

**UNIVERSITY OF KWAZULU- NATAL**

**GENETIC IMPROVEMENT OF COWPEA [*Vigna unguiculata*  
(L.) Walp.] FOR GRAIN YIELD AND YIELD COMPONENTS  
IN ZAMBIA**

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**2020**

**Genetic Improvement of Cowpea [*Vigna unguiculata* (L.) Walp.] for Grain Yield and Yield Components in Zambia**

By

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**A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of  
Philosophy (PhD) in Plant Breeding**

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Republic of South Africa

November 2020

## THESIS ABSTRACT

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Cowpea [*Vigna unguiculata* (L.) Walp.] is a multiple purpose grain legume widely cultivated in Zambia for food security and local markets. However, cowpea is yet underutilized and under researched with a relatively low market share compared with other common legumes. Farmers are using low yielding cowpea varieties such as landraces and introduced varieties that are affected by many biotic and abiotic constraints prevalent in the country. Therefore, the objectives of the study were: (1) to identify farmers' preferences and production constraints and perception on cowpea production in Zambia to guide pre-breeding, (2) to assess the genetic diversity among cowpea genotypes using phenotypic traits and single nucleotide polymorphism (SNP) markers and, to select distinct and complementary genotypes for developing improved cultivars, (3) to determine combining ability and gene action controlling yield and yield components among crosses derived from eleven-selected cowpea parents and (4) to quantify the genotype by environment interaction effect and select cowpea genotypes with high grain yield and average adaptation across selected cowpea growing environments in Zambia.

In the first study, farmers' preferences and production constraints were determined through participatory rural appraisals (PRA) research tools such as transect walks, semi-structured questionnaires and focus group discussions. Data were collected using structured questionnaires with 187 farmers and focused group discussions with 14 participants selected in Eastern, Southern, and Northern provinces of Zambia. Among the respondents, a higher proportion of respondents (93.6%) used cowpea landraces for production, while only 6.4% used improved varieties. The major farmer perceived constraints were low yielding varieties (45.1%), limited access to production inputs (14.8%), pests and diseases (18.1%). Farmer-preferred traits of cowpea varieties included high yield (46.7%), good eating quality (5%), pest and disease resistance (15%). The participant farmers indicated a willingness to adopt improved cowpea cultivars.

The second study determined the extent of genetic diversity present among a collection of 100 cowpea accessions from Zambia and Malawi using 14 phenotypic traits and 14,116 high-density single nucleotide polymorphism (SNP) markers. The number of pods plant<sup>-1</sup> (NPP), pod length (PDL), and the number of seeds pod<sup>-1</sup> (NSP) was significantly ( $P<0.05$ ) affected by genotype  $\times$  environment interaction effects. Genotypes such as CP411, CP421, CP645, CP732, Chimponongo, and MS1-8-1-4 exhibited higher grain. Grain yield had significant ( $P<0.05$ )

associations with NPP ( $r=0.50$ ), NSP ( $r=0.46$ ) and PDL ( $r=0.42$ ) useful for simultaneous selection for yield improvement in cowpea. The SNP markers revealed gene diversity and polymorphic information content of 0.22 and 0.17, respectively, showing that the tested cowpea accessions were genetically diverse. Test genotypes were classified into four genetic groups irrespective of the collection source, allowing selection and subsequent crosses to develop breeding populations for cultivar development. Genotypes Bubebe, CP411, CP421, CP645, Chimponogo, and MS1-8-1-4 were identified as the most genetically divergent and high yielding making them ideal parental lines for breeding.

The third study determined the combining ability effects, gene action controlling yield, and yield components among selected cowpea genotypes consisting of 11 parental lines and 55 progenies derived through a half-diallel mating design. Both progenies and parents exhibited significant ( $P<0.05$ ) variation for the assessed yield and yield components except for the number of pods per plant (NPP). The general combining ability (GCA) effects of parents and the specific combining ability (SCA) effects of progenies were significant ( $P<0.05$ ) for all assessed traits except days to 50% flowering (DTF) and the number of seeds per pod (NSP). Parental lines CP411 and CP732 were the best combiners for NPP, HSW, and GYD. Also, genotype CP411 exhibited desirable GCA effects for DTF and days to 90% maturity (DTM). Crosses such as CP732  $\times$  CP411, BBXSC103  $\times$  CP411, and Lutembwe  $\times$  CP645 had higher and desirable SCA effects for grain yield. Traits such as HSW, NPP, and DTM were conditioned by mainly additive gene action, while DTF, PDL, and NSP were under the control of dominant genes.

The fourth study quantified the genotype by environment interaction (GEI) of 30 selected cowpea genotypes for higher yield, yield-stability and adaptation across four environments using the additive main effects and multiplicative interaction (AMMI) and genotype and genotype by environment interaction (GGE) models. The AMMI analysis indicated that the environment, genotype, and GEI effects were highly significant ( $P<0.001$ ) and accounted for 66.97, 12.4, and 20.63% of the total variation, respectively. The test environments were delineated into three mega- environments, with Lusitu and Mansa clustered together while Nanga and SCCI were distinct individual mega-environments. The ideal environment for discriminating among the genotypes was the Lusitu site. Genotypes LT11-3-3-12, Bubebe, and Msandile were specifically adapted to the Nanga, Lusitu-mansa, and SCCI mega-environments,

respectively. Families CP421 × CP732, CP645 × MS1-8-1-4, MS1-8-1-4 × BBXSC103, and a mutant line BB10-4-2-3 were identified as high yielding with average stability across the four sites and recommended for future cowpea breeding programs.

Overall, the present study appraised the major preferences and perceived production constraints of cowpea growers to guide future breeding. Also, new cowpea breeding populations were developed with enhanced yield and yield components for further genetic advancement and multilocation selection for variety release and deployment in Zambia.

## **DECLARATION**

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I, Nelia Nkhoma, declare the following

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
  - a) Their words have been re-written but the general information attributed to them has been referenced.
  - b) Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

**Signed**

.....  
Nelina Nkhoma

As the candidate's supervisor, I agree to the submission of this dissertation

.....  
**Prof. H. Shimelis** (Supervisor)

.....  
**Prof. M. Laing** (Co-supervisor)

## ACKNOWLEDGEMENTS

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I would like to express my heartfelt and sincere appreciation to the following individuals and institutions for their contribution to the successful completion of this work.

- My major supervisor Prof. Hussein Shimelis for field supervision, guidance, genuine and constructive criticisms, suggestions, contributions and overall support. My co-supervisor, Prof. Mark Laing is sincerely thanked for his guidance and devoted support throughout the research work and thesis write up.
- My family including Pyera, Rabecca, Lamuell, Develias, Justin, Bezai, Kelvin, Letitia and Mpatso are highly appreciated for the warm support, prayers and encouragements.
- The Seed Control and Certification Institute (SCCI) and Zambia Agricultural Research Institute (ZARI) are recognised for providing research facilities that led to the successful implementation of this project.
- The Alliance for a Green Revolution in Africa (AGRA) is thanked for the financial support through the African Centre for crop improvement (ACCI).
- Rowelda Donnelly, Lydre Anderson and Susan van der Merwe from the University of KwaZulu Natal (UKZN)'s African Center for Crop improvement (ACCI) are sincerely acknowledged for the overall support during the study.
- Dr. Isack Mathew and Dr. Admire Shayanawako are thanked for their consistent proof reading, advice and encouragement during the development and write-up of the draft chapters.
- My colleagues Boster Nkukumbani, Grace Muzyamba, Yanda Shankwaya, Hardley Mwenya, Boston Mumanga, Eddy Malambo, Monty Hamunzowa, William Mwachilenga, Curtis Mweete, Sydney Mwamba and Siabusu are sincerely thanked for the technical support throughout the study.
- Drs. Patrick Chikoti, Bethseba Tembo, Richard Chanda and Kalaluka Munyinda are sincerely acknowledged for sharing the valuable experiences during the research and write-up.
- My ACCI colleagues from the 2017 Cohort, Chapwa, Esnart, Mulu, John, William, Wilson and Yared are sincerely thanked for their moral support during my study.

- Gospel impact church is thanked for their prayers.

## **DEDICATION**

This thesis is dedicated to the Almighty God for his mercies upon my life and to my son Mphatso Phiri for the unending love and joy he has brought to my life.

To my late parents, Develias and Esther Nkhoma, your hard work was never in vain.

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## Abbreviations and acronyms

AATF	African Agricultural Technology Foundation
AEC	Average environment Coordination
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive main effects and multiplication interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ASV	AMMI stability value
Beca- ILRI	Biosciences eastern and central Africa-International Livestock Research Institute
CAMV	Cowpea aphid borne mosaic virus
CGIAR	Consultative Group for International Agricultural Research
CMV	Cowpea mosaic virus
CPMoV	Cowpea mottle virus
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRSP	Bean/Cowpea collaboration Research Support Program
CV	Coefficient of variation
DArT	Diversity Arrays Technology
DF	Degree of freedom
DNA	Deoxyribonucleic acid
DTF	Days to 50% flowering
DTM	Days to 90% flowering
F	Inbreeding coefficient
F2	Second filial generation
F3	Third filial generation
FAO	Food and Agriculture Organisation of the United Nations
FGDs	Focus group discussions
GA	Genetic advance
GAM	Genetic advance as percent of mean
GART	Golden Valley Agricultural Research Trust
GBS	Genotype by Sequencing
GCA	General combining ability
GCV	Genotypic coefficient of variation
GD	Genetic Diversity
GBVs	Genomic estimated breeding value

GEI	Genotype by environment interaction	
GGE	Genotype and genotype by environment	
GMOs	Genetically Modified Organisms	
GRENEWCA	Genetic Resource Network for West and Central Africa	
GS	Genomic Selection	
GYD	Grain yield	
H <sup>2</sup>	Broad sense heritability	
HSW	Hundred seed weight	
IBPGR	International Board for Plant Genetic Resources	
IGSS	Integrated genotyping service and support	
IITA	International Institute of Tropical Agriculture	
INIA	Instituto Nacional de investigación Tecnológica Agraria Alimentaria	
IPCA	Interaction Principal Component Axis	
LED	Light-emitting diode	
LG	Linkage group	
LSD	Least significant differences	
MAF	Minor allele frequency	
MAS	Marker-assisted selection MCMC	Monte Carlo Markov Chain
NARS	National Agriculture Research Station	
NGICA	Network for Genetic Improvement of Cowpea for Africa	
NGOs	Non- governmental organisations	
NGS	Next generation sequencing	
NPGRCS	National Plant Genetic Resources Centres	
NPP	Number of pods per plant	
NSP	Number of seeds per pod	
PCA	Principal Component analysis	
PCV	Phenotypic coefficient of variation	
PDL	Pod length	
PGR	Regional Plant Genetic Resource	
PGRCU	Plant Genetic Resource for California University	
PGRFA	Plant Genetic Resource for Food and Agriculture, Brazil	
PIC	Polymorphic Information Content	
PRA	Participatory Rural Appraisal	
QTL	Quantitative Trait Loci	
RAPD	Random Amplified Polymorphic DNA	

RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant inbred lines
SADC	Southern Africa Development Community
SCA	Specific combining ability
SCAR	Sequence Characterised Amplified Region
SCCI	Seed Control and Certification institute
SDS	Seed size
SNF	Symbiotic Nitrogen Fixation
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
SSA	sub-Saharan Africa
SSR	Simple Sequence Repeat
TALENS	Transcription activator –like effector nucleases
UC	University of California
UPOV	International Union for the Protection of New Varieties of Plant
USDA	United States Department of Agriculture
ZARI	Zambia Agriculture Research Institute

## INTRODUCTION TO THESIS

---

### Background

Cowpea [*Vigna unguiculata* (L.) Walp],  $2n=2x=22$ ] is a self-pollinating legume crop mainly adapted to the tropical and subtropical regions of the world (Adigun et al. 2014). The crop is cultivated in more than 60 countries in Asia, the Middle East, Southern Europe, Africa, Central and Southern America (Singh et al. 2003). It is an important source of protein and essential nutrients such as iron (53.2 mg/kg), zinc (38.1 mg/kg), calcium (826 mg/kg) and magnesium (1915 mg/kg) useful in the food and feed industry (Boukar et al. 2019). It is valued as a companion crop in cereal based mixed cropping systems to replenish soil nitrogen through its symbiosis with the nitrogen fixing soil bacteria (Beena et al. 2018).

The global production of cowpea is estimated to be 6.5 million tons per annum on 14.5 million hectares of land (FAO, 2017). The leading world producers of cowpea are Nigeria and Niger with five and three million hectares of annual production, respectively (Singh, 2006). Cowpea is the most widely cultivated crop indigenous to Africa ranking second after groundnut (*Arachis hypogaea* L) in terms of total production (FAO, 2017). In Southern Africa, cowpea is widely cultivated by small-scale farmers in Zambia, Zimbabwe, Malawi, Namibia, Mozambique and Botswana (Horn et al., 2015; Molosiwa et al. 2016; Nkhoma et al. 2020). The average grain yield of cowpea in SSA ranges between 1 and 6 ton ha<sup>-1</sup>, which is far less than the potential yield of 3 ton ha<sup>-1</sup> reported elsewhere (Horn et al. 2015; Gerrano et al. 2017). The yield gap is attributable to a multitude of biotic and abiotic stresses and a lack of improved and high yielding cultivars in the region.

### Cowpea production constraints and breeding objectives in Zambia

The productivity of cowpea in SSA are challenged by an array of factors that included climate change-related stress, edaphic and socio-economic constraints. Climate change-related stresses such as drought and heat stresses, and poor soil fertility are among the major causes of the low crop yields in SSA (Enete and Amusa, 2016). For instance, the annual rainfall in Zambia has decreased by 50% affecting rain-fed agriculture systems (Kabisa et al. 2019). Drought and heat stress causes up to 60% reduction in grain yield of cowpea (Hampton et al., 2016). Terminal drought stress that occurs late in the growing season leads to a significant yield loss. Most

cowpea growing regions in SSA is dominated by rain-fed agriculture systems that are vulnerable to rainfall variability due to climate change. The ability of cowpea to withstand relatively high drought stress levels have rendered it suitable for cultivation in SSA to avert food security challenges. Heat stress causes failure of some of the commodity crops such as maize, rice and wheat that are highly sensitive to terminal drought. Some cowpea genotypes are highly sensitive to severe moisture stress during the vegetative and flowering stages. Boukar et al. (2018) reported that drought at the flowering stage reduced yield potential of cowpea ranging from 360 to 1000 kg ha<sup>-1</sup>. Therefore, early maturing cowpea varieties adapted to escape terminal drought and heat stresses would be recommended to improve cowpea production and productivity in SSA. Cowpea production in SSA is also challenged by poor soil fertility. Most farmers cannot access or afford inorganic fertilizers and often apply sub-optimal rates leading to poor harvests and nutrient depletion. While cowpea is a relatively efficient at nutrient mobilization and use, its yield potential is significantly reduced in nutrient deficient and acidic soils. Developing cultivars with enhanced tolerance to soil acidity and low nutrient availability will potentially improve cowpea productivity in SSA.

The most important biotic constraints of cowpea include insect pests and diseases. Aphids (*Aphis craccivora* Koch) and leafhoppers are the most important insects that affect cowpea at all stages of growth, while bud thrips (*Megalulothrips sjostedti*) (Trybom) are common during the flowering stage, and pod borers (*Maruca vitrata*) attack the pods and young shoots (Boukar et al. 2016). The most important storage pests are bruchids [*Callosobruchus maculatus* (Fabricius)]. Cowpea is affected by more than 20 viral diseases including cowpea aphid-borne mosaic virus (CABMV), cowpea mosaic virus (CMV) and cowpea mottle virus (CPMoV) that cause up to 90% yield losses (Mbeyagala et al. 2014). Bacterial pathogens such as *Xanthomonas campestris* pv. *vignicola* and *X. campestris* pv. *vignaeugiculatae* causes reduction in the number of pods per plant, seeds per pod and fodder production at 71, 68 and 53%, respectively. The most important fungal pathogens include anthracnose (*Colletotrichum lindemuthianum*), fusarium wilt (*Fusarium oxysporum* f.sp. *tracheiphilum*), foot rot (*F. solani* matt. Scc), rust (*Uromyces phaseoli* pers. wint) and scab (*Elsinoe phaseoli*) (Singh et al. 2003). Breeding for disease and insect pests resistance has been a major focus of cowpea improvement programs in SSA. Cowpea diseases may occur in combination or sequentially in a season. There are limited cowpea germplasm with durable or horizontal disease resistance. Hence,

evaluation of a diverse germplasm pool against several diseases and insect pests prevalent in the target environments is key to select breeding parents.

### **Breeding for yield and yield components**

Yield increment is the primary objective of most plant breeding programs. Breeding for yield has been pursued using conventional or marker-assisted breeding approaches. Conventional breeding entails selection of superior genotypes based on their phenotypic performance in a number of environments (Acquaah, 2015). However, direct selection for yield is difficult due to the polygenic nature of the trait and the confounding effects of the environment making conventional breeding more arduous. Many developmental traits that are controlled by many genes contribute to the final yield, which complicate direct selection for yield in multiple environments (Aliyu and Makinde, 2016). While direct selection for yield may not provide adequate selection response, the indirect selection for the complementary traits has been used effectively for yield improvement. The main yield contributing traits in cowpea include plant height, number of pod per plant, pod length, seed number per pod, hundred seed weight and grain yield (Sharma et al. 2017). Complementary traits that exhibit favourable correlations with grain yield can be used for selection under variable environments (Meena, et al. 2015). Yield and yield components are dynamic and their correlations vary across populations and prevailing environmental conditions. Therefore, it is paramount to evaluate yield and yield related components of a set of breeding population to deduce associations among yield components that could facilitate multiple traits selection in diverse environments.

