked to the marker CP171F/171R. Further studies should therefore focus on the search for other markers closer to the aphid resistance locus.

5.3 Introgression of aphid resistance locus into three farmer preferred varieties

Several adapted cowpea cultivars developed for improved yield, preferred seed colour, early maturity, abiotic stresses and high food quality by research institutions

are susceptible to aphid infestation (Asare *et al*, 2010; Kusi *et al.*, 2010). As a consequence, farmers do not attain the expected yield and quality of such cultivars.

After the identification of aphid susceptible varieties in the present work, three farmer preferred varieties were selected for improvement through marker assisted backcrosses. The varieties were Asontem, Nhyira and Asetenapa. The three aphid susceptible varieties were crossed with SARC-1-57-2 (Donor parent) to obtain F_1 hybrids as shown in Table 4.10. SARC-1-57-2 x Asetenapa had the least successful hybrids as most of the fertilized ovaries dropped few days after fertilization. This implies that there are compatibility barriers in SARC-1-57-2 x Asetenapa crosses. Other alternatives such as the use of more closely related donor parents and the use of Biotechnology should be considered for future locus introgression programmes.

The data obtained from the agro-morphological study will be used to guide selection of improved varieties that have close resemblance to their forensic parents in the course of subsequent backcrosses.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this work, 22 cowpea varieties were characterized using 20 molecular and 38 agro-morphological markers. The quantitative, qualitative and molecular data obtained were used to establish genetic diversity that existed among the 22 varieties based on principal component analysis, dissimilarity matrices, cluster analysis and correlation analysis. Variation was observed among the varieties characterized. Principal component analysis based on quantitative traits revealed that 100 seed weight, plant height, number of days to 50% flowering and Leaf Area index discriminated more efficiently among the varieties than the other quantitative parameters. In the situation where morphological markers could not separate some varieties, molecular data separated them.

The SSR marker CP 171F/172R separated aphid resistant varieties from susceptible varieties. This should however be confirmed using phenotypic approaches.

The aphid resistance locus in SARC-1-57-2 was successfully introgressed into the genome of three farmer/consumer preferred varieties which are susceptible to aphid infestation. These are Asontem, Nhyira and Asetenapa. This was done through a marker assisted backcross programme up to BC_2 where 87.5% of the genome of the recurrent parents had been recovered in less than a year.

Conventional backcross programmes require phenotypic screening at every stage to check for gene introgression to inform the breeder on which individuals to select for the next backcross. This study used the SSR marker CP 171F/172R to guide the

selection of the individuals that picked up the gene locus instead of the phenotypic screening. This system hastened the backcross process and omitted the challenges that come along with phenotypic screening.

6.2 Recommendations

Genetic diversity study in released cultivars is very important to plant breeders in order to avoid duplication and misclassification. It will therefore be important to extend this study to the other released cultivars across West Africa.

This study was conducted in the minor rainy season of Ghana under field conditions. The rainfall pattern and biotic stress might have affected the results. Future diversity studies should consider greenhouses for a more controlled environment.

The 20 SSR markers could not discriminate Videza and Asomdwoe. More polymorphic markers should be included in future molecular studies involving the two varieties.

Soronko, Tona, Adom and Songotra are farmer preferred cowpea varieties that have the potential to serve as donor parents in addition to SARC-1-57-2 for backcross programmes aimed at deploying aphid resistance based on the results obtained from this study. However, this results needs to be confirmed by phenotypic assessment.

It is also recommended that there should be a search for DNA markers that are closer to the aphid resistance locus in SARC-1-57-2 and the four varieties recommended above, than CP 171F/172R in order to enhance efficient deployment in marker-assisted selection.

Follow up studies should also consider looking at the biochemistry that underlines the resistance in all the aphid resistant varieties identified in this study. This can throw

more light on the mechanism of resistance in aphid resistant varieties and further lead to the development of biochemical assays to rapidly identify other resistant sources in the cowpea germplasm.

REFERENCES

Abdurakhmonov, I.Y. and Abdukarimov, A. (2008). Application of association mapping to understanding the genetic diversity of plant germplasm resources. *International Journal of Plant Genomics,* pp.18. http://dx.doi.org/10.1155/2008/574927.

Acquaah, G. (2012). *Principles of plant genetics and breeding second edition.* Maryland, ML: Wiley-Blackwell, USA.

Adam, J.I. and Baidoo, P.K. (2008). Susceptibility of five cowpea (*Vigna unguiculata*) varieties to attack by *Callosobruchus maculatus* (Fab.). [Coloeptera: Bruchidae]. *Journal of Ghana Science Association, 10*(2), 85-92.

Addo-Quaye, A.A., Darkwa, A.A. and Ampiah, M.P.K. (2011). Performance of three cowpea (*Vigna unguiculata* (L) Walp) varieties in two Agro-ecological zones of the central region of Ghana I: Dry matter production and Growth analysis. *ARPN Journal of Agricultural and Biological Science*, 6, 6.

Adetiloye, I.S., Ariyo, O.J., Alake, C.O., Oduwaye, O.O., Osewa, S.O. (2013). Genetic diversity of some selected Nigerian cowpea using simple sequence repeats (SSR) marker. *Afr. J. Agric. Res. 8*(7), 586-590. Available online at http://www.academicjournals.org/AJAR DOI: 10.5897/AJAR11.811.

Afiukwa C.A., Kunert K.J., Vorster J., Cullis C.A., Ubi B.E. (2011): Microsatellite association with seed protein content and flowering time in Nigerian cowpea cultivars: *African Journal of Biotechnology* Vol. 10 (79), pp. 18064-18064.

Agyemang, K., Berchie, J.N., Osei-Bonsu, L., Tetteh, Nartey, E. and Forjour, J.K. (2014). Growth and yield performance of improved cowpea (*Vigna unguiculata* L.*)* varieties in Ghana. *Agricultural Science, 2*, 44-52.

Aikins, S.H.M. and Afuakwa, J.J. (2012). Effect of four different tillage practices on soil physical properties under cowpea. *Agriculture and Biology Journal of North America, 3*(1), 17-24.

Ali, Z.B., Yao, K.N., Odeny, D.A., Kyalo, M., Skilton, R. and Eltahir, I.M. (2015). Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] accessions from Sudan using smple sequence repeat (SSR) markers. *African Journal of Plant Science, 9*(7), 293-304.

Allen, D.J., Thottappilly, A.M., Emechebe, A.M. and Singh, B.B. (1998). Diseases of Cowpea. In: Allen, D.J. and Lenne, J.M. (Eds). *The pathology of food and pasture legumes* (pp. 267 – 324). CAB International, Wallingford, WF: UK.

Amoatey, H. M. (1987) Genetic studies in some cowpea (*Vigna unguiculata* (L.) Walp*)* varieties in Ghana. *M Sc Thesis*. University of Ghana, Legon.

Andargie, M. P. (2011). Construction of a SSR-based genetic map and identification of QTL for domestication traits using recombinant inbred lines from a cross between wild and cultivated cowpea (V. *unguiculata* (L.) Walp). *Mol. Breeding, 28,*413-420.

Animasaun, D. O. (2015). Genetic variability study among ten cultivars of cowpea (*Vigna unguiculata* L. Walp) using morpho-agronomic traits and nutritional composition. *The Journal of Agricultural Sciences, 10* (2), 19 - 130.

Annan, I. B., Schaefers, G.A. and Tingey, W.M. (1996). Impact of density of *Aphis craccivora* (Aphididae) on growth and yield of susceptible and resistant cowpea cultivars. *Annals of Applied Biology*. *128* (2), 185-193.

Ansari, A.K. (1984). Biology of *Aphis craccivora* Koch and varietal resistance of cowpeas, *PHD Thesis,* University of Reading, UK, pp. 273.

