KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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COMPARATIVE STUDY OF COWPEA GERMPLASM FROM GHANA AND MALI USING MORPHOLOGICAL AND MOLECULAR MARKERS

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, FACULTY OF AGRICULTURE IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF M.Sc. AGRONOMY (PLANT BREEDING)

BY

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DECLARATION

This thesis is a presentation of my original research work and that, to the best of my knowledge, it has not been submitted anywhere for any award. Wherever contributions of others are involved, they have been acknowledged.

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DEDICATION

I dedicate this piece of work to:

- My mother Mme DOUMBIA Fanta TOGOLA: Dear mummy, this is a fruit of the tree you planted.
- My daddy El hadj Siaka DOUMBIA for his large support and blessing.
- My aunt Mme DOUMBIA Marietou DIARRA for her advice.

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ABSTRACT

Cowpea improvement can be enhanced by knowledge of the genetic diversity available between and within local and regional genebanks. Genetic diversity in the available gene pool is the foundation of all plant improvement programs. A total 94 accessions (47 from Ghana and 47 from Mali) were used for this study. CRD design was used to evaluate accessions; all stages of the study were done at CSIR-CRI, Kumasi (Ghana). Twelve qualitative and twenty quantitative traits were used to assess the collections on the basis of IBPGR cowpea descriptors. A total of 20 SSRs markers were used to assess polymorphism of the germplasm. The morphological data, results showed a relatively low level of genetic diversity between and within both germplasms (Ghana and Mali) which ranged from 1 to 0.82 and 0.99 to 0.84 for qualitative and quantitative traits respectively. Principal component analysis, similarity matrix, scatter plot matrix and clustering separated the accessions according to some qualitative and quantitative traits. Among the 20 SSRs markers screened, only 6 primer pairs were polymorphic. Seven to twenty-seven alleles per primer were detected with polymorphic information content (PIC) varying from 0.61 to 0.92 with a mean of 0.83; allele frequency from 0.17 to 0.45 with a mean of 0.28; genetic diversity from 0.66 to 0.92 with a mean of 0.84; heterozygosity from 0.00 to 0.97 with 0.47 as a mean. The diversity observed can be exploited by breeders from the two countries for cowpea improvement.

LIST OF ABBREVIATIONS

^{o}C: degree celcius

%: Percentage

AD: After Christ

AFLP: Amplified fragment length polymorphism

BC: Before Christ

CGIAR-SINGER:

Consultative Group on International Agricultural Research-System wide Information Network for Genetic Resources

cm: centimeter

CRD: Complete randomized design

D1st F: Days to 1st flower

D1st PM: Days to 1st pod maturity

D50%E: Days to 50% emergence

D50%F: Days to 50% flowering

D50%PM: Days to 50% pod maturity

DNA: Deoxyribonucleic acid

DNA Mali: Direction nationale de l'agriculture du Mali

DNSI: Direction national de la statistique et de l'information

°F: Faraday

F_2: Second filial generation from crossing.

FAOSTAT: Food and agriculture organization of United Nations statistic

FC: Flower colour

g: grams

GH: Growth habit

IBPGR: International board for plant genetic resources

IER: Institut d'Economie Rurale (Mali)

IPP: Immature pod pigmentation

IRD: Institut de recherche pour le developpement

IITA: International Institute of Tropical Agriculture

kg: kilograms

LC: Leave colour

MAS: Markers Assisted Selection

Mbp: million base pairs

mm: millimeter

MOFA: Ministry of Food and Agriculture

NL/P: Number of locule per pod

NMB: Number of main branches

NPK: Nitrogen, Phosphorus, Potassium

NP/Pt: Number of pod per plant

NPP/P: Number of pod per peduncle per plant

NS/P: Number of seed per pod

ORSTOM: Office de la recherche scientifique et technique outre-mer

PAP: Pod attachment to peduncle

PBL: Population blanc lignée

PC: Pod curvature

PCR: Polymerase chain reaction

PCR-RFLP DNA: Polymerase chain reaction-Restriction fragment length polymorphism

deoxyribonucleic acid

PDC: Pod colour

PDL/cm: Peduncle length per centimeter

PGRC: Plant genetic resources centre

PH/cm: Plant height per centimeter

PL/cm: Pod length per centimeter

PRL: Population rouge lignée

PW/g: Pod weight in grams

RAPD: Random amplified polymorphism deoxyribonucleic acid

RFLP: Restriction fragment length polymorphism

RP: Raceme position

SCC: Seed coat colour

SDS-PAGE: Sodium dodecyl sulfate- polyacrylamide gel electrophoresis

SL: Seed length

SRAC: Station de recherche agronomique de Cinzana

SS: Seed shape

SSRs: Simple sequence repeats

ST: Seed thickness

SW: Seed width

SWt/g: Seed weight in grams

RT-PCR: Reverse transcription-polymerase chain reaction

TLL: Terminal leaflet length

TLS: Terminal leaflet shape

TLW: Terminal leaflet width

TT: Testa texture

TVU: Tropical *Vigna unguiculata* (L.) Walp.

UNESCO: United Nations Educational, Scientific and Cultural Organization

USA: United State of America

USDA: United States Department of Agriculture

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CHAPTER 1

INTRODUCTION

Cowpea [(*Vigna unguiculata* (L.) Walp.)] (2n = 22) is one of the main world legumes (Pasquet and Baudoin, 1997). This legume is a major food crop in Africa, Latin America and India, because of its high protein content, acceptable palatability and low cost of production (Kareem and Taiwo, 2007; Ehlers and Hall, 1997). The annual cowpea production area in 2008 was estimated at 11.8 million ha with an annual production of 5.4 million tons of dried grains (FAOSTAT, 2010). Production in Africa represents about 91% of the global production. West Africa, with 10.7 million ha, accounts for most of Africa's production, with Nigeria and Niger being the leading cowpea growing countries (FAOSTAT, 2008).

In Mali, cowpea is the second most consumed leguminous crop after groundnut (Report Cowpea Program, 1996-1997). The main cowpea producing areas in Mali are: Kayes, Koulikoro, Mopti, Segou, and Sikasso districts. According to DNSI (2006), cowpea cultivation in Mali involves more than 4,506,000 farmers. Cowpea is grown throughout all the ten geographical regions in Ghana (MOFA, 2010).

Cowpea possesses the capacity to fix atmospheric nitrogen in poor soils and it is very adapted to arid areas and support high temperature and tolerates droughts (Ehlers and Hall, 1997). Cowpea grows quickly and permits the establishment of a good cover of the ground which decreases erosion, soil temperature and competition with weeds (Blades *et al.*, 1997).

Total protein content in seed ranges from 23% - 32% seed dry weight (Nielsen *et al.*, 1993). While cowpea leaves, green pods and green peas are consumed as human food, it is the protein rich grains, prepared in different forms in different parts of the African

continent, that constitute the main food product of the crop. Dry haulms are often fed to livestock, particularly in dry season, when animal feed is scarce making the crop an essential and integral component of sustainable crop livestock farming systems in the semi-arid and arid regions of Sub-Saharan Africa (Ortiz and Crouch, 2001).

Different forms of cowpea are cultivated in Ghana and Mali based on local preferences for growth characteristics and culinary properties. For the most part, farmers practice traditional methods of seed selection and conservation, and little effort has been made for germplasm characterization and breeding for genetic improvement within the country. Cowpeas grown are local varieties (landraces), introduced and improved varieties. The local varieties are well adapted in different agro-ecological zones and also possess the natural agronomic characteristics appreciated by farmers. They constitute the reservoir of genetic variability. Traditionally, subsistence farmers in both countries (Ghana and Mali) save seeds and rely on their own experience to select and improve their varieties (Quaye *et al.*, 2009). Conventionally, cowpeas grown in these countries are identified by local names based on morphological characteristics and agronomic attributes which are of interest to breeders (such as plant morphology, seed coat colour or other visible seed/pod characteristics). Edaphic and climatic factors can dramatically influence plant physiology and morphology, and therefore, classification schemes relying only on visible characteristics are inherently flawed.

Improvement of the local varieties and crop management practices can decrease poverty. These can help farmers and their families; making it one of the solutions to increase famers' production and productivity. The overall efficiency and effectiveness of cowpea improvement programmes can also be enhanced by knowledge of the

genetic diversity available within local and regional germplasm collection (Hegde and Mishra 2009; Hall, 2004).

Genetic characterization of germplasm is very important for gene bank managers, since it allows more efficient sampling of available resources, improves selection of desirable genotype for breeders to initiate crosses and also allows better management of available gene pool by removing obvious duplicates and setting up core collections. Genetic diversity in the available gene pool is the foundation of all plant improvement programmes. It is a source of variation, which is raw material for the improvement work. This genetic diversity is essential to decrease crop vulnerability to abiotic and biotic stresses, ensures long-term selection gain in genetic improvement, and promotes rational use of genetic resources (Barrett and Kidwell, 1998; Messmer *et al.*, 1993).

The long time of selection and strong variability of genotype, mainly due to interaction between genotype and environment, are constraints for cowpea selection in the two countries (Ghana and Mali). To overcome this limitation, it is necessary to integrate Marker Assisted Selection (MAS) into the conventional selection technique. In this way, traditional varieties adapted to different agro-ecological conditions, could be improved efficiently. In this context, good knowledge of genetic diversity in local cowpea and exotic varieties genetic diversity will be necessary. Cowpea varieties which have large variability will be good candidates for varietal improvement.

The use of molecular markers provides a much more reliable approach to distinguish cowpea genotypes for germplasm conservation, and for the identification of parental lines for use in breeding for improved cultivars in both countries, and to remove varieties which are duplicated. Several approaches were used for diversity study of

wild and cultivated cowpea germplasm which included analysis of morphological and physiological traits (Ehlers and Hall, 1996; Perrino *et al.*, 1993; Fery, 1985), allozymes (Pasquet, 1993, 1999, 2000; Vaillancourt *et al.*, 1993; Panella and Gepts, 1992), seed storage proteins (Fotso *et al.*, 1994), and chloroplast DNA polymorphisms (Vaillancourt and Weeden, 1992); random amplified polymorphic DNA (RAPD) (Zannou *et al.*, 2008; Diouf and Hilu, 2005; Xavier *et al.*, 2005;

Ba *et al.*, 2004; Nkongolo, 2003; Fall *et al.*, 2003; Mignouna *et al.*, 1998); restriction fragment length polymorphisms (RFLP) (Fatokun *et al.*, 1993); amplified fragment length polymorphisms (AFLP) (Fang *et al.*, 2007; Fatokun *et al.*, 1997); DNA amplification fingerprinting (Simon *et al.*, 2007) and analysis of simple sequence repeats (SSRs) (Uma *et al.*, 2009; Xu *et al.*, 2010; Asare *et al.*, 2010; Wang *et al.*, 2008; Ogunkanmi *et al.*, 2008;) or sequence tagged microsatellite sites (Abe *et al.*, 2003; He *et al.*, 2003; Li *et al.*, 2001; Choumane *et al.*, 2000). Of these techniques, analysis of SSRs has proven to be particularly useful since these sequences, besides being abundant and distributed throughout eukaryotic genomes, are highly polymorphic, inherited codominantly and reproducible, with simple screening requirements (Dib *et al.*, 1996). Simple sequence repeats have also been extensively used in genotype identification, seed purity evaluation and variety protection (Brown *et al.*, 1996; Senior *et al.*, 1998), pedigree analysis (Bowers *et al.*, 1999; Ayres *et al.*, 1997), and genetic mapping of simple and quantitative traits and MAS (Weising *et al.*, 1998; Blair and McCouch, 1997; Chen *et al.*, 1997).

Morphological and molecular analyses of local varieties of cowpea in Ghana and Mali are necessary. Identification and differentiation of the relatedness of cowpea germplasm will be useful for breeding in the two countries, which will contribute to

efforts aimed at maximizing the selection of diverse parent genotypes and to broaden the germplasm base for future cowpea breeding programs in developing improved cultivars. Information generated from this study will be used in identifying efficient strategies for the sustainable management of the genetic resources of cowpea from Ghana and Mali.

OBJECTIVES OF STUDY

General objective

To screen cowpea accessions from Ghana and Mali using molecular and morphological tools to select desirable varieties for breeding programs.

Specific objectives:

- To assess genetic diversity of cowpea accessions from Ghana and Mali;
- To evaluate allele and genotype frequencies between and within the accessions;
- To identify desirable varieties for crop improvement.

CHAPTER 2

LITERATURE REVIEW

2.1. Taxonomy of Cowpea

Cowpea [*Vigna unguiculata* (L) Walp.] is a dicotyledonous crop in the order *Fabaceae*, subfamily *Faboideae* (Syn. *Papillionoideae*), tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna* and section Catiang. It is a diploid plant containing 22 chromosomes (Timko and Singh, 2008) and its nuclear genome size is estimated to cover 620 million base pairs (Mbp) (Timko *et al.*, 2008). The genus was divided into sub-genera based upon morphological characteristics, the extent of genetic hybridization and geographical distribution of the species. The major groups consist of the African sub-genera *Vigna* and *Haydonia*, the Asian sub-genus *Ceratotropis*, and the American sub-genera *Sigmoidotropis* and *Lasiopron* (Timko and Singh, 2008). *V. unguiculata* sub-species *unguiculata* includes four cultivated groups: *unguiculata, biflora* (or *cylindrical*), *sesquipedalis*, and *textilis* (Ng and Maréchal, 1985). *Vigna unguiculata* subspecies *dekindiana*, *stenophylla*, and *tenuis* are intermediate wild progenitors of cultivated cowpea and form the major portion of the primary gene pool of cowpea. Fatokun and Singh (1987) pointed out that, wild subspecies like *pubescence* that do not readily hybridize and show some degree of pollen sterility form a secondary gene pool.

2.2. Origin, domestication and distribution

Cowpea (*Vigna unguiculata*) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al.*, 1974). A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia, and South America as origin (Coetzee, 1995; Tindall, 1983;

Summerfield *et al.*, 1974; Johnson, 1970). One view is that cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago (Allen, 1983). Before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia. In the 17th century AD the Spanish took the crop to West India. The slave trade from West Africa resulted in the crop reaching the southern USA early in the 18th century. Another view was that the Transvaal region of the Republic of South Africa was the centre of speciation of *V. unguiculata*, due to the presence of most primitive wild varieties (Padulosi and Ng, 1997). Presently, cowpea is grown throughout the tropic and subtropic areas of the world.

Ng (1995) postulated that during the process of evolution of *V. unguiculata*, there was change of growth habit, from perennial to annual breeding and from predominantly outbreeding to inbreeding, while cultivated cowpea (subsp. *unguiculata*) evolved through domestication and selection of the annual wild cowpea (var. *dekindtiana*). During the process of domestication and after the species was brought under cultivation through selection, there was a loss in seed dormancy and pod dehiscence, corresponding with an increase in seed and pod size. The precise location of origin of where cowpea was first domesticated is also still under speculation. The wide geographical distribution of var. *dekindtiana* throughout sub-Saharan Africa suggests that the species could have been brought under cultivation in any part of the region. However, the centre of maximum diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah region of Nigeria, southern Niger, part of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985).

2.3. Morphology and biology

Based on the investigation conducted by Padulosi and Ng (1997) and supported by Baudouin and Merechal (1985); and Padulosi 1997, about the range of variation and number of varieties found in wild cowpeas as well as their primitive characteristics, such as perenniality, hairiness, small size of pods and seeds, pod shattering with pronounced exine on the surface of the pollen, out-breeding and bearded stigma. The highest genetic diversity and most primitive forms of wild *V.unguiculata* occur in southern Africa.

Variability in morphology of different cowpea accession is very high. There are three types according to their uses: for grain, forage or dual purpose. *Vigna unguiculata* is a herbaceous trailing, prostrate, climbing, bushy, or sub erect annual plant, growing 15-80 cm high. Leaves are alternating trifoliate with petiole 5-25 cm long. The lateral leaflet is opposite and asymmetrical, while the central leaflet is symmetrical and ovate. Leaves exhibit considerable variation in size (6-16 x 4-11 cm) and shape (linear, haste, lanceolate to ovate) and they are usually dark green. The stems are striate, smooth or slightly hairy and sometimes tinged with purple. The inflorescences are racemose or intermediate at the distal ends of 5-60 cm long peduncles. The flowers are borne in alternate pairs, with usually only two flowers per inflorescence. These are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, cream, pink, pale, blue, yellow or purple. Flowers open in the early day and close at approximately midday. After blooding (opening once) they wilt and collapse. Growth pattern is either determinate or usually indeterminate under favourable conditions. Fruit are pods that vary in size, shape, colour and texture. They may be erect, crescent-shaped or coiled. They are usually slightly yellow when ripe, but may

also be brown or purple in colour. Seeds are relatively large (0.2-1.2 cm long) and weigh 5-30 g/100 seeds. They are variable in size and shape: kidney, ovoid, crowder, globose and rhomboid (IBPGR, 1983). 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. Seed shape is correlated with that of the pod. Where individual seeds are separate from adjacent ones during development, they become reniform, but as crowding within the pod increases, the seeds become globular. Pod length ranges from 8-22 cm with 10-20 seeds per pod (Chevalier, 1944).

2.4. Uses

Cowpea is a multipurpose crop, providing food for human and feed for livestock and it is a cash generating commodity for farmers, small and medium-size entrepreneurs. It has a wide variety of uses namely as a nutritious component in the human diet as well as nutritious livestock feed. Cowpea can be used at all stages of growth as a vegetable crop. The tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach. Immature snapped pods are used in the same way as snap-beans, often being mixed with other foods. Green cowpea seeds are boiled as a fresh vegetable, or may be canned or frozen. Dry mature seeds are also suitable for cooking and canning.

Cowpea can also be used as cover crop (Timko *et al.*, 2008; Langyintuo *et al.*, 2003; Singh, 2002). The very early maturity characteristics of some cowpea varieties provide the first harvest earlier than most other crops during production period. This is an important component in hunger fighting strategy, especially in Sub-Saharan Africa where the peasant farmers can experience food shortage a few months before the maturity of the new crop. Its drought tolerance, relatively early maturity and

nitrogen fixation characteristics fit very well to the tropical soils where moisture, erosion and low soil fertility is the major limiting factor in crop production (Hall, 2004; Hall *et al.*, 2002). In many areas of the world, cowpea is the only available high quality legume hay for livestock feed. Cowpea may be used green or as dry fodder. It can also be used for intercropping with the other main crops like pearl millet (*Pennisetum glaucum*), maize (*Zea maize*) or sorghum (*Sorghum bicolor*), cassava (*Manihot esculenta* L.). Cowpeas are sacred to Hausa and Yoruba tribes, and are prescribed for sacrifices to abate evil and to pacify the spirits of sickly children. Hausa and Edo tribes use cowpea medicinally; one or two seeds are ground and mixed with soil or oil to treat stubborn bowels (Nkouannessi, 2005). The protein found in cowpea is similar to the one from other legumes, rich in the essential amino acids lysine and tryptophan (Timko and Singh, 2008). However, the protein nutritive value of cowpea and other legumes is lower than that of animal proteins because they are deficient of sulfur amino acids and contain a non-nutritional factors (phytates and polyphenols), enzymes inhibitors (against trypsin, chymotrypsin and R-amylase) and hemagglutinins (Jackson, 2009). Minerals and vitamins are the other nutritional important constituents of the cowpea seeds. It has been reported that folic acid, a vitamin B necessary during pregnancy to prevent birth defect in the brain and spine content is found in higher quantity in cowpea compared to other plants (Timko and Singh, 2008; Hall *et al.*, 2003). The total crude protein in foliage ranges from 14-21% and in crop residues; it is 6-8%. The high protein content in all cowpea parts consumable by human and animal (leaves, stems, pods and seeds), is the key factor in alleviating the malnutrition among women and children and improvement of healthy status of the livestock in resource-limited households where regular access to animal protein is limited due to low economic status.

Different dishes can be prepared from cowpea. The young tender leaves can be cooked and eaten as vegetable, the green pods can be cooked and eaten just like green beans, the seeds can be cooked when fresh (semi-ripe) and, when fully matured and dry, eaten as pulses.

Wide array of legumes is produced in Ghana, but cowpea is preferred on account of its short life cycle, fodder use and quality. The dry seeds may be boiled and eaten with "Gari" (a cassava product). It is also boiled together with rice and a colouring agent to give "Waakye". The boiled seeds could also be served with fried ripe plantain (Quaye *et al.,* 2009). It is also used in preparation of weaning foods. In Ghana and other African countries like Tanzania and Niger, cowpea is used for preparation of stew that is either used together with cereal dishes or directly mixed with the cereals as maize, wheat, sorghum and rice. In Mali, cowpea is boiled and also prepared in traditional dishes called "Fary" and "Akra". The young leaves are used to prepare green sauce for different dishes. During the raining season, farmers can use immature pods to resolve their food problems before other crops are harvested.

2.5. Production status

2.5.1. Cowpea production systems

Traditionally in West and Central Africa, and Asia, cowpeas are grown on small farms often intercropped with cereals by the small scale farmers. Fertilizers and pesticides are generally not used, because they are too expensive or not available for the small farmers. In Western Africa; Burkina, Ghana, Mali, Niger and Nigeria both fodder and grain type varieties are grown sometimes as a pure crop and its commercial production is mostly done in these states. The cultivation of cowpea is

mechanized in developed countries like Georgia, California, Texas, Mississippi, Arkansas and Tennessee in the USA (Fery, 1990).

2.5.2. Cowpea production in Ghana

Cowpea is an important component of sustainable cropping system in Ghana. It is cultivated for the leaves, green pods, grain and haulm for livestock feed. Cowpea is an important source of vegetable protein and minerals for over 70% of Ghana population and it is the second most important grain legume. It is currently a food security crop (MOFA, 2010). Thus, rotating or intercropping cowpea with crops such as maize, sorghum, millet and cassava contributes to the improve soil fertility. The mean annual production of cowpea is 340 kg to 4000 kg. Sources of cowpea seeds for planting include market/traders, stored seed from own farm and from other farmers who preserve seeds for sale (ash is used to preserve seeds) (MOFA, 2005).

2.5.3. Cowpea production in Mali

Cowpea production is based generally on inter-cropping system in the large part of cowpea growing areas with maize, sorghum and millet. Famers use local, introduced and improved varieties from the market, seed companies or cowpea breeding programmes. In central part of the country, which is the most important production area, farmers practice mono-cropping system. Cowpea production is between 300 kg to 3,000 kg and intercropped system is the most developed method and accounts for 132,800 tons as against 28,538 tons produced by monocropping (DNA Mali, 2011).

2.6. Environmental requirements

2.6.1. Climate

Cowpea grows primarily under humid conditions. It is tolerant to heat and drought conditions. The crop is sensitive to frost. It germinates rapidly at temperatures above 65^{o}F; colder temperatures slow germination.

2.6.2. Soil

Cowpea is well adapted to a wide range of soils and conditions. It requires well drained sandy loams or sandy soils where the soil pH is in the range of 5.5 to 6.5 (Davis *et al.*, 1991).

2.6.3. Cultural practices

In Africa, most cowpea farmers use the following cultural practices: seedbed preparation, appropriate seedling date, appropriate method and rate of seedling, use of selective varieties with high yields and weed control.

2.7. Cowpea production constraints

2.7.1. Biotic stress

2.7.1.1. Diseases

Cowpea is susceptible to a wide variety of pests and pathogens that attack the crop at all stages of growth (Allen, 1983), for instance cowpea wilt caused by *Fusarium oscysporium* f. sp. *lycopersici*, cowpea root rust caused by a nematode (*Meloidogyne ssp)* and cowpea bacterial blight caused by *Xanthomonas vignicola*. Losses due to pest attacks or diseases can be as high as 90% (IITA, 2000). For the parasites, *Striga gesnerioides* causes important yield losses which can be between 30% to 80% (Muleba *et al.*, 1997) and sometime the entire harvest (Obilana, 1987).

