

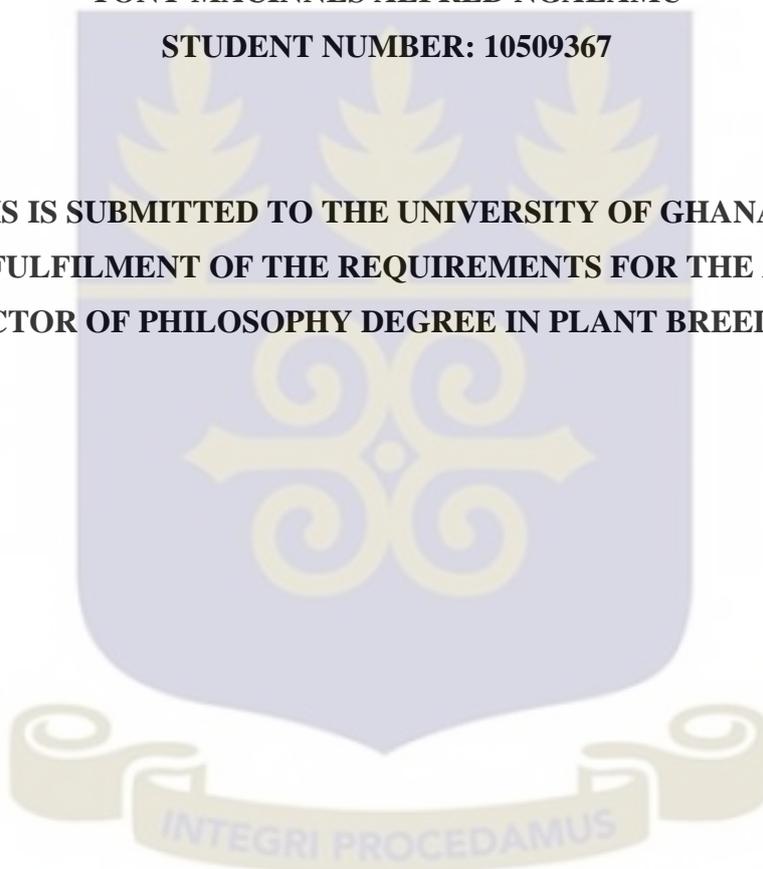
**GENETIC IMPROVEMENT OF COWPEA (*Vigna unguiculata* L. WALP) FOR  
EARLINESS AND DROUGHT TOLERANCE**

**BY**

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN  
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF  
DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING**



**WEST AFRICA CENTER FOR CROP IMPROVEMENT  
COLLEGE OF BASIC AND APPLIED SCIENCES  
UNIVERSITY OF GHANA  
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**DECEMBER, 2018**

## DECLARATION

I do hereby declare that except for citations to findings of other researchers, duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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## ABSTRACT

The number of people suffering from chronic undernourishment in sub-Saharan Africa has increased. The potential of the cowpea to be the crop of the future to address food and nutrition insecurity is evident in sub-Saharan Africa. This study was carried out to evaluate cowpea accessions for early maturity and drought tolerant genotypes with potential for higher yields for smallholder farmers in South Sudan. One hundred and six cowpea accessions were sourced from different backgrounds. Their genetic diversity was assessed using both agro-morphological traits and molecular approaches. Nine discriminatory clusters yielded cophenetic correlation coefficient values of 0.76. Results of molecular study revealed genetic divergence among the assembled cowpea panel creating an opportunity for population development through introgression of new alleles. The SNP markers used in this study could be utilized to analyse and group collections in the future. Forty-nine accessions selected from 106 genotypes were screened under well-watered and drought stressed conditions for selection of parental lines for population improvement using  $7 \times 7$  lattice square design. Five cowpea accessions from South Sudan and three from Burkina Faso, Niger and Nigeria were identified and selected for crosses. Hybridisation was carried out using hand emasculation and pollination. The North Carolina II mating design generated 15  $F_1$  populations. The  $F_1$ s were then backcrossed to the local parent to develop  $BC_1F_1$ . The 15  $BC_1F_1$  populations with their parents as checks were evaluated using an alpha lattice design to advance promising genotypes to the next round of backcrossing cycles. The resultant  $F_2$  offspring arranged in a split-plot design were evaluated to study the combining ability for early maturity and drought tolerance under well-watered and drought-stressed conditions using rainout shelters. At backcross 3, ( $BC_3F_1$ ), the population was selfed twice before running multi-location trials in Legon ( $5^{\circ}38'N$ ,  $0^{\circ}10'E$ ), Fumesua ( $6^{\circ}41'N$ ,  $1^{\circ}28'W$ ), and Nyankpala ( $9^{\circ}24'N$ ,  $0^{\circ}59'W$ ), Ghana. The study included a total population of 9000 plants, with 3000 plants per

experimental site. The trials were laid out in a 5×5 lattice square design with three replications and two watering regimes. The plot size was 1.2 m<sup>2</sup>, the distance between plants was 20 cm and 60 cm between rows. Data were collected on agronomic traits and subjected to analysis of variance and path analysis. Stability analysis identified best performers for earliness, drought tolerance, and seed yield stability. Additive main effects, multiplicative interaction (AMMI) stability analysis, and GGE biplot analysis ranked genotypes based on their performance in relation to environment, whereas Eberhart and Russell (1966) and Finlay and Wricke's (1963) stability approaches gave holistic information about those genotypes with outstanding seed yield stability. Genotype by environment analysis confirmed that environment accounted for 63.8% of the variability in the experiments, genotype accounted only for 10.8%, and the interaction between genotype and environment accounted for 24.4% of the variation. Ten genotypes, A1B×D, A1B×I, A1B×M, BA×D, BA×M, BA×I, L1B×D, TA×M, TA×D, and TA×M that mature in 60 days or less after planting were identified. Four genotypes, BC×M, L1B×I, TA×M, and A1B×M, were found to combine drought tolerance with stable yield potential. Two genotypes, A1B×M and TA×M, demonstrated both early maturity and drought tolerance. Five recommendations were drawn from this study for future research.

## ACKNOWLEDGEMENTS

My unfathomable appreciation goes to my supervisors Prof. Kwadwo Ofori, Prof. Silvestro Meseka, John Eleblu, PhD and Beatrice Ifie, PhD for methodically reading through every chapter and making valuable propositions. Additionally, being very caring and understanding during epochs I really felt distressed. I also thank Prof. Emeritus Vernon Gracen and Prof. Emeritus Glen Shinn for their undisputed support and valuable leadership. I am grateful to Mr. Christian Amenakpor, Mr. Kwabena Bediako and Mr. Michael Teye for their valuable assistance, moral support and encouragement. I am also indebted to the management of INTRA-ACP, DAAD and ACE for the scholarships, University of Ghana for admission, West Africa Centre for Crop Improvement in particular for training. I thank Prof. Eric Danquah for guidance and timely support from the inception of the study. Many thanks to the management of the University of Juba for release and support. I also wished to recognise managements of the Plant Genetic Resource Research Institute and Savanna Agricultural Research Institute (SARI), in Ghana, International Institute of Tropical Agriculture, Cowpea Improvement Unit (Kano), Nigeria, INERA (Burkina Faso) and ITRA (Togo), for providing part of the germplasms used in the study. The entire Cohort 8 member, I salute you guys. I would like to convey my sincere gratitude and thanks to all those who have been instrumental during the course of my study at the University of Ghana, and during my stay in Ghana. I thank all my friends, Dr. Itai Makanda, Eng. Julius Ngalamu, Four Cousins and Ms. Asia MacInnes for their encouragement. My sincere gratitude also goes to the two Farm Managers (Crop Science and WACCI) and their subordinates for all the assistance during the field work, the laugh and encouragement. To all members of the South Sudan community in Ghana particularly Ambassador Amoi Juma Dino, his wife Clara and children Lorika and Illam I say “Aro boya” for the comradeship and moral support. To the Love of my life Bazilika Tuna and our children Toa Ni, Yau Ni and Daniel, may the almighty God reward

you for the understanding, care, and support during the coursework, research phase of the journey and thesis preparation. Indeed, this accomplishment is a result of support and supervision from many actors. It is not possible to mention their names individually, but I dully recognised and appreciate your valuable contributions. I will conclude this acknowledgement by thanking you and asking God to richly bless you all.

## **DEDICATION**

Unreservedly I dedicate this thesis, which shows all the research work I carried out here at the University of Ghana, West Africa Centre for Crop Improvement, to God Almighty, specially my uncle and friend late Taban Juma Durufu, my lovely, compassionate and empathetic wife Mrs Basilika Tuna, and to our delightful sons Toa Ni, Daniel and daughters Mariam, Yau Ni, my siblings Katra; Joseph, Lemi, Emmanuel, Elinana and beloved parents Mr. MacInnes Ngalamu and Ms. Drusilla Galio (My Prayer Warrior).

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## LIST OF ABBREVIATIONS

AEC	Average Environment Axis
AFLP	Amplified Fragment Length Polymorphisms
AMMI	Additive Mean Effect and Multiplicative Interaction
AMP	Arithmetic Means Productivity Index
ANOVA	Analysis of Variance
AQPs	Aquaporins
BC	Backcross
BSCGD	Broad Sense Coefficient of Genetic Determination
CAPS	Cleaved Amplified Polymorphic Sequence
CCC	Cophenetic Correlation Coefficient
CGIAR	Consultative Group on International Agricultural Research
CPMV	Cophenetic Correlation Coefficient
CRI	Crops Research Institute
CV	Coefficient of Variation
DAP	Days After Planting
DF	Degree of Freedom
DII	Drought Intensity Index
DLS	Delayed Leaf Senescence
DNA	Deoxyribonucleic Acid
DS	Drought Stressed
DSI	Drought Stress Index
DSI	Drought Susceptibility Index
ENV	Environments
FAO	Food and Agriculture Organization
Fst	Fixation index
GBS	Genotype by Sequencing
GCA	General Combining Ability
GEI	Genotype by Environment Interaction
GGE	Genotype and Genotype by Environment
GLYD	Grain Yield
GMP	Geometric Mean Productivity
GR	Genotype Response
GCV	Genetic Coefficient of Variation
GWAS	Genome Wide Association Selection
HI	Harvest Index
IBOGR	International Board for Plant Genetic Resource
IDC	Iron Deficiency Chlorosis
IITA	International Institute of Tropical Agriculture
INERA	Institut de l'Environnement et de Recherches Agricoles
INRAN	Institut national de la recherche agronomique
IPCA	Interaction Principal Component
ITRA	Institut Togolaise de Recherche Agronomique
LAI	Leaf Area Index
LEA	Late Embryogenesis Abundant Proteins
LSD	Least Significant Difference
LWI	Leaf Wilting Index
MAB	Marker Assisted Breeding

MAF	Minor Allele Frequency
MAS	Marker Assisted Selection
NARO	National Agricultural Research Organisation
NCII	North Carolina II Mating Design
NDVI	Normalised Difference Vegetation Index
NJ	Neighbour-Jointing
NPP	Number of Pods Per Plant
NSCGD	Narrow Sense Coefficient of Genetic Determination
NSP	Number of Seeds Per Pod
OA	Osmotic Adjustment
PCA	Principal Component Analysis
PGRRI	Plant Genetic Resources Research Institute
QTLs	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RELP	Restriction Fragment Length Polymorphism
SARI	Savanna Agricultural Research Institute
SCA	Specific Combining Ability
SEM	Structural Equation Modelling
SI	Stress Intensity
SMC	Soil Moisture Content
SNPs	Single Nucleotide Polymorphisms
SPAD	Chlorophyll Meter
SSA	sub-Saharan Africa
SSRs	Simple Sequence Repeats
STG	Stay Green
STI	Stress Tolerance Index
TOL	Stress Tolerance
UCR	University of California, Riverside
UG	University of Ghana
UJ	University of Juba
UPGMA	Unweighted Pair-Group Average
WACCI	West Africa Centre for Crop Improvement
WW	Well-Watered
WSC	Water Soluble Carbohydrate

## CHAPTER ONE

### 1.0 GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is a diploid ( $2n = 2x = 22$ ) and an important grain legume capable of producing appreciable yields under 500 mm of rainfall in sub-Saharan Africa (SSA). It is estimated that Africa produces more than 96.1% of the cowpea in the world with a grain production of 6.99 million tonnes under 12.32 million hectares (FAOSTAT, 2016). Compared to other annual crops grown in drought-prone areas in SSA, cowpea could be one of the best options to grow, although drought stress is still a major production constraint for the crop (Fatokun *et al.*, 2012). The crop provides food for both humans and livestock and also serves as a dependable revenue generating commodity (Ajeigbe *et al.*, 2008). Cowpea is widely grown by nearly all the smallholder farmers in all agro-ecological zones across SSA for its grain. In South Sudan, cowpea ranks third and first among the legumes and is produced mostly by smallholder farmers.

Poor cowpea yield remains one of the major production problems in SSA where yields are as low as 0.4 ton/ha (Haruna and Usman., 2013). However, higher yields of 2.0 t/ha and above have been recorded when improved cultivars are grown as pure stands under high rainfall and adequate management practices (Agyeman *et al.*, 2014). Collection of adapted farmers' preferred varieties and introduction of improved varieties from international and national research institutions could provide a huge genetic pool for a functional cowpea breeding program in South Sudan.

Yield reductions in cowpea have been attributed to a number of biotic and abiotic stresses. Drought stress is the most important abiotic stress disrupting cowpea production in the SSA countries. This prevalence could be due to high variability in amount and distribution of rainfall during the cropping season. Both intermittent and terminal drought stresses occur in

South Sudan but the terminal drought is the most important because it impacts directly on pod formation and filling. Terminal drought generally leads to significant reduction in yield (Singh, 2007) and poor seed quality (Beebe *et al.*, 2013).

Extensive research efforts have been made to improve the efficiency of selection for drought tolerance based on specific physiological traits and yield. However, a good number of the approaches failed due to genotype by environment interaction effect (GEI) and the lack of precise and efficient drought screening methods. Several researchers (Mai-Kodomi *et al.*, 1999a; Singh *et al.*, 1999, Fatokun, *et al.*, 2012) have indicated that screening cowpea genotypes for tolerance to drought at seedling stage has a good potential of differentiating among contrasting entries.

The recurrent frequent occurrence of drought stress in the Iron-stone plateau, semi-arid and floodplains agro-ecological zones of South Sudan causes cowpea varieties to flower and mature faster, but with poor seed yield and low harvest index (Ngalamu *et al.*, 2015). Early maturing varieties need to be developed for both leaf and high grain yield in order to address the threat posed by drought stress. Moreover, breeding for earliness and drought tolerance should be the research focus of cowpea breeding in South Sudan where its agricultural research program and activities are still in early developmental stages. There is little information however on the genetic divergence for earliness and drought tolerance in the existing adapted varieties preferred by farmers in South Sudan (Ngalamu *et al.*, 2017).

The new developments in plant molecular genetics have provided powerful tools for diversity studies and marker-assisted selection of polygenic traits such as drought tolerance and grain yield, therefore, complimenting conventional breeding tools. There is the need to develop new varieties with farmer and consumer preferred traits as well as climate change resilient. Thus, there is a need to screen and identify drought tolerant genotypes that could be use as

the source of a gene conferring tolerance to drought stress for introgression into adapted farmers preferred early maturing varieties. Furthermore, gene action controlling earliness and tolerance to drought stress must be determined or confirmed to provide direction for improvement.

The overall research goal of the study was to identify promising cowpea genotypes that are early maturing and combining tolerance to drought with high yield to ensure food and nutrition security in South Sudan.

The specific objectives were to:

1. assess the genetic diversity among adapted farmer's preferred varieties and improved varieties from international agricultural centres for earliness and drought tolerance,
2. identify sources of drought tolerance from selected accessions for introgression into adapted cowpea genotypes,
3. introgress gene of drought tolerance into locally adapted early maturing varieties,
4. determine the gene action controlling earliness and drought tolerance, and
5. assess the performance of the backcross populations developed and their parents under drought-stressed and well-watered conditions.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Origin, Domestication Distribution and Types of Cowpea

Cowpea is a *Dicotyledonea*, belonging to the family *Leguminosae*, tribe *Phaseoleae*, genus *Vigna*, and section *Catiang* (Padulosi and Ng, 1997). The genus *Vigna* which comprises approximately of 85 species is divided into seven subgenera; *Haydonia*, *Ceratotropis*, *Macrorhycha*, *Lasiocarpa*, *Sigmoidotropis*, *Vigna* and *Plectotropis* (Marechal *et al.*, 1978). Cowpea and its cross-compatible wild relatives belong to the subgenus of *Vigna*.

The exact location or centre of cowpea origin is still difficult to determine; several assumptions are linked to it. Some studies tend to associate the centre of origin and domestication of cowpea to cytological evidence and botanical, cultural practices, geographical information (distribution) and archaeological records (Padulosi and Ng, 1997). Ng (1995) reported that the evolution process in *V. unguiculata*, change it from a perennial plant to an annual crop and from purely outcrossing to inbreed. Furthermore, Padulosi and Ng, (1997) stated that the process of evolution and domestication of cultivated cowpea (sub-specie *unguiculata*) came about as a result of successive selection of undomesticated species. Through these processes, the species lost some of its traits such as seed dormancy and pod dehiscence and in return gained improvement in the number of seeds setting per pod. To establish cowpea centre of domestication, through analysis of chloroplast deoxyribonucleic acid (DNA) polymorphism, Nigeria is suggested as centre of cowpea domestication (Vaillancourt and Weeden, 1992). However, using amplified fragment length polymorphism (AFLP), the centre of domestication was proposed to be north-east Africa (Coulibaly *et al.*, 2002). Nevertheless, the global cowpea producing countries are in Africa, with Nigeria as a leading producer and consumer, producing over 3.03 million metric tons of grain on 3.61 million hectares (ha), followed by Niger 1.99 million metric tons on 5.19 million ha

(FAOSTAT, 2016). These two countries are followed by Burkina Faso, Mali, Togo, Senegal, Ghana, Benin, Chad and Cameroon in West Africa, and Central Africa, in that order. In the East and Southern Africa, the order is Somalia, Sudan, Kenya, Uganda, Tanzania, Zimbabwe, Botswana, Zambia, and Mozambique. However, countries in Far East including India, and China to mention few do produce cowpea. West Indies, Haiti and Cuba in Central America, Brazil in South America and the USA in North America also do produce cowpea. Whereas in South Sudan, data on cowpea production level is very scarce and if available it is inconsistent and not reliable (Ngalamu *et al.*, 2015).

Cowpea exhibits a number of growth habits, erect, semi-prostrate, prostrate and climbing type. However, preference for the growth habits varies from one country, region to the other. For instance, in Niger, the prostrate type is preferred by majority of farmers because it provides more leaves as feed for livestock. Whereas, in South Sudan, growth habit is not an issue since both the leaves and grains are consumed. Wang *et al.* (2006) reported that the growth habit in cowpea is associated with competitive ability. Thus, the exhibited growth habit by cowpea made the crop competitive to weeds. In addition, cowpea can be used in soil conservation and improvement of soil fertility. Roberts *et al.* (2005) observed that cowpea suppresses nematode and other pathogens.

## **2.2 Climatic and Edaphic Factors Influencing Cowpea Production**

Cowpea thrives in broad types of soils and nutrient status and soil pH ranging from 5.5 to 8.3. Well-drained sandy loams and sandy soils were reported to be the best. It can tolerate salinity to some extent, but it tolerates soils high in aluminium (Ngalamu *et al.*, 2014). It is observed to flourish largely under humid conditions. Some genotypes were reported to be tolerant to heat and water deficit, however it is sensitive to frost (Davies *et al.*, 1991). When soil temperature is above 1.9°C, cowpea germinates rapidly, but lower temperatures were observed to slow germination. This is evident when cowpea is grown under a well-watered

and stressed condition or two irrigation regimes (Davies *et al.*, 1991). Severe drought stress is of common occurrence in the Sahel zone of SSA. Comparing the performance of cowpea, pearl millet (*Pennisetum glaucum*) and peanut (*Arachis hypogaea*) grown in the zone, cowpea was observed to continue to stay longer and produce appreciable more yield than the other two crops when they are grown in the same fields under drought condition (Singh and Matsui, 2002).

Delayed leaf senescence (DLS), an agronomic feature in cowpea, indicates a certain level of drought tolerance at the reproductive stage in erect cowpea genotypes. Flowering in cowpea starts at approximately 35 days after planting and producing a yield of 2.0 t/ha in two months. An additional 1.0 t/ha of cowpea grain could be obtained by genotypes that completes its lifecycle in 100 days from planting. These dynamics is ascribed to the ability of a cowpea plant to produce a second flush of pods (Hall *et al.*, 2003).

Photoperiod has no direct or little effect on cowpea leaf appearance (Ishiyaku *et al.*, 2005). It has no effect either on branching and internode elongation. In contrast, it was found to have great effect on the reproductive development of genotypes, although some genotypes are unresponsive to photoperiodism (Ellis *et al.*, 1994). However, cowpea responds to photoperiod in a quantitative way of short-day plants (SDP). Singh and Ntare (1985) reported that the longer the photoperiodic duration than the critical value, flowering is not affected. Thus, day neutral cowpea genotypes are preferred in breeding programs. In addition, several scientists (Craufurd *et al.*, 1997, Ishiyaku *et al.*, 2005) observed that long photoperiod during reproductive stage reduces flowering and pod production and eventually grain yield. Craufurd *et al.* (1997) reported that the neutrality of cowpea to photoperiod with respect to the onset of flowering could be markedly sensitive with pod production, although it is logical to assume that long days affect cowpea post flowering growth and development.

### 2. 3 Constraints to Cowpea Production

Despite being extensively grown in SSA, the crop is constrained by a number of insect pests such as pod suckers, aphids, thrips, and many others. Viral, bacterial and fungal diseases also devastate cowpea production. Other production constraints are drought, flood, and parasitic weeds including *Striga gesnerioides* (Wild) (Reiss and Bailey, 1998). In South Sudan, drought, lateness, low yield, lack of improved seeds and extension service were ranked as the major cowpea production constraints (Ngalamu *et al.*, 2015).

Drought stress significantly reduces number of pods per plant resulting in a reduction in the cowpea seed yield. Belko *et al.* (2013) found that an individual seed weight in cowpea was significantly higher under drought stress, but this does not compensate for the loss in the number of pods. Inherently, leaf area measured at the end of the drought stress cycle was strongly associated with seed yield (Belko *et al.*, 2013).

The detrimental effects of water deficit at any of these reproductive stages of flowering or pod filling in cowpea cannot be overcome by re-watering. Although reproductive activity in cowpea plants re-watered at the flowering stage resumes, most of the newly formed pods fail to reach maturity due to inadequate moisture supply resulting from excessive transpiration (Ahmed and Suliman, 2010). Timely planting is so important that the reproductive stages (flowering and pod filling) coincide with the period when water is most available (Ahmed and Suliman, 2010).

According to Chiulele (2010), yield reduction in cowpea due to terminal drought stress is estimated to range from 21 to 30%. However, the yield reduction in cowpea production depends on both the location and duration of the cropping season (Sabaghnia *et al.*, 2006). Most farmers' varieties are susceptible to drought, hence dry spells in farmer's field result in yield reduction. Drought can occur at anytime, anywhere and at any crop growth stage. Cowpea plants are most prone to damage due to drought stress during flowering and pod

setting stages (Bahar and Yildirim, 2010). As such, it is necessary to enhance the degree of tolerance to drought stress in the adapted cowpea genotypes in order to obtain high and stable yields.

#### **2.4 Drought Stress and Cowpea Production**

Extensive agricultural losses in the world are attributed to agricultural drought stress (Bruce *et al.*, 2002; Tuberosa and Salvi, 2006). Agricultural drought stress normally results in a reduction in grain yield. It was observed to be the correlation between the influenced stage of crop development, severity, duration of drought stress and the level of drought susceptibility of genotype (Lorens *et al.*, 1987). Effect of drought stress can be severe in field crops particularly at flowering phase where it causes a whole range of abnormality from abortion and lost pollen viability in cowpea, sterility in rice (Lafitte *et al.*, 2004), whereas in maize, growth and embryo fertilization are reduced (Bruce *et al.*, 2002; Earl and Davis, 2003). The occurrence of drought throughout the vegetative phase of the plant has a detrimental effect on yield as well. This reduction in yield is attributed to reduced leaf area which reduces the interception of photosynthetically active radiation (PAR), that in turn, truncates radiation use efficiency of the photosynthetic system and subsequent reduction in harvest index (Earl and Davis, 2003).

Yield losses in cowpea are mainly due to the adverse effects of drought stress on the photosynthetic pathways and activity. Leaf senescence tends to disrupt photosynthetic apparatus as a result photosynthesis declines during grain filling. The incidence of drought, high temperatures, and excessive irradiance was observed to worsen the degeneration of photosynthetic apparatus (Martinez *et al.*, 2003). Drought tolerant and relatively high yielding cowpea genotypes were observed to have enhanced adaptations mechanisms (morphological and physiological) that improve their photosynthesis stimulation, uptake of water and reduction of water loss under drought stress condition (de Souza *et al.*, 1997).

## 2.5 Mechanisms of Tolerance to Drought Stress

### 2.5.1 Physiological Mechanisms

According to Passioura (2002), the efforts to develop drought-tolerant genotypes in the past were obstructed by the polygenic nature of the trait as well as lack of in-depth knowledge of the physiological mechanisms influencing seed yield under water deficit condition. Breeders can focus on high yield under optimum and drought conditions to improve drought tolerance in high yielding genotypes (Danquah and Blay, 1999). As water deficit levels rise, the interaction between genotype and water deficit adversely affects plant traits that influences yield reduction. As a result, the pathway involved in the interaction could be explored in the development of a drought-tolerant population. This approach is variedly not being optimal, because of the polygenic nature of yield, high influence of genotype  $\times$  environment interaction and low heritability (Babu *et al.*, 2003). The understanding of both physiological and molecular bases may help the breeder identify and target core traits that limit yield under drought conditions.

Cattivelli *et al.* (2008) concluded that having in-depth knowledge of the molecular and physiological basis of tolerance to drought stress in field crop of interest may aid the breeder to identify the core traits that influence yield. Molecular technology is a huge asset to the conventional breeding programs and will accelerate the pace of crop improvement. The advancement in molecular biology platforms enhances the ability to locate and sequence genes of interest. Efficient utilization of the molecular tool in the introgression of quantitative trait loci (QTL), selection or genetic transformation of hard to introgress desired QTL strongly depends on plant breeders' sound thoughtful insight on the yield determining physiological procedures (Araus, 2002). Numerous traits are involved in drought tolerance and can be measured at different physiological, biochemical, and morphological levels (Hamidou *et al.*, 2007). A number of screening methods are used to assess water-use

efficiency and drought tolerance. The most common are wooden boxes, pots, hydroponic and field screenings (Singh *et al.*, 1999; Ogonnaya *et al.*, 2003). Regularly measured traits under drought-stressed conditions are the leaf area index, chlorophyll stability index, relative water content, diffusion pressure deficit, carbon isotope discrimination, and root/shoot ratio (Singh *et al.*, 1999).

Developing drought-tolerant genotypes could be through classical breeding approaches. Relying on water-use efficiency of the genotypes is the most common method used to determine early plant responsiveness to drought stress conditions. Genotypes exhibit considerable variations under drought stress which is a gene linked trait. Taiz and Zeiger (2002) observed that drought tolerant genotypes had reduced transpiration through restriction of stomatal opening or reduction of the leaf area or both. Costa *et al.* (1997) reported that genotypes that have reduced water loss and adjust their organ size or withstand the biomass production under water-limited conditions are susceptible to drought stress. Condon *et al.* (2004) proposed three key procedures to be considered in water-use efficiency hybridization in crops. These three processes are (i) enhancing level of available water uptake, (ii) boosting production of biomass per unit of water transpired, and (iii) dividing of biomass produced in the harvested product.

### **2.5.2 Genetic Mechanism**

The whole plant crop genomes are involved in the population development when using conventional breeding and then identification and selection of outstanding recombinants from among many segregating populations. This traditional approach is cumbersome and time-consuming, demanding a number of crosses, generations, and cautious field evaluation, and occurrence of tight linkage of unwanted traits with the wanted loci and makes the process hard for a breeder to accomplish a wished breeding outcome (Xu and Crouch, 2008). The advent of molecular biology resulted into development of advanced technologies, such as

numerous molecular breeding strategies and types of molecular markers which offer opportunities for geneticists and breeders to overcome many of the difficulties encountered when utilizing conventional breeding approaches. Khan *et al.* (2010) reported that field crop adaptation to drought stress is multiplex trait control by a number of gene or QTLs of small effects. Several researchers embrace the fact that breeding for drought tolerance necessitates amalgamation of both conventional and molecular approaches (Chaves *et al.*, 2003; Blum, 2011).

The molecular procedure or genomics offer an exceptional opportunity for examining quantitative traits in their single genetic determinants (QTLs), consequently laid the foundation for genetic engineering (Salvi and Tuberosa, 2005) and marker-assisted selection (MAS) (Morgante and Salamini, 2003). The application of molecular markers in plant breeding for drought tolerance can be divided into three key categories:

- 1) Fingerprinting (characterisation of germplasm);
- 2) Recognition and characterization of genome regions (QTLs) associated with the expression of the desired trait; and
- 3) Accelerated crop improvement via MAS approach.

Advancement in molecular biology has cropped new scientific disciplines such as plant functional genomics. This discipline focuses on the study of genes functions. As a result, substantial progress has been made in area of sequencing plant genome including sequencing of an approximately 620 Mbp of cowpea genome. The other advent in plant breeding molecular approaches are the detection of genome-wide single nucleotide polymorphism (SNP) by means of Genotype by Sequencing (GBS), genetic diversity analysis of a worldwide germplasm using genome-wide association (GWAS), and SNP markers linked to morpho-agronomic traits identification in crops such as cowpea, maize, rice and barley ability to withstand biotic incidences, as well as tolerance to abiotic stress. When breeding for seed

quality in cowpea, MAS and GWAS are tools a breeder can rely on for selection. Selection of some agronomic parameters such as plant growth habit, coloration of the dry pod, placement of pods, the pattern of mature seed, seed coat colour, the pattern of eye colour, flower colour, seed protein content, and sugar content can possibly be obtained through GWAS. Muchero *et al.* (2013) reported that SNP markers are also used to identify some agronomic traits: grain yield, 100-seed weight and seed size; beside the biotic and abiotic stresses such resistance/tolerance to cowpea aphid, iron deficiency chlorosis (IDC), cowpea wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*), cowpea mosaic virus (CPMV), bacterial blight (*Xanthomonas axonopodis* pv. *vignicola*), and low phosphorus uptake efficiency are being studied for relations.

## **2.6 Mechanisms of Drought Stress Avoidance**

The ability to withstand drought stress should reflect a balance amid the three mechanisms of escape; avoidance and tolerance at the same time supporting appreciable productivity (Agbicodo *et al.*, 2009).

### **2.6.1 Morphological Mechanism**

Cowpea drought tolerance morphological mechanism encompasses adjustment at tissue, molecular, physiological and whole plant system. Intrinsic single or combination of changes in cowpea determines its ability to withstand moisture stress conditions. The morphological adaptation mechanisms under drought conditions are as follows:

#### **2.6.1.1 Escape (drought avoidance)**

Escape may be defined as the preparedness of field crops to accomplish their lifespan ahead the occurrence of severe water deficit. In other words, drought avoiders, mature rapidly before the onset of drought (Agbicodo, 2009). Persistent drought incidences in cowpea producing areas have compelled farmers to plant early maturing varieties. Cowpea varieties

that are early maturing tend to best escape terminal drought, albeit they are exposed to intermittent drought stress at vegetative stage and the yield is severely affected. According to Fatokun *et al.* (2012), intermittent drought stress reduces yield by up to 50 to 67%. This has serious implication on farmers' ability to produce sufficient quality of cowpea grain for food security and household incomes.

#### **2.6.1.2 Avoidance (dehydration post-ponders)**

Dehydration avoidance is a measure of an extent to which plant water status is kept during drought stress. Drought tolerant field crops that have the ability to maintain leaf turgor through adjustment of osmotic pressure or increasing rate of water uptake and lessening the rate of water loss. The incidence of drought stress during the vegetative phase of crop development was observed to have little effect on grain production when successive conditions at the sites are favourable to encourage recovery (Fang and Xiong, 2015). Nevertheless, acute water deficit during floral initiation and flowering phase can result in almost comprehensive detachment of formed flowers and formation of immature pods, leading to 100% yield loss (Agbicodo *et al.*, 2009).

#### **2.6.1.3 Tolerance (dehydration tolerators)**

Tuberosa (2012) defined drought tolerance as the preparedness of a crop to withstand drought stress and grow and yield satisfactorily. One of the important preconditions for gainful phenotyping of drought tolerance is the identification of key functional features that contribute to tolerance. Thus, understanding the metabolic and regulatory function of drought tolerance in crop plants is likely to provide valuable information that would help in suggesting approaches for modification through genetic manipulation.

Earlier studies (Agbicodo *et al.*, 2009) have shown that drought-tolerant cowpea lines are of two types, Type 1 and Type 2. Under drought stress, in 'Type 1', plants stop growth but preserve moisture and keep all the leaves and growing tips alive for long period of time,

whereas in 'Type 2', plants assemble moisture from the lower leaves to the growing tips. This process in Type 2 results into senescence and death of lower leaves, whereas, the tips of the leaves remain alive for an even longer period of time compared to 'Type 1' plants. Knowledge about the inheritance of these two types of drought tolerance traits would facilitate their use in cowpea improvement programs.

### **2.6.2 Physiological Mechanism**

Several physiological processes are involved in plant adaptation to dry environments (Fahad *et al.*, 2017). However, cowpea exhibited little osmotic adjustment. Drought tolerance is a multiplex trait and its manifestation leans on exertion and reciprocation of different morphological characters such as early maturity, reduction of leaf area, rolling of leaves, production and deposition of wax in the epicuticular, effectual rooting system, stay-green, and yield stability. Physiological mechanisms such as reduced rate of transpiration, osmotic adjustment, high water-use efficiency, and stomatal closure were founded to be beneficial to field crops under drought stressed condition (Sinclair and Ludlow, 1986; Ludlow and Muchow, 1990). Not much was studied about the genetic mechanisms that condition these biochemical processes including proline accumulation, an increment in nitrate reductase activity and amplified storage of carbohydrate. Recognition of physiological traits that are positively and strongly correlated drought stress may be used in an index for selecting drought-tolerant genotypes. Plant physiologists have measured various plant characteristics, for example, stomatal orientation, osmotic pressure adjustment, water use efficiency, characteristics of the roots, broken off leaf water loss, and leaf water potential that correlate well with drought tolerance. Levitt (1972) grouped drought resistance into two categories based on physiological traits: dehydration avoidance and tolerance. According to Farooq *et al.* (2017), morpho-physiological traits such as osmotic adjustment (OA), deep roots architectural systems, early maturity, and wax deposition on epicuticular layer allow plants to

sustain hydration status (avoidance). On the contrary, qualities such as accumulation of molecular protectants and remobilization of stem water soluble carbohydrates (WSC) permit plants to maintain proper functionality in severely dehydrated states (tolerance) (Afshari *et al.*, 2013).

The most central establishment responsible for tolerance to drought is antioxidation, scavenging defense system, osmotic adjustment and osmoprotection. The mechanisms behind the genetic variation are not clear since it is only displayed as a response to the occurrence of drought. Nevertheless, it was assumed to be a complex physiological mechanism. High osmotic adjustment levels coupled with a low critical relative of water content of leaves are observed when drought stress mounts (Sinclair and Ludlow, 1986; Ludlow and Muchow, 1990). However, -18 bar leaf water potential was observed in cowpea under extreme dehydration avoidance (Turk and Hall, 1980). The extreme dehydration avoidance of cowpea may be explained partially by those mechanisms. Cowpea was observed to be sensitive to soil water deficit, partially closes their stomata even before the differences in the leaf water potential are identified (Bates and Hall, 1981). Additionally, OA allows plant leaf to maintain turgor at a lower water potential by decreasing the rate of decline in leaf water content (Ludlow and Muchow, 1990).

### **2.6.3 Molecular and Biochemical Mechanism**

Water stress due to water deficit is common in natural environments and occurs due to lack of rainfall. According to Nguyen and Blum (2004), drought stress results in damage of plant cell membranes (detrimental effect on the protoplasm). Removal of water from the protoplasm results in shrinkage and high concentration of solutes that may have serious structural and metabolic consequences in the plant. The integrity of the cell membrane and proteins are also affected (growth and development adversely affected). Late embryogenesis abundant (LEA) proteins, aquaporins (AQPs), and molecular chaperones play crucial roles in an osmotic

adjustment in plant cells. During seed development, abundant proteins are formed. LEA proteins are low molecular weight proteins weighing between 10 and 30 kDa. They possess essential amino acids rich in lysine, glycine, serine and lacks cysteine and tyrosine (Nguyen and Blum, 2004). They are extremely high thermal stable proteins (hyper-hydrophilic) proteins and able to survive in an aqueous state even though under boiling conditions. LEA proteins are known to be playing protection role, when plants are grown under stress condition, through the biological macromolecules produced; redirecting distribution of intracellular water and it binds to inorganic ions to escape the tissue injury as result of accumulation and high concentrations of ions under drought stress conditions (Gosal *et al.*, 2009). LEA proteins do avoid extreme desiccation of plant tissues and regulate the expression of other genes by binding to nucleic acids (Gosal *et al.*, 2009).

## **2.7 Breeding for Tolerance to Drought Stress**

Management of drought stress effects can be achieved through the development of trait specific genotypes coupled with good agronomic practices (gap), such as planting date; plant stand and soil health (Rouf *et al.*, 2012; Mariani and Ferrante, 2017). Breeding drought tolerant genotypes will help cowpea withstand drought at different stages (early, intermittent or terminal) of drought stress. Numerous breeding strategies have been developed to ensure sustainable cowpea production through genetic improvement of adapted farmers preferred high yielding genotypes. Institutions such as International Institute of Tropical Agriculture (IITA) and University of California Riverside (UCR) have extensively worked to develop genotypes with high level of water-use efficient cowpea that respond to water deficit. The best two core approaches cowpea breeders use to employ are: (1) selection of desired materials, as is the case of conventional breeding using molecular approaches, and (2) priming and hormonal applications inducing otherwise susceptible plants (Farooq *et al.*, 2009; Fathi and Tari, 2016).

### **2.7.1 Conventional Breeding Efforts**

Through conventional breeding, genetic variability for drought tolerance among genotypes can be identified and those introduced can either be adopted or introgressed into the adapted local varieties to develop what is preferred by farmers. The conventional breeding approach has a number of limitations. This approach is time-consuming because a number of selections have to be carried out and the capital cost is huge (sequential evaluation). In addition, there is a high likelihood of introgressing a number of undesired genes into the agronomically desirable genotype.

Cowpea breeders equally use physiological traits to aid in selection, designing methods for their assessments. However, a good number of the approaches failed because of the genotype by environment interaction effect (GEI) and also due to lack of precise and efficient drought screening methods. Sheshshayee *et al.* (2003) reported that selection for drought tolerance based on a physiological trait needs a comprehensive knowledge about the nature of the trait, its responsiveness to environment and contribution to seed yield. Earliness in cowpea production areas that are prone to drought stress is undoubtedly a core trait. Genotypes that mature in 50 to 60 days after planting are the most desired. In addition, traits such as the number of days to first flower, 50% flowering, first mature pod, and 95% maturity could be used in selection for earliness since it provides the required genetic information (Owusu *et al.*, 2017).