Appiah-Kubi, D., Asibuo, J.Y., Quain, M.D., Oppong, A., Akromah, R. (2014). Diversity studies on soybean accessions from three countries. *Biocatalysis and Agricultural Biolotechnology*, 198-206.

Aremu C. O., Ariyo O. J., Adewale B. D. (2007). Assessment of selection techniques in genotype x environment interaction in cowpea *Vigna unguiculata* (L.) Walp, *Afr. J. Agric. Res*. 2 352-355

Asafo-Agyei, B. Singh, B.B. and Atuahen-Amankwah, G. (2005). Registration of 'Asontem' Cowpea. *Crop Science. 45*, 2650-2651. doi: 10.2135/cropsci2004.0766

Asare, A.T., Gowda, B. S., Galyuon, K. A. I, Aboagye, L. L., Takrama, J. F. and Timko, M. P. (2010). Assessment of the genetic diversity in cowpea [*Vigna unguiculata (*L.)Walp.] germplasm from Ghana using simple sequence repeat markers. *Plant Genetic Resources: Characterization and Utilization*, *8*(2), 142-150.

Atiri, G.I. (1984). The occurrence and importance of okra mosaic virus in Nigeria weeds. *Annals of Applied Biology*, *104*, 261 – 265.

Ayres, N. M., McClung, A. M., Larkin, P.D., Bligh, H. F. J., Jones, C. A. , Park, W.D. (1997). Micro-satellites and a single-nucleotide polymorphism differenciate apparent amylose classes in an extended pedigree of US rice germ plasm. *Theor. Appl. Genet. 94,* 773-781.

Ba, F.S., Pasquet, R.S. and Gepts, P. (2004). Genetic diversity in cowpea [*Vigna unguiculata* (L) Walp.] as revealed by RAPD markers. *Genetic Resources and Crop Evolution, 51*, 539-550.

Babura, S. R. and Mustapha, Y. (2012). Screening for development of Host Plant resistance to infestation by Aphid (*Aphis craccivora* Koch) in cowpea (*Vigna unguiculata* [L] Walp). *Bayero Journal of Pure and Applied Sciences. 5*(1), 44-47.

Badiane, F.A., Gowda, B.S., Cisse, N., Diouf, D. Sadio, O. and Timko, M.P. (2012). Genetic relationship of cowpea (*Vigna unguiculata*) varieties from Senegal based on SSR markers. *Genetics and Molecular Research, 11*(1), 292-304.

Baidoo, P.K., Adam, J.I. and Sarpng-Nsiah, M. (2006). The role of extracts of *Azadirachta indica* (A. Juss) and *Lantana camara* (L) in the reduction of damage done to maize plants by Lepidopterous stem borers. *International Journal of Food and Agricultural Research, 3*(1), 66-70.

Bao-Lam, H., Timothy, J., Philip, A., Zhiqiu, H., Steve, W., Mitchell, R., Rogerio, C., Ndiaga, C., Antonio, D., Sarah, H., Christian, F., Ndeye, N, Jeffrey, D. (2013). Gene pools and the genetic architecture of domesticated cowpea, *Plant genome 6.* doi: 10.3835/plantgenome 03/0005

Bardner, R., and Fletcher, K.E. (1974). Insect infestations and their effects on the growth and yield of field crops: a review. *Bulletin of Entomological Research. 66*, 141-605.

Bata, H. D., Sing, B. B., Singh, R. S. and Ladeinde, T. A. O. (1987). Inheritance of resistance to aphid in cowpea. *Crop Science*, *27*, 892-894.

Baudoin, J.P. and Marèchal, R. (1985). Genetic diversity in Vigna. In: Singh, S.R. and Rachie, K.O. (Eds), *Cowpea research, production and utilization (*pp. 3-11). Chichester, CH: John Wiley & Sons.

Beleke, A. and Beleke, E. (2014). Overview: Morphological and Molecular Markers role in Crop Improvement Programs. *International Journal of Current Research in Life Sciences*, *3*(3), 035-042.

Bennet-Lartey S.O. and Ofori, K. (2000). Variability in some quantitative characters of cowpea (*Vigna unguiculata* (L.)Walp) landraces in Ghana. *Ghana Journal of Agric. Sci., 33*, 165-170.

Bennet-Lartey, S.O. and Ofori, K. (1999). Variability studies in some qualitative characters of cowpea (*Vigna unguiculata* (L.) Walp) accessions from four cowpea growing regions of Ghana. *Ghana Journal of Agricultural Science. 32*, 3-9.

Bhat, K. V. (1995). DNA fingerprinting techniques and cultivar identification. *National Research Centre on DNA fingerprinting*, NBPGR, New Delhi, 110 012.

Blade, S. F., Shetty, S. V., Terao, T. and Singh, B. B. (1997). Recent development in cowpea cropping research. In Singh, B. B., Mohan Raj, D. R., Dashiell, K. E. and Jackei, L. E. N. (Eds.), *Advances in Cowpea Research* (pp. 114-128). International Institute of Tropical Agriculture, Ibadan, Nigeria.

Blair M. and McCouch S.R. (1997): Microsatellite and sequence-tagged site markers diagnostic for the bacterial blight resistance gene, xa-5. *Theor. Appl. Genet. 94*, 773-784.

Bock, K.R. and Conti, M. (1974). Cowpea aphid-borne mosaic virus. *CMI/AAB Descriptions of Plant Viruses,* (134).

Botstein, D., White, R.L., Skolnick, M., Davis, R.M. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32, 314-331.

Boukar, O. (2008). *Recent progress in cowpea breeding research at IITA.* IITA – Kano. Retrieved from www.iita.org on January 14, 2015.

Boukar, O., Massawe, F. Muranaka, S. Franco, J., Maziya-Dixon, Singh, B. B., Fatokun, C. (2011). Evaluation of cowpea germplasm lines for protein and mineral grains. *Plant genet resour. 9*(4), 515-522. doi: 10.1017/S147926111000815.

Brown S.M., Hopkins M.S., Mitchell S.E., Senior M.L., Wang T.Y., Duncan R.R., Gonzalez-Candelas F. and Kresovich S. (1996). Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (Sorghum bicolor (L.) Moench). *Theor. Appl. Genet. 93*, 190-198.

Choumane W., Winter P., Weigand F. and Kahl G. (2000). Conservation and variability of sequence tagged microsatellites sites (STMSs) from chickpea (Cicer aerietinum L.) within the genus Cicer. *Theor. Appl*. Genet. 101: 269-278.

Cobbinah, F.A, Addo-Quaye, A.A, Asante, I.K. (2011). Characterization, evaluation and selection of cowpea (*Vigna unguiculata* (L) Walp) Accessions with desirable traits from eight Regions of Ghana. *ARPN J Agric Biol Sci.*, 6 (7)21-22.

CORAF/ WECARD (2011). West and Central Africa Council for Agricultural Research and Development. *Cowpea Report*.

Coulibaly, O., Alene, A.D., Manyong, V., Sanogo, D., Abdoulaye, T., Chianu, J., Kamara, A., Tefera, H. and Boukar (2009). Situation and outlook for cowpea and soybean Sub Saharan Africa: report of the Tropical legumes II Project in West and Central Africa.

Coulibaly, S., Pasquet, R.S., Papa, R. and Gepts, P. (2002). AFLP analysis of the phenetic organisation and genetic diversity of *Vigna unguiculata* (L.) Walp. Reveals extensive

gene flow between wild and domseticated types. *Theoretical and Applied Genetics 104*, 358-366.

Council for Scientific and Industrial Research - Crops Research Institute, CSIR-CRI. (2006). *Cowpea production guide: Introduction to cowpea production*. Retrieved from http://www.cropsresearch.org/publications/pdf/cowpea_Introduction.pdf.