2.7.1.2. Insects

Some of the major insect enemies of cowpea are pod borer (*Maruca vitrata*), flower thrips (*Megalurothrips sjostedti*), cowpea weevil (*Callosobruchus maculatus*), cowpea cuculus (*Chalcodermus sermus*), and the southern cowpea weevil (*Mylabris quadrimaculatus*).

2.7.2. Abiotic stress

The effects of the environment on plant growth may be divided into enforced damage effects (stress), caused by the environment, and adaptive responses, controlled by the plant (resistance) (Fitter and Hay, 1987). Damage, which may be manifested as death of all or part of the plant, or merely reduce growth rate due to physiological malfunction, is a common phenomenon and the agents are various: temperature, water availability, soil chemistry, soil physical properties and others such as air pollution, wind and diseases. However, the most important environmental agents affecting plant growth in the semi-arid tropical zone is drought. Cowpea can also exhibit incomplete emergence when soil temperatures are below 19^{o}C.

2.8. Germplasm collection

Cowpea is collected as germplasm in different part of the world with the largest collections at IITA (more than 14,000 accessions). The collection can be accessed via an electronic database maintained through the CGIAR-SINGER system (http://singer.cgiar.org). Eight thousand accessions are accessed at USDA through their Germplasm Resources Information Network or GRIN system (www.ars-grin.-gov). Five thousand accessions accessible on a Microsoft Access database are at the University of California-Riverside. The Istituto di Genetica Vegetale at Bari, Italy (www.ba.cnr.it) held a large collection of Mediterranean and African landraces (600

accessions). Other centers maintaining seed of wild and cultivated cowpeas include the following: Agricultural University-Wageningen (Wageningen, The Netherlands), Botanical Research Institute (Pretoria, South Africa), Le Jardin Botanique National de Belgique (Meise, Belgium), International Plant Genetic Resources Institute (IPGRI) in Harare (Zimbabwe), Institut Français de la Récherche Scientifique pour le Développement en Coopération (ORSTOM; now IRD) in Montpellier (France), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in Goiana (Brazil), Zentralinstitut für Genetik und Kulturpflanzenforschung (GAT) in Gatersleben (Germany), and the National Bureau of Plant Genetic Resources in New Delhi (India). In addition to the centers and facilities mentioned above, many national cowpea breeding programs in Africa (including programs in Botswana, Burkina Faso, Ghana, Kenya, Mali, Nigeria, and Senegal) also have substantial germplasm collections. The condition of some of these collections, which are important reserves of local diversity, could be improved with funding for germplasm maintenance and facility repair.

2.8.1. Germplasm evaluation

Phenotypic and genetic diversity can be evaluated using morphological characters, biochemical or molecular markers (DNA markers). Information about genetic diversity of germplasm is a useful tool in gene bank management and in planning experiments, as it facilitates efficient sampling and utilization of germplasm by identifying and/or eliminating duplicates in the gene stock and helps in the establishment of core collection (Ghafoor and Ahmad, 2005). Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up DUS (distinctness uniformity stability) test for candidate cultivar (Chowdhury *et al.*, 2002).

2.8.1.1. Germplasm evaluation by morphological characters

Traditionally, genetic diversity evaluated in crop species were based on differences in morphological characters and qualitative traits (Schut *et al.*, 1997), probably due to the fact that the assay of qualitative traits do not need any sophisticated equipment or complex experiments, they are generally simple, rapid and inexpensive to score. It has been used as a powerful tool in the classification of cultivars and also to study taxonomic status.

Like any other crop, cowpea breeding programme comprises four important procedures. The first step involves, breeders collecting and evaluation of germplasm to select the parental lines or cultivars. Second step involves, making crosses between parental lines to produce genetically variable population for selection. Selection of desirable recombinant genetic lines is the third step. Breeders must consider which effective selection procedure should be used in their breeding program. Evaluation of selected lines to find the best lines to use as new elite varieties is the fourth step in any breeding program. Morphological characterization is based on qualitative and quantitative traits.

Moalafi *et al*. (2010) undertook Germplasm evaluation and enhancement for the development of cowpea (*Vigna uncguiculata* L. Walp), crossing a set of parental lines from ARC germplasm, and evaluated the performances of F_2 families for the development of dual-purpose cowpea types (reasonable grain and fodder yields). During this study, they used some parameters to evaluate the different lines of F_2 such as number of days to flowering, number of days to maturity, 100 seed weight, suitability class, days to 50% flowering, days to pod maturity, pod length, pods/plant and grain yield (kg/ha).

Morpho-agronomic traits, days to emergence, days to flowering, days to pod formation, days to maturity, plant height (cm), number of pods/plant, 100 seeds weight (g), number of seeds/pod and grain yield (kg ha^{-1}), have been used by Asim *et al*. (2010) to evaluate seventeen genotypes for characterization and fodder production potential of local cowpea germplasm. Nkouannessi (2005), evaluated the genetic and morphological diversity of African cowpea genotypes based on 15 and 12 quantitative and qualitative traits respectively to assess 20 accessions collected from Kenya, Cameroun and South Africa.

However, the use of morphological traits depends on biochemical traits and most of them are ambiguous descriptors and have limited use for cultivar identification (Zacarias, 1997; Stegemann, 1984). Such characteristics are often controlled by multiple genes and are subject to varying degrees of environmental modification and interaction. Qualitative traits, such as yield performance and quality characters are of major importance in breeding and consequently, these traits are usually focused on during the evaluation of accessions. These traits express strong environmental effects, and often also genotype by environment interaction. Liu and Furnier (1993) emphasized the fact that many of the morphological traits are also difficult to analyse because they do not have the simple genetic control assumed by many in genetic models (Tanksley *et al.*, 1989).

2.8.1.2. Germplasm evaluation by molecular markers

Many of the cultivars being used in breeding programme have identical parentage but look very different morphologically when grown in the field. The overall efficiency and effectiveness of cowpea improvement programs would be enhanced by knowledge of the genetic relatedness/diversity available between and within local and

regional germplasm collections (Hegde and Mishra, 2009; Hall, 2004). The development and use of molecular-marker technologies, such as analysis of restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), minisatellites, and simple sequence repeats (SSRs), can facilitate the analysis of the structure of plant genomes and their evolution. This will contribute significantly to our current understanding of cowpea genome organization.

In recent years, many types of molecular markers have been used to observe variation directly at the DNA level (Nualsri and Konlasuk, 2000). Their application includes the analysis of segregating populations, multiple traits screening, selection for resistance to pest and disease, cultivar identification, germplasm characterization and estimation of genetic relatedness etc. (Langridge *et al.*, 1999).

Molecular genetics techniques using DNA polymorphism have been increasingly used to characterize and identify a novel germplasm/genetic diversity within the available germplasm collections for uses in the crop breeding process (O'Neill *et al.,* 2003). Characterization of germplasm using biochemical fingerprinting has got special attention. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Ghafoor *et al.*, 2002; Das and Mukarjee, 1995; Murphy *et al.*, 1990; Khan, 1990).

DNA based markers have been suggested as being suitable for genetic studies because they are not affected by environmental factors (Shashidhara *et al.*, 2003). Feleke *et al.* (2006) developed PCR-RFLP DNA markers that characterize domesticated cowpea and highlight its crop-weed complex. This study was aimed at analyzing geographic

and taxonomic distribution of this potentially informative mutation in a larger number of accessions. Genetic diversity within Ghanaian cowpea germplasm based on SDS-PAGE of seed proteins has been studied by Oppong-Konadu *et al*. (2005). For this study, 16 accessions from PGRC of Bunso were used and the aim was to use the SDS-PAGE technique to characterize cowpea accessions present in the germplasm pool in Ghana, and to conduct genetic analysis which determines the extent of genetic variation in the collection.

Ba *et al*. (2004) studied characterization of genetic variation in domesticated cowpea and its wild progenitor, and their relationship using RAPD. Sarutayophat (2008) also used RAPD markers for germplasm evaluation of Yardlong bean (*Vigna unguiculata ssp. sesquipedalis* (L.) Verdc). He characterized agronomic traits and determined the degree of genetic similarity among a total of 37 yardlong bean/cowpea accessions; compared the effectiveness of two selection methods (single seed descent and pedigree selection) for yield and yield components of two yardlong bean populations. Tosti and Negri (2002) evaluated efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* subsp. *unguiculata*) landraces. In this work, RAPD, AFLP, and SAMPL techniques were applied to investigate their efficiency in detecting the level of genetic diversity among cowpea (*Vigna unguiculata* subsp. *unguiculata* (L.) Walp.) landraces collected from a restricted area. Biological and molecular diagnosis of seedborne viruses in cowpea germplasm of geographically diverse origin in Sub-Saharan has been done by Salem *et al*. (2010). The objective of this study was to use a combination of grow-out/visual inspection, host range, reverse transcription polymerase chain reaction (RT-PCR) and sequence analyses to assay for seedborne viruses in cowpea accessions, with a focus on a wide selection, albeit limited in number, of seeds originating from several major cowpea-

producing regions of different agroclimatic conditions in sub-Saharan Africa. Lee *et al*. (2009) determined genetic diversity of cowpea landraces from Korea using Simple Sequence Repeats (SSR) and established a core collection of Korean cowpea landrace which will provide the formulation of appropriate strategies for conservation and germplasm management and maximize use of available germplasm. The same type of markers has been used by Asare *et al*. (2010) to assess the genetic diversity in cowpea germplasm from Ghana, and Sawadogo *et al*. (2010) to evaluate genetic diversity of cowpea (*Vigna unguiculata* L. Walp) cultivars and the applicability of using SSRs markers to characterized Burkinabe cowpea cultivars used in breeding for resistance to *S.gesnerioides*. John (2010) used SSR Assay and PCR amplification for enhancing cowpea (*Vigna unguiculata* L.) production through insect pest resistant line in East Africa.

Simple Sequence Repeats have valuable properties, such as a high level of polymorphism and information content, unambiguous designation of alleles, even distribution, selective neutrality, high reproducibility, high throughput applicability, co-dominance, and a rapid and simple genotyping assay (Timko *et al.*, 2008; Wang, 2002). The SSR markers are widely used in genotype identification, variety protection, genetic mapping, genome analysis, seed purity evaluation, germplasm conservation (Senior *et al.*, 1998; Nielsen *et al.*, 1997; Brown *et al.*, 1996), qualitative and quantitative trait locus analysis (Koh *et al.*, 1996), marker assisted breeding (Weising, 1998; Ayres *et al.*, 1997), diversity studies (Xiao *et al.*, 1996), paternity determination, and pedigree analysis (Bowers *et al.*, 1999; Ayres *et al.*, 1997; Ven and McNicol, 1996). Smith *et al.* (1997) and Senior *et al.* (1998) concluded that for measuring genetic diversity, assigning lines to heterotic groups, and fingerprinting, the discriminative power of SSRs is equal to or greater than that of RFLPs and is cost

effective. SSRs have been used to investigate genetic diversity in various crops, including cowpea (Gillaspie *et al.*, 2005; Li *et al.*, 2001), maize (Inghelandt, 2010; Senior *et al.*, 1998) rice (Xiao *et al.*, 1996; Yang *et al.*, 1994), soybean (Rongwen *et al.*, 1995), and wheat (Plaschke *et al.*, 1995).

Genetic relationship evaluation among germplasm using morphological characteristics are lengthy and costly processes (Cooke, 1984). The genetic control of many morphological characters is assumed to be complex, often including epistatic interactions, and has often not been elucidated (Smith, 1986). Many morphological markers are recessive and therefore only expressed in the homogenous condition. Most elite cultivated and breeding materials do not abound with readily observable morphological markers, a large number of which have deleterious effects on agronomic performance (Smith, 1986). Hence, morphological appearance cannot adequately describe cultivars without extensive replicated trials (Lin and Binns, 1994) and therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1989). With the current available and efficient molecular tools, breeding work has been shortened and reached a more reliable and efficient level.

The use of morphological and molecular methods to identify cowpea genotypes that will be interesting for breeding programmes should therefore be feasible. The abundance of genetic diversity in cowpea provides a great opportunity for the improvement of this crop at present and future breeding programmes. Currently available molecular tools for studying plant genome will certainly assist in the future expansion of marker-assisted selection and breeding to efficiently achieve this goal.

2.8.2. Advances in cowpea germplasm

Characterization of available germplasm is a necessary first step to facilitate breeding efforts; it especially benefits a plant breeder in choosing proper parental materials (Sarutayophat *et al.*, 2007; Cilliers and Swanevelder, 2003). Characterization can be done using morphological attributes and/or molecular markers. Information acquired during characterization has many uses apart from use by plant breeders. This includes effective management of genebank, remove duplicate copies, correct mislabeled accessions, monitoring of contamination through seed or pollen and determining future handling procedures (Chapman, 1989). It is also useful for the study of genetic variability within a sample, also useful to screen samples for traits which may be considered for breeding programmes with the aim of agricultural improvement in a given region, country or geographical area. Knowledge about interrelationships among descriptors (characteristics), from a plant breeder's perspective, aid in the selection of superior genotypes from the breeding population thus important in planning and evaluating breeding programmes (Sheela and Gopalan, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

The field experiment was conducted at the research fields of CSIR-Crops Research Institute at Fumesua in Ashanti Region (01° 36°W; 06° 43°N) from the end of major season to minor season, 2011 (July to early October). Fumesua is in the semi-deciduous forest zone with elevation of 186 m above sea level. The average total annual rainfall is about 1,727.2 mm and has a bimodal rainfall distribution. The major rainfall season is from March to July while the minor rainfall season is from August to November. The mean minimum and maximum temperatures are 21°C and 31°C respectively. The mean annual relative humidity is 95% in the morning and 61% at noon. The soil at the experimental site belongs to the Asuansi series and is classified as Ferric Acrisol (FAO/UNESCO, 1988). It has 16-20 cm thick layer of sandy loam and slope of 1-5%. The experiment was conducted on plots that had previously been planted to cowpea.

3.2. Materials

3.2.1. Plant materials

A total number of cowpea accessions used in this study was 94; 47 from Ghana and 47 from Mali. The accessions from Mali were collected in cowpea gene bank of Cinzana Agronomic Research Station (SRAC), Institut Economie Rurale (IER). This collection was comprised of 30 local varieties, 9 improved from the cowpea breeding programme and 8 introduced varieties from some Institutes which collaborate with IER. Major part of Ghanaian materials was from the Plant Genetic Resource Research Institute (PGRRI), Bunso. The composition of the materials was as follows: 39

varieties were local varieties from PGRRI (32) and CRI gene bank (7), 7 improved varieties from CSIR-Crops Research Institute (CSIR-CRI) and 1 introduced variety from IITA. The names and country of origin of the accessions are given in APPENDIX 3.1.

3.3. Methods

3.3.1. Land preparation and planting

The land was cleaned, ploughed and harrowed. The accessions were planted on 22nd July, 2011. The experiment was carried out on 338 m^2 area. CRD design was used without replication and each accession was planted on one row plot 2 m long at spacing of 20 cm within row with 1.5 m space between rows. Three seeds were sown per hill and thinned to two plants two weeks after emergence. The experimental area was surrounded by another variety which became the border.

3.3.2. Trial maintenance

Two kilograms of fertilizer NPK (15.15.15) was applied two weeks after seed emergence and first weeding. Some varieties were replanted or refilled due to poor germination, and the field was irregated as and when necessary until they became mature. Cowpea plants were treated with the dilution of 33.8 ml of Cymethoate Super EC (36 g Cypermethrin and 400g Dimethoate per litre) (NOVA AGRO HK LT, South Africa) into 507 ml of water against thrips weekly since the first flower appearance. The same amount, 33.8 ml, Pawa 2.5 EC Super EC (25 g Lamba-cyhalothrin per litre) (NOVA AGRO HK LT, South Africa) was diluted into 507 ml of water and applied against aphids since the appearance of pods and this activity continued until harvest on 2nd October 2011.

3.3.3. Evaluation of cowpea germplasm by morphological characters

Morphological characters were collected from each variety. Assessment of twelve qualitative and twenty quantitative traits was done using International Board for Plant Genetic Resources (IBPGR) cowpea descriptors.

3.3.3.1. Qualitative traits

The qualitative traits were evaluated using different scoring scales (APPENDIX 3.2).

3.3.3.2. Quantitative traits

Different characters evaluated are in APPENDIX 3.3.

3.3.4. Evaluation of cowpea germplasm by molecular characters

Genetic analysis of 94 accessions of cowpea was investigated using 20 primers of Simple Single Repeat (SSR) markers for characterization of varieties and estimated the relatedness between and within the two germplasms.

3.3.4.1. Sampling method

Young leaves of each variety were sampled on field three weeks after sowing. The leaves were cut with scissors which were held with a pair of forceps and put inside plastic bags labeled for each variety. The samples collected were placed inside the ice bag containing ice at half level. The materials used to cut the leaves (scissors and pair of forceps) were sterilized each time with 70% ethanol after taking the leaves from one variety.

3.3.4.2. DNA extraction

Two hundred grams of tissue was taken from each accession and DNA extracted according to the Qiagen protocol (2006) modified by CRI Biotechnology Laboratory (APPENDIX 3.4).

3.3.4.3. DNA quality control

After extraction, DNA was tested on agarose gel prepared from:

- Agarose: 2.4 g;
- Tris-Acetate Ethylene (TAE) solution: 300 ml;
- Ethidium bromide : 10 µl ;
- Cooking time : 3 mn.
- Running time : 40 mn.

For each samples, 1 µl was mixed with 2 µl of blue Dye and all the solution has been lodged in gel well.

3.3.4.4. DNA amplification by PCR

Ninety-four accessions of cowpea were investigated using 20 Simple Sequence Repeats (SSRs) primers pairs (forward and reverse) (Table 3.1). The same primers were used by Asare et *al*. (2010)

Table 3.1: Different primers using for molecular characterization

Name	Primers Sequence
SSR-6265F	5’-CAG AAG CGG TGA AAA TTG AAC-3’
SSR-6265R	5’-GCA TGT TGC TTT GAC AAT GG-3’
SSR-6258F	5’-GGT TTC CTA GTT GGG AAG GAA-3’
SSR-6258R	5’-ATT ATG CCA TGG AGG GTT CA-3’
SSR-6243F	5’-GTA GGG AGT TGG CCA CGA TA-3’
SSR-6243R	5’-CAA CCG ATG AAA AAG TGG ACA-3’
SSR-6218F	5’-GTG GAA GGA ATG GGT CCA G-3’
SSR-6218R	5’-AGG AAA TTT GCA TTC CCT TGT-3’
SSR-6217F	5’-GGG AGT GCT CCG GAA AGT-3’
SSR-6217R	5’-TTC CCT ATG AAC TGG GAG ATC-3’
SSR-6353F	5’-TCA TGG GTT AAA TTT GCT TCA A-3’
SSR-6353R	5’-AAA CCA TGT GGT TGT TGC AC-3’
SSR-6352F	5’-GTT GTG AGC TTC CCC AGA TG-3’
SSR-6352R	5’-AAT TTT GAA CCC ACC ACC AG-3’
SSR-6336F	5’-TGA AAA CAA CGA TAT GCA GAA-3’
SSR-6336R	5’-TCA GTC TTA GAA TTG AGT TTT C-3’
SSR-6323F	5’-CAA AGG GTC ATC AGG ATT GG-3’
SSR-6323R	5’-TTT AAG CAG CCA AGC AGT TGT-3’
SSR-6451F	5’-AAA GAG ATA CAC ATG CCT AAC-3’
SSR-6451R	5’-GAC CAA CAG CGA CTT TGA GC-3’
SSR-6277F	5’-CAC CCC CGT ACA CAC ACA-3’
SSR-6277R	5’-CAC TTA AAT TTC CAC CAG GCA T-3’
SSR-6436F	5’-CAG AAT CCT TGT GAA CCT G-3’
SSR-6436R	5’-TTT CGC AAT ATG CCC TTT TC-3’
SSR-6375F	5’-GCT CGG ATA TGG TCC TGA AA-3
SSR-6375R	5’-TCA GTG TCA GCA CCA TCC C-3’
SSR-6371F	5’-TGC TCA TCG TGC TTT GTC TT-3’
SSR-6371R	5’-CAC TTC AGA CTT AGA GCG AAG-3’
SSR-6370F	5’-CAA CTT CAC AGC CCT CAA-3’
SSR-6370R	5’-TTG AAG GTA TGG CCT TTT GTT T-3’
SSR-6356F	5’-TGC AAT ATG GAC CAG AAG AAA-3’
SSR-6356R	5’-ATG CCC CAA CAA CAA CAT TT-3’
SSR-6613F	5’-CTA TTG GAA TCT TGC CGT TG-3’
SSR-6613R	5’-CTT TAC CTT TAT GCA AAC CAA T-3’
SSR-6608F	5’-CTA AAT TAT AAT ATT CGT CGG T-3’
SSR-6608R	5’-GGT TAA GGA AAA GAG GGT AGG-3’
SSR-6603F	5’-GAG AAC TTC ACG CAC AAT AG-3’
SSR-6603R	5’-CGC GGT AGC ATG ATT GAA TTT-3’
SSR-6587F	5’-GAT ATA GAA TAG CAT ATT TAA C-3’
SSR-6587R	5’-GTT GAA AGT TTG ATA GTA AAG-3’

PCR master mix was prepared according to modified protocol, by Vos *et al*. (1995), of CRI Biotechnology Laboratory of which the different stages are the following:

PCR components	**1x**	**98x**
PCR H_2O	5.62 µl	550.76 µl
10x Buffer	1.0 µl	.....98 µl
$Mgcl_2$	0.9 µl	.....88.2 µl
dNTPs	0.4 µl	.....39.2 µl
Primer	1.0 µl	...98µl
Taq Polymerase	0.08 µl	.....7.84 µl
DNA temple	1.0 µl	882 µl/98 = 9 µl
	10 µl	

Amplification was carried out using AB Applied Biosystems (GeneAmpR PCR System 9 700 thermocycler) to amplify all the samples and the different stages for amplification are in Table 3.2.

Table 3.2: PCR Condition.

STAGES	DEGREE (°C)	TIMES (seconds)	NUMBER OF CYCLES
Initial denaturing	94	120	
Final denaturing	94	60	
Annealing	55	60	
Elongation	72	120	35
Extra time	72	600	
Conservation	4	Infinity	

3.3.4.5. Electrophoresis and gels analysis

PCR products were run on Polyacrylamid Gel (PAGE), the reagents and volumes of solution used for casting gels are in Table 3.3.

Table 3.3: Different reagents for PAGE

REAGENTS	Volumes
FADW (H_2O)	15 ml
TBE (10X)	2 ml
40% Acryl amide	3.0 ml
10% APS	200 µl
TEMED	20 µl
Total Volume	20 ml

3.3.4.6. Gel staining and scoring

Protocol established by Bassam *et al*. (1991) and modified by CRI Biotechnology Laboratory was used to stain Polyacrylamide Gel (APPENDIX 3.4) and the gel was scored according to the presence and absence of bands.

3.4. Data analysis

The data were analysed using GenStat® and the ANOVA procedure (Payne *et al.*, 2006). Morphological data were subjected to cluster analysis using Eucludien test with Group average method. For the molecular data, Jaccard test with Group average method were used to classify accessions in accordance with their similarity. For SSR analysis, based on the allelic profile, the PowerMarker program (Liu and Muse, 2005) according to Nei (1973) was used to calculate the number of alleles per locus, allele frequency, gene diversity (He), heterozygosity (Ho) and the polymorphic information content (PIC).

