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CHARACTERISATION AND DETERMINATION OF VIRUS RESISTANCE AMONG COWPEA [*Vigna unguiculata* (L.) WALP.] GENOTYPES.

A Thesis Submitted to the

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By

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DECLARATION

This thesis is the result of research work undertaken by CARLOS KWESI TETTEY in the Department of Nuclear Agriculture and Radiation Processing, of the School of Nuclear and Allied Sciences, University of Ghana, under the supervision of PROF. H. M. AMOATEY, PROF. E. ASARE-BEDIAKO and DR. A. T. ASARE. I hereby affirm that except for references to other people's works, which have been duly cited, this work is a result of my own research and that it has not been presented in part or whole for any other degree in this University or elsewhere.

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DEDICATION

This thesis work is dedicated to my siblings.

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LIST OF ABBREVIATIONS

AUDPC	Area under disease progress curve
AFLP	Amplified Frangement Length Polymorphism
bp	Base pairs
CABMV	Cowpea aphid-borne mosaic virus
CCMV	Cowpea chlorotic mottle virus
CGIAR	Consultative Group on International Agricultural Research
CIAT	CentroInternacional de Agricultura Tropical (Spanish): InternationalCenterforTropicalAgriculture
CMV	Cucumber mosaic virus
CPCMV	Cowpea chlorotic mosaic virus
CPMMV	Cowpea mild mottle virus
CPMV	Cowpea mosaic virus
CPSMV	Cowpea severe mosaic virus
CRIG	Crop Research Institute of Ghana
CSIR	Center for Scientific and Industrial Research
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
DF	Degree of freedom
DNA	Deoxyribose nucleic acid
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Corporation)
FAO	Food and Agriculture Organisation
ha	Hectare

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ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IBPGR	International Board for Plant Genetic Resources
IgG-AP	Immunoglobulin-alkaline phosphatase
IITA	International Institute of Tropical Agriculture
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter Simple Sequence Repeat
LSD	Least Significant Difference
MAS	Marker Assisisted Selection
ml	Millilitre
mm	Millimetre
ORSTOM	Office de la Recherche Scientifique et Technique d'Outre-Mer (French): Office for Overseas Scientific and Technical Research
RNA	Ribose nucleic acid
P	Probability
Pnpp	P-nitrophenyl phosphate
PROTA	Plant Resources of Tropical Africa
PSB	Phosphate saline buffer
PSB-T	Phosphate saline buffer-Tween20
PVP	Polyvinylpyrrolidone
SAFGRAD	Semi-Arid Food Grains Research and Development
SARI	Savanna Agriculture Research Institute

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SBMV	Southern bean mosaic virus
SINGER	System-wide Information Network for Genetic Resources
µl	Microliter
USDA	United States Development Agency
WAS	Weeks after sowing
WASCAL	West African Science Service Center on Climate Change and Adapted Land Use

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ABSTRACT

Several households in Ghana feed on cowpea [*Vigna unguiculata* (L.) Walp.] which serves as good source of protein. However, cowpea viral diseases and the lack of adaptable cultivars have become a limiting factor in cowpea production. This work therefore sought to explore morphogenetic diversity and viral resistance traits in cowpea germplasm to improve productivity. Thirty-eight exotic and local cowpea genotypes were cultivated at the Teaching and Research Field of the School of Agriculture, the University of Cape Coast. Two plants were maintained per stand at planting distance of 50 cm x 30 cm with three replications in a randomized complete block layout during the major (June – September) and minor seasons (November – February). The cowpea genotypes were characterised using both morphological and molecular methods to assess diversity in the coastal savanna agro-ecological zone of Ghana. They were also screened for resistance to cowpea viruses using visual scale on-field and DAS-ELISA protocol. The cowpeas showed significant ($P < 0.05$) variations in plant height, canopy diameter, number of branches, area of leaf, days to 50% flowering, days to pod maturity, pod length, number of seeds per pod and hundred seed weight. There were significant and positive correlations between pod weight and seed yield ($r = 0.985$, $P < 0.05$), plant height and canopy diameter ($r = 0.576$, $P < 0.05$), canopy diameter and number of branches ($r = 0.576$) as well as pod length and the number of seeds per pod ($r = 0.530$, $P < 0.05$). Hundred seed weight ranged from 10.03 g to 22.7 g. On the whole, 23 quantitative and qualitative parameters differentiated the cowpea genotypes into two main clusters with sub-clusters. Genomic analysis involving nine polymorphic SSR primers showed a mean genetic diversity of 0.7, polymorphic information content of 0.67 and allele frequency of 0.4 among the cowpea genotypes, which were differentiated into two

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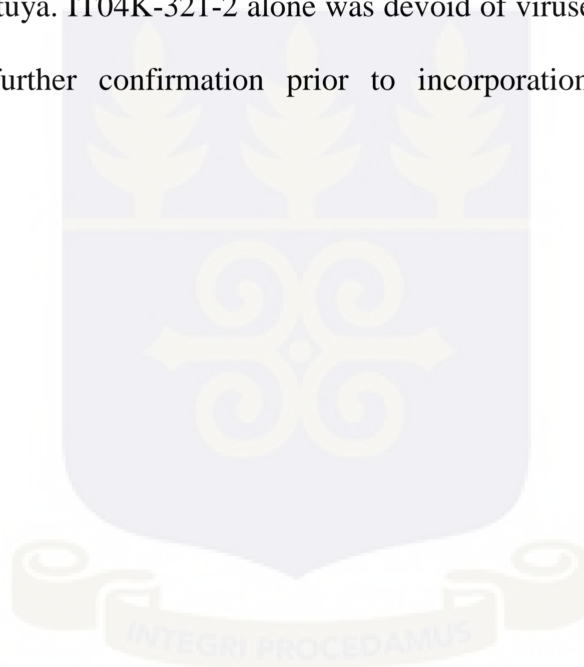
main clusters with sub-clusters. Incidence and severity of cowpea viruses in both the major and minor seasons were significantly ($P < 0.05$) different among the cowpea genotypes as well as between the two seasons with the minor season showing the higher mean incidence (53.1%) and severity (1.99) compared to 30.9% incidence and 1.49 mean severity recorded for the major season. Of the four cowpea viruses screened on 15 best performing cowpea genotypes using DAS-ELISA, two viruses, *Cowpea severe mosaic virus* (CPSMV) (1/15) and *Cucumber mosaic virus* (CMV) (13/15) were detected with one mixed infection CPSMV/CMV recorded in the local genotype Padi-tuya. IT04K-321-2 alone was devoid of viruses and could be resistant but requires further confirmation prior to incorporation into future breeding programmes.



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CHAPTER ONE

1 INTRODUCTION

1.1 Background information

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important food and forage legumes grown in the semi-arid tropics and some temperate regions of the world (Timko and Singh, 2008; Timko *et al*., 2008). West Africa has the genetically diverse forms of cultivated cowpea (Madamba *et al*., 2006). However, the controverted issue of centre of domestication event of cowpea is now recognised as occurring in the northern part of Africa (Coulibaly *et al*., 2002).

An estimated 4.5 million metric tonnes of cowpea is produced worldwide on 12 to 14 million hectares of land (Singh *et al*., 2002; Boukar *et al*., 2016). Nigeria and Niger are the leading producers of cowpea but countries such as the United States, Brazil, India, Myanmar, Sri Lanka, Australia, and Bosnia and Herzegovina also produce appreciable amount of the crop (Quinn, 1999; Timko and Singh, 2008). Nigeria alone cultivates about 4 million hectares, which yields about 45 % of the total world cowpea production (Pereira *et al*., 2001).

The cowpea grain is highly nutritious and contains about 15.06 - 38.5 % protein (this differs among cowpea varieties) (Ravelombola *et al*., 2016). Besides the numerous nutritional attributes of cowpea, it has the ability to improve soil fertility by fixing nitrogen in the soil in association with soil bacteria *rhizobia*, which benefits crops grown in succession with cowpea (Agyemang *et al*., 2014).

Cowpea is an important legume produced mainly by resource-poor smallholder farmers for livelihood purpose in Ghana. It is a source of employment for farmers in rural areas as well as cowpea traders in the urban areas (Langyintou *et al*., 2003). The bulk of the crop is produced within the Sahel and Sudan agro-ecological zones

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and the fringes of the forest agro-ecological zones (CRI, 2006) as cited by Chiamaka, 2014.

The fresh leaves, fresh pods and dry seeds are consumed in various dietary combinations throughout Ghana. The dry grains are boiled and eaten with 'gari' and fried ripened plantain. The flour obtained from the dry cowpea seeds can also be made into dough and fried in cooking oil called 'kose'.

1.2 Statement of the Problem

Despite the nutritional and economic importance of cowpea, much work has not been done in the characterization of the crop in Ghana using molecular approach. Earlier works done by Egbadzor *et al.* (2014) and Doumbia *et al.* (2014) focused mainly on the use of morphological traits in determining diversity among cowpea germplasms. Also, Ghana's recorded yield per unit area is one of the lowest in the world averaging about 0.310 tha^{-1}, which is far below the average potential yield of 0.663 tha^{-1} (FAO, 2000; Ofosu-Budu *et al*., 2007). With an annual dry grain production of 57000 t compared to an annual average demand of 169000 t, there is a deficit of 112,000 t of cowpea annually (FAO, 2000; Langyituo *et al*. 2003). Consequently, cowpea is imported from Niger (7000 metric tonnes), Burkina Faso (3000 metric tonnes) and Togo (334 metric tonnes) to augment local production, (Retieved on the November 2015 from http://www.fao.org/3/a-au994e.pdf).

The wide yield gap of the crop in Ghana is attributed to production constraints such as unavailability of improved seeds, attack by pest and diseases, poor farm practices and inability to access market (Karungi *et al*., 2000). Agents such as insect pests and disease-causing organisms such as fungi, nematodes, bacteria and viruses as well as some phyto-parasites are a major concern to cowpea producing areas (Hampton *et al*., 1997). Among these, cowpea viral diseases contribute greatly to poor cowpea

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production in major cowpea areas (Gioi *et al*., 2012). Over 20 viruses have been reported in cowpea-growing countries around the world (Thottappilly and Rossell, 1985). In Ghana, much work has not been done in assessing virus disease incidence as well as identification of cowpea viruses. Cowpea viral disease could lead to yield losses ranging from 80 - 100 % as reported elsewhere (Raheja and Leleji, 1974; Gilmer *et al*., 1974; Williams, 1977; Shoyinka *et al*., 1997).

1.3 Justification

Management of cowpea viral diseases is, therefore, crucial for yield improvement of the cowpea crop. Conventional methods used by farmers to control viruses including broad-spectrum insecticides for the control of insect vectors as well as selecting seeds from healthy looking plants are inadequate in controlling virus attack. Host plant resistance to the cowpea viral diseases is the most cost-effective approach to the management of cowpea viral diseases which is also harmless to the environment since no pesticide is required (Fraser, 1992; Aliyu *et al*., 2012). Identifying resistant varieties will lead to increased production of the crop at the national level through increased yield of the crop. It will improve farmer's income, food security as well as self-sufficiency in cowpea production, while saving the nation foreign exchange, which can be channelled into other areas of the economy.

1.4 Main Objective

The objective of the study was to characterise some cowpea genotypes and assess cowpea viral disease incidence using phenotypic and serological methods.

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1.5 Specific Objectives

The specific objectives were to:

1. Assess genetic diversity among 38 genotypes of cowpea.
2. Screen for resistance to cowpea viruses using characteristic cowpea disease symptoms.
3. Evaluate selected cowpea genotypes in the open field and by serological method.

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CHAPTER TWO

2 LITERATURE REVIEW

2.1 Taxonomy and morphological description of cowpea plant

2.1.1 Taxonomy

Cowpea [*Vigna unguiculata* (L) Walp.] is an annual leguminous crop from the genus Vigna and belongs to the family Fabaceae (ITIS report, 2017; Doumbia, 2012). The genome is made up of 22 chromosomes (2n = 2x = 22) (Timko and Singh, 2008; Doumbia, 2012). The genus *Vigna* is distributed in several tropical regions and has several species such as the mungbean (*V. radiata*), adzuki bean *(V. angularis)*, blackgram (*V. mugo*), and the bambara groundnut (*V. subterranea*) (Timko *et al.,* 2007; Timko and Singh, 2008).

V. unguiculata subspecies *unguiculata* includes four cultigroups: *unguiculata, biflora* (or *cylindrica), sesquipedalis*, and *textilis* (Timko and Singh, 2008). The primary gene pool of the genus is made up of *V. unguiculata* subspecies *dekindiana, stenophylla*, and *tenuis* (Timko and Singh, 2008). Other wild types, which hardly hybridize because they produce sterile pollen and thus require embryo rescue constitute a secondary gene pool (Timko and Singh, 2008).

2.1.2 Morphological Description

There are three types of cowpea according to their uses: for grain, forage or dual purpose (Doumbia, 2012). *Vigna unguiculata* is a herbaceous trailing, prostrate, climbing, bushy, or suberect annual plant, growing 15 - 80 cm high. Leaves are alternating trifoliate with petiole 5 - 25 cm long (Doumbia, 2012). Cowpea has tap-root and the shape of the seeds is dependent on the pod development (Davis *et al.*, 1991). Cowpea seeds are kidney-shaped if not restricted within the pod and they become progressively globular, with sizes ranging from 2 - 12 mm long and

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weighing 5 - 30 g per 100 seeds (Aveling, 1999).

Cowpea pods are cylindrical in shape and are usually curved or straight, with a variable number of seeds (8 – 18) (Timko and Singh, 2008). The seedcoat is either smooth or wrinkled (IBPGR, 1983) and of various colours such as white, cream, buff, red, brown and black seeds (Timko *et al.*, 2007; Doumbia, 2012). Cowpea seeds may have speckles, mottle patches or have some blotches (IBPGR, 1983). Some cowpeas are also referred to as "eyed" (blackeye, pinkeye, purple, hue, etc.) where the white-coloured helium is surrounded by another colour (Davis *et al*., 1991; Doumbia 2012; Timko *et al.,* 2007).

Emergence of cowpea is epigeal making it prone to damage, which may destroy the plantlets since nodes do not form below the cotyledon (Davis *et al*., 1991). The first leaves to form are a simple, opposite pair of true leaves followed by trifoliate leaves consisting of two smaller asymmetrical side leaflets and one bigger and broader central terminal leaflet, which is symmetrical. (Ige *et al.,* 2011; Pottorff *et al.,* 2012). The Leaf surface may be smooth, dull to a shiny surface, or sometimes pubescent (Pottorff *et al.,* 2012). The structure of the mature plant may be influenced by the genotype and some environmental conditions such as temperature and the photoperiods which may cause the plant to be erect, prostrate, trailing or climbing (Timko *et al.,* 2007; Timko and Singh, 2008).

Depending on the photoperiod, early flowering cowpea varieties can mature and dry within 60 days, whereas the late maturing varieties may take more than 150 days to mature and dry (Timko *et al.,* 2007). The inflorescence is axillary or terminal false raceme up to 35 cm long, with flowers clustered near the top (Madamba *et al*., 2006). Flowers are bisexual and papilionaceous with variable colours, which range from white, cream, yellow, pink to dark purple and sometimes with different

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combinations (Madamba *et al*., 2006; Ige *et al.,* 2011). The keel is boat-shaped, stamens are fused and one free, with the ovary superior (Madamba *et al*., 2006). Cowpea undergoes self-pollination with the flowers opening between 6:00 am – 6:30 am and closing by midday (11:30 pm – 12:00 pm) by which time pollination might have taken place and anthers dehisced (Ige *et al.,* 2011). Although considered self-pollinating, cowpea undergoes about 5% cross pollination (Fery, 1985; Timko *et al.*, 2007; Timko and Singh, 2008).

The display of brightly coloured flowers openly above the canopy on long peduncles and the presence of floral nectarines attracts insects (Sellschop, 1962; Timko and Singh, 2008). Depending on the prevailing growing conditions, cowpeas may have up to four or more pods per peduncle but two or three pods are the commonest (Timko and Singh, 2008). The peduncles may be hidden within or above the canopy thereby facilitating harvesting (Nkhoma, 2013).

Cowpea pods are smooth and cylindrical, straight or curved and may measure 10 and 110 cm (Doumbia, 2012). As the seeds approach the green-to-matured stage, which is the harvest stage for use as a vegetable, the colours are either green, yellow or purple (Davis *et al*., 1991). Matured cowpea pods with green and yellow pigmentation dry and change colour to a tan or brownish colour (Davis *et al*., 1991).

Cowpea varieties include both short-day and day-neutral types (Timko *et al.*, 2007; Timko and Singh, 2008). Floral bud initiation in cowpea is influenced by photoperiod sensitivity of the accession, which in turn is controlled by temperature (Timko *et al*., 2007; Timko and Singh, 2008). In West Africa, selection for adaptation to local conditions has led to the selection of cowpea genotypes which ripen by the time the cowpea growing season ends irrespective of planting date allowing pods to escape damage from excessive moisture and pathogens such us

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fungi which causes rot of pods (Timko *et al.*, 2007; Timko and Singh, 2008).

2.2 Environmental Requirements of Cowpea

2.2.1 Climate

Cowpea is adapted to different temperatures and moisture availabilities in comparison to other crops (Baidoo and Monchiah, 2014). The crop is grown with or without irrigation and is more drought tolerant than common bean (Davies *et al.*, 1991). The ability of the crop to tolerate drought is the main reason its of great importance to the underdeveloped world (Peksen, 2007). Application of water should be minimal when using irrigation system so as not cause more vegetative growth and delay (Davies *et al.*, 1991). The most important water-requiring period is just before and during flowering (Davies *et al.*, 1991; Nkomah, 2013).

2.2.2 Soil fertility and pH requirements

Cowpea grows well on well-drained sandy to sandy-loam soils with pH ranging from 5.5 – 6.5 (Davis *et al.*, 1991; Lim, 2012). It can be cultivated in marginal areas having low soil fertility because of the crops ability to fix atmospheric nitrogen through an efficient symbiotic association with mycorrhizae (Ghalmi *et al.*, 2010).

2.3 Origin and distribution of Cowpea

2.3.1 Origin

Cowpea [*Vigna unguiculata* (L.) Walp] has been used as food for a very long time with evidence showing its existence since the Neolithic period (Singh *et al.*, 2007; Akinjogula *et al.* 2008; Agyemang *et al.,* 2015). Contradictory views have been propounded by different authors as to the origin of cowpea with Africa, Asia and South America proposed to be the origin (Suliman, 2000; Coetzee, 1995; Singh, 2005; Timko *et al.*, 2007) of cowpea found in Asia are different from those in Africa implying that Asia could be an independent center of origin from African genotypes

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(Timko *et al*., 2007; Timko and Singh, 2008; Doumbia, 2012).

Current evidence on cowpea suggests that the crop originated in Africa, although the precise location on the African continent is difficult to tell (Feleke *et al*., 2006). In Ghana, the oldest evidence of wild cowpea was discovered in the Kintampo caves from archaeological findings (D'Andrea *et al.,* 2007; Agbicodo, 2009). Several parts of Africa have been suggested as being the centre of diversity of cultivated cowpea including West Africa (Otwe, 2007; Vaillancourt and Weeden 1992; Ng, 1995).

It is postulated that small seed size of wild forms of cowpea enabled its dispersal to other parts of Africa aided by agents of dispersal such as birds (Timko and Singh, 2008). Later, human quest for larger and best growth performance aided by extensive breeding work carried over several decades might have led to the diversity in cultivated and domesticated cowpeas found in Asia and Africa (Timko and Singh, 2008, IITA, 2010).

Currently, the wild cowpea, *Vigna unguiculata ssp. unguiculata var. spontanea* (also known as ssp. *dekindtiana sensu* Verdc.), is thought to be the likely progenitor of cultivated cowpea (Feleke *et al.,* 2006). Chlorplast DNA markers showed a loss of *BamHI* restriction region in 54 domesticated and 130 wild cowpea accessions from the wild progenitor and observed the loss of a *BamHI* restriction site which is a mutation in the chloroplast DNA which might have occurred before domestication of the crop and this finding confirms earlier findings by Vaillancourt and Weeden (1992), (Feleke *et al*., 2006; Timko and Singh, 2008). However, alternative halotype 1, has been observed in 40 var. *spontanea* accessions (Timko and Singh, 2008). The high frequency of the marker in West Africa could be attributed to genetic swamping of the wild or as a result of domestication focusing on fodder (Timko and Singh, 2008).

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2.3.2 Global distribution

Today, cowpea can be found growing in several countries across all continents having been carried to these new centers by explorers, traders and researchers. Cowpea may have reached India as early as the Neolithic period and preceded the Christian era and may be the center of secondary domestication (Perrino *et al.,* 1993; Timko *et al.,* 2007; Timko and Singh, 2008). Spanish explorers may have introduced the crop into the West Indies through slave trade from West Africa around the late 17th century and then into Central and South America from where it entered United states (Perrino *et al.,* 1993; Timko and Singh, 2008). Cowpea was then introduced to the south of Europe around the Mediterranean, where it was grown and used by the Greeks and Romans (Perrino *et al.,* 1993).

2.4 Economic importance of Cowpea

Cowpea is a crop of great economic importance to Africa with vast proportion of the population using it as food, animal feed and cash income (Langyintuo *et al*., 2003; Asare, 2013). The fodder derived from cowpea plant can be traded to generate extra income for cowpea farmers by 25 % (Dugje *et al*., 2009; ICRISAT, 2011). Trading in freshly produced seeds and processing of cowpea seed into food generate income for farmers and traders (Singh *et al*., 2002; Singh, 2002; Langyintuo *et al*., 2003; Ampah, 2011). Cowpea farmers provide fodder for *in situ* grazing after harvesting when fodder is scarce (Inaizumi *et al*., 1999; Nhamo and Mupangwa, 2003).

Estimating cowpea production is difficult since there has been no reliable source of data on the production since the FAO stopped publishing cowpea statistics in the mid 1980s (FAO, 2004). There is not much data on the production, trading and market access (Langyintou, *et al*., 2003) because most of the attention has been focused on cash crops such as cocoa, coffee and cereals such as maize and rice (Langyintou, *et*

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al., 2003; FAOSTAT, 2012).

2.4.1 Food and health benefits

All stages of growth of cowpea serve as food (Nielson *et al*., 1993; Singh *et al*., 1997; Ahenkora *et al.,* 1998). It is the second most important grain legume, and currently a food security crop, which provides a good and affordable vegetable protein and minerals for about 70 % of Ghanaians (MoFA, 2010; Doumbia, 2012). The mature dried seeds, immature seeds and pods and young leaves of cowpea constitute food for human consumption in Africa (Asare, 2013).

Cowpea contains high amount of protein, minerals and vitamins compared to cereal crops. The grain is about 25 % protein and 64 % carbohydrate hence a good panacea to malnutrition in the urban and rural folks (Inaizumi *et al*., 1999; Adeyanju *et al*., 2012). Most of the food consumed in many parts of Africa are mainly starchy foods (Singh *et al.,* 2002) hence the high protein content of cowpea (20 - 25 %) (Alayande *et al.* 2012) compensates for the large proportion of the carbohydrate in the diet (Lambot, 2002). Cowpea utilization is important in most parts of West and Central Africa since it provides a cheaper alternative to meat and serves as a "food security crop" (Lambot, 2002) for populations that consume cowpea as traditional staple food (Langyintuo *et al*., 2003).

In Central and West Africa, dry cowpea grains are used for a variety of dishes; the whole grain is mainly eaten with cereals or used as an ingredient in soups or stews, while milled cowpea is mostly used to make fritters or steamed cakes (Langyintuo *et al*., 2003). In Ghana, the dry grains are processed into flour and a dough made out of the flour and deep fried to prepare "agawu" and "koose" which are served with maize or millet porridge (Hausa "kooko"). The dry beans are also boiled and served with "gari" and fried ripened plantain called "ga beans" or boiled with rice popularly

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called "waakye" and "apprepensa". Others include "gable", "nyonbeeka", "tubani", "gora" and "nagbechinge" (maize and beans), which are popular in the Kumbungu district of Ghana (Quaye *et al*., 2009).

The young cowpea leaves, green pods, seeds and roots, which contain vitamins and mineral elements, constitute a source of nutrients for humans (Rachie, 1985; Ehlers and Hall, 1997). About 5 million tonnes of proteins obtained from leafy parts of cowpea is consumed yearly (Nielsen *et al*., 1997), which constitutes about 30 % of the total food legume produced in low lying areas of the tropics (Singh, 2002).

The quest for healthy 'local' diets, which have low fat content and rich in fibre and other important benefits, has made cowpea an important food crop (which is used as food) in many parts of developing and developed countries (Timko *et al*., 2007). Fat content in cowpea ranges from 1.4 - 2.7 % and fibre content about 6 % (Timko *et al*., 2007).

Cowpea is efficacious in treating worms in the stomach when boiled and eaten whereas an extract from the seed can be taken to treat amenorrhea (Chopra *et al*., 1986). The powdered roots can be added to porridge and taken to relieve menstrual pains and treat epilepsy pain in chest (Lim, 2012). The leaves can be mashed and applied on burns and the dry leaves as snuff to cure headaches (Lim, 2012). The cowpea leaves can also be used to induce vomiting in fever patients as well (Hutchings *et al.,* 1996). Cowpea leaves are also used by herbalists in the treatment of bilharzia (Nyazema, 1987; Ndamba *et al*., 1994; Kritzinger *et al*., 2004).

Cowpea contains important group of bioactive phenolic compounds such as antioxidants such as vitamin C and carotenoids which may prevent the development of atherosclerosis and (Formica and Regelson, 1995; Cai *et al*., 2003; Doblado *et al*., 2005).

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The portion of the cowpea crop aboveground except pods, serves as a useful source of nutrient-rich fodder for livestock in many areas of the world (Singh and Tarawali, 1997; Tarawali *et al*., 2002; Singh *et al.* 2003; Pele *et al*., 2016) and in West Africa, especially during the dry season when food is scarce (Fatokun *et al*., 2000; Asare *et al.,* 2010; Tarawali *et al.*, 2002; Pele *et al*., 2016)

2.4.2 Nitrogen fixation and soil fertility improvement

Cowpea has the ability to fix atmospheric nitrogen with the help of symbiotic bacteria *Rhizobia* found in root nodules, which is made available in the soil for crops grown in succession with it especially in areas where there are poor soils (Dugje *et al*., 2009; Pele *et al*., 2016). It also has the ability to form a strong and efficient symbiotic association with mycorrhizae (Pele *et al*., 2016), thereby making it adaptable to soil with low fertility and tolerant to a range of soil pH as well as drought conditions (Fery, 1990; Pele *et al*., 2016). The seedlings are used as green manure and the plant residue remaining after harvest also form organic manure. Animal droppings obtained after feeding on cowpea serve as a good organic manure to enrich the soil.

2.5 Production of Cowpea

2.5.1 Cowpea production systems

Cowpea cultivation in West Africa is based mainly on small-holder subsistence farming systems in the dry regions of Africa such as the Savanna areas which receives low rainfall (IITA, 2010). The crop is usually intercropped with sorghum [*Sorghum bicolor* (L.) Moench] or pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Ajeigbe *et al*., 2006, IITA, 2010) and also cotton (*Gossypium* sp.). In recent times, however, due to the economic importance and the demand for the crop, cowpea cultivation is moving towards a monocropping system.

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In some parts of Europe, cowpea is grown purely for the grains or as fodder with the United States of America (USA) producing on a large commercial scale using a mechanised system for local consumption and export (Imrie, 2000).

The prolonged drought experienced in the Sahelian regions of West Africa has led farmers to venture into cowpea production due to its ability to tolerate low moisture levels (Duivenboo-den *et al*., 2002; Timko *et al*., 2007). Also, the rapidly growing demand for cowpea West and Central Africa has also influenced its production by farmers (Timko *et al*., 2007; Timko and Singh, 2008).

2.5.2 World production of Cowpea

About 14.5 million hectares of land worldwide is cultivated with cowpea each year (retrieved July 14th, 2017, from http://www.cgiar.org/our-strategy/crop-factsheets/cowpea/). As at 2010, world dried cowpea production was estimated at 5.5 million metric tonnes, with Africa accounting for 94 % of production (Langyintuo *et al.,* 2003). In Nigeria 2.8 t ha^{-1} was recorded for on-farm trial (Singh *et al*., 2007) whereas less than 83 % of on-farm trial yields were reported in Burkina Faso (SAFGRAD, 1998). Nambou *et al*., (1999), also observed yield of 2.0 t ha^{-1} on farmer's field in Togo compared with 0.8 MT ha^{-1} on farmer's field observed in Ghana (SARI, 2014). Farm level yields of cowpea on area basis have remained low (600-800 kg ha-^{1}) compared to research fields (1600-2500 kg ha-^{1}) (SARI, 2014: Yirzagla *et al*., 2016).

About 38 million households made up of 194 million people cultivate the crop in sub-Saharan Africa, but productivity has been stagnant over the decades with total area, yield, and production growing by 4.3 %, 1.5 %, and 5.8 %, respectively (retrieved July 14th, 2017, from http://www.cgiar.org/our-strategy/crop-factsheets/cowpea/). The cost involved in cowpea cultivation varies depending on the tools used,

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size of cultivated field, cowpea varieties, fertiliser, soil tillage and pest control method with 70 % of the cost is for labour hence it could be profitable but the profit varies from place to place (Langyintuo *et al*., 2003).

2.5.3 Cowpea production in Ghana

Cowpea crop is second only to groundnut among the legume crops (Ogbonnaya *et al*., 2003) with an estimated annual production of about 14,3000 MT on about 156,000 ha of land making Ghana the fifth highest producer of cowpea in Africa (Doumbia, 2012), and a projected 11 % increase in production between 2010 and 2020 (ICRISAT, 2012) (Retrieved November 2015 from http//www.icrisat.org/ tropicallegumesII). In Ghana, subsistence farmers predominantly produce cowpea in the Sudan Savanna agroecological zone as the main food crop.

Ghana produces less cowpea than it consumes and this may be due to several factors mainly pest and diseases, drought, low soil fertility, the unavailability of farming inputs, improved seeds, poor cultural practices and lack of tools and equipment for large-scale production (IITA, 2010; Doumbia, 2012). To make up for the deficit, Ghana imports cowpea from Burkina Faso, Niger and Nigeria.

Ghana has the necessary agro-ecological conditions for cowpea production: warm, humid climate with annual rainfall estimated at 83 to 220 cm; relative humidity between 50 % to 80 % with mean temperatures ranging from 21°C around the coastal regions to 32°C in the northern regions (FAO, 2009). There are six agro-ecological zones in Ghana, which are grouped according to prevailing climate, natural vegetation, and soil types (Table 2.1), of which four favour cowpea production.