### **2.7.2 Molecular Approaches**

Marker-assisted breeding (MAB) is considered as one of the efficient approaches due to its ability to examine the entire genomic regions of a crop under water deficit condition. Thi Lang and Buu (2008) reported that tolerance to drought stress is governed by many minor genes (polygenes) that have an additive effect. The loci on a chromosome containing genes with a known QTL could be exploited either through direct selection under drought stressed

condition (this can occur naturally or through simulation) or via QTL mapping and the results can be used for MAS (Ashraf *et al.*, 2008). QTL mapping is feasible and allows fast-tracking of the trait, number of genes involved. Although conventional breeding is still being used but is challenging due to the complexity of tolerance to drought stress and difficulties associated with selection based phenotypic data. QTL mapping has proved to be essential for drought stress tolerance improvement. Some of the DNA used approaches are RFLPs, RAPDs, CAPS, SSRs, AFLPs, and SNPs. Its undoubted application was observed in improving crops such as maize, barley, cotton, rice and sorghum, with cowpea being intensively studied (Bernier *et al.*, 2008).

## **2.8 Significance of General and Specific Combining Ability in Crop Improvement**

Combining ability analysis is a useful biometrical tool for identifying outstanding combiners from the crosses, and aid logical selection of parental lines for crop improvement programs. However, the genetic background and performance of particular parental lines would certainly illustrate the line as a good or poor combiner. Hence, having thoughtful and logical statistical information about the nature of gene effects and how they are expressed in terms of combining ability is essential for crop improvement. Higher SCA values for a trait designates dominant gene effects, and higher values of GCA effects determines a greater role played by additive gene effects in governing the trait under study. General combining ability estimates the average contribution of an inbred to hybrid performance in a series of hybrid combinations and SCA is the contribution of an inbred to hybrid performance in a cross with a specific other inbred concerning its contribution in crosses with an array of other inbreds. If both the GCA and SCA values are not significant, then epistatic gene effects may play a vital role in determining these traits (Fehr, 1993). A significant GCA and SCA mean squares for traits studied at each site and across sites specifies the importance of both additive and non-additive gene effects in the inheritance of the traits.

### 2.8.1 Combing Ability for Early Maturity in Cowpea

Xu *et al.* (2009) reported that early maturing cowpea varieties produce pods and mature in 55 days and this pattern of maturity is essential in addressing the hunger gap period in SSA. Ojomo (1971) crossed an exotic early flowering genotype to adapted local late flowering genotypes and reported that flowering date is governed by two major gene pairs, early flowering being dominant over late flowering. Ishiyaku *et al.* (2005) reported that earliness is under polygenic control with additives  $\times$  additive (I), additive  $\times$  dominance (j), dominance  $\times$  dominance interaction effects (epistatic effects).

The genetics of earliness heritability is estimated using a simple statistical method that measures variance (phenotypic) amongst  $F_2$  population developed from the hybridization process between two sets of districted parental lines. Thus, the overall phenotypic variance among the  $F_2$  population is made up of both the genotypic and environmental variances (Xu *et al.*, 2009). Furthermore, they reported that an estimated mean of the phenotypic variance among the parental lines could be used to compute environmental variance. The difference between phenotypic and environmental variances of the  $F_2$  individuals gives the genetic variance. However, the estimated heritability may be defined as the ratio of the genetic variance to the total phenotypic variance. This type of analysis has a narrow inference space because it depends entirely on the genetic differences between two particular parental inbred lines selected. Hence, this type of heritability cannot be generalized to other populations (Lynch and Walsh, 1998). The most commonly used computation method is the method that estimates broad-sense heritability with large inference space and does not require any hybridization procedure to estimate heritability. It can be carried out by analysing multiple genotypes for traits of preference using a simple analysis of variance (Singh and Chaudhary, 1985).

### **2.8.2 Combining Ability for Drought Tolerance in Cowpea**

Knowledge of genes controlling the trait of interest is essential for crop improvement program. Hinkossa *et al.* (2013) reported that having information about the effect and extent of gene action controlling the trait of interest must be well understood and determined. Traits such as drought tolerance and yield are polygenic in nature. Numerous genes control drought tolerance and affected by environmental factors which are not transmissible from parents to offspring. It's therefore, absolutely necessary to determine the genetic factors conditioning these traits in order to establish an efficient breeding program.

Singh and Chaudhary (1985) reported that there are three types of gene effects: epistatic, additive and dominance. The dominance and epistatic effects constitute the non-additive part. The dominance gene effect can either be ambidirectional, a condition where numerous genes impact phenotype and dominance are in dissimilar directions reliant on the gene and it could either be unidirectional, dominance in one direction or positive and negative dominance at different gene loci (Kearsey and Pooni, 1996). Interaction of alleles at different loci is known as Epistasis. There are two concepts about epistatic gene action. Thus, it's important for both plant breeders and bio-statisticians to distinguish between the concepts of statistical epistasis and physiological epistasis. Physiological epistasis is referred to as the effect of interactions between loci on the phenotype of an individual. This type of epistasis is a property of particular genotypes, and its values are independent of gene frequency. Moreover, statistical epistasis is the genetic variance within a population that can be attributed to interactions among loci. This type of epistasis is a population level phenomenon, and unlike previous epistasis, the latter changes as gene frequencies change. At some gene frequencies there may be physiological epistasis, but no statistical epistasis. The other scenario is, if there is statistical epistasis there must also be physiological epistasis (Goodnight, 1995).

The effects of additive genes are reflected by the extent to which offsprings resemble their parents, as reflected in narrow-sense heritability (Derera, 2005). Estimation of relative proportion of additive genetic effects (general combining ability of a line) and non-additive genetic effects (specific combining ability of a population SCA) controlling the drought adaptive traits and their interactions with the environment is useful for designing breeding programs and assembling germplasm for population advancement (Shahi and Singh, 1985). Information about GCA and SCA of parental lines are useful and aids in the interpretation of the genetic basis underlying inheritance pattern of the trait of interest. Several researchers (Acquaah, 2012; Alidu *et al.*, 2013) reported significant GCA effect for cowpea grain yield under drought-stress condition, suggesting that yield under drought-stress condition can be improved by exploiting additive gene effects. Ayo-Vaughan *et al.* (2013), in a separate study on combining ability for seed and pod in cowpea, reported that additive gene effects significantly controlled the number of pods per plant, pod length, number of seeds per pod and 100-seed weight. Thus, upon successful introgression of drought tolerance genes into the farmers preferred varieties, South Sudan will have its first early maturing, drought tolerant lines for a future release. Being it a notable feature in self-pollinating crops such as cowpea, the skill for accurate identification of parental combinations required for generation of superior pure lines for farmers to adopt is very crucial to the success of the breeding programme. Efforts have been directed towards developing and evaluating methods of envisaging cross potential in early generations (Thurling and Ratinam, 1987). Several scientists (Khan *et al.*, 2010; Acquaah, 2012; Nduwumuremyi *et al.*, 2013) reported that proper choice of good mating designs and selection of suitable parental lines are imperative to rewarding crop improvement schemes dependent on number of dynamics, for instance, objectives of the study, cost, time, space and other biological limitations.

According to Singh and Chaudhary (1985), the mating design is a procedure for producing progenies for further selection. Theoretically and practically, plant breeders and geneticists use diverse types of mating designs and activities for targeted purposes. The postulate of mating designs facilitates: (1) provision of data on the gene controlling trait being studied; (2) populations development to be used as basis for selection and advancement progenies into prospective varieties; (3) estimation of genetic gain; and (4) provision of data required for evaluation of the parental lines used in the improvement programme (Acquaah, 2012; Nduwumuremyi *et al.*, 2013). Thus, combining ability is the capacity of an individual to transmit superior traits to its progenies. Hence it is a gene governing the trait of interest to be improved. The estimation of general combining ability for a particular genotype be governed by the mating design, but essentially it is the deviance of the progeny average from the mean of the parental lines evaluated (Acquaah, 2012). There is limited information on the combining ability of lines that have been previously used in the cowpea breeding programs in IITA for the development of drought-tolerant lines. This has tremendously resulted in the slow phase of developing improved drought tolerant cowpea genotypes (Agbicodo *et al.*, 2009).

## **2.9 Assessment of Genetic Potential of Cowpea for Breeding Program**

### **2.9.1 Genetic Diversity Studies in Cowpea**

Genetic diversity is the variation of inheritable characters existing among alleles of genes within individuals of species' populations and plays an important role in evolution by allowing adaptation in a new environment (Charles *et al.*, 1997). It is a basic requirement for any successful crop improvement program. Assemblage, introduction, and evaluation of cowpea accessions are mandatory since it avails a grander scope for using the genetic multiplicity. A quantitative assessment of the genetic divergence among the germplasm and the comparative contribution of different traits towards the genetic divergence is essential and

creates an effective genetic pool for a breeder in the hybridization program. This genetic diversity could be assessed by three approaches: physiological, morphological, or phenological approach. These criteria could equally be used to select accessions with improved adaptation to dry environments (Blum, 1988). Conventionally, genetic diversity is estimated by measuring variation in phenotypic or qualitative traits such as the number of days to first flowering, time to maturity, plant type, flower colour, seed type, seed colour, seed size, hilum colour, and quantitative agronomic traits. However, this approach is often limited and expression of quantitative traits is subject to strong environmental responsiveness (Baye *et al.*, 2011). Thus, having in-depth knowledge of phenotypic variation and relationships among genotypes will assist breeders to develop appropriate breeding strategies and to create the most adaptive and productive cultivars. The study on germplasm genetic variation exploiting, morphological, phenological and agronomic traits would be useful in the development of new varieties with better adaptation to both biotic and abiotic stress factors, as well as for high yield potential. The bigger the number of accessions collection, the higher the opportunity for selection of the most suitable parental lines for breeding objectives after a complex evaluation of samples, for adaptation capacity to broad or specific agro-ecological conditions (Hamidou *et al.*, 2007; Agbicodo *et al.*, 2009). It also provides the opportunity to identify and select accessions with good levels of tolerance to abiotic stress factors (Agbicodo *et al.*, 2009).

The inevitability for finding out genetic divergence among the genotypes are more evident because of two main reasons: i) genetically diverse parents if included in the hybridization program are likely to produce high heterotic effect; and ii) a broad spectrum of variability could be expected in the segregating generation of crosses involving distantly related parents.

### **2.9.2 Importance of Genetic Distance in Hybridisation**

The genetic distance may originate from differences in the genetic complement or genomic difference of individuals. This difference is known as a genotypic or genetic variation. It could also be induced by exposure of the individuals to different values of treatment for particular environmental parameters during development (Aremu, 2011). This type of variation is termed environmental variation and it is not a heritable component of variation. However, genotypic variation is heritable and when acted upon by selection pressure evolutions do occur (Aremu, 2011).

One of the major statistical tools used in estimating genetic distances is multivariate analysis. Multivariate analysis provides the possibility of gathering many variables into one analysis. Genetic distance based on phenotypic characters is one of the main multivariate techniques used to provide criteria for choosing parents. Genetic distance between genotypes is a way to predict the genetic variability among hybrid combinations (Cruz and Regazzi, 2001). In addition to genetic distance studies, it is worth noting that the genotypes selected for crosses should possess high individual performance, adaptability and stability features for yield. When these requirements are fulfilled, there is a high probability of selecting transgressive genotypes due to the occurrence of heterosis and the action of complementary dominant genes (Bertan *et al.*, 2007).

### **2.9.3 Genetic Information Derived from Allelic Pattern in Germplasm**

Allelic diversity is an estimate of the average number of alleles per locus. This is denoted by  $N_a = \frac{\text{Total number of alleles}}{\text{Number of a studied locus}}$ . Allelic diversity depends on the population size.  $n$  is the total number of an allele in a population, whereas  $N_a$  is the average number of alleles per locus. Thus, the genetic information provided encompasses inheritance, nature of inheritance of the trait of interest and frequency of alleles or gene of interest in the population. This implies that when the homozygosity is high, heterozygosity will be low. In

addition, the genetic relatedness of a population is normally measured by genetic distance (Jakobsson *et al.*, 2008).

#### **2.9.4 Importance of Selection Index in Crop Improvement**

The estimation of genetic progress and selection of the best genotypes can be performed using both direct and indirect selection or classical index which is based on the sum of ranks. Direct and indirect selection is applicable if gains are from a single trait targeted in the selection, and other traits of secondary significance. These secondary traits may have favourable or unfavourable effects. The classical selection index was proposed by Smith (1936) and Hazel (1943) involves a linear combination of numerous parsimoniously essential traits whose weighting coefficients are estimated in order to exploit the correlation between the genotypic aggregate and the index. The genotypic aggregate is computed by another linear combination, involving the genetic values that are weighted by their respective economic weights (Cruz *et al.*, 2012).

Estimate of heritability along with the genetic coefficient of variation (GCV) is more useful in predicting the effect of selection than heritability values alone. The GCV determines the degree of genetic variability expressed by a plant for a trait in a population (Ahsan *et al.*, 2015). Selection is the retention of desired genotypes and the elimination of undesirable ones. It is an important process in breeding for improvement of one or more plant attributes.

Drought susceptibility index (DSI), is another criterion for selection used as a degree of drought tolerance of the selected genotypes and was computed according to the procedure of Fischer and Maurer (1978) as:

$$DSI = GR/DI,$$

Where: GR = genotype response, calculated as  $GR = (1 - Y_{m1}/Y_{m2})$ ,

Where:  $Y_{m1}$  and  $Y_{m2}$  are mean yields of all genotypes in severe drought stress and well-watered conditions respectively.

DI = drought intensities, computed as  $DI = (1 - Y_1/Y_2)$ ,

Where:  $Y_1$  and  $Y_2$  are respective yields of each selected hybrid under severe drought stress and well-watered environments.

DI value ranges from 0 to 1, where lower values are considered as drought tolerant and high values as susceptible.

The Johnson *et al.* (1955) method determined the genetic correlation and analysis of variance computed each of the indices. They classified stress intensity (SI) into mild, moderate and severe. Stress intensity is mild when yield reduction is between 0 and 25%, moderate when yield reduction falls between 25 and 50% and severe when yield reduction is between 50 and 100%.

## CHAPTER THREE

### 3.0 ASSESSMENT OF GENETIC DIVERSITY AMONG COWPEA ACCESSIONS FOR EARLINESS AND DROUGHT TOLERANCE

#### 3.1 Introduction

Crop improvement requires genetic divergence, a degree of heritability and intense selection. However, the responsiveness of genotypes to selection warrants loss of variability as adaptability improves (Simmonds and Arthur, 2003). High genetic variability in a collection creates a high chance for the selection of superior cultivars as compared to low diversity populations. Charles *et al.* (1997), reported that the evolution and genetic improvement of species depend on the genetic diversity present in the germplasm assembled.

Conventionally, agro-morphological traits such as growth habit, flower colour, yield potential and tolerance to stress were exploited in cowpea diversity study. However, morphological markers do not show the inherent genetic relationships in assembled genetic stock. This is attributed to the influence of environment in the expression of some of the genes. Thus, the knowledge about the structure of the assembled germplasm is limited (Wamalwa *et al.*, 2016). Additionally, good accession collection could be maximally utilised in population development using molecular markers. The markers may be used to fingerprint the collection and also act as indicators of uniformity (Simmonds and Arthur, 2003). Therefore, efficient utilisation of phylogenetic resources by plant breeder will help agricultural producers meet the global food requirement. These resources are abundant in many undesirable genes and are primarily useful as sources of a few desirable genes they possess (Vernooy *et al.*, 2013).

In Africa and particularly in sub-Saharan countries, the majority of the population live in rural areas and rely solely on crop production for subsistence. The socio-economic development in the African rural environment tends to be stagnant due to many agricultural production constraints (Brooks, 2013). Most rural populations in SSA are constantly faced

with a reduction and erratic distribution of rainfall during cropping seasons and, as a result, their food and nutrition security status are weakened. According to Ouedraogo *et al.* (2017), the problem faced by rural subsistent farmers could be tackled through diversification of agriculture through proper utilization of all genetic resource and development of new efficient and durable production systems.

In South Sudan the food shortage created by the “Man-made famine” of 2016 to July 2017 prompted the breeding programs in the country, such as the cowpea breeding program in the University of Juba, to expedite the process of providing seed companies and farmers (community-based organisations) with improved and high yielding varieties in order to address the food crisis. Thus, genetic diversity research is a decisive base for further cowpea improvement in this defining era of climate change. Hence, it will enhance selection of parental lines having the targeted genes of interest; hence the breeding objectives can be attained in a short period of time (Collard and Mackill, 2008).

Therefore, the broad objective of this study was to characterise the assembled cowpea germplasm for useful agro-morphological traits towards the development of high yielding, early and drought tolerant cowpea varieties.

The specific objectives were to:

- a) assess genetic diversity among germplasm collection using agro-morphological traits and SNP markers; and
- b) identify early and drought tolerant accessions to be used as parent for breeding work.

## **3.2 Materials and Methods**

### **3.2.1 Experimental Materials**

One hundred and six cowpea accessions (pure lines) were collected in 2016 from six countries. Five of these countries are in West Africa and only one in East Africa (Fig. 3.1). Twenty-one of the cowpea accessions (21) were collected from the Equatoria region of South Sudan, (37) from Savanna Agricultural Research Institute (SARI), Plant Genetic Resources Research Institute (PGRRI) and West Africa Centre for Crop Improvement (WACCI) in Ghana, (10) from Institut de l'Environnement et de Recherches Agricoles (INERA) in Burkina Faso, (1) from Institut national de la recherche agronomique (INRAN) in Niger, (20) from Institut Togolaise de Recherche Agronomique (ITRA) in Togo, and (17) from IITA in Nigeria. The accessions from South Sudan were purified by planting row of each accession and rouging out off-types. Most of the accessions assembled for this study were not photo-period sensitive except for Apagu 2A from South Sudan.

### **3.2.2 Experimental Site**

The phenotypic evaluation was carried out at Crop Science Farm of the University of Ghana. One young trifoliate leaf from each plant of 106 cowpea accessions were harvested. DNA extraction was carried out in the laboratory of the Biotechnology Centre in the College of Basic and Applied Sciences, University of Ghana. The extracted DNA were then shipped to TEXAS A &M AgriLife Genomics and Bioinformatics Services, College Station, TX, USA for genotyping where Novaseq 6000 sequencing system- Illumina was used.

Table 3.1: Showing region, countries and number of cowpea genotypes assembled

<b>Region</b>	<b>Country</b>	<b>Number of Accession</b>
East Africa	South Sudan	21
West Africa	Ghana	37
	Nigeria	17
	Niger	1
	Togo	20
	Burkina Faso	10

### 3.2.3 Morphological Characterisation

The 106-cowpea diversity panel from Burkina Faso, Ghana, IITA, Niger, South Sudan and Togo were evaluated in August 2016, using an augmented design with 103 of the accessions as unreplicated entries and three (Songotra, Padi-tuya and Dan Ila) as replicated entries or checks. Each of the accessions was planted on a four-row plot with an area of 6.0 m<sup>2</sup>. The trial was planted in a field comprising of ten blocks and 13 plots per block. The crop was grown in the minor season under rain-fed conditions with supplemental irrigation, and no chemical fertilizer was applied. A botanical insecticide “Attack,” a non-systemic and highly active insecticide that controls a wide range of insect pests such as thrips, beetles, aphids was used. Its active ingredient is ‘emamectin benzoate’ which accounts only for 5 % of the ingredients was applied at 1.5 litres per hectare at all growth and development stages to reduce fall armyworm and other insect pest damage. In addition, Hercules 50 SC, a contact pesticide was used to control insect pest.

Harvesting was done as soon as pods turned yellow colour completely and were sun-dried. The experiment was repeated at the same site (University of Ghana) experimental farm in September 2017, and the average of the two evaluations was used for analysis.

### 3.2.4 Molecular Characterisation

DNA was extracted from the leaf samples using the Hexadecyltrimethylammonium bromide (CTAB) protocol (Porebski *et al.*, 1997). Leaf samples in the Eppendorf tubes were macerated into a fine paste in 500 $\mu$ L of CTAB buffer. The macerated samples were incubated in a water bath for 15 minutes at 55°C. After which the incubated samples were centrifuged at 12000 rpm for 5 min. 200  $\mu$ L of the supernatant was transferred from each tube to new Eppendorf tubes. 250  $\mu$ L of Chloroform: Iso Amyl Alcohol (24:1) was added to each tube and inverted to obtain a milky solution. The solutions were centrifuged at 13000 rpm for 1 min. After which, 200  $\mu$ L of the supernatants were transferred into a new set of Eppendorf tubes. 50 $\mu$ L of 7.5 M ammonium acetate followed by 500  $\mu$ L of ice-cold absolute ethanol were added to each tube. The tubes were inverted slowly to precipitate the DNA in the solution. The tubes were centrifuged again for 13000 rpm for 1min, and the supernatant in each of the tubes was poured entirely off so that the DNA pellets would remain at the bottom. The DNA was subsequently washed twice with 500  $\mu$ L of ice-cold 70% ethanol by centrifuging at 13000 rpm for 1 min. The DNA in the tubes were allowed to dry for about 15 minutes, after which they were dissolved in 100  $\mu$ L of 1x Tris-Acetate ethylenediaminetetraacetic acid (TAE) buffer and stored in  $-80^{\circ}\text{C}$  freezer. Sample libraries was sequenced using the Illumina HiSeq 2500. Sequence cluster identification, quality prefiltering, base calling and uncertainty assessment were done in real time using Illumina's HCS 2.2.68 and RTA 1.18.66.3 software with default parameter settings. Sequencer. bcl basecall files were demultiplexed and formatted into fastq files using bcl2fastq 2.20.0 script conFigureBclToFastq.pl.

### 3.2.5 Data Collection and Statistical Analysis

#### 3.2.5.1 Morphological data

The following quantitative data were recorded.

1. Plant height at 20 days after planting was measured using a meter ruler from the soil level to the base of meristem of the mother plant measured in centimetre (cm).
2. Number of days to first flower was recorded as number of days from sowing to the day when first flower bud opened.
3. Leaf area index (LAI) was computed as the ratio of foliage area to ground area (is a one-sided green leaf area per unit ground area in broadleaf canopies).
4. Days to 50% flowering recorded as number of days from sowing to day when 50% of plants flowered.
5. Days to first mature pods were measured as the number of days from sowing to day when 50% of plants had mature pods.
6. Pod length was recorded in cm as mean of the 10 longest mature pods from 10 randomly selected plants.
7. Number of locules per pod was counted from the 10 pods measured for pod length.
8. Days to 95% maturity was recorded from seedling emergence to harvest of each genotype per plot (duration).
9. Number of main branches computed as the average of number of branches whose origin is in the leaf axils on the main stem counted from 10 randomly selected plants recorded in the 8th week after sowing.
10. Number of pods per plant recorded as mean number of mature pods from 10 randomly selected plants.
11. Stem diameter (mm) was measured starting from early vegetative stage to the first harvest using a digital calliper from 10 randomly selected plants.

12. 100–seed weight (g) was determined by randomly counting 100 seeds from a bulked seed and weighed using a digital weighing–scale.
13. Total seed weight (GYLD) was measured on plot bases after harvest and converted into kg per hectare (kg ha<sup>-1</sup>).
14. Delay leaf senescence (DLS) as visual scoring on a scale of 1–5, where 1 = totally green and turgid and 5 = completely yellow to brown almost dead.
15. Drought stress index as a visual score on a scale of 1–9, where 1 low susceptibility, 5 medium susceptibility, and 9 highly susceptible.

Table 3. 2: Score of qualitative characteristics of cowpea accessions

S/ No	Major characteristics	When scored
01.	Growth habit	Observed in the 6 <sup>th</sup> week after planting
02.	Growth pattern	Observed in the 6 <sup>th</sup> week after planting
03.	Plant pigmentation	Observed in the 6 <sup>th</sup> week after planting
04.	Immature pod pigmentation	Observed in the 6 <sup>th</sup> week after planting
05.	Terminal leaflet shape	Observed in the 6 <sup>th</sup> week after planting
06.	Flower colour	When 50% flowering is attained
07.	Raceme position	When peduncles have reached full length
08.	Pod attachment to peduncle	When pods are full grown
09.	Pod curvature	When pods are full mature
10.	Pod colour	Mature pod
11.	Seed shape	After harvest
12.	Eye pattern	After harvest
13.	Eye colour	After harvest
14.	Testa texture	After harvest

### 3.2.5.2 Molecular data

Raw SNP reads for quality was checked using FastQC (Andrews, 2010). Then the raw data were processed by the pipeline dDocent v2.2.6 (Puritz *et al.*, 2014). The dDocent combines several developed tools into one single pipeline specifically tailored for GBS/RAD sequencing data for variation discovery. Generally, the raw sequencing data was first processed with a quality filter using the TrimGalore tool (Krueger, 2015) to remove Illumina sequencing adapter and trim the low-quality bases (Phred score < 10) on the end of reads. The quality filtered reads were mapped back to a de novo assembly reference constructed

with dDocent. Only reads with >3X coverage and that are present in more than 10% of total samples were selected for de novo assembly. For the assemblies, BLAST (version 2.6.0) with e-value =  $10^{-5}$  was used to identify and remove assemblies that matched with chloroplast or ribosomal DNA in other plant species.

Reads for each sample were mapped to the de novo assembly sequences using the BWA-MEM algorithm with the fairly conservative default parameters (Mikheenko *et al.*, 2018). Alignment files generated for each sample were then processed by the program FreeBayes (Garrison and Marth, 2012), a Bayesian-based variant detection approach, to detect SNPs from the aligned reads of all samples.

Additional quality filtering was performed to minimize the calling of false SNPs due to sequencing error, paralogs, or artifacts of library preparation. The raw variant call file (VCF) was filtered using vcftools v0.1.15 (Danecek *et al.*, 2011) to a minimum quality score of 30, with a minimum genotype depth set to 3 reads, no more than 20% missing data per SNP, and minimum mean depth of coverage (DP) of 20. Only bi-allelic SNP with a minimum minor allele frequency (MAF) of 0.05 or more were retained for downstream analysis.

### **3.2.5.3 Statistical Analysis**

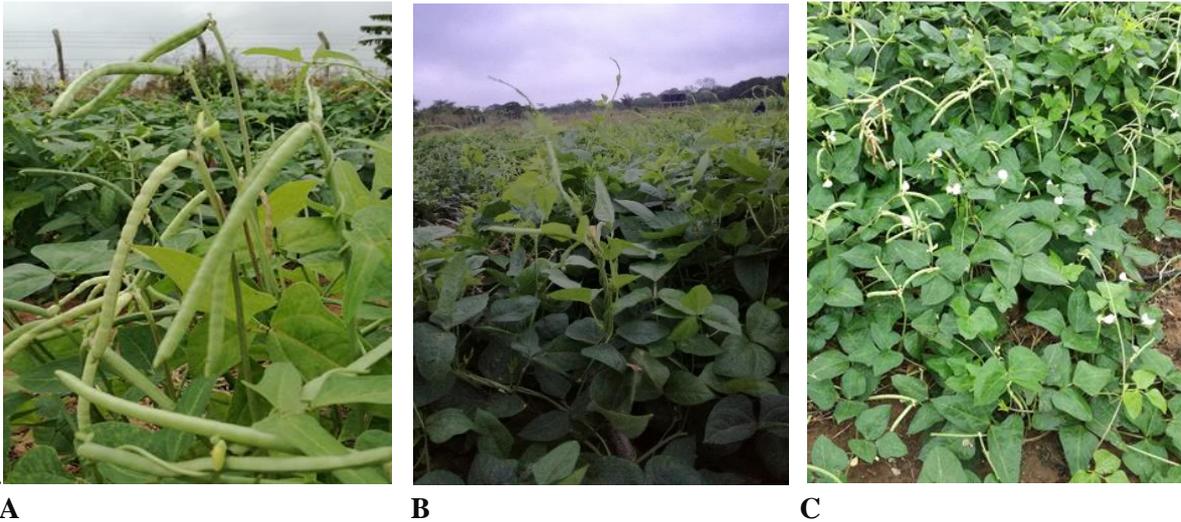
Statistical software GenStat 18<sup>th</sup> Edition was used to compute means of the traits which were then used for principal component analysis (PCA). XLSTAT (2017) version was used to generate dendrogram and clusters using the phenotypic data. The SNP data was analysed using the Darwin Software Version 6 and STRUCTURE Version 2.3.3 to assess the genetic structure of the cowpea accessions, PCA and Neighbour-Joining (NJ) tree. SNP marker data were subjected to structural analysis using data from 3148 markers to genetically characterized 106 cowpea accessions. Bayesian clustering analysis was carried out in the STRUCTURE programme (Pritchard *et al.*, 2000) to evaluate the population structure. Four

independent runs were performed for each number of assumed subpopulations (K) ranging from 1 to 12, using the admixture model correlated for all frequencies. A burn-in period of 5,000 and Monte Carlo Markov Chain (MCMC) run length of 10,000 for each run for all ranges (1 to 12) of K were carried out. Evanno's method (Earl and vonHoldt, 2012; Evanno *et al.*, 2005) was used to estimate the best number of subpopulations (K) that explains the structure of the genotypes. The run with the maximum log likelihood was used to assign genotypes into subpopulations based on their membership probability. The run was repeated for the best K at a burn-in period of 50, 000 and an MCMC run length of 100, 000 with one repeat. Genotypes with membership probability greater than or equal to 0.35 were put into same subpopulation whilst genotypes with membership probability less than 0.35 were put into mixed groups.

### **3.3 Results**

#### **3.3.1 Morphological**

The frequency distribution (percent) of variability in qualitative traits is presented in Table 3.3 as observed on the accessions studied. About ninety-six percent (96.2%) of the accessions were determinate, and only 3.8% were indeterminate. Diverse growth habits were exhibited by the various cowpea lines in the collection. Erect types were the most observed (35.8 %); semi-erect (19.8%), intermediate (18.9%), semi-prostrate (9.4%), prostrate (4.7%) and climbing (11.3%) were all observed (Plate 3.1)



**Plate 3. 1:** Plant growth habits observed: Apagu 1B, Laduni 1A and Titinwa A representing erect (A), climbing (B) and prostrate (C) types of cowpea accessions, respectively

Variable degree of plant stem pigmentation (Plate 3.2) was observed in the panel with those plants moderately pigmented at the base and tips of petioles accounting for 50%, and 10.4% were not pigmented.

Immature pods without anthocyanin pigmentation in the panel accounted for 49.1%, whereas those with solid pigmentation accounted for 12.3%. Mature and dried pods pigmentation also varied in the collection. Dark tan accounted for 60.4% of the total (Table 3.3). Accessions with white, violet and mauve-pink petals accounted for 43.4%, 55.7%, and 23.6%, respectively.

Table 3. 3: Frequency distribution of qualitative variables for cowpea accessions studied

Descriptor and classes	Frequency of class (%)										
	0	1	2	3	4	5	6	7	8	9	10
<b>Growth habit:</b> 1= Acute erect; 2= Erect; 3= Semi-erect; 4= Intermediate; 5= Semi-prostrate; 6=Prostrate; 7=Climbing	-	-	35.8	19.8	18.9	9.4	4.7	11.3	-	-	-
<b>Growth pattern:</b> 1= Determinate and 2= Indeterminate	-	96.2	3.8	-	-	-	-	-	-	-	-
<b>Plant stem pigmentation:</b> 0=None; 1= Very slight; 3= Moderate at the base and tips of petioles; 5= Intermediate; 7= Extensive; 9= Solid	10.4	21.7	-	50	-	8.5	-	6.6	-	2.8	-
<b>Immature pod pigmentation:</b> 0= None; 1= Pigmented tip; 2= Pigmented sutures; 3= Pigmented valves, green sutures; 4= Splashes of pigment; 5= Uniformly pigmented; 6= Other	49.1	26.4	12.3	-	-	12.3	-	-	-	-	-
<b>Leaf shape:</b> 1= Globose; 2= Sub-globose; 3= Sub-hastate; 4= Hastate	-	0.9	84.9	5.7	8.5	-	-	-	-	-	-
<b>Flower colour:</b> 1= White; 2= Violet; 3= Mauve-pink; 4= Other (specify in the descriptor)	-	43.4	55.7	23.6	-	-	-	-	-	-	-
<b>Raceme position:</b> 1= Mostly above canopy; 2= In upper canopy; 3= Throughout canopy	-	46.2	44.3	9.4	-	-	-	-	-	-	-
<b>Pod attachment to peduncle:</b> 3= Pendant; 5= 30 – 90° down from erect; 7= Erect	-	-	-	19.8	-	66	-	14.2	-	-	-
<b>Pod curvature:</b> 0= Straight; 3= Slightly curved; 5= Curved; 7= Coiled	6.6	-	-	67.9	-	19.8	-	5.7	-	-	-
<b>Pod colour:</b> 1= Pale tan or straw; 2= Dark tan; 3= Dark brown; 4= Black or dark purple; 5= Other	-	60.4	21.7	5.7	12.3	-	-	-	-	-	-
<b>Seed shape:</b> 1= Kidney; 2= Ovoid; 3= Crowder; 4=Globose; 5= Rhomboid	-	17	13.2	-	7.6	53.8	-	-	-	-	-
<b>Eye pattern:</b> 0= Absent; 1= Very Small; 2= Kabba group; 3= Narrow eye; 4= Small eye; 5= Holstein group; 6= Watson group; 7= Self-coloured; 8= Other	-	40.6	-	-	39.6	13.2	-	6.6	-	-	-
<b>Eye colour:</b> 0= Eye absent (white, cream); 1= Brown splash or grey; 2= Tan Brown; 3= Red; 4= Green; 5= Blue to black; 6= Blue to black spots or mottle; 7= Speckled; 8= Mottled; 9= Mottled and speckled; 10= Other	89.6	6.6	2.8	-	0.9	-	-	-	-	-	-
<b>Testa texture:</b> 1= Smooth; 3= Smooth to rough; 5= Rough; 7= Rough to wrinkled; 9= Wrinkled	-	53.8	-	26.4	-	-	-	19.8	-	-	-



**Pigmented**



**Non-pigmented**

**Plate 3. 2:** Cowpea accessions Laduni 1B (left) and Apagu 1B (right) pigmented and non-pigmented pods respectively

Accessions exhibited the three main raceme positions included mostly above the canopy, in the upper canopy and throughout canopy in the collection. These three observed raceme positions accounted for 46.2%, 44.3% and 9.4%, respectively (Table 3.3).

Four distinct terminal leaflet shapes were observed among panel members with sub-globose accounting for 84.9% of the total observation and 8.5% hastate. Similarly, hastate and globose terminal leaflets were noticeable. Still, they were less frequent than sub-globose and sub-hastate terminal leaflet orientation (Plate 3.3).



**A=Hastate**



**B=Globose**



**C=Sub-globose**



**D=Sub-hastate**

**Plate 3. 3:** Variability in cowpea terminal leaflet shapes observed

Genotypes exhibited three main types of pod attachments; pendant 30-90° and erect in the collection (Plate 3.4). The most observed pod attachment type was 30–90° for 66% of the population.

The 106 cowpea accessions showed various eye colours with 89.6% of the accessions having white or cream eye colour, 6.6% with brown splash or grey, 2.8%, and 0.9% have tan brown and green eye colour, respectively (Table 3.3). Four seed shapes were observed with rhomboid accounting for 58.8% of the collection, 17% of the accessions had kidney shape, 13.2% were ovoid, and 7.6% of the panel members had globose seed shape (Plate 3.5).



**A** **B** **C**  
**Plate 3. 4:** Cowpea accessions Apagu 1B (A), AGRAC-316 (B) and Beledi C (C), showing erect, pendant and 30°–90° types of pod attachment respectively

Four noticeable pod curvatures were observed in the collection; 67.9% were slightly curved, 19.8% curved, 6.6% with straight orientation and 5.7% were coiled (Plate 3.4).



**Plate 3. 5:** A mixture of cowpea seeds from different accessions showing diversity of seed characteristics

The assembled accessions displayed different eye patterns with 40.6% of the accession having very small eye pattern, 39.6% showed small eye pattern. Holstein group accounted for 13.2% of the variability, and 6.6% were observed to be self-coloured. About fifty-four percent (53.8%) of the accessions had smooth testa texture, 26.4% were smooth to rough, and 19.8% had rough to wrinkled testa texture (Table 3.3).

Genetic diversity analysis based on agro-morphological data of the 106 accessions using cluster analysis revealed that the accessions are genetically diverse. This genetic divergence is shown by a dendrogram based on 13 phenotypic characters by unweighted pair-group average (UPGMA) method using the overall Euclidean distance. The Euclidean dissimilarity coefficient ranged from 0.0 to 1.4 with the cophenetic correlation coefficient (CCC) of 0.76. The resultant dendrogram grouped the 106 cowpea accessions into two main clusters, with nine sub-clusters at agro-morphological level (Fig. 3.1). The smallest sub-clusters 3, 6, 8 and 9 had one accession each, cluster 5 had 2 accessions, and cluster 7 with 5 members (Table 3.4). Sub-cluster 1, 2 and 3 were the largest with 35 members in cluster 1, 39 accessions in cluster 2 and 21 members in cluster 3. Majority of cowpea accessions from South Sudan were members of sub-cluster 1 with only two accessions in sub-cluster 2. The mean performance values of measured agro-morphological traits in 106 cowpea accessions in the clusters revealed that members in cluster 1 registered the highest grain yield of 503.34 kg/ha and cluster 8 accessions had the lowest grain yield (101.00 kg/ha).