Modern molecular breeding techniques form a critical part of cowpea breeding program to expedite release of new cultivars in a short period of time. Cowpea improvement can be achieved through a combination of conventional breeding, marker-assisted selection, genomic selection, speed breeding, and high throughput phenotyping. Recent advances in bioinformatics and the advent of the next-generation sequencing techniques (NGS) has also opened opportunities for circumventing other drawbacks associated with traditional molecular makers or conventional breeding. This can reduce the breeding cycle resulting in a substantial saving in operational costs and an increase in the efficiency of the breeding program (Lorenz et al. 2011). Genomic selection helps to deal with improvement of quantitative traits by detecting quantitative trait loci (Heffner et al. 2009). High density single nucleotide

polymorphisms (SNP) are effective for prediction of genetic value making the method suitable in offering unparalleled advantage which is complementary to the conventional methods (MacLeod, et al. 2014; Ratcliffe et al. 2015). Single nucleotide polymorphism markers have been widely used in cowpea breeding programs due to their low cost, whole genome coverage, low genotyping error rate, locus specificity and co-dominance. These markers have been used to study marker-trait association and genetic diversity studies in cowpea (Fatokun et al. 2018; Nkhoma et al. 2020).

### **Rationale of this study**

There have been limited research efforts to increase cowpea production through breeding resilient varieties to multiple stresses in the country. Therefore, there is a need to develop cowpea varieties that are high yielding to increase production in marginal areas. Thus far, cowpea is considered as a minor and neglected crop in terms of research and development compared to other commodity crops such as wheat (*Triticum aestivum*), rice (*Oryza sativa*) and maize (*Zea mays*). Genetically complementary cowpea germplasm can be sourced from local farmers, cowpea growing countries and the International Institute of Tropical Agriculture (IITA) to increase the genetic diversity and prospects of identifying superior genotypes for breeding. In addition, cowpea improvement in Zambia is mainly pursued using the conventional breeding approach, which has longer breeding cycle to develop suitable cultivars. It is necessary to integrate molecular breeding techniques to improve the selection efficiency and reduce the impact of environmental variance during conventional breeding in cowpea.

### **Aim**

The aim of the study was to improve the productivity of cowpea in Zambia through breeding high yielding varieties with farmer-preferred traits.

## **Specific objectives**

The specific objectives of this study were to:

- i. Identify farmers' preferences and production constraints and perception on cowpea production in Zambia to guide pre-breeding.
- ii. Assess the genetic diversity among cowpea genotypes using phenotypic traits and single nucleotide polymorphism (SNP) markers, and to select distinct and complementary genotypes for developing improved cultivars.
- iii. Determine the combining ability effects and deduce gene action controlling yield and yield components among selected cowpea (*Vigna unguiculata* [L.] Walp.) parental lines and their progenies for breeding.
- iv. Quantify the genotype by environment interaction effect and select cowpea genotypes with high grain yield and average adaptation across selected cowpea growing environments in Zambia.

## **Research hypotheses**

- i. Farmers' preferences and production constraints and perception on cowpea production is variable in Zambia.
- ii. There exists adequate genetic diversity among cowpea genotypes that can be assessed using phenotypic and molecular markers to select breeding parents for developing improved cultivars.
- iii. The selected parents and crosses have good combining ability for yield and yield related traits.
- iv. Cowpea yield and yield related traits are affected by the change in environment.

## **Thesis outline**

This thesis consists of five chapters (Table 0.1) in accordance with the activities related to the above objectives. Chapter 1 is written as a distinct review paper while chapters 2 to 5 are in the form of discrete research papers, each following the format of a stand-alone research paper (whether or not the chapter has already been published). The literature review and the four experimental chapters of the study made the thesis chapters that were combined into discrete

but inter- dependant papers according to the University of KwaZulu-Natal’s dominant thesis format. As such, there are some unavoidable repetitions of references and introductory information among chapters. Chapter 3 was published in BioMed Central Genetics DOI: <https://doi.org/10.1186/s12863-020-00914-7>.

Table 0.1. Thesis structure

Chapter	Title
-	Introduction to the thesis
1	Breeding cowpea for climate variability and adaptation in southern African: a review
2	Diagnostic assessments of farmer preferences and production constraints of cowpea in Zambia: implications for pre-breeding
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4	Gene action and combining ability studies for grain yield and yield components in cowpea [ <i>Vigna unguiculata</i> (L.)Walp.] genotypes
5	Genotype × environment interaction effects of cowpea [ <i>Vigna unguiculata</i> (L.) Walp.] for grain yield and related traits in Zambia
-	General overview and implications of the study

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## CHAPTER 1. BREEDING COWPEA FOR CLIMATE VARIABILITY AND ADAPTATION IN SOUTHERN AFRICAN: A REVIEW

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### **Abstract**

Low yields and crop failures have increased due to biotic and abiotic factors that are exacerbated by climate changes in recent years. As a result of climate change, sub-Saharan Africa (SSA) experiences low and erratic rainfall, heat waves, and insect pest outbreaks, whose combined effects significantly reduce crop production and productivity. Use of crop cultivars that are resilient to multiple stresses ensure food production. Hence, inherently drought tolerant crops have gained recognition for their contribution to food and nutrition security enabled by their adaptation to marginal environments. Cowpea can withstand abiotic stresses notably heat and low moisture content. However, the actual yield of cowpea is very low under smallholder farmers growing condition in SSA necessitating the need to develop new and high performing cultivars. Cowpea improvement can be achieved through conventional breeding along with marker-assisted selection, genomic selection, speed breeding, and high throughput phenotyping, which are enablers for accelerated breeding. Conventional breeding is challenged by long duration required to develop unique and stable cultivars. The incorporation of next-generation sequencing (NGS) techniques in breeding programs has provided opportunities to improve the efficiency, shorten breeding cycles and reduce costs associated with molecular markers or field phenotyping in conventional breeding. It is important to evaluate the available genetic diversity, appraise new technologies and understand target markets and production constraints for effective breeding. This review aims to highlight challenges affecting cowpea production and explore possibilities for developing improved cultivars based on phenotypic and molecular markers, and to outline recent advances in cowpea breeding using conventional, genomic and NGS techniques.

**Keywords:** Climate change, conventional breeding, genetic resource, cultivar development, genetic variation, drought tolerance breeding

## 1.1 Introduction

Cowpea is a legume crop with potential to improve food and nutrition security in SSA. It is used as a vegetable crop or fodder since it contains high concentration of protein and essential nutrients such as iron (53.2 mg/kg), zinc (38.1 mg/kg), calcium (826 mg/kg) and magnesium (1915 mg/kg) (Boukar et al. 2019). In addition, the extensive root system of cowpea facilitates exploitation of scarce soil moisture and immobile nutrients (Tatsumi et al. 2019). Cowpea provides ecosystem services such as improving soil fertility through symbiosis with the nitrogen fixing *Rhizobium* bacteria (Beena et al. 2018), which enhance the productivity of subsequent crops in crop rotation systems.

The contribution of cowpea to food security in SSA has been challenged by climate-related, edaphic and socio-economic challenges. Climate change-related stress such as drought and heat stresses, and poor soil fertility are among the major challenges of crop production in SSA (Enete and Amusa, 2016). Annual rainfall has generally decreased affecting rain-fed agriculture systems while temperatures have generally increased with exacerbating effects on drought stress. The adverse effects of climate change have reduced agricultural productivity, particularly in SSA where the majority of farmers have limited coping strategies.

Cowpea has high tolerance to multiple stresses and its inclusion in agricultural systems can improve crop productivity and mitigate food insecurity amid challenges caused by biotic and abiotic stresses. The crop is relatively more tolerant to drought and heat stresses compared to other legumes such as common bean (*Phaseolus vulgaris*) and soya beans (*Glycine max*) (Tastumi et al. 2019). Despite the relatively high resilience of cowpea to harsh growing conditions, the contribution of cowpea to food production is limited by a lack of dedicated breeding programs to develop improved cowpea cultivars. Currently, most of the cultivated varieties of cowpea are landraces that are highly adapted to the local environments but have low yield potential. The maximum grain yield achieved by cowpea farmers in the southern African region is about 250kg/ha, which is very low compared to its potential yield of 5 t ha<sup>-1</sup> (Rao and Shahid, 2011). Hence, development of high yielding varieties that are also locally adopted will help to narrow the yield gap in cowpea production Zambia.

Cowpea, like other orphan legumes such as groundnut (*Arachis hypogaea*) and bambara groundnut (*V. subterranea*), suffers from neglect in terms of research and development compared to commodity crops such as wheat (*Triticum aestivum*), rice (*Oryza sativa*) and

maize (*Zea mays*) (Foyer et al. 2016). Resultantly, landraces that exhibit low yield potential are widely grown limiting the potential impact of cowpea and benefit for food security in marginal communities. There is a need to develop improved cultivars to enhance production and ensure food security. The development of improved cultivars depends on identifying sources of genetic variation, devising suitable strategies for efficient selection and executing designed crosses for generating new recombinants with superior characteristics to develop new cultivars according to the needs and requirements of the market. Cowpea breeding and cultivar development is not well developed in SSA due to limited research and development support. Therefore, the objectives of this review were to highlight challenges affecting cowpea production and explore the possibilities for developing improved cultivars based on phenotypic and molecular markers, and to outline recent advances in cowpea breeding using conventional, genomic and NGS techniques.

### **1.1.1 Constraints to cowpea production in SSA**

Moisture stress and poor soil fertility are among the most important constraints reducing production and productivity of cowpea in SSA. Drought and heat stress are currently the most important abiotic stresses limiting cowpea production, causing up to 60% reduction in grain yield and quality (Hampton et al., 2016). Rain-fed agriculture systems prevalent in SSA are vulnerable to rainfall variability. Despite its relatively high drought tolerance level, cowpea is highly sensitive to severe moisture stress during the vegetative and flowering stages. The vulnerability of rain-fed production systems has compelled farmers and breeders to identify and cultivate early maturing varieties that have enhanced potential to escape terminal drought stress (Fatokun et al. 2012). However, the flowering period, which influences varietal maturity, in cowpea is not well understood. There is a wide variation in flowering time among cowpea germplasm and Wienk (1963) reported that some cowpea genotypes exhibited photoperiod sensitivity. Other studies have also indicated that flowering and maturity are responsive to moisture and temperature variability (Ishiyaku et al. 2005; Patriyawaty et al. 2018). Similarly, improving root characteristics, selecting genotypes with high stomatal conductance and chlorophyll content have also been proposed as selection criteria for developing drought and heat stress tolerant cultivars.

Genetic variation present among cowpea genotypes for agro-morphological characteristics should be well assessed to initiating breeding programs for developing cultivars that are well adapted to prevailing conditions. Crop production in SSA is also challenged by poor soil fertility. Continuous and excessive nutrient mining through repeated crop cycles led to inadequate nutrient replenishment and has become a major cause of poor soil fertility in SSA (Bot and Benites, 2005). Globally SSA is the lowest in per capita fertilizer use. The majority of farmers do not afford to purchase fertilizer and often apply sub-optimal rates leading to poor harvests and nutrient depletion (Tadele, 2017). While cowpea is relatively efficient at nutrient mobilization and use, its yield potential is significantly reduced in nutrient deficient and acidic soils. Developing cultivars with enhanced tolerance to soil acidity and low nutrient availability will potentially improve cowpea productivity in SSA. Exploiting genetic variation for rooting traits and symbiosis with nitrogen fixing bacteria would be suitable selection criteria to increase cowpea production.

Breeding for disease resistance has been a major focus of many breeding programs aimed at improving cowpea productivity in SSA. While the diseases may occur in combination or sequentially, there may be few or no germplasm with resistance to all the diseases. It will be prudent to evaluate available germplasm for a number of the diseases endemic to the target environments for improving cowpea production. Application of crop protection chemicals is expensive and poses numerous health and environmental hazards making host plant resistance more suitable for disease control. Developing suitable and resistant cultivars will involve evaluating available germplasm including landraces and mutants for disease resistance and agronomic traits.

It is imperative to develop improved cultivars and establish efficient cultivar deployment strategies to access seed by smallholder, rural and women farmers who make up the largest proportion of cowpea farmers. These groups of farmers are usually marginalised in accessing improved and modern cultivars (Mbavai et al. 2015). Limited access to improved cultivars is partly caused by poorly developed seed systems in developing countries. For instance, Gondwe et al. (2017) reported that, the cowpea seed market in Zambia is not as organised as that of maize or other major legumes due to a lack of government support. In comparison, the international cowpea market is characterised by a well-developed and organised value chain (Hatzenbuehler, et al. 2017). Resultantly, most farmers in developing countries such as Zambia

use obsolete introduced varieties or low yielding landraces and poor crop production technologies leading to low productivity.

## **1.2 Cowpea research and development collaborating networks across the world**

Cowpea genetic resources including progenitor species, landraces, breeding populations, modern and obsolete cultivars are maintained and catalogued in several public and private gene banks. Table 1.1 summarizes some of the major gene banks of cowpea germplasm accessible to breeders. The International Institute of Tropical Agriculture (IITA) has one of the largest cowpea core-collections holding more than 15000 cowpea accessions, including wild cowpea species collected from over 100 countries. A significant proportion of the collections is made up of wild species that represent primary, secondary and tertiary gene pools (Harlan and de Wet, 1971). The IITA has a global mandate to develop and share cowpea germplasm with national agricultural research institutions. About 40% of the cowpea germplasm maintained at IITA and national gene banks has never been distributed because the germplasm is seldom requested (Singh, 2006) probably due to the gap between the available and functionally useful data.

Apart from the IITA, the Network for Genetic Improvement of Cowpea for Africa (NGICA), Genetic Resources Network for West and Central Africa (GRENEWECA), Regional Plant Genetic Resource (PGR) in West Africa, Centres of Excellence in West and Central Africa, and SADC Plant Genetic Resources Network in Zambia are some of the major cowpea genebanks in SSA. These genebanks are important in providing germplasm for addressing the global food security challenges caused by climate change and poor soil fertility stress. The plant genetic resources maintained in the genebanks provide genetic variation for developing cultivars suitable to enhance food security, energy production, provide livestock feed and other ecosystem services (Wambugu et al. 2018). However, a large proportion of the germplasm maintained in gene banks is not exploited because developing economically important cultivars from landraces and wild relatives is more difficult compared to using elite lines (Ehlers and Hall, 1997). Some of the high yielding lines developed at IITA, including TVu 201, TVu 408, TVu 410, TVu 1190, TVu 1977 and TVu 4577 are common parents in many cowpea breeding programs (Singh et al. 2014). However, the use of elite lines with a narrow genetic base as parental lines in most breeding programs has contributed to genetic bottleneck and development of cultivars that lack adaptation to harsh conditions.

Table 1.1 Major gene banks and sources of cowpea accessions across the globe

Genebank	Country	Website
African Agricultural Technology Foundation (AATF)	Kenya	<a href="http://www.aatf/africa.org">www.aatf/africa.org</a>
Agricultural Research Council- Grain Crop Institute	South Africa	<a href="http://www.arc.agric.za/arc-gci">www.arc.agric.za/arc-gci</a>
Bean/Cowpea Collaboration Research Support Program (CRSP)	United States of America	<a href="http://www.Crps.net/resources/by_crsp/bean/cowpea">www.Crps.net/resources/by_crsp/bean/cowpea</a>
Centre National de Recherche Agronomiques	Cote ' d' Ivoire	<a href="http://www.devex.com/organizations/centre-national-de">www.devex.com/organizations/centre-national-de</a>
Crops Research Institute	Ghana	<a href="http://www.cropsresearch.org">www.cropsresearch.org</a>
Institut Togolais de recherché Agronomique	Togo	<a href="http://www.goafricaonline.com/tg/2741-itra-centre">www.goafricaonline.com/tg/2741-itra-centre</a>
Instituto Nacional de Investigación Y Tecnológica Agraria Alimentaria (INIA)	Spain	<a href="http://www.inia.es/inia">www.inia.es/inia</a>
Institut National de Recherche Agricoles de Benin	Benin	<a href="http://www.asti.cgiar.org/benin/inrab">www.asti.cgiar.org/benin/inrab</a>
International Institute of Tropical Agriculture (IITA)	Nigeria	<a href="http://www.genebanks.org/resources/crops/cowpea">www.genebanks.org/resources/crops/cowpea</a>
IPK Leibniz Institute of Plant Genetics and Crop Plant Research	Germany	<a href="http://www.uibk.ac.at/botany/ecoseed/consortium/ipk">www.uibk.ac.at/botany/ecoseed/consortium/ipk</a>
N. I. Vavilov Research Institute of Plant Industry	Russia	<a href="http://www.vir.nw.ru">www.vir.nw.ru</a>
National Agrobiodiversity Centre	South Korea	<a href="http://www.slideshare.net/IITA-CO/korea-genebank">www.slideshare.net/IITA-CO/korea-genebank</a>
National Botanical Garden	Belgium	<a href="http://www.britannica.com/place/National-Botanical">www.britannica.com/place/National-Botanical</a>
National Centre for Genetic Resources and Biotechnology	Nigeria	<a href="http://unitedplantsavers.org/genetic-resources">unitedplantsavers.org/genetic-resources</a>
National Gene Bank	Kenya	<a href="http://ipgrbg.com/en/генетични-ресурси">ipgrbg.com/en/генетични-ресурси</a>
National Plant Genetic Resource Centre of Tanzania	Tanzania	<a href="http://www.fao.org/plant-treaty/countries/membership/country">www.fao.org/plant-treaty/countries/membership/country</a>
National Plant Genetic resources centre	Zambia	<a href="http://www.croptrust.org/zambia">www.croptrust.org/zambia</a>
Network for Genetic Improvement of Cowpea for Africa (NGICA)	Burkina Faso	<a href="http://www.partnership.usaid.gov&gt;partnership&gt;genetic_improvement">www.partnership.usaid.gov&gt;partnership&gt;genetic_improvement</a>
Southern Africa Development Countries Plant Genetic Resources	Zambia	<a href="http://www.sadc.int/sadc-secretariat/services-centres/spgrc">www.sadc.int/sadc-secretariat/services-centres/spgrc</a>
Southern Plant Introduction Station	United States of America	<a href="http://www.genebanks.org/resources/crops/cowpea">www.genebanks.org/resources/crops/cowpea</a>
World Vegetable Centre	Taiwan	<a href="http://www.genebanks.org/resources/crops/cowpea">www.genebanks.org/resources/crops/cowpea</a>

### 1.3 Breeding progress in cowpea

There has been significant progress in breeding cowpea varieties with tolerance to drought, insect pests and disease and low soil nutrient stresses. A breeding line IT97K-556-6 was developed for aphid resistance and genes controlling the resistance were successfully mapped (Souleymane et al. 2013) (Table 1.2). The source of aphid resistance genes was a wild relative of cowpea referred to as TVNu 1158 (Souleymane et al. 2013). This affirms the importance of

wild relatives and progenitors as sources of useful genes for insect pests and disease resistance and hence the justification for their conservation in gene banks. Similarly, accessions with confirmed resistance to flower bud thrips and *Striga* were identified in West and East Africa (Boukar et al. 2015; Boukar et al 2016).