CHAPTER 4

RESULTS

4.1. Qualitatve traits

4.1.1. Qualitative morphological character analysis

Data of twelve qualitative morphological traits of 94 accessions of cowpea studied are in APPENDIX 4.1.

4.1.1.1. Flower colour

More than two flower colours were observed for the accessions studied. Sixty-nine had violet (2) flower, 17 had white (1) flowers and 8 had other colours like white-violet (Figure 4.1).

4.1.1.2. Leaf colour

Nine cowpea accessions had pale green leaf colour (3), 13 had dark green leaves (7) and 72 intermediate green colour (5) (Figure 4.2).

4.1.1.3. Pod colour

At maturity, there were three pod colours obtained (Figure 4.4). Sixty of the varieties from Ghana and Mali had pale tan (1) colour of pods, 26 had dark tan colour (2) and 8 had dark brown colour (3).

4.1.1.4. Pod curvature

All the 94 accessions had slightly curved pods.

4.1.1.5. Pod attachment to peduncle

TVU90110, Nhyira, IT89KD-374 (Korobalen), Bengpla, KPR1-96-32, Asetenapa, KPR1-96-73, Tona, Ejura, GH5043, TVU7624, KPR1-96-54, GH7233 and Alan Cash had their pod attachment to peduncle to be erect (7); GH1619 and TVU7714 with

pendant pod to peduncle (3); the remaining accessions had their pods between 30° and 90° down from erect (5) when the pods were fully grown.

4.1.1.6. Raceme position

Sixty-seven accessions had raceme mostly above the canopy (1). Sixteen had raceme in the upper canopy (2) ; on the other hand the raceme of 11 was localized throughout the canopy (3).

4.1.1.7. Terminal leaflet shape

Thirty cowpea accessions had globose (1) terminal leaflet shape, while forms haste (4) and sub-globose (2) were for 5 and 56 genotypes respectively (Figure 4.2). Terminal leaflet shape sub-haste was attributed to 3 accessions studied.

4.1.1.8. Immature pod pigmentation

Pigmented tip (1) was found on immature pod of 60 accessions. The pigments of 3 accessions were distributed on the sutures (2) of pods, whereas 4 accessions showed pigmented valves and green sutures (3) on their immature pods. Only GH2331 had splashes of pigments (4). On the one hand, no pigmentation was found on 19 accessions. Seven genotypes had other pigmentations.

4.1.1.9. Seed shape

The seeds of GH1608, PBL 22 (Djemani), Bengpla, Parajani, TVU7699, GH3683, CZ1-94-23-1, TVU90037, PBL 112 (Dounafana) and GH2271 were ovoid shape (2) (Figure 4.3). Seeds of 32 accessions were globose (4), 23 were rhomboid (5) in shape, 17 had a crowder (3) shape. Few varieties also had kidney (1) shaped seed and they were: IT89KD-374 (Korobalen), GH2272, KPR1-96-32, KPR1-96-73, Ejura, KPR1-96-54, Alan Cash, Malam Yaya, Niebe Sucré (Sukaro-Shô), TVU90106, CZ11-94-5C and GH7226.

4.1.1.10. Testa texture

The testa texture of 72 genotypes were smooth (1) while M'Barawa, IT97K-499-35 (Djiguya), CZ1-94-23-2, Malam Yaya, CZ1-94-23-1, Niebe Sucré (Sukaro-Shô) and Apagbaala testa texture were smooth to rough (3). TN5-78 (Tieblen), PBL 22 (Djemani), IT89KD-374 (Korobalen), KPR1-96-32, Parajani, KPR1-96-73, Ejura, TVU7624, KPR1-96-54, PRL 73 (Yerewolo), Suvita-2 (Grom-Grom), Alan Cash, CZ11-94-5C, GH7226, and PBL 112 (Dounafana) were rough (5).

4.1.1.11. Growth habit

Eleven accessions had prostrate (6) growth habit, 22 had intermediate (4) growth habit. Semi-prostrate (5) growth habit was associated with 60. Only Tona had erect (2) growth habit.

4.1.1.12. Seed coat colour

Different seed coat colours were found with genotypes (Figure 4.3). Seventeen varieties had red (4) seed coat. At the maturity, 19 genotypes showed white (1) seed coat whereas 21 had brown (3) seed coat colour. Nhyira, Bengpla, TVU7681 and Marfo-Tuya had cream (2) seed coat while TVU90110 and TVU7710 had black (6) seed coat. Other colours (99) mottled brown were found with genotypes such as TVU7705, TVU7617, TVU7643, GH4529, GH7233, TVU7671, TVU7696, TVU90037, CIPEA82672, TVU7616, GH2214, TVU10377 and GH5344. Tan brown was with IAR 167B, TVU7699 and GH2290; GH7243, GH3708, GH2281, Sanzisabinli and GH2329 shown seed coat colour tan. Seeds of GH2272, TVU7714, TVU7686, IT82D-812, GH3667, CIPEA80025, CB-5 and TVU9344 were coloured mottled red whereas brown-white was for Niebe Sucré (Sukaro Shô). Ejura had white-mottled blue.

Figure 4.1: Variation in flower colour.

Figure 4.2 : Variation in leave colour and hape.

Figure 4.3: Variation in seed shape coat colour.

Figure 4.4: Variation in pod and colour

4.1.2. Qualitative principal component analysis

The first four principal components with latent roots greater than 1.0 together explained a total variation of 56.06% and each of them had percentage variation greater than 10 (Table 4.1). Scores on the first principal component (PC1) which accounted for 16.93% of the total variation was highly correlated to character related to testa texture. With 14.79% of the contribution of the traits towards the diversity of cowpea accessions revealed that in the second principal component (PC2) axis, the traits with the highest loading were immature pod pigmentation, pod attachment to peduncle and raceme position. The third principal component (PC3) explained 13.40% of the variation and was highly associated with flower colour, growth habit, leaf colour, pod colour and terminal leaflet shape. The traits flower colour, and terminal leaflet shape characterized the fourth principal component (PC4) with 10.94% of variation. The fifth principal component (PC5) and the sixth principal component (PC6) with 8.96% and 7.80% respectively as percentage of variation were

dominated by the characters that included, leaf colour and terminal leaflet shape for PC5; pod attachment to peduncle, raceme position, and seed coat colour for PC6. The principal components seven (PC7), eight (PC8) and nine (PC9) explained an additional 7.30%, 5.59% and 5.30% of total variation respectively. PC7 was correlated to characters related to seed coat colour and terminal leaflet shape while PC8 was dominated by characters that included growth habit, terminal leaflet shape and PC9 was correlated with pod attachment to peduncle and pod colour (APPENDIX 4.2).

Table 4.1: Five principal components for twelve selected qualitative traits of cowpea.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Latent roots	**1.862**	**1.627**	**1.474**	**1.203**	**0.986**
Percentage variation	**16.93**	**14.79**	**13.40**	**10.94**	**8.96**
Cumulative percent variation	**16.93**	**31.72**	**45.12**	**56.06**	**65.02**
Latent vectors (loadings)					
Flower colour	0.080	-0.153	**0.395**	**0.553**	-0.044
Growth habit	0.220	-0.067	**0.443**	-0.252	-0.557
Immature pod pigmentation	-0.221	**0.528**	0.171	0.083	-0.213
Leaf colour	0.247	0.114	**0.334**	-0.434	**0.522**
Pod attachment peduncle	0.057	**0.541**	-0.077	-0.333	0.014
Pod curvature	0.000	0.000	0.000	0.000	0.000
Pod colour	-0.325	0.052	**0.481**	-0.157	-0.147
Raceme position	-0.345	**0.483**	-0.057	0.227	-0.027
Seed coat colour	-0.412	-0.175	0.241	0.033	-0.005
Seed shape	-0.392	-0.229	0.234	-0.283	0.276
Terminal leaflet shape	0.142	0.211	**0.327**	**0.405**	**0.500**
Testa texture	**0.512**	0.150	0.217	0.055	-0.142

4.1.3. Qualitative similarity matrix

The degree of similarity between accessions ranged from 1 to 0.61 and it was highly significant for the majority of the accessions at P=0.05 probability level. For

qualitative trait at P=0.05 probability level, there was no difference between the following accessions: GH4769 and GH4526; KPR1-96-73 and KPR1-96-32. But the last two genotypes had very highly significant similarity with KPR1-96-54. Similarity matrix showed no difference between the following accessions: GH4024 and TVU7608; TVU7686 and TVU7616; GH2339 and TVU7657; CIPEA80025, TVU7696, CB-5, CIPEA82672 and TVU9344. TVU7617 and GH2293 obtained 0.63 of similarity while TVU7617 and IT89KD-374 (Korobalen) had 0.65 of similarity. The accessions Parajani and GH2293 showed 64 percent of similarity and 63 to Ejura and Marfo-Tuya. The percentage similarity was 65 for TVU7643 and GH2293 whereas Malam Yaya and GH2329 had 61 percent similarity.

4.1.4. Qualitative Scatter plot matrix

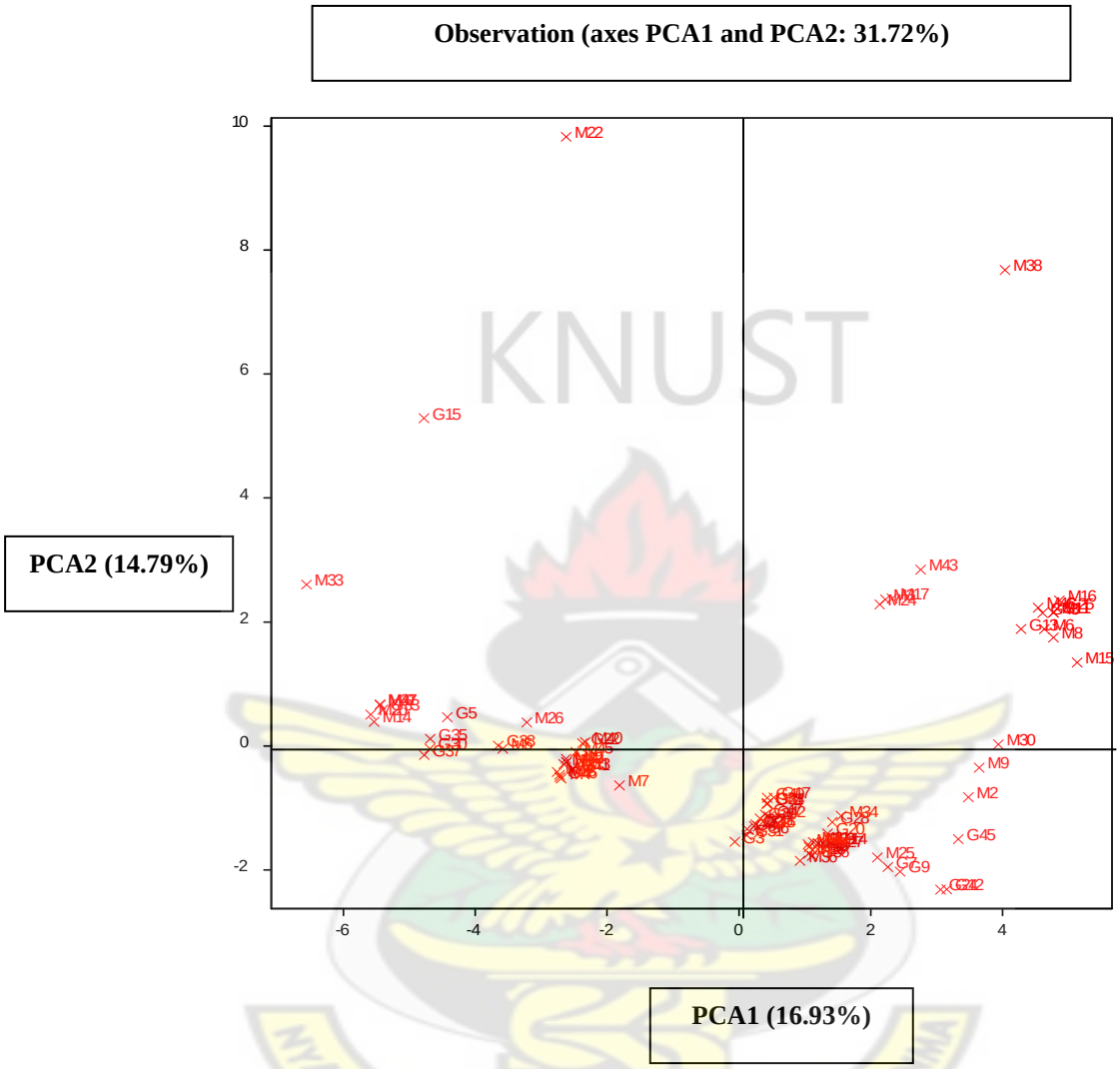

Figure 4.5: Distribution of varieties among accessions in PCA1 and PCA2 for qualitative traits.

From Figure 4.5, PCA1 and PCA2 axes for qualitative traits illustrated TVU90107 (M38) and TVU7624 (M15) are distantly related to the remaining accessions studied in the first quadrat. The second quadrat considered as the most distant IT97K-499-35 (Djiguya) (M9), GH4626 (G21) and GH2271 (G42). Thus the last two are least similar to the group. GH2329 (G37) and TVU90110 (M7) were most distant from the

others for the third while IT82D-812 (M22) and Niebe Sucré (Sukaro-Shô) were for the last quadrat.

4.1.5. Qualitative cluster analysis

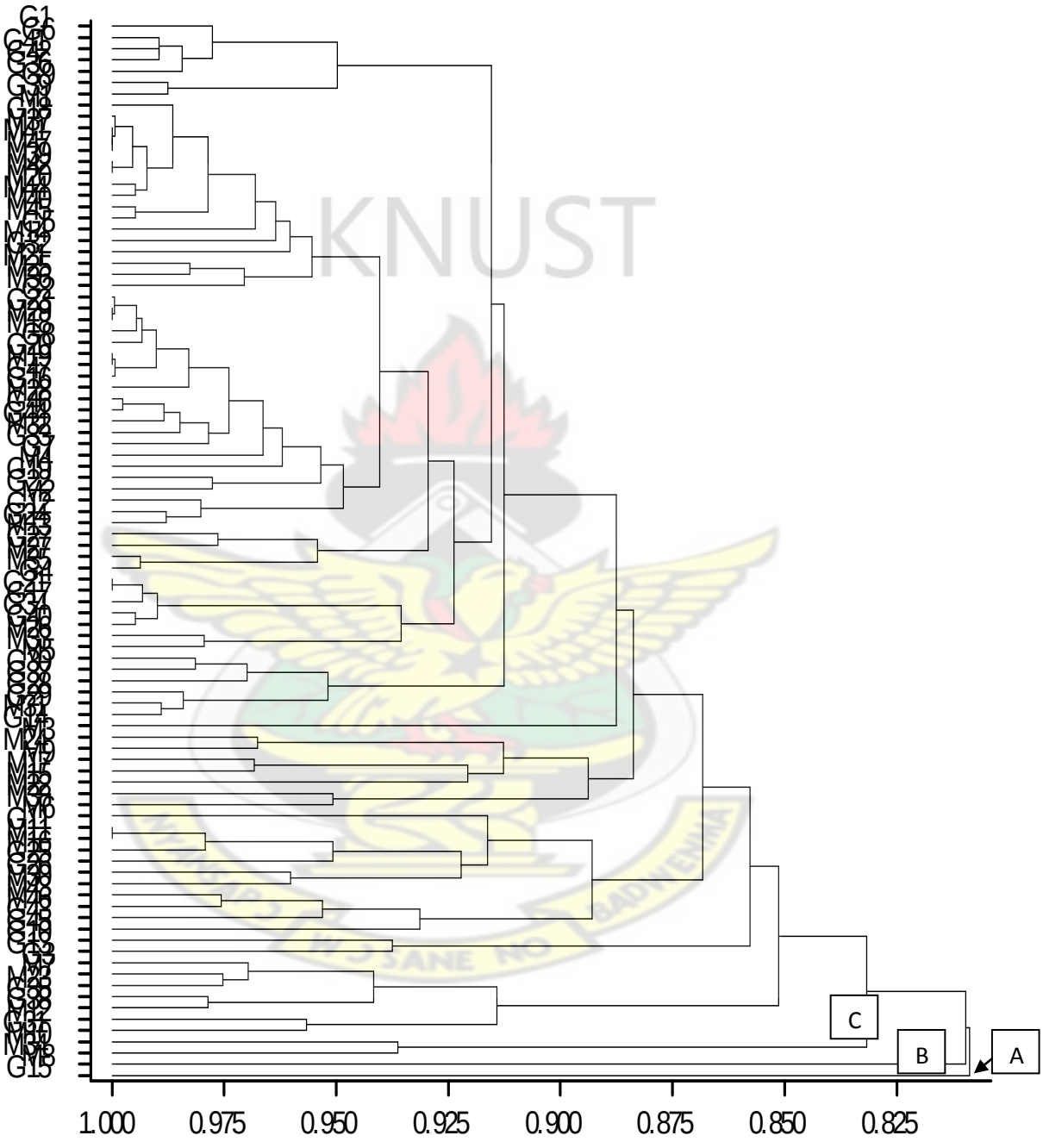

Figure 4.6: Dendrogram based on qualitative traits of 94 cowpea accessions.

NB: Identification of genotypes based on their code (APPENDIX 4.4).
Name of genotypes replaced by code M (Mali) and G (Ghana) (APPENDIX 4.11).

Based on the qualitative trait, at 0.82 level of similarity there were 3 main clusters (A), (B) and (C) (Figure 4.6) which gave different sub-cluster at 0.85 genetic distance. Distribution of the accessions into different clusters is in APPENDIXES 4.3 and 4.4. The fisrt and second clusters at 0.92 level of similarity, had one group composed of Ejura and IT97K-499-35 (Djiguya) while the third had 92 accessions. At 0.85 level of similarity, there were 2 major sub-clusters for cluster (C) which contained 2 accessions (I) and 90 accessions (II). For this cluster, there were several subgroups depending on the level of similarity chosen. Sub-cluster (II) of cluster (C) was divided into sixteen subgroups at 0.92 level of similarity. Subgroups (a), (c), (e), (h), (j) and (k) had two accessions while subgroup (b) was composed only with five accession. Subgroups (d) and (f) contained four accessions. Subgroups (g), (i) and (l) had one accession whereas subgroups (n) and (p) for the same sub-cluster contained seven accessions, all at genetic distance 0.92. Subgroup (o) was different to the others with large number of accessions (42) and six accessions were belonging to subgroup (m).

4.2. Quantitative traits

4.2.1. Quantitative morphological character analysis

Recorded data from morphological characteristics using 20 quantitative traits are in (APPENDIX 4.5).

4.2.1.1. Days to 50% emergence

Accessions emerged between 3 and 7 days with an average of 4.84. After sowing, 12 varieties emerged in 3 days, 25 varieties in 5 days, 28 varieties in 4 days, 21 varieties in 6 days and 8 varieties in 7 days.

4.2.1.2. Days to first flowering

With 35.07 days as mean, the number of day to start flowering was between 24 days and 55 days. PRL 73 (Yerewolo) took 55 days before to start flowering, TVU7705 and GH3667 matured after 24 days.

4.2.1.3. Days to 50% flowering

The mean for 50% flowering was 42 days with the range from 31 to 60 days. The varieties PRL 73 (Yerewolo), PBL 112 (Dounafana), Ejura, TVU7617, IAR 167B, GH1608, TVU7714 and Milo recorded 60 days, 56 days, 55 days, 53 days, 49 days, 48 days, 33 days and 31 days respectively to start flowering.

4.2.1.4. Days to 1st pod matured

Number of days for plants to record first matured pod differed between 44 and 76 days (Milo and PRL 73 (Yerewolo)) respectively. The average days to first pod maturity was 57 days.

4.2.1.5. Days to 50% pod maturity

The mean days to 50% pod maturity was 63. It varied from 50 days to 84 days. PRL 73 (Yerewolo) and Milo took 84 and 50 days respectively to reach 50% pod maturity.

4.2.1.6. Terminal leaflet length

For the terminal leaflet length the accessions average was 10.01 $mmx10^1$ and the widest terminal leaflet was for GH4769 and Asontem (16 $mmx10^1$) while the narrowest was for CZ1-94-23-2 and GH2290 (6 $mmx10^1$).

4.2.1.7. Terminal leaflet width

The mean terminal leaflet width was 6.28 $mmx10^1$. Two accessions (GH7875 and GH5040) had the widest terminal leaflet width of 10 $mmx10^1$. The lowest terminal leaflet width was 3 $mmx10^1$ for GH4769, GH5043, GH4526 and GH2316.

4.2.1.8. Plant height

The overall average plant height was 18.04 cm with the range between 8 cm (GH2293) and 32 cm (GH5050).

4.2.1.9. Number of pods per peduncle

The highest number of pod per peduncle was 3 for 11 genotypes whereas Alan Cash had lowest number of pod (1). The mean for this parameter was 2.11, 82 of accessions studied had 2 pods per peduncle.

4.2.1.10. Number of pods per plant

The mean number of pods per plant was 11.21 and it ranged from 3 to 22 pods per plant. Soronko, Nhyira, CZ11-94-5C and TVU7714 yielded 22 pods per plant.

4.2.1.11. Number of main branch

The average for number of main branch was 3.29 with the range from 2 to 5. According to the number of main branches counted on each plant the following groupings of the accessions were made: 4 varieties with 5 main branches, 29 varieties with 4 main branches, 51 varieties with 3 main branches and 10 varieties with 2 main branches.

4.2.1.12. Peduncle length

The range of peduncle length was from 36 mmx10^1 to 6 mmx10^1 with 20.79 as mean.

4.2.1.13. Pod length

For the length of dry mature pods, the overall average was 14.30. GH2335 had the longest pods (20 cm) while TVU7709 had shortest pods (10 cm).

4.2.1.14. Number of seeds per pod

For the number of seeds per pod, the value differed between 7 and 17 seeds per pod with an average of 13.

4.2.1.15. Number of locules per pod

The mean of locules per pod was 13.68. The highest was 18 and the lowest 9.

4.2.1.16. Pod weight

Average pod weight of ten pods was 15.51. Pod weight varied between 8 g and 26 g.

4.2.1.17. Seed length

The largest seed length was 1 mmx10^1 while the smallest was 0.6 mmx10^1 with 0.76 as average.

4.2.1.18. Seed width

The widest and smallest seed width was 0.7 mmx10^1 and 0.4 mmx10^1 respectively with 0.57 as average. Ten accessions had 0.7 mmx10^1 and 0.4 mmx10^1.

4.2.1.19. Seed thickness

The thickness measurement ranged from 0.5 mmx10^1 to 0.3 mmx10^1. The overall average was 0.42.

4.2.1.20. Seed weight

The mean hundred seed weight was 10.46 g. It varied between 6 and 19 g.

4.2.2. Quantitative principal component analysis

The first principal component (PC1) showed days to first flowering, days to first pod maturity, days to 50% flowering, days to 50% maturity mostly contributed to the divergence between the accessions at 24.45% (Table 4.2). For the second principal component (PC2), the following traits dominated at 19.95%: pods weight, seed length, seed thickness, seed width and seed weight. The principal component with 12.05% as total variation was the third and has been correlated with days to 50% flowering, number of locule per pod and number of seeds per pod. The fourth principal component (PC4) with 7.77% didn't have any particular quantitative traits which contributed positively for the divergence between the accessions. At 6.04%,

days to 50% emergence had been the most important trait for principal component five (PC5) as number of pod per peduncle for principal component six (PC6) at 5.18%. The variations were for the last three principal components 4.16%, 3.72% and 3.50% respectively. The seventh principal component (PC7) was correlated with plant height and terminal leaflet length while eighth principal component (PC8) correlated with number of main branches and terminal leaflet length. Number of main branches, and terminal leaflet width were the traits considered in principal component nine (PC9) (APPENDIX 4.6).