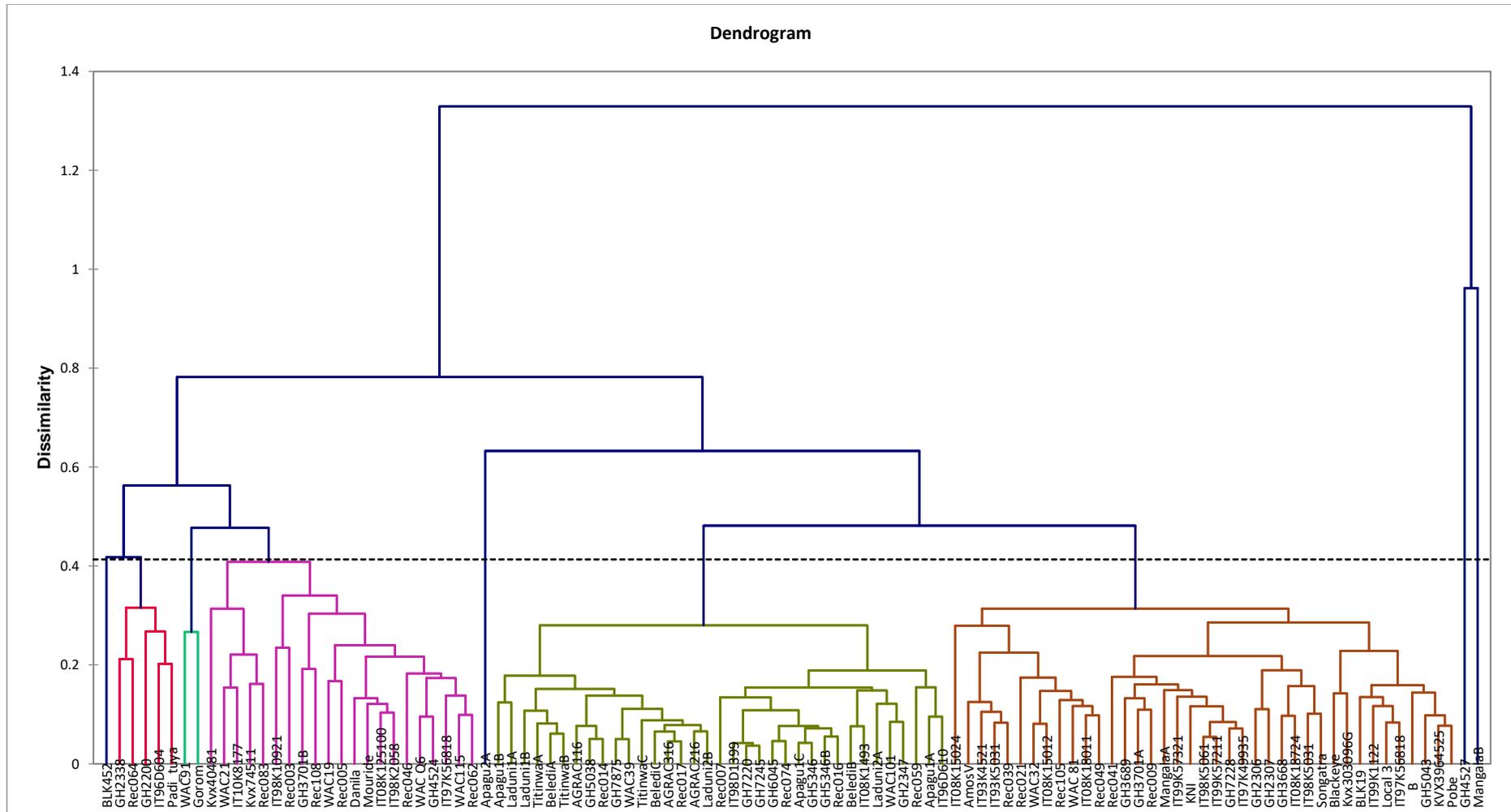


Fig. 3. 1 Dendrogram resulting from the analysis of 106 cowpea accessions (based on 13 phenotypic characters) obtained by UPGMA using the overall distance of Euclidean. The cophenetic coefficient ( $r$ ) was 0.76

Table 3. 4: Mean values of agro-morphological traits of 106 cowpea accessions

<b>Number of clusters</b>	Yield (kg/ha)	100–seed weight (g)	Plant height (cm)	Stem diameter (mm)	Leaf area index	Number of branches per plant	Days to first flower	Days to 50% flowering	Number of days first pod	Number of pods per plant	Number of locules per pod	Number of days to 95% maturity	Pod length (cm)
<b>Cluster 1 (35)</b>	503.3	13.8	12.5	12.3	0.2	5.6	36.0	38.0	42.5	42.6	15.4	61.0	15.7
<b>Cluster 2 (39)</b>	271.3	14.4	10.7	11.0	0.1	4.9	37.0	40.0	44.7	33.0	13.2	64.0	15.0
<b>Cluster 3 (1)</b>	277.0	14.4	49.0	10.3	0.2	4.8	43.0	49.0	54.0	22.0	15.5	66.0	17.7
<b>Cluster 4 (21)</b>	190.9	13.4	9.8	10.4	0.1	4.5	38.0	41.0	45.5	30.7	13.7	64.0	14.9
<b>Cluster 5 (2)</b>	158.0	17.4	9.9	11.1	0.2	4.3	38.0	43.0	47.0	16.9	11.7	63.0	12.7
<b>Cluster 6 (1)</b>	119.0	11.2	10.1	9.6	0.1	4.4	33.0	39.0	40.0	28.9	11.7	65.0	10.9
<b>Cluster 7 (5)</b>	150.0	13.7	11.2	11.2	0.1	5.2	40.0	43.0	47.3	35.7	13.2	65.0	14.7
<b>Cluster 8 (1)</b>	101.0	10.8	13.7	7.8	0.1	4.0	33.0	37.0	40.0	36.9	14.1	63.0	13.8
<b>Cluster 9 (1)</b>	146.0	13.9	23.8	9.2	0.3	11.4	42.0	45.0	49.0	14.7	14.8	76.0	15.7

Footnote: Number of entries per cluster are shown in brackets

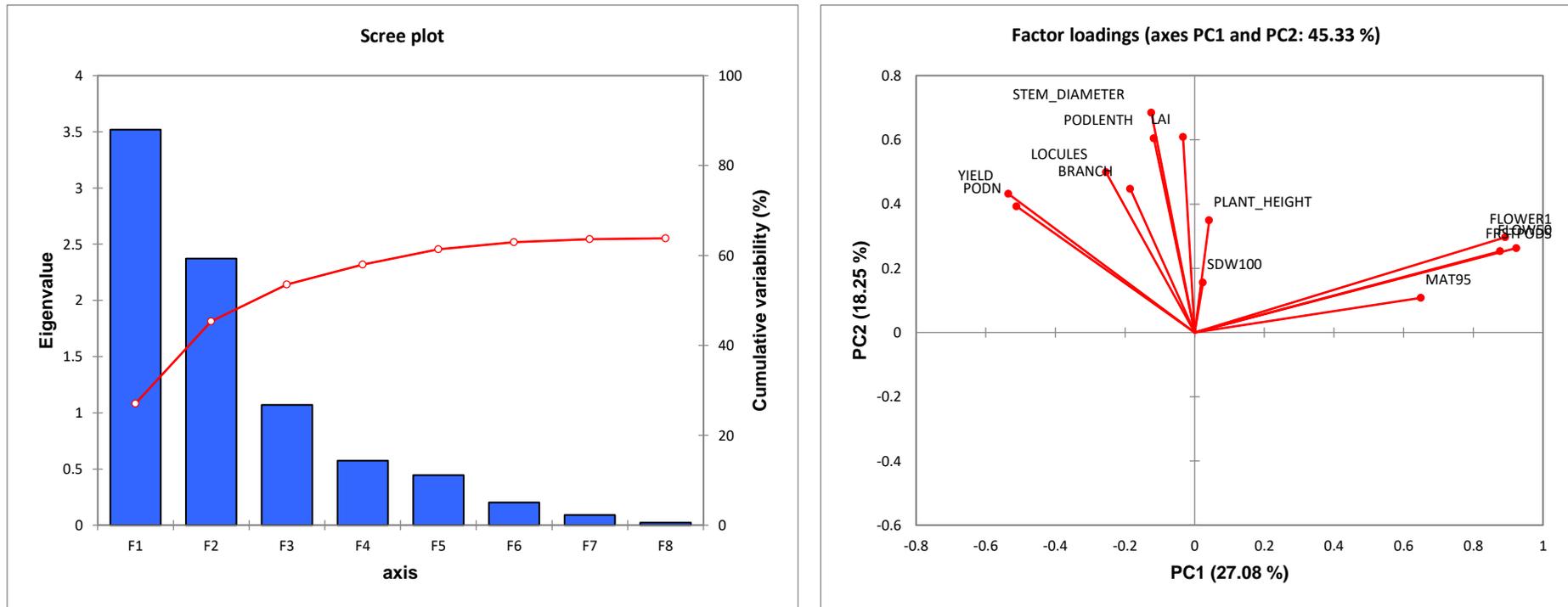
The Eigenvalues and percentage of explained variability by principal component showed that the first three principal components accounted for 56% of the total variation (Fig. 3.2 {a}). Vectors for variability were scattered on the biplot. The most discriminative traits included stem diameter, number of days to first flower, number of days to 50% flowering, number of days to first pod, pod length, leaf area index, number of locules per pod, number of pods per plant, number of branches per plant, number of days to 95% maturity and yield (Table 3.5).

Informative vectors for evaluation of cowpea accessions included vector length which was the longest relative to other vectors. Plant height at 20 days after planting and 100–seed weights were the least discriminating vectors as their vector length was the shortest relative to other vectors (Fig. 3.2 {b}). Vectors for variability were scattered on the biplot. The most discriminative traits in terms of contribution to variation among 106 cowpea accessions included stem diameter, number of days to first flower, number of days to 50% flowering, number of days to first pod, pod length, leaf area index, number of locules per pod, number of pods per plant, number of branches per plant, number of days to 95% maturity and yield (Table 3.5 and Fig. 3.2 {a}).

Table 3. 5: Principal components loading for 106 cowpea accessions variables

<b>Variables</b>	<b>Mean</b>	<b>StDev</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<b>PC4</b>	<b>PC5</b>
Yield	297.51	158.14	-0.35	-0.18	0.11	-0.13	0.05
100-seed	14.00	3.30	0.03	-0.11	0.44	-0.45	-0.09
Plant height	11.23	4.18	-0.08	-0.27	0.13	-0.05	-0.61
Stem diameter	11.10	1.79	-0.17	-0.40	-0.06	-0.14	0.45
LAI	0.145	0.05	-0.14	-0.41	-0.07	-0.00	-0.12
Branch	5.08	1.07	-0.15	-0.27	0.17	-0.24	-0.32
Flower1	37.28	2.67	0.40	-0.29	-0.11	-0.08	0.10
Flower50	40.17	2.80	0.40	-0.29	-0.08	-0.07	0.02
Firstpods	44.94	3.35	0.41	-0.25	-0.04	-0.11	0.12
NPP	35.12	11.44	-0.28	-0.14	0.11	-0.22	0.46
Locules	13.55	2.70	-0.26	-0.24	-0.25	0.45	-0.06
Mat95	63.45	4.39	0.30	-0.21	-0.22	0.09	-0.11
Podlenth	14.74	2.26	-0.21	-0.33	-0.12	0.31	0.02
DSI	3.62	2.76	-0.11	0.12	-0.52	-0.46	-0.02
DLS	2.32	0.49	-0.15	0.07	-0.56	-0.34	-0.19
<b>StDev</b>			<b>2.01</b>	<b>1.62</b>	<b>1.36</b>	<b>1.14</b>	<b>1.05</b>
<b>Variance</b>			<b>4.02</b>	<b>2.61</b>	<b>1.84</b>	<b>1.29</b>	<b>1.10</b>
<b>Prop. Var Exp</b>			<b>0.27</b>	<b>0.17</b>	<b>0.12</b>	<b>0.09</b>	<b>0.07</b>
<b>Cumulative</b>			<b>0.27</b>	<b>0.44</b>	<b>0.56</b>	<b>0.65</b>	<b>0.72</b>

Yield (grain yield), 100-seed (100-seed weight), Plant height (plant height at 21 days after planting), Stem diameter, Leaf area index, Branch (number of branches per plant), Flower1 (number of days to first flower), Flower50 (number of days to 50% flowering), Firstpods (number of days to first mature pod), NPP (number of pods per plant), Locules (number of locules per pod), Mat95 (number of days to 95% maturity), Podlenth (Pod length), DSI (Drought stress index) and DLS (delayed leaf senescence)



(a) Scree plot graph of factors responsible for variability among study accessions

(b) Projection of the 13 characteristics in axe 1 and 2

Fig. 3. 2 Eigenvalue and principal components responsible for variability among 106 cowpea accessions

### 3.3.2 Molecular Genetic Structure

The generated genetic structure from the cluster analysis of the SNPs markers gave information on the germplasm studied (Fig. 3.3). The cluster revealed the existence of a considerable degree of genetic diversity and reflecting a reasonable amount of genetic dissimilarity among the genotypes. However, there were six distinct clusters with the 20 cowpea accessions from South Sudan were genetically associated with all the members of the six clusters with only AGRAC-216 being admixture. The output of the analysis designated by each of the six colours represented a population/cluster; red is cluster 1 predominantly accessions from Burkina Faso, green is cluster 2 (Nigeria), blue is cluster 3 (Ghana), yellow is cluster 4 (South Sudan), pink is cluster 5 (Niger), and blue as cluster 6 (Togo). Cowpea accessions from South Sudan revealed relatedness to all members of the six clusters (Fig. 3.3).

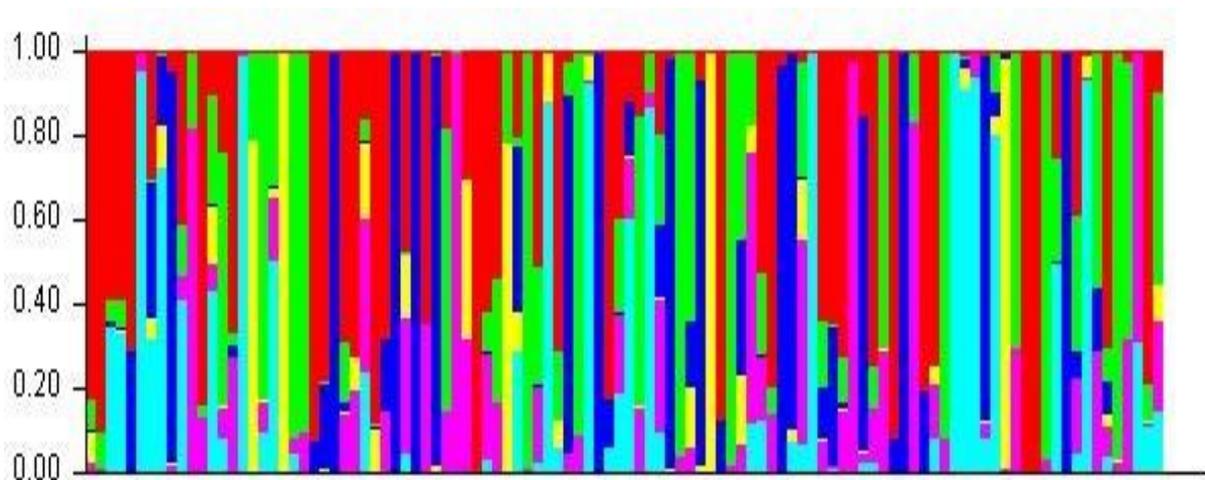


Fig. 3. 3 Display of population structure of the 106 cowpea genotypes

The highest values of expected heterozygosity approximately 0.3 were observed in cluster 5 and the lowest was in cluster 4 0.1 (Fig. 3.4). An estimated  $F_{st}$  value of 0.75 was obtained in cluster 1 and 0.28 in cluster 1 (Fig. 3.4).

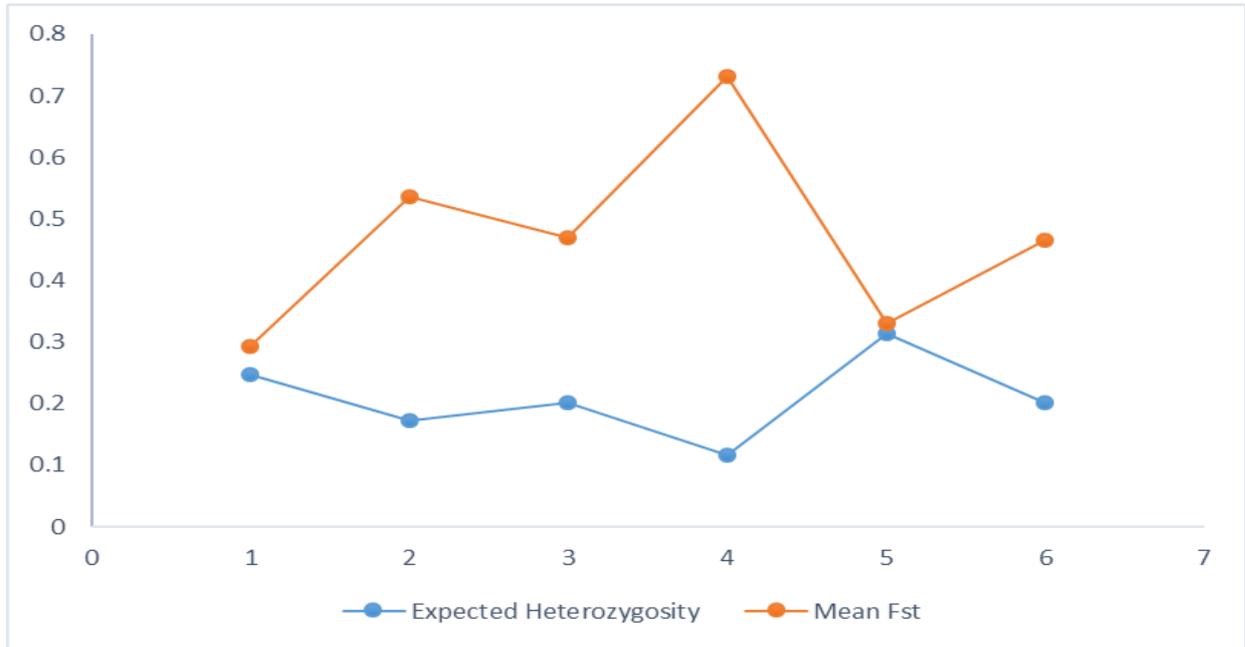


Fig. 3. 4 Expected heterozygosity and mean fixation index (Fst) for each cluster

The expected heterozygosity among members of a cluster revealed that cluster 5 had the highest value of 0.31 with cluster 4 having the lowest (0.12). The inferred inferences used in the population structure of the 106 cowpea accessions revealed a peak delta K=6. Whereas, the mean value of the fixation index of the 106 cowpea accessions members of six clusters ranged from 0.29 to 0.73 (Table 3.6).

Table 3. 6: Mean Fst value and expected heterozygosity within cluster

Cluster	Number of Members	Expected heterozygosity	Mean value of Fst
Cluster 1	30	0.25	0.29
Cluster 2	21	0.17	0.54
Cluster 3	18	0.2	0.47
Cluster 4	7	0.12	0.73
Cluster 5	13	0.31	0.33
Cluster 6	17	0.2	0.47

The analysis of allelic divergence among the clusters revealed that the longest net nucleotide distance existed between members of cluster 4 and cluster 5 (0.15). However, the shortest divergence (0.08) was observed between cluster 1 and cluster 6 (Table 3.7).

Table 3.7: Allele-frequency divergence among clusters (Net nucleotide distance)

Clusters	1	2	3	4	5	6
1	-					
2	0.09	-				
3	0.09	0.13	-			
4	0.12	0.14	0.12	-		
5	0.09	0.10	0.13	0.15	-	
6	0.08	0.10	0.10	0.12	0.10	-

The hierarchical cluster analysis of the 106 cowpea accessions revealed node numbering begun at 107. The weighted Neighbour-Joining was obtained from tree construction with a bootstrap analysis of 1000. The average edge distance between bootrapped trees was 0.40 with 0.29 as 5-percentile and 0.50 as 95-percentile. The dissimilarity index used was the Euclidean procedure and the values on the node of the phylogenetic tree are probabilities indicating relatedness of the accessions (Fig..3.5). However, there are six distinct clusters with the majority (38) of the cowpea accessions in cluster one, 20 cowpea accessions in cluster two, 16 in cluster three. Similarly, cluster four had six members, cluster five had 10 accessions, and six had six members.

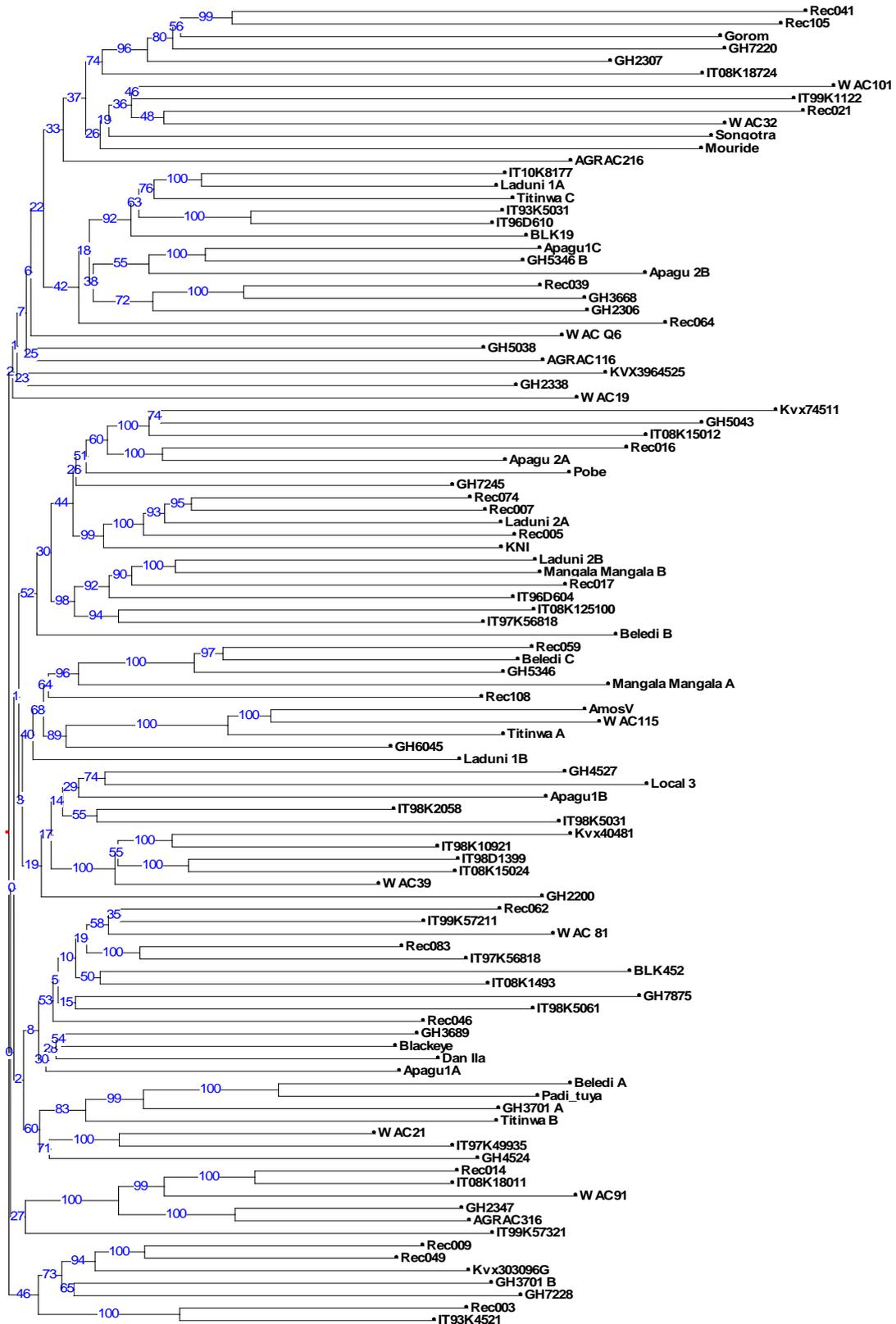


Fig. 3. 5 Phylogenetic tree depicting genetic similarities and dissimilarity among 106 cowpea accessions

The principal coordinates are calculated for the first five axes (with positive eigenvalues). Dissimilarity in the collection was calculated from the data file using Cluster Darwin V6 (Perrier *et al.*, 20003). Drought tolerant accessions grouped in one cluster; susceptible genotypes were assigned in another. Equally, high yielding accessions were in different clusters so as the late maturing group. Colour code: Togo (Black), Ghana (Blue), Niger (Brown), Nigeria (Green), Burkina Faso (Red) and South Sudan (Yellow) (Fig.3.6).



### 3.4 Discussion

The use of plant diagnostic traits to identify varieties has been a classical taxonomic approach for both varietal purity testing and identification. Plant morphological traits such as plant stem pigmentation, stem diameter, plant growth pattern, plant growth habit, plant height and the number of branches per plant were scored, measured, and analysed which facilitated classification of accessions into different groups.

Growth habits of the 106 accessions varied greatly as genotypes with the most being acute erect (35.8 %) and the least prostrate (4.7%). The majority of Ghanaian accessions had semi-prostrate growth habit, some had intermediate or prostrate growth habit, and only 1% was found to be erect. This result agrees with the finding of Doumbia *et al.* (2014) reported that the germplasm from Mali had more than 80% semi-prostrate growth habit. However, according to Wang *et al.* (2006), semi-prostrate growth habit has three benefits, competitive ability with weeds, ease of harvesting and high biomass (forage for livestock). Growth habit in cowpea is controlled by a single gene (Lachyan and Dalvi, 2013) and is easily selected for. The growth patterns of the genotypes were variable thus they were grouped into a determinate type which accounted for 96.2% of the total collection and indeterminate type constitute 3.8% of the total collection. Nkouannessi (2005) and Sarutayophat *et al.* (2007) obtained groupings of higher percentage of determinate growth type in cowpea. Similar finding was reported in green gram by Jain and Khare (2002). However, they found that determinate and indeterminate expressions of plant growth pattern were stable, but semi-determinate expression was highly influenced by environment.

Plant stem pigmentation is another qualitative trait that breeders explore in order to quantify the level of genetic divergence. The 106 cowpea accessions displayed different levels of pigmentation which varied with the stocks, the base and tips of petioles, and stem. This finding agrees with the results obtained by Cobbinah *et al.* (2011) who reported a similar

trend of variability for the trait in some cowpea genotypes evaluated in Ghana. Karkannavar *et al.* (1991) proposed a digenic inheritance mode of stem pigmentation with complementary gene action and a 17.4% crossover rate was recorded between a complementary gene for stem pigmentation and one for stipule colour.

Leaf shape is a prominent qualitative trait that breeders use as a morphological marker in diversity studies. About eighty-five percent (84.9%) of the assembled accessions had sub-globose leaf shape, 0.9% were globose. Sub-hastate leaf shape accounted for 5.7% and hastate for 8.5% of the total variability present in the collection. Doumbia *et al.* (2014) reported similar patterns of divergence in cowpea accessions from Mali with more than 50% having sub-globose leaf shape, but other accessions from Ghana were different. The Ghanaian collection was predominantly hastate and sub-hastate. Uguru (1996) found that the rhomboid and lanceolate leaf shapes were genetically stable. But Pottorff *et al.* (2012) reported that hastate leaf shape is dominant to the more common sub-globose leaf shape. They further reported that the hastate leaf shape was incompletely dominant to (ovate) globose leaf shape.

Plant height is an important characteristic which aids breeders in differentiating the genotypes. Based on this trait, genotypes were grouped into: dwarf, medium and tall. The 106 genotypes exhibited plant height ranging from 9.8 to 49.00 cm. A large percentage (66.04%) of the genotypes had medium height. Although, plant height was influenced by high additive genetic variance (Patil and Navale, 2006), it may also be influenced by environment.

Based on the number of days to 50 percent flowering which ranged from 37 days (GH 4527) to 49 days (Apagu 2A), three classes of maturity groups were identified. Among the 106 genotypes, 76 accessions which were members of cluster 1, 2, 6 and 8 matured early (<41 DAP), 29 members of cluster 4, 5 7 and 9 were medium (41–49 DAP), and the only member

of cluster 3 was late maturing (>49 DAP). This finding corroborates with the result of Aremu *et al.* (2007) who found similar trend for the trait with cowpea accessions collected from the West Africa sub-region. The variability observed in the number of days to 50% flowering could be attributed to a minor complex gene (Weiss, 1971; Adu-Dapaah *et al.*, 1988) with a tendency for dominance of early flowering. Ikram (2004) reported that earliness in cowpea was under the influence of additive gene action more than dominance effects. In addition, environmental conditions were observed to affect flowering. The number of days to 95% maturity varied from 61 to 76 days. Thirty-five accessions (cluster 1 members) were early (<60 days), one accession was late (>65 days) and remaining accessions were medium (60–65 days). Nevertheless, Patil and Navale (2006) found that maturity in cowpea is controlled by dominant gene action. In addition, environmental conditions such as soil moisture level, temperature, and relative humidity influence the growth and development of crop plants.

Based on variation observed in the flower colour, the genotypes were grouped into accessions with white flowers accounting for 43.5%, whereas 55.7% of accessions had violet flowers and 23.6% were mauve-pink. The variations in flower colour can be attributed to the genetic factors. Genes determine the colour of the petal by producing or blocking anthocyanin pigmentation. Othman *et al.* (2006) reported that flower colour was simply inherited when different types of colour were considered separately, and two genes were observed to control flower colour. Patel *et al.* (2016) found that a single recessive gene-controlled flower colour in cowpea.

Raceme position and pod attachment to peduncle in cowpea genotypes differed significantly. However, 46.2 % of the accessions had their racemes positioned above the canopy, 44.3% had their raceme in the upper canopy, and 9.4% were observed to have their raceme positioned throughout the canopy. Higher raceme position enables easy discernibility of pods

during harvest. With respect to pod attachment, 19.8% of the accessions had pendent pod attachment, 66% showed 30°–90° down from erect and remaining 14.2% had erect pod attachment. The expression of these traits is genetically controlled and heritable (Jain and Khare, 2002). Pod characteristics influence the yielding ability of cowpea.

Number of branches per plant determines the pod bearing ability which contributes to yield. Hence identification and selection of genotypes with more branching ability is desirable. In this study, genotypes exhibited varying degree of number of branching ranging from 4.0 (GH 4527) to 11.4 (Mangala Mangala B). Based on this trait, the 106 cowpea accessions were grouped into fewer (<4), medium (4-6) and high (>6) branches per plant. Accession GH 4527 had the least number of branches per plant, while one hundred and four accessions had medium number of branches per plant, and Mangala Mangala B had the highest number of branches per plant. Patil and Navale (2006) reported that number of branches per plant is controlled by dominant gene action indicating the presence of intra-allelic interaction in the trait. Thus, heterosis could be the best breeding approach for improving the number of branches per plant. Other breeding approaches such as isolation of recombinants in advance segregating population could also be useful. The distinction in the number of branches per plant is influenced by genetic factors and environmental conditions, cropping seasons, seed rate and spacing (Weiss, 1971).

Number of pods per plant were variable among the genotypes, with Laduni 1B having the highest number of pods (63). Based on the variability of this character, the genotypes were grouped as less (<15), moderate (25.7), or high (>42) pod bearing types. Patil and Navale (2006) found that number of pods per plant was governed by additive gene action. In addition, the distinction in the number of pods per plant could also be attributed to pod bearing ability of the genotype, environmental responsiveness and soil nutritional status.

Equally, pod length varied among the accessions panel, and classified the accessions into those with short pod length (<11 cm), medium (11–15 cm), and long (>15 cm) pod. Thirty-seven accessions had long pod length, while 68 were medium and one short. Singh et al. (1997) reported that pod length is moderately to highly heritable with an average value of 0.7 percent signifying pod length in cowpea is under genetic influence. Patil and Navale (2006) reported that pod length in cowpea is under the influence of high additive variance which means that the trait is controlled by additive gene action.

Pod colour at maturity is another essential trait. The results of grouping of the cowpea accessions based on pod colour at maturity showed that 60.4% of accessions were straw coloured, 21.7% had dark tan pod colour, 5.7% had dark brown, and 12.3% were black or dark purple. Similar groupings were earlier reported by Nwofia and Emeka (2014) and Sarutayophat *et al.* (2007) in cowpea, Jain and Khare (2002) in green gram. Asare *et al.* (2011) reported a dominant allelic factor controlling pod colouration.

Pod curvature ranks among the most used traits that influence producer and consumer preference when the pods are tender. Diverse pod curvatures were found in this study. Slightly curved pod orientation accounted for 67.9% of the population, 19.8% were curved, 5.7% had coil pod curvature, and 6.6% were straight. Nwofia and Emeka (2014) reported that pod curvature is controlled by dominant gene action. Furthermore, they stated that coiled pods were completely dominant over the straight pods.

Significant variation was observed in seed shape for different cowpea accessions, with 17% of the genotypes having kidney seed shape, 13.2% ovoid shaped, 7.6% globose shape and the remaining rhomboid shape. Nkouannessi (2005) and El Naim *et al.* (2012) reported that the seeds of the same cultivar might vary in shape. Furthermore, they stated that the shape may be influenced by the position of seed in the pod which may be influenced by environmental conditions during pod filling stage.

The genotypes varied in eye colour and pattern. Eye colour included white-cream eye colouration which was the most observed (89.6%), brown splash or grey eye colour, red tan brown and blue to black eye colour. The eye pattern commonly found in the 106 accession were very small eye pattern (40.6%), small (39.6%), holstein, and self-coloured (eye covers the whole seed). The seed testa texture varied significantly with 53.8% having smooth testa texture, 26.4% smooth to rough and 19.8% rough to wrinkle. Nkouannessi (2005) found seed testa texture ranging from rough to wrinkle. A similar trend of smooth to rough seed coat texture was reported by Adewale *et al.* (2011) in cowpea.

There was a significant difference among the accessions for hundred seed weight. The value ranged from 10.8 g (GH 4527) to 17.4 g (WAC91 and Gorom). Based on this, genotypes were grouped as light (<13.0 g), medium (13–15 g) and heavy (>15 g) seed weight. Among the genotypes, only one had light 100-seed weight, 103 were medium, and two cultivars had heavy 100-seed weight. This variation among the genotypes could be due to inherent genotypic differences and environment and could be used for yield improvement. Rashwan (2010) reported a cowpea population had highly significant positive additive  $\times$  dominance epistatic effect for this trait. The genotypic variation in test weight may also be due to the varied capacity of the genotype to utilize the reserved assimilates.

Seed yield per plant varied significantly within and among genotypes. Based on this, the 106 genotypes were grouped into three categories; low, moderate, and high yielders. Seed yield per hectare ranged from 503.3 kg (cluster 1 members) to 101.00 kg (cluster 8) with an average yield of 212.9 kg. Grain yield differences depend on a number of factors, and the most notable ones are number days to maturity with late and early maturity genotypes having high and low seed yield, respectively. Yield also depends on the inherent capacity of genotype, seed size, the influence of cultural practices and environmental conditions.

Siddique and Gupta (1991) reported that additive gene effects were significant in conditioning grain yield in cowpea.

Among the 13 quantitative characters studied, almost all the traits are important characters contributing to the diversity among the 106 accessions. These findings corroborate with the results obtained by Backiyarani *et al.* (2000) who found that every trait studied should add into genetic divergence displayed by accessions.

To both consumers and farmers, the most important traits of cowpea grain/seed are seed colour, seed size, cooking ability, testa and seed coat. Accessions from cluster 1 and 8 had the most desired features high yield, maturity, seed size, and colour. South Sudanese farmers and consumers preferred varieties are AGRAC-116, AGRAC-216, AGRAC-316, Apagu 1B, Titinwa A, and Titinwa B, Beledi A, Beledi C, and Laduni 1B. Accession from West Africa, especially members of clusters 2, 4 and 7 (Padi-tuya, Songotra, Black eye, IT93K-503-1, Dan Ila and Mouride) with white seed colour, distinctive black and brown eyes, smooth, rough, smooth to rough seed coat. Individually accessions with bigger seed size were AGRAC-116, AGRAC-216, Apagu 2A, Beledi A, Black eye, WAC91, Rec 003, IT08K-149-3, Pobe Local, and WAC21. The 100-seed weight of the accessions ranged between 19.0-23.0 g. Omoigui *et al.* (2006) also reported a range of 20.0-22.0 g for 100-seed weight in cowpea.

All crop improvement programs in South Sudan are still at their initial stages. This is the pioneer study that for the first time ever assessed the genetic potential of some cowpea accessions from South Sudan using molecular marker. To date, there is no genotypic information about the genetic variability existing in them. Knowledge of the genetic value of each accession will provide good evolutionary information about these accessions and is step towards an effective breeding program (Becerra *et al.*, 2017) of cowpea in South Sudan.

Huynh *et al.* (2013) reported that grouping accessions into groups of relatedness will help population development and enhance germplasm management.

The SNP marker analysis grouped the 106 cowpea accessions into six clusters in the dendrogram. However, the phenotypic analysis showed nine clusters. The differences in results between the two approaches may be attributed to the ability of molecular markers to provide insights into genetic relationships within and between sub-populations. However, morphological markers are measurable and can be influenced by GEI effect. This finding agrees with results of Karampatakis *et al.* (2017) who reported a significant difference between phenotypic and genotypic diversity within same population. The principal coordinate analysis gave a clear insight about the diversity in the 106-cowpea accession as each and every set had a unique trait (tolerance and susceptibility to drought stress, high yield and lateness. This collection may constitute a useful stock for any future cowpea improvement in the country.

The maximum likelihood and Bayesian analysis revealed that genotypes were grouped based on a threshold set. Those members with probability level greater than or equal to 0.35 were grouped into the same subpopulation (cluster), whilst genotypes with probability level less than 0.35 were put into an admixture group. The observed admixtures could be the result of inbreeding. However, one of the most important evolutionary forces, migration, was found to be responsible for the gene flow among accessions. This result agrees with the finding of Ali *et al.* (2015), who attributed diversity to exchange of materials between breeder, natural and artificial selection, genetic drift and environmental variation. This flow gives rise to individuals known as admixtures. Admixtures provide essential information about the genetic variation needed for selection. McTavish and Hills, (2014) found that, admixtures could hinder opportunities for local adaptation. Conversely, Buerkle and Lexer, (2008) reported admixtures could be utilised as basis for genetic mapping. The fixation index of the

accessions revealed wide range of genetic differentiation. Jakobsson *et al.* (2008) used  $F_{st}$  as an evolutionary parameter with an assumption of SNP markers being polymorphic taking into consideration the component of genetic variation within a cluster. The  $F_{st}$  value less than 0.05 means there was little genetic difference among members in a cluster, 0.05 to 0.15 as moderate genetic difference, 0.15 to 0.25 showing the great genetic difference and greater than 0.25 as an indication of very great genetic difference. In this study, the mean expected heterozygosity in the population was 0.21. Low heterozygosity value implies that the collection had a narrow genetic base. This finding corroborated with those of other researchers (Asare *et al.*, 2011; Ali *et al.*, 2015).

### 3.5 Conclusions

The genetic distance among and within individual accessions resulted in their separation into nine distinct clusters with a cophenetic correlation coefficient of 0.76. The first three principal components accounted for 65% of the total variability in the panel, indicating that all the traits scored varied significantly. These accessions could serve as important breeding stock for the cowpea development, particularly in South Sudan and Togo where limited breeding activities for cowpea are undertaken. The most critical yield components were the number of pods per plant, seeds per pod and 100-seed weight. Individually, high value for these traits were found in GH 4527, Laduni 1B, AGRAC-216, AGRAC-316, Titinwa A, Titinwa B, Beledi A, Beledi B and Apagu 1B and in six advanced breeding lines Songotra, Padi-tuya, Black eye, Dan Ila, Mouride, IT93K-503-1 and IT97K-499-35. The accessions that are early to medium maturing are GH 4527, BLK 452, Titinwa A, Titinwa B, Laduni 1B, Beledi A, Beledi C, AGRAC-316 and those that showed a high level of drought tolerance were IT98K-503-1, IT97K-499-35, Dan Ila and Mouride. Furthermore, the molecular study confirmed the existence of genetic divergence among the assembled cowpea panel. This panel was grouped into six: high yielding accessions, drought tolerant, susceptible, late

maturing and medium maturing. This has created an opportunity for population development through introgression of new alleles from different backgrounds into adapted local varieties. The SNP markers used in this study could be utilized to analyse and group other new set of collections in the future.

## CHAPTER FOUR

### 4.0 IDENTIFICATION OF DROUGHT TOLERANCE AMONG DIVERSE COWPEA GERMPLASM

#### 4.1 Introduction

Parental line selection in cowpea improvement is a crucial factor in population development or hybridisation process. For drought tolerance, earliness and other physiological traits such as chlorophyll content, stomatal conductance, delayed leaf senescence, stem greenness, and root architecture are essential features for parental line selection. Seed size, colour, and shape are very important traits in addition to the objective of yield improvement under drought stress conditions. For efficient drought tolerance screening, it is advisable to explore both empirical and physiological approaches either through gauging canopy temperature or root traits because there is a direct relationship between water lost through transpiration and absorption by the root system. Numerous screening methods for identification of drought-tolerant cowpea genotypes have been used. These methods include pot evaluations, wooden boxes, pin boards and field evaluations (Watanabe *et al.*, 1997; Mai-Kodomi *et al.*, 1999a; Matsui and Singh, 2003; Muchero *et al.*, 2008; Agbicodo *et al.*, 2009).

There is little information about the level of drought tolerance in the locally adapted, farmers preferred cowpea varieties in South Sudan despite research by International Institute of Tropical Agriculture (IITA), University of California, Riverside (UCR) and other National Agricultural Research Organisations (NARO) in Africa. Genotype selection for drought tolerance is very important for world food production, primarily in arid regions with erratic rain distribution. The objective of this work was to screen 49 cowpea genotypes for drought tolerance under rainout shelter, identify and select drought tolerant cowpea genotypes for genetic improvement.