Table 2.1. Characteristics of agro-ecological zones in Ghana

Zone	Rainfall (mm/year)	Portion of total Area (%)	Length of growing season (Days)	Dominant land use systems	Main food crops
Rain forest	2200	3	Major season: 150-160 Minor season: 100	Forest, plantations	Roots, plantain
Deciduous forest	1500	3	Major season: 150-160 Minor season: 90	Forest, plantations	Roots, plantain
Transition zone	1300	28	Major season: 100-150 Minor season: 60	Annual food and cash crops	Maize, roots, plantain
Guinea savanna	1100	63	180-200	Annual food and cash crops, livestock	Sorghum, maize
Sudan savanna	1000	1	150-160	Annual food crops, livestock	Millet, sorghum, cowpea
Coastal savanna	800	2	Major season: 100-110 Minor season: 50	Annual food crops	Roots, maize

Sources: http://www.fao.org/nr/water/aquastat/countries_regions/gha/GHA-CP_eng.pdf ; Otwe, 2007.

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2.5.4 Harvest and storage

Harvesting of cowpea is done by hand-picking or with the use of a combine harvester for commercial farms of which the upright cultivars with peduncles above the canopy are easy to harvest by machine (retrieved 3rd July 2017 from; http://www.agriguide.org/index.php?what=agriguide&id=161&language=en).

Young cowpea leaves grown for vegetable purposes are mainly picked by hand (Nkomah, 2013). Sowing of cowpea must be timed such that harvesting would coincide with the end of the rains so that moisture does not destroy the pods (Retrieved 3rd July, 2017 from; http://en.agriwiki.org/i ndex.php/Cow_pea_bean). However, the matured pods must be harvested in time to prevent excessive drying, which may cause pods to shatter (retrieved 3rd July, 2017 from; https://www.nqi-nigeria.org/sites/d efault/files /Quality%20and %20safety%20con trolled% 20b ean s % 2 0fr om%20 Nigeria% 20V160808a.pdf).

2.5.5 Drying and Storage

The shelf life of cowpea depends on the amount of moisture in the seeds before storage and the lower the moisture, the better the quality of seeds in storage (retrieved 3rd July, 2017 from; http://en.agriwiki.org/index. php/Cow_pea_bean). Alternatively cowpea can also be stored for a longer period by storing in temperature of about -18 ^{O}C and this can reduce pest numbers as much as 99 %. Seeds with moisture content of about 12 % can be stored for a short term whereas those seeds with moisture content of about 8 – 9 % is recommended for long-term storage (Retrieved 3rd July 2017 from; http://www.agriguide.org/index.php?what=agrigu I de&id=161&language=en). Cowpea leaves can be dried in the sun and kept for a period (Retrieved 3rd July, 2017 from; http://www.agriguide.Org/index.php?wat=agrI guide&id=161&language=en).

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2.5.6 Varieties of cowpea in Ghana

A number of improved cowpea cultivars are released yearly in different countries through local and international collaborations with about 68 countries identifying and releasing improved cowpea which are high yielding, disease and insect resistant as well as drought resistant varieties from IITA for famer cultivation (Nkomah, 2013).

Newly released cowpea lines are usually given names by the breeder and in some case, farmers may also assign names to cowpea lines to enhance farmer-farmer exchange of seeds (Jauhar and Singh, 2005; IITA, 2010). There are different varieties of cowpea in Ghana which were developed through breeding by the Crop Research Institute of Ghana and Savana Agricuture Research Institute of Ghana (CSIR-SARI) and released by the National Variety Release and Registration Committee (NVRRC). A few examples are Asontem (IT82E-16), Bengpla (IT83S-818), Marfo Tuya, Padi Tuya, Ayiyi (IT83S-728-13), Boafa (TVx 1843-1C), Soronko (TVx 2724-01F) and Apagbaala (MoFA, 2010).

2.6 Cowpea improvement and germplasmcollection

2.6.1 Cowpea improvement

Cowpea breeding programmes in Africa over the years has led to the development of early maturing cowpea cultivars with different seed characteristics which are resistant to some biotic and abiotic stresses (Odindo, 2010). Some of the cultivars developed mature within 60 - 70 days results in dry grain yields potential of about 2000 kg ha^{-1} but this yield is hardly achieved by farmers on their fields with yields as low as 300 kg ha^{-1} (Odindo, 2010). This may be as a result of some factors such as ineffective extension systems and the lack of the high-density sole-cropping (Odindo, 2010) as well as lack of proper crop management systems needed by the

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new cowpea cultivars to produce high yields (Ehlers and Hall, 1997). Cowpea farmers still rely on the poor quality local cultivars which they keep from previous harvests for sowing under low planting densities in a mixed system with other crops such as cereals leading to low yield. There is also the problem of unfavourable weather conditions, pests and diseases that the farmers may face in attempt to grow the crop (Abdelshakoor and Faisal, 2010). Cowpea under the right moisture condition undergoes flowering over a long period ensuring higher yield whereas under low moisture conditions, development of flowers is delayed or aborted and the flowering period is also short leading to low yields as is the case of the semi-arid areas (Turk *et al*., 1980; Abdelshakoor and Faisal, 2010). Also unfavourable growing environment affect seed development process leading to poor seed quality and yield (Abdou *et al.* 2013). Cowpea breeding for adaptability to different environmental stresses has been of importance to breeders who over the years have achieved some results by yield testing large populations of cowpea over several years and under variable environments (Watanabe, 1998; Ogbonnaya *et al*., 2003; Gebeyehu, 2006; Pele *et al.* 2016). This approach of screening different cowpea accessions to access variations under different environments as well as genotype-environment interaction, over a long period is cumbersome, uneconomical and time consuming. (Blum, 1988; Pele *et al.,* 2016). The most effective approach is the one that combines selection for high yield potential under favourable conditions and under controlled, repeatable stress environment for the expression of several traits of interest (Ogbonnaya *et al*., 2003; Pele *et al.* 2016).

2.6.2 Germplasm collection

Cowpea gene pool is well documented and well represented in collections around the world with IITA, Nigeria having the largest collection of more than 14,000 which is

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accessible via CGIAR-SINGER system (http://singer.cgiar.org) (Timko and Singh, 2008).

In Ghana, the Plant Genetic Resource Research Institute (PGRRI), Bunso, serves as a national germplasm conservation Institute and has a collection of germplasm that has largely been characterised using morphological markers (Bernett-Lartey, 1992). Several new varieties introduced from other cowpea-producing countries bear no descriptions, and their origin remains uncertain. Because of the informal nature of the cowpea trade and exchange of genetic materials, it is uncommon to find that morphologically similar cultivars do not bear the same name while cultivars bearing the same name may not be morphologically identical.

2.7 Molecular Phylogeny and Genome Organisation

High level of genetic variation was found to exist among 18 *Vigna* species including five from subgenus *Ceratotropis* that were screened to identify the taxonomic relatedness between members of the subgenus *Ceratotropis* and other subgenera (Fatokun *et al.*, 1993 in Timko and Singh, 2008). The allocation of species and subspecies based upon molecular taxonomy system confirmed earlier classical taxonomic criteria used, such as morphological and reproductive traits (Timko *et al.*, 2007; Timko and Singh, 2008).

Using RAPD, 23 cowpea accessions within the subgenus *Ceratotropis* were analysed Kaga *et al.* (1996) separated the cowpea lines into two groups which differ approximately 70% and this was further separated into five subgroups whose characteristics agree with recognized taxonomic classification of species (Timko and Singh, 2008). *V. unguiculata* was found to be more closely related to *V. vexillata* after assessing isozyme variation between *V. unguiculata* and its mother species in the subgenus *Vigna* (Fatokun *et al.*, 2000; Timko and Singh 2008).

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Inter-simple sequence repeats (ISSR) DNA polymorphism analysis of 18 *Vigna* species also delineated them into their various subgenus (Ajibade *et al.* 2000; Timko and Singh, 2008).

Comparison of the various markers suggests that RFLP, AFLP and SSR markers are the most efficient markers in identifying polymorphism among cowpea genotypes (Retrieved January 2016 from; http://www.fao.org/biotech/docs/Korzun.pdf/). Among this, AFLPs and SSRs are the commonly used markers due to the large quantity of DNA needed for RFLP detection and also the problem of automating RFLP analysis (Gupta *et al*. 1999).

2.8 Characterization of Cowpea

Germplasm characterisation is the recording of distinctly identifiable heritable characteristics in which characteristics of species are documented at various stages using a set of descriptors of the species (Upadhyaya *et al.*, 2008). Data are taken systematically and recorded in an orderly manner to facilitate the use of appropriate statistical tools to analyse and compare the data obtained from different regions (CIAT, 2007).

Genetic characterisation of germplasm accessions is of great importance to breeders and also allows for proper sampling of genetic materials in gene banks, improves identification of the various genetic materials and also helps in managing the available gene pool (Spiaggia *et al.*, 2009; Doumbia *et al.*, 2014). This brings out variations that exist in germplasm collections using methods that have been developed over the years, which encompass all aspects related to biodiversity. Some applicable methods in plant germplasm characterization involve morphological, molecular and biochemical markers (CIAT, 2007). These three methods can be used independently or complementarily to determine genetic diversity of germplasm

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collections and help establish the basis for their improvement. None of the available methods is superior to the other, as they all allow for the observation and recording of different parts of the total diversity available for characterization (Rao, 2004; CIAT, 2007). No technique has been identified to be enough for measuring all aspects of genetic diversity of a species (Singh *et al*., 1997; Ogunkanmi *et al.,* 2014).

2.8.1 Morphological characterization

Morphological diversity can be determined by measuring differences in the observable (phenotypic) traits of the plants (Rao, 2014). These traits may be qualitative such as flower colour, growth habit, seed coat colour, etc. and quantitative like growth and yield potential (Rao, 2004). Descriptors for a particular crop are normally used to characterise plants morphologically. In cowpea, the descriptor list by International Board for Plant Genetic Resources (IBPGR) now Biodiversity International (1983) is used in assessing variation in cowpea germplasm collections.

Morphological characterisation of germplasm is essential not only in establishing the description of each germplasm but also aids in identifying duplicates within the same collection, detect unique traits and the population structure for conservation purpose (Huamán, 1999; Rees *et al*., 2003). Duplicates must be verified using physiological, molecular or biochemical means to determine whether they are the same genotypes (Huamán, 1999). Therefore, morphological characterization needs to be complemented by either biochemical or molecular method to provide adequate information for comparison, identification and selection of genotypes.

2.8.2 Biochemical characterization

Biochemical markers such as isozymes and total proteins which occur naturally and their expression not influenced by epistatic effects are used in this method of

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characterization (CIAT, 2007).

Isozymes are molecular forms of a single enzyme coded by different gene loci or coded by different alleles from a simple locus (called allozyme) and are found in the plant tissues and organs. Isozymes are useful in that they permit comparisons between populations of a single species and help in identifying hybrids and introgressed genes. This is due to the co-dominant nature of their allelic expression. Total proteins, on the other hand, are components of plants and as genetic markers, are characterised by high level of polymorphism, simple genetic control and limited environmental influence on their electrophoretic patterns (CIAT, 2007; Oppong-Konadu *et al*., 2005).

2.8.3 Molecular characterization

Molecular studies of cultivated plants and their wild relatives, generates evidences for the establishment of breeding strategies (Spiaggia *et al*., 2009; Gao *et al*., 2015). Molecular biology has provided the novel tools, which are highly sensitive to mutations of individuals in the characterization process. This method of characterization has greatly enhanced the study the genomes of plants and how they evolved which has further enhanced our knowledge of the cowpea genome (Timko *et al*., 2007; Timko and Singh, 2008).

Molecular techniques such as Marker Assisted Selection (MAS) mostly identify specific DNA sequences within or near genes which control a specific trait which can then be used to track those genes so as to predict the phenotype of the progeny before they mature (Timko and Singh, 2008; Bian *et al*., 2013; Kainer *et al*., 2015). There are three groups of markers, morphological markers which include agronomic traits, which are visually, assessed, biochemical markers which detect translational products of specific genes and molecular markers which is used to assess DNA

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(Semagn *et al*., 2006; Mandaliya *et al*., 2010).

The development and use of molecular markers for assessing DNA polymorphism is of great significance in the field of molecular genetics (Semagn *et al*., 2006; Gao *et al.,* 2015). MAS allows for a more efficient methodin creating a pool of alleles of interest in an improved cultivar to enhance crop improvement programmes (Timko *et al*., 2007; Timko and Singh, 2008). The choice of a marker depends on the study objectives, germplasm to characterise, the cost involved and the marker's inherent characteristics. A marker, which is monomorphic, is invariable in all organisms but when a marker shows differences in molecular weight, enzyme activity, structure or restriction site, it is polymorphic and can be used as a basis for characterization (Semagn *et al.,* 2006; CIAT, 2007).

Several molecular marker techniques have been developed and applied on various crop species over the years such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites, Single Nucleotide Polymorphisms (SNPs) and others (Altpeter and Korzun, 2007; Gao, 2015). Bulk segregation analysis and the analysis of complete linkage maps have helped in identifying useful markers, which are linked to specifici traits (Korzun, 2002; Semagn *et al.,* 2006). However, the creation of partial maps and pedigree combination as well as marker information, has also contributed in identification of molecular markers linked to certain traits of interest (Korzun, 2002; Semagn *et al.,* 2006; Gao *et al*., 2015).

2.9 Disease and pest of Cowpea

Cowpea is susceptible at all stages of growth to pests and disease causing organisms (Ambang *et al*., 2009). Common diseases include scab, blight, cercospora leaf spot,

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web blight, mosaic virus and bacterial blight (Nkomah, 2013). The common pests that attack cowpeas include aphids [*Aphis craccivora* Koch (Homoptera: Aphididae)], flower beetle [*Euphoria sepulcralis* (Fabricius) (Insecta: Coleoptera: Scarabaeidae)], pod borer [*Maruca vitrata* Fabricius (syn. *M. testulalis)* (Lepidoptera: Pyralidae)], bean fly [*Ophiomyia phaseoli* (Trybon) (Diptera: Agromizidae)], leaf hopper [*Empoasca dolichi* Paoli (Homoptera: Cicadelidae)] and cowpea bruchid [*Callosobruchus* spp. (Coleoptera: Bruchidae)] (Tamo *et al*. 1997; Nkomah, 2013).

2.9.1 Viral diseases

Viral diseases are among the most important pathogens in agriculture, and have the ability to cause great economic losses to farmers by affecting the yield quality of the crop (Kehinde *et al*., 2016). Up to about 87% yield loss due to viral infection has been observed in Nigeria as well as 100% yield loss of cowpea crop in northern Nigeria due to *Cowpea aphid-borne mosaic virus* infection (Shoyinka *et al*., 1997; Aliyu *et al*., 2012; Damiri *et al*., 2013).

Over 140 viruses have been identified all over the world to infect cowpea out of which at least 11 occur in Africa (Amayo *et al*., 2012) listed nine viruses of economic importance to cowpea in Africa and they include *Bean common mosaic virus-Blackeye cowpea mosaic* (Potyvirus), *Cowpea Aphid-borne Mosaic Virus* (Potyvirus), *Cowpea Mild Mottle Virus* (Carlavirus), *Cowpea Mosaic Virus* (Comovirus), *Cowpea Chlorotic Mottle Virus* (Carmovirus), *Cowpea Golden Mosaic Virus* (Geminivirus), *Southern Bean Mosaic Virus* (Sobemovirus), *Cucumber Mosaic Virus* (Cucumovirus) and *Tobacco Mosaic Virus* (Tobamovirus) (Thottappilly and Rossel, 1992; Jeyanandarajah and Brunt, 1993; Alegbejo and Kashina, 2001; Aliyu and Balogun, 2011). A summary of the properties some of the cowpea viruses is as

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follows;

2.9.1.1 *Cowpea aphid-borne mosaic virus* (CABMV)

Cowpea aphid-borne mosaic virus belongs to the genus *Potyvirus and* was first reported in Italy in 1966 and then in Nigeria in 1976 (Thottappilly and Rossel, 1992; Shoyinka *et al.,* 1997; Taiwo, 2003). It has since been reported in many countries in different continents however, CABMV may differ among isolates worldwide (Damiri *et al*., 2013).

Among the many species of plant viruses infecting cowpea, CABMV is most significant and widespread and caused yield loss between 30 – 40 % (Damiri *et al*., 2013). Also, yield losses from this virus ranging between 87 % and 100 % were reported from Nigeria (Damiri *et al*., 2013), 13 - 87 % from Iran (Kaiser and Mossahebi, 1975) and 48-60 % from Zambia (Fuleratu, 2016; Damiri *et al*., 2013).

CABMV has flexuous rod-shaped particles 750 nm length. Its nucleic acid is a single-stranded ribonucleic acid (ssRNA), which induces cytoplasmic cylindrical inclusions made up of pinwheels and bundles in association with scrolls (Lima *et al*., 1979). The virus is transmitted in a non-persistent manner by aphids and through sap inoculation as well as (Odedara *et al*., 2009; Damiri *et al*., 2013). *Aphis craccivora* Koch., *A. gossypii* Glov., *A. fabae* Scop., *Macrosiphum euphorbiae* Thos., and *Myzus persicae* Sulz. have all been reported as vectors (Damiri *et al*., 2013).

Symptoms may vary depending on the strain and cultivars but usually forms dark green vein-banding, chlorotic spots, distortion of leaf, mottling and stunting (Hughes and Odu, 2003).

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Fig. 2.1; Cowpea plant showing typical systemic symptoms induced by mechanically inoculated cowpea cv. California Blackeye using the Moroccan isolate of CABMV-Mor. Courtesy Bashir, M. and Hampton, R. O. (1992).

2.9.1.2 *Cowpea yellow mosaic virus* (CPYMV) genus *Comovirus*

Cowpea yellow mosaic virus is the type species of the Comovirus group (Taiwo, 2003). CPYMV was first reported in Nigeria and has been reported in some African countries as well (Thottappilly and Rossel, 1992). CPYMV is a multipartite virus with isometric particles of about 25 nm diameters and the infected hosts plant contain particles, which are similar in size but different in nucleic acid content (Santa *et al.,* 1998; Taiwo, 2003). The virus is transmitted by sap inoculation and by beetles (*Ootheca mutabilis* (Schönherr)) and not through seeds (Thottappilly and Rossel, 1988). Symptoms vary from mild green mottling to a distinct yellow mosaic, leaf distortion and early death of the cowpea plant and the CPYMV is considered as one of the most important cowpea virus diseases (Taiwo, 2003). CPYMV is reported to be one of the most important viruses affecting cowpea after CABMV in Nigeria with incidences ranging from 5.2 % - 19.9 %. The virus is capable of causing 80-100 % yield losses in cowpea (Gioi, *et al*., 2012).

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2.9.1.3 *Southern bean mosaic virus* (SBMV) genus *Sobemovirus*

Southern bean mosaic virus (SBMV) belongs to the genus *Sobemovirus* and is usually found in warm temperate and tropical regions of Warm Temperate and Tropical regions of Americas (USA, Mexico and Costa Rica) and Africa (Tremaine and Hamilton, 1983). The virus is an isometric particle of about 30 nm diameter which sediments as a single component and contains a single-stranded RNA. It has a very restricted host range and can be transmitted by beetle vector (*Ceratoma trifurcate* (Forster)) in a semi-persistent manner, seed and sap inoculation (Tremaine and Hamilton, 1983; Fuleratu, 2016).

Virions (the complete, infectious virus found outside a host cell, with a core of RNA or DNA and a capsid different from 'viroid' which is an infectious agent consisting solely of a strand of RNA and is capable of causing diseases) is composed of about 21 % nucleic acid and 79 % protein (Tremaine and Hamilton, 1983). The virions are made up of a single coat protein (approximately 30 kb), a genomic RNA, and one sub-genomic RNA (sgRNA) molecule (Tamm and Truve, 2000). The genomic RNA is a single-stranded messenger-sense molecule, approximately 4 to 4.5 kb in size (Tremaine and Hamilton, 1983; Hull, 1995; Tamm and Truve, 2000).

Replication of the virus does not require the presence of a helper virus and are normally found in the mesophyll, cytoplasm and the nuclei of the host cells (Fuleratu, 2016). The virus is reported to have several strains including the cowpea strain (strain C), Ghana strain (strain G) and severe bean mosaic strain or Mexican strain (strain M) (Sehgal, 1980; Tremaine and Hamilton, 1983). The cowpea strain of SBMV is one of the several viruses that cause important diseases of cowpea (Singh and Allen, 1979) with symptoms ranging from mosaic or mottling, chlorotic spots, systemic vein clearing and banding, leaf deformation and stunting (Sehgal, 1980;

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Fuleratu, 2016).

2.9.1.4 *Cowpea mottle virus* (CPMoV) genus *Carmovirus*

Cowpea mottle virus (CPMoV) was first reported in Nigeria in 1963 (Taiwo, 2003) and it is transmitted via sap and seed and also by beetles such as *Ootheca mutabilis* (Schönherr) and *Paraluperodes quaternius* (Fairmaire) (= Luperodes lineata Kars) (Shoyinka *et al.*, 1978; Odedara *et al.*, 2009; Aliyu and Balogun, 2011). The virions are non-enveloped with diameter of 30 nm containing 20 % nucleic acid and 80 % protein and sediments as a single component at velocities of 118–130 S and densities in CsCl of 1.35 ± 0.01 g cm^{-3} (Bozart and Shoyinka, 1979; Ke *et al.*, 2004). The genome is a positive sense single-stranded RNA of 4,029 nucleotides having six major open reading frames (ORFs) (You *et al.*, 1995; Gillaspie Jr. *et al.*, 1999). *Vigna unguiculata*, *Chenopodium amaranticolor* and *C. quinoa* Willd. are effective diagnostic hosts of the virus (Bozart and Shoyinka, 1979). CPMoV replication does not depend on a helper virus as well (Sehgal, 1980; Brunt *et al.*, 1996).

The virus is transmitted in the seed of cowpea at a rate of 10 % depending on the genotype or line of the crop and the time between infection and flowering (Bozarth and Shoyinka, 1979; Gillaspie Jr. *et al.*, 1999), but many lines produced no more than 0.4 % infected seeds (Gillaspie Jr. *et al.*, 1999). The virus is extremely virulent and could spread to healthy plants in the absence of a vector making it of great importance in the international exchange of Vigna germplasm (Bozarth and Shoyinka, 1979; Sastry, 2013). Yield loss recorded as a result of the virus is more than 75 % (Shoyinka *et al.*, 1997).

Symptoms of cowpea leaves infected by this virus include chlorosis, mosaic and mosaic-mottling (Ke *et al.*, 2004) leading to about 75 % yield loss in Nigeria (Robertson, 1963). Incidences of 9.8 %, 0 %, and 7.9 % and prevalences of 29.2 %, 0

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%, and 40.9 % respectively have been reported in Nigeria within a three-year period (Shoyinka *et al*., 1997; Taiwo, 2003).

2.9.1.5 *Cowpea mild mottle virus (CPMMV) genus Carlavirus*

Cowpea mild mottle virus CPMMV is a filamentous particle of about 650 × 15 nm consisting of a coat protein of 32-36 kDa (Tavassoli *et al*., 2007). CPMMV is a member of the genus *Carlavirus* that has been classified under the plant virus family *Betaflexiviridae* (Martelli *et al*., 2007; Tavassoli *et al*., 2007). It was first observed in Ghana by Brunt and Kenten (1973) (Taiwo, 2003) and then Jeyanandarajah and Brunt (1993) but has subsequently been reported in some tropical regions of Africa (Mink and Keswani, 1987), Asia (Shahraeen, 1989; Reddy, 1991), Brazil and Argentina (Almeida, *et al*., 2005; Laguna *et al*., 2006), Nigeria in 1980 (IITA, 1980) and from La Cote d'Ivoire in diverse range of plant species including leguminous and solanaceous food crops (Tavassoli *et al*., 2007).

The genome is made up of a single-stranded RNA with size of 2.5 × 106 with six open reading frames (ORF) (Tavassoli *et al*., 2007). The virions contain 5 % nucleic acid, 95 % protein and 0 % lipid and are found in the mesophyll, epidermis and palisade parenchyma of the cytoplasm of infected cells (Brunt *et al*., 1996). CPMMV is serologically unrelated to any member of the genus (Laguna *et al*., 2006), and some isolates differ from others by being transmitted by whiteflies (Brown and Rodrigues, 2014) and their ability to form brush-like inclusions in the infected tissue (Taiwo, 2003). The isolates from are transmitted by *Bemisia tabaci* in a semi-persistent manner (Taiwo, 2003) and it is seed borne.

Symptoms of CPMMV include necrotic lesions and chlorosis on the primary leaves and necrosis on the trifoliate leaves (Taiwo, 2003). CPMMV is of no importance in some parts of Nigeria since affect few cowpea genotypes but pose a threat to other

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legumes such as groundnut, which is usually intercropped with cowpea in Ghana (IITA, 1981; Taiwo, 2003).

2.9.1.6 *Cucumber mosaic virus* (CMV) genus *Cucumovirus*

Cucumber mosaic virus is a ubiquitous found in many countries and affects several plants. It is the type member of the *Cucumovirus* group and it is characterised by isometric particles of 28 nm diameter with a tripartite genome (Taiwo, 2003) each of which is enclosed inside a coat protein (Thottappilly and Rossel, 1985). It has a total genome size of 8.621 kb which is broken into three parts; the largest part is 3.389 kb; the second largest is 3.035 kb and the third largest is 2.197 kb (Francki *et al.* 1979: ICTVdB, 2006).

CMV can be transmitted through seeds and by sap inoculation as well as several aphid species that act as vectors in a non-persistent manner (Fisher and Lockhart, 1976; Gray, 1996). As soon as they invade the host plant, the virus inhibits the plant's ability to signal for gene silencing in other tissues thereby allowing CMV to invade further into the plant. CMV replicates in the cytoplasm and cell-to-cell movement is via the plasmodesmata whereas the phloem is utilized for long distance movement within the plant (Zitter and Murphy, 2009).

Local symptoms on inoculated leaves include poorly developed chlorotic areas or reddish necrotic rings whereas systemic symptoms include mild mottle and distortions (Anderson *et al.*, 1994). About 14% yield loss in the USA has been attributed to CMV, data on the effect of the virus on yield is not available (Pio-Riberio *et al.* 1978).

2.9.2 Resistance to viral diseases

Use of resistant plants is the cheapest and environmentally friendly approach to controlling virus diseases in cowpea. This prompted studies into the possible

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inheritance of resistance against some cowpea viral diseases including *Blackeye cowpea mosaic virus*, *Cowpea aphid-borne mosaic virus*, *Cowpea mosaic virus*, *Cowpea severe mosaic virus*, *Tobacco ringspot virus*, and *Southern bean mosaic virus* (Singh *et al*., 1997). A dominant gene has been identified to confer resistance to *blackeye cowpea mosaic virus* (Singh *et al*., 1997) as discovered earlier by Fery (1985). Patel *et al*. (1982) also reported on preliminary studies of the inheritance of a recessive gene with other modifier genes, which confers both immunity and resistance to a strain of cowpea aphid-borne mosaic virus from Tanzania (Singh *et al*., 1997).

Data published in three reports suggest that a single dominant gene controls resistance of cowpea to the *cowpea mosaic virus* (Singh *et al*., 1997) and a recessive gene, *ims*, was identified by Jimenez *et al.* (1989) to confer resistance to cowpea severe mosaic virus (Singh *et al*., 1997). The resistance of a Trinidad isolate of the virus is expressed as immune, tolerant and resistant found that resistance to a Trinidad isolate of the virus is expressed as immunity, tolerance, and resistant implying that three genes acting in a dosage-dependent manner may be controlling the traits (Umaharan, 1990; Singh *et al*., 1997).

Melton *et al*. (1987) also reported that two recessive genes, *sbc-l* and *sbc-2* confer resistance to southern bean mosaic virus-cowpea strain (Singh *et al*., 1997).

Table 2.2: Properties of some viruses infecting cowpeas

Virus name	Viral group	Viral shape	Virus size (nm)	Sap	Vector	Group	Seed%	Symptoms
CABMV	*Potyvirus*	Filamentous	750	+	Aphid	ssRNA(+) virus	0-40	DGVB
CPMV	*Comovirus*	Isometric	24	+	Beetle		0	DYMo
SBMV	*Sobemovirus*	Isometric	28	+	Beetle		3-4	VoC,M,Mo
CPMoV	*Carmovirus*	Isometric	30	+	Beetle		0-10	M,BoY
CPGMV	*Geminivirus*	Geminate	20x30	-	Whitefly		0	BoY
CMV	*Cucumovirus*	Isometric	28	+	Aphid		4-26	M,Mo,R
CPMMV	*Carlavirus*	Flexious rod	650	+	Whitefly		0-90	mM

Dark green vein-banding (DGVB), Distinct yellow mosaic (DYMo), Mottle (M), Vein clearing (VoC), Mosaic (Mo), Bright yellow (BoY), Ringspot (R) and Mild mottle (mM). Source; Taiwo, 2003.

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2.9.3 Estimating Disease Incidence and Severity

Assessment of diseases is one of the most difficult aspect of studying plant diseases (Afutu *et al*., 2017). It is the cornerstone of epidemic analysis.

Development of visible symptoms is the basis of recognising a diseased plant but the challenge is with the ability to quantify the disease (Campbell and Neher, 1994). The assessment of disease incidence - which is 'the percentage of diseased plants or parts in the sample or population of plants or percentage of diseased leaves on a plant, diseased stalks or a tiller or diseased seedlings in a field' (retrieved 3rd July, 2017 from; http://www.hillagric.ac. in/edu/coa/ppath/lect/plpath 111/Lect.%209. % 20P l %20Path%20111-%20MEASUREMENT%20OF%20DISEASE.pdf; Campbell and Neher, 1994) is an apparently simple counting task but has its limitations regarding how the data is interpreted (Campbell and Neher, 1994).

A more accurate method is to assess disease severity, which is 'amount of host tissues or organ showing the symptom of the disease andis a measure of the number and size of the lesions which is used to assess the extent of damage (retrieved 3rd July, 2017 from; http://www.hillagric.ac.in/edu/coa/ppath/lect/p lpath111/Lect. % 2 09.%20Pl%20Path%20111-%20MEASUREMENT%20OF%20DISEASE.pdf; Campbell and Neher, 1994). Measurement of symptoms of a disease is limited due to error in measurement and also requires a large sample size, which is representative of the sampling area for proper conclusions to be made (Campbell and Neher, 1994). There is also the problem of inconsistency in the scoring as different evaluators may score differently and there is a problem of scoring intermediate levels of the symptoms thereby making it difficult to estimate how close estimated severity is to actual the actual severity (Campbell and Neher, 1994). Observers tend to over-estimate the intensity of the disease when using a categorical scale of disease

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assessment especially at low levels of infection (Campbel and Neher, 1994).