Council for Scientific and Industrial Research - Crops Research Institute, CSIR-CRI. (2010). *Annual report.* Ghana: Crops Research Institute.

Council for Scientific and Industrial Research - Crops Research Institute, CSIR-CRI. (2016). *Weather report: August, 2015 to January, 2016*. ICT Department. Kumasi.

Cuartera, J., Laterrot, H. and van Lenteren, J. C. (1999).Host – Plant Resistance to Pathogens and Arthropod Pests.*Integrated Pest and Disease Management in Greenhouse Crops*.Albajes, R. (Ed.), Academic Publishers. Netherlands, pp. 124-138.

Dagg, J. (2002). Strategies of sexual reproduction in aphids. *Dissertation*, Universität Göttingen, Germany, 63 pp.

Dahms, R.G. (1972). The role of host plan resistance in integrated insect control. In: M.G. Jotwani and W.R. Young, (Eds), *The control of sorghum shoot fly* (pp 152- 167). Delhi, DH: Oxford and IBH.

Davis D.W., Oelke E.A., Oplinger E.S., Doll J.D., Hanson C.V. and Putnam D.H. (1991). Alternative plant and animal products: Programs in information exchange and research. In: Janick and J.E. Simon (Eds). *Alternative Field Crops Manual forNew crops* (pp. 133-143). New York, NY: John Wiley and Sons.

De Vicente, M.C., Guzman, J., Engels, J. and Ramanatha, R. (2005). Genetic characterization and its use in decision making for the conservation of crop germplasm. *The role of Biotechnology.* Villa Gualino, Turin, Italy.

Dent, D. (1991). *Insect Pest Management*. CAB International, Wallingford, UK, 604 pp.

Department of Agriculture, Forestry and Fisheries, DAFF (2011). *Production guidelines for cowpeas.* Pretoria: Directorate of Agricultural Information Services.

Diehl, L. and Sipkins, L. (1985). The Development of Mixed Cropping Technologies in Northern Ghana. In: Ohm, H. W. and Nagy, J. G. (Eds.), *Appropriate technologies for farmers in semi-arid West Africa* (pp 260-268). International Programme in Agriculture. Purdue University. West Lafayette, Indiana, USA.

Diouf, D. and Hilu, K.M. (2005). Microsatellite and RAPD markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal.*Genet. Resour. Crop Evol. 52*, 1957-1967.

Dixon, A.F.G. 1985. *Aphids ecology*. Glasgow, GG: Blackie and Son Limited.

Doku E. V. (1970). Variability in local and exotic varieties of cowpea (*Vigna unguiculata* (L.) Walp) in Ghana. *Ghana Journal of Agricultural Science*. *3*, 139-143.

Doumbia, I. Z. (2012). Comparative Study of Cowpea Germplasm from Ghana and Mali using Morphological and Molecular markers. *Thesis: MSc. Agronomy*, Kwame Nkrumah University of Science and Technology, Ghana.

Doumbia, I.Z., Akromah, R., Asibuo, J.Y. (2013). Comparative Study of Cowpea Gerplasms Diversity from Ghana and Mali Using Morphological Characteristics. *Journal of Plant Breeding and Genetics, 1* (03), 139-147.

Doumbia, I.Z., Akromah, R.A., Asibuo, J.Y. (2014). Assessment of Cowpea Germplasms from Ghana and Mali Using Simple Sequence Repeat (SSR) Markers. *International Journal of Agriculture and Forestry*, *4*(2), 118-123.

Egbadzor, K.F, Ofori, K., Yeboah, M., Aboagye, M.L., Opoku-Agyemang, M.O., Danquah, E.Y. and Offei, S.K. (2014). Diversity in 113 cowpea [*Vigna unguiculata* (L) Walp] accessions assessed with 458 SNP markers. *Springer Plus 3*, 541.

Egbadzor, K.F., Yeboah, M., Offei, S.K., Ofori, K. and Danquah, E.Y. (2013). Farmers' key production constraints and traits desired in cowpea in Ghana. *Journal of Agricultural Extension and Rural Development, 5*(1), pp 14 - 20.

Ehlers J.D. and Hall A.E. (1997). Cowpea (*Vigna unguiculata* L. Walp). *Field Crops Res*. *53*, 187–204.

Ehlers, J.D. and Hall A.E. (1996). Genotypic classification of cowpea based on responses to heat and photoperiod. *Crop Science* 36, 673-679.

Eickhoff, T.E., Heng-Moss, T.M., Baxendale, F. P., and Foster, J.E. (2008). Levels of Tolerance, Antibiosis, and Antexenosis Among Resisitant Buffalograsses and Zoysiagrasses. *J. Econ. Entomol. 101*(2), 533 – 540.

Erbaugh, J. M., Willson, H. and Kyamanywa, S. (1995). *Participatory Appraisal: Iganga and Kumi districts, Uganda.* Working paper 95-96 of the IPM/CRSP, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. pp. 28 – 30.

Ezueh M. I. and Nwofiah G. N. (1984). Botanical observations on a local collection of Vegetable cowpea cultivars in South-Eastern Nigeria. *Tropical Grain Legume Bulletin*. *29*,2-6.

F.A.O. (2016). Food and Agriculture Organization of the United Nations, Rome. FAOSTAT Database. http://www.fao.org/faostat/en/#data/QC, accessed December 14, 2016.

Faris, D.G. (1965). The origin and evolution of the cultivated forms of *Vigna sinensis*. *Can. J. Genet.Cytol.7*, 433-452. doi: 101139/g65-058.

Fatokun C.A., Ogunkanmi A., Ogundipe O.T. and Ng, N.Q. (2008). Genetic diversity in wild relatives of cowpea (*Vigna unguiculata*) as revealed by simple sequence repeats (SSR) markers. *Journal of Food, Agriculture and Environment 6*, 3 - 4, 263 – 268.

Fatokun, C.A. (2002). Breeding cowpea for resistance to insect pest: attempted crosses between cowpea and *Vigna vexillata. Challenges and Opportunities for enhancing Sustainable Cowpea Production. Proceedings of the World Cowpea Conference III.*4 -8th September, 2000. C.A. Fatokun, S.A., Tarawali, B.B. Singh, P.M., Komawa, and M. Tamo.Eds. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 52-61.

Fery R. L. (1985). Improved cowpea cultivars for the horticultural industry in the USA. In: S.R. Singh and K.O. Rache (Eds). *Cowpea Research, Production and Utilization* (pp 129-135). John Wiley and Sons, U.K.

Fox, F. W. and Young, M.E.N. (1982). *Food from the Veld.* Delta Books, Johannesburg, South Africa. pp197.

Goenaga, R., Gillaspie, A.G. and Quiles, A. (2008). Assessing yield potential of cowpea genotypes grown under virus pressure. *Hort Science* 43:673-676.

Geissler, P.W., Mwaniki, D.L., Thiong'o, F., Michaelsen, K.F. and Friis, H. (1998). Geophagy, iron status and anemia among primary school children in Western Kenya, *Tropical medicine and International Health. 3*(7), 529-534.

Ghalmi, N, Malice, M, Jacquemin, J., Oumane, S., Mekliche, L., Baudoin, J. (2010). Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata (*L.)Walp.) landrace. *Genetic Resource Crop Evolution, 57,*371-386.

Gibbon D. and Pain A. (1985). *Crops of the drier regions of the tropics*. Longman group limited, London, U.K. pp 146.

Gomez, C. (2004). Cowpea: Post-harvest operations. In: Mejia (Ed.), *Post-harvest Compendium*, AGST, FAO. Rome, Italy.

Gullan, P.J. and Cranston, P.S. (1994). *The Insects: An Outline of Entomology*, *(2nd ed)*. New York, NY: John Willey and Sons, USA.