The IITA has spearheaded most breeding programs by providing germplasm for genetic variation used in developing cultivars with multiple stress tolerance and enhanced yield potential. The breeding lines developed at the IITA such TVu 201, TVu 4078 and TVu 1977 are commonly used as parents in many breeding programs for developing breeding populations (Singh, et al. 2014). Early maturity cowpea lines including IT82E-9, IT82E-18, IT82E-32 with yield potential between 1.5 and 2.5 t ha<sup>-1</sup> have been developed in India for drought prone production areas (Singh and Sharma, 1996). There has been progress in developing cowpea lines with improved yield potential. For instance, a variety CB50 with large white grain and resistance to fusarium wilt was released in 2008 in the Unites States (Roberts et al, 2008). In West Africa, varieties IT98K-205-8 and Melak with improved yield were released in Burkina Faso while a line known as ISRA-2065 that incorporated high yield potential and thrip and aphid resistance was released in Senegal (Roberts et al. 2008). Varieties including IT82E-16, IT82D-889 and IT85F-2020 with high yield potential have been released for the East and southern Africa region.

Despite the significant progress in developing cowpea varieties with high yield potential and resistance to different stress factors, most of the released varieties have become obsolete due to the appearance of new production challenges and poor productivity. Hence, there is a need for new varieties suitable for production under prevailing conditions. Plant breeding should be dynamic enough to respond to rapidly changing environments and meet farmer and market preferences. Genotype response to environmental conditions and farmer preferences are determined by phenotypic, biochemical and molecular markers, hence, it is important for breeders to identify suitable genotypes with characteristics that satisfy environmental, social and economic demands. The International Board for Plant genetic Resources (IBPGR, 1983) developed a suite of characteristics for evaluating cowpea germplasm and it is vital for the breeder to identify the most important target traits for their breeding objectives.

#### **1.4 Target traits for developing high yielding and climate resilient cowpea cultivars**

The cowpea germplasm maintained in gene banks around the world are vital sources of genes for key adaptive traits for developing cultivars that are tolerant to multiple stresses and with high yield potential. Many programs have targeted the development of cultivars with improved resistance to pests, diseases, heat and drought stresses (Table 1.2). Target traits for cowpea improvement include days to flowering and maturity, grain weight, pod number per plant, number of flowers per plant, pod length, seed number per pod and number of seeds per pod, number of pods per cluster, number of clusters per plant, and harvest index (Meena, et al. 2015; Sharma, et al. 2017). These traits are also contributory to the final yield productivity.

Yield is an aggregate of multiple developmental traits (Oladejo, et al. 2011; Aliyu and Makinde, 2016) and it is thus important to identify yield components that exhibit favourable association with yield for selection. Therefore, understanding the associations between yield and yield components and their underlying genetic basis is fundamental for yield improvement and drought tolerance. Earliness to flowering and maturity have been targeted to improve drought escape especially in SSA where terminal drought stress is prevalent (Owusu et al. 2018). The two traits relate to the phenology of the crop and respond to availability of soil moisture, ambient temperature and photoperiod. Early maturing genotypes have the ability to complete their growth cycle before the onset of severe moisture deficit. The goal of breeding for early maturity in cowpea is a combination of early flowering initiation and short grain filling period (Kauret et al. 2017). Early flowering and maturing genotypes are ideal for drought-prone environments. The use of early maturing varieties and manipulating planting dates is recommended to manage terminal drought stress in low rainfall environments (Abadassi, 2015). However, early maturity is associated with a yield penalty due to shortening of the vegetative and reproductive stages that may cause limited accumulation of photosynthates and grain filling (Owusu et al. 2018).

There have been efforts to increase the number of pods and seeds that contribute directly to the total yield. The number of pods per plant is related to the vegetative architecture of the crop. Selection for creeping or erect types has been practiced in cowpea breeding. However, creeping types have not been common due to mixed cropping systems prevalent in SSA, which favour erect types for intercropping with cereal crops such as maize. Since most breeding programs aim at improving the economic worth of the crop, other traits such as increased pod length can

subsequently contribute directly to the final yield. The future prospects for breeding must improve phenotyping efficiency to reduce environmental variance during selection because most of the adaptive traits are quantitative. The influence of genotype by environment interactions on quantitative has been identified as a challenge in selection, which complicates and delays breeding (Rocha et al. 2017).

Adaptive traits such as early flowering and maturity are essential conferring the ability to escape drought stress, especially terminal drought stress. On the other hand, early maturing cultivars are preferred for their role in contributing to food and nutrition security during the period before harvest of main crops such as maize. Although climate variability has resulted in unpredictable rainfall distribution resulting in a shift to the cultivation of early maturing varieties by farmers (Armah et al 2010), the preferred characteristics often vary across different regions due to environmental and socio-economic factors (Faye et al. 2004; Muranaka et al. 2015). For instance, nutritional content, grain texture and cooking time are important determinants of the market value of cowpea in SSA (Horn et al. 2015).

Table 1.1 Cowpea genetic resource reported in different regions of the world

Gene bank	Region	Characteristics	Genotype name/designation	Reference
IITA, PGRFA- Brazil	Tropical/subtropical regions	Disease resistant	IT82D-889,IT83S-818,IT86D-880,IT86D-10110,IT84S-2246-4,IT89KD-889, IT90K-59,IT90K-76,IT90K-277-2,IT90K-284-2,IT97K-207-15,IT97K-499-35, IT98K-205-8	Singh and Fatokun (2002); Van Boxtel et al. (2000); Boukar et al. (2016)
IITA	Tropical/subtropical regions	Erect plant type, early maturing and resistant to major pests	IT82E-16, IT82D-889, IT85F-2020, IT86D-1010, IT87D-611-3, IT89KD-24S, IT93K-2046-2, IT97K-568-18 IT97K-499	Singh,2006; Singh 2014
IITA	West and Central Africa		TVX3236, IT81D-985, IT81D-994, IT83S-818, IT88D-867-11, IT89KD-374-57, IT90K-76, IT90K-277-2, IT93K452-1, IT97K-499-35	
USDA- PGRCU, UC, PGRFA- Brazil	North, Central and South America		VITA1, VITA3, VITA6, VITA7, IT82E-18, IT82D-716, IT82D-789, IT82D-889, IT83D-422, IT83S-841 IT84D-449, IT84D-666, IT84S-2246-4, IT86D-314, IT86D-368, IT86D-782, IT86D-762, IT-86D-1010, IT87D-697-2, IT87D-885, IT88S-574-3, ITTVX 1836-01, IT87D-1627, IT87KD-288, IT90K-284-2, IT91K-118-2	
IITA	Tropical/subtropical regions	Drought tolerant	Danila, IT88DM-867-11, TVu 557, TVu 11982, IT98D-1399, IT98K-131-1, TVu 1438, IT97-568-19, IT98K-452-1, TVu 4574, IT98K-241-2, IT89KD-374-57, TVu 6443	Singh and Matsui, 2002; Singh 2006; Fatokun et al., 2012; Singh 2014
USDA- PGRCU, UC, PGRFA- Brazil	North, Central and South America	Heat tolerant	IT93K-452-1, IT98K-1111-1, IT93K-693-2, IT97K-472-12, IT97K-472-25, IT97K- 819-43, IT97K-499-38	
IITA	Tropical/subtropical regions	Nitrogen fixation and tolerant to low phosphorus	IT89KD-374-57, IT90K-372-1-2, IT98D-1399, IT99K-1060, IT97K-568-19, IT97K- 568-11, IT99-1148, IT97K-1069-6, IT03K-314-1, IT03K-351-2	

Table 1.2 Continued

IITA, PGRFA- Brazil	Tropical/subtropical regions	High in protein content	TVu 10425(32.2%), TVu 2822 (31.8%), TVu16581 (31.3%), TVu 450 (31.1%), TVu 16616 (31.7%)	Boukar et al. 2011
IITA, PGRFA- Brazil	Tropical/subtropical regions	High in iron content	TVu2852 (78.1mg/kg), TVu14878 (79.5mg/kg), TVu 2852 (78.7mg/kg), TVu 526 (78.1mg/kg), TVu 10342 (77.0mg/kg)	
IITA, PGRFA- Brazil	Tropical/sSubtropical regions	High in zinc content	TVu 1732 (56.1mg/kg), TVu 9576 (55.3mg/kg), TVu 2651(54.5mg/kg), TVu 1877 (54.0mg/kg)	
IITA	West Africa and North Africa	Aphid resistant	IT 97K- 556- 6, TVNu 1158	Souleymane et al 2013; Huynh et al., 2015
IITA	West and East Africa	Resistant to <i>Striga</i> <i>gesneriodes</i>	TVu 1272, TVu 16514	

Note: IITA= International Institute of Tropical Agriculture/Nigeria, UC= University of California; USDA= United States Department of Agriculture; PGRFA= Plant Genetic Resource for Food and Agriculture, Brazil

## **1.5 Breeding methods for adaptation to climate variability**

Improvement in crop performance to meet human needs can be achieved through conventional breeding assisted by molecular or mutation breeding- methodologies. Cowpea improvement in Zambia and most of the countries in SSA is usually based on conventional approaches. However, progress in developing new cultivars using conventional breeding has been constrained by the narrowing genetic diversity in cowpea germplasm. Like other self-pollinating crops, genetic diversity in cowpea is limited due to genetic erosion during selective breeding and natural selection (Iqbal et al. 2017). Over the years, landraces and wild types have been replaced with modern varieties developed from a narrow range of elite parental lines leading to narrowing of the genetic diversity. It is therefore imperative that new sources of genetic variation are identified through assembling large and diverse panels of germplasm to identify superior, divergent and complementary parental lines for breeding. Alternatively, new sources of variation must be created through crossing phenotypically and genetically divergent parental genotypes.

The other major drawback associated with conventional breeding is the relatively long time taken to develop cultivars. Conventional breeding takes more than 12 years to develop genetically distinct, uniform and stable varieties (UPOV, 2002). The period is too long to respond to rapid changes in climatic conditions experienced in different parts of the world. The rapid changes in temperatures and other climatic conditions may render newly developed cultivars obsolete if they take too long to develop. It is imperative to integrate other breeding approaches such as molecular markers, genomic selection and gene editing to expedite the breeding process in order to develop cultivars in the shortest possible time. The integration of phenotypic and molecular markers in marker-assisted breeding has potential to reduce the number of years required for cultivar development. These approaches are rarely used in developing countries due to a lack of financial and technical resources.

## **1.6 Integrated techniques for accelerated breeding**

### **1.6.1 Marker-assisted breeding for climate variability**

Identifying DNA markers for use in marker-assisted trait selection and gene cloning of adaptive traits is essential to complement conventional breeding approaches. Various DNA based

markers including simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) have been used extensively for genetic diversity studies of cowpea. For instance, 200 genic and 100 genomic SSR markers were developed by Chen et al., (2017) from cowpea unigene and genome sequences, respectively. The information generated 155 alleles with 2.9 alleles identified per marker to facilitate better understanding of the genetic relationships among lines for effective utilization. Recently, single nucleotide polymorphism (SNPs) markers have gained prominence due to their low cost, whole genome coverage, low genotyping error rates, locus specificity and co-dominance. SNP markers have been used to study marker-trait association and genetic diversity studies in cowpea (Fatokun et al. 2018; Nkhoma et al. 2020). Marker-assisted selection has also enabled plant breeders to accurately elucidate functional genes through mapping approaches including quantitative trait locus (QTL) mapping. The QTL mapping identifies regions of the genome that co-segregate with a given trait either in F2 populations or among recombinant inbred lines (RIL). To date, linkage mapping has been pivotal in discovering QTLs for resistance or tolerance to biotic and abiotic stress in cowpea improvement (Table 1.3). Despite the importance of QTL mapping in gene discovery, it has two major limitations. Firstly, only allelic diversity that segregates between the parents of the particular F2 family or within the RIL population can be assayed. Secondly, the amount of recombination that occurs during the creation of the RIL population places a limit on the mapping resolution. In addition, traditional MAS methods do not detect low copy genomic regions and small-effects loci that underpin complex polygenic traits such as grain yield, drought, and heat tolerance (Dekkers and Hospital, 2002; Crosbie et al., 2003). Also, traditional markers use electrophoretic separation of DNA, which limits the ability to detect genetic polymorphisms.

Table 1.3 Reported quantitative trait locus (QTL) and candidate genes affecting various traits in cowpea

Trait associated with gene/QTL	Pedigree	Type	Marker type	Chromosome location	Number of QTL	Reference
Aphid ( <i>A. craccivora</i> ) resistance	Apagbgala x SARC 1- 57-2	F2/ RIL	SSR and SNP	LG10	1	Kusi et al. (2017)
Cowpea bacterial blight resistance	Danlla x TVu 7778	RIL	SNP	LG3, LG5, LG9	3	Agbicodo et al., 2010
Cowpea yellow mosaic virus resistance	IT97K- 499-35 x Canapu T16	F2	AFLP	Same linkage group	3	Rodrigues et al., 2012
Charcoal rot resistance	IT93K- 503- 1 x CB46	RIL	SNP/ AFLP	LG2, LG3, LG5, LG6, LG11	9	Muchero et al., 2011
Drought- induced senescence resistance	IT93K- 503-1 x CB46	RIL	AFLP	LG1, LG2, LG3, LG5, LG6, LG7, LG9, LG10	10	Muchero et al., 2010
Foliar thrips resistance	CB46 x IT93K- 503-1 and CB27 x IT82E-18	RILs	SNP	LG2, LG4 and LG10	3	Lucas et al., 2012
Foliar thrips resistance	CB46 x IT93K- 503-1 and CB27 x IT82E-18	RILs	AFLP	LG5 and LG7	2	Muchero et al., 2010
<i>Fusarium wilt resistance (Fot race 3)</i>	CB27 x 24- 125B- 1	RIL	SNP	LG6	1	Pottorff et al., 2012b
<i>Fusarium wilt resistance (Fot race 3)</i>	IT93K- 503- 1 x CB46, CB27 x 24- 125B-1, CB27 x IT82E- 18	RIL	SNP	LG8, LG0, LG9	1	Pottorff et al., 2014
Hastate leaf shape	Sanzi x Vita7	RIL	SNP	LG15	1	Pottorff et al., 2012a
Heat tolerance	CB27 x IT82E- 18	RIL	SNP	LG2, LG5, LG6, LG7, LG8, LG10	5	Lucas et al., 2013b
Leaf senescence tolerance	ZN16 x ZJ282	RIL	SNP	LG3, LG7, LG11	3	Xu et al., 2014
Striga resistance	TVu 3236 x IT82D-849	F2	AFLP	LG1	3	Quedraogo et al., 2001
	IT84S-2246 x IT84S-2246- 4	F2	AFLP	LG1	6	Quedraogo et al., 2001
	IT84S-2246 x IT84S-2246; IT84S-2246 x IT82D- 849	F2	SCAR	LG1	2	Quedraogo et al., 2012
	IT93K- 693- 2 x IAR1696	F2	AFLP/ SCAR	Same linkage group	1	Boukar et al., 2004
Root knot nematode ( <i>Meloidgnes</i> spp.) resistance	CB27 x 24- 125B-1	RIL	SNP	LG13, LG14, LG15, LG16	4	Huynh et al., 2016
	IT84S- 2049 x UCR779	F2;3	SNP	LG19	1	
	IT93K- 503- 1 x UCR779	F2;3	SNP	LG14	1	
Root knot nematode ( <i>Meloidgnes</i> spp.)	524B x IT84S- 2049	RIL	SNP	LG9	1	Santos et al., 2018

AFLP= amplified fragment length polymorphism; F2= second filial generation; F3= third filial generation; LG= linkage group; SCAR= sequence characterised amplified region; SNP= single nucleotide polymorphism; SSR= simple sequence repeat; RIL= recombinant inbred line

## 1.6.2 Next-generation sequencing

Recent advances in bioinformatics through the advent of next-generation sequencing techniques (NGS) help to circumvent the challenges and drawbacks associated with the application of traditional molecular markers. The NGS have reduced cost of genotype by sequencing (GBS) allowing large genomes to be sequenced routinely. With GBS, a large number of single nucleotide polymorphisms (SNPs) are generated for assessing genetic diversity, constructing haplotype maps, marker-trait association mapping and genomic selection (Bhat et al. 2016).