In the current study, a modified scale by Hahn *et al*., 1980 and Gumedzoe *et al*. (1997) will be used to assess cowpea disease severity and incidence fortnightly. The numerical visual scale ranges from 1- 5 where 1 equals no symptoms and 5 referring to abundant lesions and to combine these repeated observations into a single value (Simko and Piepho, 2012), by calculating the area under the disease progress curve (AUDPC) using the trapezoidal method, to discretize the time variable (weeks) and calculate the average disease intensity between each pair of adjacent time points (Kharbika *et al*., 2015).

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CHAPTER THREE

3.0 ASSESSMENT OF GENETIC DIVERSITY AMONG 38 GENOTYPES OF COWPEA [*Vigna unguiculata* (L.) Walp].

3.1 INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important leguminous crop, which is grown in the tropics and subtropics (El Naim *et al.,* 2012; Gioi *et al.,* 2012; Doumbia *et al.,* 2014). The seeds and leaves serve as a major source of plant protein and vitamins for human and livestock as well as income for the farmer (El Naim *et al*., 2012; Imran *et al*., 2010; Mukhtar *et al*., 2010; Mbavai *et al*., 2015). It forms a close symbiotic association with soil bacteria Rhizobia, which fixes atmospheric nitrogen in poor soils hence able to adapt to poor marginal soils and is an excellent green manure as well as a good cover crop (Gioi *et al*., 2012).

About 5.4 million tonnes of dried grain is produced yearly in an area covering about 11.8 million hectares of which 91% is in West Africa (Doumbia *et al*., 2014). Nigeria is the leading cowpea growing country followed by Niger (FAO, 2010). Cowpea is consumed by over 70% of Ghana's population where it complements the staple starchy foods and serves as a cheap source of plant proteins, vitamins and minerals (MoFA, 2010; Doumbia *et al*., 2014). The crop serves as food security crop in Ghana because of its early maturing and long shelf life of the dried seeds (Doumbia *et al*., 2014). Despite the high demand, production of the crop is low and restricted mainly to the three Northern regions with average farm yield of about 0.4 t - 0.6 t ha^{-1} as compared to research fields yield of about 1.6 to 2.5t ha^{-1} (SARI, 2014). Ghana's dry cowpea seeds demand (169,000 t) exceeds production output which ranges from about 340 kg ha^{-1} to 400 kg ha^{-1} thus leaving a deficit of about 112, 000 t (FAO, 2000; Langyintuo *et al*., 2003; SARI, 2014: Yirzagla *et al*., 2016).

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The deficit in Ghana is attributed to some production constraints such as the lack of quality seeds which are high yielding and resistant to biotic and abiotic stresses which affect yield, pest and disease attacks, poor market value for the crop, among others (Adipala *et al*., 1997; Edema and Adipala 1996; Karungi *et al*., 2000; Kehinde *et al*., 2016).

In order to improve the production of the crop in Ghana, there is a need to assess the local and regional cowpea germplasms for diversities as well as identify duplicates which can help breeders in improving some local germplasms to be given to farmers to boost production and yield (Hegde and Mishra, 2009; Doumbia *et al.,* 2014). Identification of large variability among germplasms will provide good candidates for varietal improvement and decrease genetic erosion due to unfavourable abiotic and biotic stresses (Doumbia *et al*., 2014).

Some research works have been done on germplasm comparison in Ghana based on morphological characterization (Egbadzor *et al*. 2014; Doumbia *et al*. 2014). Morphological characterisation of germplasm is essential not only in establishing the description of each germplasm but also aids in detecting unique traits in the population structure for conservation purpose (Rees *et al*., 2003; Huamán, 1999). Earlier attempts by Egbadzor *et al.* (2014) and Doumbia *et al*. (2014) to characterise cowpea germplasms in Ghana was done using only morphological attributes hence there is a need to complement morphological characterization by either biochemical or molecular method to provide adequate information for comparison, identification and selection of genotypes for breeding. The use of molecular and biochemical breeding tools will help to overcome some of the limitations of breeding programmes by speeding up selection time of new varieties from crosses (Doumbia *et al*., 2014).

A range of molecular techniques have been used in assessing diversity among both

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the wild and cultivated cowpea such as Amplified Fragment Length Polymorphisms (AFLP) (Fatokun *et al*., 1997: Fang *et al.,* 2007; Doumbia *et al*., 2014); Chloroplast DNA Polymorphisms (Vaillancourt *et al.,* 1992); Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP), DNA Amplification Fingerprinting (DAF) and analysis of Simple Sequence Repeats (SSRs) (Ogunkanni *et al.,* 2008: Asare *et al.,* 2010) or Sequence Tagged Microsatellite Sites (Asare *et al*., 2010). Among these molecular techniques, SSRs markers have been the best because they are more informative, highly polymorphic, abundant, reproducible and inherited co-dominantly (Doumbia *et al*., 2012; Dib *et al*., 1996; Asare *et al*., 2010). SSRs have been applied in identification of genotypes, determination of seed purity, genetic mapping, marker assisted selection among others (Asare *et al*., 2010; Doumbia *et al*., 2012). Asare *et al*. (2010) determined genetic diversity among 141 cowpeas (*Vigna unguiculata* L. Walp.) collected from nine regions of Ghana using SSR markers alone but this study sought to assess genetic diversity among novel cowpea genotypes obtained from IITA, Nigeria using both morphological and molecular markers to augment local collections.

The objectives of this study were to:

I. Characterise 38 genotypes of cowpea using agro-morphological descriptors.

II. Assess genetic diversity among the cowpea genotypes using SSR markers.

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3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The research was carried out at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast. The location of the experimental site (5.1036^{O} N, 1.2825^{O} W) falls within the Coastal Savanna agro-ecological zone of Ghana. The experiment was conducted between June and September 2015 in the major season. The type of soil (found on the field) was sandy-clay-loamy soil, which belongs to the Benya series, and grouped as Typic Haplustult and Haplic Acrisols (Addo-Quaye *et al*., 2011; Agyeman *et al*., 2014).

3.2.2 Weather conditions

Cape Coast is in the coastal savanna agro-ecological zone hence experiences a bimodal rainfall. The major season begins from April to July with the maximum rainfall experienced in June whereas the minor season begins in September to November with the maximum in October (Table 3.1) (Addo-Quaye *et al*., 2011).

Table 3.1: Weather conditions recorded during the period of the field experiment.

Month	Temperature (^{o}C)		Precipitation/mm
	Max	Min	
June	32	25	219
July	29	23	123
August	28	22	23
September	26	21	87
October	33	25	133
November	34	25	71
December	34	30	81
January 2016	34	29	52

Source: http://www.whereandwhen.net/when/africa/ghana/cape-coast

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3.2.3 Soil Analysis

Soil samples were taken randomly from the experimental site to a depth of 0 – 30 cm using an auger. These samples were taken to the Soil Science Laboratory of the Soil Science Department, School of Agriculture, University of Cape Coast, to determine their chemical properties. The samples were dried and sieved using a 2 mm mesh and the following properties determined (Table 3.2).

Table 3.2: Mean Parameters of soil from the Experimental Site

Parameter	Quantity
pH	6.0
Available phosphorus	4.67mg/kg
Organic carbon	0.102%
Total Nitrogen	0.07%
Sand	95.4%
Silt	2.1%
Clay	2.5%

*Laboratory experiment

3.2.4 Planting materials

Thirty-eight (38) lines of cowpeas including 33 breeding lines which were identified or developed in breeding programs at the International Institute of Tropical Agriculture, Nigeria (IITA) and five (5) local varieties were studied (Table 3.3).

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Table 3.3: Description and source of cowpea genotypes used for the research

Genotype	Characteristic	Seed coat colour	Source
Apagbaala	Early maturing	White	SARI[2]
IT07K-243-1-2	Early maturing	Rough Brown	IITA
IT07K-298-9	Early maturing	White	IITA
IT07K-299-6	Early maturing	White	IITA
IT08K-125-107	Early maturing	White	IITA[1]
IT10K-836-2	Early maturing	Brown	IITA
IT10K-837-1	Early maturing	White	IITA
IT10K-843	Early maturing	White	IITA
IT10K-866-1	Early maturing	White	IITA
IT10K-973-1	Early maturing	White	IITA
IT10K832-3	Early maturing	Speckled White	IITA
IT11K-61-82	Early maturing	White	IITA
IT07-210-1-1	Medium maturing	White	IITA
IT07K-291-92	Medium maturing	White	IITA
IT07K-303-1	Medium maturing	White	IITA
IT08-125-100	Medium maturing	White	IITA
IT08-150-12	Medium maturing	White	IITA
IT08K-126-19	Medium maturing	White	IITA
IT08K-180-11	Medium maturing	White	IITA
IT09K-231-1	Medium maturing	Brown	IITA
IT10K-815-5	Medium maturing	Red	IITA
IT10K-817-7	Medium maturing	Red	IITA
IT10K-827-11	Medium maturing	White	IITA
Marfo tuya	Medium maturing	White	SARI
IT04K-321-2	Dual Purpose	White	IITA
IT07K-297-13	Dual Purpose	White	IITA
IT07K-298-15	Dual Purpose	White	IITA
IT08K-193-14	Dual Purpose	White	IITA
IT08K-193-15	Dual Purpose	White	IITA
IT09K-321-1	Dual Purpose	Brown	IITA
IT09K-456	Dual Purpose	Cream	IITA
IT10K-817-1	Dual Purpose	Red	IITA
IT10K-817-3	Dual Purpose	Red	IITA
IT10K-819-4	Dual Purpose[3]	Red	IITA
IT10K-834-3	Dual Purpose	Mottled Red	IITA
Padi tuya	Dual Purpose	White	SARI
GH 3684	*Striga*-resistant	Red	UCC/PGRRI[4]
SARC-1-57-1	Aphid-resistant	White	SARI

1. IITA- International Institute of Tropical Agriculture, Nigeria, 2. SARI- Savanna Agriculture Research and Resource Institute, Bawku, Ghana. 3. Dual Purpose- Grown for the seeds and the leaves, 4. UCC- University of Cape Coast and PGRRI- Plant Genetic Resource and Research Institute, Bunso, Ghana.

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3.2.5 Land preparation

The land was cleared using a cutlass and the thrashed weeds allowed to decay. Plots and subplots were demarcated using pegs and ropes.

3.2.6 Experimental design and field layout

The experimental outlay was based on the Randomised Complete Block Design (RCBD) with three replicates consisting of two blocks each with nineteen (19) plots of 3 m x 2 m. Each subplot consisted of four rows of cowpea plants. Three seeds were sown per hill at a spacing of 50 cm and 30 cm apart. The plots were separated by 1m alley and replicates were separated by 1.5 m alleys. Total field size was 75 m x 18 m (0.135 Ha).

3.2.7 Cultural practices

Thinning out was done two weeks after sowing, leaving two plants per hill. Weeding was done at four weeks intervals using a hoe and plants were watered using sprinkler irrigation when twice a week. Pesticide was applied once (before flower initiation) using an Emulsifier Concentrate (PAWA EC) at a rate of 35 ml per 15 L water in a knapsack sprayer.

3.2.8 Data Collection

Quantitative and qualitative data were collected on plants within the inner rows of each plot based on the comprehensive 'descriptors of Cowpea' according to Biodiversity International (1983) (See appendix 1).

3.2.8.1 Yield and yield components

When the pods dried and turned brown (Dugje *et al*., 2009), the two central rows on each subplot were harvested for the yield analysis. The pods were sampled and pod length, number of seeds per pod, 100-seed weight, and the seed yield per plot were computed.

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3.2.8.2 Pod length (cm)

A tape measure was used to determine the length of six randomly selected pods from each plot harvested.

3.2.8.3 Number of seeds per pod

The number of seeds per pod was determined by shelling the pods and counting the number of seeds in each of the six pods sampled for each plot.

3.2.8.4 100-seed weight (g)

The weight of randomly counted 100-seeds from threshed dried pods for each plot was determined by a weighing balance (PG203, Mettler Toledo, Switzerland).

3.2.8.5 Seed yield (kg ha^{-1})

Seed yield per hectare was determined by threshing the harvested plants from the central rows of each plot. These were put in labeled polythene bags and then weighed. The resulting weights, in grams per meter square were then extrapolated to obtain the average seed yield in kilogram per hectare.

3.2.9 Assessment of genetic diversity among the cowpea genotypes using SSR markers

3.2.9.1 DNA isolation using Whatman FTA (Flinders Technology Associates) Cards

The seeds of 38 cowpea genotypes were potted, and the young fresh leaves were plucked after two weeks, thoroughly washed with distilled water and blot dried with a paper towel. The leaves were surface sterilised using 75% v/v alcohol. The leaves were crushed by using a porcelain pestle and the sap collected onto FTA paper and dried for one hour at room temperature. After drying, a 2 mm sterile punch was used to obtain discs from the dried FTA paper. Discs were placed in 1.5 mL microfuge tubes and 200 µl of 75% alcohol added and vortexed for 5 minutes and decanted. This was repeated three times until the discs turned white. Hundred microliter (100

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μl) of FTA Purification Reagent was then added and incubated for 5 minutes at room temperature with moderate inversions. This was repeated twice, and the purification reagent was removed carefully using a pipette. The discs were then air dried for one hour and stored at -20 ^{O}C for subsequent use.

3.2.9.2 Polymerase Chain Reaction (PCR)

A set of 20 pairs of primers (Table 3.4) designed to amplify known Simple Sequence Repeats (SSR) regions in cowpea was used to analyse genetic diversity of 38 cowpea genotypes including local accessions and inbred lines based on modified protocol by Vos *et al.* (1995). PCR amplification was carried out in a 0.2 mL PCR tube with a reaction volume of 10 μL. The reaction contained 1 μL of 10x PCR buffer, 0.5 μl of each forward and reverse primer, 0.25 μl of dNTPs mix, 0.125 μL Taq DNA polymerase, 1 of the FTA disc DNA and 6.725 μL double distilled water. The tubes were then placed in a thermocycler programmed for initial denaturation at 94°C for 5 min followed by final denaturation for 30 s at 94°C, annealing temperature of 55°C for 30s, extension at 72°C for 1 minute, and a final extension of 10 minutes at 72°C.

3.2.9.3 Agarose gel electrophoresis

Gel electrophoresis was carried out using a modified protocol by Sambrook *et al.* (1989). A 0.8% (w/v) agarose gel was prepared by weighing 0.8 g of gel-grade agarose (Sigma) into 100 ml of 1 X TBE (Tris-Boric acid-EDTA) buffer melted in a microwave oven and allowed to cool to between 50 - 55°C. 3 μL of Ethidium Bromide solution (10 mg/mL) was then added to the molten agarose. The solution was poured into a horizontal gel electrophoresis tray mounted in a gel-casting tray fitted with two 20-tooth combs and allowed to solidify. The tray was removed from the gel-casting tray and placed in an electrophoresis tank filled with 1 x TBE to about 2 mm above the top of the gel.

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Table 3.4: Oligonucleotide primer pairs used in PCR amplifications to detect SSR Markers in the cowpea genotype.

Primer code	Primer sequences (5'–3')
SSR-6217	5'GGGAGTGCTCCGGAAAGT 3'
	5'TTCCCTATGAACTGGGAGATCTAT 3'
SSR-6218	5'GTGGAAGGAATGGGTCCAG 3'
	5'AGGAAATTTGCATTCCCTTGT 3'
SSR-6243	5'GTAGGGAGTTGGCCACGATA 3'
	5'CAACCGATGAAAAAGTGGACA 3'
SSR-6258	5'GGTTTCCTAGTTGGGAAGGAA 3'
	5'ATTATGCCATGGAGGGTTCA 3'
SSR-6265	5'CAGAAGCGGTGAAAATTGAAC 3'
	5'GCATGTTGCTTTGACAATGG3'
SSR-6277	5'CACCCCCGTACACACACAC 3'
	5'CACTTAAATTTTCACCAGGCATT 3'
SSR-6323	5'CAAAGGGTCATCAGGATTGG 3'
	5'TTTAAGCAGCCAAGCAGTTGT 3'
SSR-6336	5'TGAAAACAACGATATGCAGAAG 3'
	5'TCAGTCTTAGAATTGAGTTTTCTTCG 3'
SSR-6352	5'GTTGTGAGCTTCCCCAGATG 3'
	5'AATTTTGAACCCACCACCAG 3'
SSR-6353	5'TCATGGGTTAAATTTGCTTCAA 3'
	5'AAACCATGTGGTTGTTGCAC 3'
SSR-6356	5'TGCAATATGGACCAGAAGAAA 3'
	5'ATGCCCCAACAACAACATTT 3'
SSR-6370	5'CAACTTCACAGCCCTCAA 3'
	5'TTGAAGGTATGGCCTTTTGTTT 3'
SSR-6371	5'TGCTCATCGTGCTTTGTCTT 3'
	5'CACTTCAGACTTAGAGCGAAGAA 3'
SSR-6375	5'GCTCGGATATGGTCCTGAAA 3'
	5'TCAGTGTCAGCACCAT CCC 3'
SSR-6436	5'CAGAATCCTTGTGAACCTG 3'
	5'TTTCGCAATATGCCCTTTTC 3'
SSR-6451	5'AAAGAGATACACATGCCTAACA 3'
	5'GACCAACAGCGACTTTGAGC 3'
SSR-6587	5'GATATAGAATAGCATATTTAACATATTAG 3'
	5'GTTGAAAGTTTGATAGTAAAGTGG 3'
SSR-6603	5'GAGAACTTCACGCACAATAG 3'
	5'CGCGGTAGCATGATTGAATTTTG 3'
SSR-6608	5'CTAAATTATAATATTCGTCGGTC 3'
	5'GGTTAAGGAAAAGAGGGTAGG 3'
SSR-6613	5'CTATTGGAATCTTGCCGTTG 3'
	5'CTTTACCTTTATGCAAACCAATTC 3'

Source: Asare *et al*. (2010).

The combs were then removed carefully creating the wells. 10 μL of the PCR product was mixed with 2 μL loading dye (Bromophenol blue-Sigma) and loaded into the wells. The gel was run at a constant voltage of 100 V for one hour. The gel

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was then visualized under a high performance ultraviolet transilluminator (UVP, Cambridge, UK) and images captured with the aid of a camera. The size of DNA bands in base pairs was determined using the 100 bp DNA standard (Bioneer, Korea) marker.

3.2.10 Data Analysis

3.2.10.1 Phenotypic Data

The morphological data were analysed with GenStat 16th edition using general linear model (GLM) analysis of variance (ANOVA). Treatment means were compared for F-values showing significant difference using Duncan's Multiple Range Test (DMRT) at 5% level of probability. The linear correlation coefficient (r) among the overall means of cowpea genotypes and physiological parameters were calculated at 5%. Quantitative and Qualitative data were also further analyzed using PowerMarker, software version 3.25 (Liu *et a*l., 2005). Cluster analysis and construction of dendrogram were carried out using the Unweighted Pair-group Average Method with Arithmetic mean (UPGMA) to classify genotypes by their similarity based on Nei's Genetic Distances (Nei *et al.,* 1983) and observed in MEGA4. Principal component analysis was employed to assess the percentage contribution of each quantitative trait to variation among the genotypes.

3.2.10.2 Genotypic Data

Each SSR fragment was scored for its presence/absence (1/0), size and polymorphism using a 100bp DNA marker as a standard. Allele frequency and heterozygosity, polymorphism information content and genetic distances were also computed with the PowerMarker software version 3.25 (Liu *et al*., 2005). The cluster analysis and construction of dendrogram involved the Unweighted Pair-group Average Method with Arithmetic mean (UPGMA) to classify similar genotypes based on Nei's Genetic Distances (Nei *et al.,* 1983) and observed in MEGA4.

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3.3 RESULTS

3.3.1 Morphological characterization of 38 cowpea genotypes

3.3.1.1 Qualitative traits

3.3.1.1.1 Growth habit

The cowpea genotypes differed in growth habit with 58.7% exhibiting semi-erect growth habit (e.g. IT07K-210-1-1, IT08K-150-12, GH3684 and SARC-1-57-1) followed by acute erect (19.8%) (e.g. IT08K-125-100, IT09K-231-1, IT10K-817-7 and Marfo-tuya), erect (18.3%) (e.g. IT07K-298-9, IT10K-866-1, IT10K-817-7 and IT10K-827-11), prostrate (2.4%) (e.g. IT09K-456) and the least was climbing growth habit (0.8%) (e.g. IT07K-298-15) Fig 3.1.

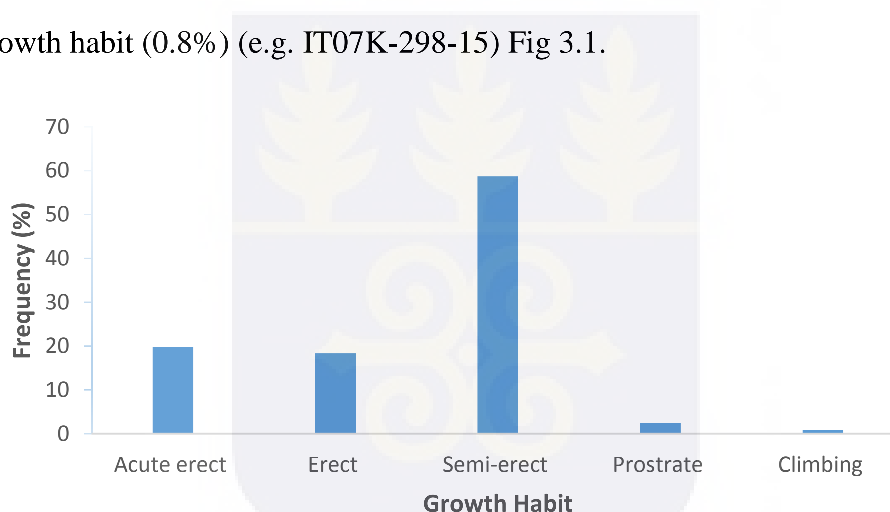

Fig 3.1: Variation in growth habit among 38 genotypes of cowpea

3.3.1.1.2 Raceme position

Fifty percent of the cowpea genotypes investigated had their raceme within the upper canopy, 26.2% mostly above the canopy and 23.8% found throughout the canopy.

3.3.1.1.3 Flower colour

Three main flower colours were observed among the cowpea genotypes. These were cream/white (61.9%), violet (11.1%) and violet-pink (27%) as shown in Fig 3.3.

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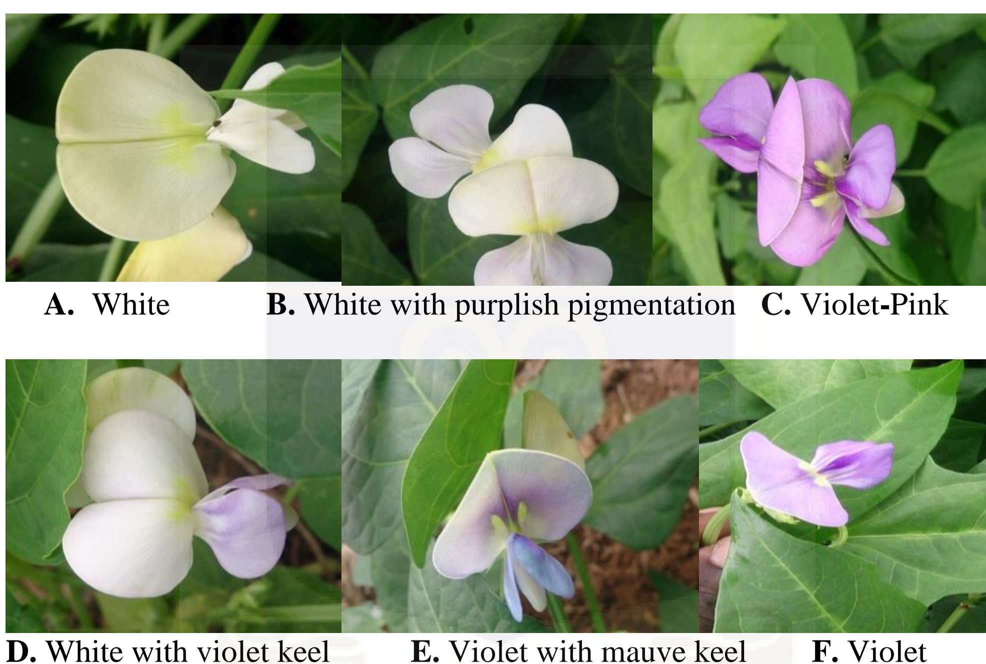
Fig. 3.2: Experimental plot at six weeks after sowing



A. White **B.** White with purplish pigmentation **C.** Violet-Pink

D. White with violet keel **E.** Violet with mauve keel **F.** Violet

Figure 3.3: Flower pigmentations observed among cowpea population.

3.3.1.1.4 Seed coat characters

Fifty percent of the cowpea genotypes had white seed coat colour, and 19% had cream, 19.1% had brown colour, and 11.9% were red.

3.3.1.2 Quantitative traits

The cowpea population exhibited varied agro-morphological characteristics. Among the 38 genotypes and local checks evaluated in the major season, differences in mean

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plant height, canopy diameter, number of branches, days to 50% flowering, days to pod maturity, and pod length were highly significant (P<0.05) (Table 3.4)

There were significant differences (P< 0.05) among the 38 cowpea genotypes for all parameters measured except the leaf area. SARC-1-57-1 recorded the highest mean plant height (42.44 cm) with the local check Apagbaala recording the lowest of 14.11 cm. IT10K-819-4 had the highest canopy diameter of 88.9 cm followed by SARC-1-57-1 with the canopy diameter of 79.4 cm while IT10K-843 recorded the least of 31.9 cm. The number of branches varied significantly (P < 0.05) among the various cowpea genotypes with SARC-1-57-1 producing the highest number of five (5) branches per plant. On the contrary, IT10K-817-7 had the least number of two (2) branches per plant. Significant (P <0.05) variation exists in the number of days to 50% flowering among the cowpea genotypes. Cowpea genotype, IT10K-836-2 recorded the least number of 36 days to 50 % flowering, and IT09K-231-1 recorded 45 days to 50 % flowering. Also, IT07K-297-13 took 66 days to reach pod maturity followed by Apagbaala, which took 65 days to reach pod maturity. SARC-1-57-1 was the first genotype to reach maturity at 53 days. There were significant differences (P < 0.05) in pod length and number of seeds per pod among the various cowpea genotypes. IT10K-819-4 had the longest pod of 21.08 cm but did not reflect the number of seeds per pod (9 seeds). Marfo tuya with a pod length of 14.5 cm produced the highest number of 15 seeds per pod. In all, IT07K-291-92 had the shortest pod length of 8.49 cm. IT07K-303-1with pod length of 11.41 cm yielded the least mean number of 7 seeds.

3.3.1.3 Yield characters

There was significant difference (P< 0.05) in 100-seed weight recorded among the cowpea population with the local control for the dual purpose genotype, Padi tuya, recording the highest of 22.66 g (Fig 3.4) followed by medium maturing genotype

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IT07K-827-11, 21 g (Fig 3.3). Marfo tuya that served as the local check for the medium maturing genotypes recorded the lowest 100-seed weight of 10.04 g. There was a significant difference (P < 0.05) in 100 seed weight with means of 18.09 g, 15.85 g, 12.81 g, 15.88 g and 18.29 g for Dual purpose, Early maturing, GH3684, Medium maturing and SARC-1-57-1 respectively.

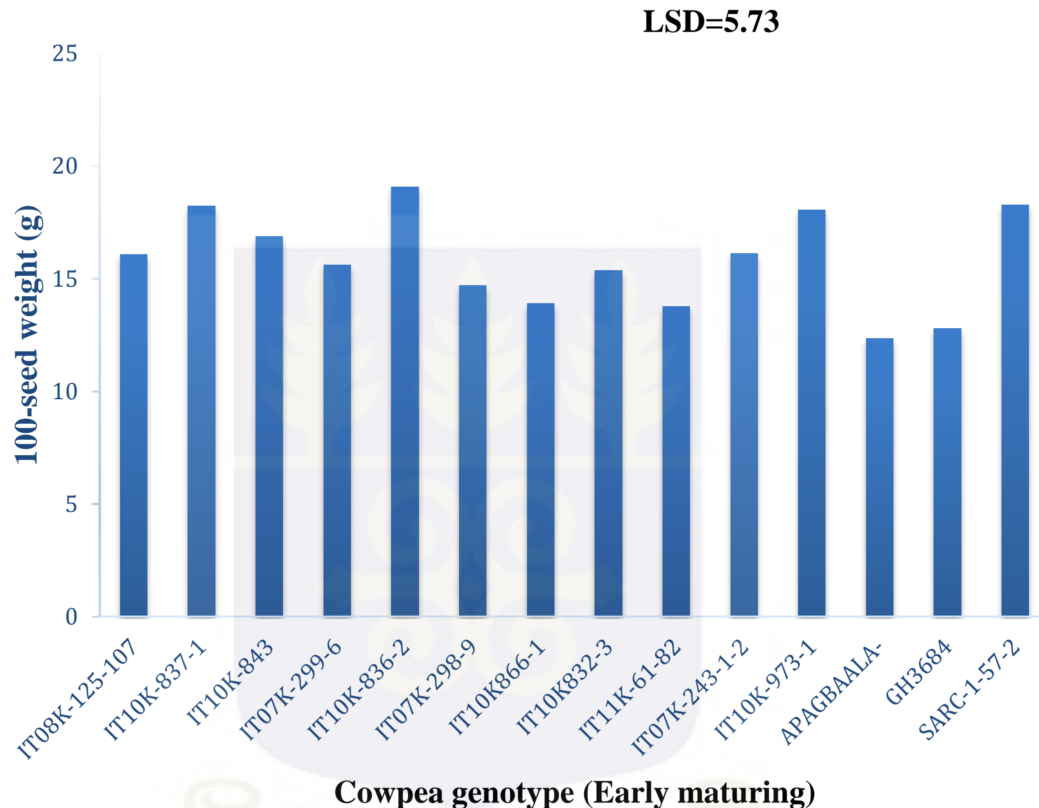


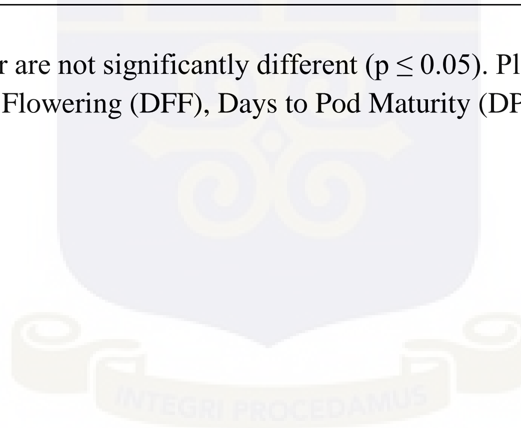
Fig. 3.4: Variation in hundred-seed weight among early maturing cowpea genotypes and the local checks.