## 4.2 Materials and Methods

### 4.2.1 Genetic Materials and Experimental Site

Forty-nine cowpea genotypes were selected after a preliminary drought tolerance screening of 106 genotypes under a rainout shelter at Crop Science Farm of the University of Ghana in May 2016. These screened genotypes were collected from farmers in South Sudan, and five research Institutes in West African countries of Togo, Ghana, Niger, Burkina Faso and Nigeria (IITA). The experiment was laid out in lattice square design (7×7) replicated twice with accessions and watering regimes as treatments. Four healthy looking seeds were planted in 10 litre grafting plastic pots with a diameter of 40 cm. Seedlings were thinned to two plants per pot eight days after planting. Each pot was filled with topsoil known as Adenta series after sieving. The soil series was analysed and found to have pH of (4.47–4.71) and 30–35 % clay, (0.5 %) loam, (5–10 %) sand and (60–62.5 %) organic matter, available P (3.26 mg/kg), (Department of Crop Science, University of Ghana). The pots were watered at field capacity two days prior to planting. Moisture stress was applied as described by Muchero *et al.* (2008).

The plants were watered to field capacity (moisture content 30%) until the reproductive stage was attained (thirty days from germination) and then water was withdrawn for three weeks for drought response measurements. During the period of stress, day and night temperatures were recorded as 22.3–28.6°C and 16.3–21.4°C, respectively using Thermo-hygrometer device. The soil moisture content during the water stress was monitored at 20 cm depth using Fieldsout TDR 150 once in a week for the second and third week of imposed drought stress. After the period of imposed drought stress, plants were watered twice a week for two weeks and the recovery scored (regrowth and stem greenness). In total, 11 variables were recorded after stressing the plants to assess drought tolerance of the 49 accessions as described in Table 4.1.

Table 4.1: Eleven variables used to categorise the drought tolerance of the 49 cowpea accessions

Variable Identifier	Description
LWI 1	Leaf wilting index after the first week of stress
LWI 2	Leaf wilting index after the second week of stress
LWI 3	Leaf wilting index after the third week of stress
IB 2	International Board on Plant Genetic Resources scale after the second week of drought stress
IB 3	International Board on Plant Genetic Resources scale after the third week of drought stress
IB 4	International Board on Plant Genetic Resources scale after the fourth week of drought stress
MAIK	Mai-Kodomi <i>et al.</i> (1999) scale after the second, third and fourth week of drought stress
SMC1	Moisture content after the second week of drought stress
SMC2	Moisture content after the third week of drought stress
STG	Stem greenness after 2 weeks of re-watering
Re-growth	Resumption of growth after 2 weeks of re-watering

#### 4.2.2 Data Collection

Data of both qualitative and quantitative traits were recorded using the cowpea descriptors developed by the International Board for Plant Genetic Resources (IBPGR, 1983).

- i. Delayed leaf senescence (DLS) as visual scoring on a scale of 1–5, where 1 = totally green and turgid and 5 = completely yellow to brown almost dead.
- ii. Stem greenness was scored using a scale of 1-5, where 1 was brown and 5 completely green.
- iii. Re-growth was scored using three categories as: 1 with no recovery, 3 recovery from axillary buds, and 5 recovery from the apical stem.
- iv. Leaf Wilting Index (LWI) was calculated using the ratio of leaves showing wilting signs and a total number of leaves per plant.
- v. Drought stress index (DSI) as a visual score on a scale of 1–9, where 1 low susceptibility, 5 medium susceptibility, and 9 highly susceptible in order to identify genotypes with ‘Type 1’ and ‘Type 2’ drought tolerance.

- vi. Soil moisture level was measured using the Fieldscout TDR 150 operation instrument.
- vii. Days to first flowering was recorded as the number of days from planting to a stage when single plants in a plastic pot began to flower.
- viii. Days to 95% maturity was scored as the number of days when the majority of the pods had attained physiological maturity.
- ix. At flowering, plant height was measured in cm on randomly selected plants in each plastic pot, taking measurements from the base of the plant to the last node on the main stem.
- x. Number of seeds and locules per pod was recorded as an average number of locules and seeds from 10 pods,
- xi. whereas the number of pods per plant was recorded as the mean of matured pods from randomly selected plants.
- xii. Number of branches per plant was counted as the branches whose origin is in the leaf axils on the main stem recorded at the 8<sup>th</sup> week after sowing.
- xiii. Pod lengths from randomly selected plants in each plastic pot were measured in cm.
- xiv. Grain yield adjusted to 12% moisture content was computed from the grain weight of harvested pods per plastic pot.

#### **4.2.3 Data Analysis**

Analyses of data combined across two contrasting environments (well-watered and drought-stressed) were performed using GenStat 18<sup>th</sup> Edition considering all effects as random except genotypes according to Vargas *et al.* (2014). META-R software was used to compute phenotypic correlation. IBM SPSS Statistics software version 22<sup>nd</sup> was used to compute cluster and dendrogram.

### 4.3 Results

#### 4.3.1 Leaf Wilting Index (LWI), Levels and Types of Drought Tolerance

Three weeks of drought stress induced highly significant differences among the genotypes for leaf wilt indices (Table 4.2). Of the 49 genotypes evaluated, 22.5% showed no sign of wilting in the first week while 77.6% showed signs of leaf wilting with GH7228 having the highest wilting ratio (0.69) and IT99K-573-11 having the lowest (0.13). Increase in LWI were observed from the second to the third week. The highest LWI was 0.96 (GH7875) and 1 for GH 7828 and Rec105, respectively. The lowest values were 0, 0.19, 0.26 and 0.33. The highest values were observed in GH7828 and Rec105 and the lowest values were registered in Mouride, IT93K-503-1, Dan Ila, K VX-404-8-1 and IT97K-499-35 in the second and third week (Table 4.2). Susceptible genotypes in the collection accounted for 14.29%. Those that showed ‘Type 1’ drought tolerance accounted for 24.49% and these are plants that cease growth but preserve moisture and keep all the leaves and growing tips alive for long period of time. Whereas, ‘Type 2’ accounted for 62.22% and they assemble moisture from the lower leaves to the growing tips remain alive for longer period of time compare to ‘Type 1’.

(Table 4.3).

Table 4. 2: Mean squares of 10 measured traits of 49 cowpea genotypes

	Traits	F	Sig.
Mean of aborted pods	55.61	2.46	0.05
Leaf wilting index 1	0.04	0.63	0.64
Leaf wilting index 2	0.02	0.35	0.84
Leaf wilting index 3	0.03	0.76	0.05
Soil moisture content 1	3.16	1.31	0.04
Soil moisture content 2	2.03	0.55	0.05
Drought stress index	4.85	1.23	0.00
Stem greenness	3.53	0.89	0.04
Re-growth	1.79	0.75	0.06
Delayed leaf senescence	2.36	2.29	0.04

F: Computed F value and Sig. Level of significance

Table 4. 3: Drought tolerance scores\* and moisture levels in 49 cowpea genotypes

Genotypes	Drought tolerance				Re-growth	Aborte d pods	Leaf wilting			Moisture level	
	DLS	DSI	type **	STG			LW1	LW2	LW3	SMC2	SMC3
AGRAC116	2	5	2	1	3	6.5	0.60	0.62	0.78	38.00	32.35
AGRAC216	2	5	1	5	5	5.0	0.53	0.73	0.85	37.50	35.75
AGRAC316	3	1	2	5	3	5.0	0.23	0.40	0.65	36.75	34.65
Amos V	3	5	2	1	3	2.5	0.34	0.77	0.84	37.50	35.40
Apagu 1A	4	5	2	1	3	12.5	0.36	0.70	0.87	37.75	34.35
Apagu 1B	2	5	S	1	1	10.5	0.29	0.76	0.82	34.75	30.65
Apagu 2A	2	1	2	5	3	6.0	0.32	0.52	0.71	38.50	36.90
Beledi A	2	5	S	1	5	9.0	0.57	0.78	0.93	38.50	35.55
Beledi C	3	5	S	1	5	5.0	0.49	0.62	0.88	37.50	36.15
BK	4	5	S	1	5	12.5	0.40	0.56	0.81	37.25	34.80
Dan Ila	1	1	2	5	5	0.5	0.00	0.26	0.47	37.50	36.05
GH7228	5	5	2	1	1	8.5	0.69	0.74	1.00	38.50	37.10
GH7875	4	5	1	1	3	10.0	0.48	0.74	0.96	36.50	34.35
Gorom	2	1	2	5	5	0.0	0.15	0.47	0.40	38.75	36.95
IT08K125100	3	1	2	5	5	0.5	0.58	0.62	0.95	36.75	35.25
IT08K15024	4	5	2	1	5	2.5	0.19	0.62	0.76	38.25	35.15
IT08K18724	5	1	1	5	1	0.0	0.45	0.66	0.81	36.75	34.75
IT10K8177	3	1	1	5	5	0.0	0.00	0.60	0.73	37.25	35.90
IT93K5031	1	1	2	5	3	0.5	0.00	0.19	0.50	38.25	34.95
IT96D604	2	5	2	5	3	0.0	0.17	0.67	0.88	38.00	36.85
IT97K49935	2	1	2	5	5	0.0	0.00	0.37	0.71	37.50	36.00
IT97K49938	3	1	2	5	5	0.0	0.00	0.53	0.59	37.25	35.35
IT97K56818	2	1	1	5	3	1.0	0.00	0.49	0.55	38.75	37.00
IT98K10921	2	1	1	5	5	0.0	0.18	0.55	0.74	37.75	36.05
IT98K2058	2	1	1	5	5	0.0	0.15	0.62	0.77	39.00	37.00
IT98K5061	2	5	2	1	3	0.0	0.00	0.42	0.79	37.50	36.70
IT99K57311	3	1	2	5	3	0.5	0.13	0.64	0.84	37.75	36.00
IT99K57321	2	1	1	5	5	0.0	0.24	0.69	0.93	36.75	34.70
KNI	3	1	2	5	1	0.0	0.28	0.42	0.59	38.00	36.80
KVX303096G	2	5	1	5	5	0.0	0.18	0.64	0.81	39.25	37.85
KVX396452D	2	1	2	5	5	0.0	0.00	0.40	0.53	39.00	36.05
KVX40481	2	1	1	5	5	0.0	0.19	0.33	0.40	38.70	33.80
KVX745118	3	1	1	5	5	0.5	0.00	0.59	0.81	38.00	36.60
Laduni1B	4	5	S	1	5	4.5	0.67	0.81	0.95	38.25	35.75
Laduni2B	4	5	2	5	3	14	0.46	0.71	0.95	38.00	35.40
Mangala Mangala A	2	5	2	5	3	8.0	0.20	0.77	0.83	39.00	34.00
Mangala Mangala B	2	1	2	5	3	4.5	0.19	0.43	0.71	37.50	34.60
Mouride	1	1	2	5	5	1.0	0.00	0.00	0.28	36.10	33.95
Padi_tuya	2	5	2	5	3	6.5	0.18	0.79	0.83	37.50	35.80
Pobe local	1	5	2	5	5	0.0	0.00	0.47	0.82	37.75	35.40
Rec016	5	5	2	1	1	6.5	0.26	0.65	0.87	37.00	33.20
Rec041	4	5	1	1	1	1.5	0.22	0.54	0.73	38.65	34.60
Rec105	3	5	2	1	5	4.0	0.31	0.71	1.00	38.70	36.85
Songotra	2	5	2	5	3	4.5	0.44	0.52	0.71	38.75	35.35
Titinwa A	3	5	S	1	5	10.5	0.33	0.65	0.95	38.75	36.25
Titinwa B	3	5	S	1	5	9.0	0.53	0.82	0.94	34.00	32.40
Titinwa C	3	5	2	1	3	8.5	0.50	0.82	0.93	38.00	34.25
WAC101	3	5	2	1	5	11.5	0.37	0.83	0.93	37.75	34.10
WAC121	4	5	2	1	1	11.0	0.41	0.79	0.95	37.25	34.45

\*Planted on September 02 2016 and stressed at reproductive stage October 02, 2016

\*\*Type 1- total plant tolerance, Type 2-moisture mobilisation from lower leaves to tips, S- Drought susceptible, DLS: Delayed leaf senescence, DSI: Score of drought stress, STG: Stem greenness, LWI: Leaf wilting index and VWC: Volumetric water content

#### **4.3.2 Wilting Scales (Delayed Leaf Senescence)**

Wilting scored according to IB scale showed a highly significant difference ( $P < 0.001$ ) among the accessions (Table 4.2). Rec016, IT08K-187-24, and GH7228 had a score of 5, indicating that these genotypes almost died. IT93K-503-1, Mouride, Pobe local, Dan Ila with score of 1 were totally green with turgescient leaves. Whereas Titinwa A showed green to yellow and wilted leaves (3), Laduni 1B had yellow-green and severely wilted leaves (4) and Beledi A, Apagu 1B and Beledi C (2) had green slightly wilted leaves.

#### **4.3.3 Score of Drought Stress Index (DSI)**

Scores of drought stress index showed highly significant differences ( $P < 0.001$ ) among the evaluated genotypes (Table 4.2). Genotypes AGRAC-116, Apagu 1B, IT98K-506-1, Rec016, Titinwa A, Titinwa B, WAC 121 and others accessions had a score of 5 indicating that these genotypes were completely dead after being stressed for three weeks. Dan Ila, IT99K-573-21, Mouride, IT93K-503-1 and many others had green turgid leaves with a score of 1.

#### **4.3.4 Soil Moisture Content (SMC)**

There were significant differences ( $P < 0.05$ ) among the genotypes relating to measured moisture and depletion in the second and third week of water stress. The highest soil moisture level in the second week of stress was recorded in Rec105 (40.58) and Titinwa B had the lowest moisture level (34.00). Whereas, in the third week, Rec105 continued to have the highest moisture level and Apagu 1B had the lowest (32.00).

#### **4.3.5 Re-growth and Stem Greenness (STG)**

Both stem greenness (STG) and re-growth in the assessed genotypes showed highly significant differences at a probability level of 0.001 (Table 4.2). For regrowth, a score of 5 was observed in AGRAC-216, IT08K-150-24, IT97K-499-35, KNI, Pobe local,

IT97K-499-38, IT93K-503-1, IT98K-109-21, K VX-303-096G, Mouride, Dan Ila, and many others indicating that these genotypes had apical meristem recovery. Apagu 1B, GH7828, Rec016, Reco41, and WAC121 had a score of 1 indicating no regrowth after the resumption of watering for two weeks. Apagu 1A, AGRAC-316, Amos V, Beledi A, Beledi C, GH7875, Mangala Mangala A and other genotypes with score 3 had auxiliary buds regrowth. For STG, almost forty-three percent of the genotypes including Apagu 1A, Apagu 1B, and AGRAC-116 had completely yellow stems. AGRAC-316, Padi-tuya, Dan Ila, Mouride, IT97K-499-35, IT93K-503-1 had completely green stem after alleviation of drought stress (Table 4.3).

Table 4. 4: Descriptive statistic for quantitative parameters measured under drought stress condition

Parameters	Mean $\pm$ SE	Range	CV (%)
Plant Height (cm)	27.17 $\pm$ 1.33	7.7 $\pm$ 82.5	44.77
Number of days to the first flower	37.76 $\pm$ 0.38	30.0 $\pm$ 49.0	10.06
Number of days to 95% maturity	62.86 $\pm$ 0.48	50.0 $\pm$ 76.0	8.40
Number of pods per plant	16.41 $\pm$ 0.63	3.3 $\pm$ 44.0	31.82
Pod length (cm)	10.29 $\pm$ 0.32	4.7 $\pm$ 27.0	23.93
Number of locules per pod	10.57 $\pm$ 0.29	3.9 $\pm$ 17.0	19.49
Number of seeds per pod	7.81 $\pm$ 0.25	2.6 $\pm$ 15.0	27.58
Yield (g)	2.31 $\pm$ 0.13	0.30 $\pm$ 6.30	47.48

SD: Standard deviation, CV%: Coefficient of variation

Significant differences in all the traits were recorded with a mean of 27.17 cm plant height under stressed conditions and 54.10 cm under well-watered conditions. The number of pods per plant was equally affected by the two treatments. Plants under optimum condition produced a higher number of pods (24.78) versus 16.41 for those under water stressed conditions. Yield under stressed conditions was 50% (2.31 g) lower than yield under well-watered conditions (5.11 g) (Table 4.5). Combined analysis of variance revealed significant differences for the interaction between genotypes and environment at  $P < 0.001$  level of significant for plant height, number of pods per plant, number of seeds per pod and yield. Whereas  $P < 0.05$  probability level was recorded for pod length and the remaining traits

(number of days for the first flower, number of days to 95% maturity and number of locules per pod) showed no significant interaction with the environment. The 49 cowpea accessions were grouped into two distinct clusters and five sub-clusters based on the Euclidean distance using Ward's method (Fig. 4.1). Accessions from South Sudan are found across sub-clusters 1, 2, 4 and 5. Sub-clusters 2 and 3 predominantly contain accessions introduced from West African countries (Table 4.6).

Table 4. 5: Mean of traits of 49 cowpea genotypes evaluated across two environments

Env		Height (cm)	First flow	95%Maturity	Number of pods	Pod length (cm)	Number of locules per pod	Number of seeds	Yield (g)
Stressed	Mean	27.17	38	63	16.41	10.29	10.57	7.81	2.31
	Std. Deviation	13.13	3.78	4.77	6.23	3.15	2.86	2.47	1.24
Watered	Mean	54.10	37	64	24.78	11.68	11.66	9.12	8.03
	Std. Deviation	19.84	2.81	4.41	10.23	2.52	3.03	2.66	5.11
Across	Mean	40.63	38	63	20.60	10.99	11.12	8.47	5.17
	Std. Deviation	21.53	3.33	4.60	9.43	2.93	2.99	2.64	4.69
CV%		47.79	7.90	7.87	19.61	20.21	17.83	23.84	62.76
LSD		3.82	1.84	1.43	2.62	3.28	2.83	2.96	4.61

Height (Plant height), First flow (number of days to first flower), 95% maturity (number of days to 95% maturity), Number of pods (number of pods per plant), Number of seeds (number of seeds per pod)

Table 4. 6: Distribution of 49 cowpea accessions in five clusters according to quantitative traits

Sub-cluster	Number of Genotypes	Attributes	Genotypes
1	10	Medium maturing	Rec041, WAC101, Rec105, GH7875, GH7228, Laduni 2B, Laduni 1B, Amos V, Rec016 and AGRAC-316
2	11	High yielding	KVX396452D, Beledi A, IT98K5061, KVX40481, Pobe local, WAC121, IT08K15024, IT08K125100, Songotra, IT08K18724, BK
3	17	Drought tolerant	Mouride, Gorom, KVX303096G, Dan lla, KVX745118, IT99K57321, IT93K5031, IT96D604, AGRAC116, KNI, AGRAC216, IT97K56818, Padituya, IT98K10921, IT97K49935, IT97K49938, IT98K2058
4	6	Susceptible to drought	Titinwa C, Beledi C, Titinwa A, Apagu 1A, Titinwa B, Apagu 1B,
5	5	Late maturing	Mangala Mangala B, IT99K57311, Mangala Mangala A, IT10K8177, Apagu 2A,

Cluster 3 had the largest number of genotypes with 17 members representing 34.7% of the genotypes. In the second position was cluster 2 whose membership accounts for 22.5% of the genotypes. The third was cluster one with 10 members representing 20.4% of the genotypes. Cluster four predominately contains accessions from South Sudan and constituted 12.2% of the total accessions. Cluster 5, with five accessions, represented 10.2% of the 49 genotypes studied (Table 4.7). Within cluster three, Dan lla stood out as a unique genotype. The dendrogram revealed that the most diverse genotypes were Apagu 2A and Mangala Mangala B (Fig. 4.1).

Cluster analysis using Ward's method of minimum variance exhibited a distinct pattern of group formation (Table 4.7). The shortest number of days characterised the genotypes in cluster IV to first flower, and the highest number of seeds per pod, number of pods per plant and grain yield. cluster V showed a longer number of days to first flower and the

highest number of seeds per pod, while members of cluster III registered the lowest grain yields.

Table 4. 7: Mean values of cowpea agronomic traits measured under drought stress and well-watered conditions

Parameters	Mean				
	Sub-cluster 1	Sub-cluster 2	Sub-cluster 3	Sub-cluster 4	Sub-cluster 5
Plant height (cm)	43.94	39.33	39.02	39.45	43.84
Number of days to the first flower	36.00	37.00	39.00	36.00	40.00
Number of days to 95% maturity	63.00	63.00	64.00	63.00	64.00
Number of pods per plant	20.71	18.26	22.27	23.48	16.37
Pod length (cm)	11.44	11.71	10.15	10.45	11.99
Number of locules per pod	11.94	11.44	9.97	11.15	12.60
Number of seeds per pod	8.66	8.76	7.61	9.02	9.67
Grain yield (g)	5.04	5.79	4.75	5.68	4.85

#### 4.3.6 Correlation between Leaf Wilting Index and Drought Tolerance Traits

Correlation analysis of drought tolerance related traits revealed significant differences at a significance level of 0.05 for various traits (Table 4.8). Drought stress index (DSI) was found to be strongly correlated with LWI and STG, but negatively correlated with regrowth. LWI was strongly but negatively correlated with regrowth and STG and regrowth positively correlates with LWI. However, stem greenness positively associates DLS and regrowth and negatively correlates with LWI 1, LWI 2 and LWI 3.

Table 4. 8: Associations among 10 drought related traits

DLS	1	-								
DSI	2	<b>0.37</b>	-							
LWI 1	3	<b>0.47</b>	<b>0.50</b>	-						
LWI 2	4	<b>0.47</b>	<b>0.52</b>	<b>0.59</b>	-					
LWI 3	5	<b>0.47</b>	<b>0.57</b>	<b>0.58</b>	<b>0.87</b>	-				
Regrowth	6	<b>-0.47</b>	<b>-0.35</b>	<b>-0.37</b>	<b>-0.32</b>	<b>-0.29</b>	-			
STG	7	<b>0.52</b>	<b>-0.60</b>	<b>-0.50</b>	<b>-0.44</b>	<b>-0.44</b>	<b>0.41</b>	-		
SMC2	8	-0.07	-0.03	-0.12	-0.11	-0.07	<b>0.33</b>	0.17		
SMC3	9	-0.03	-0.08	-0.25	-0.16	-0.09	<b>0.34</b>	0.27	<b>0.74</b>	-
		<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>

All the bold values are significant at 0.01 probability level

**Note:** Leaf wilting indices (LWI), Delayed leaf senescence (DLS), Drought stress index (DSI), soil moisture content (SMC), and stem greenness (STG)

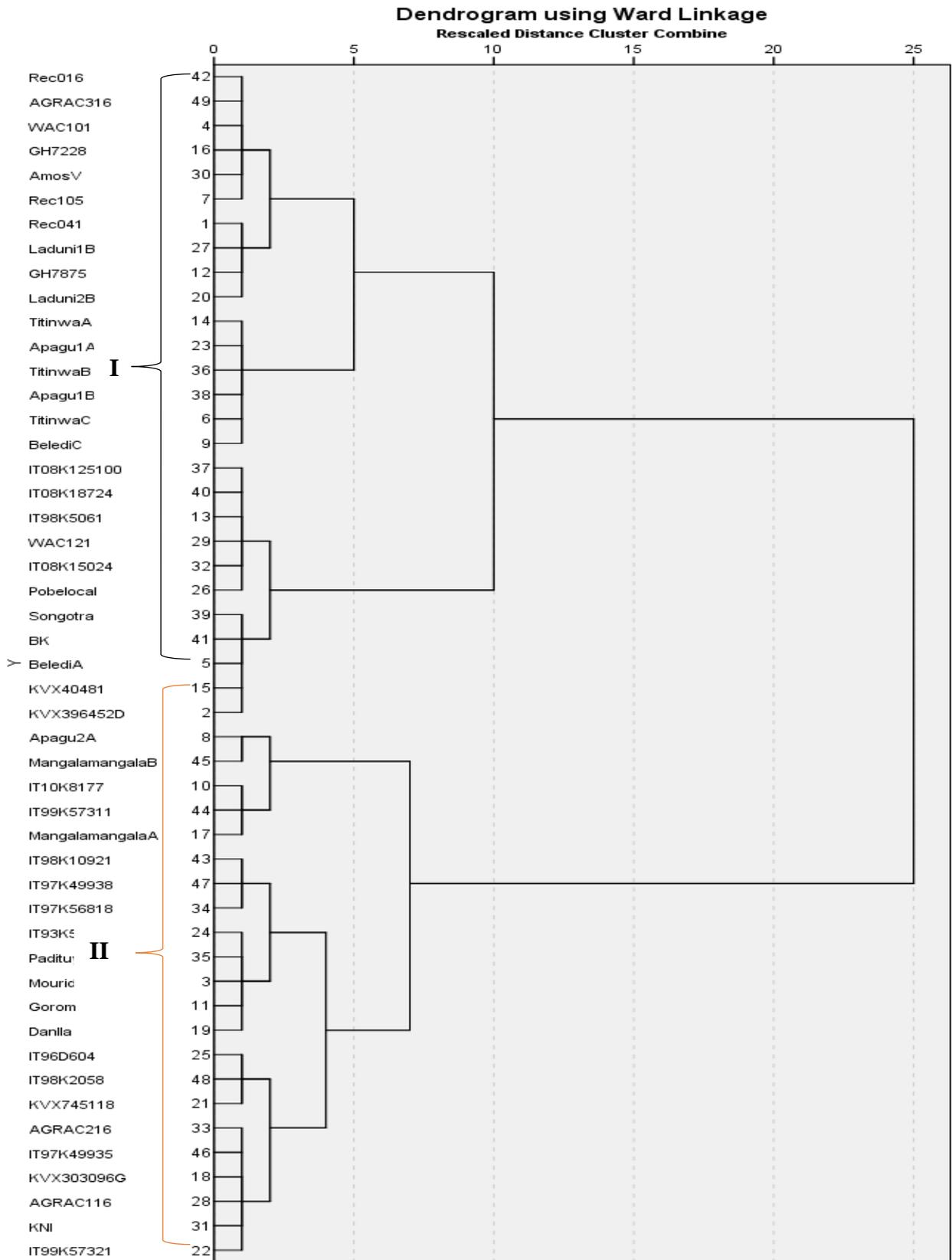


Fig. 4. 1 Dendrogram resulting from the analysis of 49 cowpea accessions grouped into **I** susceptible and **II** drought tolerant genotypes obtained by Ward method using the overall distance of Euclidean

#### 4.3.7 Phenotypic Correlation

Phenotypic correlation analysis showed a positive and strong correlation between plant height and number of seeds per pod ( $r = 0.30$ ) and grain yield ( $r = 0.31$ ) at 0.05 significance level. Whereas, number of days to 95% maturity was strongly but negatively correlated with pod length ( $r = -0.46$ ) and number of locules per pod ( $r = -0.39$ ) at  $P < 0.05$  and ( $r = -0.36$ ) number of seeds per pod at  $P < 0.01$  level of significance. Likewise, strong and positive association was found between pod length and the number of locules per pod ( $r = 0.73$ ) and number of seeds per pod ( $r = 0.67$ ) at  $P < 0.01$  and with seed yield ( $r = 0.33$ ) at  $P < 0.05$ . Number of locules per pod had a strong association with number of seeds per pod ( $r = 0.87$ ) at  $P < 0.01$  and seed yield ( $r = 0.21$ ) at  $P < 0.05$ . Positive and strong correlation was found between number of seeds per pod and grain yield. Highly significant and positive correlation was observed between number of pods per plant and grain yield ( $r = 0.29$ ) at a significance level of  $P < 0.01$  (Table 4.9).

Table 4. 9: Phenotypic correlation coefficients between morphological, grain yield and sub- yield components of cowpea genotypes measured under drought condition

Traits	Plant height (cm)	First flower	Maturity	Number pods	Pod length (cm)	Number of locules	Seeds per pod	Yield (g)
Plant height (cm)	1							
First flower	-0.05	1						
Maturity	-0.14	0.04	1					
Number pods	-0.06	0.03	-0.14	1				
Pod length (cm)	0.18	0.06	<b>-0.46*</b>	0.01	1			
Number of locules	0.21	-0.17	<b>-0.39*</b>	-0.16	<b>0.73**</b>	1		
Seeds per pod	<b>0.31*</b>	-0.13	<b>-0.36**</b>	-0.13	<b>0.67**</b>	<b>0.87**</b>	1	
Yield (g)	<b>0.30*</b>	-0.06	-0.13	<b>0.25**</b>	<b>0.33*</b>	<b>0.21*</b>	<b>0.29**</b>	1

\* Significant at 0.05 probability level and \*\* Significant at 0.01 probability level

#### 4.4 Discussion

The genetic variability amongst the 49 accessions in responsiveness to water deficit became pronounced as the duration of induced stress continued. Varietal differences were apparent in the second week of the induced stress. Apagu 1B, GH7828, Beledi A, Beledi C, BK, Laduni 1B, Titinwa A, and Titinwa B completely wilted. Whereas, the remaining 42 accessions exhibited either Type 1 or 2 of drought tolerance. Cowpea germplasms with ‘Type 1’ drought tolerance in this study are AGRAC-216, GH7875, IT08K-187-24, IT98K-503-1, IT97K-568-18, IT98K-205-8, IT99K-573-21, K VX-3030-96G, K VX-404-81, K VX-745-118, and Rec041. They stopped growing after the induction of drought stress and kept their uniformity and turgidity, but this declined with an increase in the duration of the stress, resulting in drying of the plant. These germplasms are known as dehydration avoiders, having strong stomatal sensitivity and reduced rate of growth. They could be utilised in the development of early or medium maturing varieties. These findings corroborate with the results obtained by Mai-Kodomi *et al.* (1999a) and Agbicodo *et al.* (2009) who used genotypes possessing ‘Type 1’ drought tolerance in development of early and medium maturing cowpea varieties.

AGRAC-116, Dan Ila, Mouride, IT93K-503-1, and the remaining 23 accessions showed Type 2 drought tolerance. They stayed green longer with continuous slow growth even when the severity of drought stress intensified. The mechanism of staying green and remaining active for a lengthy period of time could be due to the ability of these genotypes to drop older leaves. Members with Type 2 drought tolerance have a combination of three physiological mechanisms: selective moisture mobilisation (hygroscopic water), stomatal regulation and osmotic pressure regulation. They could be used to bred varieties that may possess the ability to withstand drought stress at both intermittent and terminal levels. Agbicodo *et al.* (2009) and Muchero *et al.* (2013) reported similar findings. These results confirm the findings of

Mai-Kodomi *et al.* (1999b) study on Type 1 and 2 of drought tolerance in cowpea. Both type 1 and 2 enhances the crops' ability to cope with prolonged drought common in Semi-arid Africa.

The phenotypic correlation coefficients between leaf wilting index, wilting scale (IB and MAIK), soil moisture content, stem greenness and regrowth are proxy traits. This agrees with Agbicodo *et al.* (2009) who used those traits as indicators of tolerance to drought stress. In addition, Pungulani *et al.* (2013) reported that leaf wilting index could fast-track breeders screening for drought tolerant genotypes. They further, reported the commonly used approaches and traits such as delayed leaf senescence and stem greenness have their limitations. The visual score of qualitative traits requires experience, LWI is easy to score since it is a ratio of wilted to the total number of leaves per plant. However, LWI should only be applied to assess crop plants that will is a good indicator of drought stress. In scenario some genotypes possess the 'Type 2' adaptability gene LWI would classify those genotypes susceptible. From the present study, the highest contributing traits to grain yield were number of seeds per pod, number of pods per plant, number of locules per pod and pod length. This confirms the findings of Jackai (1995) and Aremu *et al.* (2007) who reported that yield components were the main contributors to high yield. Number of days to 95% maturity was strongly but negatively correlated with pod length and the number of locules per pod at  $P<0.05$  and number of seeds per pod at  $P<0.01$  level of significance. This confirms the results of Aliyu and Makinde (2016). Likewise, strong and positive association was observed between pod length and the number of locules per pod and number of seeds per pod at  $P<0.01$  and  $P<0.05$ , respectively, corroborating with findings of Aliyu and Makinde (2016).

Highly significant and positive correlation was observed between number of pods per plant and grain yield at a probability level of  $P<0.01$ . Similarly, a positive and strong correlation was observed between number of seeds per pod and grain yield confirming de Almeida *et al.*

(2014). An increase in the number of pods per plant could result in an increased number of seeds per pod per plant and yield. Therefore, seed yield could be improved indirectly through selection for increased number of pods per plant.

Agglomerative hierarchical clustering with Euclidean distance matrix using Ward's Linkage method showed two distinct clusters with five sub-clusters. Cluster I members were susceptible to drought stress, early maturing and high yielding. Sub-cluster I members were medium maturing accession (60–75 days). Accessions in sub-cluster II are high yielding, and those in sub-cluster III were susceptible to drought stress. However, cluster II was made up of sub-cluster IV drought tolerant accessions, and sub-cluster V were late maturing accessions (take 90–120 days to mature). The dendrogram grouped the 49 cowpea accessions according to their responsiveness. The dissimilarity observed between a pair of varieties on the basis of a multivariate scale is useful in determination of which trait or traits are responsible for the dissimilarity and the relative contribution of these traits to total genetic variability (Tuhina-Khatun *et al.*, 2015).

Fukai and Cooper, (1995) reported that cluster analysis associates genotype more on the basis of relatedness among individuals. Satish *et al.* (2009) also reported similar findings stating that geographical distribution of accessions is not the sole reason that constitutes morphological and genetic diversity. Several other factors may be considered as causes of the genetic divergence. These factors include natural or artificial selection, exchange of breeding materials, genetic drift and environmental variations. Therefore, identification and selection of parental lines should focus more on genetic potentials rather than geographical divergence.

In the present study, sub-cluster IV was more divergent than the other sub-clusters. Genotypes in sub-cluster IV recorded the shortest number of days to first flowering as well as some yield contributing components. It is worth mentioning that breeding objectives such as

selection and hybridisation should take into consideration the genetic potentials of each cluster as well as individual genotypes within a cluster (Chahal and Gasal, 2002).

#### **4.5 Conclusions**

These materials AGRAC-216, GH7875, IT96D604 and Rec041 had 'Type 1' and 'Mouride, Padi-tuya, IT93K-503-1, Dan Ila and AGRAC-116 were possessing Type 2' drought tolerance. However, these three entries, IT93K-503-1, Dan Ila and Mouride were identified as potential parents for drought tolerance. They showed high level of delayed leaf senescence, stem greenness and were having 'Type 2' drought tolerance. However, parameters such as delayed leaf senescence, leaf wilting index, soil moisture content, drought stress index, and stem greenness are good traits for selecting parents. Emphasis should be on leaf wilting index when identifying drought tolerant genotypes in cowpea. It is easy to score and no expertise required in selection of drought tolerant genotypes.

## CHAPTER FIVE

### 5.0 COMBINING ABILITY FOR DROUGHT TOLERANCE AND EARLINESS IN COWPEA

#### 5.1 Introduction

Drought stress has an enormous effect on cowpea production and productivity, threatening nutrition and food security in SSA. It was observed that drought stress reduces water-use efficiency, disrupts photosynthetic pathways and activities, and it impairs growth and development. Hence, selection of accessions to be used as parental lines in hybridisation is one of the most important decisions faced by plant breeders. The decision has to be logical and careful thought-through, for the reason that a population with narrow genetic divergence may lead to waste of money and time. Individual performance (tolerance or resistance), wide adaptability and yield stability have constituted the base for selection (Bertan *et al.*, 2007). However, several mechanisms are involved in plants ability to cope with stress. Henceforth, it is important to distinguish between genetic and non-genetic aspects of tolerance to drought stress. Knowledge of the genetic basis of drought tolerance could be useful in developing novel varieties of cowpea with reasonably good yield even under drought conditions. Combining good seed yield with drought tolerance requires complementary gene action from parents into a common background. This will prepare the way for selection that could lead to some progenies with a good compromise of seed yield and drought tolerance.

The specific objectives of this study were to:

- (i) estimate combining ability for drought tolerance and earliness, and
- (ii) estimate heritability of earliness and drought tolerance in the progenies.

## **5.2 Materials and Methods**

### **5.2.1 Experimental Site**

The experimental site was the University farm that is located on the University of Ghana campus. The soil belongs to the Adenta series, with pH of (4.47–4.71) and 30–35 % clay, (0.5 %) loam, coarse silt, (5–10 %) sand and (60–62.5 %) organic matter, available P (3.26 mg/kg), (Department of Soil Science, University of Ghana).

### **5.2.2 Combining Ability Study for Drought-stress and Earliness**

The genetic material used included five locally adapted and farmers' preferred varieties, Titinwa A, Laduni 1B, Beledi A, Apagu 1B, and Beledi C and three drought-tolerant (Dan lla, Mouride, and IT93K–503–1) varieties selected from germplasm screening in the same location. The five adapted and farmers preferred varieties were crossed with each of the three drought tolerant genotypes in a 5 x 3 North Carolina II mating design to generate 15 F<sub>1</sub>s. Then the F<sub>1</sub> s seeds generated from the first cross were planted in the field so as to generate enough seeds for the evaluation. Twenty-five entries, made up of the 15 F<sub>1</sub>s, the 8 parents and two checks Asontem from Ghana (early maturing) and AGRAC–216 (high yielding and drought tolerant) from South Sudan (Table 5.1), were planted in pruning bags with a diameter of 20 cm under a rainout shelter from January to May 2018.

Seeds from the selfed F<sub>1</sub> generation plants were used for this study. The 25 cowpea entries were evaluated in randomized complete block design in a split-plot design fashion. The treatments were replicated four times, under two watering regimes on the 12/01/2018. These regimes are designated as drought stressed (DS) and well-watered (WW). The soil moisture content during the water stress was monitored at 20 cm depth using Fieldscout (TDR 150) once in a week for the second and third week of imposed drought stress. After the period of

imposed drought stress, plants were watered twice a week for two weeks and the recovery scored (regrowth and stem greenness).

Table 5.1: Farmers preferred drought tolerant cowpea genotypes used as parental lines in North Carolina II mating design

Parental line	Days to Maturity	Growth Habit	Botanical Name	Origin
<b>Drought tolerant (Male)</b>				
Dan Ila	73	Semi-prostrate	<i>V. unguiculata</i>	Niger
Mouride	71	Semi-erect	<i>V. unguiculata</i>	Burkina Faso
IT93K-503-1	70	Semi-erect	<i>V. unguiculata</i>	IITA/Nigeria
<b>Farmers' preferred (Female)</b>				
Beledi (C)	60	Semi-prostrate	<i>V. unguiculata</i>	South Sudan
Laduni (1B)	63	Semi-prostrate	<i>V. unguiculata</i>	South Sudan
Titinwa (A)	62	Semi-prostrate	<i>V. unguiculata</i>	South Sudan
Apagu (1B)	61	Erect	<i>V. unguiculata</i>	South Sudan
Beledi (A)	65	Semi-prostrate	<i>V. unguiculata</i>	South Sudan

### 5.2.3 Data Collection

The following variables were recorded.

1. Plant height at 20 days after planting was measured using a meter ruler from the ground level to the base to the tip of the plant meristem expressed in cm.
2. Number of days to first flower: recorded as the number of days from sowing to stage when plants had open flowers.
3. Number of days to 50% flowering: recorded as the number of days from sowing to stage when 50% of plants have flowered.
4. The number of days to first mature pods: recorded as the number of days from sowing to stage when 50% of plants had mature pods.
5. Pod length (cm): computed as the mean of 10 fully mature pods randomly selected.
6. Number of locules per pod: mean number of the 10 pods counted.
7. Days to 95% maturity: was recorded as the number of days from seedling emergence to harvest of each genotype per plot (duration).