Genomic selection (GS) has more practical application for breeding cowpea cultivars with multiple traits. The GS approach predicts the performance of progenies by calculation of genomic estimated breeding values (GEBVs) from parental lines having genotypic data using a predictive model derived from individuals having both phenotypic and GBS data (Bhati et al. 2016). The GEBVs are then used to advance genotypes in the breeding cycle without phenotyping. This can increase genetic gains during breeding for tolerance to drought and heat stresses, which is currently challenged by spatial variation in imposing uniform stress on large populations. The GS can reduce the breeding cycle by 50%, resulting in substantial reduction in operational costs and an increase in the efficiency of the breeding program (Lorenz et al. 2011). The application of GS and other NGS based breeding approaches is limited in Africa due to the initial capital investment and operational costs associated with running NGS platforms. However, the Consultative Group on International Agricultural Research (CGIAR) has since established NGS sequencing facilities accessible to the National Agriculture Research Station (NARS). The most notable among them being the Biosciences eastern and central Africa – International Livestock Research Institute (BecA-ILRI) hub based in Kenya. The BecA-ILRI hub, through its Integrated Genotyping Service and Support (IGSS) has so far provided GBS data to over 20 NARS at a subsidized cost (Yao et al. 2016). Ultimately, the advent of NGS has increased the capacity to generate large amounts of genetic data that can be integrated with different breeding methods to increase selection efficiency and accelerate the breeding process.

### **1.6.3 Integration of speed breeding, high throughput phenotyping and genetic engineering**

To develop cultivars that respond to rapid changes in the environment, there is a need to reduce the breeding time through integration of speed breeding, high throughput phenotyping, and genetic engineering. Speed breeding shortens the generation time and accelerates the breeding process (Watson et al. 2017). It involves reducing the generation time of long or day-neutral plants by extending photoperiod duration, temperature, and humidity to hasten physiological growth for early seed harvesting. The environmental conditions can be altered artificially to stimulate and accelerate phenological development. Controlled environment growth chambers can provide infrared growth lights and optimum heat units, which accelerate plant development and maturity. Exposure to 22 hours of light at 22 °C, and 2 hours dark period at 17 °C achieved up to six generations per year for wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), and pea (*Pisum sativum*) (Watson and Ghosh et al. 2017).

The response of other legumes such as chickpea and pea to speed breeding shows that there is potential to accelerate breeding in cowpea. The NARS without access to growth chambers can supplement the lighting in glasshouses with a low-cost infrared light-emitting diode (LED) to extend the photoperiod exposure (Watson et al., 2017). Speed breeding is efficient when large population panels are phenotyped to increase the chances of identifying the best parents with desirable traits. Hence, speed breeding would be effective when implemented in conjunction with high throughput phenotyping. High-throughput phenotyping technologies involve the use of automated systems for capturing, storage, and statistical analysis of large volumes of data (Mir et al. 2019). This allows for fast and precise large-scale quantification and monitoring of various phenotypic traits. Satellite imagery, remote sensing techniques, drones, and automated camera systems are reported to precisely measure various phenotypic traits (Mir et al. 2019). Abdelrahman et al (2018) found that cost-effective phenotyping has been used in legumes such as cowpea and beans to increase the accuracy, precision, and throughput of measurements.

### **1.6.4 Prospects of genetic engineering for future cowpea improvement**

The dependence on naturally available genetic variation or sexually created recombinants through various mating designs limits prospects of identifying genotypes with high yield

potential or stress resistance genes. There may be no genetic variation within a germplasm to respond to environmental stimuli leading to limited genetic variability of a crop. Modern techniques are required to alter the fixed genetic constitution and create the required variation. For the past few years, genetic engineering to generate novel gene combinations has been tested to develop climate-resilient cowpea cultivars (Xie and Yang, 2013; Ji et al., 2019).

Recently, gene editing with transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) has been used to make specific deletions or insertions at specific loci that underpin trait expression in crop plants. The CRISPR-associated 9 (Cas9) –holds a potential in modifying the plant genome. The Cas9 make it possible to target a predefined region of DNA and minimize hazards associated with the disruption of genes (EFSA, 2012). For example, Li et al. 2019 used Cas9 to efficiently disrupt the representative symbiotic nitrogen fixation (SNF) gene in cowpea. The integration of conventional and molecular breeding and genetic modification has a potential impact on crop breeding amidst climate change, insect pests and disease, and population growth challenges. There has been evidence that use of genetically modified organisms (GMOs) positively influence farmers' incomes, access to food, and increased tolerance of crops to various biotic and abiotic stresses (Muzhinji et al. 2020). However, the public and civil organizations in Africa have been highly critical and sceptical of GMOs (Schmidt et al. 2020). Nevertheless, the legislation and regulation of genome-edited plants in many countries, including Africa, are evolving rapidly as authorities realise the potential role of genetic engineering in enhancing food security.

## **1.7 Conclusion**

Cowpea is an indigenous legume that is inherently adapted to marginal environments. It is a key food security crop in drought prone areas due to its ability to withstand multiple stresses such as heat and low soil moisture. The low average cowpea yield in SSA (< 250 kg/ha) necessitates the need to develop new cultivars to improve productivity. It is thus imperative to assess the genetic diversity present in cowpea germplasm collections and develop breeding strategies to exploit the available genetic diversity. A wide range of breeding techniques comprising of conventional breeding, marker-assisted selection, genomic selection, speed breeding, and high throughput phenotyping can be used for accelerated breeding and to

enhance adaptation to climate variability through unique crop varieties. Given the rapid increase in climate change related stresses, human population pressure and a change in life style to plant-based protein sources, there is need to increase the efficiency and precision in cowpea breeding to deliver market-preferred varieties in Africa.

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## CHAPTER 2. DIAGNOSTIC ASSESSMENTS OF FARMER PREFERENCES AND PRODUCTION CONSTRAINTS OF COWPEA IN ZAMBIA: IMPLICATIONS FOR PRE-BREEDING

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### Abstract

Cowpea is a relatively drought tolerant and multipurpose crop valuable in cereal-based cropping systems. In Zambia, cowpea is a valuable food security crop. However, its production and productivity under the smallholder production systems, is affected by an array of biotic and abiotic factors and socio-economic constraints. The objective of this study was to identify farmers' preferences and production constraints and perception on cowpea production in Zambia to guide pre-breeding. A participatory rural appraisal (PRA) research tools were used and data collected from three major cowpea production areas of Zambia namely Eastern, Southern and Northern provinces during the 2017/18 cropping season. Data were collected involving 187 farmers, while 43 additional farmers participated in focus group discussions. Results showed that considerably larger proportion of females (44%) were involved in cowpea production in the surveyed provinces. Higher proportion of respondents (93.6%) use unimproved local cowpea landraces for production, while only 6.4% respondents used few introduced varieties during the survey. The major farmer perceived constraints to cowpea production were low yielding varieties (reported by 45.1% of respondents), a limited access to production inputs (14.8%), a lack of extension support service (3.3%), weed infestation (2.2%), poor soil fertility (1.1%), a lack of production labour (9.3%), and pests and diseases (18.1%). Farmer-preferred traits of cowpea varieties in the study areas included high yield (reported by 46.7% of respondents), good eating quality (5%) and pest and disease resistance (15%). All participant farmers' indicated their persuasive willingness to adopt improved cowpea cultivars. Therefore, cowpea breeding programs in Zambia should consider the aforementioned farmer-preferred and essential traits to develop improved cultivars to enhance sustainable production for food security and market.

**Key words:** cowpea breeding, farmer perceptions, landrace varieties, participatory rural appraisal, Zambia

## 2.1 Introduction

Cowpea is a relatively drought and heat stress tolerant (Awika and Duodu, 2017) making it a suitable crop for development and cultivation in the drier regions characterized by erratic rainfall and heat stress due to global climate change. It is a highly nutritious crop with higher grain content of 17.5 – 32.5% (Boukar et al., 2011), carbohydrates of 50-60% and about 1% fat (Kirse and Karklina, 2015). The International Institute of Tropical Agriculture (IITA) maintained 15,122 cowpea genetic resources collected from 88 countries (IITA, 2019).

In SSA cowpea supports over 110 million people who are the most impoverished smallholder communities (Jukanti et al. 2016). In the region both the grain, matured fresh pods and young succulent leaves are consumed and marketed. The global annual production of cowpea exceeds 5.4 million tonnes (FAOSTAT, 2017). SSA contributes over 96% of the total world cowpea production (Neduraman et al. 2015). Most households in SSA are largely dependent on starch-based diets often leading to malnutrition among children and pregnant mothers (Maziya-Dixon et al. 2017). Thus, inclusion of cowpea in household diets has diverse health benefits. Bioactive polyphenols and peptides are present in cowpea with anti-inflammatory properties against cancer, cardiovascular, diabetes and other human diseases (Awika and Duodu, 2017, Jayathilake et al., 2018).

Cowpea is a cheap source of fodder for animals making it a useful component in mixed livestock-crop production systems (Samireddypalle et al. 2017). It is useful in crop rotation systems with maize (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench), finger millet (*Eleusine coracana* L.) and sunflower (*Helianthus annuus* L.). This enhances soil fertility through atmospheric nitrogen fixation and ensuring sustainable crop production and productivity through optimal land uses (Beena et al. 2018).

In the past decade there has been considerable yield gains reported in cowpea such as reported in SSA countries attributed to the development and deployment of improved cultivars. These cultivars are reported to have superior agronomic traits, drought tolerance (Boukar et al. 2018) and resistance to *Striga gesneriodes* (Timko, 2017). Nonetheless, the yield levels of cowpea have remained low and stagnant (0.3 t ha<sup>-1</sup>) in most SSA countries including Zambia (Shanko et al. 2014). This is due to a lack of locally adapted and high yielding varieties, use of traditional farming systems, biotic stress (e.g. diseases, pests and weeds]) and abiotic stresses (e.g. low

soil fertility) and socio economic constraints (e.g. a lack of extension service and lack of access to finance).

In Zambia cowpea is largely cultivated by smallholder women farmers (Gondwe et al. 2017). It is cultivated under intercropping systems with cereal crops such as maize, sorghum or millet. In the country farmers use unimproved landrace varieties with low yield potential. The government research institutions bred and released only three cowpea cultivars such as ‘Lutembwe’, ‘Msandile’ and ‘Bubebe’ in Zambia (Kannaiyan et al. 1986). These varieties regularly succumbed to pests and diseases leading to low yields and occasional crop failures. Also, the levels of adoption of the released varieties was low due to a lack of farmer-preferred traits needing demand-led cowpea breeding in the country (Langyintuo et al. 2004).

Studies have shown that consultation of farmers and their value chains led to higher adoption rate of crop cultivars (Chambers, 2014, Reij and Waters-Bayer, 2014). Engaging farmers during the initial phase of breeding is an important step to supporting pre-breeding initiatives through targeting specific traits and attributes as required by farmers and clients (Shimelis and Laing, 2012; Persley and Anthony, 2017). This model reduces the gap between farmers, science and technology and plant breeders. It has a functional approach and improves research efficiency by involving beneficiaries at different stages of variety development. Thus, it has the potential to generate appropriate and relevant research products (crop varieties or cropping management practises) that are compatible and consistent with farmers’ needs and requirements.

Various methods including participatory rural appraisal (PRA) are used to capture farmers’ knowledge, attitudes and constraints. PRA can be conducted through focus group discussions (FGDs), interviews (structured or semi-structured), matrix ranking, transect walks among other tools. Sperling et al. (1993) reported that breeders’ insights were enhanced and complemented during variety development and deployment by indigenous knowledge of the farmers who are the end users of any production technology. This assisted in accelerating the acceptance and adoption of newly developed varieties (Maurya et al. 1988, Prain et al. 1992, Joshi and Witcombe, 1996, Franzel et al. 2001). In Zambia, there are limited recent PRA studies that documented the production status, constraints and utilization of cowpea. There is a need for a well-structured survey using the PRA approach in order to discover the major production problems and constraints affecting the production of cowpea in the major growing zones in

Zambia. This will enable to guide cowpea pre-breeding and breeding in the country. Therefore, this study was conducted to identify farmers' preferences and production constraints and perception on cowpea production in Zambia to guide pre-breeding.

## **2.2 Materials and methods**

### **2.2.1 Description of study sites**

The PRA was conducted in the following three provinces of Zambia: Southern, Eastern and Northern (Figure 2.1) in 2018. These provinces are situated in the three diverse agro-ecological regions of Zambia. Southern Province is situated in Region I, Eastern Province in Region II and Northern Province in Region III. The descriptions and agro-ecological characteristics of the selected research areas is presented in Table 2.1. The provinces were selected among the 10 provinces of Zambia based on the importance of cowpea and other food crop production.

### **2.2.2 Sampling procedure**

The study used a purposive sampling procedure. Sampling followed the hierarchy of administration system in Zambia that included Province, District, Block and Camp. Briefly, the sampling was done as follows; from Eastern Province, two representative districts were sampled namely Chipata and Mambwe (Table 2.1). From Chipata District, Chiparamba agricultural block was sampled comprising of two agricultural camps (Kalichero and Chawa). In Mambwe district, Masumba agricultural block was sampled with two agricultural camps namely Masumba and Ncheka (Table 2.1). The sampled districts from Northern Province were Mpika and Kasama with agricultural blocks Mpika Central and Kasama Central sampled, respectively. The sampled agricultural camps from Mpika were Mpika Central and Chishibeso, while from Kasama, Kasama Central and Kasonde were sampled (Table 2.1). Three districts were sampled from Southern Province including Mazabuka, Gwembe and Chirundu. In Mazabuka district, three agricultural blocks were sampled (Ngwezi, Magoye and Dumba). The Ngwezi and Dumba agricultural blocks each were represented by Ngwezi and Munjile agricultural camps, respectively. The Magoye block was represented by two agricultural camps (Maunga and Magoye). In Gwembe and Chirundu Districts, only one agricultural block was sampled namely Gwembe and Lusitu, respectively (Table 2.1). An agricultural camp

comprised of three to five villages. The number of villages sampled from each agricultural camp varied from two to five villages.

The number of participants during the study are presented in Table 2.2. In the study, 187 farmers were sampled with good representation of gender and age groups. Samples represented youths, elderly men and women. PRA participants comprised of farmers, agricultural officers, civic leaders (such as ward councillors) and traditional leaders (such as headmen) with different roles in cowpea production. Agricultural officers and civic leaders were contact personnel for the survey. Officers were also helpful in explaining how farmers acquired their production inputs and indigenous agricultural methods used in the areas. Traditional leaders gave permission for the villagers to be interviewed and had to be present in all group discussions to make sure that all the norms are followed. They were useful in explaining how traditional land was acquired for crop production in their areas. To aid this study, different cowpea grains were displayed on trays. In addition, different potted cowpea plants were displayed to the farmers for selection. Farmers were allowed to select their preferred grains and plant types of different varieties.

### **2.2.3 Data collection**

Data were collected using different methods. These included focus group discussions (FGDs), interviews using semi-structured questionnaires and transect walks. The FGDs involved different categories of farmers to discuss different topics on cowpea. Two to three farmers were selected from each agricultural camp and 15 to 25 farmers participated in each focus group. In total 14 focused group were available for the study. The selection of farmers for FGDs was done in conjunction with agricultural extension officers and other traditional leaders.

The semi-structured questionnaire was administered and quantitative and qualitative data collected on demographic composition of the cowpea farmers, cowpea production practices, significance of cowpea in the study areas. Also, important diseases and pests of cowpea, other production constraints, preferred and non-preferred traits of cowpea were collected through the questionnaire. The researcher, agricultural officers and farmers participated during transect walks to validate data collected during FGDs and interviews.