Table 4.2: Five principal components for twenty selected quantitative traits of cowpea.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Latent roots	4.890	3.990	2.410	1.553	1.208
Percentage variation	24.45	19.95	12.05	7.77	6.04
Cumulative percent variation	24.45	44.40	56.45	64.22	70.26
Latent vectors (loadings)					
Day 1st flowering	**0.360**	0.014	0.296	0.129	0.049
Day 1st pod maturty	**0.388**	0.047	0.266	0.133	-0.019
Day 50% emergence	0.037	-0.023	-0.006	-0.197	**0.741**
Day 50% flowering	**0.350**	0.075	**0.330**	0.174	0.049
Day 50% maturity	**0.398**	0.037	0.247	0.118	-0.025
Number locule/pod	-0.324	-0.053	**0.307**	0.283	-0.035
Number main branches	-0.004	0.179	0.137	-0.317	-0.400
Number pod/peduncle	0.043	0.110	0.072	0.005	0.113
Number pod/plant	0.055	0.184	0.090	-0.373	-0.404
Number seeds/pod	-0.299	-0.081	**0.349**	0.267	-0.044
Peduncle length/mmx10[1]	-0.148	0.221	0.207	-0.310	0.117
Plant height/cm	-0.084	0.263	0.246	-0.320	0.092
Pod length/cm	-0.318	0.131	0.162	0.250	-0.082
Pods weight/g	-0.234	**0.310**	0.076	0.245	0.082
Seed length/mmx10[1]	0.092	**0.368**	-0.234	0.096	0.009
Seed thickness/mmx10[1]	0.069	**0.365**	-0.146	0.171	-0.015
Seed width/mmx10[1]	0.133	**0.360**	-0.263	0.156	-0.061

Seed weight/g	-0.010	**0.373**	-0.227	0.178	0.103
Terminal leaf length/ mmx10[1]	-0.118	0.281	0.185	0.063	0.032
Terminal leaf width/ mmx10[1]	-0.087	0.233	0.237	-0.267	0.234

4.2.3. Quantitative similarity matrix

The coefficient of similarity was highly significant for the majority of the varieties studied but between the accessions it was less than 100%. Apart from TVU7705 and PRL 73 (Yerewolo) with 0.63 of similarity, TVU7705 and Ejura with 0.62 of similarity between them, the remaining of genotypes had been either highly or very highly correlated at P=0.05 probability level.

4.2.4. Quantitative scatter plot matrix

The two components, PC1 and PC2, from quantitative data explain cumulative variability of 44.4% (Figure 4.7). Based on the distribution of varieties, cultivars TVU90106 (M34) and Ejura (G15) were the most distantly related to that group while the second group showed cultivars PRL 73 (Yerewolo) (M17) and Bengpla (G9) were distantly related to the others. The most distantly related cultivars from the third quadrat were cultivars GH2329 (G37) and Soronko (G10). The last quardrat identified GH7233 (G22) and Milo (G8) to be distantly related to the rest of the group.

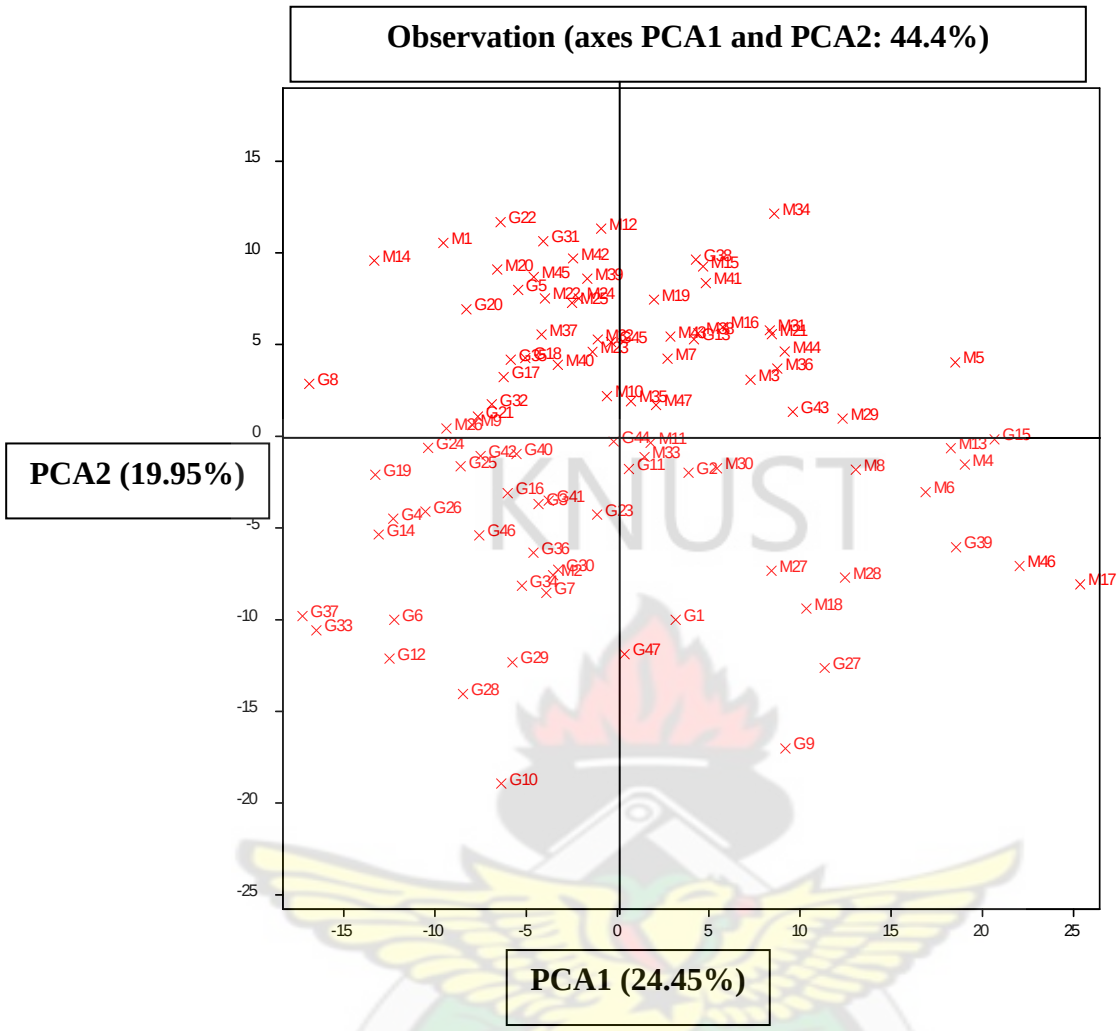

Figure 4.7: Distribution of varieties among accessions in PCA1 and PCA2 for quantitative traits

4.2.5. Quantitative cluster analysis

The data collected from quantitative characters shown in the dendrogram consisted of two majors' clusters (A) and (B) at similarity coefficient 0.84 (Figure 4.8). Cluster (A) consisted of 5 accessions (Marfo-Tuya, PRL 73 (Yerewolo), PBL 22 (Djemani), PBL 112 (Dounafana) and Ejura). Cluster (B) consisted of 89 accessions. APPENDIXES 4.7 and 4.8 indicate the various accessions in sub-clusters. At genetic distance 0.88, cluster (B) had two sub-clusters (I and II). One accession, Alan Cash was in sub-cluster I and 88 were in sub-cluster II. There were several subgroups within sub-cluster II.

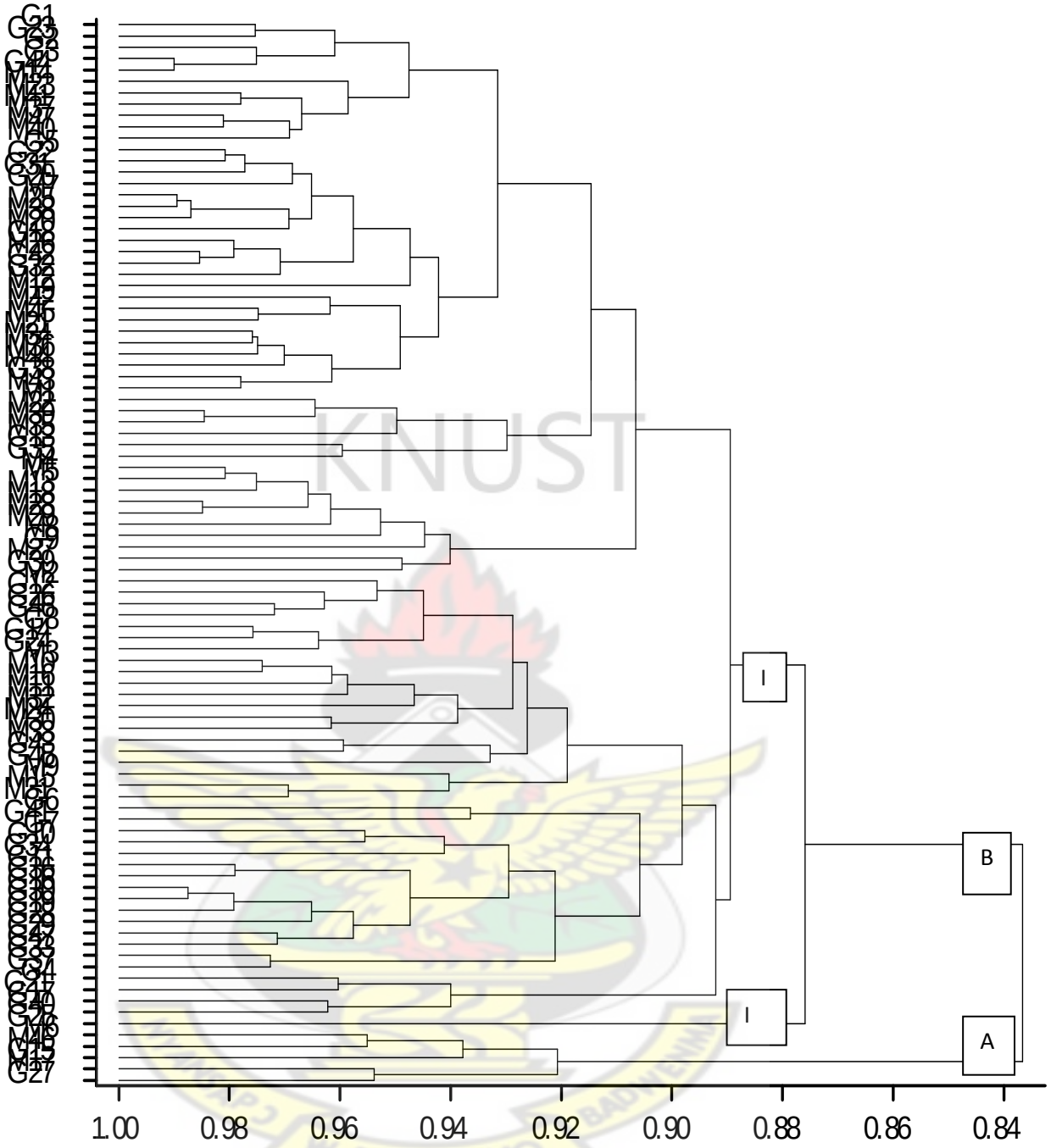

Figure 4.8: Dendrogram based on quantitative traits of 94 cowpea accessions.

NB: Identification of genotypes based on their code (APPENDIX 4.8).
Name of genotypes replaced by code M (Mali) and G (Ghana) (APPENDIX 4.11).

4.3. Molecular characterization

Figure 4.9 is result of SSR6261 from amplification of 94 cowpea genotypes. The lanes from left to right correspond with the order of arrangement in Appendix 4.7.

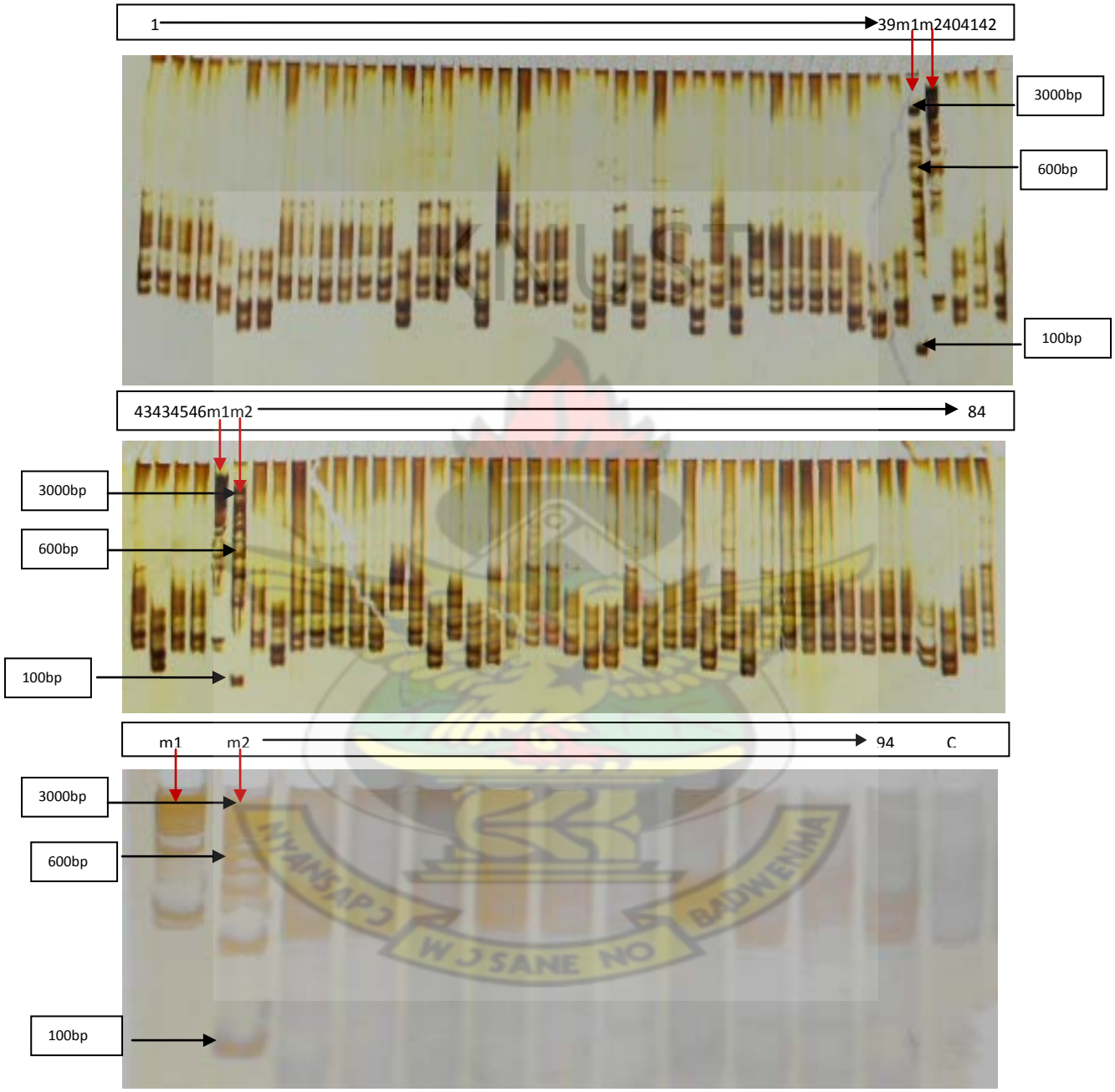

Figure 4.9: Silver stained acrylamide gel showing PCR products from primer SSR6261.

NB: m1: Ladder 1kb; m2: Ladder 100Kb; c: test control without DNA.

4.3.1. Cluster analysis

The combined data of six polymorphic primers which delineated the 94 accessions into three major clusters (A), (B) and (C) at 0.125 similarity coefficient (APPENDIXES 4.9 and 4.10) is showed in Figure 4.10. Cluster (A) consisted of 10 accessions, seven of them came from Ghana and 3 from Mali. Cluster (B) consisted of several subgroups. There were 42 accessions in the cluster, 18 were from Ghana and 24 were from Mali. Clusters C contained 42 accessions, 21 were from both Ghana and Mali.

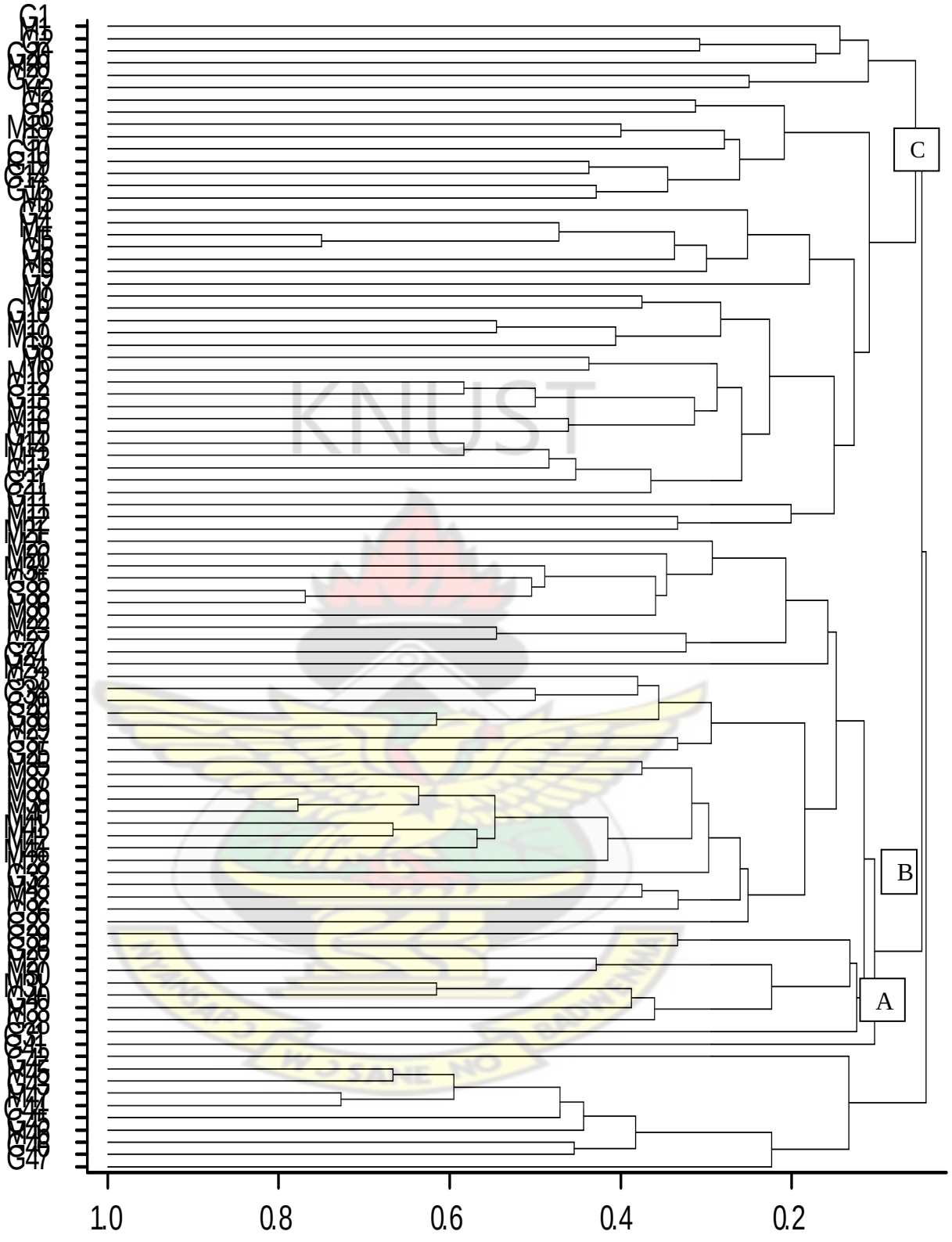

Figure 4.10: Dendrogrm based on quantitative traits of 94 cowpea accessions.

NB: Identification of genotypes based on their code (APPENDIX 4.10).
Name of genotypes replaced by code M (Mali) and G (Ghana) (APPENDIX 4.11).

4.3.2. Genetic characterization based on SSR markers

Twenty pairs (forward and reverse) primers were used to analyse genetic diversity of 94 cowpea varieties. These primers generated a total of 108 bands across the selected genotypes. Fourteen SSR primers did not show any polymorphism between varieties, and therefore, they were excluded in the analysis. The size of amplified alleles ranged from 100 to 3000 bp as shown in Table 4.3. The primers SSR6261 and SSR6356 amplified the highest (386) and lowest (34) number of polymorphic bands respectively, across the DNA samples. The number of alleles varied from 7 (SSR6353) to 27 (SSR6261). The allele frequency ranged from 0.15 (SSR6353) to 0.45 (SSR6261) with a mean of 0.28. The polymorphic information content (PIC) representing the allele diversity for a specific locus varied from 0.61 (SSR6353) to 0.92 (SSR6261) with a mean of 0.83. Gene diversity ranged from 0.66 in SSR6353 to 0.92 in SSR6261 with an average of 0.84. Variation in heterozygosity among cowpea SSRs increased from 0.0 (SSR6217 and SSR6353) to 0.97 (SSR6261) with an average occurrence of 0.47.

Table 4.3 : Size range, allele frequency, number of allele, gene diversity, heterozygosity and polymorphic information content (PIC) values generated from SSR data.

Marker	Allele size range (bp)	Allele Frequency	Number of Allele	Gene Diversity	Heterozygosity	PIC
SSR6217	130-285	0.24	13.0	0.89	0.00	0.88
SSR6353	100-110	0.15	7.0	0.66	0.00	0.61
SSR6243	100-280	0.17	21.0	0.91	0.73	0.91
SSR6352	100-3000	0.34	21.0	0.85	0.61	0.84
SSR6356	130-690	0.33	19.0	0.83	0.50	0.81
SSR6261	110-620	0.45	27.0	0.92	0.97	0.92
Mean		**0.28**	**18.0**	**0.84**	**0.47**	**0.83**

CHAPTER 5

DISCUSSION

Earlier studies on cowpeas using morphological traits have been carried out by many researchers. These traits were found to be of great importance to distinguish genetic variability, and have lead to a better classification of cowpea genotypes (Apte *et al.*, 1987; Roquid and Patnaik, 1990; Nkouannessi 2005; Adewale *et al.*, 2011). Emebiri (1989) also characterized cowpea cultivars using their flower size and style length, and also reported that both characters were highly heritable. As in previous studies, this study also found that agro-morphological traits (quantitative and qualitative) are still valuable tools for cowpea genetic diversity studies. Evaluations of some of these traits were done with meter rule or calipers for most of the quantitative traits and physical observation for most of qualitative traits.

5.1. Qualitative morphological analysis

The observed colours of the accessions used in this study showed that about 73% of the accessions produced violet flowers, 19% produced white flowers and 8% produced white-violet flowers which is in agreement with the observation of Cobbinah *et al.* (2011) who found in their study the percentage of flower colour distribution as follows: 80.6% violet colour, 13.4% white flowers and 6% for violet-pink. The only difference in this study was in the white-violet flowers; apart from that the majority of the accessions had violet flower colours followed by white flowers in the two studies. This may be attributed to the relatively large number of accessions studied in their work as compared to the number used in the present study. In contrast, Ezueh and Nwoffiah (1984), and Bennett-Lartey and Ofori (1999), observed that accessions with purple flowers ranked the highest. Therefore, the results of the present study showed two flower colours documented by IBPGR (1982). Studies done by Gibbon and Pain

(1985) reported of additional flower colours such as pale blue, yellow and pink, which were not observed in this study.