Hall, A.E. and Patel, P.N. (1985). Breeding for resistance to drought and heat. In: Singh S.R., Rachie, K.O. eds. *Cowpea Research, Production, and Utilization.* New York: John wiley and Sons, pp. 137-151.

Hall, A.E., Cisse, N., Thiaw, S., Elawad H.O.A., Ehlers J.D., Ismail, A., Fery, R., Roberts, P., Kitch, L.W., Murdock L.L., Boukar, O., Phillips, R.D. McWatters K.H. (2003). Development of cowpea cultivars and germplams by the Bean/Cowpea CRSP. *Field Crops Research 82*,103-134.

Hoogendijk, M. and Williams, D. (2001). Characterizing genetic diversity of home garden crop species: some examples from Americas. In: *Proceedings of the Second International home Gardens Workshop*, 17-19 July 2001, Witzenhausen, Federal

Republic of Germany. Home gardens and in situ conservation of plant genetic resources in farming systems. Eds. J.W. Watson and P.B. Eyzaguirre. pp 34-40. http//hdl.handle.net/123456789/1172

Hordzi, W. (2011). Insects observed on cowpea flowers in three districts in central region of Ghana. *African Journal of Food, Agriculture, Nutrition and Development, 11(*3), 1-16.

International Board for plant Genetic Resources. 1983. *Descriptors for cowpea*. Rome, IBPGR.

International Fund for Agricultural Development, IFAD. (2000). *Report and recommendation of the President to the executive board on proposed technical assistance grants for Agricultural research and training* by CGIAR – Supported International Centres. 69/R.27

International Institute of Tropical Agriculture, IITA (1982). *Cowpea Production Training Manual,* Manual series No. 11.

Jackai, L.E.N, and Dacoust, R.A. (1986). Insect pests of cowpeas. *Annual Review of Entomology, 31*, 95-119.

Jackai, L.E.N. and Adalla, C.B. (1997). The management practices in cowpea: a review. In: *Advances in cowpea research*, B.B. Singh, D.R. Mohan Raj, K.E. Dasiell and L.E.N. Jackai (Eds), Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS), IITA Ibadan, Nigeria, pp. 240-258.

Javanmardi J., Khalighi A. K., Bais H. P. and Vivanco J. M. (2002). Chemical Characterization of Basil (*Ocimum basilicum* L.). *J. Agric. Food Chem*, *50,* 5878-5883.

Kay, D.E. (1979). *Food Legumes*. Tropical Development and Research Institute, London. UK.

Kogan, M. and Omar, E.E. (1978). Antixenosis – a new term proposed to replace painter's 'Nonpreference' modality of resistance . *ESA Bulletin,* 24

Kumar, L.S. (1984). *Insect pest control with special reference to African Agriculture*. London: Edward Arnold, pp. 298.

Kurt, W., Hilde, N., Kirsten, W. and Gunter, K. (2005). *DNA fingerprinting in plants: Principles, methods, and applications, 2nd edition.* Taylor and Francis Group, USA.

Kusi, F. (2014). Deployment of the cowpea aphid resistance gene for cowpea improvement in Ghana. *Thesis: PHD-Crop Science (Entomology)*. University of Ghana, Legon.

Kusi, F., Obeng-Ofiri, D., Asante, S. K. and Padi, F. K. (2010a). New sources of resistance in cowpea to the cowpea aphid (*Aphis craccivora* Koch) (Homoptera: Aphididae). *Journal of Ghana Science Association*, *12*(2), 95 – 104.

Kusi, F., Obeng-Ofori, D., Asante, S.K., Padi, F.K. (2010b). Compensatory and susceptive responses of cowpea varieties to infestation by *Aphis craccivora* Koch. *Journal of Science and Technology, 30* (3).

Lesly, W.D. (2005). Characterization and Evaluation of Cowpea [*Vigna unguiculata* (L.)Walp] Germplasm. *Thesis for Master of Science*. University of Agricultural Sciences, Dharwad.

Li, C.D., Fatokun, C.A., Ubi, B., Singh, B.B..and Scoles, G.J. (2001). Determining genetic similarities and relationship among cowpea breeding lines and cultivars by microsatellite markers. *Crop Science 41*,189-197.

Mackean, D.J. (2006). *Biology teaching resources*. http://www.biology-resources.com/insect-01.html, Retrieved on 17th April, 2016.

Magloire N. (2005). The Genetic, Morphological and Physiological Evaluation of African Cowpea varieties.*Thesis: MSc Plant Science.* University of Free State.

Marèchal, R., Mascherpa, J.M. and Stainier, F. (1978). Etudè taxonomique d'un groupe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base donnèes morphologiques et polliniques, traitèes pour l'analyse informatique. *Boissiera* 28, 1-273

Martin, J.M., Blake, T.K. and Hockett, E.A. (1991). Diversity among North American Spring Barley cultivars based on coefficient of parentage. *Crop. Sci. 31*, 1131-1137.

Menendez, C.M., Hall, A.E, Gepts, P. (1997). A genetic linkage map of cowpea (Vigna unguilata) developed from a cross between two inbred, domesticated lines. *Spinger-Velag 95*, 1210 – 1217.

Meyer, J.R. (2003). *Pest control Tactics*. North Carolina State ENT 425 Chapter 19. http://www.cals.ncsu.edu/course/ent425/text19/cultural.html

Miah, G., Mohd, Y.R., Ismail, M.R., Puteh, A.B., Rahim, H.A. and Mohammed, L.A. (2015). Recurrent parent genome recovery analysis in a marker-assisted backcrossing program of rice (*Oryza sativa* L.). *Elsevier*. *338,* 83-94.

Milee, A. Neeta, S. and Harish, P. (2008). Advances in molecular techniques and their applications in plant sciences. *Plant Cell Reports* 27, 617-631.

Ministry of Food and Agriculture - MOFA (2010). *Facts and Figures*. Accra, Ghana: MOFA Publication.

Mohan, M., Nair, S. Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R., and Sasaki, T., (1997). Genome mapping, molecular markers and marker assisted selection in crop plants. *Molecuar breed., 3,*87-103.

Monfankye, R. (2014). The Management of Field Pests on Cowpea (*Vigna unguiculata* (L.) Walp) using Botanicals [Tobacco (*Nicotiana tabacum*) Leaves, Neem (*Azadirachta indica*) Leaves, Ginger (*Zingiber officinale*) Rhizomes and Onion (*Allium cepa*) Bulbs]. *Thesis for Master of Science (Environmental Science).* Kwame Nkrumah University of Science and Tachnology (KNUST).

Morris, M., Dreher, K., Ribau, J.M. and Khairallah, M. (2003). Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker assisted selection. *Molecular Breeding, 11*, 235-247.

Montimore, M.J., Singh, B.B., Harris, F. and Blade, S.F. (1997). Cowpea in traditional cropping systems. In: Singh, B.B., Mohan-Raj, D.R., Dashiell, K.E., Jackai, L.E.N. (Eds.), *Advances in cowpea research*, Co publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS) IITA, Ibadan, Nigeria. pp. 99 -113.

Munyuli, T. (2009). Effects of naïve insect predators on population densities of *Aphis craccivora* and yields of *Vigna unguiculata* and *Arachis hypogeae* grown under

various cropping systems, in Kivu province, Eastern Democratic Republic of Congo. *Tunisian Journal of Plant Production, 208* (4), 197-210.

Nei, M., Tajima, F. and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J Mol Evol. 19* (2), 153-70.

Ng, N.Q. (1990). Recent developments in cowpea germplasm collection, conservation, evaluation and research at the Genetic Resources Centre. In: N. Q. Ng and L.M. Monti (Eds) *Cowpea Genetic Resources*, Ibadan, Nigeria IITA. pp. 13-28.