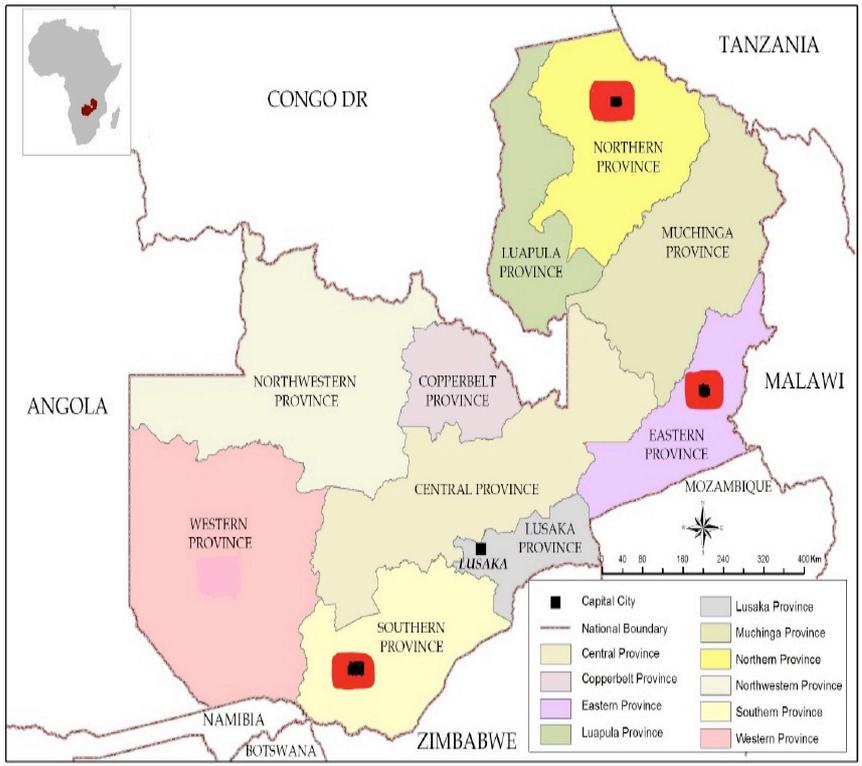


Figure 2.1 Map of Zambia showing the survey areas (Source: Geology.com 2018)

Table 2.1. Description of the study sites in three agro- ecological regions of Zambia

Province	District	Block	Camp	AER	Alt (m)	GPS	Rainfall (mm)	Temp (°c)	Dominant soils	
<b>Eastern</b>	Chipata	Chiparamba	Kalichero	2	900-1300	-14.367, 31.682	800-1000	32	Highly leached red to brown clay and shallow to gravel soils	
			Chawa	2	900-1300	-13.610, 32.469	800-1000	32		
	Mambwe	Masumba	Masumba	2	900-1300	-13.258,31.931	800-1000	32		
			Ncheka	2	900-1300	-10.803, 24.277	800-1000	32		
<b>Northern</b>	Mpika	Mpika central	Chishibeso	3	<1300	-11.837, 31.441	<1000	28	Clay to loamy soils, slightly leached clay soils and Kalahari soils	
	Kasama	Kasama central	Kasonde	3	<1300	-10.283,31.051	<1000	28		
<b>Southern</b>	Mazabuka	Ngwezi	Ngwezi	1	300- 900	-17.527, 24.277	>800	38	Loamy and clay soils with coarse to fine loam top soils and reddish coarse sandy soils	
			Magoye	Maunga	1	300- 900	-18.185, 23.887	>800		38
				Magoye	1	300- 900	-18.185, 23.887	>800		38
	Dumba	Munjile	1	300- 900	-15.381, 28.322	>800	38			
		Gwembe	Gwembe	Gwembe Central	1	300- 900	-16.625, 27.655	>800		38
				Chirundu	lusitu	Lusitu	1	300- 900		-15.433, 28.290

AER agro-ecological region, GPS global positioning system, Alt (m) Altitude (metres), Temp (°c) Temperature (degree Celsius)

Table 2.2. Description of participants during the survey

Province	District	Block	Camp	Village	Female	Male	Total	
Eastern	Chipata	Chiparamba	Kalichero	Katema	2	4	6	
				Majuku	2	3	5	
				Gampany	4	2	6	
				Pepe	1	3	4	
			Chawa	Kambani	0	1	1	
				Kasinje	0	1	1	
				Chikalawa	0	1	1	
				Beni	0	1	1	
				Chiloba	1	0	1	
	Mambwe	Masumba	Masumba	Maulidi	0	1	1	
				Teddy	1	0	1	
				Kango	2	0	2	
				Chiulumo	1	0	1	
				Katungu	1	0	1	
				Chilumba	1	0	1	
				Ncheka	Mnkhanya	0	3	3
					Kapantha	0	1	1
					Muchochoma	0	1	1
					Kasinje	0	1	1
Total					16	24	40	
Northern	Mpika	Mpika central	Chishibeso	Mubanga	3	7	10	
				Chibansa	2	5	7	
	Kasama	Kasama central	Kasonde	Chilebela	3	6	9	
				Kasonde Mutukwa	3	6	9	
				Chileshe Mkulu	2	4	6	
Total					13	29	41	
Southern	Mazabuka	Ngwezi	Ngwezi	Simuzila	2	1	3	
				Magoye	Maunga	2	0	2
					Magoye	3	2	5
		Dumba	Munjile	1	1	2		
	Gwembe	Gwembe	Gwembe Central	Mweemba	1	2	3	
				Hadyabantu	10	8	18	
				Moonde	8	5	13	
				Hagwanama	5	10	15	
				Choolwe	5	12	17	
				Hademaunda	8	8	16	
	Chirundu	Lusitu	Lusitu	Chitalika	7	5	13	
Total					52	53	106	
Grand total					81	106	187	

## 2.2.4 Data analysis

Data was compiled, organised and analysed using the Statistical Package for the Social Sciences (SPSS) version 24 (SPSS 2017). Descriptive statistics and frequencies were performed and pairwise comparisons between variables were achieved through cross-tabulations. Statistical inferences were made based on the Pearson Chi-square test statistics.

## 2.3 Results

### 2.3.1 Demographic description of the study areas

The demographic profiles of participants are presented in Table 2.3. Male participants were slightly higher (56%) than females (44%). There was non-significant difference ( $P > 0.05$ ) among the age group of respondent farmers. Farmers' with age group between 35 and 49 years represented over 65% of the participants, followed by age group between 20 and 34 years at 28%. Interestingly, all age groups actively participated in crop production activities. The respondents did not own private land but used traditional land (allocated by Chiefs) for all agricultural activities. The proportion of land allocated to cowpea varied from 0.125 ha to 1.0 ha in the surveyed areas.

Table 2.3. Gender and age representation of farmers interviewed in the survey areas

Variable	Class	Province (%)			Total (%)	df	Chi-square	P-value
		Eastern	Southern	Northern				
Gender	Male	8.80	38.50	8.80	56.00	2	13.06	0.001
	Female	10.40	18.70	18.70	44.00			
Age (years)	15 – 19	1.10	4.40	0.00	5.49	6	12.06	0.60
	20 – 34	6.04	19.23	3.30	28.57			
	35 – 49	12.10	33.00	20.33	65.38			
	> 50	0.00	0.55	0.00	0.55			

df= degrees of freedom; P-value= probability value

### 2.3.2 Importance of cowpea

In the study areas, farmers perceived cowpea as an important source of food and cash income. Results indicated significant differences ( $p < 0.000$ ) among respondents in the use and value of

cowpea in their livelihoods (Table 2.4). Participants in Southern Province had recorded the highest percent in using cowpea for cash income (25.4%) followed by for home consumption (24.9%). Cowpea was traded for its grain for food and for seed. 14.9% of respondent farmers in the Northern Province grew cowpea for cash income and 2.2% for food, while in Eastern Province 7.7% cultivate the crop for food and 9.9% for cash income. Other uses of cowpea included as food supplement for the sick (2.2%), livestock feed (1.7%), soil fertility improvement through crop rotation (5.6%) and a source of manure (2.8%). Figure 2.2 shows farmers discussing the types of cowpea varieties and their usage.

Table 2.4. Uses and significance of cowpea in the surveyed areas

Uses	Province (%)			Total	df	Chi-square	P-value
	Eastern	Southern	Northern				
Human food	9.9	24.9	2.2	37			
Livestock feed	0	1.7	0	1.7			
Cash income	7.7	25.4	14.9	48.1			
Dowry	0	0.6	0	0.6	14	48.3	0.001
Manure	0.6	1.1	1.1	2.8			
Soil fertility	1.1	3.3	1.2	5.6			
Seed	0	0	2.2	2.2			
Food supplement	0	0	2.2	2.2			

df= degrees of freedom; P-value = probability value



Figure 2.2 Farmers discussing uses of different cowpea varieties

### 2.3.3 Constraints to cowpea production

Tables 2.5 and 2.6 present farmers' perceived production constraints in the three studied provinces. Farmers in all the selected surveyed areas identified several common or specific cowpea production constraints. The results indicated non-significant differences ( $P>0.05$ ) among Provinces in the occurrence of both insect pests and diseases. Black aphids (*Aphis craccivora* Koch) and Cowpea aphid borne mosaic virus (CABMV) disease were highly common in the surveyed provinces. Most respondent farmers (46%) use seeds of unimproved local cowpea varieties for production. Other important insect pests in the study areas included pod borers (*Maruca vitrata*) and thrips (*Megalulothrips sjostedti trybom*). Limited access to production inputs (reported by 18.1% respondents), lack of knowledge on cowpea agronomy management (14.8%), lack of labour (9.3%), lack of credit facilities (3.3%), weed pressure

(2.2%) and limited access to extension services (0.5%) were other constraints limiting sustainable cowpea production.

Table 2.5. Important cowpea insect pests and diseases reported in the surveyed areas

Insect/disease	Name	Province (%)				df	Chi- square	P- value
		Eastern	Southern	Northern	Total			
Insect pests	Leafhoppers	1.1	3.8	1.1	6	8	13.02	0.22
	Aphids	17	52.7	20.3	90.1			
	Thrips	0	0.5	0	0.5			
	Beetles	0.5	0	1.1	1.6			
	Pod borers	0.5	0	1	1.5			
Diseases	Anthracnose	6	11.5	2.2	19.8	8	15.25	0.12
	CABMV	12.1	42.9	17.6	72.5			
	Ascochyta blight	0	1.1	1.1	2.2			
	Cercospora	0	0.5	1.1	1.6			
	Crown root	1.1	1.1	1.6	3.8			

df = degrees of freedom; P-value=probability value; CABMV = Cowpea aphid borne mosaic virus

Table 2.6. Major constraints to cowpea production in the surveyed areas

Constraint	Province (%)				df	Chi- square	P- value
	Eastern	Southern	Northern	Total			
Lack of improved seed	12.10	29.70	3.30	45.10	16	59.48	0.001
Lack of labour	1.60	6.00	1.60	9.30			
Pests and diseases	3.30	11.50	3.30	18.10			
Limited access to inputs	0.50	5.50	8.80	14.80			
Lack of knowledge on cowpea agronomy	1.10	0.00	3.80	4.90			
Weed pressure	0.00	1.10	1.10	2.20			
Lack of extension services	0.50	0.00	0.00	0.50			
Limited access to credit	0.00	2.20	1.10	3.30			
High labour costs	0.00	0.50	0.00	0.50			
Poor seed germination	0.00	0.50	0.50	1.10			

df =degrees of freedom; P- value = probability value

### 2.3.4 Farmers' preference of cowpea varieties

Results from direct observation through the transect walks indicated that farmers are interested on cowpea varieties with various production attributes including eating quality or taste. They preferred cultivars with good taste, early maturity, large seed size and high tolerance to pests and diseases (Table 2.7). Other preferences were province - specific that included white seed colour mostly liked in Northern and Southern Provinces and determinate growth type for Eastern and Southern Provinces.

The study found highly significant differences ( $P < 0.000$ ) among respondent farmers with regards to their choice of desirable cowpea characteristics (Table 2.8). The selected good traits were seed colour, big seed size, early maturity and ability to make thick sauce. High yielding, drought tolerance, pests and disease tolerance were among the most preferred characteristics required by farmers. These results were consistent across all the three provinces. Undesirable attributes of cowpea reported by respondent farmers included; leaf bitterness, tough leaves, late maturity, long cooking time, small seed size, black seed coat colour, short shelf life, susceptibility to pests and diseases, poor eating quality and taste (Table 2.8).

Table 2.7. Some desirable attributes of cowpea reported by respondent cowpea farmers during focus group discussion in the three provinces of Zambia

Attributes							
Province	Palatability	Maturity (days)	Seed colour	Seed size	Growth type	Tolerance to pests/diseases	Yield potential
Eastern	Ability to make thick sauce	Medium (January to April)	red	large	determinate	very tolerant	high
Northern	Ability to make thick sauce	Medium (January to April)	white	large	indeterminate	very tolerant	high
Southern	Ability to make thick sauce	Early (January to March)	white	large	determinate	very tolerant	very high

Table 2.8. Desirable and undesirable attributes of cowpea reported by interviewed farmers across the surveyed areas

Traits	Characters	Province (%)				df	Chi- square	P- value
		Eastern	Southern	Northern	Total			
Desirable	High yielding	12.2	33.3	1.1	46.7	24	93.3	0.001
	Big leaf size	0.6	0	0	0.6			
	Early maturity	1.1	0	0	1.1			
	Drought tolerance	1.1	12.8	3.9	17.8			
	Resistance to pests and diseases	1.1	8.3	5.6	15			
	Good leaf taste	0.6	1.1	3.3	5			
	White seed coat colour	1.1	0.6	1.1	2.8			
	Medium seed size	0	0	0.6	0.6			
	Smooth texture	0.6	1.7	1.7	3.9			
	Easiness to harvest	0.6	0	0	0.6			
	Good pod taste	0	0	2.2	2.2			
	Good shelf life	0	0	1.1	1.1			
	Good grain taste	0.6	0	2.2	2.8			
Undesirable	Leaf bitterness	10.5	27.1	7.2	44.8	28	45.01	0.02
	Tough leaves	0	1.7	0	1.7			
	Long cooking time	3.9	1.7	1.1	6.6			
	Very small seed size	1.7	8.3	7.7	17.7			
	White seed colour	0.6	1.1	1.7	3.3			
	Susceptibility to pests and diseases	2.2	4.4	1.7	8.3			
	Difficult to harvest	0	0	0.6	0.6			
	Difficult to remove pods	0.6	0.6	0	1.1			
	Long maturity	0	5.5	2.2	7.7			
	Short shelf life	0	2.2	0.6	2.8			
	Susceptibility to drought	0	1.7	0	1.7			
	Long maturity but low yield	0	1.7	0.6	2.2			
	Not tolerance to floods	0	0.6	0	0.6			
	Mixed grain colour	0	0.6	0	0.6			
	Selective to soil types	0	0	0.6	0.6			

df =degrees of freedom; P- value = probability value

### 2.3.5 Cowpea production practices and sources of seed

Table 2.9 contains data on cowpea varieties and sources of seed in the surveyed areas. There were significant differences ( $P < 0.000$ ) among cowpea varieties grown in the surveyed areas. Some introduced cowpea varieties were cultivated in the surveyed areas. Variety Lutembwe

was the most common cultivated introduced variety grown by 46.2% of respondent farmers, followed by local landraces (19.2%). Other varieties widely grown in the surveyed regions included Bubebe, Msandile, Namuseba and Mtilizi (Table 2.9). Zambia Research Institute developed Bubebe, Msandile and Namuseba in 1995, 2004 and 2011 respectively. These varieties are widely grown in Eastern province and known for their desirable white seed coat colour. Mtilizi was also developed by Zambia research Institute and released in 2017 for its tolerance to bruchid.

Through observations made during the transect walk, farmers revealed that cowpea was grown in fields that were located near their homesteads, while other crops such as sunflower, cotton and sweet potatoes were grown in remote fields.

Results indicated significant difference ( $P < 0.001$ ) among respondents in terms of sources of cowpea seed for production (Table 2.9). The common source of seed was farm saved seed (34.1%); followed by seed companies (24.2%), non-governmental organisations (13%) and the government through farmer support program (11.2%).

Table 2.9. Common cowpea varieties grown in the study areas and sources of seed for production in the surveyed areas

Parameter	Name/description	Province (%)			Total	df	Chi- square	P- value
		Eastern	Southern	Northern				
Variety	Mtilizi	3.80	1.10	0.00	4.90	10	113.19	0.001
	Lutembwe	7.10	34.10	4.90	46.20			
	Msandile	2.70	4.90	3.30	11.00			
	Bubebe	0.50	10.40	0.50	11.50			
	Namuseba	2.70	1.60	0.00	4.40			
	Landrace	2.1	4.90	14.80	21.90			
Sources of seed	Exchange with other farmer with other crops	1.10	1.10	0.00	2.20	10	24.50	0.01
	Farm saved seed	3.30	18.10	12.60	34.10			
	Seed companies	3.80	13.70	6.60	24.20			
	NGOs	3.30	9.90	0.50	13.70			
	Government	2.20	6.60	2.70	11.50			
	Other organisations	5.50	7.70	1.10	14.30			

df =degrees of freedom; P-value =probability value, NGOs non-governmental organisations

### 2.3.6 Cowpea cropping systems

Cowpea cropping systems in the surveyed provinces are presented in Table 2.10. There were significant differences ( $P < 0.000$ ) among the cowpea cropping systems used by respondent. Maize is the leading crop intercropped with cowpea (66.5%) followed by sorghum (18.1%) and finger millet (6%). Also, other crops such as sunflower (3.8%), sweet potatoes (*Ipomoea batatas*) (3.8 %) and cassava (*Manihot esculenta crantz*) (0.5%) were intercropped with cowpea. Farmers indicated that intercropping with traditional varieties of cowpea had little resource competition with companion crops. Table 2.11 presents direct matrix ranking of different crops during the FDGs across the three surveyed provinces. Cowpea was ranked as the fourth most important crop after maize, groundnut and dry bean.

During the transect walk, it was observed that in areas where cowpea was grown for the market, the crop was grown as a sole stand. Farmers indicated that crops grown as pure stand were more prone to pests and diseases than grown as cowpea-cereal intercrop systems. They also reported preferring intercrop systems because it saved time and resources in a form of labour for land preparation and weeding. Intercropping also reduced weed incidences since there is no space left between crop plants.