Table 3.5: Variation in morphological characters evaluated on 38 cowpea genotypes during the major season.

Genotypes	Character	PH/cm	CD/cm	NB	LA/cm^2	DFF	DPM	PL/cm	SNP	SY
Apagbaala	Early maturing	14.1^{d}	42.0^{c}	2.67^{bc}	57.2	42.00^{bc}	64.67^{ab}	11.94^{d}	7.56^{bc}	1543^{ab}
GH3684	Striga resistant	32.07^{bc}	51.2^{bc}	3.59^{bc}	94.0	40.00^{cd}	60.33^{b}	17.14^{bc}	11.83^{ab}	**8010**^{a}
IT07K-243-1-2	Early maturing	27.58^{c}	47.5^{bc}	2.94^{bc}	58.9	40.67^{cd}	53.67^{c}	14.82^{dc}	9.44^{bc}	1733^{ab}
IT07K-298-9	Early maturing	22.92^{cd}	53.8^{bc}	3.06^{bc}	**127.6**	38.67^{d}	53.33^{c}	14.29^{dc}	10.56^{bc}	2100^{ab}
IT07K-299-6	Early maturing	27.31^{c}	50.1^{bc}	3.50^{ab}	81.3	38.00^{d}	56.33^{bc}	15.43^{c}	10.17^{bc}	2077^{ab}
IT08K-125-107	Early maturing	26.92^{c}	63.6^{bc}	4.06^{ab}	73.1	42.00^{bc}	58.00^{bc}	15.47^{c}	5.94^{c}	**4010^{ab}**
IT10K-832-3	Early maturing	22.28^{cd}	62.7^{bc}	3.56^{ab}	88.5	44.67^{ab}	60.00^{bc}	13.87^{dc}	9.67^{bc}	3277^{ab}
IT10K-836-2	Early maturing	21.50^{cd}	58.4^{bc}	3.44^{ab}	69.5	**35.67^{e}**	56.00^{bc}	14.95^{dc}	12.00^{ab}	3257^{ab}
IT10K-837-1	Early maturing	21.34^{cd}	55.2^{bc}	3.72^{ab}	59.0	40.67^{cd}	54.33^{c}	13.83^{dc}	6.33^{c}	3483^{ab}
IT10K-843	Early maturing	16.32^{d}	39.1^{c}	2.89^{bc}	40.0	43.67^{ab}	60.00^{bc}	14.87^{dc}	5.28^{c}	3150^{ab}
IT10K-866-1	Early maturing	20.97^{cd}	78.9^{ab}	4.17^{ab}	62.7	40.00^{cd}	59.33^{bc}	14.18^{dc}	9.00^{bc}	1313^{b}
IT10K-973-1	Early maturing	14.69^{d}	46.8^{bc}	2.44^{bc}	65.2	39.33^{cd}	55.33^{bc}	15.41^{c}	8.28^{bc}	3057^{ab}
IT11K-61-82	Early maturing	27.17^{c}	66.0^{b}	3.94^{ab}	97.6	39.33^{cd}	55.67^{bc}	14.93^{dc}	12.22^{ab}	3207^{ab}
IT07-210-1-1	Medium maturing	22.17^{cd}	69.5^{ab}	3.39^{ab}	59.6	41.00^{c}	63.00^{ab}	16.14^{c}	10.17^{bc}	4063^{ab}
IT07K-291-92	Medium maturing	23.00^{cd}	52.1^{bc}	3.72^{ab}	123.5	41.67^{bc}	57.33^{bc}	8.49^{e}	9.27^{bc}	1350^{ab}
IT07K-303-1	Medium maturing	23.78^{cd}	59.0^{bc}	2.89^{bc}	53.2	41.67^{bc}	56.33^{bc}	11.41^{d}	6.50^{c}	1873^{ab}
IT08-125-100	Medium maturing	24.26^{cd}	55.0^{bc}	2.50^{bc}	77.4	40.67^{cd}	62.67^{ab}	13.61^{dc}	8.83^{bc}	2513^{ab}
IT08-150-12	Medium maturing	27.03^{c}	61.4^{bc}	2.89^{bc}	88.9	41.33^{bc}	60.33^{b}	14.97^{dc}	9.94^{bc}	**6223^{ab}**
IT08K-126-19	Medium maturing	23.88^{cd}	60.2^{bc}	3.83^{ab}	72.3	43.33^{ab}	57.33^{bc}	14.62^{dc}	9.89^{bc}	3463^{ab}
IT08K-180-11	Medium maturing	24.42^{cd}	57.9^{bc}	3.06^{bc}	70.4	44.33^{ab}	59.67^{bc}	12.37^{d}	6.28^{c}	2767^{ab}
IT09K-231-1	Medium maturing	23.17^{cd}	53.0^{bc}	2.22^{bc}	40.8	**45.33^{a}**	62.33^{ab}	14.26^{dc}	11.94^{ab}	3600^{ab}
IT10K-815-5	Medium maturing	26.42^{c}	72.9^{ab}	4.44^{ab}	70.6	40.33^{cd}	55.33^{bc}	16.69^{bc}	9.61^{bc}	4400^{ab}
IT10K-817-7	Medium maturing	22.15^{cd}	58.6^{bc}	**1.56^{c}**	71.8	43.33^{ab}	63.00^{ab}	19.09^{b}	9.06^{bc}	2153^{ab}
IT10K-827-11	Medium maturing	16.34^{d}	41.8^{c}	3.06^{bc}	60.7	40.33^{cd}	58.67^{bc}	13.54d^{c}	8.61^{bc}	2290^{ab}
Marfo tuya	Medium maturing	28.67^{bc}	69.0^{ab}	3.11^{bc}	102.0	42.67^{bc}	60.67^{b}	14.5^{dc}	15.06^{a}	4747^{ab}
IT04K-321-2	Dual purpose	31.8^{bc}	63.3^{bc}	4.67^{ab}	56.2	41.67^{bc}	61.00^{ab}	14.06^{dc}	9.77^{bc}	3370^{ab}
IT07K-297-13	Dual purpose	29.97^{bc}	63.0^{bc}	4.78^{ab}	87.4	39.33^{cd}	**66.33^{a}**	14.52^{dc}	9.33^{bc}	4033^{ab}
IT07K-298-15	Dual purpose	26.39^{c}	67.2^{b}	3.06^{bc}	125.4	39.33^{cd}	61.67^{ab}	15.46^{c}	8.44^{bc}	4707^{ab}
IT08K-193-14	Dual purpose	22.78^{cd}	63.2^{bc}	4.89^{ab}	74.0	44.33^{ab}	62.33^{ab}	15.57^{c}	10.17^{bc}	6867^{ab}
IT08K-193-15	Dual purpose	26.11^{c}	63.9^{bc}	3.67^{ab}	97.6	42.33^{bc}	61.67^{ab}	16.53^{bc}	12.39^{ab}	5717^{ab}

Table 3.5 cont'd

IT09-456	Dual purpose	28.52^{bc}	65.0^{bc}	4.12^{ab}	55.4	43.33^{ab}	59.00^{bc}	15.72^{c}	10.37^{bc}	6770^{ab}
IT09K-321-1	Dual purpose	26.22^{c}	62.8^{bc}	3.17^{bc}	98.6	44.33^{ab}	64.00^{ab}	15.18^{dc}	11.67^{ab}	5697^{ab}
IT10K-817-1	Dual purpose	26.73^{c}	74.1^{ab}	3.28^{b}	82.7	40.00$^{c.d}$	57.33^{bc}	17.82^{bc}	11.11^{b}	5073^{ab}
IT10K-817-3	Dual purpose	23.21^{cd}	69.4^{ab}	4.22^{ab}	70.2	41.00^{c}	60.33^{b}	16.66^{bc}	12.00^{ab}	5713^{ab}
IT10K-819-4	Dual purpose	30.83^{bc}	**88.9^{a}**	3.33^{ab}	71.1	40.00^{cd}	61.00^{ab}	**21.08^{a}**	8.78^{bc}	5627^{ab}
IT10K-834-3	Dual purpose	31.5^{bc}	68.5^{ab}	3.61^{ab}	120.9	40.67^{cd}	62.67^{ab}	15.78^{c}	11.72^{ab}	**7040^{ab}**
Padi tuya	Dual purpose	31.72^{bc}	69.2^{ab}	3.17^{bc}	86.9	39.33^{cd}	59.67^{bc}	15.97^{c}	9.5^{bc}	5027^{ab}
SARC-1-57-1	Aphid resistant	**41.00^{a}**	77.10^{ab}	4.61^{ab}	81.43	39.00	53.33	15.42	9.03^{bc}	5593^{ab}
Grand mean		26.27	61.4	3.53	82.4	41.08	59.37	15.14	9.75	**4022**
P-value		<. 001	0.002	0.007	0.081	<.001	<.001	<.001	0.002	<.001



Means in same column followed by same letter are not significantly different ($p \leq 0.05$). Plant Height (PH), Canopy Diameter (CD), Number of Branches (NB), Leaf Area (LA), Days to 50% Flowering (DFF), Days to Pod Maturity (DPM), Pod Length (PL), Number of Seeds per Pod (SNP) and Seed Yield (SY).

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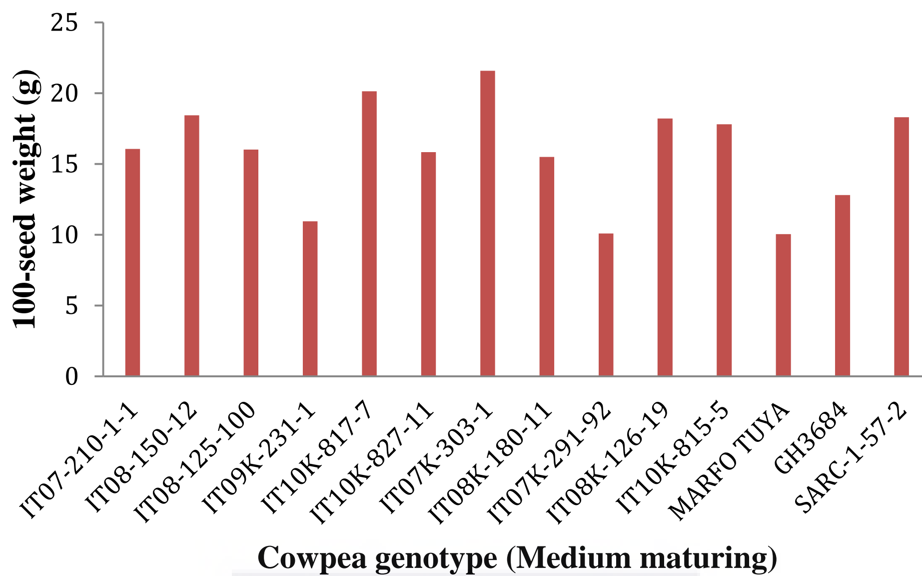

Fig. 3.5: Variation in hundred seed weight among medium maturing cowpea genotypes and the local checks.

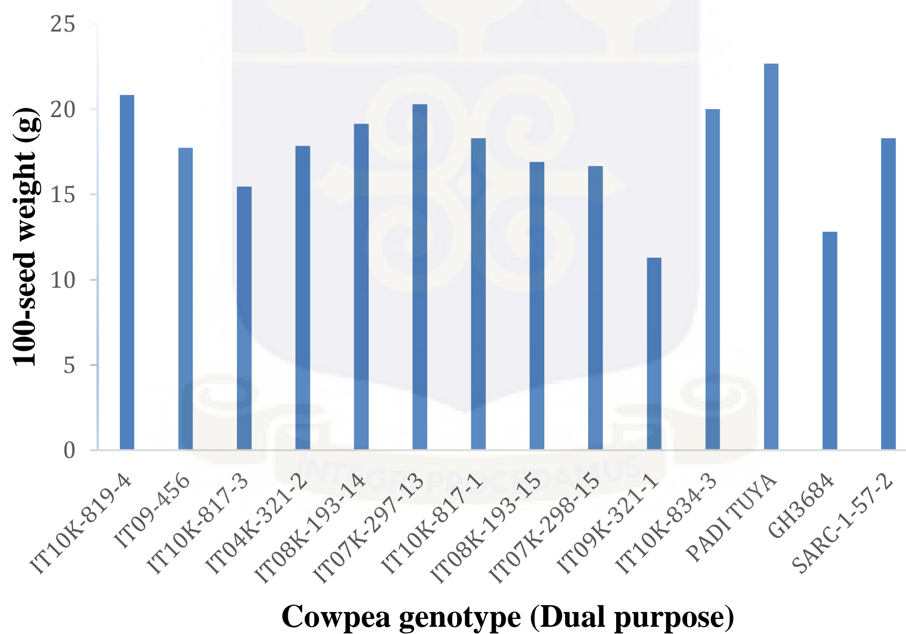

Fig. 3.6: Variation in hundred seed weight among dual purpose cowpea genotypes and the local checks.

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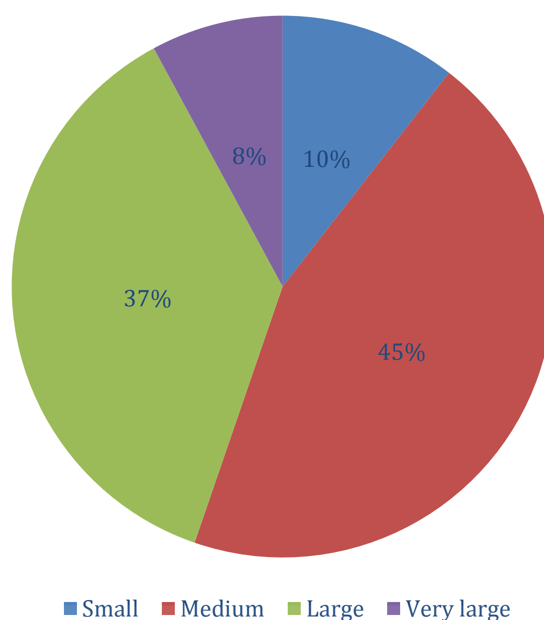

Fig. 3.7: Variation in seed size among 38 cowpea genotypes.

Figure 3.3 compares the 100-seed weight among the early maturing cowpea genotypes and the local checks. IT10K-836-2 had the highest 100-seed weight of 19.09 g whereas the local check Apagbaala gave the lowest 100-seed weight of 12.11 g. Among the medium maturing genotypes, IT07K-303-1 had the highest seed weight of 21.58 g followed by IT10K-817-7 with seed weight of 20.13 g. The local check Marfo Tuya recorded the lowest seed weight of 10.04 g (Fig. 3.4). Another local check Padi tuya recorded the highest seed weight among the dual purpose genotypes (Fig. 3.5) with a weight of 22.66 g. IT10K-819-4, IT07K-297-13 and IT07K-297-13 also recorded high seed weights of 20.82 g, 20.27 g and 20.01 g respectively. IT09K-321-1 also recorded the least 100-seed weight of 11.28 g. Differences among the various genotypes and the local checks were significant (P< 0.05). Out of the 38 cowpea genotypes, 10% were small size, 45% were medium sized, 37% large size and 8% very large size (Figure 3.6). There was evidence in seed shapes, colour and texture among the cowpea population (Fig 3.7).

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A.IT10K-819-4 (Red) **B.**IT07K-298-15 (DP) **C.** IT11K-61-82 (EM)

D. IT10K-832-3 (EM) **E.** IT07K-243-1-2 (White) **F**. IT09K-321-1 (Brown)

G. IT10K-834-3 (DP) **H.** IT09K-456 (Cream) **I.** IT08K-180-11 (MM)

Fig 3.8: Seed coat variation among the cowpea genotypes

White seeds with brown eyes or black eyes to brown seeds and red seeds with varying seed coat characteristics were observed among the cowpea population. IT09K-546, a dual purpose genotype had a cream seed coat colour with brown eyes. IT10K-832-3 showed some amount of 'dirty' brown speckling colour (Figure 3.7).

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3.3.1.4 Cluster Analysis

The results of cluster analysis constructed using Neil's method based on 23 parameters are presented in Fig. 3.8. The 38 cowpea genotypes were grouped into two major clusters when the dendrogram was cut at a dissimilarity of 38%. Cluster I was the least weighted comprising one genotype (IT10K-817-1), which is early maturing and has a wide canopy, broad leaves, long pods and moderately large seeds. Cluster II comprised 37 cowpea genotypes made up of four-member out-group consisting of the aphid resistant genotype (SARC-1-57-1), *Striga* resistant genotype (GH3684) and two medium maturing genotypes (IT09K-231-1 and IT08K-126-19). Cluster II is further grouped into six sub-clusters with the first consisting of two genotypes (IT07K-298-15 and IT10K-817-3) which are both dual purpose cowpea genotypes and have similar characteristics of broad leaves and canopy diameter. The second sub-cluster consists of six medium maturing genotypes and one dual purpose line. Third and fourth sub-clusters comprised five and seven early maturing genotypes. The fifth sub-cluster comprises one medium maturing and three dual purpose genotypes while the sixth sub-cluster comprised three medium maturing and five dual purpose genotypes.

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3.3.1.5 Principal Component Analysis

The results of the MINITAB-mode principal component analysis (PCA) are presented in Table 3.5. Five principal components were obtained based on Eigen values greater than 1. The first principal component (PC1) explained 23.4% of the total variance observed and this was correlated to variation in eye colour and seed texture. Second principal component (PC2) accounted for 21.7% of the variations with pods per peduncle, eye colour, seed shape and eye pattern being the traits with the highest loadings. The third (PC3) accounted for 12.2% of the variation and was highly associated with eye colour and seed shape. The fourth (PC4) accounted for 9.8% of the variation and was associated with number of pods per peduncle, eye colour, seed shape and eye pattern and the fifth (PC5) accounting for 5.1% of the variation with the pods per peduncle, eye colour, leaf colour and eye pattern having the highest loading.

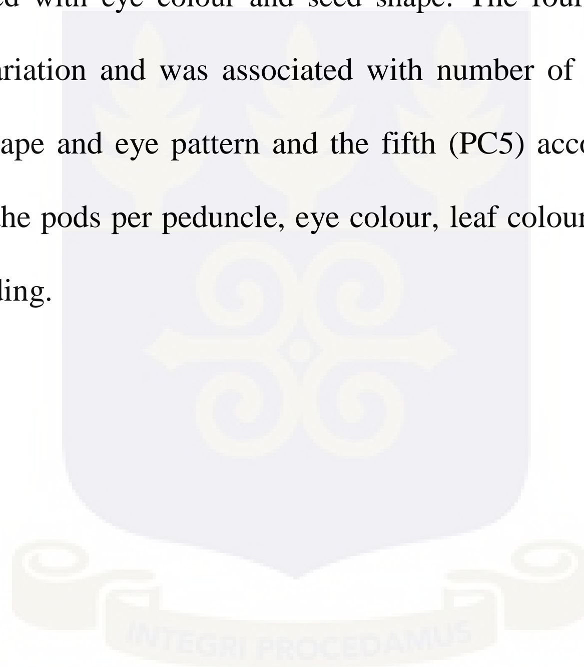

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Table 3.6: Four-factor model explaining 62.28% of the total variance for traits

Variables	PC1	PC2	PC3	PC4	PC5
GH	0.019	-0.002	0.002	-0.020	0.039
PL	0.021	-0.010	-0.022	0.040	-0.014
CD	-0.003	0.021	0.001	0.022	0.000
TLA	0.025	0.012	0.055	-0.037	-0.081
PoL	-0.004	-0.004	-0.010	0.000	-0.032
NSP	-0.002	-0.011	-0.030	0.025	-0.066
NB	0.019	0.019	0.005	0.045	0.006
TPW	0.005	0.110	-0.040	0.031	-0.126
TSW	-0.001	0.115	-0.037	0.047	-0.114
DFF	-0.004	0.005	0.000	0.002	-0.002
DPM	0.001	0.008	-0.002	-0.007	-0.013
HSW	-0.021	0.037	0.020	-0.006	-0.011
PPP	-0.109	-0.215	0.126	**0.461**	**0.711**
FC	0.030	-0.021	-0.014	-0.026	-0.047
SCC	0.028	-0.057	-0.087	0.006	0.097
PC	0.013	-0.031	-0.004	-0.067	0.009
RP	0.012	0.125	-0.002	0.018	-0.126
TLS	0.016	0.040	-0.141	-0.083	-0.062
EC	0.299	-0.668	0.485	0.243	-0.384
SS	-0.016	-0.458	**-0.830**	0.218	-0.162
LC	-0.168	-0.004	0.071	-0.222	-0.336
ST	**-0.930**	-0.192	0.137	0.038	-0.137
EP	-0.023	0.459	0.012	**0.780**	-0.338
Eigen Value	4.646	4.148	2.325	2.325	2.325
Percentage of total variance	24.300	21.700	12.200	9.800	5.100
Cumulative percentage of variance	24.3	46.1	58.2	68.1	77.3

Growth habit (GH), plant length (PL), canopy diameter (CD), terminal leaf area (TLA), pod length (PoL), number of seeds per pod (NSP), number of branches (NB), total pod weight (TPW), total seed weight (TSW), days to fifty percent flowering (DFF), days to pod maturity (DPM), hundred seed weight (HSW), pods per peduncle (PPP), flower colour (FC), seed coat colour (SCC), pod colour (SCC), raceme position (RP), Terminal leaf shape (TLS), eye colour (EC), seed shape (SS), leaf colour (LC), seed texture (ST) and eye pattern (EP).

3.3.2 Molecular Characterization of 38 cowpea genotypes using SSR Markers

Of the twenty pairs of SSR primers used to assess genetic diversity among the 38 cowpea genotypes, eleven (55%) (SSR primers) did not show any polymorphism (across the genome of the 38 cowpea genotypes) and therefore, they were excluded from the analysis. The remaining nine primers generated 285 bands across the

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genome of the 38 cowpea genotypes with sizes of amplified allelic loci ranging from 280 to 520 bp. The primer pair, SSR-6258 amplified the highest number of DNA polymorphic bands, and the lowest number of DNA polymorphic bands was observed for SSR-6218 (17). The number of alleles varied from 4 (SSR-6218, SSR-6258 and SSR-6323) to 10 (SSR-6375). Allele frequency ranged from 0.2368 (SSR-6375) to 0.5526 (SSR-6218) with a mean of 0.4035. Polymorphic information content (PIC), which used to assess the allele diversity at a specific locus, varied from 0.5276 (SSR-6218) to 0.8285 (SSR-6375) with a mean of 0.6683. Gene diversity ranged from 0.5928 in SSR-6218 to 0.8463 in SSR-6375 with an average of 0.7124 as shown in Table 3.7.

Table 3.7: Assessment of genetic diversity among 38 cowpea genotypes using nine cowpea primers resolved on 2% agarose gel.

Primer	Sample size	Allele Frequency	Allele No.	Availa bility	Genetic Diversity	PIC	No. of Bands
SSR-6218	38.00	0.55	4.00	1.00	0.59	0.53	17
SSR-6243	38.00	0.53	6.00	1.00	0.65	0.62	19
SSR-6258	38.00	0.50	4.00	1.00	0.64	0.58	51
SSR-6323	38.00	0.42	4.00	1.00	0.63	0.56	22
SSR-6356	38.00	0.34	6.00	1.00	0.76	0.73	35
SSR-6375	38.00	0.24	10.00	1.00	0.85	0.83	34
SSR-6436	38.00	0.34	8.00	1.00	0.77	0.73	41
SSR-6451	38.00	0.29	8.00	1.00	0.80	0.77	32
SSR-6587	38.00	0.42	6.00	1.00	0.72	0.68	34
Mean	38.00	0.40	6.22	1.00	0.71	0.67	31.67

3.3.2.1.1 Cluster Analysis

The dendrogram (Fig 3.7) illustrates the combined data obtained from the nine polymorphic primers, which grouped the genome of the 38 cowpea genotypes into two major clusters at about 40 % dissimilarity coefficient. Cluster 1 was made up of 34.2 % of the cowpea genotypes, which was further separated into two sub-clusters (1A and 1B) with 1A consisting of two early maturing genotypes IT11K-61-82 and IT07K-299-6 at a dissimilarity coefficient of about 17 %. The second branch

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consisted of two early maturing genotypes and the local check, Apagbaala with IT07K-243-1-2 delineating itself at about 12 % dissimilarity coefficient. Cluster 1B also regrouped into two sub-clusters with the first group consisting of a local check Marfo tuya that delineated itself at dissimilarity coefficient of 24 % from the other genotypes. This group also contained two medium maturing genotypes, IT09K-456 and IT10K-819-4, which had the least coefficient of dissimilarity of about 5 %. The second group consisted of three dual purpose genotypes and a medium maturing genotype.

Cluster 2A has two sub-groups with the first consisting of the Striga resistant GH3684 which delineated itself from the other two genotypes at dissimilarity coefficient of 31%. The other subgroup consisting of IT10K-832-3 and the aphid resistant genotype SARC-1-57-2 that grouped together at about 23% dissimilarity coefficient. The second sub-cluster consists of IT10K-843 and IT08K-125-100 which both delineated themselves at 35% and 31.5% dissimilarity coefficients respectively.

Cluster 2B is made up of 15 genotypes which are sub-grouped into three comprising six medium maturing, eight dual purpose and a local check for dual purpose (Padi Tuya) at 37% dissimilarity index and regrouped into two at dissimilarity index of 38%. The majority of the dual purpose genotypes were found in cluster 2 and 1 with eight and three respectively. IT07K-299-6 and IT07K-210-1-1 were the most widely separated among the cowpea genotypes.

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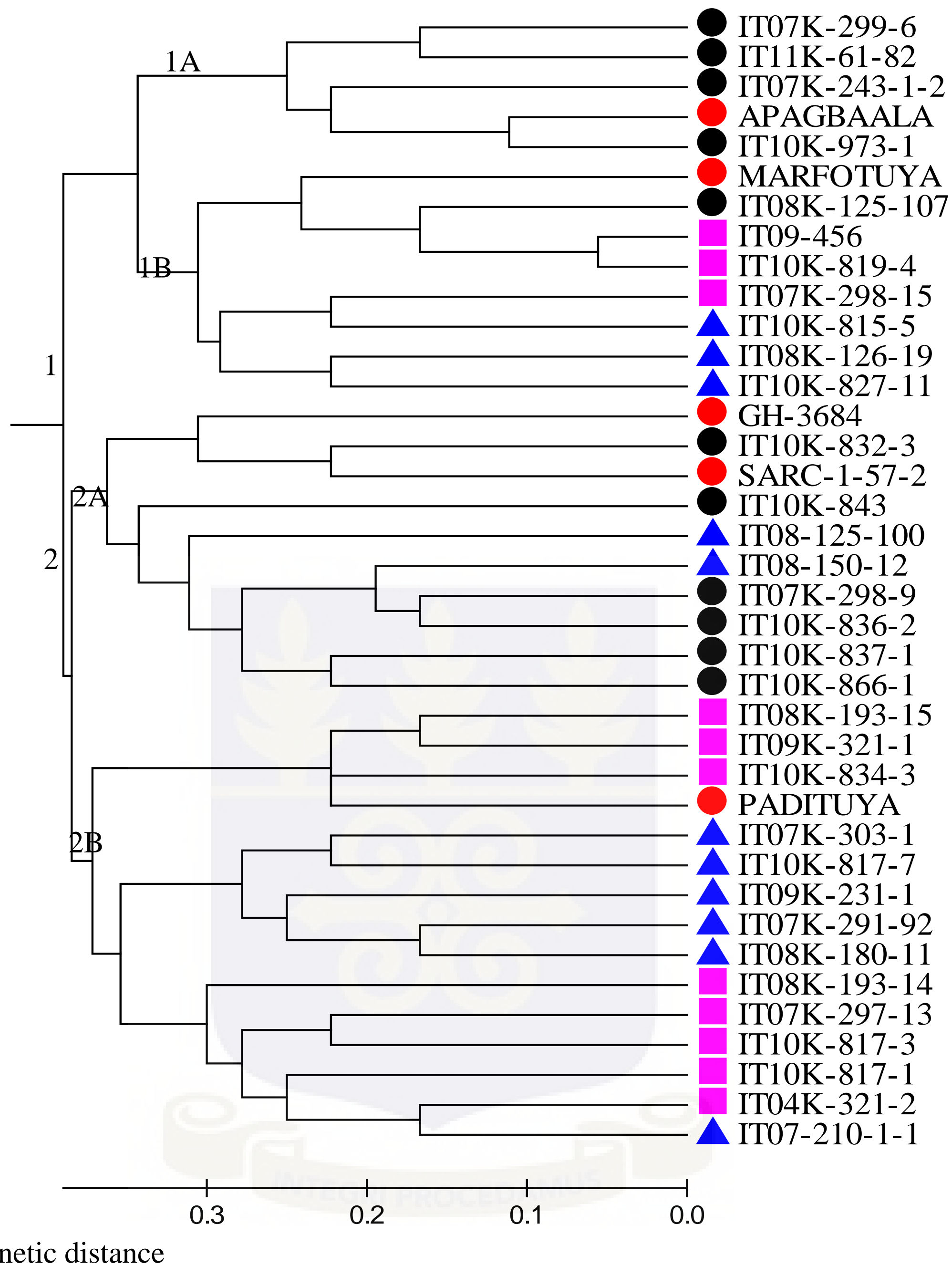

Genetic distance

Fig. 3.10: A dendrogram showing the relationship among 38 cowpea genotypes generated using ninepolymorphic SSR markers and the sequential clustering algorithm (UPGMA) based on genetic similarity (Nei andLi, 1983)

The cowpea genotypes are defined as in Table 3.2. The key to the groupings is as follows:

Medium maturing — Dual purpose

Early maturing — Local check

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3.3.3 Correlation Analysis

Table 3.8 represents correlation coefficient value among twelve (12) quantitative traits of the various cowpea genotypes. Total pod weight and total seed weight showed very high positive or negative correlation among all the traits (0.985). Canopy diameter and plant height (0.58), canopy diameter and number of branches (0.58), pod length and number of seeds per pod (0.53) were moderately positively correlated among all the traits. Number of branches and plant height (0.45), total pod weight and canopy diameter (0.32), total seed weight and canopy diameter (0.32), total pod weight and pod length (0.41), total seed weight and pod length (0.40), total pod weight and number of seeds per pod (0.41), total seed weight and number of seeds per pod (0.35), total pod weight and number of branches (0.41), total seed weight and number of branches (0.41) showed low positive/negative correlation with all the traits.