Intensity of green colour of the leaves varied between pale green, dark green and intermediate green. Most of the accessions which had violet flower had the same leaf colour (intermediate green). Others varieties showed either pale green or dark green leaves.

For the matured pod pigmentation pattern, more than 60% of the accessions were pale tan, 28% dark tan and 6% dark brown. In accordance with cowpea descriptor, all colours were identified except black or dark purple and other colours such mottled red, mottled tan. There was little difference between the 94 accessions with regard to pod curvature; they were slightly curved.

Phenotypic observation of raceme position showed that 73% of the accessions had their raceme positions mostly above the canopy while 18% had the raceme positions in upper canopy, 9% had their raceme positions throughout the canopy. All raceme positions were represented. This parameter is important since it facilitates pods harvesting. It was pointed out that when the racemes are held at the same level or within the canopy the pods also become hidden and strenuous in harvesting. It was mentioned by Pandey and Ngarm (1985) and Bennett-Lartey and Ofori (1999) that varieties with their raceme above the canopy are easier and cheaper to harvest than those with racemes below the canopy. Such accessions will also enhance the use of mechanical harvesters since they will not require the pulling up of the whole plant during harvesting. Porter *et al*. (1974) observed that accessions with racemes within the canopy were the commonest among 4 000 accessions they studied.

Recorded at mature leaf in the 6th week after sowing, majority of the genotypes from Mali had terminal leaflet shape sub-globose and this form characterized more than 50% of the accessions studied. The second group had terminal leaflet shape globose. The forms hastate and sub-hastate were predominantly from Ghana.

Apart from uniform pigmentation, all the characteristics of immature pod pigmentation was found with the accessions and around 67% had pigmented tips; within this group, the number of varieties from Ghana was greater than those from Mali. Pods of accessions that had pigmented valves and green sutures were 5% while 1% was for splashes of pigment. Around 17% of the genotypes did not show any pigmentation on their pods. Pigments were distributed on the sutures of 2% of the samples whereas 8% had other pigmentations such as pigmented valves and green sutures. Pigmented valves, green sutures and splashes of pigment of pod were found in collection from Ghana. For the same parameter, Nkouannessi (2005) had four types of pod pigmentation at full grown immature pod stage whereas accessions in this study were distributed between six types of immature pod pigmentations. The study revealed that the pattern of pigment distribution of full grown immature pods varied; pods of most of the immature accessions in this study were not pigmented even though Porter *et al*. (1974) had reported of six different patterns of pod pigmentation. These results confirmed result of Cobbinah *et al*. (2011) who found in their investigation higher percentage of accessions with pigments at the tips and lower percentage of accessions with splashes of pigment on the immature pods. According to Singh and Rachie (1985), Fery (1985) and Bennet-Lartey and Ofori (1999) the pods of many cowpea cultivars contain anthocyanin which is either partially or wholly purple. The results obtained from this study could be attributed to the fact that the

accessions studied lack or have low concentration of anthocyanins pigment in the pods at immature state.

The kidney, ovoid, globose, rhomboid and crowder seed shape were observed in this study. Compared with the study done by Musvosvi (2009), none globose seed shape was found with the accessions he worked on. This could be due to the small size of sample used in that study. No genotype studied had rough to wrinkled and wrinkled seed coat. Seed testa texture ranged from smooth, smooth to rough and rough. Additionally the result from Nkouannessi (2005) study showed that seed texta texture ranged from rough to wrinkled. Smooth to rough seed testa texture were reported in accessions evaluated by Adewale *et al*. (2011).

On the seven growth habit patterns of the International Board for Plant Genetic Resources IBPGR (1982) cowpea descriptor, four of them were observed with all the accessions. Germplasm from Mali had shown more than 80% semi-prostrate, 10% prostrate and 5% intermediate. Majority of Ghanaian accessions had semi-prostrate growth habit, some with intermediate or prostrate growth habit. For all the genotypes together, it was observed that 73.2% of the accessions studied produced semi-prostrate growth habit, 17.8% had intermediate growth habit and 8% were prostrates. Only 1% of the accessions studied showed erect growth habit. Regarding the results of this study, the difference with IBPGR may be attributed to the large number of cowpea genotypes assessed which were collected from major cowpea growing areas of the world as compared to this study. Touré *et al*. (1999) found some improved varieties such as PBL 22 (Djemani), PRL 73 (Yerewolo), PBL 112 (Dounafana), CZ1-94-23-1, CZ1-94-23-2 and CZ11-94-5C to be prostrate. These differences may be due to the acclimatization of the genotype or genetic make up of the tested

genotypes. Bennett-Lartey and Ofori (1999) obtained semi-prostrate, prostrate and intermediate between accessions from four cowpea-growing regions of Ghana. Growth habit is very important in the cropping system of cowpea in Ghana and Mali. Doku (1970) and Rachie and Rawal (1976) observed that most landraces show the prostrate and climbing cowpea types, and that the prostrate types are used by peasant farmers in mixed cropping.

Variability was found between and within the two cowpea germplasms for seed coat colour. Accessions have shown the following seed coat colour: red, white, brown, cream, black and other colours. Between the different seed coat colour, many accessions from both germplasms had other seed coat colours like mottled brown, tan brown, tan, mottled red, brown-white and white mottled. This variability was not observed with the samples used by Aremu *et al*. (2007) who observed seed coat colour range from white, brown, milk, dark and red. The assessment of the same parameter was slightly different from Musvosvi (2009) with seed coat colour green and buff.

5.2. Quantitative morphological analysis

Data of twenty quantitative traits were collected from ten plants within the row for some of the parameters. The number of days to 50% maturity may be depended on the number of days to 50% emergence. Some genotypes which germinated earlier showed days to 50% maturity too late and the same has been for the materials that emerged late. For instance, IT97K-499-35 (Djiguya) germinated 7 days after sowing but got 50% maturity in 56 days; for Apagbaala it was 3 days to 50% emergence and 78 days to 50% pods maturity. It was only PRL 73 (Yerewolo) which kept it lateness, 7 days

to reach 50% emergence and 84 days to reach 50% pods maturity. This variation may be due to some environmental factors which can affect the genotypes.

Number of days for the accessions to initiate flowering was between 24 days (TVU7705 and GH3667) and 55 days (PRL 73 (Yerewolo)). The greatest number of days to 50% flowering has been with PRL 73 (Yerewolo) with 60 days while TVU7714 had the earliest (33 days). TVU7714 took the same days to attain 50% flowering with Milo. From sowing date to first pod maturity, the number of days was between 76 for PRL 73 (Yerewolo) and 44 for Milo.

Days to maturity among cowpea genotypes ranged from 50 to 84 days. Milo took fewer days to mature (50 days) and was an extra early maturing genotype followed by TVU7714 and GH2329 (53 days). PRL 73 (Yerewolo) was observed as the late maturing genotype (84 days). Breeding for earliness is an important breeding objective in cowpea as breeder and famers always require the early maturing genotypes for crop improvement and yield. Fifty percent of the genotypes from Ghanaian accessions matured between 50-60 days whereas 10 varieties were only from Mali. The genotypes studied showed variation in maturity between and within both germplasms. Such variation in maturity of genotypes may be due to climatic conditions or genetic make up of the tested genotypes. These results are in conformity with those of Amanullah *et al*. (2000), who studied 20 genotypes and found significant variations for days to maturity. For Sanjeev *et al*. (2010) study, number of accessions was found to be significantly early compared to the check variety C152 (81.5). Sarvamangala (2005) also observed some early maturing cowpeas maturity date ranging from 71-78 days. Singh *et al*. (2007) and Dugje *et al*. (2009) had classified cowpea varieties that matured in 60 days as extra- early, 61-80 days as early

and > 80 days as late. Majority of accessions from this study were early maturing whilst PRL 73 (Yerewolo) was the only late maturing variety.

Alternative and trifoliate, longer terminal leaflet was associated with Asontem and GH4769 with 16 mmx10^1. CZ1-94-23-2 and GH2290 had the smallest size (6 mmx10^1) for the same parameter. More than half the collection from Ghana had terminal leaflet length ≤10 mmx10^1. The value obtained for the width of leaf was 10 mmx10^1 for GH7875 and GH5040 which was the broadest terminal leaflet of all accession. The narrowest value (3 mmx10^1) for the same character was for GH4769, GH5043, GH4526 and GH2316. For Nkouannessi (2005), the size of terminal leaflet length was between 34.1 mm and 89.3 mm while for terminal leaflet width was 7.2 mm to 59.64 mm. According to Summerfield *et al.* (1974), Kay (1979) and Fox and Young (1982), leaves exhibit considerable variation in size (6-16 x 4-11 cm). In this study, data on terminal leaflet length had been within this interval. For terminal leaflet width some varieties had less than 4 cm. Cobbinah *et al.* (2011) observed from their study that in accessions where leaf shedding did not occur and most leaves remained on the plant, harvesting of the matured pods (when the pods were 85% to 90% dry) became strenuous because these mature pods were not exposed enough to facilitate easy harvesting.

The highest height of plant at 4 weeks after planting, and also between the highest numbers of pods per peduncle, was for GH5050 with 32 cm and 3 pods respectively. Across the ninety-four accessions, the least height (8 cm) was obtained by GH2293. In accordance with Akbar *et al*. (2010), genotypes with early maturity produced dwarf plants, while genotypes with late maturity showed highest plant height. The same results were reported by Ram *et al*. (1994). In this investigation, some earlier

materials had high plant height and vice-versa. This variation might be attributed to the differences in the genotypes or might be due to environmental fluctuations. Plant height and canopy spread reflects contribute to the photosynthetic activity of the plant, having indirect effect on seed yield. Plant with the spreading nature covers the ground and reduce moisture loss. Plants which have tall stature and large canopy spread are desirable areas where there is scarcity of water.

Majority of the accessions studied showed number of pods per peduncle to be greater than one, with 3 being the highest for TN5-78 (Tieblen), GH4769, GH5050, Asetenapa, KPR1-96-73, PRL 73 (Yerewolo), TVU7608, Marfo-Tuya, TVU7657, TVU7710 and GH2214. Alan Cash had the lowest number of pod per peduncle (1), but generally it was not the variety with least number of pods per plant. For this parameter, our results were close to those obtained by Goenaga *et al*. (2008), who observsed more than 80% of the lines had 2 as number of pods per peduncle.

There was no correlation between the parameters ; number of pods per peduncle and number of pods per plant because any of the varieties with high number of pods per peduncle didn't get the highest number of pods per plant. With two pods per peduncle for Soronko and TVU7714, number of pods per plant was 2 and 3 for TVU7714 and Soronko respectively.

There were differences between and within the two germplasms in number of main branches. Majority of the collections from Mali and Ghana had three main branches per plant. The lowest value obtained from the mean of the main branches of ten selected plants was 2 for GH7178, IT97K-499-35 (Djiguya), CZ1-94-23-2, TVU7686, Alan Cash, GH2331, TVU90037, CB-5, CIPEA82672 and CZ11-94-5C. On the other hand varieties GH7875, Niebe Sucré (Sukaro-Shô), GH2329 and GH7226 were

different from the others with high number of main branches (5). These varieties had the same number of pods per peduncle with Soronko which showed the highest number of pods per plant. Number of main branches produced in Shiringani study (2007) ranged between 3 and 10 branches. Environmental fluctuation or acclimatization may be the basis for the large difference between the two studies.

In terms of peduncle length, certain varieties from Mali (CZ1-94-23-2 and TVU90106) were the only accessions which gave peduncle length of less than 10 cm. The shortestt length had been shown by TVU90106 (6 mmx10^1) while M'Barawa and Soronko had the highest length of peduncle (36 mmx10^1). The accessions from Ghana did better than those from Mali because more than 80% had peduncle length beyond 20 mmx10^1. Cobbinah et *al*. (2011) evaluated the performance of some accessions of cowpea from Ghana at two different locations (Pokuase and Bunso) and found mean peduncle length of 250.11 mm and 294.20 mm. Peduncle length determines the position of the pods on the plant and thus becomes an important character with respect to harvesting in cowpea. Mature pods are normally held on the peduncle, which reflects the position of pods on the plant. Pandey and Ngarm (1985) stated that for easy harvesting of cowpea, the peduncle length should be intermediate and above the canopy to hold the pods above canopy to enhance easy visibility. It was observed that the plants with extra long peduncle were easily lodged by strong winds causing other problems such as rotting and rodent attack.

In accordance with Chevalier (1944), cowpea pods length ranges from 8-22 cm with 10-20 seeds per pod. Our study showed the size of pods between 10-20 cm which were inside the interval determined by the previous author. GH2335 (20 cm) gave larger sizes than the other accessions whereas TVU7709 had the smallest pod size (10

cm). Pod length is important for the seed per pod and this affects seed yield. Variation was observed between and within cowpea from the countries. Similar results were reported by Muhammad *et al*. (1994), who studied six different genotypes under medium rainfall conditions and reported significant variation for pod length among the genotypes. Highly significant ($p \leq 0.01$) variations were observed for the same traits among 26 genotypes (Akbar Khan *et al.*, 2010). Bennett-Lartey and Ofori (2000) found significant ($p \leq 0.05$) variability in some quantitative characters of cowpea landraces in Ghana. They thought this character was genetically controlled to a large extent; potential genetic variability for selection was low and hence may lead to very little change.

Data collected from number of seeds per pod was found between the range of 7-17 seeds. Two lines from each germplasms (GH1619, GH2339, TVU7678 and CIPEA80025) produced maximum number (17) of seeds per pod, while genotype Ejura produced 7 as the lowest number of seeds per pod. There were some variability in terms of number of seed per pods within and between the germplasms. Akbar Khan *et al*. (2010) realized that larger pods produced maximum number of seed per pods, while smaller pod produce less. These results were reported by Muhammad *et al*. (1994) and Amanullah *et al*. (2000). Thiyagarjan and Rajasekaran (1993) found that plant height also affect number of seeds per pod. In our study, there was no relationship between these parameters. Moreover this variation might be due to different genotypes or environmental differences which promoted early maturity; minimum time for seed setting and development. It also might be due to lack of rainfall which can prevent the accessions to grow to their full genetic potential.

Number of locule per pods may not depend on number of seeds per pod. Some lines which had higher numbers in terms of seeds per pod did not attain the same position with number of locules per pods; among these lines were GH2339 and TVU7687, with 18 locules per pod. CZ1-94-23-1 and PBL 112 (Dounafana) had the least number of locules per pods (9).

After harvesting, the weight of ten randomized pods selected was between 8 g x 10^{-1} and 26 g x 10^{-1}. Ten accessions from Ghana's collection took the highest weight than the others and they were us follows 26 g x 10^{-1} for GH4769, GH3667 and GH2339; 25 g x10^{-1} for GH4526 and GH3683; 24 g x10^{-1} for Soronko, GH7875, Asontem and GH5040; 23g x10^{-1} for GH1619. Most important variety from Malian collection in term of pods weight was low; TVU90037 (22 g x 10^{-1}) while TVU7616 had 8 g x 10^{-1} as pod weight. All the lines from Ghana showed pod weights above or equal to 10 g x 10^{-1}. This difference between the two germplasm collections might be due to lack of acclimatization of genotypes from Mali which were grown for their first time in Ghana.

The lines TVU7705, Parajani, IT82D-812 and TVU7696 gave the smallest sizes for the three traits about seed length, seed width and seed thickness of 0.6 mmx10^{1}, 0.4 mmx10^{1} and 0.3 mmx10^{1} respectively. Conversely, Ejura and CZ1-94-23-1 had 1 mmx10^{1}, 0.7 mmx10^{1} and 0.5 mmx10^{1} respectively. The size of the grain is very important for a consumer, that's why one of the breeding objectives is to select plants with bigger seeds and at the same time with good technological traits. One variety from Ghanaian accessions (Ejura) had the highest 100 seed weight of 19 g followed by CZ1-94-23-1 (18 g). The varieties TVU7617, TVU7687 and TVU7616 recorded the lowest weight for 100 seeds (6 g).

In terms of hundred seed weight, less than ten grams was given by the majority of the accessions from Malian collection. These differences in seed weight might be the time factor for the accumulation of assimilates in the seeds and differences in the genetic make up of different genotypes. Acclimatization factor might also be responsible for higher seed weight. Similar variations were reported by Amanullah *et al*. (2000). This result also support the work done by Khan *et al*. (2010) who found highly significant variation for 100 seed weight in 24 exotic cowpea genotypes. Olge *et al*. (1987) classified cowpea varieties into size categories based on their 100-seed weight. Varieties with seed weight between 10-15 g were described as small; 15.1-20 g were medium size seed while large seed have 20.1-25 g. Seed weights over 25 g were described as very large seeds. Thirty-seven of the accessions studied had 100-seed weight less than 10 g; twenty-five varieties from this group were from the Malian collection. Classifying the study collection, the thirty-seven accessions will be classified as very small seeds. More than 50% of Ghanaian materials had 100 seed weight between 10-15 g, which were small in seed size. Around 43% of the materials from Mali were described as small in terms of 100 seed weight. Medium size has been attributed to Ejura and CZ1-94-23-1 which had 19 g and 18 g respectively.

5.3. Qualitative and quantitative traits principal component analysis

The main reason for plant collection is to obtain raw material that can be useful for providing germplasm pools for crop improvement. Therefore, PCA is perhaps the most useful statistical tool for screening multivariate data with significantly high correlations (Johnson, 1998). Information obtained from principal component analysis (PCA) may assist the plant breeders to identify limited traits for use in hybridization and selection programs. From the PCA of the qualitative traits, the most effective characters for distinguishing among the cowpea accessions included flower colour,

growth habit, leaf colour, pod attachment to peduncle, pod colour, raceme position, seed coat colour and terminal leaflet shape. The quantitative characters such as days to first flowering, days to first pod maturity, days to 50% flowering, days to 50% maturity, days to 50% emergence, number of locule/pod, number of main branches, number of pod/peduncle, number of seeds/pod, plant height (cm), pods weight (g), seed length (mm) $x10^1$, seed thickness (mm) $x10^1$, seed width (mm) $x10^1$, seed weight (g), terminal leaflet length (mm) $x10^1$ and terminal leaflet width (mm) $x10^1$ highly contributed for divergence between the study accessions. For the two morphological characters, majority of the traits contributed to the divergence between and within both germpalsms. Comparative study on the phenology and yield components of ten cowpea varieties by Manggoel *et al*. (2011) showed that out of the nine characters assessed, six had contributed for divergence between the accessions.

5.4. Qualitative and quantitative similarity matrix

The degree of similarity between accessions ranged from 0.61 to 1.0 and it was highly significant for the majority of the accessions with qualitative characters. The correlation level in this study between KPR1-96-73, KPR1-96-32 and KPR1-96-54 was supported by Toure *et al*. (1999) and these varieties are improved varieties from the same crossing parents. The genotypes which had no difference in terms of similarity between and within both germplasm may be attributed either to the collection of the same varieties at different geographical location with different names or introductions from the same gene bank as IITA.

For quantitative data, coefficient of similarity was highly significant for the majority of the varieties studied but between the accessions it was less than 100%. The significant correlation within Ghana collection was between GH4542 and GH7178 at

0.99 similarity level. The same level was for TVU90107 and TVU90110 from Mali collection.

5.5. Qualitative and quantitative scatter plot matrix

The first two principal components (PCA1 and PCA2) for qualitative traits explaned 31.72% of the total variation and was highly associated with seed testa texture, immature pod pigmentation, pod attachement to peduncle and raceme position, made some varieties from the study accessions were distant to the others. The varieties in the first quardrat will be good candidates for genetic improvement. For axemple, M6 (PBL 22 (Djemani)) with prostrate growth habit and white seed coat and M15 (TVU7624) with semi-prostrate growth habit, G13 (Parajani) with white seed coat colour and intermediate growth habit, can be intersting to improve cowpea varieties which will be usefull for intercropping systems.

The results of the fisrt two principal components analysis from quantitative traits showed that the genotypes divergence from the others was based on the contribution of the following reproductive traits and seed components: days to first flowering, days to first pod maturity, days to 50% flowereing, days to 50% maturity, pod weigth, seed length, seed width, seed thickness and 100 seeds weight. The results of this study were not different from those of Manggoel *et al*. (2011) who found contribution of days to first flowering, days to 50% flowering and 100 seed weight for the divergence between the accessions from the fisrt two principal components analysis. According to them, the number of days from sowing to flowering is of great importance in cowpea as it affects pod set and crop yield. Sulnathi *et al*. (2007) 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 this study and supported by

these authors, these three characters should be considered while selecting the parents for hybridization programme in yield improvement of cowpea. Accessions grouped in the first quardrat are the best samples for variety improvement. G15 (Ejura) with seed length, seed width, seed thickness, pod weight, 50% maturity, 100 seed weight and number of mean branches; M34 (TVU90106) with 50% emergence, 50% maturity and number of main branches; M5 (IAR 167B) with plant height; G38 (GH2290) with 50% emergence, 50% maturity, number of pods per plant, number of pods per peduncle and number of main branches; can be used for crossing to improve some varieties from the two germplasms (Ghana and Mali).

5.6. Cluster analysis

The analysis suggests that when numerous characters are considered simultaneously, Principal Component Analysis (PCA) alone may not give an adequate character representation in terms of their relative importance and hence it needs to be complemented with other techniques such as Single Linkage Cluster Analysis (SLCA) which provides a clear and more informative display of the relative positions of the accessions. Cluster analysis decreases the number of individual variable units by classifying such variation into groups which are translated into a dendrogram using the coefficient of similarity (Sneath and Sokal, 1973; Tatineni *et al.*, 1996). It presents patterns of relationships between genotypes and hierarchical mutually exclusive grouping such that similar descriptions are mathematically gathered into same cluster (Hair *et al.*, 1995; Aremu, 2007).

5.6.1. Qualitative traits

The agglomerative hierarchical clustering dendrogram illustrates with data collected, shown the relationship among the accessions. At 0.97 level of similarity, almost all the 94 accessions were distinct from each other while at 0.95 levels, all the accessions were similar to each other. The cluster analysis separated the 94 accessions as different genotypes with Euclidien similarity distance ranging from 0.99 to 0.82. The dendrogram at similarity distance 0.82 identified three major clusters according to the morphological characters associated with them. The first two clusters contained only one accession from the two countries. Cluster (A) had Ejura which had distinct seed coat colour (white-black), number of seeds per pod and 100 seed weight. For cluster (B), IT97K-499-35 (Djiguya) was different from the others because it was the only accession which had white flower and white seed coat colour with rhomboid seed shape. Apart from the two accessions of clusters (A) and (B), the rest of the accessions fell under cluster (C).

5.6.2. Quantitative traits

From hierarchical cluster analysis of quantitative data, cutting of the dendrogram at 0.84 similarity level gave two major clusters designated (A) and (B). Cluster (A) consisted of Marfo-Tuya, PRL 73 (Yerewolo), Ejura, PBL 22 (Djemani) and PBL 112 (Dounafana). Cluster (B) consisted of 89 accessions. At 0.88 similarity coefficient of cluster (B) was divided into 2 sub-clusters, the first sub-cluster (I) had only Alan Cash which showed disimilarity in number of pods per peduncle (1) and the other sub-cluster (II) with 88 accessions.