Nielson, S.S., Brandt, W.E., Singh, B.B. (1993). Genetic variability for nutritional composition and cooking time of improved cowpea lines. *Crop Science 33,* 469-472.

Nkouannessi M. (2005). The genetic, morphological and Physiological evaluation of African Cowpea genotypes: *MSc. thesis*, University of the Free State, Bloemfontein.

Nualsri, C., Potarot, S., Jansod, J., Milosevic, D., Wuttiwong, K., Benchasri, S. and Maneelert, V. (2010). Evaluation and utilization of Cowpea (*Vigna unguiculata* (L.) Walp.) Germplasm for Varietal Improvement of Resistance to Cowpea Aphid (*Aphis craccivora* Koch.) in Thailand.

Obeng-Ofiri, D. (2007). Pests of grain legumes. In: *Major pests of food and selected fruit and industrial crops in West Africa*, D. Obeng-Ofori (ed.) the City publishers Ltd, Accra. pp. 81-112.

Ofuya, T.I. (1988). Antibiosis in some cowpea varieties resistant to the cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae). *Integrated Pest Control, 30*, 68-69.

Ofuya, T.I. (1993). Evaluation of selected cowpea varieties resistance to *Aphis craccivora* Koch (Homoptera: Aphididae) at the seedling and podding phase. *Ann. Appl. Biol. 123*, (1) 19-23.

Ofuya, T.I. (1997). Control of cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) in cowpea, *Vigna unguiculata* (L.) Walp. *Integrated Pest Management Reviews*. *2*, 199-207.

Olatunde, J.A., Odebiyi, Chiang, H.S. and Jackai, L.E.N. (1991). Identification of sources of resistance in cowpea, *Vigna unguiculata* L. Walp. of *Clavigralla tomentosicollis.* Stal. (Hemiptera: Coreidae). *Insect Sci. Applic., 12*(4) 455-461

Ombakho, G.A., Tyagi, A.P. and Pathak, R.S. (1987). Inheritance of resistance to the cowpea aphid in cowpea. *Theoretical and Applied Genetics 74*, 817-819.

Omongo, C.A., Ogenga-Latigo, M.W., Kyamanywa, S. and Adipala, E. (1997). Effects of seasons and cropping systems on occurrence of cowpea pests in Uganda. *African Crop Science Conference Proceedings*, *3*, 1111 – 1116.

Osseo-Asare, F. (2015). *Ghana Cookbook.* Hippocrene Books. New York

Painter, R.H. (1951). *Insect resistance in crop plant*. New York, NY: Macmillan. p. 520.

Panella, L. and Gepts, P., (1992). Genetic relationships within *Vigna unguiculata* (L.) Walp. Based on isozyme analyses. *Genet. Res. Crop Evolution*. *39*, 71-88.

Panella, L., Kami, J. and Gepts, P. (1993). Vignin diversity in wild and cultivated taxa of *Vigna unguiculata* (L.)Walp. (Fabaceae). *Econ. Bot. 47*, 371-386

Pant, K.C., Chandel, K.P.S. and Joshi, B.S., (1982). Analysis of diversity in Indian cowpea genetic resources. *SABRO J., 14,*103-111.

Pasquet, R.S. (1999). Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. *Theor. Appl. Genet. 98*, 1104-1119.

Pasquet, R.S. (2000). Allozyme diversity of cultivated cowpea *Vigna unguiculata (*L.) Walp.*Theor. Appl. Genet. 101,* 211-219. Pathak, R.S. (1988). Genetics of resistance to aphid in cowpea. *Crop Science*. *28*, 474-476.

Peksen, Aysun and Pekson, Erkut. (2013). Agronomic and morphological characters of newly registered Peksen and Reyhan Vegetable Cowpea Cultivars in Turkey. *International Journal of Current Microbiology and Applied Sciences, 2* (9), 133-140.

Perrier, X. and Jaccquemoud-Collet, J.P. (n.d.). DARwin software. http://darwincirad.fr/darwin.

Piper, C.V. (1913). The wild prototype of cowpea. *USDA Bureau of Plant Industry.* Circular No. 124. Miscellaneous papers. Washington, Government Printing Office, 29-32

Rachie, K.O. and Rawal, K.M. (1976). Integrated approaches to improving cowpea (*Vigna unguiculata* (L) Walp). *IITA Technical Bulletin* No. 5. pp. 1-13.

Richard, A. (1847). *Tentamen florae abyssinicae, volume primum.* Arthus Bertrand, Paris.

Ross, M. (2000). Cowpea Aphid (*Aphis craccivora* Koch). *Agricultural Pests of the Pacific*.6.

Sawadogo, M., Ouedraogo, J.T., Gowda, B.S., Timko, M.P. (2010). Genetic diversity of cowpea (Vigna unguiculata L. Walp.) cultivars in Burkina Faso resistant to *Striga gesnerioides*. *Afr. J. Biotechnol. 9*(48), 8146-8153.

Schreiner, I. (2000). *Cowpea aphid* (*Aphis craccivora* Koch.). Agricultural Pests of the Pacific, Agricultural Development in the American Pacific, College of Tropical Agriculture and Human Resources, University of Hawaii at Manao.

Senior, M.L., Murphy, J.P, Goodman, M.M. and Stuber, C.W. (1998). Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Sci. 38*, 1088-1098.

Selvam, Y.A., Manivannan, N., Murugan, S., Thangavelu, P., and Ganesan J. (2000). Variability Studies in cowpea [*Vigna unguiculata* (L.) Walp], *Legume Res.*, 23:279 – 280.

Sharma, H.C. (2009). *Biotechnological approaches for pest management and ecological sustainability*. CRC Press. Boca Raton.

Singh, B.B. (2004). *Achievements so far in improveing cowpea productivity through conventional breeding.* A conference proceeding at an internatioanal workshop for strategies for application of molecular technologies to the breeding of cowpea in Africa for increased productivity.Jointly convened by AATF, NGICA and Kirkhouse Trust.15-17 November, 2004, Cresta Royal hotel- Accra, 6 pp.

Singh, B.B. and Ajeigbe, H. (2007). Improved Cowpea-Cereals-Based Cropping Systems for Household Food Security and Poverty Reduction in West Africa. *Journal of Crop Improvement*, *19,* 1-2.

Singh, B.B. and Tarawali, S.A. (1997). Cowpea and its improvement: key to sustainable mixed crop/livestock farming systems in West Africa. In: Renard C. (ed) *Crop Residues in sustainable mixed crop/livestock farming systems.*CAB in association with ICRISAT and ILRI, Wallingford, UK, pp. 79 -100.

Singh, B.B., Ehlers, J.D., Sharma, B., Freire-Filho, F.R. (2002). Recent progress in cowpea breeding, In: Fatokum, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M., Toma, M. (eds) Challenges and opportunities for enhancing sustainable cowpea production. International institute of Tropical Agriculture, Ibadan, Nigeria. pp. 22-40.

Singh, S.R. (1990). *Insect pests of Tropical food legumes*. John Wiley and Sons Ltd, New York, S.R. Singh (ed.). pp 43 -89.

Singh, S.R. and Allen, D.J. (1980). Pests, diseases, resistance and protection of Vigna unguiculata (L.) Walp.*Advances in legumes science.* R.J. Summerfield and H.A. Bunting. Eds. London: Royal Botanical Gardens, and Ministry of Agriculture, Fisheries and Food. pp 419-443.

Singh, S.R. and Jackai, L.E.N. (1985). Insect pests of cowpea in Africa; Their life cycle, economic importance, potential for control. In: *Cowpea Research, Production, and Utilization*. Singh, S.R. and Rachie, K. O. (Eds.). Pp. 217 -231.