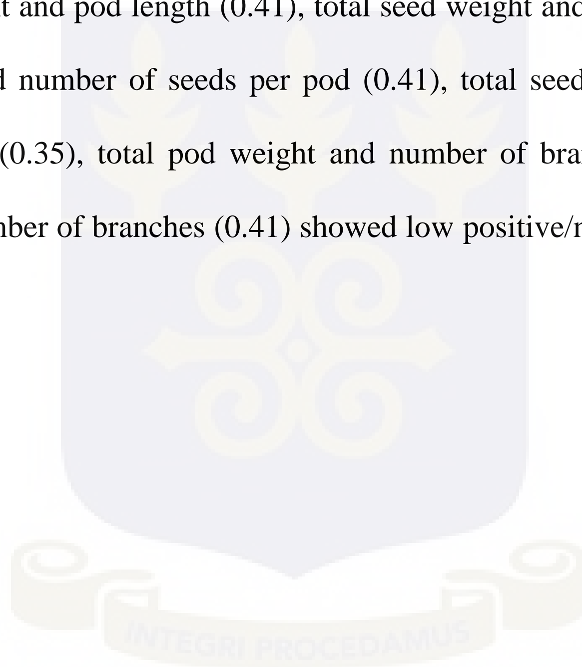

Table 3.8: Association of twelve (12) quantitative traits among 38 cowpea genotypes

	PH	CD	LA	PL	NSPP	NB	TPW	TSW	FPF	DPM	HSW	PPP
CD	0.576**											
LA	0.223	0.268										
PL	0.149	0.159	0.194									
NSPP	0.079	0.046	0.101	0.530								
NB	0.448*	0.576**	0.068	-0.039	0.008							
TPW	0.214	0.316	0.148	0.414	0.414	0.414						
TSW	0.224	0.321	0.137	0.402	0.352	0.408	0.985***					
FPF	-0.247	-0.186	-0.147	-0.117	-0.078	-0.110	0.085	0.079				
DPM	-0.169	-0.155	-0.097	0.025	-0.005	-0.198	-0.069	-0.086	0.248			
HSW	0.124	0.206	0.084	0.283	-0.069	0.024	0.138	0.151	-0.173	-0.036		
PPP	0.149	-0.155	0.006	0.144	0.188	-0.110	-0.022	-0.056	-0.149	0.068	-0.252	-0.313

Plant height (PH), canopy diameter (CD), leaf area (LA), pod length (PL), number of seeds per pod (NSPP), number of branches (NB), total pod weight (TPW), total seed weight (TSW), days to pod maturity (DPM), hundred seed weight (HSW) and pods per peduncle (PPP).

Size of correlation	**Interpretation**
±1	Perfect positive/Negative correlation
±.90 to±.99	Very high positive/ Negative correlation
±70 to ±.90	High positive/Negative correlation
±.50 to ±.70	Moderate positive/ Negative correlations
±.30 to ±.50	Low positive/Negative correlation
±.10 to ±.30	Very low positive/ Negative correlation
±.00to±.1	Poor positive/Negative correlation

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3.4 DISCUSSIONS

3.4.1 Phenotypic characterization

Accurate description of cowpea genotypes is crucial towards making a decision to release a variety as well as conservation of germplasm. The identities of 38 cowpea genotypes were established by using morphological characteristics described as cowpea descriptors (Makanur *et al.,* 2013). Both quantitative and qualitative parameters considered in the current work showed variations among the 38 cowpea genotypes.

Variation in growth habit was evident among the cowpeas. The semi-erect growth habit was predominantly expressed in 58.7 % of the cowpea genotypes studied. However, 19.8% had acute erect growth habit, 18.3 % erect growth habit, 2.4 % prostrate growth habit and the least was climbing growth habit, 0.8 %. The early maturing cowpea genotypes exhibited only semi-erect and acute erect forms of growth habit. Cobbinah *et al.,* (2011) also observed 30.6 % acute erect growth habit, 22.4 % intermediate growth habit, 19.4 % semi-prostrate, 17.2 % prostrate growth habit, 0.8 % erect growth habit and 9.7 % also showing semi-erect growth habit. The differences observed may be as a result of genetic variation (varietal differences) as well as environmental differences (growing conditions).

Cowpea exhibit growth habits ranging from climbing, prostrate, semi-prostrate, intermediate, semi-erect, erect and acute erect (IBPGR. 1982). Five of these growth habits were expressed among the cowpea genotypes in this study maybe due to the low number of genotypes used compared to that used by IBPGR. Growth habit is a very important feature of cowpea plants because of its influence on harvesting of the crop (Cobbinah *et al.,* 2011). Prostrate cowpea is difficult to harvest by hand as one

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is expected to bend down very low to harvest the pods compared to the erect and semi-erect genotypes (Aryeetey, 1971; Cobbinah *et al.,* 2011).

Raceme position is a good attribute since those that are held above the canopy aids easy visibility of pods for harvesting using mechanical tools as compared to those held within canopy (Pandey and Ngarm, 1985; Cobbinah *et al*., 2011) In the current research, 26.2 % of the cowpea genotypes studied had raceme above the canopy, 50 % in the upper canopy and 23.8 % throughout the canopy. On the other hand, Cobbinah *et al.* (2011) reported 59.7 % accessions with the raceme position above the canopy, 29.8 % at the same level as the canopy and 10.4 % within the canopy for 134 cowpea accessions studied. This implies that more selection over the years may have been in favour of cultivars with the raceme above the canopy to ensure uniform drying of pods and easy harvesting.

Flower colour variations were expressed among the cowpea genotypes. On the whole, the predominant flower colour was white (61.9 %) whereas 27 % of the cowpeas produced violet-pink flowers while 11.1 % produced violet flower colour. This does not conform to earlier observations by Ezueh and Nwoffiah (1984) as well as Bennett-Lartey and Ofori (1999) who observed that cowpea accessions with purple flowers ranked the highest. Other flower colours such as pale blue, yellow and pink have also been reported on cowpea (Gibbon and Pain, 1985), which were not observed in the current work This variation between studies could be attributed to sample size, genotype, and factors of location. According to Purseglove (1968), the commonest flower colours found among cowpeas are yellow, white and purple, and this imply monogenic inheritance The variation in flower colour could imply that different forms of alleles may be controlling flower pigmentation.

Cowpea seed coat colour is an important feature consumers consider in chosing

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cowpea in West Africa (Egbadzor *et al*., 2014). Cowpea seed coat colour may be smooth or wrinkled red, mottled, black, brown, green, buff or white as dominant full coloure, spotted, marbled, speckled, eyed, or blotched (Owusu, 2015). Anthocyanin and flavonoids present in plants are responsible for the various pigmentations in plants and different anthocyanin genes control different pigments in various parts of the plants (Egbadzor *et al*., 2014). In the current research, half (50%) of the cowpea genotypes had white seed coat colour while 19% had cream and 19.1% brown of varying shades and speckles with 11.9% being red. This variation in seed coat colour observed for the cowpea genotypes grown under the same environmental conditions may be attributed to inherent genetic differences and also an indication of selection, which favour white seeds.

Mean plant height varied significantly ($P \leq 0.05$) from 14.11 cm in the early maturing local check Apagbaala to 42.44 cm in SARC-1-57-1 in the major season. On the whole, the average heights of cowpea plants in the major season were higher than the mean height recorded in the minor season. Values determined for mean plant height in the present study are within the ranges reported in earlier studies by Aboyomi *et al*. (2008) who recorded values between 20.21 cm and 59.12 cm in cowpea genotypes but lower than those reported by Peksen and Artık (2004) and also Khan *et al.* (2010). Plant height varied significantly among the genotypes of cowpea grown under the same conditions on the field. The observed variation in plant height may be attributed to genetic differences among the cowpeas.

The number of primary branches (NB) determines ultimately, the pod bearing ability of the cowpea plant, which in turn contributes to yield. Hence, identification and selection of cowpea accessions with more branching ability are necessary (Makanur, 2013). In the present study, the cowpea accessions exhibited varied branching ability

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ranging from an average of 1.56 cm (IT10K-817-7) to 4.94 cm (SARC-1-57-1). There was a moderately positive correlation between NB and CD (r = 0.58) and a low positive correlation between the NB and PH (r = 10.45), NB and SY (r = 0.41), NB and PY (0.41). The positive correlation between the number of branches and number of seeds per pods might have accounted for the high yield in SARC1-57-1 The variation observed in the number of branches could partly be due to genetic factors (Magani and Kuchinda, 2009) and environmental conditions (Marie-Hrbne and Bertrand, 1997).

Most cowpeas exhibit photoperiod sensitivity whiles others are day neutral when it comes to floral bud initiation and development of flowers (Timko *et al.*, 2007; Timko and Singh, 2008). The length of reproductive period varied significantly among genotypes with early flowering genotype, IT10K-836-2 taking 36 days to attain 50% flowering. The rest of the cowpea genotypes took between 36 and 46 days to flower which is contrary to expected 30 days from planting to flowering for early maturing and 90 - 100 days for late cultivars as reported by Madamba *et al.*, (2006). In choosing cowpea accessions for the different agro-ecological zones, the photosensitivity status of the accessions must be established so as to synchronise the maturity time with the climate to ensure pods mature and ripen when there is low moisture (Timko *et al.*, 2007; Timko and Singh, 2008). This will allow pods to dry well and avoid rot as a result of excessive moisture, which is conducive for some pathogens, which can affect the yield (Timko *et al.*, 2007; Timko and Singh, 2008).

Cowpea exhibits high variability regarding both pod length (PL) and the number of seeds per pod (NSP) (Basaran *et al*. 2011). In the current study, IT10K-819-4 had the longest pod of 21.08 cm, but this did not translate into the highest number of seeds per pod, as the genotype record only nine seeds per pod whereas Marfo tuya with

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pod length of 14.5 cm yielded the highest NSP of 15. There was a moderate correlation analysis showed moderate correlation between PL and SNP in this study. Different authors have reported different PL values in separate experiments; Basaran *et al*. (2011) recorded PL values averaging 14.4 cm and 14.2 cm respectively for two cultivars studied whereas Khan *et al*. (2010) also reported a range of 10 cm to 38 cm for PL and 7 to 21 for SNP among 24 genotypes. PL was also observed to vary between 9.2 cm to 43.7 cm in more than 400 cowpea genotypes studied by Pasquet (1998). In this respect, the ranges for PL (8.49 cm to 21.08 cm) and SNP (5 - 15) observed among the cowpea genotypes in this study are within those recorded elsewhere. There was, however, a poor correlation between the pod length and the number of seeds per pod and this may be due to unfavorable growth conditions which disfavoured pod and seed formation.

Variation in PL and SNP may be as a result of both genetic and abiotic factors influencing seed yield. Fery (1985) showed that pod length was highly heritable with average heritability estimated at 75.2 %. Availability of moisture at the time of pod formation and maturity might have also influenced larger and longer pods as observed by Aliyu & Makinde, (2016) in the major season as compared to the minor season where grand means recorded were 15.14 cm and 14.9 cm respectively. Since all the plants were exposed to similar growing conditions, any variation in PL and SNP in the population may be genetic in nature.

Hundred-seed weight (HSW) among the cowpea genotypes ranged from 10.04 g to 22.66 g with the highest achieved by local check Padi-Tuya. Regarding average SY, the results of this study agree with previous results by several workers (Peksen and Artık, 2004; Vural and Karasu, 2007; Akande and Balogun, 2009). They also compare favourably with data on varieties released by CSIR, which were obtained

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from IITA (Catalogue of crop varieties released and registered in Ghana, 2015). Asare (2013) in a study of variation in 100-seed weight among cowpea genotypes classified seed weight as follows: 10 – 15 g (small-sized seeds), 15.1 - 20 g (medium-sized seeds), 20.1 – 25 g (large seeds) and 25.1 g and above (very large seeds). From the current studies, 10.53 %, of the cowpea genotypes were small sized, 44.74 % were medium-sized, 36.84 % were large seeds and 7.89 % were very large. The low number of large seeds in the population could be attributed to low sample size (Doebley, 1989; Pasquet, 2000; Coulibaly *et al*., 2002; Ba *et al*., 2004).

Also, the average seed yield (SY) for the various genotypes was 4022 kg ha^{1}. This falls outside the range obtained by Basaran *et al.* (2011) who observed seed yield between 1,170 kg ha^{-1} and 1,420 kg ha^{-1}. Seed yield observed in the current research ranged from 1313 to 8010 kg ha^{-1} which was greater than that reported by Peksen and Artık (2004) who recorded SY of between 680 to 1,120 kg ha^{-1} in six cowpea genotypes grown in Black Sea coastal ecology. Also, SY in current research was higher than reported by Khan *et al*. (2010) who recorded SY ranging from 317 to 3,550 kg ha^{-1}among 24 cowpea genotypes in Pakistan ecology. Moreover, in a study conducted with ten genotypes in different conditions (in Nigeria) for two years at three locations, SY varied from 915 to 1,173 kg ha^{-1} (Akande and Balogun, 2009). This differences may be attributed to the genotypes and the environmental conditions under which the cowpeas were grown. The high yield obtained in the current research may not be the case on farmer fields if the timing of sowing to coincide with adequate rain coupled with proper farm practices and monitoring. There was abundance of food during the period of maturation hence birds did not destroy pods as may be the case when grown in the minor season.

There was a low positive correlation between SY and number of branches (NB) (r =

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0.41), SY and pod length (PL) (r = 0.40) but a highly positive correlation (r = 0.98) with pod yield (PY) (Table 3.8). The fact that the total seed yield per plant varied significantly ($P \leq 0.05$) among the accessions of cowpea and correlated positively with NB, PL, CD and PY emphasise their importance as indices of selection for high yield. Similar correlations in cowpea parameters were observed by Manggoel and Uguru (2011). They recorded a significantly positive relationship between grain yield and number of peduncles per plant, the number of flowers per plant, number of pods per plant and pod length. They concluded that there is a positive association between grain yield and yield attributes. However, grain yield in the cowpea genotypes might have been influenced by genetic and environment interactions.

Principal component analysis (PCA) presented yielded five principal components based on components with Eigen values greater than 1 and loading factor of ±0.3 explaining 73.1 % of the total variance. The first principal component (PC1) explained 23.4 % of the total variance observed and this was mainly due to the high negative factor loading of pods per plant (PPP), seed texture (ST) and leaf colour (LC) and high positive factor loading of the eye colour (EC). Second principal component (PC2) accounted for 21.7 % of the total variance observed, and this was negatively correlated with PPP, EC and seed shape (SS) and a high positive factor loading of eye pattern (EP). Earlier work by Doumbia (2012) also identified seed texture, leaf colour and seed coat colour to be of high loading value. Chiorato *et al.* (2006) suggested that the greatest loading coefficient in the least component indicated a redundancy of the descriptor (trait) associated with the component and therefore, the number of pods per peduncle (PPP) may be described as a redundant descriptor in the description and characterization of the cowpeas evaluated.

Some interrelationships among agronomic traits of cowpea have been reported using

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correlation and regression analysis (Ogunbodede, 1989; Musvosvi, 2009). Correlation measures the level of association between these traits (Steel and Torrie, 1984). Many of the traits assessed on the cowpea genotypes were related to one another. All positively correlated traits show close relation as an increase in one, results in an increase of the other. On the other hand, negative correlation implies that a decrease in one trait results in an increase in the other. Hence, the essence of correlation study is to ascertain the mutualistic traits on which selection can be based. The analysis revealed the highest significant correlation between total pod weight and total seed weight (r = 0.985), followed by number of branches and canopy diameter (r = 0.576) and the number of seeds per pod and pod length (r = 0.530). This result is similar to that of Nakawuka and Adipala (1999) who reported that there was a highly significant positive correlation between number of seeds per pod and pod length. Kamai *et al*. (2014) also observed a positive correlation between grain yield and number of seeds per pod, pod length but observed a negative correlation between grain yield and number of branches. This is an indication that grain yield is a function of different yield-attributing characters. These could be the major grain determinants that contributed positively to the superior grain yield of the genotypes evaluated in this study.

Phenotypic differences may be a good pointer to genetic differences among germplasm. Autrique *et al.* (1996), Johns *et al.* (1997) and Van Beuningen and Bush (1997) used morphological, developmental and physiological traits to create distances that were used in evaluating genetic diversity in a large collection of germplasm. The significant ($P \leq 0.05$) differences in quantitative traits among plants of the various genotypes studied are indicative of genetic diversity among the cowpea genotypes.

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Cluster analysis helps by decreasing some individual variables by putting such variation into clusters and this presented in the form of a dendrogram using the coefficient of similarity-dissimilarity (Doumbia *et al*., 2014). It shows a hierarchical representation of how related the genotypes are by grouping them such that those with similar descriptions are mathematically grouped into the same cluster (Doumbia *et al*., 2014). The results of the cluster analysis based on Nei's Genetic Distances (Nei *et al.,* 1983) method using 23 morphological traits grouped the 38 cowpea genotypes into two main clusters with six out-groups at a dissimilarity coefficient of 38 %. Cluster one consisted of only one genotype, IT10K-817-1. This genotype had a wide canopy, broad leaves, early maturing, long pods and moderately large seeds. Cluster two showed some clear patterns of groupings of the lines which showed close relatedness implying some amount of diversity based on morphological data.

3.4.2 Genotypic analysis of SSR markers across the genome of 38 genotypes of cowpea.

The 20 SSR primer pairs used in the current study gave amplification products with 45 % exhibiting polymorphism. Also, 4 and 10 alleles were detected among the 38 cowpea genotypes by the selected polymorphic primer pairs with an average of 6.22 alleles per primer. This is similar to results obtained by Li *et al.* (2001) who assessed genetic diversity in 48 wild lines of cowpea using SSR primers and detected between 4 and 13 alleles with an average of 7.5 alleles per primer. Also, Diouf and Hilu, (2005), observed number of alleles ranged from 1 to 9 allele number per SSR primer combination in cowpea germplasm from Senegal. An average of 8.2 bands per primer pair was obtained for sixteen SSR primers, which generated allele numbers ranging between 5 and 12 fragments among cowpea genotypes (Sawadogo *et al*., 2010). Asare *et al.* (2010), using 25 polymorphic primer pairs to analyse Ghanaian

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cowpea collections, had allele number ranging between 1 and 9 with a mean of 3.8 alleles. The current study detected a mean of 6.22 alleles in the cowpea germplasm obtained from IITA, which could enrich the local germplasm in Ghana through hybridization.

The mean PIC value (0.67) recorded in the current study compares favourably with results obtained from previous reports by Kuruma *et al*., (2008) who obtained polymorphic information content (PIC) ranging between 0.09 and 0.87 with a mean of 0.34, and Doumbia *et al*. (2014) who had PIC of 0.61-0.92 with a mean of 0.83. Genetic diversity in the current study also ranged from 0.59 (SSR-6218) to 0.85 (SSR-6375) with an average of 0.71, which is higher than the earlier report by Asare *et al*., (2010) who obtained a range of 0.12 to 0.68 with an average of 0.44. These variations in genetic diversity and PIC values obtained may be as a result of differences in the sample size studied as well as variation in growth conditions experienced during the various experiments.

The use of a large number of molecular markers in determining genetic relationship among cowpea accessions helps to bring out the genomic differences among germplasms collections (Asare *et al*., 2010). The highest heterozygosity of 0.85 indicated by primer SSR-6375 may suggest variations in the cowpea genome which even though is self-pollinating but might have undergone some amount of hybridization under open field cultivation (Asare *et al*., 2010). Nonetheless, it was possible to roughly place the various cowpea genotypes studied into several main groups and sub-groups which indicate their origins and relatedness (Asare *et al.,* 2010).

The dendrogram generated from molecular weights of SSR markers showed some genetic relationship among the 38 cowpea genotypes studied. Results obtained

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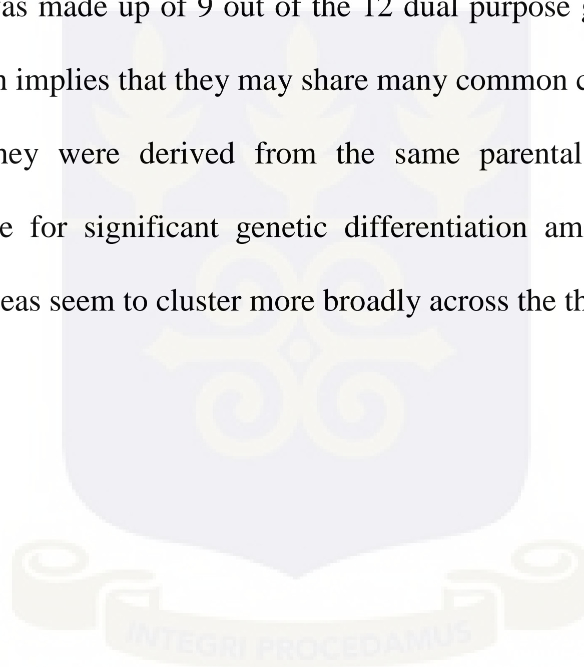
revealed two main clusters at a dissimilarity coefficient of 40 % for all the cowpea genotypes. The coefficient of dissimilarities among the cowpea genotypes was low, indicative of some bottleneck effect during some stage of the domestication process, which has been maintained by self-pollination mechanism of the crop (Ali *et al*., 2015). Most of the cowpea genotypes, which clustered together based on morphological similarities, re-grouped into different clusters and sub-clusters using the SSR markers. Within each of the larger groups, there were distinct sub-clusters at varying coefficients of dissimilarities that reflected the groupings they belong. Cluster three was made up of 9 out of the 12 dual purpose genotypes implying low diversity, which implies that they may share many common characteristics. This may suggest that they were derived from the same parental stock (as a result of inadequate time for significant genetic differentiation among them). The other groups of cowpeas seem to cluster more broadly across the three major clusters.



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CONCLUSION

In all, there is low genetic variability among the 38 genotypes of cowpea based on the morphological characterization. Twenty-three quantitative and qualitative traits distinguished the 38 cowpea genotypes to predict genetic diversity but could not identify duplicates whereas the nine SSR markers grouped the 38 cowpea genotypes into two clusters with IT07K-299-6 and IT07-210-1-1having the least dissimilarity coefficient of about 5% implying that they may be duplicates. The mean genetic diversity (0.71) and PIC (0.67) suggest that a broad genetic base in the genotypes studied which can be incorporated into local cowpea germplasm of Ghana.

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CHAPTER FOUR

4.0 PHENOTYPIC SCREENING OF COWPEA GENOTYPES FOR RESISTANCE TO COWPEA VIRAL DISEASES UNDER FIELD CONDITIONS

4.1 INTRODUCTION

Cowpea, [*Vigna unguiculata* (L) Walp], is an important legume crop in Ghana (Cobbinah *et al.,* 2011). It is commonly grown for its dry grains but the fresh leaves and pods are also consumed on-farm or sold whereas the hay also serves as a good source of proteins (Tarawali *et al*., 1997). The crop is an important source of nutrition for both poor rural and resource - poor urban households who normally feed on starchy foods such as maize, cassava, yam, millet and sorghum (Bresanni, 1985). It is usuallyconsumed directly after cooking or as a component or complement of food made from cereals or root crops (Adekola and Oluleye, 2007; Timko and Singh, 2008). In Ghana, 'gari and beans' a popular meal of boiled cowpea and roasted cassava dough (gari), is consumed in all second cycle institutions and also a delicacy among both rich and poor in most parts of the country.

Cowpea possesses several advantages for farmers in Ghana over other grain legumes and vegetables because of its adaptability to poor soils, improvement of soil fertility through nitrogen in the soil (60 - 70 $kg{\cdot}N{\cdot}ha^{-1}$ available to crops grown in rotation with cowpea), hence doesn't require much fertilizer application, and high yield (Singh *et al*., 2011). The crop has high tolerance to unfavourable conditions of drought and high temperatures compared to other legumes (Craufurd *et al*., 1997). These unique attributes of cowpea make it an excellent food security crop in Ghana especially in the northern regions and the coastal Savanna areas where drought and high environmental temperatures are common during part of the year and adversely

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affect crop production.

In spite of the significant contribution of cowpea to nutrition and food security in Ghana, yields and the production of the crop in the country are very low and continue to decline over the years. The current average yield of cowpea of 1.3 mt ha^{-1} is lower than the achievable yield of 2.6 mt ha^{-1} (MoFA, 2013).

The wide yield gap has been attributed to several abiotic and biotic constraints of which pests and diseases are major ones. Cowpea viral diseases have considerable impact on cowpea productivity in both tropical and subtropical regions of the world where cowpea is cultivated (Mali and Thottappilly, 1986) including Ghana (Zettler and Evans, 1973; Lamptey and Hamilton, 1974; Fuleratu, 2016). About 140 cowpea viruses have been reported worldwide, of which only nine are known to occur in Africa (Timko and Singh, 2008). These include *Cowpea aphid-borne mosaic virus* (CABMV, genus (Potyvirus), *Cowpea mild mottle virus* (CMMV, genus Carlavirus), *Cowpea mosaic virus* (CoMV, genus Comovirus), *Cowpea chlorotic mottle virus* (CCMV, genus Carmovirus), *Cowpea golden mosaic virus* (GPGMV genus Geminivirus), *Southern bean mosaic virus* (SBMV, genus Sobemovirus), *Cucumber mosaic virus* (CMV, genus Cucumovirus) and *Tobacco mosaic virus* (TMV, genus Tobamovirus) (Thottappilly and Rossel, 1992; Alegbejo and Kashina, 2001; Aliyu and Balogun, 2011). Cowpea viruses reported so far in Ghana include Southern bean mosaic virus (SBMV), Cowpea aphid-borne mosaic virus (CABMV) (Lamptey and Hamilton, 1974), Blackeye cowpea mosaic virus (BICMV) (Zettler and Evans, 1973; Fuleratu, 2016), Bean common mosaic virus (BCMV) (Fuleratu, 2016) and Cowpea mild mottle virus (CPMMV) (Brunt and Kenten, 1973; Jeyanandarajah and Brunt, 1993).

Symptoms of viral diseases in cowpea include mottling of the leaves, yellow mosaic

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patterns on the leaves, vein clearing, chlorosis, necrotic spots and stunting (Thottappilly and Rossel, 1985). Yield losses of cowpea as a result of viral infections are estimated to be between 10% and 100% and complete loss of irrigated cowpeas has been recorded in northern Nigeria, which was attributed to virus infection (Aliyu and Balogun, 2011).

Effective management of these viral diseases is quite important to improve yields of cowpea. Conventional methods used by farmers to control viruses including broad-spectrum insecticides for the control of vectors that transmit the viruses are inadequate and not cost-ineffective to the peasant farmers. The use of host plant resistance is therefore considered to be the most economical and environmentally friendly approach in the management of viral diseases (Fraser, 1992). Identifying sources of resistance in cowpea germplasms is an important objective of cowpea breeding programmes (Timko and Singh, 2008; Kehinde *et al*., 2016).

The main objective of the study was to assess the reaction of 38 cowpea genotypes to virus infection under field condition both in the major and minor cropping seasons to identify sources of resistance.

Specific objectives:

1. To screen the cowpea genotypes against cowpea viral infection under natural conditions in the field.
2. To assess the effect of viral infection on grain yield in cowpea genotypes

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4.2 MATERIALS AND METHODS

4.2.1 Planting material

The 38 cowpea genotypes used in Chapter Three Section 3.2.4 were used for this experiment.

4.2.2 Experimental design and field layout

The experiment was laid out in a Randomised Complete Block Design (RCBD) with three replicates as described in chapter three section 3.2.6. The experiment was repeated in the minor season.

4.2.3 Data collection

Data were collected on dry seed yield (kg ha^{-1}), disease incidence and severity. Data were collected on ten plants in the middle row, and the means were taken. Disease assessment was done fortnightly starting four weeks after sowing (WAS) till senescence.

Disease incidence was based on disease symptoms described by Gumedzoe *et al.* (1997). Percentage disease incidence (DI) was then determined using the formula:

$$DI(\%) = \frac{number\ of\ plants\ infected\ in\ the\ inner\ rows}{total\ number\ of\ plants\ in\ the\ two\ inner\ rows} X\ 100$$

The severity of viral diseases was assessed based on the intensity of disease symptoms using a modified 1-5 visual scale by Hahn *et al.* (1980) and Gumedzoe *et al.* (1997) (Table 4.1).

Data on mean severity scores were used to calculate Area Under Disease Progress Curve (AUDPC = this is a useful quantitative summary of disease commonly used to quantify disease intensity over time by integrating the amount of disease over time, rather than at a particular time) for each of the cowpea lines using the formula of Campbell and Madden (1990):

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AUPDC=$\sum_{i=1}^{n-1}((y_i + y_i - 1)/2)(t_i + 1 - t_i))$

Where "t" is the time of each reading (days after planting), "y" is the percent of affected foliage at each reading and "n" is the number of readings.

Table 4.1: Visual scales for rating severity of cowpea viral diseases in the open fields

Scale	Symptom description
1	Unaffected shoots, no symptoms.
2	Mild chlorosis, mild distortions at the base of leaves, while the remaining parts of the leaves and leaflets appear green and healthy.
3	Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets.
4	Severe mosaic distortion of two-thirds of most leaves and general reduction of leaf size and stunting of shoots.
5	Very severe mosaic symptoms on all leaves, distortion, twisting, misshapen and severe leaf reductions of most leaves accompanied by severe stunting of plants.

Modified from Sseruwagi *et al*., (2004) and Gumedzoe *et al*. (1997).

Plants with a mean AUDPC of less than five (< 5) were classified as resistant (R), AUDPC of 5 - 10 were moderately resistant (MR), and AUDPC score > 10 were classified as susceptible (S).

4.2.4 Data Analyses

Data on disease incidence were arcsine-transformed to homogenise variances and reduce error before subjecting them to analysis of variance (ANOVA). The other quantitative data (AUDPC, final severity, plant height and seed yield) were subjected to ANOVA and the means separated by the least significant difference (LSD) method at 5% level of probability. Pearson's correlation coefficients among the

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parameters (disease incidence, final severity and AUPDC) were calculated. All statistical analyses were performed using GenStat Release 10.3 (VSN International).

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4.3 RESULTS

4.3.1 Disease Symptoms observed

Figure 4.1 shows the symptoms of virus disease observed. Eight different symptoms were observed. Over 80% of the genotypes showed mosaic and mottling followed by chlorotic spots (34.2 %), vein clearing (28.9 %), leaf curl (26.3 %), necrotic lesions (15.8 %) whereas stunting was the least (10.5 %).