8. Number of main branches: computed as the number of branches whose origin is in the leaf axils on the main stem; recorded in the 8<sup>th</sup> week after sowing. Mean of 10 randomly selected plants computed was recorded for each experimental unit.
9. Leaf chlorophyll content: SPAD Meter was used to measure chlorophyll content at 30, 45 and 60DAP.
10. Number of pods per plant: mean number of mature pods from 10 randomly selected plants.
11. 100–seed weight (g): determined by randomly counting 100 seeds from a bulked seed and weighed using a digital weighing scale.
12. Seed yield (g): determined by measuring bulked seed using a digital weighing-scale
13. Delay leaf senescence (DLS): visual scored on a scale of 1–5, where 1 = totally green and turgescient and 5 = completely yellow to brown almost dead.
14. Drought stress index: visual scored on a scale of 1– 9, where 1 low susceptibility, 5 medium susceptibility, and 9 highly susceptible.

#### 5.2.4 Data Analysis

The model for split-plot design used was as follows:

$$X_{ijk} = Y \dots Mi + B_j + d_{ij} + S_k + (MS)_{jk} + e_{ijk} \dots \dots \dots (1)$$

Where  $X_{ijk}$  = mean observation,  $Y$ = the experimental mean,  $Mi$  =the main plot factor effect,  $B_j$  = replication or block effect,  $d_{ij}$  = mean plot error equally known as error a,  $S_k$  =the subplot factor effect,  $(MS)_{jk}$  =the mean plot and subplot treatment interaction effect,  $e_{ijk}$  =the subplot error also known as error b.  $i$ = a particular main plot factor,  $j$ = a particular number of replication or block,  $k$ = a particular subplot factor.

Drought intensity index (DII) was quantified according to Fischer and Maurer (1978).

$$DII = 1 - \frac{X_s}{X_p}$$

Where  $X_s$  and  $X_p$  were the mean seed yield of all the genotypes under well-watered and drought stressed conditions respectively.

Data collected were subjected to analysis of variance using the GenStat 18<sup>th</sup> edition of VSN international. The means of 15 F<sub>2</sub> populations, two checks, and eight parents were compared in analysis of variance (ANOVA) by means of a linear model and AGD-R version 3 Software. Combined analysis across the contrasting watering regime was carried out. The phenotypic variation was partitioned into males and females and their interactions. The individual responsiveness  $k$  of the 15 F<sub>2</sub>s resulting from the hybridization between male  $i$  and female  $j$  is modeled as:

$$Y_{ijk} = \mu + r_k + m_i + f_j + (mf)_{ij} + e_{ijk} \dots \dots \dots (2)$$

Where:

- $Y_{ijk}$  is the progeny observed value of the  $i^{th}$  male crossed with  $j^{th}$  female in the  $k^{th}$  replication
- $\mu$  is the overall population mean
- $m_i$  is general combining ability of the  $i^{th}$  males
- $f_j$  is the general combining ability of the  $j^{th}$  females
- $(mf)_{ij}$  is the specific combining ability of the  $i^{th}$  X  $j^{th}$  cross
- $r_k$  is the replication effect
- $e_{ijk}$  is the experimental error

Table 5.2: Format of analysis of variance for North Carolina mating design II

Source of Variation	D.F	Mean Square (MS)	Expected mean squares	Genetic variance Components ( $\sigma^2$ )
Replication	$r-1$			
GCA <sub>Male</sub>	$m-1$	$MS_m$	$\sigma_e^2 + r\sigma_{fm}^2 + rf\sigma_m^2$	$\sigma_m^2 = (MS_m - MS_{mf})/rf$
GCA <sub>Female</sub>	$f-1$	$MS_f$	$\sigma_e^2 + r\sigma_{fm}^2 + rm\sigma_f^2$	$\sigma_f^2 = (MS_f - MS_{mf})/rm$
SCA=M x F	$(m-1)(f-1)$	$MS_{mf}$	$\sigma_e^2 + r\sigma_{fm}^2$	$\sigma_{fm}^2 = (MS_{mf} - MS_e)/r$
Error (e)	$mf(r-1)$	$MS_e$	$\sigma_e^2$	$\sigma_e^2 = MS_e$
Total	$rmf-1$			

$r$  = number of replications;  $m$  = number of males lines;  $f$  = number of females lines,  $\sigma_e^2$  = environmental variance,  $\sigma_f^2$  = variation between females,  $\sigma_m^2$  = variation between males,  $\sigma_{fm}^2$  = variation due to interaction between females and males

Source: Kearsy and Pooni, (1996)

a) The additive ( $\sigma^2_A$ ) and dominance ( $\sigma^2_D$ ) variance components were estimated as follows:

$$\sigma^2_A = 4\sigma^2_m \quad \text{and} \quad \sigma^2_D = 4\sigma^2_{mf}$$

b) The general combining ability for both female and male was computed as follows:

$$GCA_f = X_f - \mu \quad \text{and} \quad GCA_m = X_m - \mu$$

Where:

$X_f$  and  $X_m$  = Mean of male and female parents, respectively

$GCA_m$  and  $GCA_f$  = General combining ability of male and female parents, respectively

$\mu$  = Overall mean of crosses in the trial.

c) Specific combining ability was computed using the formula proposed:

$$SCA_x = X_x - E(X_x) = X_x - [GCA_f + GCA_m + \mu]$$

Where:

$X_x$  = Observed mean value of the cross

$E(X_x)$  = Expected mean value of the cross based on the 2 GCAs of its parents

$SCA_x$  = specific combining ability of the cross x

Ozimati *et al.* (2014) estimation formulae based on genotype mean was used to compute broad and narrow sense coefficient of genetic determination (BSCGD and NSCGD).

$$BSCGD = \frac{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCAfmf}}{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCAfm} + \sigma^2_e/r}$$

$$NSCGD = \frac{\sigma^2_{GCAf} + \sigma^2_{GCAm}}{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCAfm} + \sigma^2_e/r}$$

$$\text{Bakers ratio} = \frac{\sigma^2_{GCAf} + \sigma^2_{GCAm}}{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCAfm}}$$

### 5.3 Results

#### 5.3.1 Response of F<sub>2</sub> Populations and Parental Lines to Drought Stress

The combined analysis of variance for the number of pods per plant, number of seeds per pod, 100-seed weight and seed yield for the parental lines, checks, and F<sub>1</sub> populations showed significant differences at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively. The watering regimes exhibited significant mean squares for yield and yield component (Table 5.3).

Table 5.3: Mean squares for responses of 15 F<sub>2</sub> populations, eight parental lines and two checks subjected to drought stress and well-watered conditions at reproductive stage

Source of Variation	D.F	NPP	NSP	100 SWT	Seed Yield
Replications	3	2.5	9.7	2.8	357.7
Watering Regime	1	75.2*	115.0**	202.2*	104868.3***
Main Plot Error	3	7.2	2.3	28.7	800.9
Genotypes	24	10.3***	8.5***	17.5***	463.8**
Watering Regime × Genotypes	24	6.2 <sup>ns</sup>	5.1*	5.8 <sup>ns</sup>	417.1*
Sub Plot Error	144	4.5	2.9	4.7	282.5
Total	199				

\*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability levels respectively; DF, degree of freedom; NPP, number of pods per plant, NSP, number of seeds per plant; 100 SWT, 100 seed weight; ns = non-significant at  $P > 0.05$

The estimated mean squares for delayed leaf senescence (DLS) was highly significant for both GCA and SCA effects. The values of  $GCA_f$  and  $GCA_m$  were bigger than  $SCA_{f,m}$  and this implies that additive gene effects were more important than non-additive effects for DLS (Table 5.4).

Table 5.4: Estimated mean squares for GCA and SCA for delayed leaf senescence

Source	D.F	S. S	M.S	F-value
$GCA_{male}$	2	30.40	15.20	8.87**
$GCA_{female}$	4	27.73	6.93	4.04**
$SCA_{(male, female)}$	8	100.27	12.53	7.31**
Error	42	72.00	1.71	

\*\*Significant at the  $\leq 0.01$  level of probability

The mean squares of the 15 F<sub>2</sub> populations, 8 parents and 2 checks showed a significant difference ( $P < 0.01$ ) for the number of pods per plant, seeds per pod, pod length and 100-seed weight at and grain yield. Chlorophyll content at 30DAP, 45DAP and 60DAP were significantly different (Table 5.5). Mean performance of the crosses for number of pods per plant and number of seeds per pod were higher than the average of the parents expects for 100-seed weight and seed yield (Table 5.6).

At the commencement of drought stress conditions, TA×I registered the highest normalised vegetation index value of 50.2 whereas, BA×D had the lowest index value of 37.23. At 45DAP, Asontem had the highest value for chlorophyll content (58.65) and BC×M had the lowest value of 35.5. At 60DAP, TA had the lowest chlorophyll content (34.9), whereas IT93K–503–1 had the highest concentration of 60.53 (Fig. 5.1).

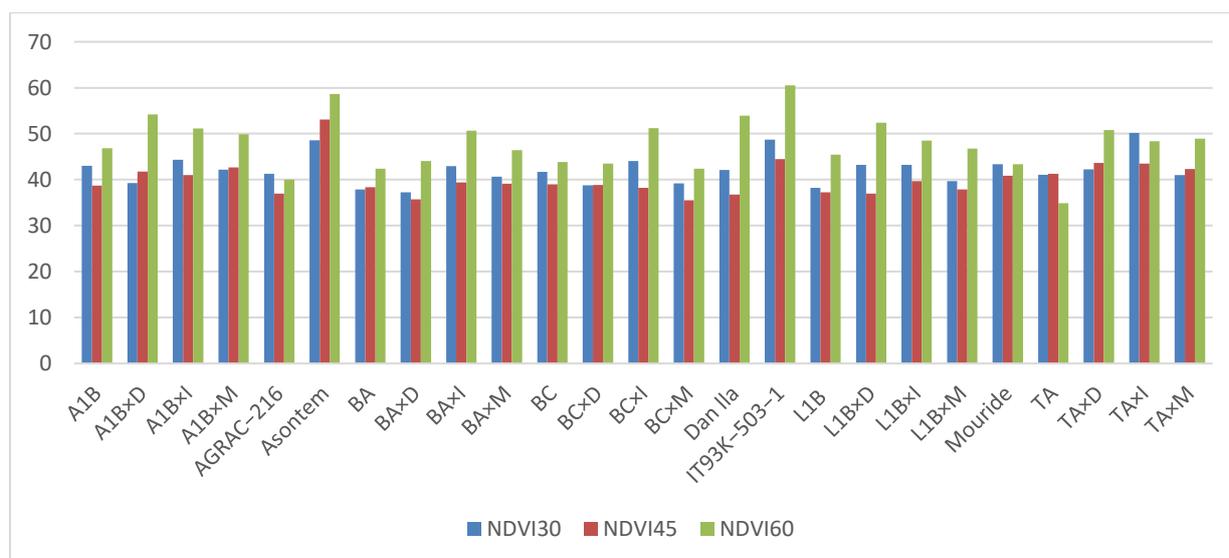


Fig 5. 1 Chlorophyll content of the genotypes under drought stress condition and re-watering at 30, 45 and 60 days after planting

Table 5.5: Mean squares for 15F<sub>2</sub> cowpea population, eight parents and two checks subjected to drought stress at reproductive stage

Source of Variation	D.F	NPP	NSP	Pod length	100–seed weight	Seed Yield	NDVI 30	NDVI 45	NDVI 60
Replication	3	4.54	7.77	2.30	12.25	357.7	10.59	10.94	47.02
Genotype	24	8.83**	7.30**	15.58**	12.90 **	463.8**	43.45*	54.32*	131.33*
Error	72	3.52	3.24	3.91	4.87	293	23.05	31.73	72.95
Total	99								

\*Significant at P < 0.05, \*\*Significant at P < 0.01, ns: non-significant, NPP: number of pods per plant, NSP: number of seeds per pod and NDVI: SPAD chlorophyll meter reading at 30, 45, 60DAP

Table 5.6: Means of yield and yield components in F<sub>2</sub> cowpea population and their eight parents evaluated under drought-stress and well-watered conditions

Watering Regimes		DS	WW	DS	WW	DS	WW	DS	WW
Population	Genotype	NPP	NPP	NSP	NSP	100–seed	100–seed	Seed Yield (g)	Seed Yield (g)
<b>F<sub>2</sub></b>	A1B×D	5.75	6.75	8.75	10.30	13.30	14.98	21.30	69.80
	A1B×I	7.00	7.25	9.53	9.90	11.70	15	28.00	77.5
	A1B×M	6.00	7.00	9.58	10.8	13.75	15.5	23.30	75.00
	BA×D	4.00	6.00	8.60	8.18	13.10	15.7	31.80	55.00
	BA×I	4.75	5.75	6.68	8.90	14.50	17.45	22.30	57.50
	BA×M	5.75	4.25	5.63	9.03	12.80	17.65	23.40	55.00
	BC×D	3.50	5.75	6.98	10.35	14.73	16.13	24.80	72.50
	BC×I	4.25	7.25	6.45	10.23	13.40	16.93	23.50	87.20
	BC×M	5.25	6.50	8.88	10.33	14.93	16.5	24.80	77.50
	L1B×D	7.75	6.50	8.38	10.85	13.58	17.9	26.30	82.50
	L1B×I	4.25	9.50	8.73	7.80	13.73	15.43	23.40	64.80
	L1B×M	4.25	5.75	9.03	12.00	11.65	17.4	15.90	75.00
	TA×D	7.25	7.50	7.85	9.08	13.93	18.38	30.50	62.50
	TA×I	8.50	8.25	6.40	9.50	16.00	17.88	30.70	72.50
TA×M	6.00	5.75	7.08	8.28	15.00	17.2	18.00	35.00	
<b>Mean</b>		<b>5.62</b>	<b>6.65</b>	<b>7.90</b>	<b>9.70</b>	<b>13.74</b>	<b>16.67</b>	<b>24.53</b>	<b>67.95</b>
<b>Parents</b>	A1B	6.53	7.50	8.83	9.20	14.38	14.13	32.50	57.50
	BA	4.00	8.75	8.75	9.10	13.35	15.90	20.30	67.50
	BC	5.50	8.00	7.68	11.35	12.68	13.45	27.40	78.30
	Dan Ila	5.75	6.38	7.80	7.10	17.10	16.80	23.70	80.00
	IT93K–503–1	4.95	5.75	6.40	8.63	16.65	16.23	27.30	70.50
	Mouride	4.50	6.50	6.19	7.90	17.63	17.90	30.50	62.50
	L1B	6.00	8.25	10.85	11.10	16.10	15.70	28.70	99.80
	TA	3.75	6.50	6.53	10.68	15.00	16.83	22.30	67.50
<b>Mean</b>		<b>5.18</b>	<b>7.14</b>	<b>7.88</b>	<b>9.42</b>	<b>15.18</b>	<b>15.96</b>	<b>26.36</b>	<b>72.39</b>
<b>LSD (5%)</b>		2.75	3.38	2.57	2.23	3.20	3.09	9.90	32.83
<b>Geno. Probability</b>		*	ns	**	***	**	ns	*	ns

\*Significant at P < 0.05, \*\*Significant at P < 0.01, \*\*\*Significant at P < 0.001, NPP: Number of pods per plant, NSP: Number of seeds per pod and 100–seed: 100–seed weight, DS: Drought Stress and WW: Well-Watered conditions

Mean squares of the F<sub>1</sub> crosses were significantly different under drought stress and well-watered conditions (Table 5.7) for number of pods per plant, number of seeds per pod, 100–seed weight, seed yield and chlorophyll content at different number of days after planting.

Under drought stress condition, mean squares for male parental lines were significantly different at  $P < 0.05$  for seed yield and chlorophyll content at 30DAP and were highly significantly different ( $P < 0.01$ ). Whereas, female parents GCA were highly significant at  $P < 0.001$  for number of pods per plant, 0.05% for number of seeds per pod and chlorophyll content at 30 and 45DAP (Table 5.7). However, the Baker's ratio and broad sense coefficient of genetic determination (BSCGD) were high for number of pods per plant, the number of seeds per pod, 100-seed weight and seed yield. Hence narrow sense coefficient of genetic determination (NSCGD) for these traits was moderate to low ( $< 40\%$ ) (Table 5.7).

Table 5.7: Mean squares, variance components for the responses of 15 F<sub>2</sub> population evaluated under well-watered and drought stress condition

Drought-Stressed (DS) Condition								
Source of Variation	D.F	NPP	NSP	100–Seed Weigh (g)t	Seed Yield (g)	NDVI30	NDVI45	NDVI60
GCA Male	2	1.80	1.81	0.29	188.21**	141.69**	5.54	50.04
GCA Female	4	14.04***	12.85*	9.67**	37.39	32.29*	78.16*	65.55
SCA (Male, Female)	8	6.84**	4.30	5.01*	73.93*	17.00	9.08	36.62
Genotypes	14	8.18**	6.39	5.67**	79.81*	39.18*	28.31	46.80
Error	42	3.18	3.59	5.07	60.13	20.63	35.01	52.57
Genotype Variance		0.21	0.33	0.10	0.94	3.53	3.06	1.62
Additive Variance		0.85	1.33	0.42	3.75	14.12	12.24	6.48
Dominance Variance		3.66	0.70	0.00	13.80	0.00	0.00	0.00
Environmental Variance		12.74	14.37	20.26	240.50	82.52	140.04	210.26
<b>The coefficient of genetic determination on entry mean</b>								
Bakers ratio		0.70	0.77	0.67	0.75	0.91	0.90	0.76
BSCGD		0.86	0.84	0.75	0.83	0.90	0.73	0.74
NSCGD		0.45	0.45	0.40	0.44	0.47	0.41	0.41
Well-Watered (WW) Condition								
Source of Variation	D.F	NPP	NSP	100–Seed Weight	Seed Yield (g)	NDVI30	NDVI45	NDVI60
GCA Male	2	17.92*	3.40	0.54	356.55	81.48**	9.86	40.66
GCA Female	4	13.85*	7.77*	11.25*	1419.94**	39.59*	100.86***	38.04
SCA (Male, Female)	8	2.63	4.67*	3.33	441.34	13.46	34.46**	12.94
Genotypes	14	8.02	5.37	5.19	708.83*	30.64	49.92	24.07
Error	42	5.92	2.38	4.88	451.32	15.21	11.43	33.63
Genotype Variance		0.86	0.11	0.30	42.56	2.73	2.46	1.77
Additive Variance		3.43	0.45	1.19	170.22	10.94	9.84	7.08
Dominance Variance		0.00	2.28	0.00	0.00	0.00	23.03	0.00
Environmental Variance		23.67	9.53	19.51	1805.30	60.85	45.72	134.51
Bakers ratio		0.93	0.71	0.78	0.80	0.90	0.93	0.73
BSCGD		0.85	0.87	0.76	0.83	0.90	0.73	0.75
NSCGD		0.79	0.61	0.59	0.67	0.81	0.71	0.63

\*Significant at P < 0.05, \*\*Significant at P < 0.01, \*\*\*Significant at P < 0.001, ns: non-significant, NPP, number of pods per plant, NSP, number of seeds per pod and NDVI: SPAD chlorophyll meter reading at 30, 45 60 DAP, BSCGD, Broad sense coefficient of genetic determination; NSCGD, Narrow sense coefficient of genetic determination

### 5.3.2 Estimates of General and Specific Combining Ability Effects

Female parents Beledi A and Titinwa A showed desirable significant positive GCA for seed yield (Table 5.8). Beledi C and Titinwa A had desirable GCA for 100–seed weight. Whereas, parental lines Apagu 1B and Laduni 1B showed desirable, significant GCA for number of seeds per pod. Apagu 1B had significant positive GCA for normalised difference vegetation index (NDVI) at 45 and 60DAP while Titinwa A had a similar effect at 45DAP under drought-stressed condition. The parent Laduni 1B showed significant negative GCA effect for grain yield, 100–seed weight, and NDVI at 45DAP. Male parents, Dan Ila and IT93K–503–1, had desirable positive GCA for seed yield, whereas Mouride showed similar trend but negative GCA effect (Table 5.8). Additionally, IT93K–503–1 had significant positive GCA effect for NDVI at 30DAP.

Beledi A  $\times$  Dan Ila showed significant positive SCA effect for seed yield and number of seeds per pod, whereas Laduni 1B  $\times$  Dan Ila had significantly positive SCA effect for grain yield and number of pods per plant. The cross Apagu 1B  $\times$  IT93K–503–1 had significant positive SCA effect for seed yield and a negative SCA effect for 100–seed weight (Table 5.9). Titinwa A  $\times$  IT93K–503–1 had significantly positive SCA effect for grain yield, the number of pods per plant, 100–seed weight and drought stress index. The cross Apagu 1B  $\times$  Mouride had significantly positive SCA effect for 100–seed weight and seed yield as well as a desirable negative SCA effect for drought stress index. While Beledi C  $\times$  Mouride showed desirable, significant positive SCA effect for grain yield, the number of pods per plant and number of seeds per pod (Table 5.9). Four crosses, Beledi A  $\times$  IT93K–503–1, Apagu 1B  $\times$  Dan Ila, Laduni 1B  $\times$  Mouride and Titinwa A  $\times$  Mouride, had desirable negative SCA effect for seed yield, while Beledi C  $\times$  IT93K–503–1 Beledi C  $\times$  Dan Ila, Titinwa A  $\times$  Dan Ila, Laduni 1B  $\times$  IT93K–503–1 and Beledi A  $\times$  Mouride showed no desired SCA effect for seed yield and some of the yield components measured (Table 5.9).

Table 5.8: Estimates of general combining ability effects of yield and yield components for both male and female

Donor Parents	Well-Watered Condition			Drought Stress Condition							
	NPP	NSP	100-SWT	Seed Yield	NDVI 30	NDVI 45	NDVI 60	NPP	NSP	100-SWT	Seed Yield
Mouride	-0.67*	0.39	0.18	-4.45***	-1.34*	-0.24	-1.74*	-0.3	0.14	-0.11	-3.46***
IT93K-503-1	1.08*	-0.43	-0.13	3.95**	3.06***	0.60	1.36*	0.01	-0.35	0.13	1.06*
Dan Ila	-0.42	0.05	-0.05	0.50	-1.73*	-0.37	0.38	0.3	0.21	-0.01	2.4**

Recurrent Parents	Well-Watered Condition				Drought Stress Condition							
	NPP	NSP	100-SWT	Seed Yield	NDVI 30	NDVI 45	NDVI 60	NPP	NSP	100-SWT	Seed Yield	
Apagu 1B	0.48	0.63	-1.51*	6.13**	0.04	2.06**	3.13**	0.50	1.38**	-0.82*	-0.33	
Laduni 1B	0.73*	0.52	0.24	6.13**	0.16	-1.57*	0.62	-0.33	0.81*	-0.76*	-2.66**	
Beledi A	-1.85**	-1	0.27	-12.12***	-1.60	-1.66*	-1.60*	-0.25	-0.93*	-0.27	1.28*	
Beledi C	-0.02	0.6	-0.15	11.13***	-1.21	-2.24**	-2.92**	-1.42**	-0.47	0.61	-0.19	
Titinwa A	0.65*	-0.75	1.15*	-11.28***	2.59	3.40***	0.77	1.50**	-0.79*	1.24**	1.9*	

\*Significant at  $P < 0.05$ , \*\*Significant at  $P < 0.01$ , \*\*\*Significant at  $P < 0.001$ , NPP: Number of pods per plant, NSP: Number of seeds per pod, 100-SWT: 100-seed weight and NDVI: Normalised difference vegetation index

Table 5.9: Estimates of specific combining ability effects for crosses under well-watered and drought stress condition

Population	Genotype	Drought-Stressed Condition					Well-Watered Condition			
		Yield	NPP	NSP	100SDWT	DS	Yield	NPP	NSP	100WT
F2	Apagu 1B × Dan Ila	-5.28***	-0.80	-0.74	0.40	0.53	-4.83**	0.17	-0.08	-0.13
	Beledi A × Dan Ila	3.56**	0.20	1.42*	-0.35	0.20	-1.33	-0.25	-0.58	-1.18*
	Beledi C × Dan Ila	-1.97	-1.13	-0.67	0.39	-0.13	-7.08***	-0.33	0.00	-0.34
	Laduni 1B × Dan Ila	2.00*	2.03**	-0.54	0.61*	-0.47	7.92***	-0.33	0.58	1.04*
	Titinwa A × Dan Ila	1.69	-0.30	0.53	-1.04**	-0.13	5.33***	0.75	0.07	0.61
	Apagu 1B × IT93K-503-1	2.76*	0.75	0.59	-1.34**	0.53	-0.53	-0.83	0.00	-0.03
	Beledi A × IT93K-503-1	-4.60**	-0.75	0.05	0.91*	-0.80*	-2.28*	0.00	0.63	0.65
	Beledi C × IT93K-503-1	-1.91	-0.08	-0.64	-1.08**	-0.13	4.22**	-0.33	0.36	0.54
	Laduni 1B × IT93K-503-1	0.49	-1.17	0.36	0.62*	-0.47	-13.28***	1.17*	-1.98*	-1.35*
	Titinwa A × IT93K-503-1	3.26**	1.25*	-0.36	0.90*	0.87*	11.88***	0.00	0.98	0.19
	Apagu 1B × Mouride	2.52*	0.05	0.16	0.95*	-1.07**	5.37***	0.67	0.08	0.16
	Beledi A × Mouride	1.04	0.55	-1.48*	-0.55	0.60	3.62**	0.25	-0.06	0.53
	Beledi C × Mouride	3.88**	1.22*	1.31*	0.69*	0.27	2.87*	0.67	-0.36	-0.20
	Laduni 1B × Mouride	-2.49*	-0.87	0.18	-1.22**	0.93*	5.37***	-0.83	1.40*	0.31
	Titinwa A × Mouride	-4.95**	-0.95	-0.17	0.14	-0.73*	-17.22***	-0.75	-1.06*	-0.80
<b>Standard Error of the Mean</b>		3.14	0.96	0.76	0.82	0.60	7.67	0.59	0.79	0.67

\*Significant at  $P < 0.05$ , \*\*Significant at  $P < 0.01$ , \*\*\*Significant at  $P < 0.001$ , NPP: Number of pods per plant, NSP: Number of seeds per pod, 100SWT: 100-seed weight

### 5.3.3 Computation of Earliness in Cowpea

Mean square value of the 15 F<sub>2</sub> population, 8 parents and 2 checks showed significant ( $P < 0.01$ ) differences among the genotypes for number of days to first flower, number of days to 50% flowering, number of days to first mature pod, number of days to 95% maturity, number of seeds per pod, pod length and 100-seed weight and chlorophyll content at 30 DAP, 45 DAP and 60 DAP, whereas number of pods per plant showed no significant difference among the 25 entries (Table 5.10).

The estimates of GCA effects of the parents revealed that Mouride, Apagu 1B, and Beledi C exhibited negative and significant values for number of days to the first flower, number of days to 50% flowering, number of days to first mature pod and number of days to 95% maturity. While Laduni 1B exhibits significant negative GCA effects for the number of days to first flower and number of days to 50% flowering, Titinwa A showed negative, but significant GCA values for number of days to first flower and number of days to 95% maturity (Table 5.11).

Negative estimates of SCA effects for number of days to first flower was observed in nine of the fifteen crosses (Table 5.13). However, significant and negative SCA effects estimates for the trait is observed in Apagu 1B × Dan Ila, Laduni 1B × Dan Ila, Beledi A × IT93K-503-1, Beledi C × IT93K-503-1, Beledi A × Mouride, Laduni 1B × Mouride and Titinwa A × Mouride (Table 5.11).

The estimates of negative SCA for number of days to 50% flowering, seven of the crosses indicated significant negative SCA. These hybrids in this category were Titinwa A × Dan Ila, Apagu 1B × IT93K-503-1, Beledi A × IT93K-503-1, Beledi A × Mouride and Laduni 1B × Mouride. For number of days to 50% flowering, estimates of SCA was observed in eight of the fifteen combiners. Five of the eight hybrids that exhibited significant negative SCA were

Beledi C × Dan Ila, Titinwa A × Dan Ila, Beledi C × IT93K-503-1, Apagu 1B × Mouride and Laduni 1B × Mouride (Table 5.11).

Negative estimates of SCA effect for the number of days to 95% maturity were observed in seven of the fifteen crosses. Five out of the seven crosses exhibited significant negative SCA for this trait. The hybrids exhibiting significant negative SCA effects were Titinwa A × Dan Ila, Beledi C × Dan Ila, Apagu 1B × IT93K-503-1, Titinwa A × IT93K-503-1 and Laduni 1B × Mouride (Table 5.11).

Table 5.10: Mean squares for 15 F<sub>2</sub> population, eight parents and two checks measured under well-watered condition

Source of Variation	D.F	Firstflo	50%flo	FMP	MT	NPP	NSP	PL	100sdwt	NDVI30	NDVI45	NDVI60
Replication	3	2.73	3.81	1.93	5.37	5.16	4.22	6.19	19.20	11.98	55.20	65.53
Genotype	24	22.73**	36.36**	49.63**	51.52**	7.76 <sup>ns</sup>	6.32**	7.33**	10.35*	33.75**	94.85**	90.88**
Error	72	6.54	7.58	11.00	10.66	5.37	2.64	1.89	4.58	13.60	19.89	31.41

\*Significant at P < 0.05, \*\*Significant at P < 0.01, ns: non-significant, Firstflo, number of days to first flower, NPP, number of pods per plant, NSP, number of seeds per pod, FMP, first mature pod, MT, number of days to 95% maturity, 50%flo, number of days to 50% flowering, PL, pod length, 100sdwt, 100-seed weight and NDVI: SPAD chlorophyll meter reading at 30, 45 60DAP

Table 5.11: Estimates of general and specific combining ability among eight parental lines and their F<sub>2</sub> for the number of days to the first flower, days to 50 % flowering, days to the first mature pod and 95% maturity in cowpea

Parent GCA	Number of days to the first flower	Number of days to 50% flowering	Number of days to first mature pod	Number of days to 95% maturity
Dan Ila	0.48*	0.43*	0.87*	0.35
IT93K-503-1	-0.07	0.03	-0.18	-0.10
Mouride	-0.42*	-0.47*	-0.68*	-0.25*
Apagu1B	-0.97*	-1.15**	-0.43*	-0.77*
Beledi A	2.62**	3.02**	2.82**	2.57**
Beledi C	-1.13**	-1.48**	-1.77*	-1.35**
Laduni1B	-0.47*	-0.40*	0.48*	0.23*
Titinwa A	-0.05	0.02	-1.10**	-0.68**
<b>F<sub>2</sub> Population SCA</b>				
Apagu1 B × Dan Ila	-0.48*	0.15*	0.38*	0.57*
Beledi A × Dan Ila	1.93**	1.73**	0.13	0.23
Beledi C × Dan Ila	-0.32	-0.52*	-0.78*	-2.10**
Laduni 1B × Dan Ila	-0.98**	-0.35*	0.72*	1.82*
Titinwa A × Dan Ila	-0.15	-1.02**	-0.45*	-0.52*
Apagu 1 B × IT93K-503-1	0.32	-0.45*	-0.07	-0.73*
Beledi A × IT93K-503-1	-1.27**	-1.37**	-0.07	-0.32
Beledi C × IT93K-503-1	-0.77**	-0.12	-0.48**	2.35**
Laduni 1B × IT93K-503-1	1.57**	1.55**	0.52*	1.27*
Titinwa A × IT93K-503-1	0.15	0.38*	0.10	-2.57**
Apagu 1 B × Mouride	0.17	0.30*	-0.32*	0.17*
Beledi A × Mouride	-0.67*	-0.37*	-0.07	0.08
Beledi C × Mouride	1.08**	0.63*	1.27*	-0.25
Laduni 1B × Mouride	-0.58*	-1.20*	-1.23**	-3.08**
Titinwa A × Mouride	-0.43*	0.63*	0.35*	3.08**

\*Significant at P < 0.05, \*\*Significant at P < 0.01,

The means square for performance and variance for all the parental lines and their progenies were significantly different for number of days to the first flower, number of days to 50% flowering, number of days to first mature pod and number of days to 95% maturity. The broad sense heritability of these traits was equally high (Table 5.12).

Table 5.12: Mean square for performance and genetic components for days to the first flower, days to 50 % flowering, maturity in 15 crosses of cowpea

Source of variation	D.F	Days to the first flower	Days to 50% flowering	Days to first mature pod	Days to 95% maturity
Rep	3	21.17	30.14	48.55	68.17
Genotypes	14	21.19*	23.33*	23.52*	33.60*
Male	2	7.62	9.39	7.45*	7.20
Female	4	51.51***	54.93**	61.17*	97.79*
Male × female	8	9.42	11.01	8.70	8.10*
Error	42	7.18	7.51	6.84	4.69
Additive Variance (D)		7.49	7.84	9.43	9.94
Dominance Variance (H)		2.24	3.51	1.87	6.67
Environmental Variance (E)		1.79	1.88	1.71	2.61
Narrow-sense ( $H_N$ ) (%)		65	59	72	52

\*Significant at  $P < 0.05$ , \*\*Significant at  $P < 0.01$ , and \*\*\*Significant at  $P < 0.001$

#### 5.4 Discussion

Climate influences the amount of water available in the soil profile for agricultural production. Thus, creation of genetic variability for traits under selection is crucial (Falconer and Mackay, 1996). The computed drought intensity index (DII) value of 0.64 was adequate to explain the degree of reduced cowpea yield and its components. Miklas *et al.* (2006) reported that drought stress is considered severe when the intensity index value exceeds 0.7. Thus, the computed DII in the present study of 0.64 is adequate to distinguish drought susceptible and tolerant genotypes.

The crosses and parental lines were significantly different for traits studied under induced drought stress at the reproductive stage. This difference demonstrates the level of genetic variability among the genotypes for drought tolerance. The mean performance of yield and yield components of the hybrids were higher than most of the parental lines except for 100–seed weight. This difference indicates segregation as a result of gene introgression. Marame *et al.* (2009) reported that complementary gene action influences performance as a result of transgressive segregation which suggests polygenic inheritance of gene for drought tolerance. The best breeding approaches for polygenic traits are backcross, pedigree and recurrent selection methods. These methods allow increase of frequency of the desirable alleles.

Mean number of pods per plant, number of seeds per pod, 100–seed weight and seed yield were higher under well-watered conditions than the mean of parental lines and crosses under drought stress conditions, indicating that drought stress significantly reduces yield and yield components of the progenies and their parents. Both additive and non-additive effects contributed to the heritability of these traits.

Combining ability analysis identifies the breeding value of parental lines to produce hybrid progenies (Sprague and Tatum, 1942; Griffings, 1956). The results of this study revealed that both GCA and SCA effects were significant for DLS trait, indicating that both additive and non-additive gene actions were important and contributed to the expression of DLS.

The GCA mean squares for the traits seed yield, 100–seed weight, number of seeds per pod and number of pods per plant were greater than SCA across the two watering regimes. This implies that additive genetic effects were more important than non-additive genetic effects in the inheritance of these traits. Chiulele (2010) reported a predominance of non-additive gene action over additive genetic effects for seed yield and some yield components including number of pods per plant, number of seeds per pod, 100-seed weight, DLS and NDVI which could be used for indirect selection in cowpea improvement program under drought stress conditions.

The high value of Baker's ratio and narrow sense coefficient of genetic determination for the traits seed yield, 100–seed weight, and number of pods per plant, implies that there could be an equally high probability of hybrid performance from the parental lines GCA effects for these traits. This ratio indicates the breeding value (additive genetic variance) acts as the main determinant of response to selection (Falconer and Mackay, 1996). These traits could be improved by simple selection methods such as mass and pedigree selection which agrees with the report of Chiulele (2010). Furthermore, the non-additive genetic effects due to dominance and/or epistasis indicated preponderance of SCA effects and its contribution in the total genetic variation in seed yield, 100–seed yield and number of pods per plant. The existence of non-additive gene action hampers the process of early generation selection (Olajide and Ilori, 2017) and provides the avenue for hybridisation.

The BSCGD was high for all the traits across the contrasting environments. The high estimate of BSCGD could have been caused by lower environmental variance, interaction

between genotypes and environment or higher additive gene action. Acquah (2012) reported that environmental variance, interaction between genotype and environment and level of additive gene action contributed to high score of BSCGD. From the results of this study, NSCGD was moderate to low (<40%) under drought stress conditions. Narrow sense coefficient of genetic determination is a powerful statistical tool that could help breeders in the estimation of a gene transmitted to progenies. Low NSCGD suggests the presence of dominant gene action. This finding agrees with the results reported by Abney *et al.* (2001) who found that both narrow and broad sense heritability of quantitative traits are fundamental in studying a founder population.

However, selection of crosses with favourable estimates of SCA genetic effects should be prioritised and the cross combinations should involve at least one parental line that has shown favourable GCA effect. The hybrids Titinwa A  $\times$  IT93K-503-1, Apagu 1B  $\times$  Mouride Laduni 1B  $\times$  Dan Ila and Beledi C  $\times$  Mouride showed desirable, positive and significant SCA effects for seed yield and its components. This implies that the developed populations performed better than the prediction based on their parental GCA effects. The mean performance of Beledi A  $\times$  Dan Ila, Titinwa A  $\times$  IT93K-503-1, and Beledi C  $\times$  Mouride under drought stress conditions was higher than the mean seed yield of all the crosses. Falconer (1989) attributed the superiority of such crosses over their parents to complementary and duplicate gene action. This sets the precedence for the development of desirable segregants that could be utilised in cowpea improvement programs.

The best combiner for seed yield and number of pods per plant was IT93K-503-1. Its outstanding performance could be attributed to high seed yield and number of pods across the contrasting environments as well as positive GCA effects. These results suggest that IT93K-503-1 could be the most desirable donor parent for seed yield improvement in water-

limited environments. Hence, number of pods per plant could be used as an important index for indirect selection. This finding confirms the study carried out by Muchero *et al.* (2013).

## 5.5 Conclusions

Having adequate knowledge about the heritability of the numerous traits can help breeders design an efficient crop improvement program where several traits are to be improved concurrently. The study assessed the environmental responsiveness of 15 F<sub>2</sub>s and 8 parents under well-watered conditions for earliness in cowpea. Both additive and non-additive gene effects were important in governing early maturity in cowpea. Improving genotypes for earliness should consider both types of gene effects. The gene action conditioning genotype responsiveness and adaptation to drought stress environments are present in the selected donor parents. Both additive and non-additive effects controlled the transmission of genes responsible for drought tolerance in cowpea. However, non-additive (dominance) gene actions were more important than additive gene actions as shown by the outstanding performance of the inbreds in specific combinations. Thus, cowpea improvement for drought tolerance could be done by selecting progenies with desirable and positive SCA effects and subsequent advancement to later generations. The crosses Titinwa A × IT93K-503-1, Apagu 1B × Mouride, Laduni 1B × Dan Ila, and Beledi C × Mouride showed desirable positive significant SCA value for seed yield and its components. The parent IT93K-503-1 could be recommended as the most desirable donor for yield improvement in water-limited environments and as a tester for new hybrid development. The F<sub>2</sub> progenies advanced from the crosses Beledi A × Dan Ila, Titinwa A × IT93K-503-1, Apagu 1B × Mouride, Laduni 1B × Dan Ila and Beledi C × Mouride, Apagu 1B × IT93K-503-1, Laduni 1B × IT93K-503-1, Beledi A × IT93K-503-1, Laduni 1B × Mouride were promising combinations with significant and positive SCA effects for yield and yield components. These combinations should be subjected to further selection that would eventually result in release of improved

drought-tolerant cowpea variety (varieties). However, progeny testing should be done on the  $F_2$  population, where segregation is expected to be maximum, before carrying out the final selection. Based on the analysis of general combining ability for the four traits, Beledi C is recommended as a desirable source of gene conditioning earliness traits in cowpea.