Singh, S.R. and Ntare, B.R. (1985). Development of improved cowpea varieties in Africa. In S.R. Singh and K.O. Rachie (eds.). *Cowpea Research, Production and Utilization*. Chichester: John Wiley & Sons, pp. 106-115.

Singh, S.R. and Rachie, K.O. (1985). *Cowpea research, production and utilization.* John Wiley and Sons, UK. pp.65-101.

Singh, S.R.(1977). Cowpea cultivars resistant to insect pests in world germplasm collection. *Tropical Grain Legume Bulletin, 9, 3-7.*

Singh, S.R., Jackai, L.E.N., Dos Santos, J.H.S. and Adalia, C.B. (1990). Insect pests of cowpea. In: S.R. Singh (ed.) *Insect Pests of Tropical Food Legumes*. Chichester, CH: John Wiley & Sons, pp. 43-89.

Smith, O.S. and Smith, J.S.C. (1992). Measurement of genetic diversity among maize hybrids – a comparison of isozymic, RFLP, pedigree and heterosis data. *Maydica, 37*, 53-60.

Snelling, R.O. (1941). Resistance of plants to insect attack. *Botanical Research, 7*, 543-548.

Souleymane, A., Aken'Ova, M.E., Fatokun, C.A and Alabi, O.Y. (2013). Screening for resistance to cowpea aphid (*Aphis craccivora* Koch) in wild and cultivated cowpea (*Vigna unguiculata* L. Walp) accessions. *International Journal of Science and Environment, 2*(4), 611 - 621.

Souza, R.P., Machado, E.C., Silva, J.A.B., Lagoa, A.M.M.A., Silveira, J.A.G. (2004). Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environmental and Experimental Botany, 51*(1), 45-56.

Steele, W.M. (1976). Cowpea, *Vigna unguiculata* (Legumimosae-Papillionatae). In: Simmonds NW (ed) Evolution of Crop Plants. Longman, London, pp 183-185.

Sulnathi G., Prasnthi L. and Reddy Sekhar M. (2007). Character contribution to diversity in cowpea. *Legume Res*., 30 :70-72.

Summers, C.G., Godfrey, L.D., Rethwisch, M., Goodell, P.B. and Long, R.F. (2006). *UC IPM Pest Management Guidelines*. UC ANR Publication, pp 3430.

Tatineni V., Cantrell R.G. and Davis D. (1996). Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPD. *Crop. Sci.* 36: 136-196.

Thottappilly, G. and Rossel, H.W. (1985). World-wide ccurence and distribution of virus diseases. In: S.R. Singh and K.O. Rachie (Eds). *Cowpea research, production and utilization* (pp.155-171). Chichester CH: John Wiley & Sons, UK.

Timko, M.P., Ehlers, J.D. and Roberts P.A. (2007). Cowpea. In: Kole C (eds) Pulses, Sugar and Tuber Crops, Genome Mapping and Molecular Breeding in Plants. Vol.3 Berlin/Heidelberg: *Springer-Verlag,* pp 49-67.

Timko M.P. (2009). *The Cowpea Genoics Knowlegde Base (CGKB)*. University of Virginia. http://cowpeagenomics.med.virginia.edu/CGKB/index.pl?gene_space_read_primers

Tweneboah C.K. (2000). *Modern agriculture in the tropics with special reference to Ghana food crops*. Cowood Publishers 2000.

Vaillancourt, R.E., and Weeden, N.F. (1992). Chloroplast DNA polymorphism suggests a Nigerian center of domestication for the cowpea, *Vigna unguiculata* (Leguminosae). *Am J Bot, 79*, 1194-1199.

Vaillancourt, R.E., Weeden, N.F., Bernard, J.D. (1993). Isozyme diversity in the cowpea species complex. *Crop Science 33*, 606-613.

Vavilov, N.I. (1926). Studies on the origin of cultivated plants. *Bulletin of Applied Botany and Plant Breeding 16*, 1-248.

Wein, H.C., and Summerfield, R.J. (1980). Adaptation of cowpeas in West Africa: Effects of photoperiod and temperature responses in cultivars of diverse origin. In: Summerfield RJ, Bunting AH (Eds.) *Adavance in Legume Science* (pp. 405-417). Royal Botanic Gardens, Kew, UK.

Weising, K., Nybom, H., Wolff, K. and Gunter, K. (2005). *DNA Fingerprinting in Plants Principles, Methods, and Applications, (2nd Ed).* Boca Raton, BR: Taylor & Francis.

Wellings, P.W., Leather, SR. and Dixon, A.F.G. (1980). Seasonal variation in reproductive potential: a programmed feature of aphid life cycles. *J. Anim. Ecol. 49*, 975-985.

Whitworth, R.J and Ahmad, Aqeeel. (2009). *Cowpea Aphid*. Kansas Crop Pests, MF-2865.

Wilhem, W. W., Ruwe, K., Schlemmer, M.R. (2000). "Comparisons of three Leaf Area Index Meters in a Corn Canopy". *Crop Science, 40,* 1179-1183.

Wu, and Tanksley (1993). Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen Genet, 241,*225.

Xiao J., Li J., Yuan L., McCouch S.R. and Tanksley S.D. (1996). Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. *Theor Appl Genet, 92*, 637-643.

Youdeowei, A. (1989). Major arthropod pests of food and industrial crops in Africa and their economic importance. In: J.S. Yaninek and H.R. Herren (eds) *Biological Control: A sustainable solution to crop pests' problem in Africa* (pp.31 -50), Ibadan, IITA.

Young, N.D.A. (1999). Cautiously iptimistic vision for marker-assisted breeding. *Molecular Breeding*, *5*, 505-510.

APPENDICES

Appendix 1: Weather parameters for August to November, 2015

	August	September	October	November
Rainfall range, mm	0.2 - 5.41	0.2 - 6.6	0.2 - 24.21	0.2 - 17.2
Min Temp, °C	25.96565	25.29194	25.95008	26.35772
Max Temp, °C	29.12813	28.46358	29.13655	29.68991
Max Relative Humidity, %	58.3	64.8	64	62.6
Mean Solar Radiation, W/m²	143.2792	122.2793	162.7185	161.5717
Mean Water Content, m³/m³	-222.51	-0.38851	-0.39626	-0.39646
Mean Pressure, mbar	979.944	980.1606	979.954	979.1671
Mean Wind Speed, m/s	0.64796	0.782577	0.694355	0.617611
Mean Gust Speed, m/s	2.279974	2.683129	2.504435	2.143361
Mean Wind Direction, ø	177.2575	200.1117	172.9095	152.7839

Source: CSIR-CRI, 2016

Appendix 2: Summary of Cluster analysis based on qualitative data

Cluster	Sub-cluster	Rescaled Distance	Number of accessions
A		25	10 (Hewale; Hansadua; Soronko; Agyenkwa; Apabgaala; Songotra; Padi-Tuya; Bra-01; Zayura and Sanzi)
B		25	12 (Asontem; Adom; Tona; Zamzam; Nhyira; Bengpla; Ayiyi; SARC-1-57-2; Hewale; Videza; Asondwoe and Asetenapa)

Generated with SPSS Version 16.0

Appendix 3: Summary of Cluster analysis based on quantitative data

Cluster	Sub-cluster	Similarity coefficient	Number of accessions
A		0.85	1 (Soronko)
B		0.85	2 (Asontem and Adom)
C	I	0.86	4 (Nketewade, Hansadua, Ayiyi and SARC-1-57-2)
	II	0.86	4 (Bengpla, Tona, Asetenapa and Hewale)
	III	0.86	3 (Bra-01, Zaayura and Padi-Tuya)
D	I	0.875	7 (Apabgaala, Zamzam, Agyenkwa, Songotra, Nhyira, Asomdwoe and Videza)
	II	0.875	8 (Sanzi)