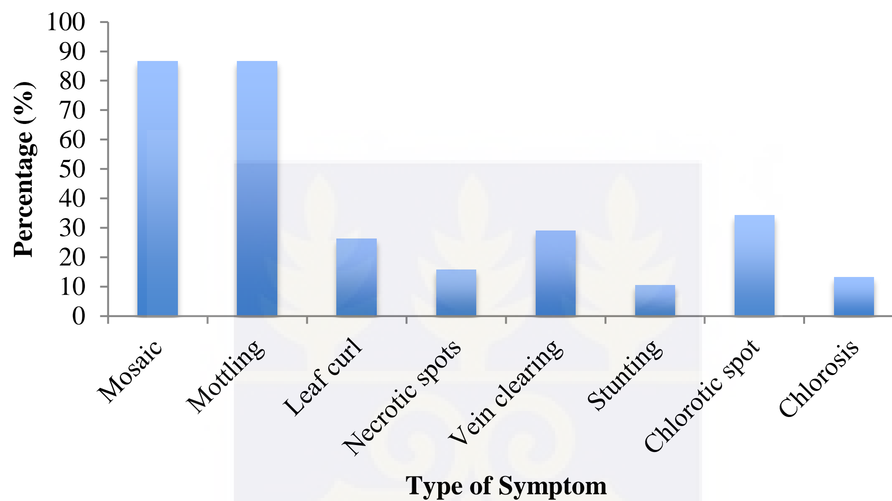

Fig 4.1: Characteristic cowpea viral disease symptoms (%) observed during the major growing season.

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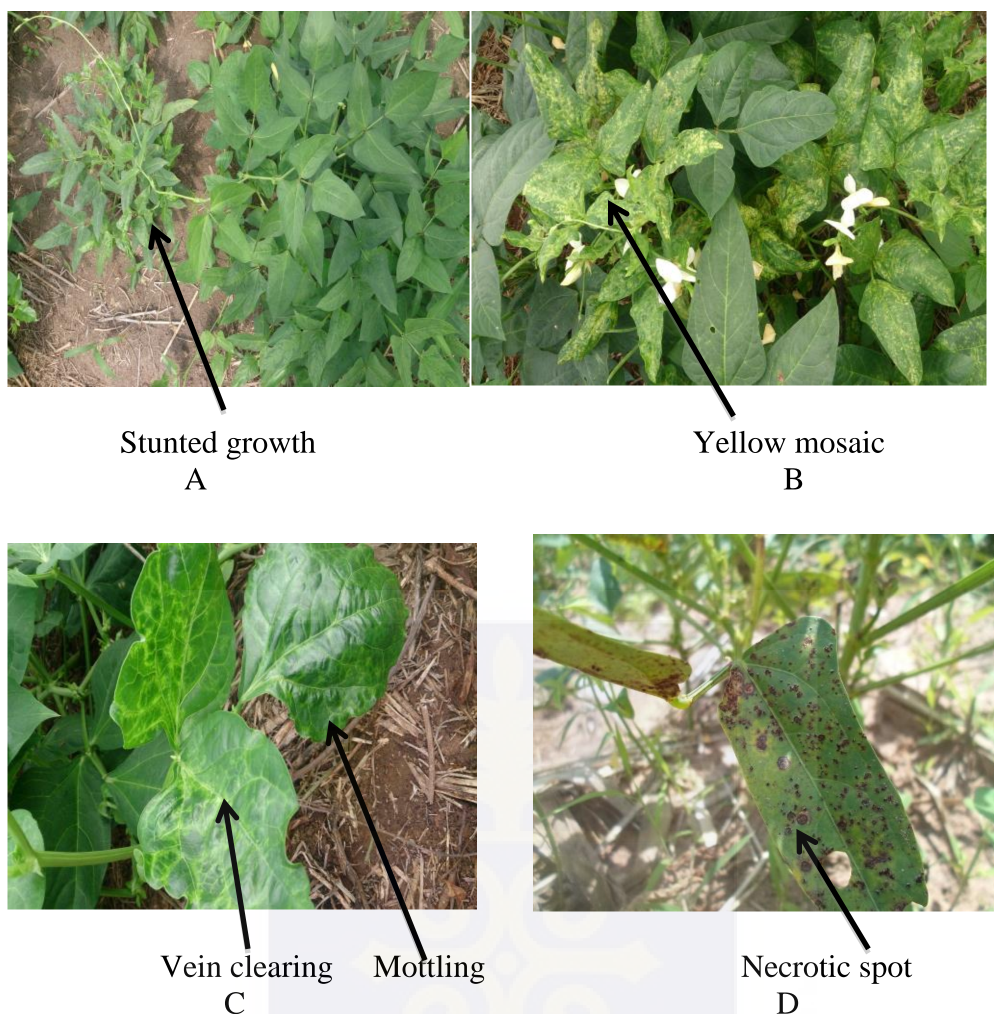

Fig.4.2: Viral disease symptoms observed in the open field; **A** = stunted growth (Left) compared to a healthy plant (Right) of the same genotype. **B** = yellow mosaic leaves and **C** = vein clearing and mottling of the leaves. **D** = necrotic lesions on leaves.

4.3.2 Incidence of viral diseases

Mean incidence (%) of viral diseases on the 38 cowpea genotypes are shown in Table 4.2. Generally, for all 38 cowpea genotypes, the incidence of viral diseases increased from 4 to 8 WAS, with overall mean incidences increasing from 17.7 % to 29.2 % in the major season and from 34.4 % to 53.1 % in the minor season.

ANOVA showed no significant differences in the mean incidence of viral diseases during the major season among the cowpea genotypes at 4 WAS ($F_{41, 84} = 1.17$; $P = 0.274$), but differed significantly amongst them at 6 WAS ($F_{41, 84} = 2.61$; $P < 0.001$)

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Table 4.2: Mean incidence of viral diseases among 38 cowpea genotypes during the major and minor seasons

Cowpea Genotypes	Disease incidence					
	Major season			Minor season		
	4WAS	**6WAS**	**8WAS**	**4WAS**	**6WAS**	**8WAS**
Apagbaala	26.1	57.8^{ab}	52.8^{ab}	23.1	60.0	51.5
GH3684	**0.0**	39.1^{bcdefg}	45.0^{abcde}	11.8	41.8	56.75
IT04K-321-2	17.7	8.9^{hi}	8.9^{fgh}	26.8	56.8	60.0
IT07-210-1-1	8.9	8.9^{hi}	8.9^{fgh}	31.1	53.0	45.0
IT07K-243-1-2	36.1	21.9^{efghi}	19.9^{defgh}	41.7	38.0	30.0
IT07K-291-92	13.1	25.4^{cdefghi}	21.1^{cedfgh}	26.8	63.7	60.0
IT07K-297-13	17.7	**0.0**^{i}	6.1^{gh}	16.1	26.3	41.8
IT07K-298-15	19.2	27.8^{cdefghi}	32.2^{bcdefg}	45.5	51.5	63.3
IT07K-298-9	**54.1**	48.9^{abcde}	51.1^{ab}	43.8	58.5	73.9
IT07K-299-6	23.1	30.3^{bcdefgh}	37.2^{abcdef}	51.6	58.9	78.3
IT07K-303-1	17.2	26.2^{cdefghi}	36.9^{abcdef}	48.3	63.7	55.7
IT08-125-100	6.1	47.3^{abcdef}	46.9^{abcd}	49.8	60.0	63.3
IT08-150-12	6.1	36.8^{cdefgh}	45.0^{abcde}	41.7	60.0	41.3
IT08K-125-107	6.1	52.1^{abcd}	54.1^{ab}	**55.2**	48.3	58.5
IT08K-126-19	17.2	28.1^{bcdefghi}	28.1^{bcdefgh}	41.3	55.7	62.2
IT08K-180-11	12.3	26.1^{cdefghi}	21.1^{cdefgh}	16.1	45.0	60.0
IT08K-193-14	18.4	17.7^{fghi}	17.2^{efgh}	26.3	45.0	48.3
IT08K-193-15	21.1	8.9^{hi}	8.9^{fgh}	30.0	48.7	60.0
IT09-456	13.1	17.2^{ghi}	8.9^{gh}	37.0	38.0	37.0
IT09K-231-1	15.0	37.2^{bcdefgh}	30.3^{bcdefg}	33.3	45.5	62.2
IT09K-321-1	8.9	13.1^{ghi}	18.9^{defgh}	33.3	41.8	51.5
IT10K-815-5	15.0	11.1^{ghi}	11.1^{fgh}	33.3	70.2	63.3
IT10K-817-1	8.9	**0.0**^{i}	8.9^{fgh}	45.0	51.5	58.5
IT10K-817-3	17.2	17.2^{ghi}	19.9^{defgh}	34.8	52.0	45.5
IT10K-817-7	12.3	8.9^{hi}	6.1^{gh}	36.5	53.5	63.3
IT10K-819-4	13.1	**0.0**^{i}	**0.0**^{h}	26.8	46.1	49.8
IT10K-827-11	6.1	13.1^{ghi}	19.2^{defgh}	45.0	48.3	90.0
IT10K-832-3	33.0	36.1^{bcdefgh}	36.8^{abcdef}	11.7	30.0	43.9
IT10K-834-3	11.1	**0.0**^{i}	33.0^{bcdefg}	26.8	54.7	70.2
IT10K-836-2	26.1	8.9^{gi}	19.2d^{efgh}	48.6	**82.0**	63.3
IT10K-837-1	19.2	**71.1**^{a}	**62.7**^{a}	**54.4**	26.8	30.0
IT10K-843	6.1	49.6^{abcde}	53.9^{abdefgh}	41.7	38.0	38.0
IT10K-866-1	45.0	53.9^{abc}	30.3^{bcdefg}	30.0	45.0	38.0
IT10K-973-1	41.1	19.9^{efghi}	47.7^{abcd}	31.5	38.5	52.0
IT11K-61-82	27.3	27.3^{cdefghi}	18.9^{defgh}	38.1	48.7	48.3
Marfo tuya	**0.0**	27.3^{cdefghi}	53.9^{ab}	15.0	15.0	11.8
Padi Tuya	15.0	23.9^{defghi}	36.1^{abcdef}	26.8	33.2	33.7
SARC-1-57-1	17.7	30.8^{bcdefgh}	50.8^{abc}	48.7	67.0	52.0
Grand mean	17.7	25.7	29.2	34.4	49.5	53.1
P	0.27	<. 001	<. 001	0.547	0.118	0.077

Each value is the mean of 3 replicates. In each column, means followed by the same letter are not significantly different ($P < 0.05$) according to LSD test at 5% level of probability.

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and 8 WAS ($F_{41, 84} = 2.71$; $P < 0.001$). At the final observation (8 WAS), IT10K-837-1 had the highest mean incidence (62.7 %) in the major season whereas IT10K-819-4 showed no disease symptom (0 %) (Table 4.2).

On the contrary, in the minor season, ANOVA did not show any significant differences in the mean incidence of viral diseases among the cowpea genotypes at 4 WAS ($F_{41, 84} = 0.96$; $P = 0.547$), 6WAS ($F_{41, 84} = 1.36$; $P = 0.118$) and 8 WAS ($F_{41,84} = 1.45$; $P = 0.077$). However, at 8 WAS, the genotype IT10K-827-11 had the highest mean disease incidence (90 %) while Marfo tuya had the lowest mean incidence of 11.8 % (Table 4.2).

4.3.3 Severity of viral diseases and area under disease progress curve

The cowpea genotypes showed significantly varying levels of disease severity ($F_{37, 74} = 4.0$; $P < 0.001$) at the final observation (8 WAS) during the major season (Table 4.3). Marfo tuya recorded the highest symptom severity score of 2.5 whereas IT10K-836-2, IT11K-61-82 and IT04K-321-2 did not show any symptom. On the contrary, in the minor season, there was no significant difference in the levels of viral symptom severity scores recorded for the cowpea genotypes ($F_{37, 74}$=1.0; P= 0.45). Genotype IT07K-298-9 had the highest mean symptom severity score of 2.4 whereas Marfo tuya had the lowest disease severity score of 1.11, at 8 WAS (Table 4.3). The AUDPC recorded for the 38 cowpea genotypes in the major season varied significantly among them ($F_{37, 74} = 4.0$, $P < 0.001$). The highest AUDPC of 9.1 was recorded for Apagbaala whereas IT10K-819-4 had the lowest (4.1). There was, however, no significant difference ($F_{37, 74} = P > 0.05$) amongst the cowpea genotypes regarding AUDPC values in the minor season (Table 4.3).

Table 4.3: Mean Viral Disease Severity Scores, Area Under Disease Progress Curve (AUDPC), and Mean Seed Yield for 38 Cowpea Genotypes Screened during Major and Minor Seasons.

	Major Season					Minor season				
Genotype	Plant height/cm	Final Severity	AUDPC	Seed yield /kg ha^{-1}	Disease reaction	Plant height/cm	Final Severity	AUDPC	Seed yield /kg ha^{-1}	Disease reaction
Apagbaala	14.11^{d}	2.9^{a}	9.1^{a}	1543^{ab}	MR	15.63^{b}	1.7	5.0	N/A	R
GH3684	32.07^{bc}	1.6^{cd}	$6.2^{bcdefghi}$	8010^{a}	MR	20.79^{a}	1.9	6.6	N/A	MR
IT04K-321-2	27.58^{c}	1.1^{cd}	4.3^{kl}	3370^{ab}	R	17.14^{b}	1.9	5.3	N/A	MR
IT07-210-1-1	22.92^{cd}	1.1^{cd}	4.6^{hijkl}	4063^{ab}	R	18.94^{a}	1.5	4.9	N/A	R
IT07K-243-1-2	27.31^{c}	1.5^{cd}	$5.8^{defghijkl}$	1733^{ab}	MR	20.41^{a}	1.4	4.5	N/A	R
IT07K-291-92	26.92^{c}	1.1^{cd}	4.9^{ghijkl}	1350^{ab}	R	18.94^{a}	2.2	5.7	N/A	MR
IT07K-297-13	22.28^{cd}	1.0^{d}	4.4^{jkl}	4033^{ab}	R	16.86^{b}	1.4	4.0	N/A	R
IT07K-298-15	21.50^{cd}	1.4^{cd}	$5.4^{efghijkl}$	4707^{ab}	MR	15.82^{b}	1.8	5.1	N/A	MR
IT07K-298-9	21.34^{cd}	1.7^{cd}	7.1^{bcde}	2100^{ab}	MR	13.98^{b}	2.4	6.8	N/A	MR
IT07K-299-6	16.32^{d}	1.4^{cd}	$5.5^{efghijkl}$	2077^{ab}	MR	15.56^{b}	2.4	6.1	N/A	MR
IT07K-303-1	20.97^{cd}	1.4^{cd}	$5.8^{efghijkl}$	1873^{ab}	MR	16.54^{b}	2.1	6.1	N/A	MR
IT08-125-100	14.69^{d}	2.0^{bc}	7.7^{abc}	2513^{ab}	MR	18.46^{a}	2.1	5.7	N/A	MR
IT08-150-12	27.17^{c}	2.1^{bc}	6.8^{bcdef}	6223^{ab}	MR	21.07^{a}	1.6	5.3	N/A	MR
IT08K-125-107	22.17^{cd}	1.8^{c}	6.8^{bcdef}	4010^{ab}	MR	21.46^{a}	1.6	4.6	N/A	R
IT08K-126-19	23.00^{cd}	1.7^{cd}	$6.4^{bcdefgh}$	3463^{ab}	MR	18.91^{a}	2.0	5.3	N/A	MR
IT08K-180-11	23.78^{cd}	1.2^{cd}	$5.2^{fghijkl}$	2767^{ab}	MR	14.42^{b}	2.1	5.3	N/A	MR
IT08K-193-14	24.26^{cd}	1.1^{cd}	4.6^{ijkl}	6867^{ab}	R	15.76^{b}	1.7	4.6	N/A	R
IT08K-193-15	27.03^{c}	1.1^{cd}	4.4^{jkl}	5717^{ab}	R	13.29^{b}	2.1	5.2	N/A	MR
IT09-456	23.88^{cd}	1.1^{cd}	4.5^{jkl}	6770^{ab}	R	16.36^{b}	1.5	4.4	N/A	R
IT09K-231-1	24.42^{cd}	1.4^{cd}	$5.5^{efghijkl}$	3600^{ab}	MR	22.03^{a}	2.2	5.6	N/A	MR
IT09K-321-1	23.17^{cd}	1.3^{cd}	4.8^{ghijkl}	5697^{ab}	R	17.39^{a}	1.7	4.9	N/A	R
IT10K-815-5	26.42^{c}	1.1^{cd}	4.7^{ghijkl}	4400^{ab}	R	17.59^{a}	2.1	5.6	N/A	MR

Table 4.3 cont'd

IT10K-817-1	22.15^{cd}	1.1^{cd}	4.2^{l}	5073^{ab}	R	18.08^{a}	1.9	5.3	N/A	MR
IT10K-817-3	16.34^{d}	1.2^{cd}	4.8^{ghijkl}	5713^{ab}	R	12.54^{b}	1.7	5.0	N/A	R
IT10K-817-7	28.67^{bc}	1.1^{cd}	4.4^{jkl}	2153^{ab}	R	14.81^{b}	1.9	5.5	N/A	MR
IT10K-819-4	31.80^{bc}	1.0^{d}	4.1^{l}	5627^{ab}	R	18.18^{a}	1.7	4.8	N/A	R
IT10K-827-11	29.97^{bc}	1.1^{cd}	4.7^{hijkl}	2290^{ab}	R	17.48^{a}	2.7	6.0	N/A	MR
IT10K-832-3	26.39^{c}	1.4^{cd}	6.1^{bcdefghij}	3277^{ab}	MR	14.86^{b}	1.9	5.2	N/A	MR
IT10K-834-3	22.78^{cd}	1.2^{cd}	5.2^{fghijkl}	7040^{ab}	MR	15.53^{b}	2.4	6.4	N/A	MR
IT10K-836-2	26.11^{c}	2.8^{b}	4.5^{ijkl}	3257^{ab}	R	15.76^{b}	2.2	5.6	N/A	MR
IT10K-837-1	28.52^{bc}	2.2^{bc}	9.0^{a}	3483^{ab}	MR	21.8^{b}	1.4	4.6	N/A	R
IT10K-843	26.22^{c}	1.5^{cd}	7.6^{abcd}	3150^{ab}	MR	16.13^{b}	1.4	3.9	N/A	R
IT10K-866-1	26.73^{c}	1.6^{cd}	6.4^{bcdefg}	1313^{b}	MR	14.68^{b}	1.5	4.8	N/A	R
IT10K-973-1	23.21^{cd}	1.3^{cd}	5.1^{fghijkl}	3057^{ab}	MR	16.23^{b}	1.9	5.1	N/A	MR
IT11K-61-82	30.83^{bc}	1.1^{cd}	4.5^{ijkl}	3207^{ab}	R	14.97^{b}	1.9	5.3	N/A	MR
Marfo tuya	31.50^{bc}	2.2^{bc}	7.8^{ab}	4747^{ab}	MR	22.66^{a}	1.1	3.9	N/A	R
Padi tuya	31.72^{bc}	1.6^{cd}	6.0^{cdefghijk}	5027^{ab}	MR	15.39^{b}	1.5	4.2	N/A	R
SARC-1-57-1	41.00^{a}	1.6^{cd}	6.3^{bcdefgh}	5593^{ab}	MR	17.73^{a}	1.8	5.2	N/A	MR
Grand mean	**26.27**	**1.47**	**5.71**	**4022**		**17.2**	**1.71**	**5.32**		
P-value	**<. 001**	**<. 001**	**<. 001**	**<. 001**		**<. 001**	**0.92**	**0.52**		

Each value is the mean of 3 replicates. In each column, means followed by the same letter are not significantly different (P < 0.05) according to Least Significant Difference (LSD) test at P< 0.05. Moderate resistance (MR), Resistance (R). N/A-Yield was not assessed for the dry season due to lost of flowers and pods to birds.

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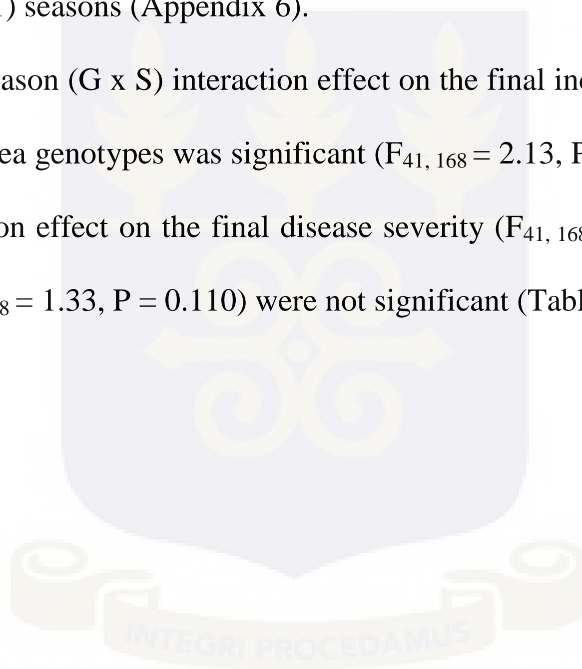
4.3.4 Genotype by season interaction effect

A 2-way ANOVA revealed a highly significant difference in disease incidence ($F_{1, 168} = 76.14$, $P < 0.001$) between the major and minor seasons. The minor season, which had a higher overall mean incidence of 53.1 %, compared to the major season recorded 30.9 % (Appendix 6). The mean disease severity score recorded in the minor season (1.99) was also significantly higher ($F_{1, 168} = 10.66$, $P = 0.001$) than that of the major season (1.49) (Table 4.4). The ANOVA also revealed highly significant differences ($F_{1, 168} = 40.12$: $P < 0.001$) in AUDPC values between the major (4.07) and minor (5.31) seasons (Appendix 6).



Genotype by season (G x S) interaction effect on the final incidence of viral diseases on the 38 cowpea genotypes was significant ($F_{41, 168} = 2.13$, $P < 0.001$). However, the G x S interaction effect on the final disease severity ($F_{41, 168} = 1.24$, $P = 0.177$) and AUDPC ($F_{41, 168} = 1.33$, $P = 0.110$) were not significant (Table 4.4).

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Table 4.4: Mean sum of squares for viral disease incidence, final severity and AUDPC of viral diseases among the 38 genotypes of cowpea grown in major and minor seasons

		Mean square		
Source	**Df**	**Disease incidence**	**Virus severity**	**AUDPC**
Genotype (G)	41	633.50^{ns}	1.71^{ns}	3.00^{ns}
Season (S)	1	30835.70***	16.16**	96.62***
G x S	41	863.20***	1.87^{ns}	3.19^{ns}
Residue	168	405.00^{ns}	1.52^{ns}	2.41^{ns}

** Significant at P < 0.01; *** significant at P < 0.001; ns = not significant (P > 0.05); Degree of freedom (Df).

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4.3.5 Correlation Analysis

Table 4.5 shows the correlation coefficients among the parameters studied. Incidence of viral disease in the major season was significantly and positively correlated with disease severity ($r = 0.7721$; $P < 0.05$) and AUDPC ($r = 0.8801$; $P < 0.05$) but negatively and not significantly correlated with plant height ($r = -0.1320$; $P > 0.05$) and seed yield ($r = -0.1296$; $P > 0.05$). Plant height was not significantly correlated ($r = 0.0580$; $P > 0.05$) with disease severity but significantly and positively correlated with seed yield ($r = 0.5340$; $P < 0.05$).

Incidence of viral disease in the minor season was also significantly and positively correlated with disease severity ($r = 0.9243$, $P < 0.05$) and AUDPC ($r = 0.4722$; $P < 0.05$) but not significantly correlated with plant height ($r = -0.2749$; $P > 0.05$). Disease severity was significant and positively correlated with AUDPC ($r = 0.5178$; $P < 0.05$) but non-significantly correlated with plant height ($r = -0.2975$; $P > 0.05$) (Table 4.5).

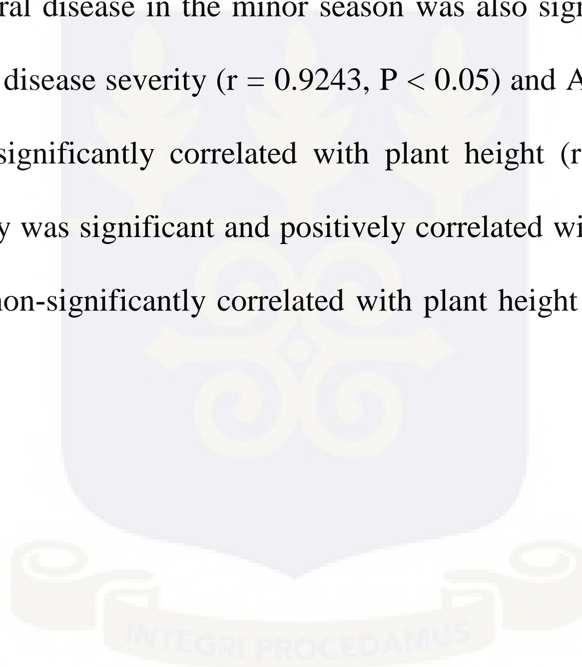

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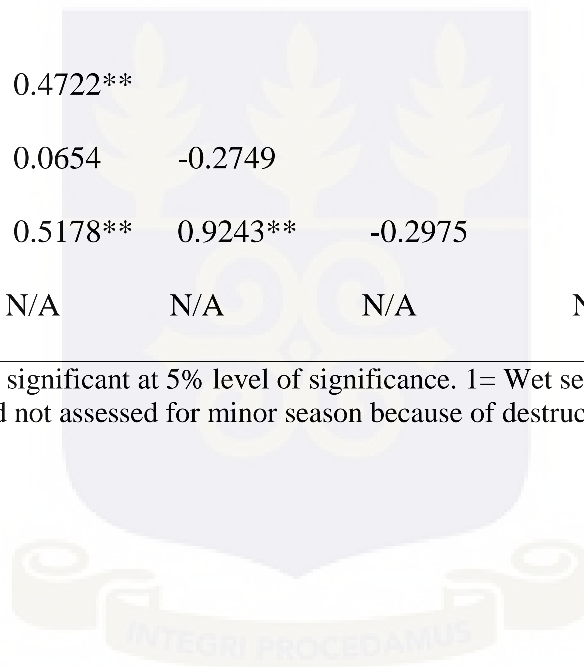
Table 4.5: Association between the disease severity, incidence, AUDPC, plant height and seed yield among the cowpea genotypes in the major and minor seasons

Major season	**AUDPC**	**Incidence**	**Severity**	**Plant height**	**Seed yield**
AUPDC					
Incidence	0.8801**				
Severity	0.8924**	0.7721**			
Plant height	-0.2163	-0.1320	0.0580		
Seed yield	-0.2397	-0.1296	-0.0368	0.5340**	

Minor season	**AUDPC**	**Incidence**	**Plant height**	**Severity**	**Seed yield**
AUPDC					
Incidence	0.4722**				
Plant height	0.0654	-0.2749			
Severity	0.5178**	0.9243**	-0.2975		
Seed yield	N/A	N/A	N/A	N/A	



** Correlation is significant at 5% level of significance. 1= Wet season and 2 = dry season.
N/A = Seed yield not assessed for minor season because of destruction of pods by birds.

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4.4 DISCUSSION

The study revealed that some of the cowpea genotypes were susceptible to virus infection and exhibited varying symptoms. Major symptoms observed during both the major and minor season field trials were leaf mottling, leaf mosaic, chlorotic spots, vein clearing, leaf curl, necrotic lesions with the least common being stunting. Similar symptoms were reported elsewhere on legumes affected by viral diseases (Thottappilly and Rossel, 1985; Vanderborght and Baudoin, 2001; Akinjogunla, 2005; Aliyu *et al*., 2012; Amayo *et al*., 2012). The symptoms observed in the present study are consistent with those associated with viral infection of cowpea in Ghana caused by BICMV, CABMV, CMEV and CYMV (Timko and Singh, 2008; Fuleratu, 2016). The various cowpea symptoms observed in the field may be due to factors such as the type of viral strains, cowpea cultivar, the time of infection of the virus pathogen, mixed infections and the presence of unknown viruses (Jones *et al.*, 1991; Timko and Singh, 2008). Variations in the cowpea viral disease symptoms development may also be as a result of factors such as changes in plant nutritional status, physiological age of the plant and some environmental factors (Hewings *et al*., 1990; De Koeijer and van der Werf, 1995; Gaunt, 1995; Bachand and Castello, 1998).

Differences in disease incidence observed among the cowpea genotypes in the major season, suggests significant host-virus interaction effects. This variation in the host-virus interaction effects could be due to the differences in the genetic makeup of the different cowpea genotypes and viral species as reported in tomato-*Tomato yellow leaf curl virus* pathosystem (Navas-Castillo *et al*., 1999; Delatte *et al*., 2006; Azizi *et al*., 2008; Abu *et al*., 2011). It has been reported by Booker *et al.* (2005) and Van Loon (1983) that viral disease progression in a plant occurs only when new leaves

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develop, since symptoms cannot be expressed in already expanded leaves. This might have accounted for the increase in the mean disease incidence among the cowpea genotypes from 17.7 % to 29.2 % and 34.4 % to 53.1 % for the major and minor seasons respectively. But the level of disease incidence in some cowpea genotypes decreased between 4 WAS and 8 WAS. For instance, the level of disease incidence recorded for genotype IT07K-243-1-2 decreased from 36.1 % to 19.9 % between 4 WAS and 8 WAS (Table 4.2). This could be due to the fact that shortly after anthesis, some plants tend to become more resistant to viral infection and the number of healthy plants available for new infections also decreases as the season progresses (Thresh, 1974; Vacke, 1983).

On the whole, viral disease incidence, severity and AUDPC were significantly higher in the minor season than in the major season. This observation could be due to the higher temperatures and low relative humidities experienced during the minor season compared to the major season which might have influenced rapid disease development (Schuerger and Hammer, 1995) and suppressed the plasticity and recovery rate of the cowpea genotypes (Booker *et al*., 2005). Higher temperatures experienced in the minor season might have favoured the rapid development of aphid vectors, and hence increased the chances of transmitting viral diseases in the cowpea genotypes. A close relationship between aphids and viral incidences has been reported elsewhere (Vacke, 1983; Bukvayová *et al*., 2006). Edema *et al*. (1997) and Shoyinka *et al*. (1997) also reported the effect of varying weather conditions between seasons as a cause of the significant differences in the disease severity and incidence observed in the two growing seasons.

The high plant growth and spread of the cowpea plants observed on the field during the major season might have created microclimate which adversely affected the

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dynamics of virus vectors that transmit viruses to the cowpea plants (Ogenga-Latigo *et al*., 1992; Orawu *et al*., 2001). Hence the relatively lower incidence and severity of viral disease recorded in the major season than in the minor season. Vectors that transmit viruses are normally found in weeds around the field especially during the minor growing (Atiri *et al*., 1986), which may have accounted for the greater incidence and severity recorded during the dry season.

The study showed a significant genotype-by-season interaction effect on the incidence and severity as well as the AUDPC. This shows the inconsistent performance of these cowpea genotypes in the two seasons as reported also by Afutu *et al*. (2016). Consequently, it has been suggested that for the purpose of breeding, different varieties have to be developed for different environments (Acquaah, 2007).