5.7. Molecular characterization

5.7.1. Cluster analysis

The selected microsatellite significantly differentiated the cowpea accessions; they clustered the accessions differently from the morphological classification. The results from SSR markers showed lower level of similarity between and within the germplasms (Ghana and Mali). From 1 to 0.85 level of similarity, the accessions were different and their high level of similarity started around 0.4 genetic distance. The accessions from the two germplasms which were cluster based on their morphological data, belonged to different cluster or different sub-cluster using SSRs. The results from this study were different from those obtained by Asare *et al*. (2010) who reported lower level of genetic variability among Ghanaian cultivated genotypes. Oppong-Konadu *et al*. (2005) also examined high degree of homogenisity using Ghanaian collection on the basis of stored seed protein banding patterns by SDS-PAGE techniques.

5.7.2. Genetic characterisation based on SSR markers

Simple sequence repeat markers have been used to evaluate genetic diversity and phylogenetic relationships of cowpea genotypes (Badiane *et al.*, 2012; Afiukwa *et al.*, 2011 ; Asare *et al.*, 2010; Sawadogo *et al.*, 2010; Choumane *et al.*, 2000). All of the 20 SSR primer combinations used gave amplification products with 26% being polymorphic. Li *et al*. (2001) used 12 cowpea derived SSR primers to assess the genetic similarities and relationships and detected between 4 and 13 alleles among 48 wild cowpea lines with average of 7.5 alleles per primer. According to Diouf and Hilu (2005), the number of alleles ranged from 1 to 9 alleles per SSR primer combination in germplasm from Senegal. Sawadogo *et al*. (2010) obtained from 16 SSR primers a

range of alleles between 5 and 12 fragments with an average of 8.2 bands per primer combination among cowpea lines. Asare *et al*. (2010) used 25 informative SSR primer combination to analyze Ghanaian germplasm and yielded 1 to 6 alleles per primer pair with a mean of 3.8. The results from this study were in agreement with recent reports on the number of alleles detected using SSR markers in other legumes, such as, 14 to 67 alleles in chickpea (Upadhyaya *et al.*, 2008), 9 to 14 in alfalfa (Mengoni *et al.*, 2000), 1 to 9 in yardlong bean (Tantasawat *et al.*, 2010), 3 to 12 in pea (Sarikamis *et al.*, 2010), 11 to 26 in soybean (Rongwen *et al.*, 1995). Kuruma *et al*. (2008) in their study observed polymorphic information content (PIC) ranging between 0.09 to 0.87 with a mean of 0.34. For the same parameter, Fatokun *et al*. (2008) in their study found PIC ranging from 0.29 to 0.87 with a mean of 0.68 among the 48 cowpea lines. The mean PIC value (0.83) recorded in the current study compared favorably with results obtained from these two reports. Genetic diversity in Asare *et al*. (2010) study shown a range from 0.12 to 0.68 with an average of 0.44; 0.01 to 0.84 for variation heterozygosity with a mean of 0.19. On a basic of SSRs used in this study, the mean values from genetic diversity (0.84) and heterozygosity among the accessions were twice the values obtained by Asare *et al*. (2010). High levels of heterozygosity was observed ranging from 0.50 (SSR6356) to 0.97 (6261) whereas some of the markers never detected any heterozygosity.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Morphological and molecular characterisations were used in the current analysis of genetic diversity of cowpea. An overall relatively high level of similarity was observed among the accessions for most of the morphological traits (qualitative and quantitative) analyzed between and within germplasms from two countries (Ghana and Mali). This study also revealed that some morphological traits (qualitative and quantitative) discriminated more efficiently between the accessions than others. It would be very important to identify beforehand the right agro-morphological characters (those with high discriminative capacity) before undertaking genetic diversity studies based on morphological traits. Some groups of accessions (PBL 22 (Djemani), TVU7624, Parajani, Ejura, TVU90106, IAR 167B, GH2290) were different from the other accessions for some important traits such as growth habit, seed coat colour, seed length, seed width, seed thickness, pods weight, 50% maturity, 100 seed weight, mean number of branches, 50% emergence, plant height, number of pods per plant and number of pods per peduncle.

Simple sequence repearts data were able to separate accessions into different genotypes to show low level of similarity between and within both germplasms. Results from this study were different from those reported by Kuruma *et al*. (2008) who found low level of genetic diversity among cowpea accessions in Kenya for both morphological and molecular markers. For them, the resemblance of cowpea types among regions is an indication of both high levels of gene flow between regions and inadequate time for significant genetic differentiation along geographical lines.

Twenty of the study materials, from Ghana, were used by Asare et *al*. (2010) which supported some of the recent study results. For axample, GH2316 and GH2329, GH2339 and GH3708 were found to belong to the same subgroups on the basis of morphological assessment. Genotypes GH7226 and GH7178 have been confirmed to cluster based on morphological and molecular data.

In this study, results from molecular data indicate there is enough genetic diversity within and between Ghanaian and Malian accessions cowpea germplasms which could be exploited for improved genotypes for cowpea production. The current study also found that morphological descriptors, even though easy to use and readily available, may lead to mislabeling, particularly where certain varieties can be identified as the same on the basis of morphological data.

Therefore, it will be better, if morphological characterisation should be backed with DNA markers for accurancy and reliable genetic diversity assessment and germplasm management.

6.2. Recommendations

Because of importance of diversity study in cowpea germplasm, it will be important to replant the same collection during the major season and also evaluated these collections at the second location, Mali. The low level of polymorphism among the 14 SSRs might have affected the results. More polymorphic markers should be used for validation of the results.

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APPENDIX

APPENDIX 3.1: Source of different cowpeas used for germplasm comparison

Number	Accessions	Source	Original sources
1	**GH1608**	PGRRI*	GHANA
2	**TVU7705**	SRAC*	MALI
3	**GH7178**	PGRRI*	GHANA
4	**M'Barawa**	SRAC*	MALI
5	**GH3684**	PGRRI*	GHANA
6	**TN5-78 (Tieblen)**	SRAC[b]	Niger
7	**GH4769**	PGRRI*	GHANA
8	**TVU7617**	SRAC*	MALI
9	**IAR167B**	SRAC[b]	NIGARIA
10	**GH7243**	PGRRI*	GHANA
11	**PBL22 (Djemani)**	SRAC[a]	MALI
12	**GH5050**	PGRRI*	GHANA
13	**TVU90110**	SRAC*	MALI
14	**Nhyira**	CRI[a]	GHANA
15	**Milo**	CRI*	GHANA
16	**IT89KD-374 (Korobalen)**	SARC[b]	NIGERIA
17	**Bengpla**	CRI[b]	GHANA
18	**IT97K-499-35 (Djiguya)**	SRAC[b]	NIGERIA
19	**Soronko**	CRI[a]	GHANA
20	**GH2272**	PGRRI*	GHANA
21	**KPR1-96-32**	SRAC[a]	MALI

22	**Asetenapa**	CRI[a]	GHANA
23	**Parajani**	CRI*	GHANA
24	**KPR1-96-73**	SRAC[a]	MALI
25	**Tona**	CRI[a]	GHANA
26	**CZ1-94-23-2**	SRAC[a]	MALI
27	**Ejura**	CRI*	GHANA
28	**TVU7643**	SRAC*	MALI
29	**GH1619**	PGRRI*	GHANA
30	**TVU7714**	SRAC*	MALI
31	**GH5043**	PGRRI*	GHANA
32	**TVU7624**	SRAC*	MALI
33	**KPR1-96-54**	SRAC[a]	MALI
34	**GH3708**	PGRRI*	GHANA
35	**PRL73 (Yerewolo)**	SRAC[a]	MALI
36	**GH4529**	PGRRI*	GHANA
37	**TVU90035**	SRAC*	MALI
38	**GH4024**	PGRRI*	GHANA
39	**TVU7608**	SRAC*	MALI
40	**GH4526**	PGRRI*	GHANA
41	**TVU7686**	SRAC*	MALI
42	**GH7233**	PGRRI*	GHANA
43	**TVU7709**	SRAC*	MALI
44	**GH2339**	PGRRI*	GHANA
45	**IT82D-812**	SRAC[b]	IITA/NIGERIA

46	**TVU7677**	SRAC*	MALI
47	**Wantele**	CRI*	GHANA
48	**Suvita-2 (Grom-Grom)**	SRAC[b]	Burkina Faso
49	**Alan Cash**	CRI*	GHANA
50	**TVU7681**	SRAC*	MALI
51	**Malam Yaya**	CRI*	GHANA
52	**TVU7699**	SRAC*	MALI
53	**Marfo-Tuya**	CRI[a]	GHANA
54	**GH3683**	PGRRI*	GHANA
55	**Niban**	SRAC*	MALI
56	**GH7875**	PGRRI*	GHANA
57	**TVU7671**	SRAC*	MALI
58	**GH2281**	PGRRI*	GHANA
59	**TVU7657**	SRAC*	MALI
60	**GH2293**	PGRRI*	GHANA
61	**CZ1-94-23-1**	SRAC[a]	MALI
62	**TVU10362**	SRAC*	MALI
63	**GH2331**	PGRRI*	GHANA
64	**TVU7710**	SRAC*	MALI
65	**GH3667**	PGRRI*	GHANA
66	**Niebe Sucré (Sukaro-Sho)**	SRAC[b]	IITA/NIGERIA
67	**Asontem**	CRI[a]	GHANA
68	**TVU90106**	SRAC*	MALI
69	**Sanzisabinli**	CRI*	GHANA

70	**GH2335**	PGRC*	GHANA
71	**TVU7687**	SRAC*	MALI
72	**GH2329**	PGRRI*	GHANA
73	**TVU7708**	SRAC*	MALI
74	**GH2290**	PGRRI*	GHANA
75	**CIPEA80025**	SRAC*	MALI
76	**TVU90107**	SRAC*	MALI
77	**TVU7696**	SRAC*	MALI
78	**TVU90037**	SRAC*	MALI
79	**CB-5**	SRAC[b]	BOSTWANA
80	**CIPEA82672**	SRAC*	MALI
81	**CZ11-94-5C**	SRAC[a]	MALI
82	**Apagbaala**	CRI[a]	GHANA
83	**TVU7616**	SRAC*	MALI
84	**GH2316**	PGRRI*	GHANA
85	**GH2214**	PGRRI*	GHANA
86	**GH2271**	PGRRI*	GHANA
87	**GH7226**	PGRRI*	GHANA
88	**GH4542**	PGRRI*	GHANA
89	**TVU10377**	SRAC*	MALI
90	**GH7185**	PGRC*	GHANA
91	**PBL 112 (Dounafana)**	SRAC[a]	MALI
92	**GH5344**	PGRRI*	GHANA
93	**TVU9344**	SRAC*	MALI
94	**GH5040**	PGRRI*	GHANA

NB: *: landrace; a: improved variety; b: introduced variety.

APPENDIX 3.2: Different parameters assessed for qualitative traits

TRAITS	DIFFERENT PATHS OF SCORING
1- Growth habit	1 =Acute erect; 2 = Erect; 3 = Semi-erect; 4 = Intermediate; 5 = Semi–prostrate; 6= Prostrate; 7= Climbing.
2- Terminal leaflet Shape	1 = Globose; 2 = Sub-globose; 3 = Sub-hastate; 4 = Hastate.
3- Raceme position	1 = Mostly above canopy; 2 = In upper canopy; 3 = Throughout canopy.
4- Pod attachment to peduncle	3 = Pendant; 5 = 30 – 90° down from erect; 7 = Erect.
5- Pod curvature	0 = Straight; 3 = Slightly curved; 5 = Curved; 7 = Coiled.
6- Immature pod pigmentation	0 = None; 1 = Pigmented tip; 2 = Pigmented sutures; 3 = Pigmented valves, green sutures; 4 = Splashes of pigment; 5 = Uniformly pigmented.
7- Seed shape	1 = Kidney; 2 = Ovoid; 3 = Crowder; 4 = Globose; 5 = Rhomboid.
8- Testa texture	1 = Smooth; 3 = Smooth to rough; 5 = Rough; 7 = Rough to wrinkled; 9 = Wrinkled.
9- Leaf colour	3 = Pale green; 5 = Intermediate green; 7 = Dark green.
10- Flower colour	1= White; 2= Violet; 3= Mauve-pink; 4= Other colour.
11- Pod colour	1= Pale tan or straw; 2= Dark tan; 3= Dark brown; 4= Black or dark purple; 5= Other colour.
12- Seed coat colour	1 = White; 2 = Cream; 3 = Brown; 4 = Red; 5 = Purple; 6 = Black; 99 = Other colour.

APPENDIX 3.3: Different parameters assessed for quantitative traits

TRAITS	STAGE/MANNER OF SCORING
1-Days to 50% emergence	From sowing to stage when 50% of the seeds are germinated.
2- Days to first flowering	From sowing to stage when plants have begun to flower.
3- Days 50% flowering	From sowing to stage when 50% of plants have begun to flower.
4- Days to first mature pods	From sowing to stage when plants have begun to ripe.
5- Days 50% mature pods	From sowing to stage when 50% of plants have ripen.
6- Seed weight (g)	Weight of 100 seeds randomly selected after husking pods.
7- Terminal leaflet length (mm)	Mean length of 10 terminal leaflets from 10 randomly selected plants.
8- Terminal leaflet width (mm)	Mean width of 10 terminal leaflets measured on the broadest part of 10 randomly selected plants.
9- Number of main branches	Mean of 10 randomly selected plants.
10- Number of pods per peduncle	Mean of 10 randomly selected peduncles.
11- Peduncle length (mm)	Recorded when peduncles have reached full

	length.
12- Number of pods per plant	Mean number of mature pods from 10 randomly selected plants.
13- Pod length (cm)	Mean of the 10 longest mature pods from 10 randomly selected plants.
14- Plant height (cm)	Recorded in the 8th week after sowing.
15- Pod weight (g)	Mean weight of the 10 longest mature pods from 10 randomly selected plants.
16- Number of seeds per pod	Mean number of seeds of the 10 longest mature pods from 10 randomly selected plants.
17- Number of locules per pod	Recorded after husking pods
18- Seed length (mm)	Mean of 10 mature seeds excluding those from the extremities of pods
19- Seed width (mm)	Mean width from hilum to keel of the 10 seeds measured for length in 6.3.1.
20- Seed thickness (mm)	Mean thickness of the 10 seeds measured for length in 6.3.1; measured perpendicular to length and width.

APPENDIX 3.4: Protocol of DNA extraction

1. Weigh 200 g of tissue into 2 ml eppendorf tubes.
2. Grind to fine powder with liquid nitrogen.
3. Add 400 µl Buffer AP1, 4 µl RNase A (100 mg/ml) and vortex vigorously.
4. Incubate at 65ºC for 10 mins; mix 2- 3 X by inversion during incubation.
5. Add 130 µl Buffer AP2 to lysate, mix and incubate on ice for 5 mins.
6. Centrifuge at 14,000 rpm for mins.
7. Pipette the lysate into QIA shredder mini spin column.
8. Centrifuge at 14,000 rpm for 2 mins.
9. Transfer flow-through fraction into a new eppendorf tube without disturbing the cell debris pellet.
10. Add 1.5 volumes of Buffer AP 3/E to lysate and mix by pipetting immediately.
11. Pipette 650 µl of mixture including any precipitate that may have formed into the DNeasy mini spin column.
12. Centrifuge at 8,000 rpm for 1 min.
13. Discard the flow-through, reuse the collection tube, and repeat steps 11-13 with remaining sample.
14. Discard flow-through and collection tube.
15. Place the DNeasy mini spin column into a new 2 ml collection tube, add 500 µl Buffer AW.
16. Centrifuge at 8,000 rpm for 1 min, discard flow-through and reuse collection tube.
17. Add 500 µl Buffer AW to the DNeasy mini spin column.
18. Centrifuge at 14,000 rpm for 2 mins.
19. Empty spin at 5 mins.

20. Transfer the DNeasy mini spin column to a 1.5/2 ml tube; pipette 50 µl Buffer AE directly into the DNeasy membrane.
21. Incubate at room temperature for 10 mins.
22. Centrifuge at 8,000 rpm for 1 min to elute.
23. Repeat by adding 50 µl Buffer AE to DNeasy membrane.

APPENDIX 3.5: Steps for Silver Staining

STEPS	PROCEDURE	TIME
1. Fix	Fix solution: 10% Acetic acid	20-30mins
2. Wash	Water distilled	5-10secs.
3. Wash	1.5% Nitric acid	5mins
4. Stain	Silver staining	30mins
5. Wash	Water distilled	5-10secs
6. Developer	Developer solution	5-10mins (visual control
7. Stop	Acetic acid	10mins
8. Wash	Water distilled	5-10secs
9. Store	Tray containing distilled water	

APPENDIX 4.1: Mean scores of twelve qualitative traits of cowpea

ACCESSIONS	FC	LC	PDR	PC	PAP	RP	TLS	IPP	SS	TT	GH	SCC
GH1608	2	3	1	3	5	2	1	1	2	1	4	4
TVU7705	2	5	2	3	5	2	2	1	4	1	5	99
GH7178	2	5	1	3	5	1	1	1	4	1	5	4
M'Barawa	2	5	1	3	5	2	1	1	5	3	4	1
GH3684	2	5	3	3	5	3	1	2	5	1	5	4
TN5-78 (Tieblen)	4	5	1	3	5	1	2	0	5	5	6	3
GH4769	2	5	1	3	5	1	4	1	3	1	4	4
TVU7617	2	5	1	3	5	1	2	0	5	1	5	99
IAR 167B	2	5	1	3	5	3	2	2	4	1	5	99
GH7243	2	7	2	3	5	1	1	1	4	1	5	99
PBL22 (Djemani)	2	7	1	3	5	1	2	0	2	5	6	1
GH5050	2	3	1	3	5	1	1	1	3	1	4	4
TVU90110	2	5	3	3	7	2	2	0	5	1	5	6
Nhyira	2	5	1	3	7	1	1	3	4	1	4	2

Milo	2	5	1	3	5	1	1	1	4	1	4	3
IT89KD-374 (Korobalen)	2	5	1	3	7	2	2	2;3	1	5	5	1
Bengpla	2	3	1	3	7	1	1	1	2	1	4	2
IT97K-499-35 (Djiguya)	1	7	1	3	5	2	2	0	5	3	4	1
Soronko	2	5	1	3	5	1	1	0	3	1	4	3
GH2272	2	5	3	3	5	1	2	2;3	1	1	5	99
KPR1-96-32	1	5	1	3	7	1	2	1	1	5	6	1
Asetenapa	1	5	1	3	7	2	1	1	4	1	4	1
Parajani	4	5	1	3	5	1	3	0	2	5	4	1
KPR1-96-73	1	5	1	3	7	1	2	1	1	5	6	1
Tona	2	7	1	3	7	1	1	1	4	1	2	3
CZ1-94-23-2	1	5	2	3	5	3	2	2;3	4	3	6	3
Ejura	4	5	1	3	7	3	2	0	1	5	4	99
TVU7643	2	5	1	3	5	1	2	0	4	1	5	99
GH1619	2	5	1	3	3	1	1	1	3	1	4	4
TVU7714	1	5	2	3	3	1	2	1	4	1	5	99

GH5043	2	5	1	3	7	1	4	1	3	1	4	4
TVU7624	2	7	2	3	7	2	2	1	5	5	5	0
KPR1-96-54	1	7	1	3	7	1	2	1	1	5	6	1
GH3708	2	5	2	3	5	1	2	2;3	4	1	5	99
PRL 73 (Yerewolo)	2	7	1	3	5	1	2	0	5	5	6	3
GH4529	2	7	1	3	5	1	1	1	5	1	6	99
TVU90035	2	5	1	3	5	1	1	1	5	1	5	3
GH4024	2	5	1	3	5	1	1	1	3	1	5	3
TVU7608	2	5	1	3	5	1	2	1	3	1	5	3
GH4526	2	5	1	3	5	1	4	1	3	1	4	4
TVU7686	2	5	2	3	5	1	2	1	5	1	5	99
GH7233	2	7	1	3	7	1	2	1	4	1	5	99
TVU7709	2	5	2	3	5	1	2	0	5	1	5	3
GH2339	2	5	1	3	5	1	1	1	4	1	5	3
IT82D-812	2	5	2	3	5	1	2	1	4	1	5	99
TVU7677	2	5	3	3	5	2	2	1	4	1	4	3
Wantele	1	5	1	3	5	2	2	1	4	1	4	1

Suvita-2 (Grom-Grom)	4	5	2	3	5	1	2	1	5	5	6	3
Alan Cash	1	7	1	3	7	2	3	1	1	5	4	1
TVU7681	2	5	2	3	5	1	2	1	4	1	5	2
Malam Yaya	1	7	2	3	5	1	3	2;3	1	3	4	1
TVU7699	2	5	1	3	5	1	2	1	2	1	5	99
Marfo-Tuya	1	5	1	3	5	2	1	0	4	1	4	2
GH3683	2	5	1	3	5	3	2	1	2	1	5	3
Niban	1	5	2	3	5	2	2	0	3	1	5	3
GH7875	2	5	1	3	5	3	1	1	3	1	5	4
TVU7671	2	5	1	3	5	1	1	0	5	1	5	99
GH2281	2	5	1	3	5	3	1	1	5	1	4	99
TVU7657	2	5	1	3	5	1	1	1	4	1	5	3
GH2293	2	5	3	3	5	3	2	2;3	4	1	5	4
CZ1-94-23-1	1	5	2	3	5	3	2	0	2	3	5	1
TVU10362	2	5	1	3	5	3	2	1	4	1	5	3
GH2331	2	5	2	3	5	1	2	4	4	1	5	4
TVU7710	2	5	1	3	5	2	2	2;3	5	1	5	6

GH3667	2	5	1	3	5	1	1	1	4	1	5	99
Niebe Sucré (Sukaro-Shô)	1	5	1	3	5	1	2	1	1	3	5	99
Asontem	1	5	1	3	5	1	4	1	3	1	5	4
TVU90106	2	5	3	3	5	2	2	0	1	1	5	3
Sanzisabinli	2	5	3	3	5	1	2	3	5	1	5	99
GH2335	2	3	1	3	5	1	1	1	4	1	5	3
TVU7687	1	5	2	3	5	1	2	0	4	1	5	4
GH2329	2	3	1	3	5	3	1	1	5	1	5	99
TVU7708	2	3	2	3	5	1	2	1	5	1	5	3
GH2290	2	7	3	3	5	1	2	3	5	1	5	99
CIPEA80025	2	5	2	3	5	1	2	1	4	1	5	99
TVU90107	2	5	1	3	5	1	2	1	4	1	5	3
TVU7696	2	5	2	3	5	1	2	1	4	1	5	99
TVU90037	2	5	2	3	5	1	2	1	2	1	5	99
CB-5	2	5	2	3	5	1	2	1	4	1	5	99
CIPEA82672	2	5	2	3	5	1	2	1	4	1	5	99
CZ11-94-5C	4	5	1	3	5	1	2	1	1	5	5	3

Apagbaala	2	3	1	3		1	1	1	3	3	4	1
TVU7616	2	5	2	3	5	1	2	1	5	1	5	99
GH2316	2	5	1	3	5	1	4	1	3	1	5	4
GH2214	2	3	1	3	5	1	1	1	3	1	5	99
GH2271	2	3	1	3	5	1	1	1	2	1	5	4
GH7226	4	5	2	3	5	1	2	3	1	5	5	1
GH4542	2	5	1	3	5	1	2	1	5	1	5	4
TVU10377	2	5	2	3	5	1	2	1	3	1	5	99
GH7185	4	7	1	3	5	1	2	2	3	1	6	1
PBL 112 (Dounafana)	4	5	1	3	5	1	2	0	2	5	6	1
GH5344	2	5	1	3	5	1	1	1	5	1	5	99
TVU9344	2	5	2	3	5	1	2	1	4	1	5	99
GH5040	2	5	1	3	5	1	1	1	3	1	5	4