Generated with Genstat 12th edition

Appendix 4: Summary of Cluster analysis based on molecular data

Cluster	Sub-cluster	Similarity coefficient	Number of accessions
A		0.2	1 (Hewale)
B	I	0.125	1 (Zaayura)
	II	0.125	3 (Asomdwee, Videza)
C	I	0.13	2 (SARC-1-57-2, Sanzi)
	II		7
	a	0.15	1 (Bra-01)
	b	0.15	6 (Padi-Tuya, Nketewade, Agyenkwa, Zamzam, Apabgaala, Ayiyi)
D	I		
	a	0.11	2 (Adom, Asontem)
	b	0.125	3 (Tona, Nhyira, Soronko)
	II	0.13	3 (Hansadua, Bengpla, Songotra)

Generated with DARwin 6.0.010

Appendix 5: Dissimilarity matrix based on qualitative data

Variety	Soronko	Nketewa	Hansadua	Bengpla	Tona	Apabgaala	Bra 01	Videza	Zaayura	Asetenapa	Asontem	Zamzam	SARC-1-57-2	Ayiyi	Asomdwoe	Sanzi	Agyenkwa	Songotra	Padi-Tuya	Nhyira	Adom	Helewa
Soronko	0.000																					
Nketewa	0.058	0.000																				
Hansadua	0.072	**0.000**	0.000																			
Bengpla	0.353	0.309	0.381	0.000																		
Tona	0.122	0.165	0.237	0.144	0.000																	
Apabgaal	0.158	0.058	0.129	0.324	0.122	0.000																
Bra 01	0.216	0.173	0.129	0.568	0.281	0.259	0.000															
Videza	0.129	0.129	0.216	0.223	0.108	0.201	0.360	0.000														
Zaayura	0.309	0.223	0.108	0.518	0.417	0.396	0.209	0.281	0.000													
Asetenap	0.281	0.223	0.295	0.201	0.345	0.453	0.453	0.165	0.374	0.000												
Asontem	0.683	0.525	0.583	0.360	0.460	0.597	0.971	0.295	0.475	0.489	0.000											
Zamzam	0.173	0.129	0.230	0.108	**0.007**	0.129	0.403	0.072	0.453	0.281	0.295	0.000										
SARC-1-5	0.381	0.353	0.338	0.273	0.245	0.410	0.266	0.223	0.245	0.273	0.561	0.324	0.000									
Ayiyi	0.432	0.360	0.417	0.108	0.194	0.417	0.647	0.245	0.424	0.396	0.165	0.144	0.309	0.000								
Asomdwo	0.108	0.108	0.180	0.072	0.072	0.209	0.324	0.065	0.331	0.129	0.446	0.079	0.245	0.180	0.000							
Sanzi	0.446	0.388	0.374	0.568	0.568	0.604	0.374	0.245	0.209	0.223	0.669	0.576	0.223	0.647	0.353	0.000						
Agyenkw	0.058	0.029	0.101	0.367	0.209	0.201	0.187	0.101	0.309	0.151	0.698	0.201	0.309	0.489	0.094	0.302	0.000					
Songotra	0.144	0.115	0.101	0.367	0.281	0.187	0.173	0.216	0.266	0.252	0.712	0.273	0.338	0.532	0.194	0.245	0.158	0.000				
Padi-Tuya	0.338	0.194	0.223	0.460	0.417	0.309	0.194	0.410	0.331	0.317	0.892	0.468	0.403	0.669	0.302	0.324	0.252	0.151	0.000			
Nhyira	0.273	0.216	0.331	0.151	0.108	0.273	0.475	0.187	0.583	0.295	0.424	0.043	0.338	0.187	0.137	0.676	0.230	0.403	0.496	0.000		
Adom	0.957	0.784	0.727	0.417	0.619	0.770	0.899	0.612	0.504	0.662	0.273	0.568	0.475	0.424	0.662	0.683	**1.000**	0.784	0.763	0.655	0.000	
Helewa	0.216	0.245	0.230	0.165	0.065	0.317	0.230	0.144	0.223	0.266	0.396	0.129	0.079	0.115	0.108	0.374	0.259	0.302	0.410	0.173	0.468	0.00
Maximum	0.957	0.784	0.727	0.568	0.619	0.770	0.971	0.612	0.583	0.662	0.892	0.576	0.475	0.669	0.662	0.683	1.000	0.784	0.763	0.655	0.468	0.00
Minimum	0.058	0.000	0.101	0.072	0.007	0.129	0.173	0.065	0.209	0.129	0.165	0.043	0.079	0.115	0.094	0.245	0.158	0.151	0.410	0.173	0.468	0.00

Generated with SPSS v. 16.0

Appendix 6: Dissimilarity matrix based on quantitative data

Variety	Soronko	Nketewa	Hansadu	Bengpla	Tona	Apabgaa	Bra 01	Videza	Ayiyi	SARC-1-	Zaayura	Asetena	Asontem	Zamzam	Asomdwo	Sanzi	Agyenkw	Songotr	1Padi-Tu	Nhyira	Adom	Helewa
Soronko	0.0																					
Nketewa	0.343	0.000																				
Hansadu	0.579	0.045	0.000																			
Bengpla	0.152	0.058	0.238	0.000																		
Tona	0.263	0.007	0.112	0.028	0.000																	
Apabgaa	**1.000**	0.185	0.072	0.488	0.260	0.000																
Bra 01	0.428	0.035	0.069	0.142	0.066	0.201	0.000															
Videza	0.256	0.069	0.088	0.137	0.092	0.318	0.123	0.000														
Ayiyi	0.401	0.016	0.095	0.084	0.032	0.199	0.110	0.163	0.000													
SARC-1-	0.694	0.081	**0.001**	0.296	0.167	0.066	0.088	0.157	0.114	0.000												
Zaayura	0.228	0.035	0.130	0.068	0.032	0.308	0.028	0.077	0.089	0.168	0.000											
Asetena	0.144	0.041	0.177	0.017	0.005	0.389	0.109	0.074	0.080	0.249	0.028	0.000										
Asontem	0.105	0.090	0.204	0.064	0.073	0.475	0.171	0.071	0.113	0.268	0.061	0.032	0.000									
Zamzam	0.381	0.012	0.018	0.132	0.057	0.161	0.033	0.027	0.075	0.048	0.035	0.078	0.106	0.000								
Asomdwo	0.138	0.120	0.217	0.124	0.101	0.462	0.168	0.033	0.209	0.305	0.056	0.041	0.057	0.089	0.000							
Sanzi	**0.943**	0.194	0.126	0.456	0.259	0.034	0.290	0.348	0.145	0.123	0.332	0.372	0.438	0.195	0.460	0.000						
Agyenkw	0.119	0.067	0.188	0.061	0.050	0.421	0.116	0.053	0.125	0.264	0.019	0.007	0.026	0.074	0.003	0.405	0.000					
Songotr	0.205	0.022	0.144	0.036	0.003	0.309	0.081	0.096	0.041	0.203	0.014	**0.000**	0.049	0.062	0.063	0.279	0.009	0.000				
Padi-Tu	0.213	0.074	0.223	0.060	0.039	0.393	0.080	0.174	0.086	0.263	0.009	0.033	0.076	0.113	0.114	0.380	0.046	0.015	0.000			
Nhyira	0.263	0.028	0.091	0.097	0.029	0.232	0.082	0.048	0.058	0.140	0.018	0.024	0.055	0.021	0.038	0.212	0.017	0.007	0.052	0.000		
Adom	0.103	0.127	0.282	0.085	0.081	0.516	0.214	0.147	0.130	0.360	0.076	0.033	0.019	0.168	0.067	0.449	0.020	0.030	0.057	0.058	0.000	
Helewa	0.073	0.125	0.265	0.079	0.081	0.537	0.204	0.067	0.192	0.372	0.077	0.018	0.033	0.138	0.005	0.512	**0.002**	0.048	0.101	0.060	0.028	0.00
Min	0.073	0.007	0.001	0.017	0.003	0.034	0.028	0.027	0.041	0.048	0.009	0.000	0.019	0.021	0.003	0.212	0.002	0.007	0.052	0.058	0.028	0.00
Max	1.000	0.194	0.282	0.488	0.260	0.537	0.290	0.348	0.209	0.372	0.332	0.372	0.438	0.195	0.460	0.512	0.046	0.048	0.101	0.060	0.028	0.00