Table 2.10. Crops intercropped with cowpea and list of other main crops cultivated in the surveyed areas

Variable	Crop	Province (%)				df	Chi- square	P- value
		Eastern	Southern	Northern	Total			
Intercropped crops	Maize	9.9	42.3	14.3	66.5	12	58.54	0.00
	Sunflower	0.5	0.5	2.7	3.8			
	Finger millet	3.3	2.2	0.5	6			
	Sorghum	4.4	12.1	1.6	18.1			
	Cassava	0	0	3.8	3.8			
	Sweet potato	0	0	0.5	0.5			
	Cotton	1.1	0	0	1.1			
Main crops	Maize	8.2	30.2	6	44.5	18	61.66	0.00
	Cowpea	4.4	5.5	0	9.9			
	Soybean	0	1.6	2.2	3.8			
	Sorghum	3.3	11.5	4.4	19.2			
	Sunflower	0	0.5	0.5	1.1			
	Cassava	0	0	4.4	4.4			
	Sweet potato	0	0.5	0	0.5			
	Dry bean	0	1.6	2.7	4.4			
	Groundnut	2.2	4.9	2.7	9.9			
	Pigeon pea	0	0.5	0	0.5			

df= degrees of freedom; P-value= probability value

Table 2.11. Ranking of crops based on their importance during focus group discussion in the survey areas

Name of crop	Frequency	Ranking	Percent	Cumulative Percent
Maize	84	1	44.90	44.90
Groundnut	37	2	19.80	64.70
Beans	19	3	10.20	74.90
Cowpea	18	4	9.60	84.50
Cassava	8	5	4.30	88.80
Sunflower	8	5	4.30	93.10
Finger millet	7	6	3.70	96.80
Sweet potato	3	7	1.60	98.40
Sorghum	2	8	1.10	99.50
Pigeon pea	1	9	0.50	100.00
Total	187		100.00	

## 2.4 Discussion

### 2.4.1 Demographic description of the study

The results showed that cowpea production was relatively male-dominated (56%). Despite the narrow margin, cowpea is the woman's crop in Zambia and other sub-Saharan African countries. There are several reports that document cowpea as a woman's crop in Africa (Gómez, 2004, Murdock and Sithole-Niang, 2013, Murdock and Baoua, 2014, Togola et al. 2017, Ouédraogo et al. 2018). The relatively male dominance of cowpea production is due to the high price on the market attracting male growers (Gondwe et al. 2017). The shift in male participation of cowpea production in Zambia has also been as a result of government interventions and awareness campaigns to motivate orphan crop growers. These crops are admired for their economic value and for nutritional and income generation. Males in Zambia have access for more traditional land for agricultural activities, and hence there was a shift in cowpea production to males. During FGDs and interviews, both males and females showed interest in participating in the study. In this study there was active participation of people of 15 to above 50 years of age (Table 2.3), showing the importance of the crop for income generation and subsistence. Koutsou et al. (2014) argued that the involvement of young farmers did not only improve productivity but also brought out many good innovations in the industry. Cowpea

is a versatile crop and plays an important role in alleviating poverty and famine at household levels especially in the dry areas of country.

#### **2.4.2 Importance of cowpea for food security and income generation**

Two major uses of cowpea were identified: income generation (48%) and food (37%). For the people living in harsh and drier environments where other crops such as maize cannot do well, cowpea is grown as a cash crop. It becomes a cash crop that brings resources that could be used to pay for children's school as well as medical fees. Cowpea as a food crop plays an important role in providing nutritional elements such as antioxidants that are good for malnourished children and expecting mothers (Jimenez-Lopez and Clemente, 2019). The authors added that cowpea is low priced compared to other sources of proteins. Cowpea is considered as meat of the poor because it provides a cheap source of protein where animal protein sources are scarce. Silva (2018) reported that cowpea production is a strategic venture for the promotion of food security and health of populations in Africa.

#### **2.4.3 Constraints to cowpea production**

In all the three surveyed provinces, the most important production constraints to cowpea identified by farmers included aphids as main pre-harvest pest, cowpea aphid borne mosaic virus as an important field disease, bruchids as the main post-harvest pest and a lack of improved varieties. A report by Singh et al. (1997) and Baidoo and Mochiah (2014) indicated that aphids were the most important constraint of cowpea production in Southern Africa. Farmers reported that cowpea was susceptible to various pests in the field and storage. Pests cause severe grain damage and a farmer may end up with nothing in a short space of time if not treated with agrochemicals. Most farmers intercrop cowpea with crops such as maize, sorghum and cassava for pest and disease management of the main crops. Tiroesele et al. (2015) also lamented that cowpea weevil is a cosmopolitan storage pest and is ranked as the principal post-harvest pest in the tropics because it causes substantial quantitative and qualitative losses manifested by seed perforation as well as reductions in weight, market value and germination ability of seeds. Responded farmers could not name the actual name of CABMV disease but they were able to describe it by its symptoms such as yellowing, stuntedness and dying out of

leaves as the most notorious disease. This indicated a need for awareness of diseases with their symptoms and causal agents of cowpea in different parts of the country. Respondent farmers complained that they had limited access to improved varieties and most of the varieties used in their areas were landraces that are mostly prone to pests, diseases and extended maturity. Ficiciyan et al. (2018) reported that modern varieties are preferred due to their high yields and tolerance to pests and disease resistant.

#### **2.4.4 Farmers' preferences of cowpea varieties**

Farmers' preferences of a cowpea variety across all the study areas included large seeded grain, white seed coat, early maturity, high yield potential, good palatability and tolerance to pests and diseases resistance. Figure 2.3 shows farmers selecting cowpea varieties of according to their preference. High yield was the major factor in choice of cultivars for cultivation. The results are in line with a previous study by Abudulai et al (2016) in Ghana where farmers preferred high yielding varieties of cowpea with good taste and other economically important characteristics such as pest tolerant, disease resistance and drought tolerance. Taste, short cooking time and ability to make thick sauce were also among the important characteristics preferred by farmers. In SSA, quality and taste are growing needs in plant breeding programs. Research in rice has also reported aspects related to taste and palatability as important traits to include when developing farmer-preferred cultivars (Aoki et al. 2017, Assmann, 2017). Eating quality is as important as high yield and local adaptation in a cowpea variety. The results suggest that these characteristics had to be prioritised when improving cowpea varieties. Coulibaly and Lowenberg-DeBoer (2002) concluded that knowledge of the preferred attributes by farmers played a vital role in developing improved cowpea varieties. In the same vain, Persley and Anthony (2017) added that breeders are required to know the important and preferred characteristics for quick acceptance and adoption of their varieties along the value chain.

The cowpea variety Msandile was highly valued by farmers in Chipata and Mambwe districts. Farmers indicated their need for more characteristics in the same variety such as good grain to make thicker sauce and bigger leaf size as leaf vegetable. In Southern Province, the choice of varieties Namuseba and Bubebe was mainly for income generation. These varieties have high quality of grain such as white testa colour and large sized grain. This was in agreement with

the study conducted in West Africa by Langyintuo et al. (2004) who reported that large white grain seed colour with tolerance to pests especially bruchid spp. were highly considered by farmers hence, used as acceptability criteria in breeding of cowpea varieties. The varied choices of varieties by farmers seemed to have been influenced by both local and international market demand. Therefore, market-driven quality attributes are critical in demand-led cowpea breeding programs.



Figure 2.3 Farmers selecting cowpea varieties according to their preference

#### **2.4.5 Cowpea production practices and sources of seed**

Among the released cultivars, Lutembwe was the most popular (56%) and its seed was found in all the study areas compared to the other four. However, most farmers rely on traditional landraces that are easily accessible to produce the crop. The popular landraces are known by different names such as “Nyemu”, “Kobwe” and “Chimponongo”. The landraces Kobwe and

Nyemu are comprised of a mixture of colours such as black, purple (violet) and light brown. Traditional landraces have varied benefits such as good adaptation to marginal agricultural environments and being a source of resistance to bacterial blights such as caused by the cowpea bacterial blight (*Xanthomonas axonopodis* pv. *Vignicola*) (Durojaye et al. 2019). However, landraces have challenges of being very low yielding and take long to mature. de Freitas et al. (2012) reaffirmed that in spite of the traditional landraces having genetic diversity, good specific environmental adaption and low input systems, they have limited yield gains.

Farmers in the study areas obtain cowpea seed from different sources such as farm saved, seed companies, non-governmental organisations (NGOs) and government support program. Cowpea being a self-pollinated crop, most farmers are able to recycle the seed and use it in the subsequent years. Zambia has more than ten seed companies but very few (less than 4) seed companies are producing self-pollinated crops. However, non- governmental organisations and the government of Zambia through government support program provide farmers, especially in drier areas, with cowpea seed.

#### **2.4.6 Cowpea cropping systems**

The cropping systems in Zambia showed that farmers prefer to intercrop cowpea with maize, sorghum and millet among other crops. The cowpea's compatibility with other important crops offers better land use value, increased soil fertility and reduced pests and disease incidences. Adeniyani et al. (2011) highlighted that intercropping as an agricultural system provide farmers with a steady income and leads to increased food security. Olufajo and Singh (2002) and Masvaya et al. (2017) indicated that intercropping legumes with cereals would lead to profit maximisation, risk reduction in case of failure of the main crop, soil fertility improvement and better weed control. Farmers indicated that the difference in growth period and maturity of mixed crops enabled them to have constant supply of food throughout the year. The farming systems were characterised by non-use of chemical fertilisers and supplementary irrigation. Thus, cowpea can contribute to soil fertility amelioration, and being drought-tolerant leads to improved food security in the region. Other crops grown in the study areas included maize, groundnuts, cassava, beans, sorghum, sunflower, Bambara nuts, millet and sweet potatoes. Cowpea ranked fourth after maize, groundnuts and beans.

The results obtained in this study are key for future demand-led cowpea breeding and variety adoption. The study indicated that participatory rural appraisal approach is an important pre-breeding tool for improved food and nutritional security in SSA region. Kitch et al. (1998) and Chambers (2014) confirmed the method to clearly demonstrated the effectiveness and efficiency in utilisation of knowledge from the indigenous farmers. In the study areas, the PRA approach brought openness and friendly atmosphere for farmers to engage in discussions on a number of important matters concerning cowpea production and its challenges.

## **2.5 Conclusions**

The aim of the study was to investigate farmers' preferences, constraints and perception on cowpea production in Zambia. Zambia, like many other SSA countries depend heavily on cereals especially maize and sorghum as food staples. These crops are mainly poor in protein and other micronutrients. Millions of people are at risk of malnutrition because of the heavy dependence on starch-based staple foods. The cultivation of legumes, especially cowpea offers a huge incentive to farmers in SSA, as it boosts nutritional productivity and food security. Cowpea has vast uses and varied significance levels in the region ranging from source of income, human food, soil fertility and being livestock feed. Both males and females participated in the cowpea production, with the former dominating the cropping systems, which was contrary to the expectations. Farmers identified major production constraints as pest, disease and use of unimproved seed. Priority traits preferred by farmers were high yield, pest tolerance and disease resistance as well as good leaf taste for leaf vegetable. Unimproved/ local cultivars were dominant in the production systems despite there being low yielding and their susceptibility to pests and diseases. Farmers preferred several of these traits to be incorporated into one cultivar. The development of high yielding cowpea cultivars require a good selection of high yielding genotypes from both landraces and exotic varieties for introgression of farmer preferred traits. The preferred grain traits across the three agro- ecological regions were large seed, white seed coat, good taste and ability to provide thick sauce with tolerance to insect pests and diseases. The mentioned characteristics should therefore be included in the cowpea breeding programmes for ease of adoption and acceptance of newly developed varieties by farmers across the country.

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### CHAPTER 3. ASSESSING THE GENETIC DIVERSITY OF COWPEA [*VIGNA UNGUICULATA* (L.) WALP.] GERMPLASM COLLECTIONS USING PHENOTYPIC TRAITS AND SNP MARKERS

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#### Abstract

**Background:** Productivity of cowpea in sub-Saharan Africa is curtailed by a lack of farmer-preferred and improved cultivars and modern production technologies. The objectives of the study were to determine the extent of genetic diversity present among a collection of cowpea accessions from Zambia and Malawi using phenotypic traits and single nucleotide polymorphism (SNP) markers and, to select distinct and complementary parental lines for cultivar development. One hundred cowpea genotypes were evaluated for agronomic traits in two selected sites in Zambia, using a 10×10 alpha lattice design with two replications. Ninety-four of the test genotypes were profiled with 14,116 SNP markers.

**Results:** Number of pods plant<sup>-1</sup> (NPP), pod length (PDL), and number of seeds pod<sup>-1</sup> (NSP), were significantly ( $p<0.05$ ) affected by genotype × environment interaction effects. Genotypes such as CP411, CP421, CP645, CP732, Chimponongo, and MS1-8-1-4 exhibited higher grain yield of > 1200 kg/ha with excellent performance in yield components such as NSP, PDL, HSW and GYD. Grain yield had significant ( $p<0.05$ ) associations with NPP ( $r=0.50$ ), NSP ( $r=0.46$ ) and PDL ( $r=0.42$ ) useful for simultaneous selection for yield improvement in cowpea. The SNP markers revealed gene diversity and polymorphic information content of 0.22 and 0.17, respectively, showing that the tested cowpea accessions were genetically diverse. Test genotypes were classified into four genetic groups irrespective of source of collection allowing selection and subsequent crosses to develop breeding populations for cultivar development.

**Conclusions:** Genotypes Bubebe, CP411, CP421, CP645, Chimponongo and MS1-8-1-4 were identified to be the most genetically divergent and high yielding making them ideal parental lines for breeding. This study provided a baseline information and identified promising cowpea genetic resources for effective breeding and systematic conservation.

**Key words:** cowpea, genotypic diversity, phenotypic traits, SNP makers, population structure, yield components, Zambia.

Note: This chapter was published in BioMed Central Genetics Journal Nkhoma, N., H Shimelis, M Laing, A Shayanowako, I Mathew 2020 Assessing the Genetic Diversity of Cowpea [*Vigna unguiculata* (L.) Walp ] Germplasm Collections using Phenotypic Traits and SNP Markers DOI: <https://doi.org/10.1186/s12863-020-00914-7>

### 3.1 Background

The Southern African Development Community (SADC) gene bank in Lusaka/Zambia conserve diverse cowpea germplasm collections. The country serves as Plant Genetic Resources Centre coordinating the works of some 16 National Plant Genetic Resources Centres (NPGRCs) in southern Africa [1]. Farmers in southern Africa widely grow unimproved landraces due to a lack of improved and locally adapted farmer-preferred cultivars. Landraces exhibit low yield potential, heterogeneous in flowering and maturity, poor processing quality, and low palatability and digestibility [2]. Low palatability and digestibility are adaptive traits against field and storage pests, traits resulted from repeated cycles of natural and artificial selection. The low palatability and digestibility of landraces reduce their utility for human consumption due to prolonged cooking time and reduced bioavailability of essential nutrients. Therefore, the cowpea genetic resources found in the region can be explored as a novel source of genetic variation for breeding programs.

A well-characterised crop genetic resource is a precondition for effective breeding and genetic conservation. Genetic diversity is assessed using phenotypic traits and molecular markers. Phenotypic characterisation in the target production environment enables identification and quantification of genetic variation for key qualitative and quantitative traits for ideotype breeding. Knowledge of phenotypic variation and traits relationship assist crop breeders to develop the most adaptive and productive cultivars [3]. The genetic diversity of cowpea for phenotypic traits is assessed using standard descriptors developed by the International Board for Plant Genetic Resource [4]. Key phenotypic traits include days to flowering, time to maturity, growth habit, flower colour, number of pod plant<sup>-1</sup>, pod length, number of seeds pod<sup>-1</sup>, seed colour, seed size, hundred seed weight and grain yield [4].

Various DNA markers such as the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD) and single nucleotide polymorphisms (SNP) have been used in cowpea genetic diversity analysis [5; 6; 7]. SNPs are markers of choice in genetic diversity analysis because they are widely distributed throughout the genome and their detection is amenable to automation [8]. In addition, SNP markers are increasingly time and cost efficient to genotype large populations with a relatively higher throughput [9]. SNP markers were applied in genetic diversity analysis of cowpea [5].

Cowpea is one of food security crops in Zambia widely cultivated in the eastern, southern and western regions. Hitherto, only seven cowpea varieties were released in the country that are relatively poor performers (< 700 kg/ha) and largely succumbed to emerging pests and diseases. The genetic diversity present among the germplasm collections conserved in the gene bank and landraces cultivated by smallholder farmers in Zambia can be explored for cowpea breeding and new cultivar deployment. Therefore, the objectives of the present study were to determine the extent of genetic diversity present among a collection of cowpea accessions from Zambia and Malawi using phenotypic traits and SNP markers, and to select distinct and complementary parental lines for cultivar development in Zambia.

## **3.2 Materials and Methods**

### **3.2.1 Plant materials**

The study used 100 cowpea germplasm collections acquired from different sources (Supplementary Table 1). The germplasm included 29 advanced breeding lines and released cultivars from Malawi, 21 genotypes from the University of Zambia, 15 landraces collected from smallholder farmers in Zambia and 35 genotypes from National Gene Bank/Zambia. The 21 genotypes from the University of Zambia included 14 mutant lines (initially derived from three parental lines; Lutembwe, Bubebe and Msandile), five released cultivars (Namuseba, Mtilizi, Lutembwe, Bubebe and Msandile) and two accessions originally sourced from the International Institute of Tropical Agriculture (IITA)/Nigeria. The accessions from IITA and the released cultivars were used as standard checks.