Based on the AUDPC values obtained, none of the cowpea genotypes studied was immune to cowpea viral disease in both the major and minor seasons. From the current study, AUDPC data for wet season showed 42.1 % of the cowpea genotypes to be resistant (i.e. AUDPC of ≤ 5) and 57.9 % of the cowpea genotypes as moderately resistant (i.e. AUDPC of 5 - 10). For the dry season, 39.5 % were resistant, and 60.5 % were moderately resistant. This difference in the disease reactions for plants grown under different environmental conditions may be due to inherent difference in factors controlling the ability of the cowpea genotypes to withstand different environmental conditions, viral infection and viral strains as postulated by Jones *et al.* (1991). This suggests it did not exhibit a stable genotype-virus-environment interplay. In all, there was no significant correlation between the cowpea virus disease incidence and the seed yield observed. Hence the various genotypes may have reacted differently to the viral infections.

Soil moisture stress influences shoot growth and this might have accounted for the

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difference in growth among the cowpea genotypes in the major and minor season (Sangakkara, 1998). SARC-1-57-1 had mean plant height of about 41 cm in the major season but had mean plant height of 17.7 cm in the minor season. Overall average for the major season was much higher (26.27 cm) than that of the minor season (17.20 cm). The difference in the mean growth rate in the major and minor seasons may be due to the favourable environmental conditions of moisture during this period as well as the inherent differences among the cowpea genotypes. The low moisture and high temperatures recorded in the minor season affected the growth of the cowpea genotypes hence the lower mean plant height recorded.

Yield was also not recorded during the minor season due to unfavourable environmental factors which caused inflorescence to drop and also the continuous feeding on the fresh pods by birds due to lack of food during this period. Incidence of viral disease was also high which affected growth as well as pod formation. A preliminary experiment conducted during the minor season of 2002 by Taiwo and Akinjogunla, (2006) resulted in complete loss in pod and seed yield, which was attributed to the hot and low soil moisture level associated with the minor season, which is known to influence virus multiplication in cowpea. Hughes and Shoyinka, (2003) also indicated that yield loss in sub-Saharan Africa could be attributed to virus mixtures and cultivar-environment interactions. This finding thus shows a genotype-virus-environment interaction effect; hence genotypes that showed stable viral disease reaction over the two seasons as well as high yield could be selected for further evaluation.

Incidence of viral disease in the major season was significantly and positively correlated with disease severityand AUDPC but negatively correlated with plant height and seed yield. IT10K-819-4 and Marfo tuya had the least disease incidences

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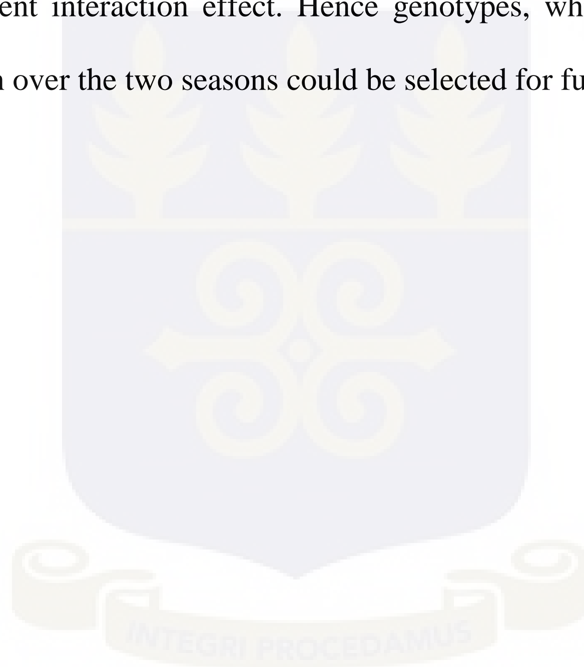
respectively for the major and minor seasons, but these did not translate into the highest yield compared to GH3684, which recorded the highest yield of 8010 kg ha^{-1} with a disease incidence and severity of 57.3 % respectively as well as AUDPC of 6.2. This may be an indication of the fact that GH3684 is more tolerant to the cowpea diseases and hence better adapted to the field conditons than IT10K-819-4 and Marfo tuya (which yielded dry cowpea seeds of 5,627 kg $ha^{-1}$and 4,747 kg ha^{-1} respectively). Even so, all three genotypes exceeded the achievable yield of 2,600 kg ha^{-1} estimated by MoFA (2013). This is a reflection of the presence of a genotype-virus-environment interaction effect. Hence genotypes, which showed stable viral disease reaction over the two seasons could be selected for further evaluation.



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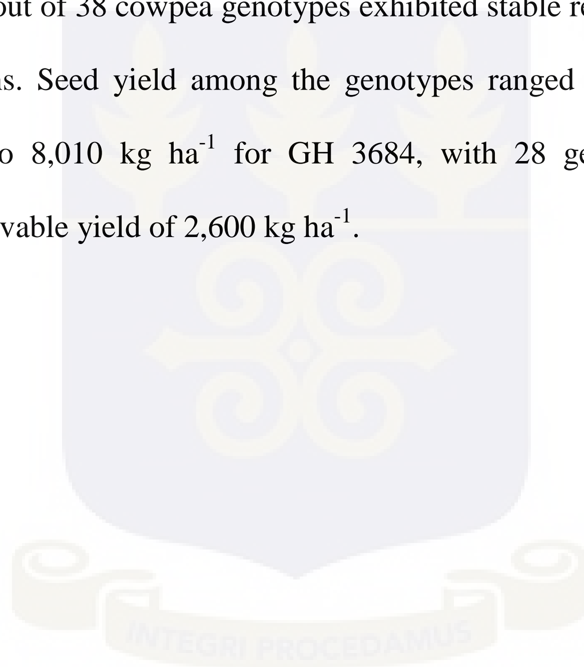
CONCLUSION

The 38 cowpea genotypes exhibited varying degrees of cowpea viral infection in the field, both in the major and minor seasons. The symptoms include mosaic, mottling, necrosis, and deformation and stunting. Disease incidence and severity were significantly higher in the dry season than the wet season. The cowpea genotypes also showed significant genotype versus season interaction effect on disease incidence and severity as well as the Area Under Disease Progress Curve (AUDPC). Six (IT07-210-1-1, IT07K-297-13, IT08K-193-14, IT09-456, IT10K-817-3 and IT10K-819-4) out of 38 cowpea genotypes exhibited stable resistance both in the wet and dry seasons. Seed yield among the genotypes ranged from 1,313 kg ha^{-1} for IT10K-866-1 to 8,010 kg ha^{-1} for GH 3684, with 28 genotypes exceeding the estimated achievable yield of 2,600 kg ha^{-1}.



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CHAPTER FIVE

5.0 PHENOTYPIC AND SEROLOGICAL EVALUATION OF SELECTED COWPEA GENOTYPES FOR RESISTANCE TO VIRAL DISEASE

5.1 Introduction

Most of the world's cowpea production is in Africa, with Nigeria, Kenya, Niger, Burkina Faso, Ghana, and Uganda being major producers (Bennett-Lartey and Ofori, 2000). However, the yield of the crop in the growing areas of Africa is about 45% of what is produced in the developed countries (Akibode, 2011). Cowpea Aphid Borne Mosaic Virus alone has been implicated in yield losses between 87 % to 100 % in Nigeria, 13-87 % in Iran 48 to 60 % Zambia (Fuleratu, 2016) and 20 - 60 % yield loss due to viruses across several countries (Taiwo, 2003; Bashir and Hampton, 1996; Ouzounov, 1988; Rachie, 1985). Under favourable conditions for virus spread, overall cowpea loss may not appear to be much even though local areas may be severely affected (Webster *et al*., 2004).

Though over 140 viruses have been identified to affect cowpea (Hughes and Shoyinka, 2003; Shoyinka *et al*., 1997), relatively few are of economic importance in Africa (Thottappilly and Rossel, 1992; Alegbejo and Kashina, 2001). Some of the major ones include CpMoV, CYMV, CABMV and SBMV, which have been reported severally on cowpea in Nigeria (Allen *et al.*, 1982; Aliyu *et al.*, 2012), Senegal (Ndiaye *et al.*, 1993) and Pakistan (Bashir and Hampton, 1996) among other countries. Four cowpea viruses have been reported in Ghana and this include *Southern bean mosaic virus* (SBMV), *Cowpea aphid-borne mosaic virus* (CABMV), *blackeye cowpea mosaic virus* (BICMV) and *Cowpea mild mottle virus* (CPMMV) (Zettler and Evans, 1973; Lamptey and Hamilton, 1974; Jeyanandarajah and Brunt, 1993). Fuleratu (2016) identified some multiple virus infections in a study carried out in cowpea growing areas of Ejura and Mampong in the Ashanti region as well as

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Atebubu and Nkoranza in the Brong Ahafo regions of Ghana. This gives an indication of the presence of virus-complex infections within the study area and possibly within the country. Higher disease severity and seed yield reduction have been reported in mixed virus infections (Taiwo and Akinjogunla, 2006; Kareem and Taiwo, 2007).

Despite the high yield loss associated with cowpea viral disease, control of the cowpea viruses in Ghana is limited to some agronomic practices such as using symptom-free propagules, roguing of infected individuals, elimination of alternative hosts and vectors by weeding, burning and use of pesticides, and by quarantine practices (Webster *et al*., 2004). Accurate disease diagnosis and mapping of the geographical and temporal distribution of the cowpea viral diseases is very essential in the establishment of control measures (Naidu and Hughes, 2003).

The most economical and effective method disease control method in managing cowpea disease is by the establishment of resistant cultivars through screening, which is resistant to the predominant virus strain and also adaptable to a particular region (Taiwo, 2003). An efficient screening method is therefore needed to identify resistant varieties of cowpea. Visual assessments, which are primarily based on observable symptoms and cannot determine whether all genotypes being screened have been exposed to viral inoculum are considered as major limitations in screening for virus resistance under field conditions (Taiwo, 2003). A more sensitive method of screening cowpea is therefore needed which can detect specific viruses. Serological or molecular tests are examples of very sensitive and specific methods, which can detect viruses, which do not present symptoms for visual assessment, and also the specific virus causing the disease since some different viruses may cause similar symptoms in the same plant (Walkey, 2012).

Efficient and accurate diagnosis is therefore important in the managing the

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consequences associated with the transmission of viruses in cowpea (Odedara *et al*., 2009). Among the screening methods, Enzyme-Linked Immunosorbent Assays (ELISA) have become the primary virus detection method, which is highly sensitive, simple to use, low cost and suited for large scale testing (Ojuederie *et al*., 2009; Dhanasekar and Reddy, 2015).

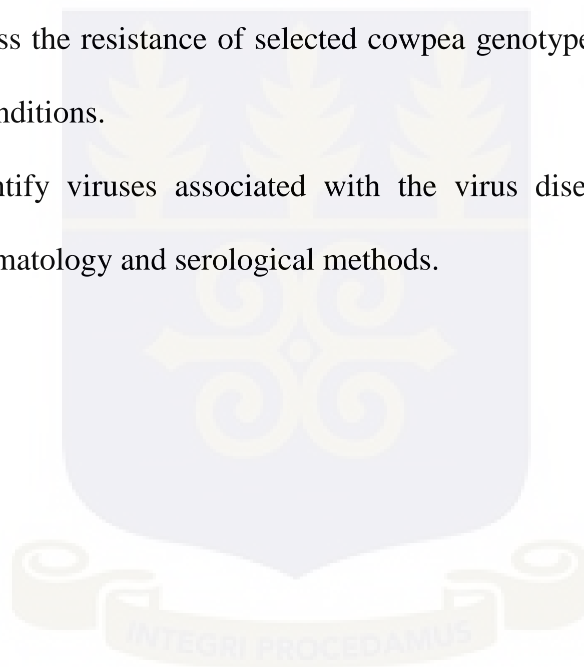
Objective of Study

The main objective of this study was, therefore, to evaluate selected cowpea genotypes for resistance to viral disease and to identify the associated viruses.

Specific Objectives;

1 To assess the resistance of selected cowpea genotypes to viral disease under field conditions.

2 To identify viruses associated with the virus disease on the field using symptomatology and serological methods.



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5.2 METHODOLOGY

5.2.1 Plant material

Fifteen cowpea genotypes, each possessing large seeds, high seed yield per unit area and high to moderate resistance to cowpea viral diseases observed in the previous field trials were selected for further evaluation in the field during the 2016 major cropping. This was done to ensure the development of most stable virus-resistant cowpea cultivars under a more rigorous screening regime. The cowpea genotypes comprise four early maturing, four medium maturing, and seven dual purpose genotypes. The names of the cowpea genotypes and their growth habits are indicated in Table 5.1.

Table 5.1: Cowpea genotypes selected for evaluation to assess cowpea viral disease

Genotypes	Character	Disease severity	100 SW/g	Seed yield/ kg ha^{-1}
Apagbaala	Early maturing	2.2	12.4	1543
IT04K-321-2	Dual purpose	1.0	17.8	3370
IT07K-210-1-1	Medium maturing	1.2	16.1	4063
IT07K-297-13	Dual purpose	1.3	20.3	4033
IT08K-125-107	Early maturing	1.5	16.1	4010
IT08K-126-19	Medium maturing	2.0	18.2	3463
IT08K-150-12	Medium maturing	2.1	18.4	6223
IT08K-180-11	Medium maturing	1.4	15.5	2767
IT08K-193-14	Dual purpose	1.2	19.1	6867
IT09K-456	Dual purpose	1.1	17.7	6770
IT10K-817-3	Dual purpose	1.2	15.5	5713
IT10K-819-4	Dual purpose	1.1	20.8	5627
IT10K-832-3	Early maturing	1.4	15.4	3277
IT11K-61-82	Early maturing	1.0	13.8	3207
Padi tuya	Dual purpose*	1.8	22.7	5027

*Dual purpose = leaves and seeds are used as food.

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5.2.2 Experimental site

The evaluation was carried out at the Teaching and Research Farm of the School of Agriculture, College of Agriculture and Natural Sciences (CANS) of the University of Cape Coast.

5.2.3 Design and layout of experiment

The Randomised Complete Block Design (RCBD) with four replications involving 15 treatments was used for this experiment. There were 60 subplots, each measuring 3 × 5 m with 1 m between subplots and 0.5 m between plots within a block and 1 m between replicates. The plots measured 7.0 m x 6.4 m in size.

5.2.4 Cultural practices

Thinning out, weeding and pesticide application was done as described in the first experiment.

5.2.5 Data collection

Data were collected on disease incidence, disease severity, AUDPC and seed yield. In each case data were collected from ten tagged plants from the middle of each plot and the means were calculated.

5.2.6 Viral disease incidence, severity scoring and AUDPC

Symptom severity of viral disease was assessed using a modified scale of 1-5 by Hahn *et al*. (1980) as described in previous chapter. Disease incidence per plot was assessed as the percentage of plants out of the ten plants showing disease symptoms as described by Gumedzoe *et al*., (1997). Data on mean severity scores were used to calculate Area Under Disease Progress Curve (AUDPC) for each of the cowpea lines using the formula of Campbell and Madden (1990) as shown in previous chapter.

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5.2.7 Viral detection by Double Antibody Sandwiched Enzyme-Linked Immunosorbent Assay (DAS-ELISA).

The presence or absence of the virus in the plants was assessed serologically by using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) of Clark and Adams (1977). Polyclonal antisera specific for *Cowpea aphid-borne mosaic virus* (potyvirus), *Cowpea severe mottle virus* (carlavirus), *Cowpea mosaic virus* (comovirus), *Southern bean mosaic virus* (sobemovirus) and *Cucumber mosaic virus* (cucumovirus) (DSMZ, Germany) were used. This experiment was carried out at the Virology Laboratory of Cocoa Research Institute of Ghana (CRIG), Tafo.

Leaf samples (both symptomatic and asymptomatic) were collected from each cowpea genotype from top, middle and lower parts of each plant. The leaf samples were kept at -80 ^{O}C until they were used.

One gram (1 g) of leaf sample of each cowpea genotype was crushed in extraction buffer (8.0 g NaCl, 0.2 g KH_2PO_4, 1.1 g Na_2HPO_4, 0.2 g KCl L^{-1}, pH 7.4) containing 0.05 % v/v Tween 20, and 2 % w/v polyvinyl-pyrolidone) at a ratio of 1:10.

Serological testing was carried out following manufacturer's protocol. A negative control (extraction buffer without plant sap) and positive control (infected sap from DSMZ) were included in the test to ensure the reliability of results. The antibody IgG was diluted in the coating buffer (Na_2CO_3 + $NaHCO_3$ + NaN_2) at a recommended dilution of 1:1000 according to manufacturer's instructions and 200 µL was added to the wells and incubated at 37 ^{O}C for 4 hours.

The plates were then washed three times with phosphate buffered saline + Tween 20 (0.05 %) (PBS-T). A 200 µL volume of each sample, the positive and negative controls were added to the coated wells. All samples and controls were tested in

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duplicates and incubated at 4 ^{O}C overnight after which the plates were washed thrice with buffer PBS-T.

After washing the plates three times with PBS-T, 100 µL of the antibody-enzyme conjugate (alkaline phosphatase conjugate, diluted at 1/1000 in PBS-T + BSA + NaN_2) was added to each test well and incubated at 37°C for 2 hours. The plates were then washed four times to ensure that all unbound antibody-enzyme conjugate was removed from the wells. The plates were then incubated for one hour at room temperature with the freshly prepared phosphate substrate solution (100 µL per well). The substrate was a p-nitrophenyl phosphate (pNPP) tablet (Sigma-Aldrich Co. LLC) applied at 1.0 mg mL^{-1} in 9.87 % diethanolamine, pH 9.8 until the colour (yellow) developed as signal for presence of virus.

5.2.7.1 Seed yield

Seed yield per hectare (kg ha^{-1}) and hundred seed weight was determined after harvest (plants from the central one square meter of each plot). The resulting weights, in grammes per meter square (g m^{-1}) were then extrapolated to kilogramme per hectare (kg ha^{-1}) basis to get the average seed yield per hectare. Also hundred seeds for each genotype was counted and weighed (g).

5.2.7.2 Data analysis

Data on disease incidence was Arcsine transformed to homogenise the variances and reduce error. The other quantitative data (severity, AUPDC, seed yield and hundred seed weights) were subjected to ANOVA and the means separated using l.s.d at 5 % level of probability. Microsoft Excel was also used to determine the proportions of virus present after the serological test and plot bar graphs.

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5.3 RESULTS

5.3.1 Disease incidence and severity

There was a significant difference in the incidence and severity among the fifteen cowpea genotypes ($P < 0.001$) in the second, fourth and sixth week after sowing. Apagbaala and IT08K-193-14 showed no viral symptoms during the period of study whereas IT08K-180-11 had the highest incidence and severity (Table 5.1).

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Table 5.2: Incidence and severity of cowpea viral diseases on 15 cowpea genotypes grown in the open field.

	Incidence (%)			**Severity score**		
Genotype	**2WAS**	**4WAS**	**6WAS**	**2WAS**	**4WAS**	**6WAS**
Apagbaala	0.0^{d}	7.2^{d}	0.0^{d}	1.0^{e}	1.0^{c}	1.0^{d}
IT04K -321 - 2	41.1^{c}	32.8^{c}	31.3^{c}	1.4^{c}	1.3^{b}	1.3^{c}
IT07 - 210 - 1 - 1	13.0^{d}	7.2^{d}	0.0^{d}	1.1^{d}	1.1^{c}	1.0^{d}
IT07K - 297 - 13	13.0^{d}	18.4^{cd}	10.4^{d}	1.1^{d}	1.1^{c}	1.1^{d}
IT08K - 126 - 19	18.6^{d}	13.0^{cd}	13.0^{d}	1.1^{d}	1.1^{c}	1.1^{d}
IT08K - 180 - 11	77.0^{b}	90.0^{a}	68.8^{b}	2.3^{a}	2.2^{a}	2.1^{a}
IT08K - 193-14	0.0^{d}	7.2^{d}	0.0^{d}	1.0^{e}	1.0^{c}	1.0^{d}
IT08K- 150 - 12	13.0^{d}	18.4^{cd}	13.0^{d}	1.1^{d}	1.1^{c}	1.1^{d}
IT09K - 456	18.4^{d}	13.0^{cd}	13.0^{d}	1.1^{d}	1.1^{c}	1.1^{d}
IT10K - 817 - 3	26.2^{cd}	31.3^{c}	23.6^{c}	1.0^{e}	1.0^{c}	1.0^{d}
IT10K - 819 - 4	82.8^{ab}	79.6^{a}	74.1^{a}	2.0^{b}	1.9^{a}	1.9^{b}
IT10K - 823 - 3	90.0^{a}	77.0^{b}	77.0^{a}	2.0^{b}	1.9^{a}	1.9^{b}
IT10K -125 - 107	18.4^{d}	18.4^{cd}	18.4^{c}	1.1^{d}	1.1^{c}	1.1^{d}
IT11K - 61 - 82	7.2^{d}	7.2^{d}	21.2^{c}	1.0^{e}	1.1^{c}	1.2^{cd}
Padi tuya	31.1^{cd}	36.4^{c}	36.4^{c}	1.1^{d}	1.1^{c}	1.1^{d}
Grand mean	30	30.5	26.7	1.3	1.3	1.3
F-Value	<.001	<.001	<.001	<.001	<.001	<.001
SE±	15.55	16.55	15.31	0.06	0.08	0.10

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5.3.2 Area Under Disease Progress Curve (AUDPC)

ANOVA revealed a highly significant difference ($P < 0.001$) in the AUPDC amongst the cowpea genotypes over the period of study (Figure 5.1). Apagbaala and IT10K-817–3 showed no symptoms after six weeks of sowing and this translated into the lowest mean AUPDC of 4.1 and 4.0 respectively. The highest mean AUPDC was recorded by IT08K-180–11 (8.8) followed by IT10K-823–3 (7.7) and IT10K-819–4 (7.6). In all 26.7 % of the cowpea were classified as moderately resistant (AUDPC ranging from 5 - 10) and 73.3 % were classified as resistant (AUDPC < 5) (Fig. 5.1).

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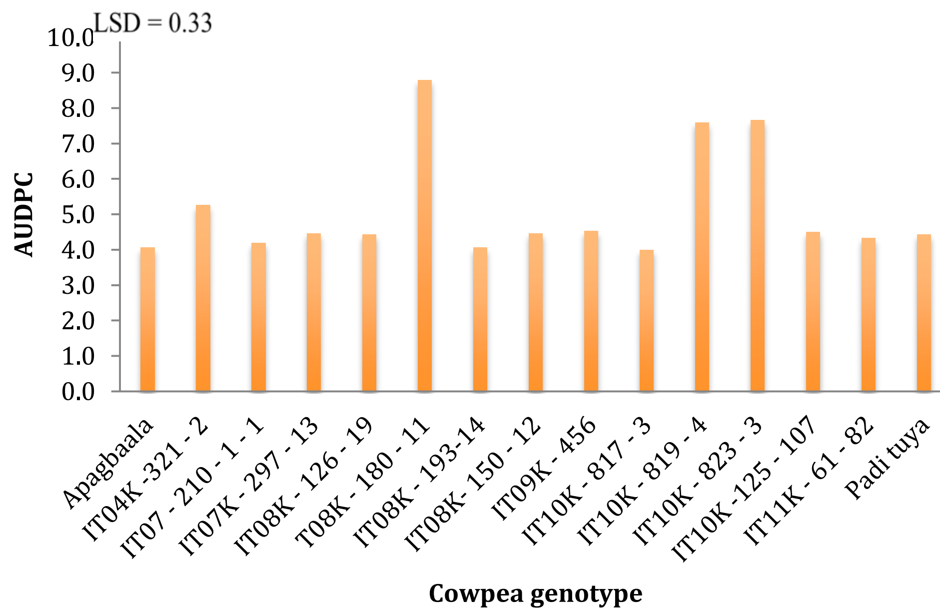
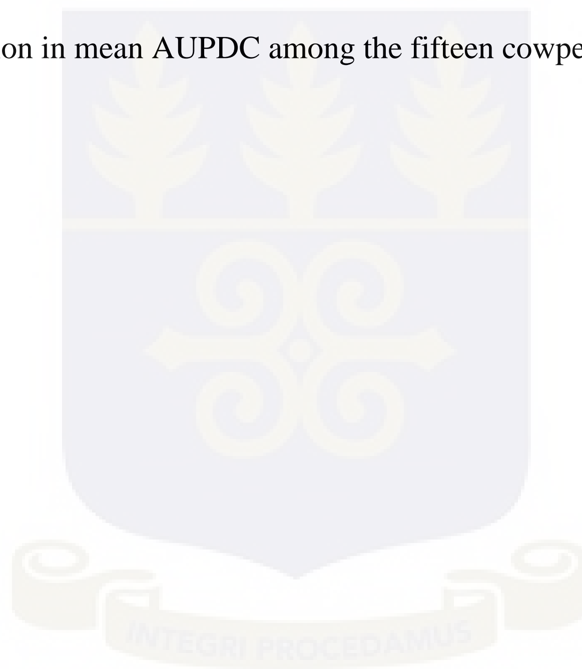


Fig.5.1: Variation in mean AUPDC among the fifteen cowpea genotypes evaluated.



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5.3.3 Hundred seed weights

There was a significant difference ($P < 0.001$) in hundred-seed weights among the fifteen cowpea genotypes evaluated. Genotype IT10K-819–4 had the highest seed yield of 21.5 g whereas the local variety, Apagbaala had the lowest (12.75 g) (Fig.5.2).

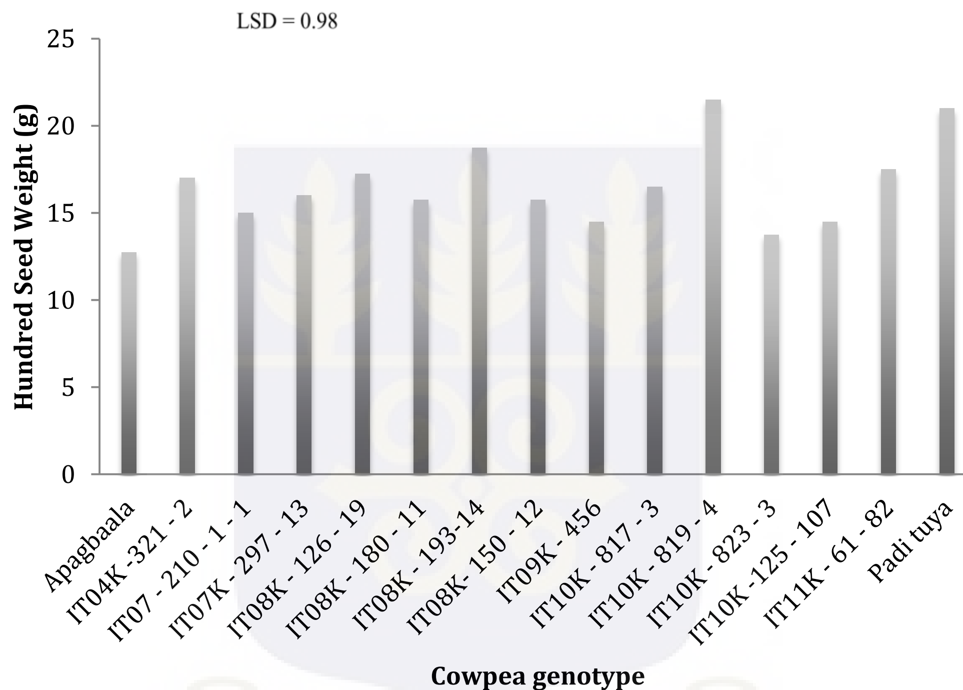

Fig. 5.2: Variation in hundred seed weights (g) among the fifteen cowpea genotypes evaluated.

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5.3.4 Seed yield

There was significant difference ($P < 0.001$) in the seed yield recorded for the various cowpea genotypes evaluated (Figure 5.2). Local variety Padi tuya had the highest dry seed yield of 5862 kg ha^{-1}, followed by IT11K-61-82 with seed yield of 4383 kg ha^{-1}whereas genotype IT10K-125-10 had the lowest (1850 kg ha^{-1}).

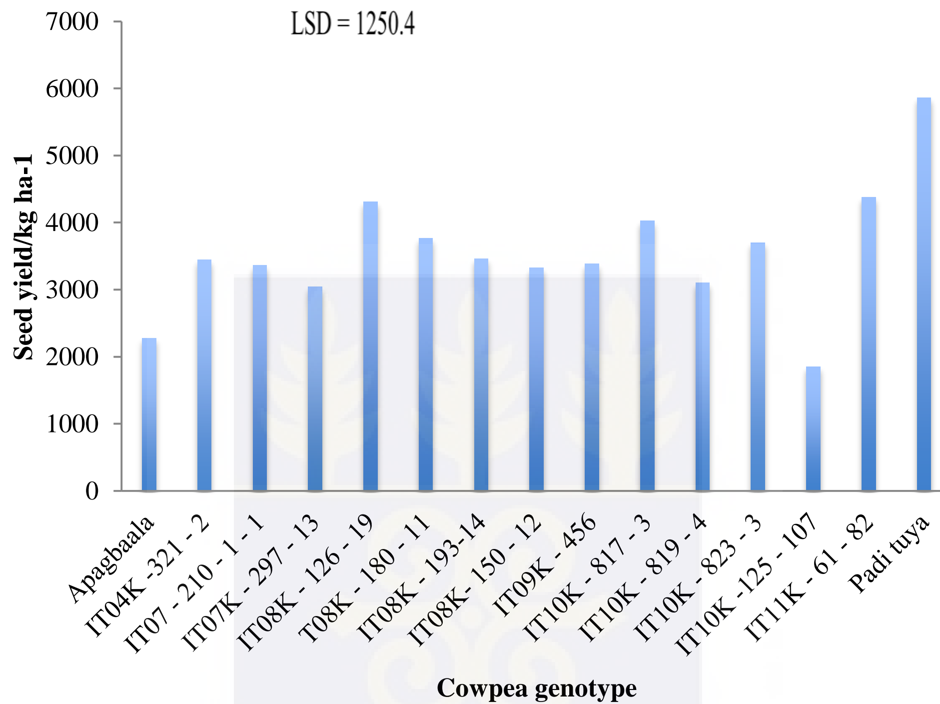

Fig.5.3: Variation in dry seed yield (kg ha^{-1}) among the fifteen cowpea genotypes evaluated.

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5.3.5 Virus detection by DAS-ELISA

The DAS-ELISA detected *Cucumber Mosaic Virus* (CMV) and *Cowpea Severe Mosaic Virus* (CSMV) in the cowpea samples, and all the cowpea genotypes were susceptible to one or both viruses except IT04K-321-2 although the distribution of the viruses varied across the different genotypes. CMV was detected in all the cowpea genotypes except IT04K-321-2 and IT10K-819-4, suggesting that it is the most common viruses infecting cowpea in the study area. CSMV was detected in only two cowpea genotypes (IT10K-819-4 and Padi tuya) indicating that it is rare. *Southern bean mosaic virus* (SBMV) and *Cowpea mosaic virus* (CMoV) were not detected in any of the cowpea genotypes. There was a single case of mix infection with CSMV + CMV in the local variety Padi tuya.