Generated with Genstat 12th edition

Appendix 7: Dissimilarity matrix based on molecular data

Variety	Adom	Agyenk	Apabg	Aseten	Asomd	Asonte	Ayiyi	Bengp	Bra-01	Hansa	Hewal	Nhyira	Nket	Padi-Tu	S1-57-2	Sanzi	Songo	Soronko	Tona	Videza	Zamzam
Adom																					
Agyenk	**0.652**																				
Apabgaala	0.565	0.304																			
Asetenapa	0.326	0.543	0.457																		
Asomdwoe	0.326	0.500	0.413	0.130																	
Asontem	0.217	0.478	0.391	0.413	0.370																
Ayiyi	0.587	0.283	0.196	0.478	0.435	0.457															
Bengpla	0.478	0.435	0.478	0.500	0.457	0.304	0.413														
Bra-01	0.413	0.370	0.326	0.435	0.391	0.326	0.348	0.500													
Hansadua	0.522	0.304	0.391	0.587	0.500	0.391	0.413	0.348	0.457												
Hewale	0.413	0.630	0.587	0.391	0.435	0.370	**0.652**	0.543	0.565	0.543											
Nhyira	0.457	0.413	0.413	0.522	0.522	0.239	0.522	0.370	0.391	0.370	0.435										
Nketewade	0.630	0.152	0.370	0.435	0.435	0.543	0.304	0.413	0.261	0.326	0.609	0.565									
Padi-Tuya	0.565	0.261	0.261	0.500	0.457	0.348	0.326	0.391	0.370	0.348	0.587	0.283	0.370								
S1-57-2	0.522	0.261	0.348	0.587	0.500	0.391	0.457	0.522	0.370	0.391	0.587	0.413	0.370	0.348							
Sanzi	0.370	0.457	0.413	0.391	0.391	0.326	0.391	0.500	0.304	0.587	0.565	0.478	0.391	0.457	0.283						
Songotra	0.457	0.413	0.370	0.391	0.348	0.413	0.391	0.326	0.522	0.370	0.565	0.391	0.435	0.326	0.413	0.478					
Soronko	0.391	0.522	0.435	0.630	0.630	0.304	0.587	0.391	0.457	0.391	0.543	0.196	0.587	0.391	0.391	0.457	0.370				
Tona	0.413	0.457	0.413	0.478	0.478	0.283	0.435	0.326	0.478	0.370	0.435	0.217	0.565	0.370	0.413	0.478	0.304	0.283			
Videza	0.326	0.500	0.413	0.130	**0.000**	0.370	0.435	0.457	0.391	0.500	0.435	0.522	0.435	0.457	0.500	0.391	0.348	0.630	0.478		
Zamzam	0.587	**0.109**	0.239	0.478	0.435	0.457	0.217	0.413	0.261	0.283	**0.652**	0.435	0.130	0.239	0.326	0.478	0.348	0.500	0.435	0.435	
Zaayura	0.413	0.413	0.326	0.261	0.261	0.326	0.391	0.457	0.391	0.457	0.435	0.348	0.478	0.370	0.543	0.435	0.391	0.500	0.391	0.261	0.391
Min	0.217	0.109	0.196	0.130	0.000	0.239	0.217	0.326	0.261	0.283	0.435	0.196	0.130	0.239	0.283	0.391	0.304	0.283	0.391	0.261	0.391
Max	0.652	0.630	0.587	0.630	0.630	0.543	0.652	0.543	0.565	0.587	0.652	0.565	0.587	0.457	0.543	0.478	0.391	0.630	0.478	0.435	0.391

Generated with DARwin 6.0.010

Appendix 9: Anova Tables (Generated with Genstat 12th edition)

Appendix 9a: Leaf area index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.3273	0.1637	0.4	
Rep x Variety	21	41.337	1.9684	4.83	<.001
Residual	42	17.1115	0.4074		
Total	65	58.7758			

Appendix 9b: Leaf length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	2.139	1.07	0.47	
Rep x Variety	21	265.79	12.657	5.58	<.001
Residual	42	95.334	2.27		
Total	65	363.264			

Appendix 9c: Leaf width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.0039	0.002	0	
Rep x Variety	21	126.7309	6.0348	6.08	<.001
Residual	42	41.7027	0.9929		
Total	65	168.4376			

Appendix 9d: Number of locules

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.636	0.318	0.13	
Rep x Variety	21	176.258	8.393	3.5	<.001
Residual	42	100.697	2.398		
Total	65	277.591			

Appendix 9e: Number of peduncles per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.485	0.242	0.03	
Rep x Variety	21	419.091	19.957	2.46	0.006
Residual	42	340.182	8.1		
Total	65	759.758			

Appendix 9f: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	34.94	17.47	0.81	
Rep x Variety	21	8435.82	401.71	18.63	<.001
Residual	42	905.73	21.56		
Total	65	9376.48			

Appendix 9g: Pod length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	7.303	3.6515	25.43	
Rep x Variety	21	26.9242	1.2821	8.93	<.001
Residual	42	6.0303	0.1436		
Total	65	40.2576			

Appendix 9h: Number of pods per peduncle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	7.303	3.6515	25.43	
Rep x Variety	21	26.9242	1.2821	8.93	<.001
Residual	42	6.0303	0.1436		
Total	65	40.2576			

Appendix 9i: Number of pods per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	4.76	2.38	0.16	
Rep x Variety	21	1653.09	78.72	5.3	<.001
Residual	42	623.91	14.85		
Total	65	2281.76			

Appendix 9j: Seed length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.007576	0.003788	0.89	
Rep x Variety	21	0.952727	0.045368	10.64	<.001
Residual	42	0.179091	0.004264		
Total	65	1.139394			

Appendix 9k: Seed width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.005758	0.002879	0.9	
Rep x Variety	21	0.579394	0.02759	8.63	<.001
Residual	42	0.134242	0.003196		
Total	65	0.719394			

Appendix 9l: Number of seeds per pod

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.576	0.288	0.12	
Rep x Variety	21	215.333	10.254	4.19	<.001
Residual	42	102.758	2.447		
Total	65	318.667			

Appendix 10a: Chi squared test for BC1 derived from (F1 and Asontem)

	E	O	O-E	(O-E)2	(O-E)^2/E	X^2	df	p
Homozygous (susceptible)	23.5	25	1.5	2.25	0.096	0.191	1	<0.75
Heterozygous	23.5	22	-1.5	2.25	0.096			

Appendix 10b: Chi squared test for BC1 derived from (F1 and Nhyira)

	E	O	O-E	(O-E)2	(O-E)^2/E	X^2	df	p
Homozygous (susceptible)	23.5	22	-1.5	2.25	0.096	0.191	1	<0.75
Heterozygous	23.5	25	1.5	2.25	0.096			

Appendix 10c: Chi squared test for BC1 derived from (F1 and Asetenapa)

	O	E	O-E	(O-E)2	(O-E)^2/E	X^2	df	p
Homozygous (susceptible)	18	17	1	1	0.059	0.118	1	<0.75
Heterozygous	16	17	-1	1	0.059			