NB: FC: Flower colour; **GH:** Growth habit; **IPP:** Immature pod pigmentation; **LC:** Leave colour; **PAP:** Pod attachment to peduncle; **PC:** Pod curvature; **PCR:** Pod colour; **RP:** Raceme position; **SCC:** Seed coat colour; **SS:** Seed shape; **TLS:** Terminal leaflet shape; **TT:** Testa texture

APPENDIX 4.2: Principal components for twelve selected qualitative traits of cowpea

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Latent roots	1.862	1.627	1.474	1.203	0.986	0.858	0.803	0.615	0.584
Percentage variation	16.93	14.79	13.40	10.94	8.96	7.80	7.30	5.59	5.30
Cumulative percent variation	16.93	31.72	45.12	56.06	65.02	72.82	80.12	85.71	91.01
Latent vectors (loadings)									
Flower colour	0.080	-0.153	**0.395**	**0.553**	-0.044	0.174	-0.499	-0.032	0.153
Growth habit	0.220	-0.067	**0.443**	-0.252	-0.557	0.196	0.106	**0.510**	-0.004
Immature pod pigmentation	-0.221	**0.528**	0.171	0.083	-0.213	-0.411	-0.068	0.235	-0.367
Leaf colour	0.247	0.114	**0.334**	-0.434	**0.522**	-0.015	-0.153	-0.054	-0.427
Pod attachment peduncle	0.057	**0.541**	-0.077	-0.333	0.014	**0.415**	0.002	-0.030	**0.528**
Pod curvature	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pod colour	-0.325	0.052	**0.481**	-0.157	-0.147	-0.409	0.130	-0.491	**0.35**6
Raceme position	-0.345	**0.483**	-0.057	0.227	-0.027	**0.327**	-0.253	-0.041	-0.185

Seed coat colour	-0.412	-0.175	0.241	0.033	-0.005	**0.532**	**0.469**	-0.204	-0.336
Seed shape	-0.392	-0.229	0.234	-0.283	0.276	0.094	-0.444	0.227	0.162
Terminal leaflet shape	0.142	0.211	**0.327**	**0.405**	**0.500**	-0.046	**0.451**	**0.307**	0.243
Testa texture	**0.512**	0.150	0.217	0.055	-0.142	0.145	-0.098	-0.499	-0.146

APPENDIX 4.3: Cluster distribution of 94 cowpea accessions based on twelve qualitative traits

Cluster	Sub-cluster	Similarity of coefficient	Number of accessions
A		**0.82**	
	I	**0.85**	1 (Ejura)
B		**0.82**	
	I	**0.85**	1 (IT97K-499-35 (Djiguya))
C		**0.82**	
	I	**0.85**	2(TVU90106, KPR1-96-32)
	II	**0.85**	
	a	0.92	2(GH2293, CZ1-94-23-2)
	b	0.92	5(GH2290, Sanzisabinli, TVU7677, TVU90110, GH3684)
	c	0.92	2(Soronko, Parajani)
	d	0.92	4(GH7185, GH7226, PBL 112 (Dounafana), CZ11-94-5C)
	e	0.92	2(CZ1-94-23-1, Malam Yaya)
	f	0.92	4(Alan Cash, KPR1-96-73, KPR1-96-54, GH2272)
	g	0.92	1(PBL 22 (Djemani))
	h	0.92	2(TVU90107, IT82D-812)
	i	0.92	1(TVU7624)
	j	0.92	2(PRL 73 (Yerewolo), IT89KD-374 (Korobalen))

	k	0.92	2(TN5-78 (Tieblen), Suvita-2 (Grom-Grom)
	l	0.92	1(Tona)
	m	0.92	6(TVU10362, GH7875, GH3683, GH2329, GH2281, IAR 167B)
	n	0.92	7(Niebe Sucré (Sukaro-Shô), TVU7699, GH2316, Asontem, GH5043, GH4526, GH4769)
	o	0.92	42(TVU7687, Niban, Marfo-Tuya, TVU7643, Wantele, Asetenapa, M'Barawa, GH7233, GH4529, TVU7617, Nhyria, GH3667, TVU7710, GH4542, GH5344, TVU7671, GH1619, GH5040, TVU7608, GH4024, Milo, TVU90035, TVU7657, GH2339, GH7178, TVU7708, TVU7681, TVU7709, GH2331, TVU7714, GH7243, TVU10377, TVU90037, TVU7616, TVU7686, CIPEA82672, TVU7696, TVU9344, CB-5, CIPEA80025, GH3708, TVU7705)
	P	0.92	7(Apagbaala, Bengpla, GH2335, GH2271, GH2214, GH5050, GH1608)

APPENDIX 4.4: Identification of genotypes based on their code for qualitative traits

```
** Levels   100.0  90.0  80.0
G1           1  ..
G6          12  ..)
G41         85  ..)
G42         86  ..)
G36         70  ..)
G9          17  ..)
G39         82  ..)..
M1           2  ..   )
G18         34  ..)  )
M37         75  ..)  )
M41         79  ..)  )
M47         93  ..)  )
M39         77  ..)  )
M42         80  ..)  )
M20         41  ..)  )
M44         83  ..)  )
M40         78  ..)  )
M45         89  ..)  )
G5          10  ..)  )
M14         30  ..)  )
G32         63  ..)  )
M21         43  ..)  )
M25         50  ..)  )
M36         73  ..)..)
G2           3  ..   )
G23         44  ..)  )
M29         59  ..)  )
M18         37  ..)  )
G8          15  ..)  )
G20         38  ..)  )
M19         39  ..)  )
G47         94  ..)  )
G16         29  ..)  )
M28         57  ..)  )
G46         92  ..)  )
G44         88  ..)  )
M32         64  ..)  )
G33         65  ..)  )
G7          14  ..)  )
M4           8  ..)  )
G19         36  ..)  )
G22         42  ..)..)
M2           4  ..   )
G12         22  ..)  )
G24         47  ..)..)
M13         28  ..   )
G27         53  ..)  )
M27         55  ..)  )
M35         71  ..)..)
G4           7  ..   )
G21         40  ..)  )
G17         31  ..)  )
G34         67  ..)  )
G40         84  ..)..)
M26         52  ..   )
M33         66  ..)..)
```

```
M5           9  ..   )
G30         58  ..)  )
G37         72  ..)  )
G28         54  ..)  )
G29         56  ..)  )
M31         62  ..)..)..
G14         25  ........)
M3           6  ..      )
M24         48  ..)..   )
M9          18  ..   )  )
M17         35  ..)..)  )
M15         32  .....)..)
M22         45  ..      )
M38         76  ..).....)
M6          11  .....   )
G11         21  ..   )  )
M11         24  ..)  )  )
M16         33  ..)  )  )
G25         49  ..)..)  )
G26         51  ..   )  )
M30         61  ..)..)..)
M43         81  ..      )
M46         91  ..)     )
G43         87  ..)..   )
G45         90  .....)..)
G10         19  .....   )
G13         23  .....)..)
G3           5  ..      )
M7          13  ..)     )
M23         46  ..)..   )
G35         69  ..   )  )
G38         74  ..)..)  )
M12         26  ..   )  )
G31         60  ..)..)..)..
M10         20  .....     )
M34         68  .....).....)
M8          16  ..........)
G15         27  ..........).........
```

APPENDIX 4.5: Mean scores of twenty quantitative traits of cowpea

ACCESSIONS	D 50 % E	D 1st F	D 50 % F	D 1st PM	D 50 % PM	TLL/ mmx 10^1	TLW/ mmx 10^1	PH/ Cm	NP/ P	NP/ Pt	NMB	PDL/ mmx 10^1	PL/ cm	NS/ P	NL/ P	PW/ G	SL/ mm x10^1	SW/ mm x10^1	ST/ mm x10^1	SWt/ g
GH1608	3	37	48	59	68	11	8	24	2	4	3	30	17	15	16	16	0.7	0.5	0.4	8
TVU7705	5	24	35	46	55	7	4	12	2	4	3	19	16	15	17	14	0.6	0.4	0.3	8
GH7178	4	34	47	58	65	10	7	23	2	7	2	21	14	14	15	13	0.8	0.5	0.4	8
M'Barawa	4	34	43	55	61	9	5	14	2	12	4	36	15	11	11	14	0.8	0.6	0.5	14
GH3684	4	28	38	54	63	10	7	26	2	9	3	21	15	13	14	19	0.8	0.6	0.4	12
TN5-78 (Tieblen)	4	33	44	61	70	10	6	15	3	12	3	15	16	12	14	15	0.9	0.7	0.5	13
GH4769	4	27	37	52	57	16	3	22	3	6	3	23	18	16	17	26	0.8	0.6	0.5	14
TVU7617	6	49	53	70	77	12	6	21	2	15	3	15	11	12	14	10	0.7	0.5	0.4	6
IAR 167B	6	43	49	66	78	10	6	22	2	8	3	10	13	11	12	10	0.7	0.5	0.4	7

GH7243	5	30	37	52	57	9	7	14	2	11	3	18	14	12	13	12	0.7	0.5	0.4	8
PBL 22 (Djemani)	5	45	52	64	76	10	6	24	2	5	4	20	11	9	11	10	0.9	0.7	0.5	8
GH5050	4	31	37	49	55	11	9	32	3	17	3	27	17	16	16	15	0.8	0.6	0.4	11
TVU90110	5	33	37	59	68	8	5	17	2	10	3	21	12	11	11	10	0.7	0.6	0.4	8
Nhyira	3	36	42	56	61	10	6	22	2	21	4	26	14	13	14	19	0.7	0.6	0.4	11
Milo	5	28	31	44	50	11	7	19	2	10	3	21	14	8	12	21	0.8	0.6	0.4	14
IT89KD-374 (Korobalen)	6	49	54	64	70	9	5	15	2	15	3	17	12	11	11	17	0.8	0.6	0.4	14
Bengpla	6	48	54	66	72	10	8	24	2	13	3	32	15	13	13	19	0.6	0.5	0.4	12
IT97K-499-35 (Djiguya)	7	32	37	51	56	9	7	15	2	18	2	24	15	11	11	14	0.8	0.6	0.5	14
Soronko	6	38	43	57	62	9	6	25	2	22	4	36	16	13	14	24	0.8	0.6	0.4	11
GH2272	4	38	46	59	64	10	7	10	2	14	4	23	19	16	16	20	0.8	0.6	0.4	11
KPR1-96-32	4	34	43	55	62	8	6	16	2	9	3	18	14	13	14	19	0.9	0.7	0.5	13
Asetenapa	4	28	36	50	56	10	7	25	3	18	4	35	14	12	12	17	0.9	0.6	0.5	13

Parajani	5	36	43	60	65	9	6	10	2	13	4	21	12	12	13	10	0.6	0.4	0.3	8
KPR1-96-73	6	36	43	60	65	9	6	17	3	9	4	21	13	9	12	18	0.9	0.7	0.5	13
Tona	5	29	36	50	55	13	8	20	2	10	3	28	15	13	13	22	0.8	0.6	0.4	13
CZ1-94-23-2	5	33	38	55	61	6	5	15	2	9	2	9	13	11	11	17	0.7	0.6	0.4	14
Ejura	5	49	55	71	79	9	6	12	2	6	3	16	11	7	10	18	1	0.7	0.5	19
TVU7643	5	48	54	67	76	9	5	15	2	12	3	19	13	12	12	11	0.7	0.6	0.4	7
GH1619	5	30	40	56	61	12	9	18	2	10	3	22	15	17	17	23	0.7	0.6	0.5	12
TVU7714	5	26	33	48	53	8	6	13	2	3	3	17	17	14	16	20	0.7	0.6	0.4	12
GH5043	7	33	41	51	58	15	3	11	2	4	3	21	16	14	14	22	0.8	0.6	0.4	13
TVU7624	7	35	41	57	65	7	5	14	2	11	3	14	13	10	10	10	0.7	0.6	0.5	10
KPR1-96-54	5	36	42	61	68	10	7	14	2	10	3	14	14	10	11	15	0.9	0.7	0.4	12
GH3708	6	34	39	54	59	9	6	12	2	10	3	22	14	14	16	16	0.8	0.6	0.4	8
PRL 73 (Yerewolo)	7	55	60	76	84	9	7	16	3	9	3	22	13	11	12	16	0.9	0.7	0.5	13
GH4529	4	29	36	48	54	11	8	22	2	10	3	23	16	16	18	20	0.8	0.6	0.5	12

TVU90035	6	42	50	63	73	12	7	20	2	12	4	28	13	12	13	14	0.7	0.5	0.4	10
GH4024	7	25	35	48	56	10	6	16	2	8	4	19	13	13	13	14	0.8	0.6	0.4	10
TVU7608	4	33	38	57	65	10	5	20	3	7	3	15	11	12	12	10	0.6	0.5	0.4	8
GH4526	3	30	38	52	59	15	3	19	2	6	4	17	17	12	15	25	0.7	0.6	0.5	13
TVU7686	6	29	36	51	56	8	5	15	2	6	2	20	14	10	13	10	0.7	0.5	0.4	8
GH7233	5	30	36	51	56	8	6	13	2	8	3	13	14	12	13	15	0.8	0.6	0.4	12
TVU7709	4	39	44	61	68	10	6	17	2	16	3	13	10	12	12	10	0.7	0.6	0.4	9
GH2339	3	39	44	58	63	13	8	21	2	12	3	23	17	17	18	15	0.8	0.5	0.4	9
IT82D-812	6	35	39	52	58	9	7	19	2	8	3	15	14	15	16	12	0.6	0.4	0.3	7
TVU7677	5	35	42	57	62	7	4	13	2	7	3	19	16	15	17	17	0.6	0.5	0.4	12
Wantele	4	26	35	51	56	9	6	19	2	13	3	24	13	10	14	18	0.8	0.6	0.5	14
Suvita-2 (Grom-Grom)	3	30	36	54	61	9	5	16	2	13	4	15	12	8	11	13	0.9	0.7	0.5	14
Alan Cash	6	31	36	52	57	12	7	21	1	6	2	29	14	8	10	12	0.9	0.6	0.4	12
TVU7681	5	32	38	54	59	10	5	20	2	9	3	16	11	11	12	10	0.7	0.6	0.4	9
Malam Yaya	4	29	36	51	56	13	7	20	2	12	4	30	14	13	13	13	0.9	0.6	0.5	13

TVU7699	6	31	37	51	57	10	7	16	2	10	3	25	17	15	16	17	0.7	0.5	0.4	8
Marfo-Tuya	7	45	52	68	73	11	9	22	3	11	3	31	12	9	10	14	0.8	0.6	0.5	12
GH3683	6	31	42	54	60	10	8	27	2	14	4	29	19	15	17	25	0.8	0.6	0.5	13
Niban	4	34	41	70	76	12	6	19	2	16	4	28	12	8	11	14	0.8	0.6	0.4	8
GH7875	4	30	42	55	62	12	10	21	2	18	5	29	16	13	17	24	0.7	0.6	0.5	12
TVU7671	6	40	52	65	72	10	7	21	2	14	4	26	12	11	11	11	0.7	0.6	0.4	8
GH2281	5	36	43	57	63	12	9	19	2	12	3	26	17	15	16	21	0.8	0.6	0.5	12
TVU7657	5	43	50	63	71	11	6	19	3	13	4	15	13	14	14	11	0.7	0.6	0.4	9
GH2293	5	29	36	53	59	7	5	8	2	14	4	17	14	13	13	13	0.8	0.6	0.4	9
CZ1-94-23-1	4	36	46	56	66	11	6	18	2	17	3	17	14	8	9	17	1	0.7	0.5	18
TVU10362	6	39	43	60	70	10	7	16	2	9	4	14	11	11	13	12	0.7	0.6	0.5	12
GH2331	6	35	38	52	58	10	7	14	2	12	2	23	15	13	13	18	0.9	0.5	0.4	8
TVU7710	5	35	39	56	61	9	6	19	3	16	3	15	13	13	14	12	0.8	0.6	0.5	7
GH3667	7	24	34	49	54	12	9	27	2	17	4	27	15	14	16	26	0.8	0.6	0.4	13
Niebe Sucré (Skaro-Shô)	5	36	42	59	65	13	9	22	2	10	5	18	14	11	13	15	0.8	0.6	0.5	15

Asontem	4	36	41	57	62	16	4	20	2	20	3	23	17	16	16	24	0.8	0.6	0.4	12
TVU90106	3	41	44	60	67	8	5	14	2	13	3	6	13	13	13	12	0.7	0.5	0.4	7
Sanzisabinli	3	30	36	52	58	7	4	15	2	14	4	24	14	11	12	10	0.6	0.5	0.3	9
GH2335	5	36	42	56	62	11	7	21	2	11	4	25	20	16	16	20	0.9	0.6	0.4	11
TVU7687	6	47	42	54	64	8	5	14	2	13	4	24	14	17	18	10	0.6	0.5	0.3	6
GH2329	6	25	34	49	53	12	9	30	2	12	5	26	17	14	14	26	0.7	0.5	0.4	11
TVU7708	4	40	45	62	70	9	5	19	2	6	3	17	11	12	14	11	0.7	0.5	0.4	9
GH2290	4	37	42	59	64	6	4	12	2	12	3	15	13	11	12	10	0.8	0.6	0.4	8
CIPEA80025	3	27	39	56	61	8	6	12	2	7	3	19	16	17	17	18	0.7	0.5	0.4	10
TVU90107	6	36	41	59	67	8	6	19	2	6	3	16	12	12	12	12	0.7	0.6	0.4	9
TVU7696	7	36	41	56	60	8	5	12	2	6	3	18	13	16	16	13	0.6	0.4	0.3	7
TVU90037	5	35	42	58	61	10	7	12	2	7	2	17	18	16	17	22	0.7	0.6	0.4	12
CB-5	4	37	43	59	67	8	5	12	2	5	2	15	15	14	16	14	0.7	0.5	0.4	9
CIPEA82672	4	28	40	53	58	8	5	15	2	7	2	17	13	14	15	9	0.6	0.5	0.4	7
CZ11-94-5C	4	38	42	56	63	9	5	12	2	19	2	17	12	10	11	12	0.8	0.6	0.4	10

Apagbaala	3	49	54	72	78	10	5	17	2	16	3	22	13	11	11	13	0.7	0.5	0.4	10
TVU7616	3	38	47	60	68	10	5	17	2	9	4	17	15	12	12	8	0.7	0.5	0.4	6
GH2316	5	34	41	55	59	14	3	19	2	8	3	22	16	15	15	18	0.8	0.6	0.5	13
GH2214	6	27	41	53	60	11	9	25	3	10	3	23	15	12	14	13	0.7	0.5	0.3	13
GH2271	6	35	38	52	57	10	7	20	2	12	3	27	14	14	15	11	0.7	0.5	0.4	7
GH7226	5	38	46	60	70	10	7	18	2	18	5	13	14	10	12	14	0.9	0.6	0.4	13
GH4542	4	34	42	55	63	10	7	23	2	11	3	18	14	14	15	15	0.8	0.6	0.4	10
TVU10377	4	27	36	53	59	8	5	13	2	8	4	21	13	13	15	9	0.6	0.5	0.4	7
GH7185	4	36	39	56	62	10	6	14	2	20	4	17	19	12	12	10	0.8	0.6	0.4	8
PBL 112 (Dounafana)	4	45	56	72	80	9	6	21	2	17	4	18	11	9	9	14	0.9	0.7	0.5	14
GH5344	4	35	39	53	58	11	8	27	2	15	4	22	16	15	15	17	0.8	0.6	0.5	11
TVU9344	3	36	45	60	65	10	7	15	2	9	3	17	16	16	17	19	0.7	0.6	0.4	11
GH5040	3	37	46	60	67	12	10	25	2	11	4	25	16	15	17	24	0.8	0.6	0.5	12

Min.	3	24	31	44	50	6	3	8	1	3	2	6	10	7	9	8	0.6	0.4	0.3	6
Mean	4.84	35.07	42.00	56.89	63.41	10.01	6.28	18.04	2.11	11.21	3.29	20.79	14.30	12.63	13.68	15.51	0.76	0.57	0.42	10.64
Max.	7	55	60	70	84	16	10	32	3	22	5	36	20	17	18	26	1	0.7	0.5	19
SDV	1.17	6.45	5.92	6.20	7.02	2.00	1.55	4.79	0.34	4.27	0.71	5.85	2.11	2.40	2.28	4.72	0.10	0.07	0.06	2.70

NB: D1st F: Days to 1st flower; **D1st PM:** Days to 1st pod matured; **D50%E:** Days to 50% emergence; **D50%F:** Days to 50% flower; **D50%PM:** Days to 50% pod matured; **NL/P:** Number of locule per pod; **NMB:** Number of main branches; **NP/Pt:** Number of pod per plant; **NPP/P:** Number of pod per peduncle; **NS/P:** Number of seed per pod; **PDL:** Peduncle length; **PH/G:** Plant height per grams; **PL:** Pod length; **PW/G:** Pod weight per grams; **SL:** Seed length; **ST:** Seed thickness; **SW:** Seed width; **SWt/g:** Seed weight per grams; **TLL:** Terminal leaflet length; **TLW:** Terminal leaflet width ; **Min.:** minimal; **Max.:** maximal, **SDV :** standard of deviation

APPENDIX 4.6: Principal components for twenty selected quantitative traits of cowpea

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Latent roots	4.890	3.990	2.410	1.553	1.208	1.037	0.833	0.744	0.699
Percentage variation	24.45	19.95	12.05	7.77	6.04	5.18	4.16	3.72	3.50
Cumulative percent variation	24.45	44.40	56.45	64.22	70.26	75.44	79.60	83.32	86.82
Latent vectors (loadings)									
Day 1st flowering	**0.360**	0.014	0.296	0.129	0.049	-0.132	-0.122	-0.025	-0.103
Day 1st pod maturty	**0.388**	0.047	0.266	0.133	-0.019	-0.033	0.017	0.008	0.017
Day 50% emergence	0.037	-0.023	-0.006	-0.197	**0.741**	-0.146	-0.367	0.305	0.060
Day 50% flowering	**0.350**	0.075	**0.330**	0.174	0.049	-0.085	-0.027	-0.017	-0.012
Day 50% maturity	**0.398**	0.037	0.247	0.118	-0.025	-0.007	0.067	0.035	0.079
Number locule/pod	-0.324	-0.053	**0.307**	0.283	-0.035	0.075	-0.082	-0.042	0.191
Number main branches	-0.004	0.179	0.137	-0.317	-0.400	-0.030	-0.128	**0.597**	**0.506**
Number pod/peduncle	0.043	0.110	0.072	0.005	0.113	**0.861**	-0.320	0.113	-0.167

Number pod/plant	0.055	0.184	0.090	-0.373	-0.404	-0.104	-0.467	-0.220	-0.338
Number seeds/pod	-0.299	-0.081	**0.349**	0.267	-0.044	0.070	-0.175	-0.072	0.045
Peduncle length/mmx10^{1}	-0.148	0.221	0.207	-0.310	0.117	-0.181	-0.036	0.018	-0.330
Plant height/cm	-0.084	0.263	0.246	-0.320	0.092	0.224	**0.421**	-0.137	-0.029
Pod length/cm	-0.318	0.131	0.162	0.250	-0.082	-0.137	-0.221	-0.043	-0.088
Pods weight/g	-0.234	**0.310**	0.076	0.245	0.082	-0.192	-0.103	0.130	-0.006
Seed length/mmx10^{1}	0.092	**0.368**	-0.234	0.096	0.009	-0.059	-0.064	-0.199	-0084
Seed thickness/mmx10^{1}	0.069	**0.365**	-0.146	0.171	-0.015	0.171	0.103	-0.035	0.193
Seed width/mmx10^{1}	0.133	**0.360**	-0.263	0.156	-0.061	0.042	-0.136	-0.052	0.118
Seed weight/g	-0.010	**0.373**	-0.227	0.178	0.103	-0.121	-0.058	0.085	0.023
Terminal leaf length/ mmx10^{1}	-0.118	0.281	0.185	0.063	0.032	-0.006	**0.441**	**0.380**	-0.371
Terminal leaf width/ mmx10^{1}	-0.087	0.233	0.237	-0.267	0.234	-0.015	0.034	-0.497	**0.477**