### **3.2.2 Phenotyping**

#### **3.2.2.1 Description of the study sites**

The 100 genotypes were field evaluated during the 2017/2018 main crop season at the following two sites: the Seed Control and Certification Institute (SCCI) in Chilanga and Golden Valley Agricultural Research Trust (GART) in Chisamba/Zambia. The study was undertaken in two sites, only in one growing season due to the nature of the crop that is self-pollinating that depend less on external factors, and there are no new character introductions to the

offspring. The SCCI site is situated at a latitude of 15° 32'S and a longitude of 28°11'E with an altitude of 1206 meters above sea level. The total mean annual rainfall at the SCCI site is 1092 mm, while the mean daily minimum and maximum temperatures were 12°C and 26°C, respectively. The GART site is situated at a latitude of 14° 96'S and a longitude of 28°10'E and an altitude of 1103 meters above sea level. The GART site receives a total mean annual rainfall of 884 mm with mean daily minimum and maximum temperatures of 10°C and 30°C, respectively. The soils at both sites are classified as Haplustalf clays with pH of 5.8 and 5.2 at SCCI and GART, respectively [31].

### 3.2.2.2 Trial design, field planting and management, and data collection

The experiments were laid out in a 10×10 alpha lattice design with two replications due to limited germplasm. Each genotype was sown in a plot with two rows of 5m long. The plot area was 3.75m<sup>2</sup>. The inter-row and intra-row spacings were 75 and 45 cm, respectively. Two seeds were sown per station at a depth of 2cm and later thinned to one plant two weeks after emergence. Basal fertiliser (N: P: K), containing 20% nitrogen, 10% phosphorus and 20% potassium, was applied at a rate of 200 kg ha<sup>-1</sup> prior to planting. All other agronomic practices for cowpea production were followed as recommended for Zambia [32]. The crops were grown under rain-fed conditions and both sites received an annual rainfall of 850mm during the study.

### 3.2.2.3 Data collection

Data was collected from six qualitative and eight quantitative traits following the descriptors of the [4] and [33]. The list of traits and details of data collection and units are provided in Table 3.1. Grain yield was determined in kg ha<sup>-1</sup> based on the following formula:

$$\frac{\text{plot weight}}{\text{plot area}} \times \frac{100-14}{100-mc} \times 10,000$$
 where; mc is moisture content measured at harvesting, 14% is standard constant moisture content for legumes [33] and 10,000 is a conversion factor for a hectare.

Table 3.1 Qualitative and quantitative traits of cowpea assessed during the study.

No	Trait	Abbreviation	Trait description
<b>Qualitative traits</b>			
1	Flower colour	FLC	Flower colour intensity: violet-1, yellow -2, white- 3
2	Leaf green colour	LGC	Colour intensity: light-1, medium -2, dark- 3
3	Growth pattern	GTH	Type 1 - determinant, type 2- indeterminate, type 3- creeping
4	Pod colour	PDC	Pod colour intensity; light green-1, deep green -2, purple- 3
5	Seed coat colour	STC	Primary colour intensity of the seed coat; reddish- brown -1, white - 2, purplish- brown -3, brown - 4, black - 5,
6	Leaf size	LFS	Size of the most tip leaf ; small -1, medium -2, big -3
<b>Quantitative traits</b>			
1	Days to 50% flowering	DTF	The number of days from sowing until 50% of the plants in a plot have visible flowers
2	Days to 90% maturity	DTM	Days from date of sowing to the date when 90% of pods in a plot turn yellowish brown
3	Number of pods per plant	NPP	Mean number of mature pods from 10 randomly selected and tagged plants in a plot
4	Pod length	PDL	Mean length of 10 mature pods from randomly selected and tagged plants
5	Number of seeds per pod	NSP	Mean weight of seed from mature pods of 10 randomly selected and tagged plants
6	Seed size	SDS	Mean length of 10 randomly selected seed measured in millimetres
7	Hundred seed weight	HSW	Weight of one hundred randomly selected seeds of a genotype measured in grams
8	Grain yield	GYD	The average grain yield per plot and converted into kg ha <sup>-1</sup> using the formula given above.

### 3.2.2.4 Data analysis

The frequency of test genotypes displaying the assessed qualitative traits were summarised and statistical significant tests conducted using the cross tabulation procedure with the Statistical Package for the Social Sciences (SPSS) version 24 [34]. The quantitative data was subjected to analysis of variance (ANOVA) using the alpha-lattice procedure in GenStat® version 18

[35]. A combined analysis of variance was conducted after detecting significant differences among tested genotypes in each location. The following linear model was used for the combined analysis of variance:  $B_{ijk} = \mu + G_i + E_j + G_i * E_j + E_i(rk)(b) + \epsilon_{ijk}$ , where;  $\beta_{ijk}$ =observed response;  $\mu$ =grand mean  $G_i$  = the effect of  $i^{\text{th}}$  genotype;  $E_j$ =the effect of  $j^{\text{th}}$  location,  $G_i * E_j$ = the genotype x location interaction effect;  $E_j(rk)(b)$ =error associated with  $k^{\text{th}}$  replication in blocks in the  $j^{\text{th}}$  location and  $\epsilon_{ijk}$ =experimental error. The blocks within replications were considered as random factor, while genotypes and locations were fixed factors. Trait means of test genotypes were separated using the Tukey's honestly significant difference test at  $p \leq 0.05$  significance level. Genotypic, genotype by location interaction and phenotypic variances were computed from the expected mean squares of the analysis of variance as follows;  $\sigma^2g = \frac{msg - mse}{lr}$ ;  $\sigma^2gl = \frac{msgl - mse}{r}$ ;  $\sigma^2p = \sigma^2g + \sigma^2e + \sigma^2gl$ , where;  $\sigma^2g$  = genotypic variance,  $\sigma^2gl$  = genotype by location interaction variance,  $\sigma^2p$  = phenotypic variance, msg = mean square of genotype, mse = mean square of error, l = number of location and r = number of replication. Heritability in broad sense ( $H^2$ ) was computed according to [36], (1989);  $H^2 = \frac{\sigma^2g}{\sigma^2p} \times 100$  where;  $\sigma^2g$  is genotypic variance and  $\sigma^2p$  is phenotypic variance. Heritability was categorized as low (0–0.30), moderate (0.30–0.60) and high (>0.60) following [37]. A covariance analysis was performed to calculate coefficient of variations. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) expressed in percent were computed as described by [38] as follows:  $GCV = \left( \frac{\sqrt{\sigma^2g}}{\bar{x}} \right) \times 100$ ;  $PCV = \left( \frac{\sqrt{\sigma^2p}}{\bar{x}} \right) \times 100$ , where  $\sigma^2g$  = genotypic variance,  $\sigma^2p$  = phenotypic variance,  $\bar{x}$  = grand mean. Genetic advance was calculated following [50] as follows:  $GA = (k) (\sigma_p) (h^2)$ , where, GA = Genetic advance;  $k$  = selection differential at 5% selection intensity;  $\sigma_p$  = phenotypic standard deviation;  $h^2$ = broad sense heritability; Genetic advance as a per cent of mean (GAM) was computed following [51]:  $GAM = \left[ \frac{GA}{\bar{x}} \right] \times 100$ , where, GA (%) = Genetic advance as a per cent of mean; GA= Genetic advance;  $\bar{x}$  = Grand mean. Genetic advance as a per cent of mean was classified and rated based on the scales given by [41] as low (<10 %), moderate (10–20 %) and high (>20 %).

The magnitude of traits relationship was determined using Pearson's correlation coefficients ( $r$ ) using the SPSS version 24 [34]. Principal component analysis (PCA) was performed using the same software to examine the number principal components and trait associations. The principal components (PCs) with Eigen-values  $\geq 1.0$  were considered to explain the variation in phenotypic traits among the genotypes. PCA biplots were constructed in GenStat [35] to depict the relationships among the studied genotypes and traits.

### **3.2.3 Genotyping**

#### **3.2.3.1 DNA isolation and genotyping**

Ten seeds of each cowpea genotype were planted in a plastic pot. The seedlings were allowed to grow to the three-leaf stage before fresh leaves were harvested for DNA extraction. Leaves were sampled from each genotype for DNA extraction. Fifty milligrams of total genomic DNA was extracted from the ten well-developed trifoliolate leaves with the NucleoSpin plant II kit (Macherrey- Nagel, Duren, Germany) using the Lysis Buffer 1 (based on the CTAB method) according to the manufacturer's procedures. The DNA concentration of each sample was measured using a NanoDrop 1000 (Invitrogen, California, USA). For verifying DNA integrity, 2  $\mu\text{L}$  of DNA were subjected to gel electrophoresis on 1.0% (w/v) agarose gel, stained with ethidium bromide. Subsequently, 40  $\mu\text{L}$  of a 50ng/ $\mu\text{L}$  DNA of each sample were genotyped with Illumina Cowpea iSelect Consortium Array using Diversity Arrays Technology (DArT) markers. In total, 94 cowpea genotypes were genotyped by the genotyping by sequencing (GBS) technology as described by [42] with 20,000 DArT markers. The markers were integrated into a linkage map by inferring marker order position from the consensus Dart map. Genotyping of the materials was carried out at the Biosciences eastern and central Africa-International Livestock Research Institute (BeCA- ILRI) in Kenya.

### **3.2.3.2 Data analyses**

#### **3.2.1.1.1 SNP filtering**

For quality control, DArTseq SNP derived markers were filtered to remove bad SNPs and genotypes using the software's PLINK 1.9 in MS window and R statistical package. Markers and genotypes with >20% missing data were eliminated. Rare SNPs with <5% minor allele frequencies were also pruned from the data. After data imputation, only 14,116 informative DArTseq-derived SNP markers and 90 genotypes were used for analysis while 14240 SNPs were filtered. Four genotypes, CP1, CP2, CP479, and CP2223 were removed due to extreme heterozygosity (<90%), duplication or high levels of missing data (>20%).

#### **3.2.1.1.2 Population structure and genetic diversity analysis**

The Bayesian clustering method was used for inferring the population structure of the germplasm using the STRUCTURE version 2.3 software [43]. The STRUCTURE settings were set at a burn-in period of 5000 and 5000 Monte Carlo Markov Chain (MCMC) iterations with an admixture model to deduce the number of clusters using K values between 1 and 10. The best K- value for estimating a suitable population size was identified by the Evanno method in the online based Structure Harvester program [44]. After estimating the best K, a new run using a burn-in period of 100,000 and 100,000 MCMC was performed to assign accessions to sub-populations. The accessions with a membership probability lower than 0.80 of a sub-population were assigned to an admixture group. Population differentiation to genetic structure was assessed using a Neighbour Joining tree method [34]. Principal component analysis was conducted in TASSEL v.5 [57] using the 14,116 SNPs and plotted using TIBCO spotfire 6.5.0. A dendrogram was generated using hierarchical clustering method [47]. The expected heterozygosity ( $H_e$ ) and polymorphism information content (PIC) were calculated using [48].

### 3.3 Results

#### 3.3.2 Analysis of variance based quantitative phenotypic traits across locations

The combined analysis of variance revealed that the genotype  $\times$  site interaction effects were significant ( $P < 0.05$ ) for PDL, NPP and NSP (Table 3.2). DTF, DTM, PDL and NPP varied significantly ( $P < 0.05$ ) between the two sites. The genotypes had varied flowering and maturity date as revealed by the significant ( $p < 0.05$ ) genotypic effect. Similarly, there was significant ( $P < 0.05$ ) genotype difference for PDL, NSP, HSW and GYD.

#### 3.3.3 Mean performance of cowpea genotypes

The mean days to flowering of the test genotypes was 41 days. DTF varied from 22 days (for the genotype BB10-4-2-5) to 59 days (Kapita black) (Table 3.3). The mean DTM of test genotypes was 74 days. Genotype ZM2960 was relatively early maturing with 60 days to maturity. Other early maturing genotypes included BB10-4-2-5 (62 days), Lutechipata and ZM6680 (63 days). The number of pods per plant varied from 13 to 33. Genotypes MS1-8-1-4, CP411, BBXSC103 and Kapita black had the highest NPP ( $> 30$  pods plant<sup>-1</sup>). Pod length varied amongst genotypes. The longest pod were recorded for BBXSC13 and MS1-8-1-4 with a mean of 21 cm. The genotypes that recorded higher number of seeds per pod were Bubebe, CP421 and CP 3422 with 18.50, 18.25 and 18.25 seeds per pod, respectively.

Heavier hundred seed weight was recorded for the genotypes Kapita (15.95 g/100 seed), CP2980 and ZM6680 (15.55). There existed significant genotype difference for GYD ranging from 87 kg ha<sup>-1</sup> (for genotype ZM 6680) to 2197.7 kg ha<sup>-1</sup> (CP411). The overall mean GYD of test genotypes was 748.56 kg ha<sup>-1</sup>. Genotypes Chimponongo (with mean GYD of 2093.2 kg ha<sup>-1</sup>), CP645 (1899 kg ha<sup>-1</sup>) and MS1-8-1-4 (1779.80 kg ha<sup>-1</sup>) were among the top yielding selections. Overall, the following test genotypes were selected: Bubebe, BBXSC13, Chimponongo, CP411, CP645 and MS1-8-1-4 based on suitable and complementary quantitative agronomic traits. These genotypes are recommended as breeding parents to develop cowpea-breeding populations.

Table 3.2 Mean squares and significant tests among 100 cowpea germplasm collections evaluated based on eight quantitative agronomic traits in two locations in Zambia.

Source of variation	DF	DTF	DTM	PDL	NPP	NSP	SDS	HSW	GYD
Location (L)	1	702.20*	13806.30***	542.61***	2550.25***	29.7	0.01	6.30	107770.00
Rep(R)	2	8.30	1731.08***	56.94**	20.91	1.72	2.40	0.67	388357.00
Block (B)	18	196.90	167.30	8.84	61.44	14.77*	5.09*	13.25*	1070734.00***
Genotype (G)	99	242.50*	154.70*	14.02*	64.44	11.93*	2.10	10.26*	532280.00***
Genotype × location	99	161.00	124.30	15.13*	71.32*	11.24*	1.06	8.15	233499.00
Residual	180	148.00	107.20	10.66	50.41	8.66	1.72	7.07	207464.00
Total	399	177.60	168.40	14.08	65.69	10.41	1.80	8.38	334119.00

Note: \*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively; ns = not significant; DF: degrees of freedom; DTF: days to flowering; DTM: days to maturity; PDL: pod length (cm), NPP: number of pods per plant; NSP: number of seeds per pod, SDS: seed size; HSW: hundred seed weight (g) and GYD: grain yield in kg per hectare

Table 3.3 Mean values for grain yield and yield components of 100 cowpea genotypes showing the top 10 and bottom 5 ranked genotypes based on grain yield (kg/ha) when assessed in two locations in Zambia

Genotype	DTF	DTM	PDL	NPP	NSP	SDS	HSW	GYD
Top 10 genotypes								
CP411	34.50	73.75	20.15	32.25	16.50	3.00	13.48	2197.70
Chimponongo	51.50	79.50	19.52	26.75	16.75	5.00	20.95	2093.20
CP645	51.00	73.25	20.68	28.00	17.25	4.50	13.48	1899.30
MS1-8-1-4	39.75	68.50	21.20	33.25	15.50	5.00	15.03	1779.80
CP732	34.75	81.00	17.02	22.25	16.00	4.50	15.50	1672.40
BB14-16-2-2	36.75	74.00	19.70	25.25	15.50	3.00	11.25	1501.90
ZM3003	39.00	74.50	16.68	18.50	13.50	6.00	14.08	1454.10
CP421	44.75	72.75	19.90	24.00	18.25	3.00	16.23	1328.20
CP2	39.50	75.00	17.53	26.75	16.25	4.50	11.75	1252.70
CP601	40.00	73.50	17.85	22.25	16.75	5.50	13.83	1237.80
Bottom 5 genotypes								
ZM2966	38.50	74.50	17.25	18.75	13.75	4.00	13.43	227.10
CP2231	45.75	77.00	15.41	16.75	12.75	6.00	15.13	225.40
ZM2954	47.00	73.75	15.90	19.25	13.50	5.50	14.68	188.20
CP1769	35.25	73.00	18.63	21.25	17.25	5.00	13.55	126.00
ZM6680	29.25	62.75	12.30	16.50	11.25	5.00	15.55	87.00
Mean	41.10	73.86	17.98	21.40	15.60	4.20	12.93	748.56
SE	8.60	7.32	2.31	5.02	2.08	0.92	1.88	322.10
THSDT (5%)	16.97	14.50	4.56	9.90	4.11	1.83	3.71	635.50
CV (%)	29.60	14.02	18.16	33.18	18.86	31.09	20.55	60.85

Note: CV: coefficient of variation; LSD: tukey's honestly significant difference test; SE: standard error; DTF: days to flowering; DTM: days to maturity; PDL: pod length (UNIT?); NPP: number of pods per plant; NSP: number of seeds per pod; SDS: seed size (mm); HSW: hundred seed weight (g/100 seed); GYD: grain yield in kg ha<sup>-1</sup>

### 3.3.4 Variation based on qualitative phenotypic traits

There were significant differences ( $P < 0.00$ ) among test genotypes for key qualitative traits (Supplementary Table 2). For growth habit, 43 of the accessions were indeterminate, 39 determinate and 18 creeping types. Genotypes with predominantly upright growth type and short plant height were Bubebe, Namuseba, Msandile and MS1-8-1-4. Chimponongo and BBXSC13 had creeping growth type. Forty-nine accessions had brown and 21 black seed coat

colour, while the rest of the genotypes had 12 purple- brown, 10 white and 8 red- brown. Based on leaf colour genotypes were assorted into light green (26 genotypes), light green (35) and dark green (39). Pod colour was variable varying from deep green (52 genotypes), light green (30) and purple (18). There were three classes of genotypes based on flower colour: 95 genotypes displayed violet flower, while four had yellow and one had white. Therefore, a combination of the assessed qualitative traits are useful markers for genotype selection in cowpea improvement programs.