## CHAPTER SIX

### 6.0 YIELD AND YIELD STABILITY OF COWPEA GENOTYPES

#### 6.1 Introduction

Traits that are of economic importance such as drought tolerance and yield are polygenic in nature, and they are sensitive to environmental differences. Most breeding programs conduct experiments in a number of contrasting sites to assess polygenic traits such as drought tolerance and grain yield. In those trials, variations in the performance of genotypes across diverse environments are consistently noticed. This is known as genotype by environment interaction (GEI), useful for improving quantitative traits (Bernardo, 2002). GEI makes it difficult to identify genotypes that are more stable and high yielding (Yan and Hunt, 1999). Analysis of GEI effects permits grouping of genotypes for their responsiveness in dissimilar conditions indicating whether they are stable or adaptable to broader or specific locations. Stability refers to the stability of performance exhibited by genotypes across locations. Yan and Kang (2003) grouped stability into two types, the dynamic (agronomic) and static (biological) stability. Plant breeders are mostly interested in agronomic stability. Agronomic stability requires that the genotypes are stable over testing sites (Yan and Kang, 2003; Frutos *et al.*, 2014). Adaptability is an organism adjustment to its environment. A high yielding genotype in a specific agro-ecological condition could be a poor yielder in other environments (Casanoves *et al.*, 2005). Numerous statistical methods are available to analyse GEI. Some of the frequently used methods are combined analysis of variance (ANOVA), multivariate and stability analyses.

Combined ANOVA is one of the often-used statistical approaches in the identification of GEI in trials across locations. The drawback associated with combined ANOVA is the assumption of homogeneity of variance among environments, which is a core requirement for determining genotype dissimilarities. However, this type of analysis permits determination of the variances of those constituents from diverse factors: genotype, environment, and GEI, but

it does not allow breeders to see the non-additive responses of the genotypes evaluated (Zobel *et al.*, 1988; Gauch *et al.*, 2008).

Analysis of genotype stability offers an opportunity for assessing the environmental responsiveness of the genotypes to changes in the environment. Cox (1997) suggested an analysis of linear regression as a statistical approach for gauging stability. The analytical approaches proposed and revised by Finlay and Wilkinson (1963), Eberhart and Russell (1966), Francis and Kannenberg (1978), Lin (1985), and Crossa (1990) have been widely used.

Genotypic stability analysis encompasses regression of the mean of genotypes on an environmental index (mean seed yield of all the entries evaluated across locations) known as stability index. Core biological drawback shows up when only a handful of genotypes with very high and low yields from different locations are involved in the stability analysis. Another problem with this approach is the hypothesis of a linear association between environmental means and GEI while the actual genotypic responses to the locations are multi-variate (Crossa, 1990). There are three main reasons for carrying out multivariate analysis when GEI effect is the core interest: (a) removal of "noise" in a data set (distinguishing systematic and non-systematic variation); (b) information summary; and (c) to reveal association in the data set (Crossa, 1990; and Gauch, 1992). The multivariate analysis models are built on PCA, such as Site regression (SREG) and Additive main effects and multiplicative interaction (AMMI). There are both linear and bilinear models with the main effect of the environment or genotypes (additive component) and GEI (multiplicative component).

AMMI analysis is an amalgamation of ANOVA, the core for analysing genotypes and environmental effects, and PCA of GEI (Zobel *et al.*, 1988; Gauch *et al.*, 2008). AMMI models depend on the number of principal components (PCs) used in the interaction between genotype and environment known as AMMI (1), AMMI (2) ...AMMI (n) (Hongyu *et al.*,

2014). This results in biplots a graphical representation of the interaction in biplot (Gabriel, 1971) which allows (1) display of the interaction of environments (vectors) and genotypes (points) in the same graph, and (2) examination of GEI effects. The angles between the environments and genotypes represented in the biplot graph gives the level of interaction between genotype and environment. The distances from the origin of the graph to the average environment axis (AEC) indicate the level of interaction that the genotypes display across locations.

Site regression analysis, also known as genotype main effect and genotype and GEI analysis, is both a linear and bilinear model that eliminates the effect of site and states the response simply as a function of the effect of genotypes and the GEI (Yang *et al.*, 2010; and Crossa *et al.*, 2015). This model is commended when the locations are the main cause of variation and it contributes to the genotypes and GEI effect taking into consideration the total disproportion of both environmental and genotypic contributions to the total variations (Casanoves *et al.*, 2005). Moreover, GGE is quite different from the AMMI model. The GGE analysis considers the GEI effects as crossover effects arising from pronounced variations in the performance of genotypes across locations (Yan *et al.*, 2000).

According to Yan *et al.* (2000), the use of GGE biplot graphical analysis has helped researchers to understand the behaviour and interactions of genotypes without the effect of the environment. Furthermore, they postulated that the first principal component (PC 1) usually accounts for responses of the genotypes that are comparative to the locations linked with the GEI without alteration of the assortment. Whereas, the second principal component (PC 2) offers evidence about cultivation sites that are not proportionate to the locations, showing attributes that are accountable to the G×E crossover interaction. The GGE biplot also permits identification and grouping of the environment into mega-environments, which entails those portions of the testing site of genotypes that show homogeneous agro-ecological conditions, where performances of some genotypes are consistent over the periods of

evaluation (Gauch and Zobel, 1988). In each designated mega-environment, genotype by location interaction effects are inadequate or insignificant (Yan and Hunt, 2000).

The objectives of this study were, therefore, to 1) assess GEI effects using GGE and AMMI approaches for yield and yield components of drought-tolerant early maturing cowpea populations, and 2) determine the yield stability for seed yield of 25 cowpea genotypes grown under drought-stressed and well-watered conditions during off-seasons in three contrasting locations.

## **6.2 Materials and Methods**

### **6.2.1 Population Development**

The genetic material used in the development of the genotypes for this study included five farmers' preferred varieties, Titinwa A, Laduni 1B, Beledi A, Apagu 1B, and Beledi C, and three drought-tolerant varieties, Dan Ila, Mouride, and IT93K-503-1. The five early maturing farmers' preferred varieties were crossed with each drought-tolerant material in a 5 x 3 North Carolina II mating design to generate 15 early droughts tolerant first filial ( $F_1$ s) generation. Beledi C, Laduni 1B, Beledi A, Apagu 1B and Titinwa A (recurrent parents) and three other varieties, Dan Ila, Mouride, and IT93K-503-1 (donor parents), were crossed in September 2016 at WACCI farm. The 15  $F_1$ s were backcrossed to their donor parents three times. The crossing block was set up on the 5<sup>th</sup> and 13<sup>th</sup> September 2016 to synchronize the flowering pattern of the genetic materials. Each of the breeding materials (recurrent/ donor parent) was planted in a three-row plot of 4 m long with an inter-row spacing of 1 m and intra-row spacing of 1 m between hills. Two seeds were planted per hill and each plot had a total population density of 30 plants. Each male parent was planted in one row of 20 plants along with two rows of the female parent. The male parents were planted seven days earlier than the female parents to flower about the same time (nicking). Standard cultural practices of pesticide, and weeding were applied to all the plots to keep the field free of weeds and pests. Necessary precautions were taken to avoid the contamination of the parental lines at

the time of crossing. Hand emasculation of the receptive floral buds and pollination were carried out by skilled workers under the management of the researcher according to IITA guide. Pollination commenced on 13<sup>th</sup> October 2016 and lasted for four weeks. After emasculation (cutting half of the upper petal of the female parent and removal of stamens), pollen was collected from a freshly opened male parent flowers and dusted on the stigma of the female parent. The crosses were harvested on 24<sup>th</sup> of November 2016.

Backcross breeding method was employed to recover the recurrent parent's performance with an added drought tolerance attribute. Fifteen F<sub>1</sub>s and five recurrent parents were planted on the 22<sup>nd</sup> November 2016. Emasculation and pollination started on the 26<sup>th</sup> December 2016 for four weeks and harvest of the backcross one F<sub>1</sub>s (BC<sub>1</sub>F<sub>1</sub>s) was on the 17<sup>th</sup> of January 2017. Subsequently, the BC<sub>1</sub>F<sub>1</sub>s seeds and donor (female parents) were plant on 18<sup>th</sup> March 2017 to develop BC<sub>2</sub>F<sub>1</sub>s. Crossing started on the 24<sup>th</sup> April 2017 for four weeks and BC<sub>2</sub>F<sub>1</sub>s seeds were harvested from a single plant on the 17 May 2017. The crossing block for developing BC<sub>3</sub>F<sub>1</sub>s was set up on 29<sup>th</sup> and 30<sup>th</sup> June 2017. Crossing commenced on the 10<sup>th</sup> of August 2017 for four weeks (9<sup>th</sup> of September 2017). BC<sub>3</sub>F<sub>1</sub>s seeds were harvested on the 6<sup>th</sup> of October 2017. The BC<sub>3</sub>F<sub>1</sub>s were selfed twice from 16<sup>th</sup> September 2017 to 10<sup>th</sup> January 2018 to generate BC<sub>3</sub>F<sub>3</sub> and ensure purity.

### **6.2.2 Genetic Materials and Environment of the Test Locations**

The 15 BC<sub>3</sub>F<sub>3</sub>s, their eight parents and two checks (AGRAC-216 and Asontem) were evaluated during the offseason of 2018 in three testing sites: Coastal Savanna Agro-ecological Zone (WACCI farm/Legon, Accra), Guinea Savanna Agro-ecological Zone (SARI/Nyankpala, Tamale), and Forest-Savanna Agro-ecological Zone (CSIR-Crops Research Institute CRI/, Fumesua, Kumasi) (Table 6.2).

### **6.2.3 Experimental Layout**

The genetic materials consisting of 25 entries arranged in  $5 \times 5$  lattice square design with three replications were evaluated across three test locations (Nyankpala, Fumesua, and Legon) in Ghana. The trial was planted in two adjustment blocks spaced 50 m apart denoting two water regimes. The first block (Block 1) was designated as well-watered (WW) and the second (Block 2) as severe stress (SS). Plants planted in Block 2 were subjected to severe water stress-imposed by withdrawing irrigation water for three weeks at flowering stage. Then watering was resumed for two weeks, twice a week, in order to score recovery and regrowth parameters of the genotypes.

Table 6.1: List of 15 BC<sub>3</sub>F<sub>3</sub> cowpea genotypes, eight parents and two checks evaluated in three locations in 2018

<b>Pedigree</b>				
<b>Genotype</b>	<b>Female</b>	<b>Male</b>	<b>Attribute</b>	<b>Source</b>
BC×M	Beledi C	Mouride	Developed for drought tolerance and earliness	Breeders material
BC×D	Beledi C	Dan Ila	Developed for drought tolerance and earliness	Breeders material
BC×IT	Beledi C	IT93K-503-1	Developed for drought tolerance and earliness	Breeders material
BA×D	Beledi A	Dan Ila	Developed for drought tolerance and earliness	Breeders material
BA×IT	Beledi A	IT93K-503-1	Developed for drought tolerance and earliness	Breeders material
BA×M	Beledi A	Mouride	Developed for drought tolerance and earliness	Breeders material
TA×M	Titinwa A	Mouride	Developed for drought tolerance and earliness	Breeders material
TA×IT	Titinwa A	IT93K-503-1	Developed for drought tolerance and earliness	Breeders material
TA×D	Titinwa A	Dan Ila	Developed for drought tolerance and earliness	Breeders material
A1B×M	Apagu 1B	Mouride	Developed for drought tolerance and earliness	Breeders material
A1B×D	Apagu 1B	Dan Ila	Developed for drought tolerance and earliness	Breeders material
A1B×I	Apagu 1B	IT93K-503-1	Developed for drought tolerance and earliness	Breeders material
L1B×I	Laduni 1B	IT93K-503-1	Developed for drought tolerance and earliness	Breeders material
L1B×D	Laduni 1B	Dan Ila	Developed for drought tolerance and earliness	Breeders material
L1B×M	Laduni 1B	Mouride	Developed for drought tolerance and earliness	Breeders material
Mouride			Drought tolerant	Burkina Faso
Dan Ila			Drought tolerant	Niger
IT93K-503-1			Drought tolerant	Nigeria
Laduni 1B			Preferred and adaptable	South Sudan
Titinwa A			Preferred and adaptable	South Sudan
Apagu 1B			Preferred and adaptable	South Sudan
Beledi C			Preferred and adaptable	South Sudan
Beledi A			Preferred and adaptable	South Sudan
AGRAC-216			High yielding (check)	South Sudan
Asontem			Early maturing (check)	Ghana

#### 6.2.4 Data Collection

Data were collected on an individual plant basis in accordance with the International Plant Genetic Resources cowpea descriptors (1983).

- i. number of days to 50% flowering was recorded as the number of days from planting to a stage when 50% of plants in a plot had begun to flower, and
- ii. number of days to 95% maturity was scored when 95% of the plants in a plot has attained maturity.

At physiological maturity, individual plants were harvested separately, then the mean of each trait measured on individual plant was computed to determine yield and yield components:

- iii. number of pods per plant recorded as the mean of matured pods from 20 plants,
- iv. number of seeds per pod recorded an average number of seeds from 20 pods, whereas,
- v. pod length was measured in centimetre as the mean of 20 pod length.
- vi. 100–seed weight was computed from 10 randomly selected plants in each plot weighed and recorded as the weight of 100–seed in grams, and
- vii. seed yield harvested at 12% moisture content was computed from the weight of harvested pods per plot ( $\text{kgha}^{-1}$ ), and
- viii. harvest index was computed as ratio of seed yield to biological yield.

Table 6.2: Description of test sites for 25 cowpea genotypes evaluated across three diverse agro-ecologies in Ghana

Environment	Agro-ecological Zone	Soil Type	Altitude (masl)	Average Rainfall (mm)	Temperature (°C)		Geographic Location		DII	STI	TOL
					Min	Max	Latitude	Longitude			
Legon	Coastal Savanna	Adenta series	97	809	23.8	31.2	5° 38' N	0° 10' E	0.31	0.69	675.47
Fumesua	Forest Savanna	Fumesua series	286	1500	22.4	32.1	6°41' N	1° 28' W	0.29	0.39	523.10
Nyankpala	Guinea Savanna	Tolon series	171	1091	23.9	35.3	9°24' N	0°59' W	0.61	0.39	612.70

Source: Climate-data.org, STI: Stress tolerance index, TOL: Drought tolerance index and DII: Drought intensity index

### 6.2.5 Data Analysis

The data were analysed by using GenStat 18<sup>th</sup> edition, to compute mean performance and mean separation. IBM SPSS 22<sup>nd</sup> edition was used to generate the regression graphics and GEA-R for stability analysis using AMMI, and GGE biplot analysis models, respectively.

#### Selection Index

Harvest index was computed using Donald and Hamblin (1976) formula

$$\text{Harvest index (\%)} = \frac{\text{Grain Yield}}{\text{Biological Yield}} \times 100$$

The percent reduction due to moisture stress and drought susceptibility index was computed using the formula suggested by Fischer and Maurer (1978).

$$\text{Percent reduction} = \frac{\text{Yield under non-stress} - \text{Yield under stress}}{\text{Yield under non-stress}} \times 100$$

$$\text{Drought susceptibility index (DSI)} = \frac{(1 - Y_d / Y_p)}{\bar{Y}_p}$$

$$\text{Drought index (DII)} = 1 - \frac{\text{Mean grain yield of all genotypes under drought stress condition}}{\text{Mean grain yield of all genotypes under well-watered condition}}$$

Where,

$Y_d$  = Grain yield of genotypes under severe moisture stress condition.

$Y_p$  = Grain yield of genotypes under well-irrigated condition.

### 6.3 Results

The environmental conditions of the three testing sites coupled with induced drought stress were significant enough to show the degree of drought severity on seed yield as illustrated in

Fig. 6.1.

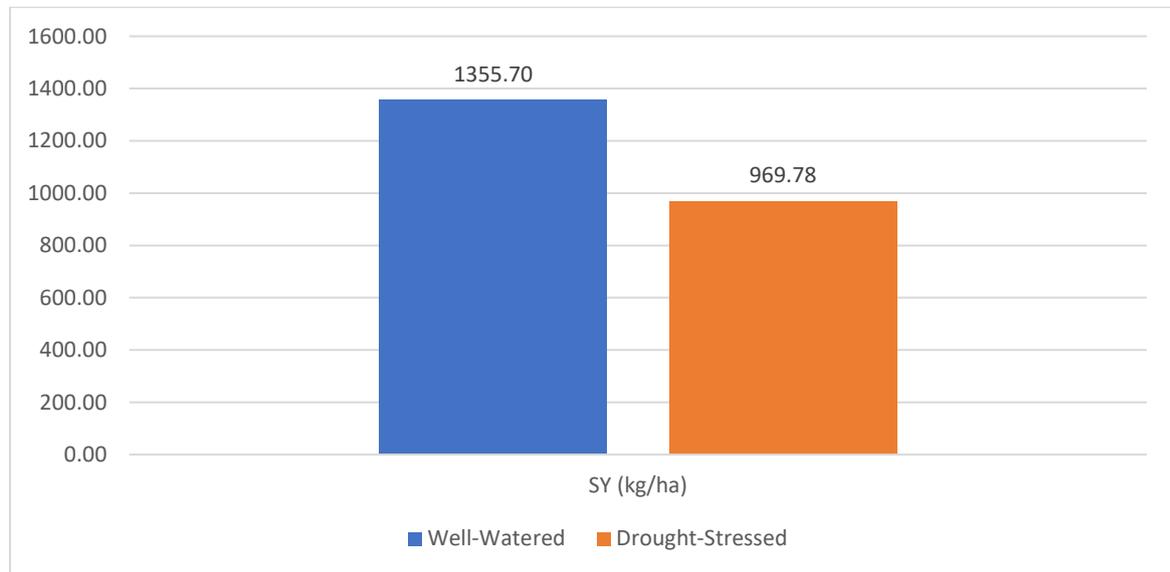


Fig. 6. 1 Estimated means for the twenty-five genotypes selected under drought stress and well-watered conditions at ( $P < 0.05$ )

The computed drought intensity index (DII) revealed that Legon, Tamale, and Kumasi were significantly ( $P < 0.05$ ) different. Hence, this index could be used for selecting genotypes for drier environments by comparing their DII with performance under both well-watered and drought stress conditions (Table 6.2).

As shown in Fig. 6.2, the comparison of genotypes in terms of seed yield under drought stress condition at Legon, Fumesua and Nyankpala revealed that the genotypes generally gave a higher yield in Fumesua than in Legon and Nyankpala. This was supported by the numerical measures of seed yield. The median seed yield in Nyankpala ( $300 \text{ kg ha}^{-1}$ ) was lower than both Legon ( $800 \text{ kg ha}^{-1}$ ) and Fumesua ( $1200 \text{ kg ha}^{-1}$ ). It was observed that the third quartile of Nyankpala ( $600 \text{ kg ha}^{-1}$ ) was lower than the seed yield median for Legon.

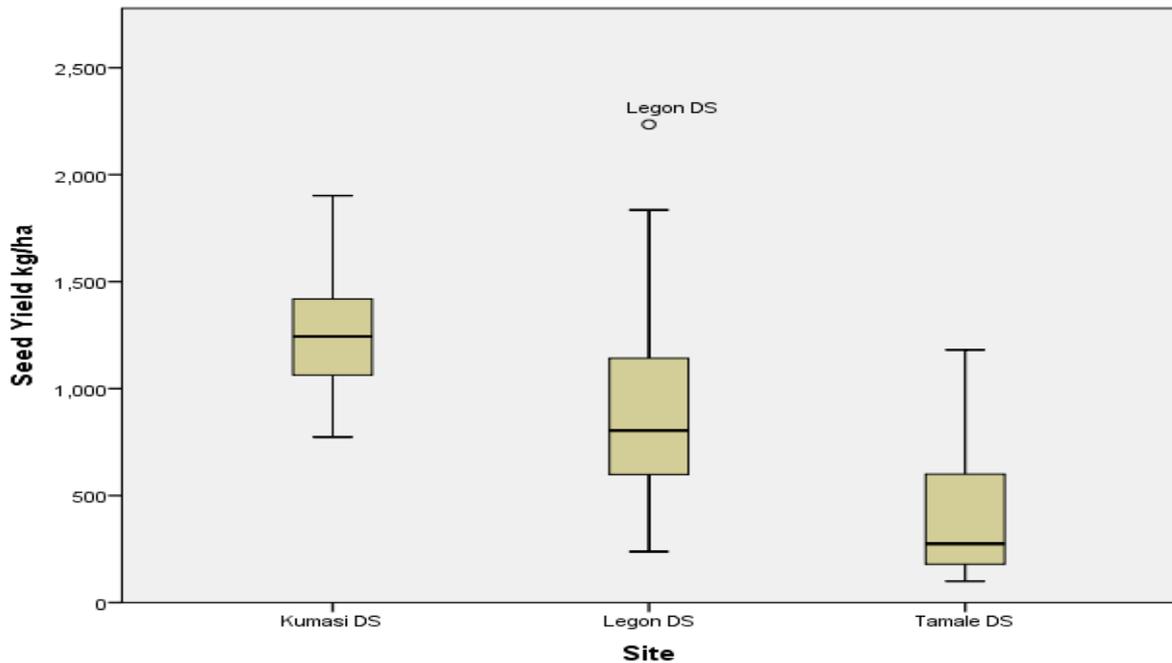


Fig. 6. 2 Boxplot estimated means for the significant effects under drought stress (DS) conditions at ( $P < 0.05$ )

### 6.3.1 Crop Performance at Nyankpala

The overall drought tolerance index (DII) at Nyankpala (Tamale) was 0.61, stress tolerance index (STI) 0.39 and stress tolerance (TOL) was 612.7 (Table 6.2). Genotype Dan Ila, a donor parent, had the highest yield of  $849.1 \text{ kg ha}^{-1}$  under drought stress condition (Table 6.3). BC×M the second-best performer yielded  $839.9 \text{ kg ha}^{-1}$  under drought stress condition. This genotype had stress tolerance index (STI) of 0.5, mean productivity index (AMP) 1191.2, and geometric mean productivity (GMP) of 1138.2. Additionally, BC×M had TOL of 702.5 and DII of 0.46. BA×D had the lowest yield of  $129.3 \text{ kg ha}^{-1}$  among the families despite having STI value of 0.1, an AMP (589.2), GMP was 368.3 and TOL was 919.8 with DII of 0.88 (Table 6.3). The computed mean squares at Nyankpala showed that under drought stress condition, Dan Ila had the heaviest 100–seed weight (16.1 g) and BA×D registered the lightest (5.4 g) 100–seed weight under drought stress condition (Table 6.4). Whereas, under well-watered condition, genotype Mouride, a non-recurrent parent, had the heaviest 100–seed

weight (17.4 g), while BC×D had the lightest seed weight (7.5 g) under well-watered condition (Table 6.4).

Asontem, a check in this study, had the highest number of seeds per pod (12.4) and TA×M the lowest (7.5) under the well-watered conditions. IT93K-503-1 registered the highest seed count of 8 seeds per pod, Whereas TA×I and TA×M both had the lowest number of seeds per pods (4.1) under drought stressed conditions. Beledi C had the highest number of pods per plant (14.6) and AGRAC-216 had the lowest (6.5) pods per plant under well-watered conditions at Nyankpala. Laduni 1B registered the highest value of 7.3 pods and BA×M had the lowest number of pods per plant (0.8) under drought stressed environment (Table 6.4).

BA-I registered the longest number of days to reach 95% (91 days) and Laduni 1B had the shortest (73.8) days to attain 95% maturity under drought stressed condition. AGRAC-216 took the longest number of days (80.0) to reach 95% maturity under a well-watered condition, while Laduni 1B had the shortest number of days (69.0) to attained 95% maturity under well-watered condition. The number of days to attain 50% flowering was another trait for computing earliness in cowpea. AGRAC-216 had the longest number of days to 50% flowering (58 days), whereas, Laduni 1B had the shortest (43 days) under drought stress condition. Mouride, took 56 days to 50% flowering and Titinwa A, a recurrent parent, had the shortest number of days (40 days) to attain 50% flowering under well-watered conditions. Under drought stress conditions, BC×M had the highest harvest index (HI) of 55.6% and AGRAC-216 registered the lowest value of 0.2. Titinwa A had the highest percentage of 85.1% for HI and L1B×D had the lowest value of 9.2% under well-watered conditions (Table 6.4).

Table 6.3: Seed yield performance of 25 genotypes and other agronomic traits measured under drought stressed and well-watered conditions at Nyankpala

Attribute	Genotype	WW	DS	STI	AMP	GMP	TOL	DII
High Yielding Ability	Dan Ila	1004.4	849.1	0.8	926.8	923.5	155.3	0.15
	BC×M	1542.4	839.9	0.5	1191.2	1138.2	702.5	0.46
Medium Yielding Ability	BA×M	604.8	588.3	1.0	596.6	596.5	16.5	0.03
	TA×I	695.4	567.8	0.8	631.6	628.4	127.6	0.18
	BC×I	1121.7	545.3	0.5	833.5	782.1	576.4	0.51
	A1B×M	898.9	513.1	0.6	706.0	679.1	385.8	0.43
	A1B×I	881.8	472.5	0.5	677.2	645.5	409.3	0.46
	IT93K–503–1	1425.1	459.7	0.3	942.4	809.4	965.4	0.68
	TA×D	846.4	448.2	0.5	647.3	615.9	398.2	0.47
	Mouride	1700.4	412.6	0.2	1056.5	837.6	1287.8	0.76
	A1B×D	950.9	397.6	0.4	674.3	614.9	553.3	0.58
	L1B×I	1052.9	394.3	0.4	723.6	644.3	658.6	0.63
Low Yielding Ability	L1B×M	688.8	361.1	0.5	525.0	498.7	327.7	0.48
	Asontem	989.3	359.6	0.4	674.5	596.4	629.7	0.64
	BC×D	780.5	351.7	0.5	566.1	523.9	428.8	0.55
	BA×I	1052	309.1	0.3	680.6	570.2	742.9	0.71
	TA×M	824.4	284.5	0.3	554.5	484.3	539.9	0.65
	Laduni 1B	1161.7	260.3	0.2	711.0	549.9	901.4	0.78
	Beledi C	800.3	246.4	0.3	523.4	444.1	553.9	0.69
	Apagu 1B	630.2	227.1	0.4	428.7	378.3	403.1	0.64
	L1B×D	1288.4	212.1	0.2	750.3	522.8	1076.3	0.84
	Titinwa A	1352	201.5	0.1	776.8	521.9	1150.5	0.85
	Beledi A	996.5	163.3	0.2	579.9	403.4	833.2	0.84
	BA×D	1049.1	129.3	0.1	589.2	368.3	919.8	0.88
	AGRAC–216	641	67	0.1	354.0	207.2	574	0.90
Mean		999.2	386.5					
CV%		39.80	62.20					
Significance		ns	*					

WW: well-watered condition, DS: Drought-stressed condition, STI: Stress tolerance index, AMP: Arithmetic means productivity, GMP: Geometric means productivity, TOL: Drought tolerance index, DII: Drought intensity index

Table 6.4: Means from analysis of variance measured under drought stress and well-watered conditions at Nyankpala 2018

Genotype	Env 1 (Nyankpala Well-Watered)								Env 4 (Nyankpala Drought-Stressed)							
	HI	SY (kg/ha)	100swt (g)	NSP	NPP	95%mat	50%flo	HI	SY (kg/ha)	100swt (g)	NSP	NPP	95%mat	50%flo		
A1B×D	38.2	950.9	8.2	9.8	11.2	70.0	44.0	38.9	397.6	7.5	5.5	3.5	76.0	49.0		
A1B×I	68.7	881.8	8.8	10.9	10.6	70.0	46.0	44.7	472.5	8.0	5.2	5.2	81.0	48.0		
A1B×M	21.5	898.9	12.3	9.3	9.9	71.0	47.0	27.8	513.1	10.4	5.3	2.6	82.0	44.0		
AGRAC-216	11.5	641.0	14.5	8.0	6.5	80.0	53.0	0.2	67.0	10.8	5.4	3.2	81.0	58.0		
Apagu 1B	47.9	630.2	8.7	11.0	7.7	70.0	46.0	26.3	227.1	8.4	6.1	5.8	75.0	46.0		
Asontem	41.3	989.3	12.3	12.4	7.3	73.0	50.0	20.4	359.6	12.1	6.4	5.4	81.0	53.0		
BA×D	20.3	1049.1	9.1	9.1	9.5	77.0	49.0	1.8	129.3	5.9	7.0	4.3	87.0	50.0		
BA×I	11.4	1052.0	11.1	8.5	7.9	76.0	45.0	13.0	309.1	11.1	5.3	1.9	91.0	54.0		
BA×M	18.1	604.8	10.7	7.7	7.9	76.0	47.0	20.8	588.3	9.4	4.6	0.8	88.0	53.0		
BC×D	69.5	780.5	7.5	10.7	10.8	72.0	44.0	14.3	351.7	8.4	5.1	2.6	82.0	45.0		
BC×I	57.0	1121.7	11.8	9.9	11.2	72.0	42.0	28.4	545.3	8.5	4.5	4.0	82.0	45.0		
BC×M	52.9	1542.4	10.7	10.1	13.3	72.0	43.0	55.6	839.9	9.4	6.0	2.2	82.0	45.0		
Beledi A	20.9	996.5	8.8	10.5	12.6	77.0	47.0	4.3	163.3	9.6	6.5	3.3	83.0	49.0		
Beledi C	43.6	800.3	7.8	12.1	14.6	73.0	42.0	14.4	246.4	8.0	6.6	4.0	85.0	45.0		
Dan Ila	29.3	1004.4	16.7	10.8	10.8	77.0	54.0	49.6	849.1	16.1	7.6	6.5	65.0	55.0		
IT93K-503-1	69.3	1425.1	14.3	11.1	11.1	74.0	48.0	12.8	459.7	13.4	8.0	4.8	88.0	51.0		
L1B×D	9.2	1288.4	12.3	9.0	8.3	72.0	42.0	13.6	212.1	9.9	5.2	3.8	76.0	44.0		
L1B×I	51.1	1052.9	10.9	8.4	7.9	71.0	43.0	42.2	394.3	8.5	5.1	2.8	76.0	43.0		
L1B×M	27.1	688.8	12.0	8.8	7.9	72.0	43.0	14.6	361.1	11.4	4.8	3.5	76.0	45.0		
Laduni 1B	52.7	1161.7	11.2	12.2	10.6	69.0	42.0	12.6	260.3	11.5	5.3	7.3	74.0	43.0		
Mouride	44.7	1700.4	17.4	9.1	10.8	77.0	56.0	15.3	412.6	16.0	6.0	4.1	87.0	53.0		
TA×D	44.4	846.4	12.4	8.2	9.6	76.0	45.0	25.4	448.2	9.2	4.9	4.3	84.0	46.0		
TA×I	38.4	695.4	9.5	8.1	10.5	72.0	42.0	36.5	567.8	10.1	4.1	3.2	82.0	44.0		
TA×M	51.3	824.4	13.5	7.5	9.5	71.0	42.0	13.9	284.5	13.8	4.1	3.0	83.0	44.0		
Titinwa A	85.1	1352.0	12.7	9.9	13.6	74.0	40.0	13.6	201.5	10.1	4.5	3.6	83.0	44.0		
<b>Mean</b>	<b>41.0</b>	<b>999.2</b>	<b>11.4</b>	<b>9.7</b>	<b>10.1</b>	<b>73.0</b>	<b>46.0</b>	<b>22.4</b>	<b>386.5</b>	<b>10.3</b>	<b>5.6</b>	<b>3.8</b>	<b>81.0</b>	<b>48.0</b>		
CV%	64.9	39.8	11.4	13.5	26.4	2.9	6.4	72.2	62.2	22.2	28.9	43.9	12.6	6.4		
Lsd p<0.05	45.5	680.4	2.2	2.2	4.5	3.6	5.0	27.8	411.4	3.9	2.7	2.9	17.5	5.2		

Hi: Harvest index, SY: Seed yield, 100swt: 100-seed weight, NPP: Number of pods per plant, NSP: Number of seeds per pod, 95%mat: 95% maturity and 50%flo: 50% flowering

### 6.3.2 Crop Performance at Legon

The overall DII of the test site Legon (Accra) was 0.31, STI was 0.69 and TOL was 675.47 (Table 6.2). At Legon, genotype AGRAC-216 had the highest 100-seed weight of 21.9 g and 21.5 g under both drought-stressed and well-watered conditions, respectively. A1B×D had the lowest 100-seed weight (7.4 g) under drought stress and Beledi C had the lowest 100-seed weight (8.9 g) under well-watered conditions (Table 6.5).

Under drought stress condition, Mouride, a non-recurrent parent, yielded 1375.1 kg $ha^{-1}$  followed by BC×M with the second highest seed yield producing 1365.3 kg $ha^{-1}$ . Genotype Beledi A had the lowest seed yield (375.2 kg $ha^{-1}$ ) under drought stress condition. Whereas, under well-watered condition, IT93K-503-1 yielded 2559.0 kg $ha^{-1}$  and BC×D had the lowest seed yield of 796.0 kg $ha^{-1}$  (Table 6.4). Among the progenies evaluated, BC×M had STI of 1.3, AMP of 1203.3 and GMP of 1192.1. This genotype also had TOL of -324.2 and DII of -0.3. BC×M had the highest seed yield (1365.2 kg $ha^{-1}$ ) while BA×D had the lowest seed yield (466.2 kg $ha^{-1}$ ) under drought stress condition. However, BA×D had STI value of 0.3, AMP (10221.1), GMP was 857.7 and TOL of 1111.8 with DII of 0.77. Parent Beledi A had the worst performance under both conditions producing seed yield below the overall mean despite having drought tolerance index of 0.5 (Table 6.5).

Laduni 1B had 16 seeds per pod under stressed condition and TA×I had the lowest number of seeds per pods (10.8) under drought-stress condition. Nevertheless, L1B×I had the highest number of seeds per pod (16.6) and TA×I had the lowest number of seeds per pod (10.4) under well-watered condition. Beledi C had the highest number of pods per plant, while Beledi A had the lowest number of pods per plant and under drought stressed condition. IT93K-503-1, a non-recurrent parent, had the highest number of pods per plant and Asontem had the lowest number of pods per plant under well-watered condition (Table 6.6).

One of the earliness traits, number of days to 95% maturity, revealed that Dan Ila took the longest number of days to attain 95% maturity (72 days) and A1B×I registered 51 days for the trait under drought stress conditions. Under well-watered conditions, Dan Ila also took 71 days to attain 95% maturity and progeny TA×M took only 54.0 days to reach 95% maturity under optimum conditions. Dan Ila took 49.0 and 49.0 days to reach 50% flowering under both drought-stressed and well-watered conditions, respectively. A1B×M had the shortest number of days to attain 50% flowering (35 and 34 days) under both drought-stressed and well-watered conditions. Under drought stress conditions, A1B×I had the highest HI, while Beledi A scored the lowest HI value. Asontem had the highest HI value and Mouride registered the lowest value under well-watered conditions (Table 6.6).