APPENDIX 4.7: Cluster distribution of 94 cowpea accessions based on twenty quantitative traits

Cluster	Sub-cluster	Similarity Coefficient	Number of Accessions
A		**0.84**	
	I	**0.88**	5(Marfo-Tuya, PRL 73 (Yerewolo), Ejura, PBL 22 (Djemani), PBL 112 (Dounafan))
B		**0.84**	
	I	**0.88**	1(Alan Cash)
	II	**0.88**	
	a	0.92	4(GH2316, GH5043, GH4526, GH4769)
	b	0.92	13(GH2329, GH3667, GH5040, GH7875, GH3683, GH2281, GH4529, GH1619, GH2272, GH2335, Asontem, Soronko, Nhyria)
	c	0.92	2(Gh5050, GH2214)
	d	0.92	3(TVU7624, TVU10362, IT97K-499-35 (Djiguya))
	e	0.92	17(GH7185, GH7226, Niebe Sucré (Sukaro-Shô), CZ1-94-23-1, Suvita-2 (Grom-Grom), TVU7710, KPR1-96-73, KPR1-96-54, KPR1-96-32, TN5-78, Wantele, Tona, Milo, Asetenapa, M'Barawa, Malam Yaya, GH5344)
	f	0.92	10(Apagbaala, Niban, Bengpla, IT89KD-374 (Korobalen), TVU7657, TVU7671, TVU90035, TVU7643, IAR 167B, TVU7617)
	g	0.92	6(Parajani, Sanzisabinli, TVU7687, TVU7696,

			IT82D-812, TVU7705)
	h	0.92	33(CZ11-94-5C, GH2290, TVU7616, TVU7708, TVU90106, TVU7709, TVU10377, CIPEA82672, TVU7608, CZ1-94-23-2, GH2331, GH2271, TVU7699, GH3708, TVU7686, TVU90107, TVU7681, TVU90110, GH4024, GH2293, GH7233, GH7243, TVU90037, TVU9344, CIPEA80025, CB-5, TVU7677, TVU7714, GH4542, GH3684, GH7178, GH2339, GH1608)

APPENDIX 4.8: Identification of genotypes based on their code for quantitative traits

```
** Levels   100.0  90.0  80.0

G1           1  ..
G23         44  ..)
G2           3  ..)
G3           5  ..)
G44         88  ..)..
M14         30  ..   )
M23         46  ..)  )
M41         79  ..)  )
M37         75  ..)  )
M47         93  ..)  )
M40         78  ..)..)
G5          10  ..   )
G22         42  ..)  )
G31         60  ..)  )
G20         38  ..)  )
M7          13  ..)  )
M25         50  ..)  )
M38         76  ..)  )
M20         41  ..)  )
G18         34  ..)  )
M26         52  ..)  )
G42         86  ..)  )
G32         63  ..)..)
M12         26  .....)
M19         39  ..   )
M42         80  ..)  )
M45         89  ..)  )
M21         43  ..)  )
M34         68  ..)  )
M36         73  ..)  )
M44         83  ..)  )
G38         74  ..)  )
M43         81  ..)..)
M1           2  ..   )
M22         45  ..)  )
M39         77  ..)  )
M35         71  ..)..)
G13         23  ..   )
G35         69  ..)..)
M4           8  ..   )
M5           9  ..)  )
M13         28  ..)  )
M18         37  ..)  )
M28         57  ..)  )
M29         59  ..)  )
M8          16  ..)..)
G9          17  .....)
M27         55  .....)
```

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G39         82  .....)..
M2           4  ..      )
G12         22  ..)     )
G26         51  ..)     )
G46         92  ..)..   )
G8          15  ..   )  )
G14         25  ..)  )  )
G24         47  ..)..)  )
M3           6  ..   )  )
M10         21  ..)  )  )
M16         33  ..)  )  )
M11         24  ..)..)  )
M32         64  .....)  )
M24         48  ..   )  )
M30         61  ..)..)  )
M33         66  ..   )  )
G43         87  ..)..)  )
G45         90  .....)  )
M9          18  .....)  )
M15         32  ..   )  )
M31         62  ..)..)..)
G6          12  .....   )
G41         85  .....)  )
G7          14  ..   )  )
G10         19  ..)..)  )
G34         67  .....)  )
G11         20  ..   )  )
G36         70  ..)..)  )
G16         29  ..   )  )
G30         58  ..)  )  )
G19         36  ..)  )  )
G28         54  ..)  )  )
G29         56  ..)  )  )
G47         94  ..)..)  )
G33         65  ..   )  )
G37         72  ..)..)..)
G4           7  ..      )
G21         40  ..)..   )
G17         31  ..   )  )
G40         84  ..)..)..)
G25         49  ........)..
M6          11  ..         )
M46         91  ..)..      )
G15         27  .....)     )
M17         35  ..   )     )
G27         53  ..)..).....)..........
```

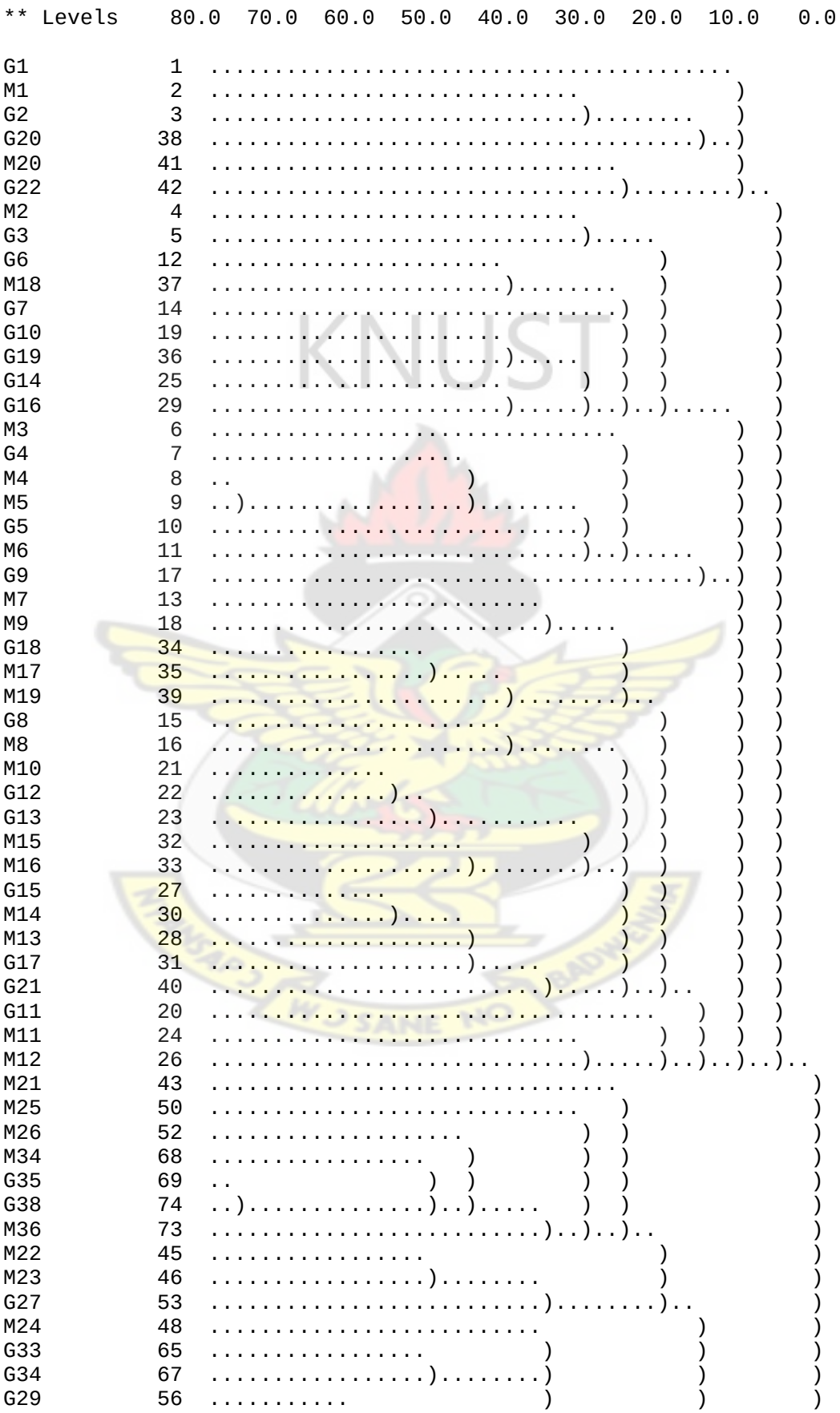
APPENDIX 4.9: Cluster distribution of 94 cowpea accessions based on molecular data

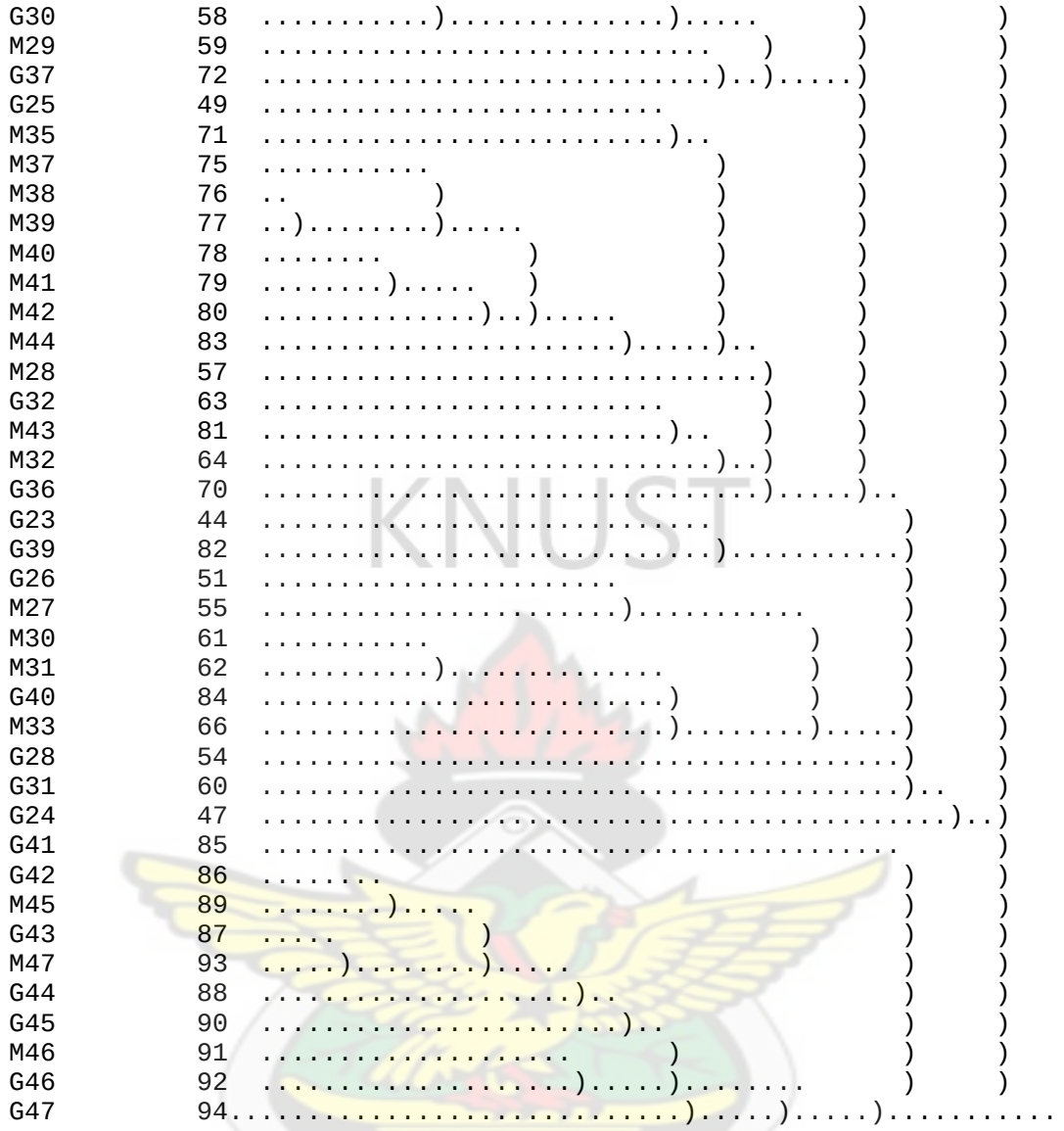
Cluster	Sub-cluster	Similarity Coefficient	Number of Accessions
A		**0.125**	
	I	**0.2**	
	a	0.4	1(GH5040)
	b	0.4	2(GH5344, PBL 112 (Dounafana))
	c	0.4	6(GH4542, GH7185, TVU9344, GH7226, TVU10377, GH2271)
	II	**0.2**	1(GH2214)
B		**0.125**	
	I	**0.2**	1(GH2293)
	II	**0.2**	1(GH3683)
	III	**0.2**	1(Niebe Sucré (Sukaro-Shô)
	a	0.4	1(GH2316)
	b	0.4	2(TVU10362, CZ1-94-23-1)
	c	0.4	2(Niban, Malam Yaya)
	d	0.4	2(Apagbaala, GH2339)
	IV	**0.2**	1(GH2335)
	V	**0.2**	
	a	0.4	1(TVU7710)
	b	0.4	1(CZ11-94-5C)
	c	0.4	1(GH2331)

	d	0.4	1(TVU7671)
	e	0.4	8(CIPEA8272, CB-5, TVU90035, TVU7696, TVU90107, CIPEA80025, TVU7687, TVU7616)
	f	0.4	1(Alan Cash)
	g	0.4	1(GH2329)
	VI	**0.2**	
	a	0.4	1(TVU7657)
	b	0.4	2(GH2281, GH7875)
	c	0.4	2(Asontem, GH3667)
	d	0.4	1(Suvita-2 (Grom-Grom))
	e	0.4	1(Wantele)
	VII	**0.2**	
	a	0.4	1(Marfo-Tuya)
	b	0.4	2(TVU7677, IT82D-812)
	c	0.4	1(TVU7708)
	d	0.4	4(GH2290, Sanzisabinli, TVU90106, TVU7699)
	e	0.4	1(TVU7681)
	f	0.4	1(TVU7709)
C		**0.125**	
	I	**0.2**	2(CZ1-94-23-2, KPR1-96-73)
	II	**0.2**	1(GH2272)
	III	**0.2**	
	a	0.4	1(GH4526)
	b	0.4	4(GH5043, TVU7643, TVU774, Ejura)
	c	0.4	2(KPR1-96-54, TVU7624)

	d	0.4	3(Parajani, Asetenapa, KPR1-96-32)
	e	0.4	2(IT89KD-374 (Korobalen), Milo)
	f	0.4	3(TVU7608, PRL 73 (Yerewolo), GH3708)
	g	0.4	1(IT97K-499-35 (Djiguya)
	h	0.4	1(TVU90110)
	IV	**0.2**	1(TVU90110)
	V	**0.2**	
	a	0.4	1(PBL 22 (Djemani))
	b	0.4	1(GH7243)
	c	0.4	3(IAR 167B, TVU7617, GH4769)
	d	0.4	1(TN5-78 (Tieblen)
	VI	**0.2**	
	a	0.4	2(GH1619, Tona)
	b	0.4	2(GH4529, Soronko)
	c	0.4	1(Nhyira)
	d	0.4	2(TVU90035, GH5050)
	e	0.4	1(GH3684)
	f	0.4	1(M'Barawa)
	VII	**0.2**	2(GH7233, TVU7686)
	VIII	**0.2**	1(GH4024)
	VIIII	**0.2**	2(GH7178, TVU7705)
	X	**0.2**	1(GH1608)

APPENDIX 4.10: Identification of genotypes based on their code for molecular data




G30 58
M29 59
G37 72
G25 49
M35 71
M37 75
M38 76
M39 77
M40 78
M41 79
M42 80
M44 83
M28 57
G32 63
M43 81
M32 64
G36 70
G23 44
G39 82
G26 51
M27 55
M30 61
M31 62
G40 84
M33 66
G28 54
G31 60
G24 47
G41 85
G42 86
M45 89
G43 87
M47 93
G44 88
G45 90
M46 91
G46 92
G47 94

APPENDIX 4.11: Code adopted to the different accessions in accordance to the countries

ACCESSIONS	CODES	ACCESSIONS	CODES
GH1608	G1	Suvita-2 (Grom-Grom)	M24
TVU7705	M1	Alan Cash	G25
GH7178	G2	TVU7681	M25
M'Barawa	M2	Malam Yaya	G26
GH3684	G3	TVU7699	M26
TN5-78 (Tieblen)	M3	Marfo-Tuya	G27
GH4769	G4	GH3683	G28
TVU7617	M4	Niban	M27
IAR 167B	M5	GH7875	G29
GH7243	G5	TVU7671	M28
PBL 22 (Djemani)	M6	GH2281	G30
GH5050	G6	TVU7657	M29
TVU90110	M7	GH2293	G31
Nhyira	G7	CZ1-94-23-1	M30
Milo	G8	TVU10362	M31
IT89KD-374 (Korobalen)	M8	GH2331	G32
Bengpla	G9	TVU7710	M32
IT97K-499-35 (Djiguya)	M9	GH3667	G33
Soronko	G10	Niebe Sucré (Skaro-Shô)	M33
GH2272	M10	Asontem	G34
KPR1-96-32	G11	TVU90106	M34

Asetenapa	G12	Sanzisabinli	G35
Parajani	G13	GH2335	G36
KPR1-96-73	M11	TVU7687	M35
Tona	G14	GH2329	G37
CZ1-94-23-2	M12	TVU7708	M36
Ejura	G15	GH2290	G38
TVU7643	M13	CIPEA80025	M37
GH1619	G16	TVU90107	M38
TVU7714	M14	TVU7696	M39
GH5043	G17	TVU90037	M40
TVU7624	M15	CB-5	M41
KPR1-96-54	M16	CIPEA82672	M42
GH3708	G18	CZ11-94-5C	M43
PRL73 (Yerewolo)	M17	Apagbaala	G39
GH4529	G19	TVU7616	M44
TVU90035	M18	GH2316	G40
GH4024	G20	GH2214	G41
TVU7608	M19	GH2271	G42
GH4526	G21	GH7226	G43
TVU7686	M20	GH4542	G44
GH7233	G22	TVU10377	M45
TVU7709	M21	GH7185	G45
GH2339	G23	PBL 112 (Dounafana)	M46
IT82D-812	M22	GH5344	G46
TVU7677	M23	TVU9344	M47
Wantele	G24	GH5040	G47

APPENDIX 4.12 : Preparation and dilution of some reagents used in molecular study

Preparation of 2X loading Dye from 6X Loading Dye:

- 6X Loading Dye : 133.3 µl
- NSFW : 266.7 µl
- Final volume : 400 µl

Preparation of 1X TE (Tris-Ethylenediaminetetraacetic): total volume 500 ml

- 1M Tris HCL (ph8) : 5.0 ml
- 0.5M EDTA (ph8) : 1.0 ml
- Top with FADW to 500 ml

Preparation of 50X TAE (Tris Acetate-Ethylenediaminetetraacetic): total volume 500 ml

- Tris base : 121.0g
- Glacial acetic acid : 28.55 ml
- 0.5M EDTA : 50 ml (ph8)
- Top up to 500 ml with distilled water.

Preparation of 6% of Polyacrylamide Gel Electrophoresis (PAGE) (volume: 70 ml):

- FADW (H_2O): 52.5 ml
- TBE (10X) : 7 ml
- 40% Acrylamide: 10.5 ml
- 10% APS : 700µl
- TEMED : 70µl

TEMED: N,N,N',N'-tetramethylethylenediamine

Preparation of 10% APS (Ammonium persufate) (volume: 10ml)

- 10ml=1 g
- 10 ml?
- Take 1g APS and dissolve in 10 ml of distilled water.

Preparation of 10X TBE (Tris-Borate Ethylenediaminetetraacetic):

- Weigh 108g of Tris Base (M.W=121.14, $C_4H_{11}NO_3$)
- Weigh 55.0g Boric Acid (M.W=61.83, CH_3BO_3)
- Measure 40 ml of 0.5M EDTA (ph8)
- Dissolve all of them into 1 liter (1000 ml)

Preparation of 10mM dNTP Mix final volume 1000µl:

Concentration of Stock (dATP, dCTP, dGTP and dTTP)= 100mM

- $C_1V_1=C_2V_2$; C_1= 100mM; V_1?; C_2=10 and V_2= 1000µl
- $V_1=\frac{10mM \times 1000\ \mu l}{100mM}$

  V_1= 100µl
- Volume to be taken for each dNTP= $\frac{100\mu l}{4}$ = 25µl

Preparation of 1X TBE (Tris-Borate Ethylenediaminetetraacetic) from 10X TBE:

- Measure 100 ml 0f 10X TBE
- Top up with water to 1000 ml

Preparation of Developer Stain: total volume= 2000 ml

- Na_2CO_3 : 60g
- 37% Formaldehyde : 3.0 ml
- Stock solution (Sodium thiosulphate 10mg/ml) : 0.5 ml
- Top the solution with water to 2000 ml

Preparation of Nitric Acid: total volume= 2000 ml

- 1.5% of Nitric Acid : 30 ml
- Water : 1970 ml

Preparation of Silver Staining: total volume= 2000 ml

- Silver Nitrate : 2.0g
- Water : 2000 ml
- Formaldehyde: 3.0 ml

Preparation of 10% Glacial Acetic Acid: total volume= 2000 ml

- Acetic Acid: 200 ml
- Top the solution with water to 2000 ml

Preparation of 1kb Ladder: total volume= 500µl

- 1kb Ladder : 50µl
- 6X Loading dye : 80µl
- Water : 370µl

Preparation of 10mg/ml Ethidium Bromide:

- 10mg= 1ml

- X= 50ml

- X=10mg x 50ml= 500mg=0.5g

  1ml

- Steps:

  - ✓ Weigh 0.5g of Ethidium Bromide powder into container
  - ✓ Add 50ml of nuclease free sterile water and dissolve

Preparation of 1kb Ladder: total volume 500µl

- 1kb Ladder : 50µl
- 6X Loading dye: 80µl
- Water distilled : 370µ
- Final volume : 500µl

Primer Stock Solution Preparation (100µM)

Molar solution concentration equation: $C = \frac{m}{V} \times \frac{1}{MW}$

C is the molar Conc. In mol/L

M is the mass

V is the volume of solution in litre (L)

MW is the molar weight in g/mol

Eg. Substance 'A' has a molecular weight of 5604.7g/mol and mass of 0.71 mg. How much solvent will be needed in millitres (ml) to make a 100 micro molar (µM) solution of substance A?

$C = \frac{m}{V} \times \frac{1}{MW} \longrightarrow 0.00001M = \frac{0.00071g}{Vol} \times \frac{1}{5604.7}$

Vol= 0.0012668L= 1.26679ml