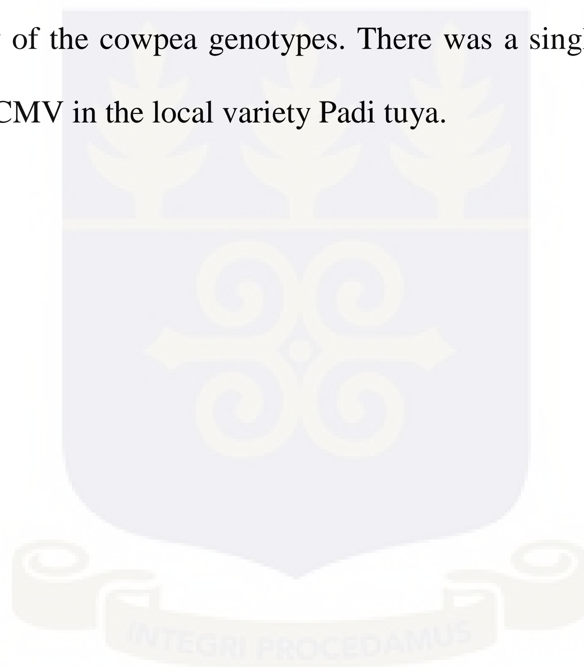

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Table 5.3: DAS-ELISA detection of cowpea viruses among 15 selected cowpea genotypes.

Genotype	CMoV	CSMV	CMV	SBMV
IT07-210-1-1	-	-	+	-
IT10K-819-4	-	+	-	-
IT10K-832-3	-	-	+	-
IT10K-817-3	-	-	+	-
IT11K-61-82	-	-	+	-
IT08K-180-11	-	-	+	-
IT08K-125-107	-	-	+	-
Apagbaala*	-	-	+	-
IT08K-126-19	-	-	+	-
IT07K-297-13	-	-	+	-
IT08-150-12	-	-	+	-
IT09K-456	-	-	+	-
IT08K-193-14	-	-	+	-
Padi tuya*	-	+	+	-
IT04K-321-2	-	-	-	-
Positive control	+	+	+	+
Proportion of plants infected	0/15	2/15	13/15	0/15

*Local genotypes. + denotes presence of virus; - denotes absence of virus

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5.4 DISCUSSION

During the evaluation, low incidence and severity of virus symptoms among the genotypes were observed suggesting that there were probably a low population of virus vectors and consequently, low inoculum source to cause high virus incidence in the fields of cowpea or better still, the genotypes were resistant (Edema *et al*. (1997) and Shoyinka *et al*. (1997). In the current study, it was also observed that the progress of virus infection developed slowly in most cowpea genotypes with some recovering after six weeks, hence low mean AUPDCs recorded. This suggests that the cowpea genotypes exhibited field resistance, thus confirming the virus-resistance identified in chapter 4. Gumedzoe *et al*. (1997) implicated genetic variations within the host plants, age, the architecture of the plant and environmental factors as factors that influence incidence and severity of viral diseases in plants. The detection of two viruses CMV and CSMV in the cowpea genotypes by DAS-ELISA suggests that none of the cowpea lines that exhibited field resistance was immune to virus infection.

Even though this study did not identify genotypes with complete immunity based on visual assessment, several lines with good levels of resistance were identified. Thirteen out of fifteen cowpea genotypes were infected with CMV whereas only two genotypes were infected with CSMV, suggesting that CMV may be the commonest virus infecting cowpea in the study area. This finding is consistent with the report of van Regenmortel *et al*. (2000) which states that *Bromoviridae* including CMV, is one of the most important widespread groups of viruses in the world infecting the largest number of plant species hence its high incidence in the cowpea sampled in our study. The study also revealed co-infection of one cowpea genotype with CMV and CSMV, an indication of the presence of virus-complex infection within these cowpea genotypes. This is consistent with the study of Fuleratu (2016) who also reported the

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presence of multiple virus infections among the cowpea accessions screened. Several reports have shown that multiple virus infections are usually associated with higher disease severity and yield reduction (Taiwo and Akinjogunla, 2006; Kareem and Taiwo, 2007).

Viruses that were not detected among the cowpea genotypes may not necessarily be absent from the study area, but rather due to their low concentrations in the leaf samples (Fuleratu, 2016). It may also be due to the presence of serologically variable strains of the viruses and the non-availability of antibodies specific to them (Aliyu *et al.*, 2012).

Infection in the field with other viruses is difficult to avoid, especially if viruses have similar symptoms (Orawu, 2007). Based on the findings from field assessments and ELISA tests, Oruwa (2007) categorized cowpea genotypes with respect to their levels of resistance. In his classification, genotypes with less than 10.0 % disease severity that reacted negatively to the ELISA tests were categorised as resistant whereas those in the range between 10-20 % of disease severity or very weakly to the ELISA was categorised as moderately resistant as long as the ELISA tests showed a negative reaction (Orawu, 2007). Using this classification, the genotype IT04K-321-2 may be classified as partially resistant. Similarly, the genotypes IT07K-297–13, IT08K-126–19, IT08K-150–12 and IT09K–456 may be categorized as moderately resistant.

However, some plants exhibited symptoms that did not test positive for any of the above viruses. Bachand and Castello (1998) pointed out that if the virus concentration in the seedlings does not exceed 5-25 ng g^{-1}, then an ELISA test would assess the plant samples as negative. Hence the virus concentration in the cowpea genotypes used for ELISA may be too low to be detected by the ELISA technique used. A more sensitive and robust technique for detecting viruses is required,

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especially with the use of specific primers in polymerase chain reaction (PCR), to amplify the viruses present.

Great variability in plant morphology was observed in the cowpea genotypes studied. One hundred-seed weight observed during the evaluation (12.7 g) was consistent with result obtained in chapter 3 in which Apagbaala recorded the least hundred-seed weight of 12.11 g. IT10K-819-4 had highest seed weight (21.5 g) comparable to that observed in the first experiment (20.8 g). This implies that seed size may be a stable trait hence could serve as a basis for selection during breeding.

Seed yield was high compared to that obtained by others in various agro-ecological zones with Padi tuya, a local variety yielding dry seeds of 5,862 kg ha^{-1} which is comparable to that obtained in the first experiment in the major season (5,027 kg ha^{-1}). Apagbaala which yielded the least in the first experiment (1,543 kg ha^{-1}) had 2,275 kg ha^{-1} during the evaluation which was higher than that obtained by IT10K-125-10 (1,850 kg ha^{-1}) which yielded the least during the evaluation. These values are however higher than those obtained by Basaran *et al* (2011) who observed seed yield between 1,170 kg ha^{-1} and 1,420 kg ha^{-1}. Yield figures obtained in the current study are also higher than that obtained by Akande and Balogun, (2009) who studied cowpea genotypes in different agro-ecological conditions in three locations within Nigeria for two years and observed yield ranging from 915 to 1,173 kg ha^{-1}.

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CONCLUSION

Field evaluation of the fifteen cowpea genotypes showed low levels of disease incidence and severity as well as AUDPC. Based on the AUDPCs, three genotypes were moderately resistant with the rest resistant. DAS-ELISA tests were able to detect two viruses (CSMV and CMV) with one mixed infections detected (CSMV/CMV). No virus was detected in genotype IT04K-321-2 by DAS-ELISA, which may imply resistance to the cowpea viruses tested. This genotype can further be screened against other cowpea viruses so as to confirm its status, after which it may serve as source of resistant genes for future breeding work as well as planting material for farmers.

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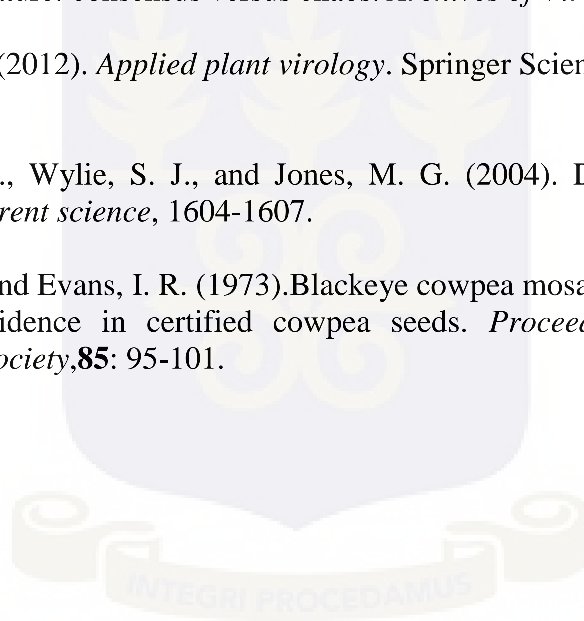

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CHAPTER SIX

6 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

A series of experiments were carried out to characterise and evaluate the performance of 38 cowpea genotypes against some cowpea viruses in the coastal savanna agroecological zone of Ghana.

The first experiment sought to characterise 38 cowpea genotypes using some qualitative and quantitative cowpea descriptors followed by the determination of genetic relationship among the 38 genotypes of cowpea using SSR primers and gel electrophoresis. The third experiment assessed the incidence and severity of cowpea virus among the 38 genotypes of cowpea and identified potential lines which are resistant to viral diseases. The fourth experiment sought to further evaluate selected cowpea genotypes with good potential on the field and the laboratory using DAS-ELISA. The following are the main conclusions:

Chapter 3

a. Cluster analysis based on 27 morphological traits grouped the cowpea genotypes into two main clusters at dissimilarity coefficient of 0.42 where IT10K-817-1 and IT08K-195-15 were the most diverse genotypes observed.

b. GH3684 recorded the highest dry seed yield of 8010 kg ha^{-1} and Apagbaala recorded the least (1543 kg ha^{-1}).

c. Principal Component Analysis (PCA) showed characters contributing differently to the 73.1 % of the total variability with PC1 contributing 23.4 % of the total variability.

d. Total seed yield (TSY) showed low to very high positive correlation with Total Pod Weight (TPW), Number Seeds Per Pod (NSPP), Canopy Diameter (CD), Pod Length (PL) and Number of Branches (NB).

e. The nine SSR primers used generated a total of 285 bands across the selected

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cowpea genome with sizes of amplified allelic loci ranging from 280 to 520 bp revealing three major clusters at a dissimilarity index of 40% for all the cowpea genotypes.

f. IT07K-299-6 and IT07K-210-1-1 were the most widely separated (distantly related) among the cowpea genotypes.

Chapter 4

a. Characteristic cowpea virus disease symptoms were observed in the field among which leaf mosaic and mottling (86.67 %) were the most common symptoms observed followed by chlorotic spots (34.21 %), vein clearing (28.95 %), leaf curl (26.32 %), necrotic lesions (15.79 %) and the least was stunting (10.53 %).
b. The overall mean AUDPC (5.31) recorded in the minor season was higher than that of the major season (4.07).
c. There was a highly significant difference in disease incidence between the major and minor seasons, with the minor season having the highest overall mean incidence of 53.1 % compared to 30.9 % in the major season.

Chapter 5

a. DAS-ELSA detected two out of the four cowpea viruses tested. These were Cowpea severe mosaic virus (CSMV) and Cucumber mosaic virus (CMV). A mixed infection of CPSMV/CMV was recorded in the local cultivar Padi tuya.
b. None of the viruses tested was detected in IT04K-321-2, which maybe resistant.

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6.2 RECOMMENDATIONS

6.2.1 Future breeding work

Based on the morphological characterization as well as response to virus infection, one genotype IT04K-321-2 may be recommended for use in future breeding work. This genotype is a dual-purpose cowpea whose leaves and seeds (white) are both used for food. It is high yielding with large seeds and resistant to the four viruses tested.

6.2.2 Future research areas

Based on the current study, the following research areas are recommended

1. Further morphological and molecular characterization should be done to determine the correlation between the planting density, viral disease incidence and seed yield.
2. A nation-wide survey should be done to map the cowpea viruses present among the local cowpea cultivars.
3. Genetic diversity studies should be carried out on the two viruses detected to ascertain their strains.
4. Imported cowpea varieties should screened to assess their virus load status.
5. Screen house experiment should be carried out on the identified cowpea genotype during which it will be challenged (inoculated) with various strains of the viruses to confirm its resistance.
6. Reverse transcription PCR should be used to screen the 15 selected cowpea genotypes to detect any cowpea viruses that might be present in these cowpea genotypes, which may have been missed by the DAS-ELISA.

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APPENDICES

APPENDIX 1

List of cowpea descriptors used in the morphological study

1. Growth habit

1.Acute erect (branches form acute angles with main stem), 2. Erect (branching angle less acute than above), 3. Semi-erect (branches perpendicular to main stem, but do not touch the ground), 4. Intermediate (lower branches touch the ground), 5. Semi-prostrate (main stem reaches 20 or more centimetres), 6. Prostrate (plants flat on ground; branches spread several metres) 7. Climbing

2. **Days to 50% flowering-** Number of days from sowing until 50% of the plants have begun to flower. Recorded for plants with the same sowing date at the same location.

3. **Pod length** [cm]- Average length of the 10 longest mature pods from 10 randomly selected plants

4. **Days to pod maturity-**Number of days from sowing until 95% of the plants have mature pods

5. **Testa texture-** 0. Smooth, 1. Smooth to rough 2. Rough (fine reticulation) 3. Rough to wrinkle, 4. Wrinkled (coarse folds on the testa)

6. **100-Seed weight** [g] - Weight of 100 seeds with 12% moisture content

7. **Seed coat colour -**Recorded at maturity; 1. White, 2. Cream 3. Brown. 4. Red, 5. Purple, 6. Black, 99. Other (i.e. ‘yellow’ or ‘blue’, specify)

8. **Flower colour-**1. White, 2. Violet, 3. Mauve-pink, 99. Other (specify in the descriptor)

9. **Number of pods per peduncle -**Recorded under total insect control. Average number of 10 randomly selected peduncles

Pod colour of mature pod – 1. Pale tan or straw, 2. Dark tan, 3. Dark brown, 4. Black or dark purple, 99. Other (specify in the descriptor **Notes**)

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APPENDIX 2

Cowpea genotypes and their sources

Genotype	Characteristic	Seed coat colour	Source
Apagbaala	Early maturing	White	SARI[2]
IT07K-243-1-2	Early maturing	Rough Brown	IITA
IT07K-298-9	Early maturing	White	IITA
IT07K-299-6	Early maturing	White	IITA
IT08K-125-107	Early maturing	White	IITA[1]
IT10K-836-2	Early maturing	Brown	IITA
IT10K-837-1	Early maturing	White	IITA
IT10K-843	Early maturing	White	IITA
IT10K-866-1	Early maturing	White	IITA
IT10K-973-1	Early maturing	White	IITA
IT10K832-3	Early maturing	Speckled White	IITA
IT11K-61-82	Early maturing	White	IITA
IT07-210-1-1	Medium maturing	White	IITA
IT07K-291-92	Medium maturing	White	IITA
IT07K-303-1	Medium maturing	White	IITA
IT08-125-100	Medium maturing	White	IITA
IT08-150-12	Medium maturing	White	IITA
IT08K-126-19	Medium maturing	White	IITA
IT08K-180-11	Medium maturing	White	IITA
IT09K-231-1	Medium maturing	Brown	IITA
IT10K-815-5	Medium maturing	Red	IITA
IT10K-817-7	Medium maturing	Red	IITA
IT10K-827-11	Medium maturing	White	IITA
Marfo tuya	Medium maturing	White	SARI
IT04K-321-2	Dual Purpose	White	IITA
IT07K-297-13	Dual Purpose	White	IITA
IT07K-298-15	Dual Purpose	White	IITA
IT08K-193-14	Dual Purpose	White	IITA
IT08K-193-15	Dual Purpose	White	IITA
IT09K-321-1	Dual Purpose	Brown	IITA
IT09K-456	Dual Purpose	Cream	IITA
IT10K-817-1	Dual Purpose	Red	IITA
IT10K-817-3	Dual Purpose	Red	IITA
IT10K-819-4	Dual Purpose[3]	Red	IITA
IT10K-834-3	Dual Purpose	Mottled Red	IITA
Padi tuya	Dual Purpose	White	SARI
GH 3684	*Striga*-resistant	Red	UCC/PGRRI[4]
SARC-1-57-1	Aphid-resistant	White	SARI

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APPENDIX 3

Buffers used in Genetic Analysis

Stocks:
CTAB: for 1L of CTAB buffer
100 ml of 1 M Tris, pH 8.0
280 ml of 5 M NaCl
40 ml of 0.5 M EDTA
20 g of CTAB (Cetyltrimethyl ammonium bromide, Amresco cat#:0833-1Kg)

TE buffer: [Final] for 1L use:
10 mM = 10 ml of 1 M Tris, pH 8.0
1 mM = 2 ml of 0.5 M EDTA

1 M Tris, pH 8.0: for 1 L
121.1 g Tris (Fisher Cat#: BP152-5)
700 ml ddH_2O
Dissolve tris and bring to 900 ml. pH to 8.0 with concentrated HCl (will need ~50ml)
Bring to 1 L.

0.5 M EDTA pH 8.0: for 1 L
186.12 g of EDTA (Fisher Cat#: BP120-1)
750 ml ddH_2O
Add about 20 g of NaOH pellets
Slowly add more NaOH until pH is 8.0,
EDTA will not dissolve until the pH is near 8.0.

5 M NaCl: for 1 L
292.2 g of NaCl (Fisher Cat#: BP358-10)
700 ml ddH_2O
Dissolve and bring to 1 L.

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APPENDIX 4

DAS ELISA BUFFERS

Coating Buffer (Carbonate Buffer)

Sodium carbonate 1.59g
Sodium hydrogen carbonate 2.93g
Make up to 1 litre with dH20. The pH of this buffer is 9.6 and does not require to be adjusted.

Phosphate buffered saline (PBS) x10
Sodium chloride 80g
Potassium diHydrogen orthophosphate 2g
diSodium Hydrogen orthophosphate 11.5g
Potassium chloride 2g
Make up to 1 litre with dH20. The pH of this solution when diluted to 1xs is 7.2

Wash buffer (PBS + Tween 20)
Phosphate buffered saline 1litre
Tween 20 0.5 ml

General Extraction Buffer
Polyvinylpyrrolidone (PVP) 20g
Ovalbumin 2g
Sodium sulphite (anhydrous) 1.3g
Sodium azide 0.2g
Tween 20 0.5ml
Sodium chloride 8g
Potassium diHydrogen orthophosphate 0.2g
diSodium Hydrogen orthophosphate 1.15g
Potassium chloride 0.2g
Make up to 1 litre with distilled/deionised water. This buffer can be difficult to get into solution and it is easier if the PVP is mixed into a "paste" with a small volume of water before adding the other components and the remainder of the water.

Conjugate buffer
Bovine serum albumin 0.2g
PBST 100ml

Substrate buffer (Diethanolamine buffer 1M)
Diethanolamine 90.39g
Diethanolamine-HCl 19.82g
Magnesium chloride 0.1g
Make up to 1 litre with dH20. The pH of this buffer is 9.8 and it does not require to be adjusted. (The diethanolamine and diethanolamine-HCl are liquids however; it is easier to weigh them out than to measure their volumes, as they are extremely viscous.)
pNPP is added to the above buffer at 1mg/ml to make up the substrate for alkaline phosphatase.

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APPENDIX 5

ANOVA table for 100-Seed Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.037	2.519	0.63	
Rep.*Units* stratum					
Code	41	1157.887	28.241	7.06	<.001
Residual	82	328.236	4.003		
Total	125	1491.159			

ANOVA table for Days 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.587	0.294	0.17	
Rep.*Units* stratum					
Code	41	519.206	12.664	7.41	<.001
Residual	82	140.079	1.708		
Total	125	659.873			

ANOVA table for Canopy Diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1729.8	864.9	5.06	
Rep.*Units* stratum					
Code	41	15112.8	368.6	2.16	0.002
Residual	82	14020.3	171.0		
Total	125	30862.9			

ANOVA table for Days to 50 % Pod Maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	287.25	143.63	11.33	
Rep.*Units* stratum					
Code	41	1438.54	35.09	2.77	<.001
Residual	82	1039.41	12.68		
Total	125	2765.21			

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ANOVA table for GrowthHabit

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.6190	0.3095	0.51	
Rep.*Units* stratum					
Code	41	87.4286	2.1324	3.54	<.001
Residual	82	49.3810	0.6022		
Total	125	137.4286			

ANOVA table for Leaf Area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3289.	1644.	0.67	
Rep.*Units* stratum					
Code	41	144544.	3525.	1.44	0.081
Residual	82	200833.	2449.		
Total	125	348666.			

ANOVA table for Number of Seeds per Pod

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		23.357	11.679	1.86	
Rep.*Units* stratum						
Code	41		545.897	13.315	2.12	0.002
Residual	80	(2)	503.014	6.288		
Total	123	(2)	1071.943			

ANOVA table for Number of Branches

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	22.9805	11.4902	11.51	
Rep.*Units* stratum					
Code	41	77.4260	1.8884	1.89	0.007
Residual	82	81.8380	0.9980		
Total	125	182.2444			

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ANOVA table for Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	258.46	129.23	4.01	
Rep.*Units* stratum					
Code	41	4884.87	119.14	3.70	<.001
Residual	82	2641.40	32.21		
Total	125	7784.73			

ANOVA table for Pod length

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.684	0.342	0.11	
Rep.*Units* stratum						
Code	41		513.14	12.516	3.90	<.001
Residual	81	(1)	259.87	3.208		
Total	124	(1)	772.379			

ANOVA table for Disease Incidence in the minor season (4WA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4500.1	2250.1	4.14	
Rep.*Units* stratum					
PLOTS	37	14592.5	394.4	0.73	0.857
Residual	74	40224.1	543.6		
Total	113	59316.7			

ANOVA table for Disease Incidence in the minor season (6WAS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7108.7	3554.4	7.53	
Rep.*Units* stratum					
PLOTS	37	15904.2	429.8	0.91	0.616
Residual	74	34941.5	472.2		
Total	113	57954.5			

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ANOVA table for Disease Incidence in the minor season (8WAS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3072.9	1536.5	3.17	
Rep.*Units* stratum					
PLOTS	37	19444.4	525.5	1.09	0.375
Residual	74	35839.1	484.3		
Total	113	58356.4			

ANOVA table for Disease Severity in the minor season (4WAS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.5490	0.7745	4.81	
Rep.*Units* stratum					
PLOTS	37	7.4863	0.2023	1.26	0.201
Residual	74	11.9191	0.1611		
Total	113	20.9544			

ANOVA table for Disease Severity in the minor season (6WAS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.6440	0.8220	2.99	
Rep.*Units* stratum					
PLOTS	37	5.4825	0.1482	0.54	0.979
Residual	74	20.3251	0.2747		
Total	113	27.4516			

ANOVA table for Disease Severity in the minor season (8WAS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.1184	0.5592	2.28	
Rep.*Units* stratum					
PLOTS	37	11.7225	0.3168	1.29	0.176
Residual	74	18.1866	0.2458		
Total	113	31.0276			

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ANOVA for Season/genotype interaction for Disease Severity (8WAS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Code	41	69.941		1.706	1.12	0.297
Season 1	16.164	16.164		10.66	0.001	
Code. Season	41	76.835		1.874	1.24	0.177
Residual	168	254.763		1.516		
Total 251		417.703				

ANOVA showing seasonal variation in AUDPC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Code	41	122.997		3.000	1.25	0.169
Season 1	96.624	96.624		40.12	<.001	
Code.Season	41	130.916		3.193	1.33	0.110
Residual	168	404.571		2.408		
Total 251	755.107					

Evaluation

ANOVA table for Disease Incidence at 2WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
accession	14	49287.8		3520.6		14.56	<.001
Residual	45	10883.8		241.9			
Total	59	60171.6					

ANOVA table for Disease Incidence at 4WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
accession	14	45506.6		3250.5		11.87	<.001
Residual	45	12326.8		273.9			
Total	59	57833.5					

ANOVA table for Disease Incidence at 6WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
accession	14	39005.8		2786.1		11.89	<.001
Residual	45	10542.8		234.3			
Total	59	49548.7					

ANOVA table for Disease Severity at 2WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
accession	14	10.533333		0.752381	188.10		<.001
Residual	45	0.180000		0.004000			
Total	59	10.713333					

ANOVA table for Disease Severity at 4WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
accession	14	7.621926		0.544423		91.87	<.001
Residual	45	0.266667		0.005926			
Total	59	7.888593					

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ANOVA table for Disease Severity at 6WAS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
accession	14	8.19437	0.58531	54.12	<.001
Residual	45	0.48667	0.01081		
Total	59	8.68104			

ANOVA showing Variation in 100-Seed Weight in evaluated genotypes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	1.8000	0.6000	1.28	
REP.*Units* stratum					
ASSESSIONS	14	341.5000	24.3929	52.01	<.001
Residual	42	19.7000	0.4690		
Total	59	363.0000			

Two-sided test of correlations different from zero probabilities

AUPDC					
Incidence	0.0000				
Severity	0.0000	0.0000			
Plant height	0.1921	0.4296	0.7294		
Seed yield	0.1471	0.4380	0.8262	0.0006	
	AUPDC	Incidence	Severity	Plant height	Seed yield

AUDPC for evaluated cowpea genotypes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.52578	0.17526	3.26	
rep.*Units* stratum					
Variety	14	134.65481	9.61820	178.68	<.001
Residual	42	2.26089	0.05383		
Total	59	137.44148			

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APPENDIX 6

Seasonal effects on the final incidence and severity of viral diseases on 38 cowpea genotypes

Season	Final Incidence (%)	Final severity	AUDPC
Major	30.9	1.49	4.07
Minor	53.1	1.99	5.31
Grand mean	42.0	1.74	4.69
$LSD_{0.05}$	5.01	0.31	0.39
P value	<0.001	0.001	<0.001
$F_{1,168}$	76.14	10.66	40.12

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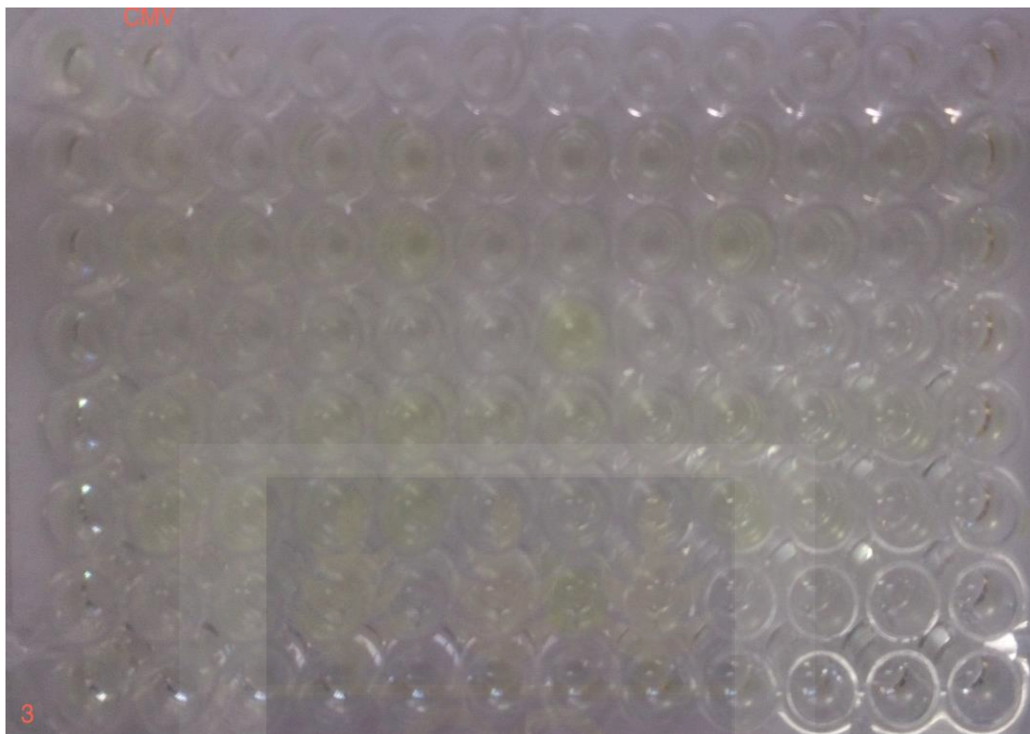
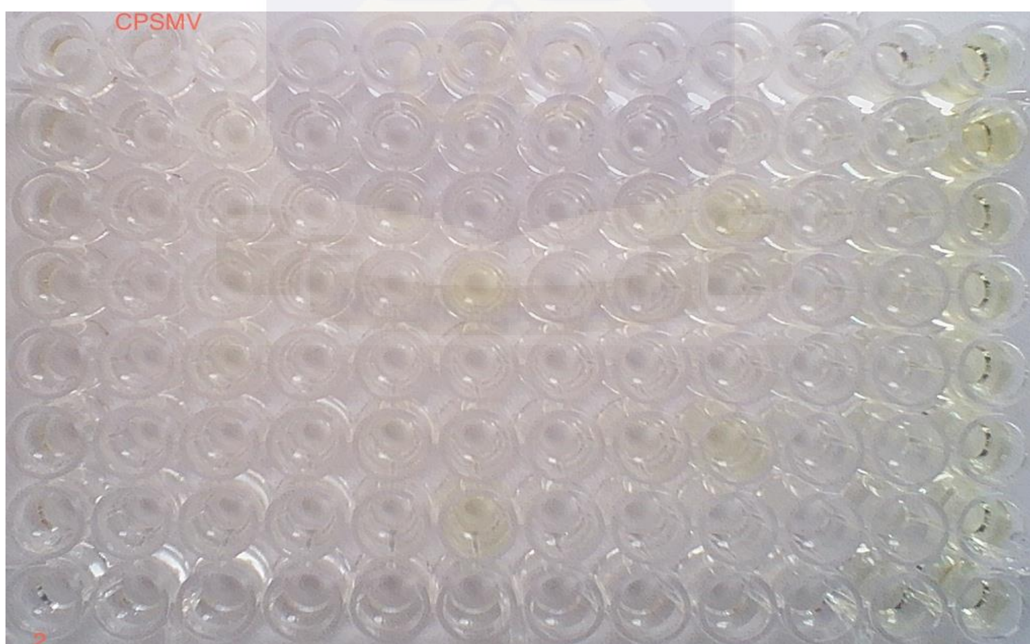
APPENDIX 7

Plates showing DAS-ELISA detection of cowpea viruses from 15 cowpea genotypes.



A: **Detection of *Cucumber mosaic virus* (CMV)**



B: Detection of Cowpea severe mosaic virus