Table 6.5: Seed yield performance of 25 genotypes and other agronomic traits measured under drought stressed and well-watered conditions at Legon

Attribute	Genotype	WW	DS	STI	AMP	GMP	TOL	DII
High Yielding Ability	L1B×M	1814.0	1064.2	0.6	1439.1	1389.4	749.8	0.4
	Beledi C	778.0	1283.7	1.7	1030.9	999.4	-505.7	-0.7
	L1B×D	1368.0	959.1	0.7	1163.6	1145.4	408.9	0.3
	Titinwa A	1357.0	985.0	0.7	1171.0	1156.1	372.0	0.3
	TA×M	1344.0	1234.3	0.9	1289.2	1288.0	109.7	0.1
	L1B×I	1312.0	1115.7	0.9	1213.9	1209.9	196.3	0.1
	AGRAC-216	1265.0	989.0	0.8	1127.0	1118.5	276.0	0.2
	Dan lla	1244.0	1096.8	0.9	1170.4	1168.1	147.2	0.1
	A1B×M	1232.0	1163.7	0.9	1197.9	1197.4	68.3	0.1
	A1B×I	1065.0	1039.2	1.0	1052.1	1052.0	25.8	0.0
	BC×I	1064.0	1012.0	1.0	1038.0	1037.7	52.0	0.0
	BC×M	1041.0	1365.2	1.3	1203.1	1192.1	-324.2	-0.3
	Mouride	1022.0	1375.7	1.3	1198.9	1185.7	-353.7	-0.3
A1B×D	2222.0	897.5	0.4	1559.8	1412.2	1324.5	0.6	
Medium Yielding Ability	Laduni 1B	1309.0	854.2	0.7	1081.6	1057.4	454.8	0.3
	Apagu 1B	1164.0	839.7	0.7	1001.9	988.6	324.3	0.3
	TA×I	885.0	610.7	0.7	747.9	735.2	274.3	0.3
	TA×D	1311.0	657.8	0.5	984.4	928.6	653.2	0.5
	Asontem	904.0	625.0	0.7	764.5	751.7	279.0	0.3
Low Yielding Ability	IT93K-503-1	2559.0	453.7	0.2	1506.4	1077.5	2105.3	0.8
	BA×D	1578.0	466.2	0.3	1022.1	857.7	1111.8	0.7
	BA×M	1313.0	534.3	0.4	923.7	837.6	778.7	0.6
	BA×I	1335.0	567.1	0.4	951.1	870.1	767.9	0.6
	Beledi A	802.0	375.2	0.5	588.6	548.6	426.8	0.5
	BC×D	796.0	518.2	0.7	657.1	642.3	277.8	0.3
Mean		1283.4	883.3					
CV%		33.70	33.43					
Significance		***	***					

WW: well-watered condition, DS: Drought-stressed condition, STI: Stress tolerance index, AMP: Arithmetic means productivity, GMP: Geometric means productivity, TOL: Drought tolerance index, DII: Drought intensity index

Table 6. 6: Means from analysis of variance measured under drought stress and well-watered conditions at Legon 2018

Genotype	Env 3 (Legon Well-Watered)								Env 6 (Legon Drought-Stressed)							
	HI	SY (kg/ha)	100swt (g)	NSP	NPP	95% mat	50% flo	HI	SY (kg/ha)	100swt (g)	NSP	NPP	95% mat	50% flo		
A1B×D	22.7	2222.0	9.6	12.7	16.5	56.0	38.0	28.7	976	7.4	13.3	11.0	56.0	39.0		
A1B×I	17.4	1065.0	9.4	13.4	14.6	56.0	38.0	30.3	1028	9.0	14.9	9.3	51.0	38.0		
A1B×M	8.6	1232.0	13.3	12.9	16.8	56.0	34.0	17.0	1301	12.8	13.2	8.3	530	35.0		
AGRAC-216	9.1	1265.0	21.5	11.9	11.6	59.0	45.0	8.6	1847	21.9	11.9	4.8	57.0	44.0		
Apagu 1B	15.2	1164.0	8.6	14.3	20.7	56.0	38.0	25.8	1052	7.5	13.9	9.9	54.0	38.0		
Asontem	27.5	904.0	15.2	15.1	6.9	58.0	45.0	5.5	1648	14.9	11.7	4.3	56.0	44.0		
BA×D	13.9	1578.0	9.2	14.6	20.6	60.0	39.0	6.2	1279	10.4	11.7	4.5	59.0	40.0		
BA×I	9.1	1335.0	12.0	14.8	15.4	55.0	40.0	5.2	1274	11.3	14.0	4.4	59.0	39.0		
BA×M	8.3	1313.0	11.0	13.4	21.6	57.0	37.0	3.9	1171	10.2	13.2	4.9	61.0	38.0		
BC×D	9.3	796.0	9.9	12.1	13.7	58.0	40.0	17.6	818	8.0	13.6	5.8	55.0	40.0		
BC×I	7.7	1064.0	10.0	10.9	21.9	57.0	39.0	21.2	1491	10.1	14.0	8.8	55.0	40.0		
BC×M	8.8	1041.0	12.5	11.1	17.6	61.0	37.0	24.6	1052	10.5	12.9	12.0	54.0	39.0		
Beledi A	7.2	802.0	10.1	13.0	16.0	59.0	40.0	3.7	1057	10.5	12.4	3.3	59.0	41.0		
Beledi C	22.7	778.0	8.9	11.0	14.3	61.0	38.0	20.9	905	9.2	13.7	12.1	53.0	38.0		
Dan Ila	26.1	1244.0	19.0	11.0	13.1	71.0	49.0	7.5	1495	17.2	12.3	6.2	72.0	49.0		
IT93K-503-1	18.5	2559.0	13.6	16.1	29.3	67.0	44.0	6.3	1739	14.9	12.5	2.9	69.0	47.0		
L1B×D	13.2	1368.0	10.0	16.4	18.4	55.0	37.0	12.9	1211	11.6	14.3	6.9	53.0	38.0		
L1B×I	12.2	1312.0	11.7	15.7	16.9	59.0	36.0	10.4	1378	8.3	16.2	9.7	54.0	36.0		
L1B×M	14.4	1814.0	11.9	16.6	17.2	58.0	38.0	10.7	1272	11.0	15.3	7.5	56.0	39.0		
Laduni 1B	18.6	1309.0	10.5	16.1	20.6	57.0	40.0	12.6	1454	11.2	16.0	5.7	59.0	38.0		
Mouride	6.3	1022.0	18.2	12.6	11.9	66.0	44.0	12.6	1464	17.0	12.3	7.8	63.0	48.0		
TA×D	16.3	1311.0	11.7	13.1	15.9	59.0	38.0	11.6	1126	11.3	10.8	6.9	55.0	37.0		
TA×I	9.2	885.0	10.5	10.4	17.1	58.0	38.0	14.6	1213	10.6	12.0	5.8	55.0	39.0		
TA×M	11.7	1344.0	13.3	13.8	17.5	54.0	35.0	18.1	1175	14.8	13.5	7.0	53.0	37.0		
Titinwa A	14.6	1357.0	11.0	13.6	20.2	55.0	37.0	28.1	1110	11.5	12.8	8.0	56.0	37.0		
<b>Mean</b>	<b>13.9</b>	<b>1283.4</b>	<b>12.1</b>	<b>13.5</b>	<b>17.1</b>	<b>59.0</b>	<b>39.0</b>	<b>14.6</b>	<b>1262</b>	<b>11.7</b>	<b>13.3</b>	<b>7.1</b>	<b>57.0</b>	<b>50.0</b>		
CV%	79.8	33.7	12.5	17.3	29.3	5.8	5.3	46.4	33.4	11.8	12.4	28.6	4.3	4.9		
Lsd p<0.05	19.04	740.2	2.6	4.0	8.5	5.9	3.5	11.6	505.6	2.4	2.8	3.5	4.2	3.3		

Hi: Harvest index, SY: Seed yield, 100swt: 100-seed weight, NPP: Number of pods per plant, NSP: Number of seeds per pod, 95% mat: 95% maturity and 50% flo: 50% flowering

### 6.3.3 Crop Performance at Fumesua

The overall drought tolerance index of the test site at Fumesua (Kumasi) was 0.29, STI was 0.71 and TOL was 523.1 (Table 6.2). Under drought stressed condition, genotype AGRAC-216 gave the highest yield (1847.2 kg ha<sup>-1</sup>) and BC×D gave the lowest yielder (818.0 kg ha<sup>-1</sup>). Among the progenies evaluated under drought stress, BC×I gave the best yield producing 1491 kgha<sup>-1</sup> of seed yield, while BC×D had the lowest seed yield (818.0 kg ha<sup>-1</sup>). Under well-watered condition, Asontem had the highest yield of 2750.0 kgha<sup>-1</sup> and A1B×D had the lowest yield of 1211.0 kgha<sup>-1</sup> (Table 6.7). AGRAC-216, which gave the highest yield under drought stress, had stress STI value of 0.7, AMP of 2169.6, and GMP of 2145.5. However, among the progenies, BC×I had TOL value of -57.6 and DII of 0.0 despite having STI value of 1.0, AMP of 1461.8, with GMP of 1461.5 and DII of 0.0 (Table 6.7).

AGRAC-216 had the highest 100-seed weight of 20.1 and 20 g, respectively, under both drought-stress and well-watered conditions. A1B×D registered the lowest 100-seed weight (8.6 g) under drought stress, while Beledi C had the lightest 100-seed weight (7.4 g) under well-watered conditions (Table 6.8).

Under drought stress conditions, Asontem gave the highest number of seeds per pod (17) compared to AGRAC-216 and BC×D with the lowest number of seeds (11.7) per pods. Apagu 1B had the highest number of seeds per pod (16) and Mouride registered the lowest number of seeds per pod (11) under well-watered condition. BC×I recorded the highest number of pods per plant (11.7) and Dan Ila registered the lowest numbers of pods per plant (8.4) under drought stress environment. Whereas IT93K-503-1 had the highest number of pods per plant (39.4) and TA×D had the lowest pod number per plant (20.9) under well-watered condition. (Table 6. 8).

In terms of maturity, Mouride took the longest number of days (64 days) to maturity and Titinwa A had the shortest number of days (53 days) to reach 95% maturity (extra early)

under drought stress condition. Dan Ila took the longest number of days (71 days) to attain 95% maturity and A1B×D had the shortest number of days (54.0 days) to reach 95% maturity under optimum condition. Under drought stress condition, Dan Ila and Mouride had the longest number of days (53 days) to attain 50% flowering, whereas BC×M had the shortest number of days (42 days) to reach 50% flowering. Dan Ila took 48.0 days to achieve 50% flowering whereas both TA×D and TA×M attained 50% flowering at 37.0 days under well-watered condition. Under drought stress condition, both Asontem and IT93K-503-1 had the highest HI value (44%), while L1B×D had the lowest HI value (9%). Asontem had the highest HI (31%) and BA×D registered the lowest HI value (9%) under the well-watered condition (Table 6.8).

Table 6. 7: Seed yield performance of 25 genotypes and other agronomic traits measured under drought stressed and well-watered conditions at Fumesua

Attribute	Genotypes	WW	DS	STI	AMP	GMP	TOL	DII
High Yielding Ability	AGRAC-216	2492.0	1847.2	0.7	2169.6	2145.5	644.8	0.3
	IT93K-503-1	2698.0	1739.2	0.6	2218.6	2166.2	958.8	0.4
	Asontem	2750.0	1648.0	0.6	2199.0	2128.8	1102.0	0.4
	Dan Ila	2070.0	1494.8	0.7	1782.4	1759.0	575.2	0.3
	BC×I	1433.0	1490.6	1.0	1461.8	1461.5	-57.6	0.0
	Mouride	2073.0	1464.0	0.7	1768.5	1742.1	609.0	0.3
	Laduni 1B	1833.0	1454.4	0.8	1643.7	1632.8	378.6	0.2
	L1B×I	1711.0	1377.8	0.8	1544.4	1535.4	333.2	0.2
	A1B×M	1884.0	1301.2	0.7	1592.6	1565.7	582.8	0.3
	BA×D	1282.0	1279.2	1.0	1280.6	1280.6	2.8	0.0
Medium Yielding Ability	BA×I	2504.0	1274.2	0.5	1889.1	1786.2	1229.8	0.5
	L1B×M	1901.0	1272.2	0.7	1586.6	1555.1	628.8	0.3
	TA×I	1403.0	1213.4	0.9	1308.2	1304.8	189.6	0.1
	L1B×D	1647.0	1211.4	0.7	1429.2	1412.5	435.6	0.3
	TA×M	1798.0	1174.6	0.7	1486.3	1453.2	623.4	0.3
	BA×M	1991.0	1171.4	0.6	1581.2	1527.2	819.6	0.4
	TA×D	1387.0	1126.0	0.8	1256.5	1249.7	261.0	0.2
	Titinwa A	1839.0	1110.0	0.6	1474.5	1428.7	729.0	0.4
	Beledi A	1384.0	1056.6	0.8	1220.3	1209.3	327.4	0.2
	BC×M	2028.0	1052.2	0.5	1540.1	1460.8	975.8	0.5
Low Yielding Ability	Apagu 1B	1296.0	1051.8	0.8	1173.9	1167.5	244.2	0.2
	A1B×I	1303.0	1028.2	0.8	1165.6	1157.5	274.8	0.2
	A1B×D	1211.0	976.2	0.8	1093.6	1087.3	234.8	0.2
	Beledi C	1315.0	905.4	0.7	1110.2	1091.1	409.6	0.3
	BC×D	1381.0	818.2	0.6	1099.6	1063.0	562.8	0.4
Mean		1784.6	1261.5					
CV%		25.30	15.82					
Significance		***	***					

WW: well-watered condition, DS: Drought-stressed condition, STI: Stress tolerance index, AMP: Arithmetic means productivity, GMP: Geometric means productivity, TOL: Drought tolerance index, DII: Drought intensity index

Table 6. 8: Means from analysis of variance measured under drought stress and well-watered conditions at Fumesua 2018

Genotype	Env 2 (Fumesua Well-Watered)							Env 5 (Fumesua Drought-Stressed)						
	HI	SY (kg/ha)	100swt (g)	NSP	NPP	95%mat	50%flo	HI	SY (kg/ha)	100swt (g)	NSP	NPP	95%mat	50%flo
A1B×D	16.8	1211.0	9.1	13.0	24.1	54.0	41.0	16.9	976.0	8.6	13.2	10.2	63.0	47.0
A1B×I	20.9	1303.0	9.6	13.8	24.9	56.0	40.0	13.8	1028.0	9.7	13.5	9.5	60.0	46.0
A1B×M	20.1	1884.0	11.0	13.7	24.7	56.0	38.0	24.8	1301.0	10.8	14.2	10.1	58.0	43.0
AGRAC-216	19.9	2492.0	20.0	12.7	21.8	59.0	46.0	38.8	1847.0	20.1	11.7	9.6	54.0	43.0
Apagu 1B	15.4	1296.0	9.3	16.0	23.0	56.0	42.0	30.9	1052.0	9.1	13.8	10.1	57.0	46.0
Asontem	31.3	2750.0	14.6	14.6	30.6	58.0	48.0	9.7	1648.0	13.5	17.0	8.7	60.0	49.0
BA×D	8.9	1282.0	9.9	13.0	26.0	60.0	42.0	12.7	1279.0	10.6	13.5	10.6	63.0	45.0
BA×I	15.3	2504.0	11.5	12.7	37.3	55.0	41.0	10.8	1274.0	11.0	13.3	10.4	59.0	42.0
BA×M	13.5	1991.0	10.8	13.8	32.3	57.0	40.0	22.4	1171.0	10.9	13.2	9.8	62.0	50.0
BC×D	21.8	1381.0	9.7	12.8	26.0	59.0	39.0	16.1	818.0	8.8	11.7	9.6	61.0	46.0
BC×I	13.3	1433.0	10.7	12.4	27.1	59.0	39.0	21.9	1491.0	11.4	13.6	11.5	59.0	42.0
BC-M	18.2	2028.0	9.7	12.0	31.9	60.0	38.0	24.6	1052.0	9.6	12.4	10.6	58.0	46.0
Beledi A	10.5	1384.0	11.0	12.8	26.4	59.0	42.0	23.7	1057.0	10.6	13.3	8.9	61.0	52.0
Beledi C	12.4	1315.0	7.4	11.3	32.0	59.0	40.0	24.0	905.0	9.0	12.1	10.0	54.0	42.0
Dan Ila	18.7	2070.0	16.1	12.0	24.8	71.0	48.0	35.7	1495.0	16.2	13.2	8.4	63.0	53.0
IT93K-503-1	30.0	2698.0	13.6	13.8	39.4	67.0	47.0	43.7	1739.0	15.4	13.3	10.4	61.0	48.0
L1B×D	14.4	1647.0	10.8	14.8	25.9	55.0	38.0	8.7	1211.0	10.5	14.4	9.5	58.0	42.0
L1B×I	24.9	1711.0	10.8	15.5	23.0	59.0	37.0	33.9	1378.0	10.8	15.8	9.7	58.0	46.0
L1B×M	14.0	1901.0	11.1	14.5	25.9	58.0	38.0	13.4	1272.0	11.1	14.9	9.2	60.0	48.0
Laduni 1B	24.4	1833.0	11.6	15.7	26.7	57.0	41.0	24.8	1454.0	13.3	13.4	9.8	52.0	43.0
Mouride	24.4	2073.0	15.8	11.0	24.7	66.0	49.0	18.9	1464.0	16.0	12.0	9.2	64.0	53.0
TA×D	13.5	1387.0	11.9	13.3	20.9	59.0	37.0	15.3	1126.0	10.8	13.5	9.4	63.0	47.0
TA×I	15.2	1403.0	11.1	12.7	25.3	58.0	38.0	19.3	1213.0	11.1	13.6	9.6	60.0	45.0
TA×M	17.9	1798.0	10.6	13.5	23.9	55.0	37.0	24.2	1175.0	10.7	13.5	9.6	59.0	44.0
Titinwa A	17.8	1839.0	10.9	13.3	30.7	55.0	39.0	24.2	1110.0	11.5	12.5	9.3	53.0	42.0
<b>Mean</b>	<b>18.1</b>	<b>1784.6</b>	<b>11.5</b>	<b>13.4</b>	<b>27.2</b>	<b>59.0</b>	<b>41.0</b>	<b>22.1</b>	<b>1261.4</b>	<b>11.7</b>	<b>13.5</b>	<b>9.7</b>	<b>59.0</b>	<b>46.0</b>
CV%	43.7	25.3	6.9	1.9	18.3	5.9	4.0	53.0	15.8	9.9	7.4	8.3	7.6	10.2
Lsd p<0.05	13.6	773.1	1.4	8.2	8.5	5.9	2.8	20.1	341.7	2.0	1.7	1.4	7.7	8.04

Hi: Harvest index, SY: Seed yield, 100swt: 100-seed weight, NPP: Number of pods per plant, NSP: Number of seeds per pod, 95%mat: 95% maturity and 50%flo: 50% flowering

### 6.3.4 Combined Analysis of Variance

Combined analysis of variance across the three test locations revealed highly significant ( $P < 0.001$ ) differences among the genotypes for all measured traits under both watering regimes (Table 6.9). Mean seed yield, 100–seed weight, number of pods per plant, number of seeds per pod, pod length, number of days to 95% maturity, number of days to 50% flowering and harvest index were significantly different among the cowpea genotypes under each watering regime. Environmental differences were significant ( $P < 0.001$ ) for all the measured traits. Watering regime was the main source of variation among the genotypes for seed yield and most of its components measured in this study showing highly significant ( $P < 0.001$ ) differences among the genotypes (Table 6.9 and Fig. 6.3).

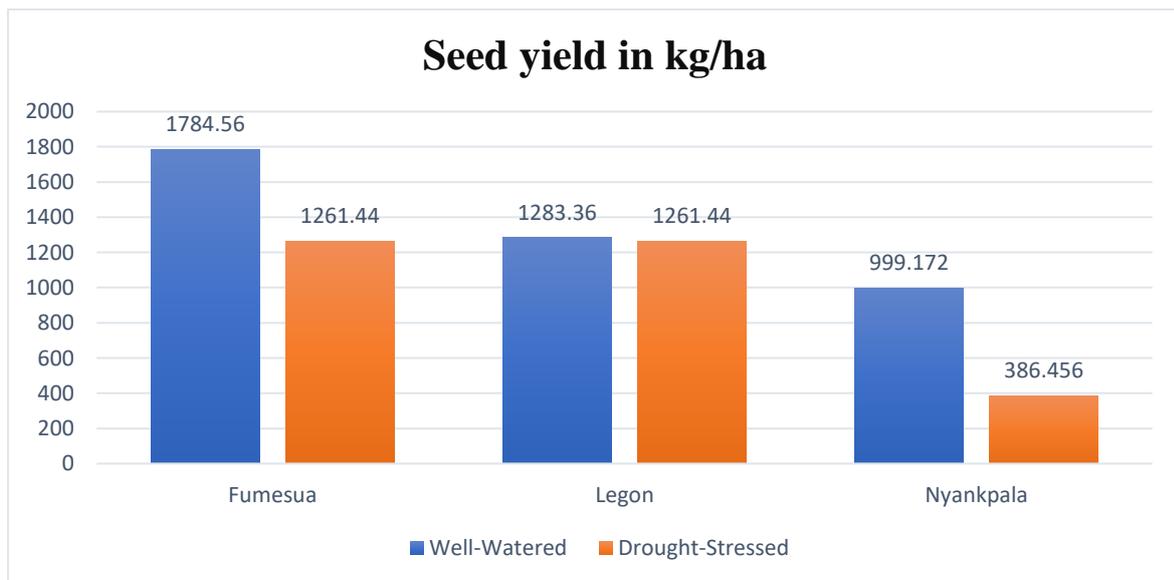


Fig. 6. 3 Estimated mean squares of seed yield across sites under drought stress (DS) and well-watered (WW) conditions

The structural equation modelling (SEM) and the extension of several multivariate analyses explained the relationships among variables. Number of days to 50% flowering and 95% maturity are exogenous variables and tend to be negatively related with number of pods per plant similar to the association between HI and number of seeds per pod. HI was positively associated with seed yield similar to the relationship between number of pods per plant and number of seeds per pod. The result also revealed that number of seeds per pod and number pods per plant had a positive influence on seed yield (Fig. 6.4).

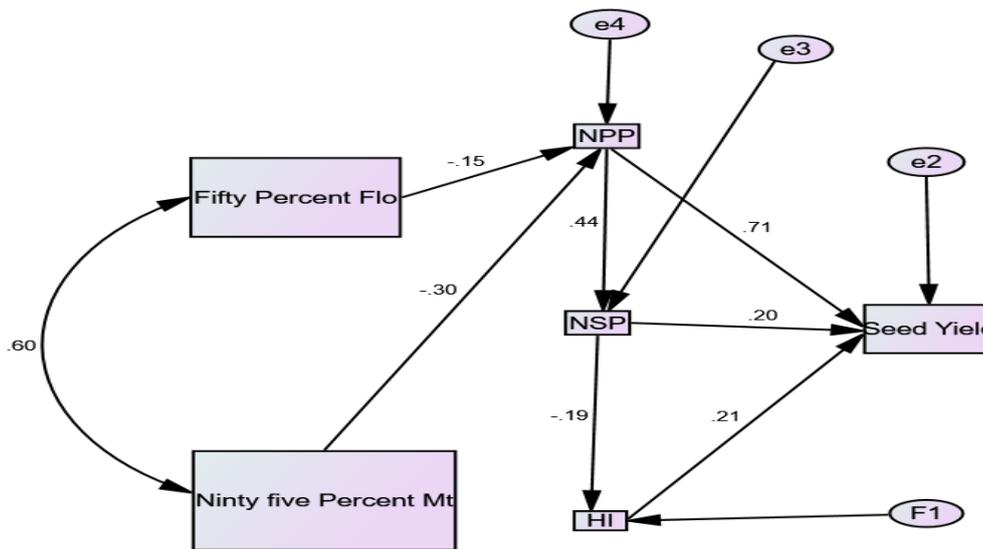


Fig. 6. 4 Path diagram cause (traits studied) and effect (seed yield) relationship of 25 cowpea genotypes

Table 6.9: Mean squares from the combined analysis of variance of seed yield and yield components measured under drought stress and well-watered conditions

Source of Variation	DF	Seed Yield (kgha <sup>-1</sup> )	100SWT (g)	NSP	Pod length (cm)	NPP	95%mat	50% flowering	HI (kgha <sup>-1</sup> )
<b>Drought–Stressed Condition</b>									
Bloc	4	81346	8.0	4.6	11.3	14.6	119.6	109.6	314
Rep	2	215751	1.0	0.0	0.5	0.7	390.3	133.0	578.5
Geno	24	199113***	65.3***	4.2**	26.82***	9.3***	69.0*	91.9***	440.7***
Env	2	14445667***	47.9***	1527.6***	652.0***	659.5***	13279.2***	1311.4***	1489.1***
Geno × Env	48	172397***	6.6***	4.1***	4.2***	8.9***	44.0*	18.0*	250.6**
Error	144	69975.0	2.9	2.1	1.0	2.7	43.4	13.6	166.5
Total	224	235618.0	10.9	16.4	10.5	10.8	171.1	37.3	232
<b>Well–Watered Condition</b>									
Bloc	4	332390	23.5	1.4	8.1	74.1	51.2	62.5	281.2
Rep	2	387552	0.6	19.6	2.3	21.9	6.1	114.5	2258.2
Geno	24	699491***	63.3***	10.4***	30.4***	67.6***	88.3***	106.0***	502.9*
Env	2	11858298***	10.2***	339.7***	223.1***	5550.5***	5516.4***	845.4***	15923.7***
Geno × Env	48	361764***	3.8***	5.5***	2.3***	39.0***	14.0*	6.9*	319.6*
Error	144	183543	1.6	2.7	1.2	19.5	9.5	6.7	320.5
Total	224	385732	9.1	7.3	6.7	79.2	68.8	26.8	495.8

\*, \*\*, \*\*\* Significantly different at 0.05, 0.01 and 0.001 levels of probability, respectively, 100SWT: 100–seed weight, NSP: Number of seeds per pod, NPP: Number of pods per plant, 95% mat: Number of days to 95% maturity, 50% flowering: Number of days to 50% flowering and HI: Harvest index

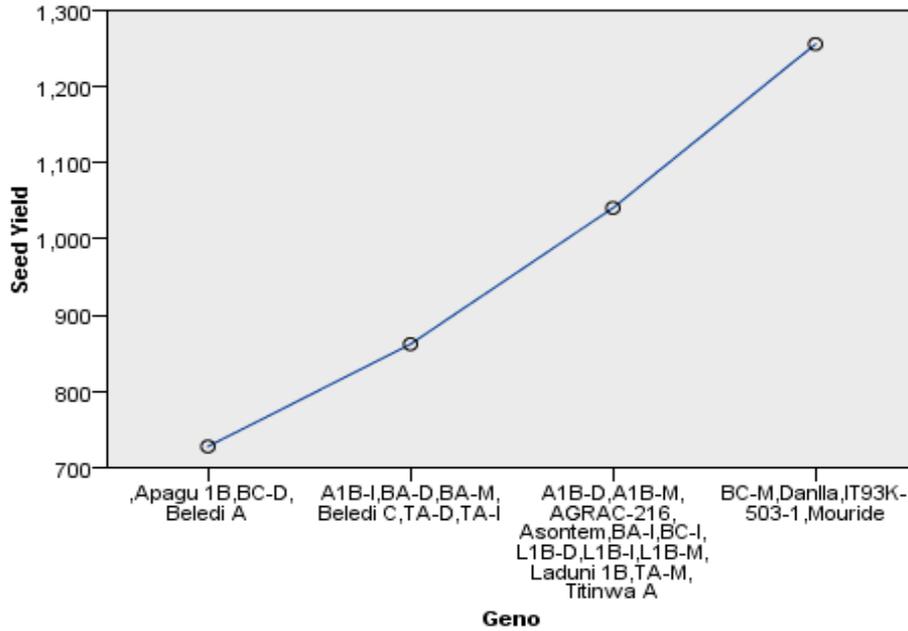


Fig. 6. 5 Estimated means of overall seed yield of 25 cowpea genotypes at ( $P < 0.05$ )

**NB:** Geno (genotypes) and seed yield ( $\text{kg ha}^{-1}$ )

In the combined analysis of variance across stress and non-stressed environments, genotype, environment (individual water regimes considered as environment) and genotype by environment interactions were significant ( $P < 0.001$ ) sources of variation among genotypes for seed yield and yield components (Table 6.10). Significant differences were found for genotype by environment interaction for HI and number of pods per plant. Genotype and genotype by environment interaction were not significantly ( $P < 0.05$ ) different for the number of days to 95% maturity and the number of days to 50% flowering. However, Apagu 1B, Beledi C  $\times$  Dan lla and Beledi A had the lowest yield. Whereas, Beledi  $\times$  Mouride, Dan lla, IT93K–503–1 and Mouride were reported to be high yielding across the testing sites (Fig. 6.5).

Table 6.10: Mean squares from the combined analysis of variance for seed yield and yield components of 25 cowpea genotypes measured across three test sites under drought stress and well-watered conditions

Source of Variation	D.F	Seed Yield (kgha <sup>-1</sup> )	100SWT (g)	NSP	Pod length (cm)	NPP	95%mat	50% flowering	HI
Bloc	4	301939	25.8	4.6	117.9	42.8	109.96	192.4	359.8
Rep	2	67698	2.1	5.6	22.7	16.4	167.8	224.7	487.1
Geno	24	607686***	120.955***	10.7***	55.4**	34.7***	145.03***	187***	750.8***
Env	5	16418495***	23.016***	827.5***	706.4***	5190.6***	7915.1***	1057.5***	7449.5***
Geno × Env	120	270609***	4.789***	3.9***	25.8*	30.5***	36.53ns	11.1ns	273.1ns
Pooled Error	294	129843	2.5	2.4	31.8	13.7	30.31	10.0	255.1
Total	449	375650	9.9	12.5	39.7	77.2	127.23	34.0	368.5

\*, \*\*, \*\*\* Significantly different at 0.05, 0.01 and 0.001 levels of probability, respectively, 100SWT: 100–seed weight, NSP: Number of seeds per pod, NPP: Number of pods per plant, 95% mat: Number of days to 95% maturity, 50% flowering: Number of days to 50% flowering and HI: Harvest index

### 6.3.5 AMMI Stability Analysis

The seed yield AMMI analysis showed significant ( $P < 0.001$ ) differences for genotype, environment, and genotype by environment interaction. Environment, genotype, and GEI accounted for 63.8, 10.8% and 25.4% of the total sum of squares, respectively. A high proportion of the sum of squares of the AMMI model was due to environment and GEI effects. Additionally, the model divided the GEI sum of squares into interaction principal component axes (IPCA) and residual. The mean squares of the first three IPCA were significantly different and accounted for 83.5% of the total variation (Table 6.11).

The graphical representation of the AMMI analysis in the biplot revealed that genotype environmental responsiveness is enormous with each environment influencing a set of genotypes (Fig. 6.6). The AMMI analysis for the first singular axis captured the highest percentage (38.9%) of the variability. The values of PC1 component showed that genotypes designated as 4 (AGRAC-216), 6 (Asontem), 8 (BA×I) and 16 (IT93K-503-1) were the best and equally productive under both well-watered and drought stressed conditions. The most stable genotypes were IT93K-503-1, Beledi C × Mouride and Mouride producing seed yield above average trial mean ( $995.54 \text{ kg ha}^{-1}$ ).

Table 6. 11: AMMI analysis of variance for seed yield across locations

Source of Variation	D.F	SS	MS	Sum of Squares Explained		
				%Total	%G × E	%G × E Cumulative
Env	5	82092476.1	16418495.2***	63.8		
Geno	24	13879110.0	578296.3***	10.8		
Env × Geno	120	32672984.1	272274.9***	25.4		
IPCA 1	28	12491716.6	446132.7***	39.8	39.8	39.8
IPCA 2	26	9790907.4	376573.4***	31.2	31.2	71.0
IPCA 3	24	3930368.2	163765.3	12.5	12.5	83.5
IPCA 4	22	3225490.7	146613.2	10.3	10.3	93.8
IPCA 5	20	1955458.4	97772.9	6.2	6.2	100.0
Residuals	300	40022335.3	133407.8			

\*\*\* Significantly different 0.001 level of probability

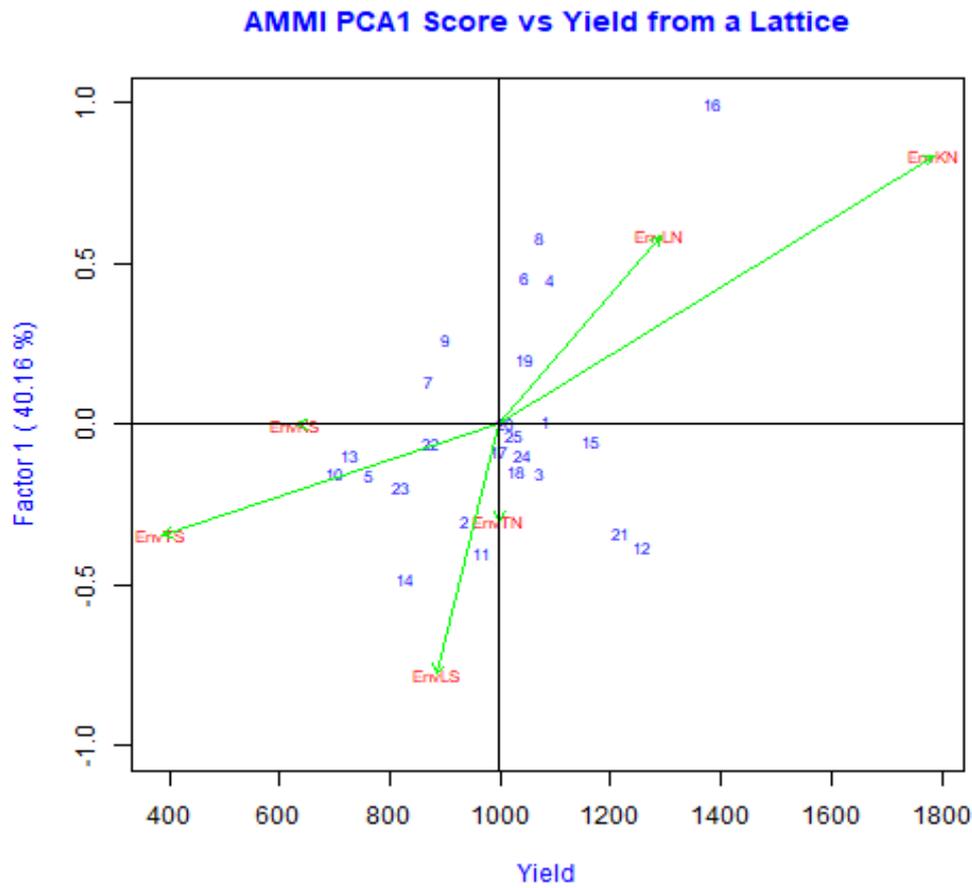


Fig. 6. 6 AMMI 1biplot for seed yield of 25 cowpea genotypes denoted by numbers and six environments using genotypic and environmental scores

**ENVIRONMENTS:** ENVLN: Legon well-watered, ENVLS: Legon drought stressed, ENVKS: Fumesua well-watered, ENVKT: Fumesua drought stressed, ENVTN: Nyankpala well-watered and ENVTS: Nyankpala drought stressed

**GENOTYPES:** Dan Ila (15), Asontem (6), AGRAC-216 (4), IT93K-503-1 (16), Mouride (21), BC×M (12), Beledi C (14), BC×D (10), BA×I (8), BA×D (7), Laduni 1B (12), Titinwa A (25), TA×M (24), L1B×I (18), L1B×M (19), L1B×D (17), BA×M (9), BC×I (10), Beledi A (13), TA×D (22), Apagu 1B (5), TA×I (23), A1B×M (3), A1B×D (1) and A1B×I (2)

### 6.3.6 GGE Biplot Analysis

The first two principal components of the biplot explain 62.6% of the variability among the genotypes across the test environments. There were a few of the genotypes designated by numbers on the crests of the polygon. Genotypes at the vertex of the polygon were 16 (IT93K-503-1), 21 (Mouride), 12 (BC×M), 14 (Beledi C), 10 (BC×D), 8 (BA×I), and 7 (BA×D). The majority of the genotypes were found within the polygon (Fig. 6.7).

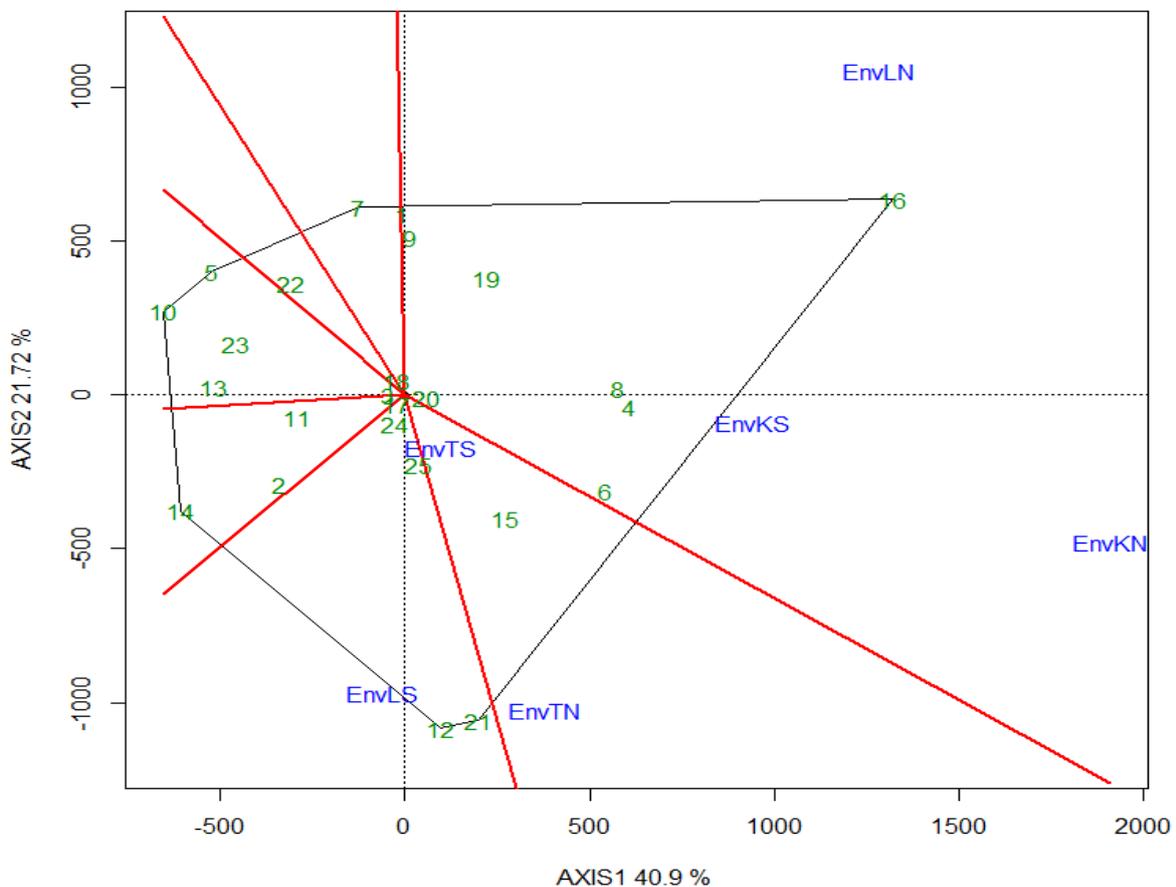


Fig. 6. 7 Polygon view of genotype by environment interaction of 25 cowpea genotypes

Genotypes on the peak had a long detachment from the origin of the biplot. Genotype 16 (IT93K-503-1) in ENVLN (Legon well-watered) is a vertex genotype with the highest seed yield. Environments Nyankpala drought stress (ENVTS), Fumesua drought stress (ENVKS),

Fumesua well-watered (ENVKN) and Nyankpala well-watered (ENVTN) were laid on the same portion of the graph, suggesting that these environments do not differ significantly in terms of seed yield. Genotypes 12 (BC×M) and 21 (Mouride) were the only two genotypes in the environment ENVTN (Fig..6.7). Genotypes 25 (Titinwa A), 24 (TA×I), and 18 (L1B×I) were less responsive and low yielding compared to the vertex genotypes. The best genotypes were 16 (IT93K–503–1), 21 (Mouride), and 12 (BC×M) (Fig. 6.7).

The discrimination pattern of the genotypes and environmental representativeness showed that ENVTS and ENVTN were more representative environments for testing cowpea genotypes and likewise ENVKS and ENVKT. Environments with long vectors, ENVLS, ENVLN, ENVTN, and ENVKN, were more discriminating than those with short vectors, indicating their ability for genetic discrimination (Fig..6.8).

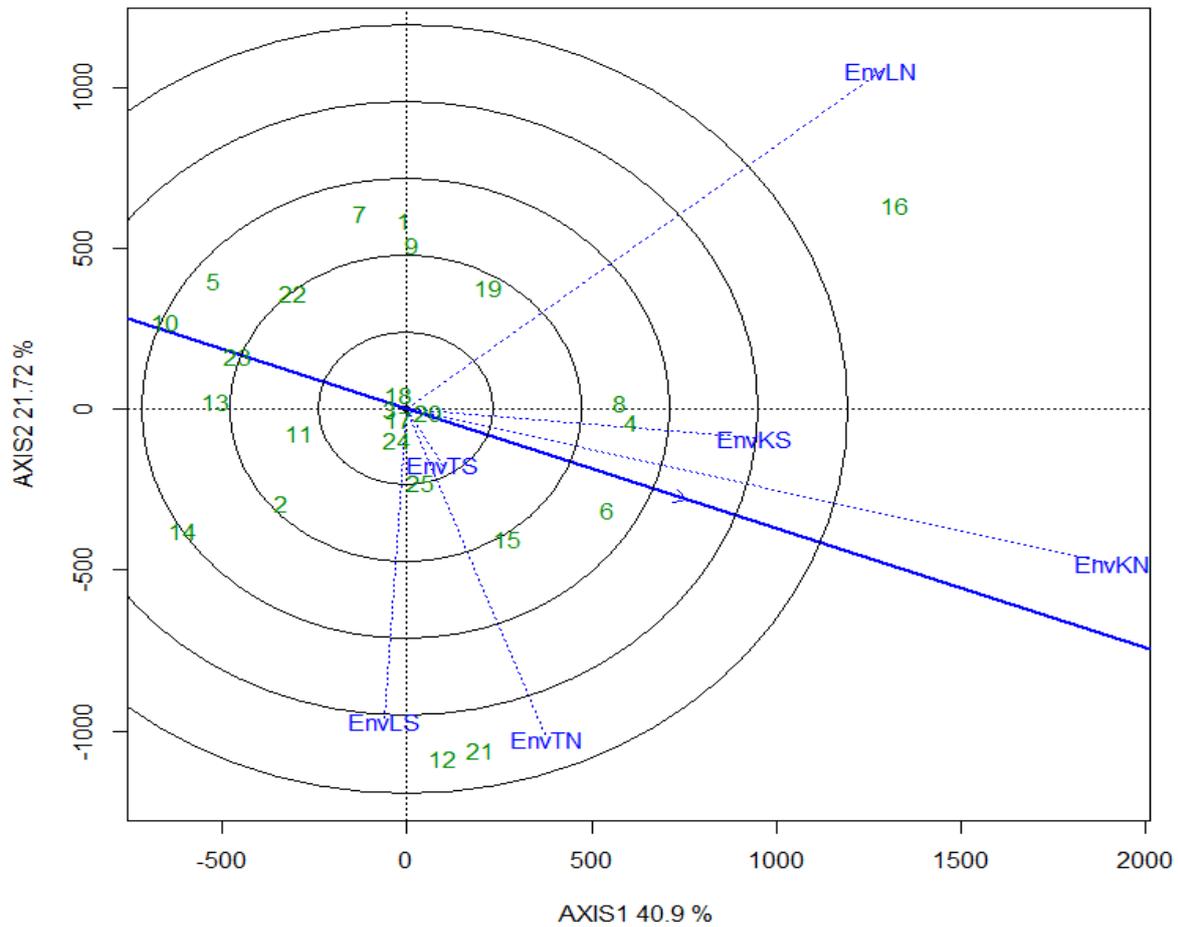


Fig. 6. 8 Discrimination power and representativeness of test sites

The rationale behind the visual display of mean vs stability in GGE biplot analysis is to help breeders compare genotypes based on performance and stability across testing sites. The equality lines which connected lines between genotypes also aid in grouping the genotypes into a specific environment based on their responsiveness. Among the 25 cowpea genotypes evaluated across six environments, genotype 6 (Asontem) was the most stable and high yielding followed by genotypes 4 (AGRAC-216) and 8 (BA×I). However, genotype 16 (IT93K-503-1) was high yielding but unstable across test environments. Genotype 20 (Laduni 1B) was found to be a low responsive genotype across the test environments, but suitable only for the unfavourable environments (Fig..6.9).

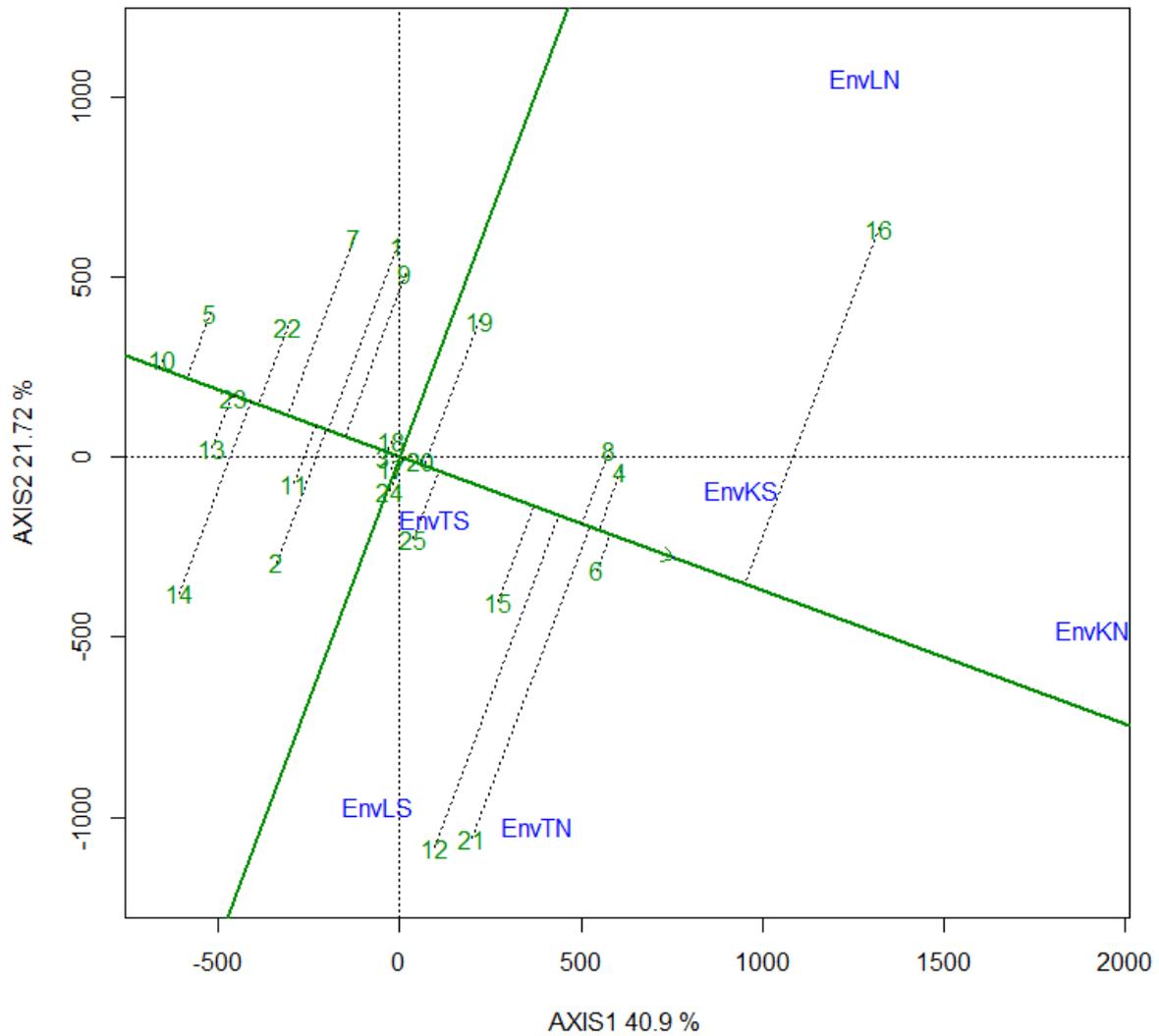


Fig. 6. 9 Mean vs Stability view of 25 cowpea genotype main effect and GEI effect

### 6.3.7 Adaptability and Yield Stability Analyses

With respect to seed yield, genotypes BC×M, Dan Ila, TA×M, L1B×I, A1B×D Mouride and A1B×M had good performance and were stable across the six test environments. They had higher mean seed yield; their  $b_i$  values were less than one and had non-significant  $s^2d_i$  values (Table 6.8). Mouride, IT93K-503-1, L1B×D, L1B×M, Laduni 1B, Titinwa A, AGRAC-216, Asontem and BA×I had higher mean seed yield and non-significant  $s^2d_i$ , but their  $b_i$  values were either equal or more than ( $b_i \leq 1.0$ ). Based on Wricke's Ecovalence analysis of adaptability and stability, Laduni 1B was found to be stable, and AGRAC-216 and BA×I were adaptable (Table 6.8). Laduni 1B had the lowest value, followed by L1B×D, and L1B×I (99150.2). Asontem registered the highest Ecovalence value of 1174065.

Genotypes that are high yielding across sites with low coefficients of variability ( $CV_i$ ) are considered to be stable. Accordingly, A1B×M, BC×M, Dan Ila, and L1B×I had  $CV_i$  values lower than 40% and seed yield of more than  $1000 \text{ kg ha}^{-1}$ , whereas BA×I had  $CV_i$  value of 68.7% with seed yield of  $1180.6 \text{ kg ha}^{-1}$  (Table 6.12).

Table 6.12: Means estimates of adaptability and phenotypic stability for 25 cowpea genotypes evaluated across three locations under drought stressed and well-watered conditions

Genotype	Code	Mean	Rank	Eberhart & Russell				Wricke's Ecovalence		Francis and Kannenberg		
				bi	S <sup>2</sup> di	Rank	R <sup>2</sup>	Wi	Sd	CV (%)	Rank	
A1B×D	1	1168.6 abcd	9	0.7	210679.9	2	0.4	1083814	569.5	48.7	16	
BC×D	10	766.6 e	25	0.7	-30663.2	24	0.9	140384.5	345.8	45.1	12	
BC×I	11	1090 bcde	16	0.6	-6174.8	14	0.7	348026.3	316.7	29.1	1	
BC×M	12	1346.4 ab	2	0.6	126614.3	4	0.4	812650.8	476.7	35.4	4	
Beledi A	13	814.7 de	24	0.9	22510.1	11	0.8	271734	477.5	58.6	21	
Beledi C	14	904.4 cde	22	0.7	61269.2	6	0.6	506372.2	440.4	48.7	15	
Dan Ila	15	1288 abc	4	0.9	-9956.2	16	0.9	134438.9	467	36.3	6	
IT93K-503-1	16	1526.1 a	1	1.8	223245.7	1	0.8	1747226	956.9	62.7	22	
L1B×D	17	1100.4 bcde	15	1.1	-21834.3	22	0.9	86270	512.9	46.6	13	
L1B×I	18	1139.3 abcde	11	0.9	-20576.6	21	0.9	99150.2	440.4	38.7	7	
L1B×M	19	1151.2 abcde	10	1.2	35789.1	8	0.8	342413	600.1	52.1	18	
A1B-I	2	1021.6 bcde	17	0.7	-17817.2	19	0.9	187867.3	363	35.5	5	
Laduni 1B	20	1125.8 bcde	13	1.1	-36059.3	25	1	28299.8	497.6	44.2	11	
Mouride	21	1342.7 ab	3	1	123347.9	5	0.6	663875.9	583.5	43.5	10	
TA×D	22	961.7 bcde	20	0.8	-17129.1	18	0.9	157783.4	389.1	40.5	8	
TA×I	23	919.9 cde	21	0.6	-13501.1	17	0.8	283249	323.5	35.2	3	
TA×M	24	1132.8 abcde	12	0.9	-8580.7	15	0.9	138622.7	472.6	41.7	9	
Titinwa A	25	1117.8 bcde	14	1.1	-5730.8	13	0.9	168855.9	561.8	50.3	17	
A1B×M	3	1177.4 abcde	8	0.7	-25143	23	0.9	148802.4	361.9	30.7	2	
AGRAC-216	4	1240.7 abc	5	1.7	34643.4	10	0.9	836004.3	831.4	67	23	
Apagu 1B	5	843.4 de	23	0.8	-17842.1	20	0.9	140399.9	402.1	47.7	14	
Asontem	6	1183.3 abcd	6	1.6	139600.7	3	0.8	1174065	856.9	72.4	25	
BA×D	7	976.9 bcde	19	1	35441.5	9	0.8	312233.8	541.9	55.5	19	
BA×I	8	1180.6 abcd	7	1.7	9740.1	12	0.9	708310.3	810.8	68.7	24	
BA×M	9	996.7 bcde	18	1.1	46530.1	7	0.8	367308.6	581.3	58.4	20	
<b>Overall mean</b>		<b>1100.4</b>						<b>4435526</b>				

Means followed by a similar letter belongs to the same class (P<0.05), bi: regression coefficient R<sup>2</sup>: regression coefficient of determination Wi: Ecovalence Sd: deviation from the regression, CVi: Francis and Kannenberg's Coefficient of variability

The mean AMP (Arithmetic means productivity) for seed yield across six environments was 1104.0 kgha<sup>-1</sup>. Genotype IT 93K-503-1 had the highest AMP value of 1554.0 kgha<sup>-1</sup>, whereas BC×D had the lowest value of 748 kgha<sup>-1</sup>. A similar trend was observed in GMP for these genotypes. IT93K-503-1 had the highest GMP value (1398.8 kgha<sup>-1</sup>) and BC×D scored the lowest value (722.9 kgha<sup>-1</sup>). AGRAC-216 had the highest GMP (GMP: Geometric means productivity) value (18.0) for 100-seed weight, while A1B×D had the lowest (8.3). L1B×D had the highest 100-seed weight percent reduction index of 19.9 and Beledi C had the lowest value of -8.3 (Table 6.13). L1B×I had the highest GMP (12.9) for the number of seeds per pod, whereas TA×I had the lowest GMP value of 10.1. Percent reduction for HI revealed that Laduni 1B had the highest index of 20.3, while BC×I had the lowest value of 2.7. Beledi C had the highest GMP of 13.4 for number of pods per plant and AGRAC-216 had the lowest value (8.9). The highest percent reduction (60.7%) seed yield was observed in IT93K-503-1 and TA×I had the lowest value (18.0%) (Table 6.13).

Table 6.13: Overall mean performances of 25 cowpea genotypes evaluated across sites under drought stressed and well-watered conditions

Genotype	NPP				NSP				100–seed yield (g)				Seed yield kg <sup>ha</sup> <sup>-1</sup>					
	WW	DS	GMP	PR%	WW	DS	GMP	PR%	WW	DS	GMP	PR%	WW	DS	AMP	GMP	PR%	STI
<b>Parents</b>																		
Apagu 1B	17.0	8.5	12.1	50.0	13.6	11.1	12.3	18.4	8.9	8.5	8.7	4.5	1007	685.6	846	830.9	31.9	0.3
Laduni 1B	19.4	7.6	12.1	60.8	14.7	11.7	13.1	20.4	11.1	11.9	11.5	-7.2	1444	868.8	1156	1120.1	39.8	0.6
Mouride	15.5	7.0	10.4	54.8	11.0	10.2	10.6	7.3	17.1	16.3	16.7	4.7	1604	1078	1341	1315	32.8	0.9
Beledi A	17.9	5.3	9.7	70.4	12.1	10.9	11.5	9.9	10.1	10.1	10.1	0.0	1044	548	796	756.4	47.5	0.3
Beledi C	20.7	8.7	13.4	58.0	11.5	10.7	11.1	7.0	8.2	8.9	8.5	-8.5	999	795.4	897	891.4	20.4	0.4
Dan Ila	16.4	7.0	10.7	57.3	11.3	10.9	11.1	3.5	17.3	16.6	17	4.0	1435	1112.2	1274	1263.3	22.5	0.8
IT93K–503–1	26.7	6.0	12.7	77.5	13.8	11.2	12.4	18.8	13.9	14.7	14.3	-5.8	2232	876.5	1554	1398.7	60.7	1
Titinwa A	21.5	7.0	12.3	67.4	12.2	10.0	11.0	18.0	11.4	11	11.2	3.5	1487	774.7	1131	1073.3	47.9	0.6
<b>Mean</b>	<b>19.4</b>	<b>7.1</b>	<b>11.7</b>	<b>63.4</b>	<b>12.5</b>	<b>10.8</b>	<b>11.6</b>	<b>13.6</b>	<b>12.3</b>	<b>12.2</b>	<b>12.3</b>	<b>0.8</b>	<b>1406.5</b>	<b>842.4</b>	<b>1124</b>	<b>1081.1</b>	<b>40.1</b>	<b>0.6</b>
<b>Crosses</b>																		
A1B×D	17	8.4	11.9	50.6	11.9	10.9	11.4	8.4	9	7.6	8.3	15.6	1472	807.9	1140	1090.5	45.1	0.7
A1B×I	16.5	7.9	11.4	52.1	12.6	11.1	11.8	11.9	9.3	9	9.1	3.2	1068	826.9	947	939.7	22.6	0.5
A1B×M	16.8	7	10.8	58.3	12.1	10.8	11.4	10.7	12.1	11.4	11.8	5.8	1374	986.8	1180	1164.4	28.2	0.8
BA×D	19.1	6.5	11.2	66.0	12.2	10.9	11.5	10.7	9.5	8.8	9.1	7.4	1303	656	980	924.5	49.7	0.5
BA×I	20.4	5.8	10.9	71.6	11.8	11	11.4	6.8	11.4	11.1	11.3	2.6	1622	748.4	1185	1101.8	53.9	0.7
BA×M	20.4	5.2	10.3	74.5	11.8	10.4	11.1	11.9	11	10.2	10.6	7.3	1306	774.1	1040	1005.5	40.7	0.6
BC×D	17.1	5.9	10	65.5	11.7	10.1	10.8	13.7	8.7	8.4	8.6	3.4	940	556	748	722.9	40.9	0.3
BC×I	20	8	12.7	60.0	11	10.7	10.8	2.7	10.6	9.9	10.3	6.6	1202	1017.8	1110	1106.1	15.3	0.7
BC×M	21	8.2	13.1	61.0	11.1	10.3	10.7	7.2	10.9	10	10.4	8.3	1553	1062.7	1308	1284.7	31.6	1
L1B×D	17.7	6.9	11.1	61.0	13.5	11.5	12.4	14.8	11.1	10.5	10.8	5.4	1474	830.6	1152	1106.5	43.6	0.7
L1B×I	15.8	7.3	10.7	53.8	13.3	12.5	12.9	6.0	11.4	9.1	10.2	20.2	1365	955.1	1160	1141.8	30.0	0.8
L1B×M	16.7	6.6	10.5	60.5	13.2	11.6	12.4	12.1	11.7	11	11.4	6.0	1438	897.2	1168	1135.9	37.6	0.8
TA×D	15.5	6.8	10.2	56.1	11.4	9.6	10.4	15.8	11.8	10.7	11.2	9.3	1168	702.3	935	905.7	39.9	0.5
TA×I	17.6	6.2	10.4	64.8	10.4	9.9	10.1	4.8	10.2	10.5	10.3	-2.9	976	800.5	888	883.9	18.0	0.5
TA×M	17	6.4	10.4	62.4	11.6	10.4	10.9	10.3	12.3	13.1	12.7	-6.5	1304	895	1100	1080.3	31.4	0.7
<b>Mean</b>	<b>17.9</b>	<b>6.9</b>	<b>11</b>	<b>61.5</b>	<b>12</b>	<b>10.8</b>	<b>11.3</b>	<b>10.0</b>	<b>10.7</b>	<b>10.1</b>	<b>10.4</b>	<b>5.6</b>	<b>1304.3</b>	<b>834.5</b>	<b>1069</b>	<b>1039.6</b>	<b>36.0</b>	<b>0.6</b>
<b>Checks</b>																		
AGRAC–216	13.6	5.8	8.9	57.4	11	9.6	10.3	12.7	18.7	17.6	18.2	5.9	1521	963.4	1242	1210.5	36.7	0.6
Asontem	14.8	6.2	9.6	58.1	14.1	11.6	12.8	17.7	14.2	13.7	13.9	3.5	1554	874	1214	1165.4	43.8	0.6
<b>Mean</b>	<b>14.2</b>	<b>6</b>	<b>9.2</b>	<b>57.7</b>	<b>12.5</b>	<b>10.6</b>	<b>11.5</b>	<b>15.2</b>	<b>16.5</b>	<b>15.6</b>	<b>16.1</b>	<b>5.5</b>	<b>1537.5</b>	<b>918.7</b>	<b>1228</b>	<b>1188</b>	<b>40.2</b>	<b>0.6</b>
<b>Overall mean</b>	<b>18</b>	<b>6.9</b>	<b>11.1</b>	<b>61.7</b>	<b>12.2</b>	<b>10.8</b>	<b>11.5</b>	<b>11.5</b>	<b>11.8</b>	<b>11.4</b>	<b>11.6</b>	<b>3.4</b>	<b>1362.2</b>	<b>846.1</b>	<b>1104.0</b>	<b>1068.9</b>	<b>37.9</b>	<b>0.6</b>

WW: Well-watered condition, DS: Drought-stressed condition, NPP: Number of pods per plant, NSP: Number of seeds per pod, GMP: Geometric productivity mean, Arithmetic mean productivity, STI: Stress tolerance index and PR%: Percent reduction

#### 6.4 Discussion

The analyses of multi-location trials conducted in Nyankpala, Fumesua and Legon revealed that genotype, environment, and genotype by environment interactions were significant thus influencing the performance of the studied traits. This result corroborates with other reports (Temesgen *et al.*, 2015, Sharifi *et al.*, 2017 and Dia *et al.*, 2018), who conducted trials under contrasting condition and reported effects of environment and GEI on their performance.

Drought and high-temperature stresses were pronounced during the field evaluation in Nyankpala and seed yield was reduced by 38.7%. This finding agrees with results of a study conducted by Devasirvatham and Tan (2018) who reported that drought and heat stresses could reduce seed yield of field crops by 50%. Although the results obtained through boxplot analysis revealed the impact of drought stress on the genotypes evaluated in Nyankpala evidently high, the site could still be a good testing environment for drought tolerance study. Equally, it may be concluded that Legon and Fumesua could be good test sites performance study for optimum growing conditions.

The path analysis aspect of the study revealed a total positive association of  $r = 0.91$  between number of pods per plant, number of seeds per pod, harvest index and seed yield. This association implies that an increment of an independent variable by a unit will increase the other dependent variable (seed yield). Thus, high seed yield could be obtained through selection for increased HI, number of pods per plant, number of seeds per pod. These results agree with the findings of Sousa *et al.* (2015) who used yield components for indirect selection.

Site-specific environmental influences on the performance of the traits were also observed. A1B×D, the highest yielding genotype under an optimum condition at Legon in the Coastal Savanna agro-ecological zone, outperformed the two checks and parent IT93K-503-1. However, it gave an appreciable yield above the average under drought stress condition. This

implies that the environment had an impact on yielding ability and maturity and drought tolerance of the genotypes evaluated in this study. Thus, this genotype should be tested across multiple sites to confirm the consistency of its yield performance.

Environmental responsiveness of the genotypes showed their levels of tolerance using different indices. The same pattern of variation was observed at Nyankpala in the Guinea Savanna agro-ecological zone, where BC×M out yielded all the other genotypes with the exception of IT93K-503-1. This performance could be a result of genotype, environment, and genotype by environment interaction effects. Genotypes evaluated at Fumesua in the Forest agro-ecological zone registered the highest seed yield under both drought stress and well-watered conditions, suggesting that Fumesua is an ideal environment for growing cowpea.

This trend in the performance of traits across the test sites suggests genotypic differences which influenced the expression of the traits. The variation exhibited by the genotypes revealed a significant amount of variability that could be exploited to improve early maturing cowpea genotypes with higher levels of tolerance to drought for farmers. Some of the identified early maturing genotypes, especially those combining earliness with drought tolerance, could be advanced through further yield and on-farm trials for production in drought-prone areas.

Ten progenies (A1B×D, A1B×I, A1B×M, BA×D, BA×M, BA×I, L1B×D, TA×M, TA×D, and TA×M) attained 95% maturity at 54 and 57 days in two environments (ENVKS and ENVLS) indicating that they are extra-early in maturity. Such genotypes are important for farmers to provide food during the period of hunger gap when planted early and also have the advantage of producing seed yield under terminal drought when planted late in the season. This finding agrees with results obtained by Cláudio *et al.* (2003) who classified cowpeas into those that matured in 60 days after planting as extra-early and those that matured

between 61 to 70 days after planting as early maturing genotypes. The medium-early varieties take between 71 to 80 days to mature and medium-late are those that matured in 81 to 90 days after sowing. The late maturing varieties attained maturity in 91 days after planting.

Farmers in drought-prone areas cultivate cowpea as an insurance crop, particularly if they expect rainfall too low for growing maize; they resort to growing crop varieties that are early and medium early maturing in 60 to 80 days. Of the fifteen progenies evaluated in this study, ten demonstrated earliness with good levels of tolerance to drought completing their life cycle in less than 60 days. Dias *et al.* (2009) reported that earliness can best be evaluated through the duration from seedling emergence to an indication of the first flower. Adeyanju and Ishiyaku (2007) and Hall (2012) reported that erect and short cycle cowpeas are good for the drought-prone Sahel zones of West Africa. Padi (2007) stated that earliness and seed size are important traits for cowpea adoption in the Savannas of West Africa.

The link between time to 50% flowering and drought stress was also observed in this study. Drought stress delayed the number of days to 50% flowering and number of days to 95% maturity to great extent. This result agrees with the findings of Kazan and Lyons (2016) who observed that abiotic stresses such as drought stress influence flowering time.

The use of the established biometrical tools to assess the adaptability of genotypes and their yield and yield components stability is crucial, particularly when the varieties to be evaluated are newly developed populations. In this study, the AMMI stability analysis identified some genotypes including AGRAC-216, BA×I, BC×I and Laduni 1B as adaptable and stable though some of these genotypes were low yielding. Environment accounted for 63.8% of the total variability in the experiments, while genotype accounted for only 10.8% and the genotype and environment interaction had slightly higher (24.4%) contribution to the total variation. This result showed that environment explained the highest portion of the observed variance and therefore largely influenced the performance of genotypes. Thus, more test sites

should be considered for logical inferences about the performance of these genotypes. The results of this study agree with the findings of Rashidi *et al.* (2013) who reported slightly higher values for environment (81.2%). Genotypes G7 (BA×D), G9 (BA×M), G10 (BC×D), G13 (Beledi A), G22 (TA×D), G5 (Apagu 1B), G23 (TA×I), G14 (Beledi C) and G2 (A1B×I) showed specific adaptability to drought-stress environments. Test environments ENVLS, ENVKS, and ENVTS had seed yield less than the trial mean (995.6 kgha<sup>-1</sup>) suggesting that these genotypes demonstrated specific adaptability. They expressed good performance with high seed yield in a specific test environment(s).

In this study, GGE biplot explained 62.5 % of the total yield variation in the test environments. However, IT93K-503-1 could be planted by farmers under conditions such as EVNLN (Legon well-watered), ENVKN (Fumesua well-watered), and ENVKS (Fumesua drought-stressed) and obtained high yields. Mouride and BC×M showed specific adaptability thus, these two genotypes could only yield to their potential at Nyankpala under well-watered (ENVTN) and Legon drought stressed (ENVLS) conditions. AGRAC-216 was relatively responsive across the testing locations, but had low grain yield compared to Dan Ila, IT93K-503-1 and BC×M. Thus, testing this genotype across other contrasting sites could result into assigning it to a specific location. The remaining genotypes were not responsive and expressed low yield potential, far below the average trial mean yield (1104.0 kg ha<sup>-1</sup>). The poor performance of these genotypes may be attributed to GEI effects and their genetic backgrounds.

In the polygon view of the discriminating environments, ENVLN (Legon well-watered), ENVTN (Nyankpala drought-stressed), ENVKS (Fumesua well-watered) and ENVLS (Legon drought-stressed) were strongly correlated and most discriminatory, suggesting that removing one of the locations as a test environment would not lead to any loss of information. This could cut down on resources that could be put to better use in other

locations (Meseka *et al.*, 2016). This finding confirms the proposal of Yan and Tinker (2005) who postulated that test environments that are none discriminating provide no information on the genotypes and therefore should not be used as test environments.

Seed yield of 25 cowpea varieties was affected linearly by the amount of water applied. Drought stress clearly reduced seed yield of the 25 genotypes, suggesting that inducing drought at the reproductive phase of the crop disrupts reproductive mechanisms including floral and pod development, number of ovules per locules and pod filling contributing to high seed yield. Although drought stress negatively affects the three phases, the degree of the effect is determined by the severity and duration of drought stress. In this study, a yield component, number of pods per plant was most severely affected by drought stress. On an average, drought stress reduced the number of pods per plant by 61%. This high level of reduction could be attributed to the environmental responsiveness of the genotypes. These findings agreed with the results obtained by Pandey *et al.* (1984) who reported a similar trend of reduction of yield components in cowpea.

## **6.5 Conclusions**

The genotype and environment main effects and genotype by environment interaction effects were significant for seed yield and other agronomic traits in 25 cowpea genotypes evaluated in this study. Five of the best ten progenies, in terms of seed yield across the environments, were A1B×D, BC×M, L1B×M, A1B×M, and BA×D. Some of these genotypes out yielded their parents as well as the checks. Additionally, seven of the 25 genotypes showed stability and adaptability, qualifying them as good performers across the test environments. Genotypes BC×M, L1B×I, TA×M and A1B×M could be further tested under drought stress to confirm their ability to tolerate drought stress and produce appreciable seed yield, which could provide the opportunity for their possible release to farmers.

## CHAPTER SEVEN

### 7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

#### 7.1 General Conclusions

A genetic divergence study was carried out in 106 cowpea germplasm collections using phenotypic and molecular approaches. There was high cophenetic correlation coefficient (CCC) of 0.76 resulting from nine discriminatory clusters using morphological data. However, molecular data indicated a higher level of genetic divergence among the assembled cowpea panel compared to morphological data, suggesting that a good opportunity exists for population development through introgression of new alleles from different backgrounds. The SNP markers used in this study could be utilized to analyse and group new cowpea collections in the future. The cowpea accessions from South Sudan were closely related to the materials from West Africa. This could reasonably be attributed to an early 1980's IITA's germplasm expedition to South Sudan who collected some cowpea accessions from the Equatoria region of South Sudan. The evaluation of the 106 accessions revealed that Dan Ila, Mouride, and IT93K-503-1 were drought tolerant with both Type 1 and 2 drought tolerance adaptabilities. Five farmers' preferred accessions from South Sudan, Titinwa A, Beledi A, Beledi C, Laduni 1B, and Apagu 1B, were susceptible to drought stress. However, the progenies from the crosses between drought tolerant and farmers' susceptible varieties were evaluated under well-watered and drought stressed conditions. Three outstanding combinations, Beledi A × Dan Ila, Titinwa A × IT93K-503-1, and Beledi C × Mouride, with desirable, significant and positive SCA effects under drought stress were identified. The negative SCA effects detected in Titinwa A × Dan Ila; Beledi A × IT93K-503-1, Laduni 1B × Mouride and Beledi C × Dan Ila crosses suggested that they were early maturing.

Drought stress is highly heterogeneous in time and space and is unpredictable. This trait makes it hard to identify representative drought stress conditions. Environmental influence is associated with drought stress since phenotype is the ultimate expression of the interaction between genotype and environment. The unpredictable and variable forms in which drought stress manifests itself make the selection of individual genotypes and breeding for drought tolerance difficult. Multiple stresses occur, as was the case in Nyankpala (Tamale), where drought and heat stress were difficult to separate. The results of stability analysis revealed that BC×M, BC×I, L1B×I, TA×M and A1B×M were the highest yielding genotypes across the test environments. Determining useful selection indicators (traits) for yielding ability, drought tolerance and earliness in cowpea may be useful in varietal selection. These genotypes would be further assessed for earliness and drought tolerance and released to smallholder farmers growing cowpea in drought-prone areas.

## 7.2 Recommendations

- Heat tolerance should be considered as an important component when breeding cowpea for tolerance to drought.
- The four early-maturing and drought-tolerant genotypes exhibiting high and stable yields would be further evaluated in the farmers' fields to validate their performance for possible release.
- A genome-wide association study on the cowpea populations used in this study would be useful to identify loci associated with drought tolerance.
- Genomic data (SNPs) available from this study could be used for both background selection and diversity studies.

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## APPENDICES

## Appendix III: Genetic clustering of the cowpea accessions

Table 3.1 Distribution of 106 in nine clusters accessions of cowpea

Cluster	Number of Genotypes	Genotypes
1	35	AGRAC116, AGRAC216, AGRAC316, Apagu1A, Apagu1B, Apagu1C, Beledi A, Beledi B, Beledi C, GH5346B, WAC101, GH2347, GH5038, GH5346, GH6045, GH7220, GH7245, GH7875, IT08K1493, IT96D610, IT98D1399, Laduni1A, Rec017, Rec059, Laduni1B, Laduni2A, Laduni2B, Rec007, Rec014, Rec016, Titinwa A, Titinwa B, Titinwa C, WAC39 and Rec074
2	39	Amos V, B, Blackeye, GH3701A, WAC 81, BLK19, WAC 32, GH2306, GH2307, GH3668, GH3689, GH5043, GH7228, IT08K15012, IT08K15024, IT08K18011, IT08K18724, IT93K4521, IT93K5031, IT97K49935, IT97K56818, IT98K5031, IT98K5061, IT99K1122, IT99K57211, IT99K57321, KNI, Kvx303096G, Kvx3964525, Local 3, Mangala Mangala A, Pobe, Rec009, Rec021, Rec039, Rec041, Rec049, Rec105, Songotra
3	1	Apagu 2A
4	21	Dan lla, GH3701B, WAC19, WAC21, WAC115, WAC Q6, GH4524, IT08K125100, IT10K8177, IT97K56818, IT98K10921, IT98K2058, Kvx40481, Kvx74511, Mouride, Rec003, Rec046, Rec062, Rec083, Rec005, Rec108
5	2	WAC91 and Gorom
6	1	BLK452
7	5	GH2200, GH2338, IT96D604, Padi_tuya, and Rec064
8	1	GH4527
9	1	Mangala Mangala B

Table 3.2. Grouping of the genotypes into inferred clusters based on probabilities of association of greater than or equal to 35% to the 6 clusters.

Genotypes	Populations						Inferred Cluster
	1	2	3	4	5	6	
Songotra	0.828	0.072	0.001	0.071	0.026	0.001	1
Dan Ila	0.902	0.084	0.000	0.000	0.010	0.003	1
Padi_tuya	0.592	0.049	0.013	0.000	0.001	0.345	1
Beledi A	0.588	0.067	0.009	0.001	0.000	0.335	1
IT08K1493	0.712	0.000	0.286	0.001	0.001	0.001	1
Titinwa A	0.001	0.000	0.001	0.001	0.040	0.957	6
AGRAC116	0.306	0.002	0.321	0.053	0.003	0.316	Admixture
AGRAC216	0.007	0.003	0.163	0.102	0.001	0.724	6
Laduni_1A	0.046	0.002	0.929	0.002	0.020	0.001	3
IT08K18724	0.408	0.125	0.000	0.000	0.055	0.411	6
IT08K15024	0.001	0.178	0.000	0.000	0.820	0.000	5
Apagu1A	0.838	0.032	0.000	0.000	0.129	0.001	1
Laduni_1B	0.106	0.252	0.011	0.134	0.064	0.434	5
Titinwa B	0.236	0.604	0.002	0.007	0.069	0.081	2
Black eye	0.666	0.028	0.032	0.001	0.273	0.000	1
Beledi C	0.001	0.001	0.004	0.001	0.002	0.991	6
AGRAC316	0.001	0.207	0.000	0.791	0.000	0.001	4
Laduni_2A	0.001	0.823	0.001	0.007	0.073	0.096	2
Mangala_Mangala A	0.004	0.317	0.001	0.022	0.149	0.507	6
IT08K18011	0.002	0.000	0.002	0.994	0.001	0.000	4
IT08K15012	0.002	0.915	0.000	0.003	0.037	0.043	2
Apagu_2A	0.001	0.902	0.000	0.001	0.095	0.001	2
IT99K57211	0.928	0.000	0.069	0.001	0.001	0.001	1
IT97K56818	0.785	0.001	0.204	0.003	0.000	0.007	1
IT10K8177	0.001	0.001	0.995	0.002	0.000	0.001	3
IT97K49935	0.689	0.144	0.022	0.006	0.134	0.005	1
IT99K57321	0.722	0.001	0.000	0.080	0.196	0.000	1
Local_3	0.159	0.050	0.007	0.177	0.366	0.242	5
IT98K5061	0.885	0.007	0.001	0.100	0.005	0.002	1
IT99K1122	0.681	0.001	0.172	0.001	0.139	0.006	1
IT96D610	0.002	0.000	0.995	0.002	0.001	0.000	3
IT98K5031	0.477	0.003	0.000	0.154	0.318	0.047	1
Titinwa C	0.001	0.000	0.996	0.002	0.000	0.000	3
IT98K2058	0.645	0.000	0.000	0.000	0.355	0.000	1
IT93K5031	0.007	0.001	0.976	0.011	0.004	0.001	3
IT96D604	0.182	0.673	0.000	0.000	0.144	0.000	2
IT98K10921	0.000	0.001	0.000	0.000	0.996	0.002	5
GH4527	0.302	0.001	0.001	0.379	0.310	0.008	4
GH7228	0.997	0.001	0.000	0.001	0.001	0.000	1
GH2338	0.615	0.097	0.007	0.001	0.248	0.032	1
GH3689	0.538	0.293	0.000	0.000	0.168	0.000	1
GH2347	0.003	0.213	0.000	0.781	0.001	0.001	4
GH5038	0.207	0.019	0.393	0.089	0.003	0.289	3
Mangala Mangala B	0.001	0.989	0.000	0.001	0.009	0.001	2
GH4524	0.508	0.281	0.006	0.001	0.182	0.022	1
GH2307	0.002	0.001	0.003	0.110	0.001	0.883	6
GH3701_B	0.708	0.168	0.001	0.064	0.001	0.058	1
GH2306	0.026	0.079	0.848	0.001	0.046	0.000	3
GH5043	0.002	0.909	0.001	0.001	0.082	0.005	2
GH7220	0.005	0.003	0.004	0.059	0.002	0.927	6
GH3668	0.001	0.000	0.995	0.000	0.003	0.000	3
GH7875	0.826	0.001	0.110	0.002	0.000	0.062	1
GH3701_A	0.396	0.225	0.001	0.001	0.186	0.192	1
GH6045	0.118	0.002	0.129	0.003	0.141	0.606	6
GH7245	0.154	0.684	0.004	0.001	0.156	0.001	2
GH5346	0.002	0.091	0.000	0.003	0.037	0.867	6

GH2200	0.198	0.214	0.167	0.013	0.311	0.098	Admixture
GH5346_B	0.019	0.001	0.972	0.001	0.005	0.002	3
Rec005	0.001	0.959	0.000	0.000	0.039	0.000	2
IT97K56818	0.003	0.634	0.161	0.142	0.060	0.000	2
Apagu_2B	0.068	0.001	0.910	0.019	0.001	0.001	3
WAC91	0.002	0.000	0.003	0.990	0.001	0.004	4
WAC_81	0.875	0.001	0.118	0.005	0.001	0.000	1
Laduni_2B	0.002	0.977	0.001	0.001	0.018	0.001	2
IT08K125100	0.005	0.442	0.318	0.164	0.070	0.001	2
WAC101	0.007	0.166	0.001	0.064	0.642	0.121	5
WAC19	0.522	0.199	0.001	0.003	0.152	0.123	1
WAC21	0.796	0.062	0.001	0.001	0.139	0.001	1
Apagu1C	0.029	0.000	0.967	0.001	0.000	0.002	3
BLK19	0.006	0.001	0.889	0.030	0.000	0.074	3
Apagu1B	0.024	0.274	0.004	0.147	0.483	0.068	5
WAC115	0.001	0.003	0.001	0.001	0.001	0.993	6
WAC_Q6	0.642	0.157	0.120	0.003	0.077	0.002	1
BLK452	0.650	0.001	0.329	0.001	0.011	0.009	1
IT93K4521	0.722	0.108	0.017	0.004	0.143	0.007	1
WAC39	0.025	0.000	0.000	0.000	0.973	0.000	5
WAC32	0.153	0.001	0.793	0.010	0.015	0.028	3
Rec003	0.748	0.096	0.001	0.002	0.128	0.024	1
Rec007	0.001	0.706	0.000	0.001	0.290	0.002	2
Rec062	0.917	0.000	0.080	0.001	0.001	0.001	1
Rec021	0.003	0.001	0.994	0.001	0.001	0.001	3
IT98D1399	0.001	0.167	0.000	0.000	0.831	0.001	5
Rec083	0.802	0.000	0.195	0.001	0.001	0.000	1
Rec046	0.745	0.001	0.001	0.040	0.132	0.081	1
Rec016	0.005	0.914	0.000	0.001	0.079	0.000	2
Amos V	0.001	0.001	0.000	0.000	0.000	0.997	6
Rec105	0.012	0.009	0.018	0.050	0.003	0.907	6
Rec059	0.001	0.001	0.000	0.000	0.060	0.937	6
Rec064	0.008	0.001	0.867	0.003	0.040	0.082	3
Rec041	0.005	0.091	0.057	0.040	0.002	0.805	6
Rec014	0.002	0.000	0.016	0.968	0.014	0.000	4
Rec074	0.001	0.700	0.001	0.001	0.297	0.000	2
Rec049	0.995	0.001	0.000	0.001	0.003	0.000	1
Rec009	0.997	0.001	0.001	0.000	0.001	0.000	1
Rec017	0.003	0.962	0.001	0.001	0.033	0.000	2
Rec108	0.253	0.243	0.005	0.001	0.003	0.494	6
Rec039	0.002	0.000	0.996	0.001	0.001	0.000	3
Mouride	0.387	0.321	0.068	0.001	0.177	0.048	1
Gorom	0.009	0.001	0.002	0.052	0.003	0.933	6
Pobe	0.001	0.559	0.151	0.001	0.288	0.000	2
KVX3964525	0.706	0.079	0.074	0.029	0.075	0.037	1
Kvx74511	0.007	0.963	0.001	0.007	0.021	0.002	2
KNI	0.025	0.655	0.000	0.000	0.319	0.000	2
Kvx40481	0.001	0.001	0.001	0.001	0.687	0.309	5
Kvx303096G	0.788	0.089	0.000	0.002	0.011	0.109	1
Beledi B	0.096	0.458	0.002	0.081	0.215	0.148	2