### **3.3.5 Variance components and heritability of quantitative agronomic traits**

Phenotypic coefficient of variation (PCV) values were higher than genotypic coefficient of variation (GCV) for all the traits (Table 3.4). The GCV values ranged from 0 to 14.6%, while the PCV ranged from 0 to 21.56%. Larger discrepancies between GCV and PCV estimates were observed for all assessed traits. The genotypic variance accounted for  $\geq 50\%$  of the total variation for grain yield. Low heritability ( $\leq 30$ ) estimates were recorded for days to maturity, hundred seed weight, number of seed pod<sup>-1</sup> and pod length and number of pod plant<sup>-1</sup>. The heritability estimates for days to flowering and seed size were moderate (30-60%), while grain yield recorded heritability estimates above 60% that will enhance the response to selection and breeding gains. Genetic advance ranged from 0 to 20.58%. Seed size and days to flowering had moderate GA% (10- 20%).

Table 3.4 Estimates of variance components and genetic parameters for yield and yield components among 100 cowpea genotypes evaluated in two locations in Zambia

Component	DTF	DTM	PDL	NPP	NSP	SDS	HSW	GYD
Genotype (G)	21.75	9.27	0.00	0.00	0.42	0.38	0.57	0.10
Location (L)	148.31	125.56	11.10	50.46	8.65	1.78	7.48	0.23
G x L	6.32	0.00	2.01	10.43	1.30	0.00	0.34	0.00
Total (G + L + G x L)	176.38	134.83	13.12	60.89	10.37	2.17	8.39	0.34
Phenotypic variance	61.99	40.66	3.78	17.83	3.23	0.83	2.61	0.16
Heritability (%)	35.00	23.00	0.00	0.00	13.00	46.00	22.00	64.00
GCV (%)	11.35	4.12	0.00	0.00	4.14	14.68	5.82	0.04
PCV (%)	19.16	8.63	10.82	19.73	11.52	21.58	12.48	0.05
GA	5.69	2.99	0.00	0.00	0.48	0.87	0.72	0.53
GA (%)	13.85	4.05	0.00	0.00	3.07	20.58	5.60	0.07

GCV: genotypic coefficient of variation; PCV: phenotypic coefficient of variation; GA: genetic advance; GA (%): genetic advance as a percentage of the mean; DTF: days to flowering; DTM: days to maturity; PDL: pod length; NPP: number of pods per plant; NSP: number of seeds per pod; SDS: Seed Size; HSW: hundred seed weight (g); GYD: grain yield in kg per hectare

### 3.3.6 Correlations among quantitative traits

Phenotypic correlation coefficients among assessed quantitative traits is summarised in Table 3.5. Grain yield showed significant ( $P \leq 0.05$ ) correlations with PDL ( $r=0.42$ ), NPP ( $r=0.50$ ) and NSP ( $r=0.46$ ). The following traits exhibited significant ( $P \leq 0.05$ ) correlations: DTF and DTM ( $r=0.66$ ), PDL with NPP ( $r=0.44$ ) and NSP ( $r=0.64$ ). NPP and NSP were significantly correlated ( $r=0.38$ ), while HSW and SDS exhibited a relatively stronger association ( $r=0.51$ ).

Table 3.5 Correlation coefficients of grain yield and yield components among 100 cowpea genotypes evaluated at two locations in Zambia

<b>Traits</b>	<b>DTF</b>	<b>DTM</b>	<b>PDL</b>	<b>NPP</b>	<b>NSP</b>	<b>SDS</b>	<b>HSWT</b>	<b>GYD</b>
DTF	1							
DTM	0.66**	1						
PDL	-0.05	0.01	1					
NPP	-0.05	-0.05	0.43**	1				
NSP	-0.05	0.03	0.64**	0.38**	1			
SDS	-0.01	0.00	-0.04	-0.09	-0.30**	1		
HSW	0.01	-0.06	0.07	-0.09	-0.12	0.51***	1	
GYD	-0.05	-0.07	0.42**	0.50**	0.46**	-0.12	0.04	1

Note: \*, \*\*, \*\*\* = Significant at 10%, 5% and 1% respectively; DTF: days to flowering; DTM: days to maturity; PDL: pod length (cm), NPP: number of pods per plant; NSP: number of seeds per pod, HSW: hundred seed weight (g), GYD: grain yield in kg per hectare

### 3.3.7 Principal component (PC) and bi-plot analyses

The first three PCs with Eigen-values greater than 1 accounted for 71.25% of the total variation exhibited by the assessed quantitative traits (Table 3.6). The first principal component (PC1) accounted for 31.5%, while PC2 and PC3 contributed to 20.97 and 18.78%, respectively, of the total variation. The highest contributing traits correlated with PC1 were PDL (0.84), NSP (0.82), GYD (0.75), and NPP (0.72). The loadings on PC2 were mostly contributed by DTF (0.84) and DTM (0.87), while HSW (0.80) and SDS (0.78) had the largest contributions to the variation correlated with PC3.

The relationships among the different traits and genotypes and their association with the respective principal components are further illustrated by the principal component biplot presented in Figure 3.1. The biplot dimension vectors showed a high positive correlation among traits GYD, NPP, NSP and PDL, as well as among DTF, DTM, HSW, and SDS. Most of the tested accessions were scattered in the positive side of the first principal component, with genotypes E10 (CP411), E71 (LT16-7-2-5), E13 (CP421) and E20 (CP645) excelling in grain yield and yield components.

Table 3.6 Eigen values, variances and loading scores of eight quantitative traits among 100 cowpea genotypes assessed in two locations in Zambia

<b>Traits</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>
Eigen-values	2.52	1.68	1.50
Proportion variance (%)	31.49	20.97	18.78
Cumulative variance (%)	31.49	52.46	71.25
DTF	-0.09	0.88	-0.22
DTM	-0.03	0.87	-0.27
PDL	0.84	0.06	0.04
NPP	0.72	0.02	0.13
NSP	0.82	-0.01	-0.21
SDS	-0.25	0.18	0.78
HSW	0.02	0.31	0.80
GYD	0.75	0.11	0.27

DTF: days to flowering; DTM: days to maturity; PDL: pod length (cm), NPP: number of pods per plant; NSP: number of seeds per pod, HSW: hundred seed weight (g), GYD: grain yield in kg per hectare PC=principal component

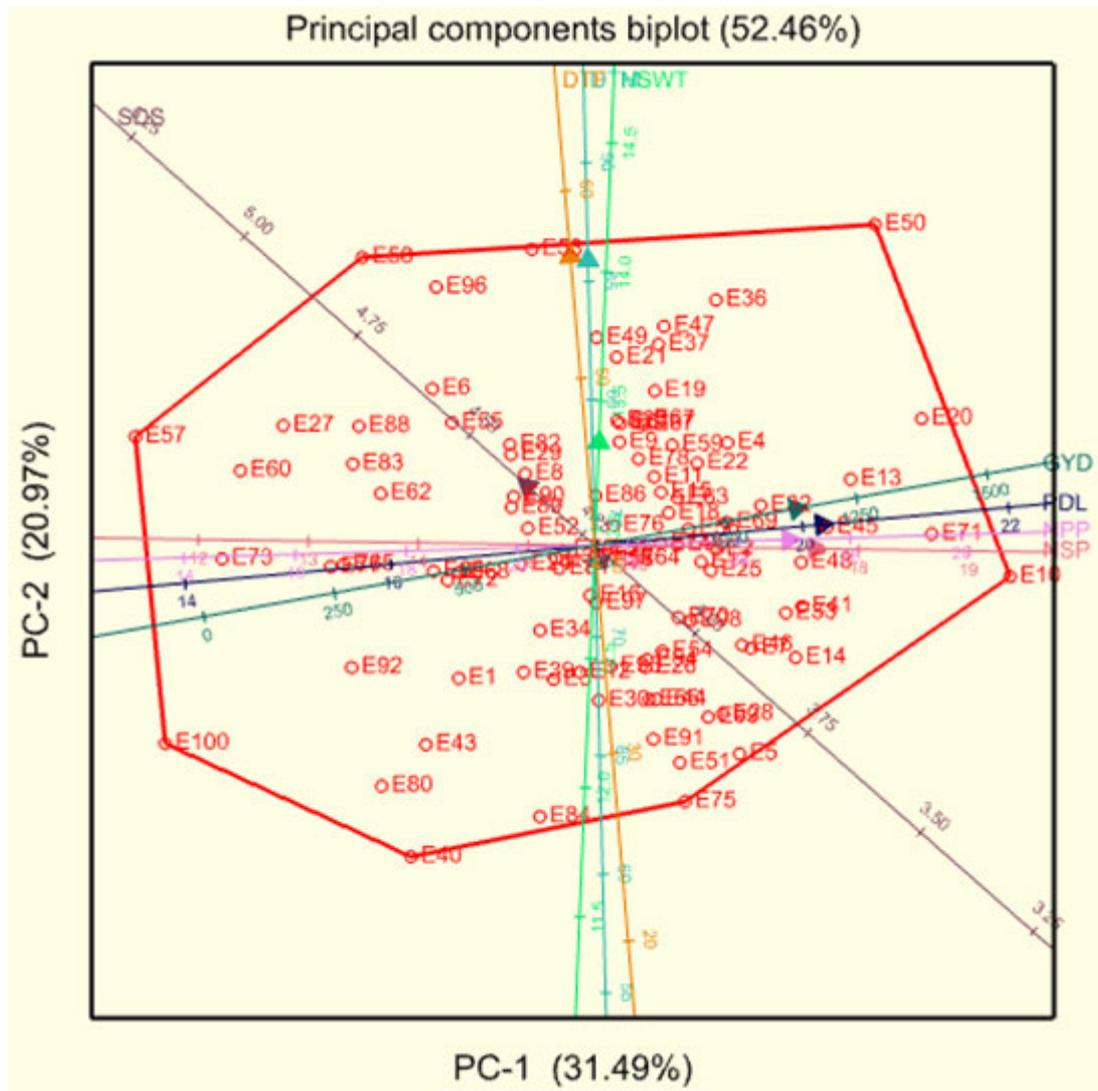


Figure 3.1 Genotype-trait biplot showing association of eight quantitative traits in 100 genotypes of cowpea assessed in two loactions.

Note: DTF: days to flowering; DTM: days to maturity; PDL: pod length (cm), NPP: number of pods per plant; NSP: number of seeds per pod, HSW: hundred seed weight (g), GYD: grain yield in kg per hectare PC-1 and PC-2: principal component 1 and principal components 2, respectively

### 3.3.8 Genetic diversity and population structure

The tested SNP markers were fairly highly polymorphic with a mean PIC value of 0.17 (Table 3.7). The PIC values varied from 0.01 to 0.38. The gene diversity (GD) varied from 0.01 to 0.50 with a mean of 0.22. The highest minor allele frequency was 0.50 with a mean of 0.18. The presently tested cowpea populations had high heterozygosity (0.30). The heterozygosity

values fell within a range of 0.25 and 0.34 showing a level of inbreeding owing to the inherent nature of self-pollination in cowpea. The mean inbreeding fixation index was -0.35.

Table 3.7 Genetic parameters of 90 cowpea germplasm collections assessed based on 14,116 SNP markers

Parameter	<b>GD</b>	<b>PIC</b>	<b>MAF</b>	<b>Ho</b>	<b>F</b>
Mean	0.22	0.17	0.18	0.3	-0.35
Lower	0.10	0.10	0.10	0.25	-0.52
Upper	0.5	0.38	0.5	0.34	-0.13

GD= genetic diversity; PIC= polymorphic information content; MAF= minor allele frequency, Ho= observed heterozygosity; F= inbreeding coefficient

The structure analysis based on the Evanno method allocated the test genotypes into four main clusters with the highest value of  $\Delta K$  that occurred at  $K=4$  (Figure 3.2A). Genotypes that scored  $<0.80$  were considered as pure line populations, while those that were  $>0.80$  as admixtures (Figure 3.2A). The model-based clustering using the 90 accessions showed the four admixture sub-populations (Figure 3.2C). Sub-population I was composed of 16 accessions (17.7%) that were sourced from Malawi and the University of Zambia. About 22 accessions (24.4%) were allocated in sub-population II and these genotypes were mainly acquired from Malawi, the National Gene Bank of Zambia and the University of Zambia. Sub-population III was the largest group, consisting of 35 accessions (38.9%). Members of this sub-population were landraces and elite lines sourced from the National Gene Bank, and the University of Zambia. Sub-population IV consisted of 17 accessions (18.9%) obtained from the University of Zambia and the National Gene Bank. The sub-population II (University of Zambia) and III (National Gene Bank) were characterized by mean  $F_{st}$  values of 0.57 and 0.69, respectively. Principal component analysis (PCoA) assigned the accessions to four admixture groups. In particular, sub-populations I and II were clustered in PC1, while sub-populations III and IV were dominant in PC2 (Figure 3.2B).

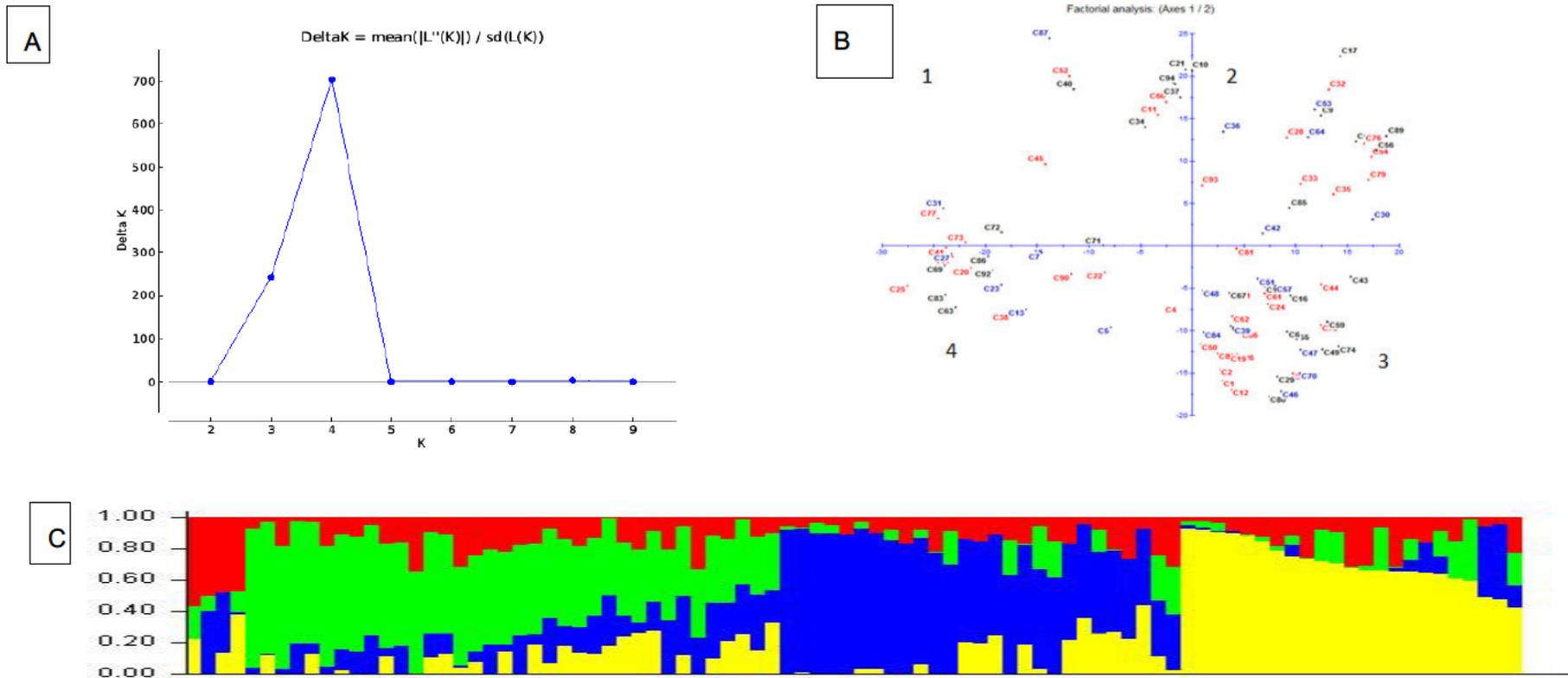


Figure 3.2 Subpopulation inference among the 90 cowpea accessions based on 14166 SNPs showing (A) likelihood and delta K values for different number of assumed clusters, (B) principal component analysis clustering of the genotypes and (C) population structure at  $K = 4$  with the different colours representing different populations.

The neighbour-joining tree revealed three heterogeneous clusters of the test genotypes (Figure 3.3). The clusters were designated as A, B and C. Each main cluster was further subdivided into two subgroups. Cluster A1 comprised of genotypes from all sources, while Cluster A2 comprised of elite lines from Malawi, University of Zambia and the National Gene Bank. Accessions from the National Gene Bank, Malawi and the University of Zambia were clustered in B1. About 35% of the accessions in B2 were acquired from the University of Zambia and the National Gene Bank. Accessions in cluster C1 were acquired from Malawi, the National Gene Bank and the University of Zambia. About 61% of accessions in cluster C2 were from the National Gene Bank, while the rest genotypes were collection from the smallholder farmers, the University of Zambia and Malawi. The red coded genotypes performed identified to be used for both green vegetables due to the stay green characteristic.

The analysis of molecular variance (AMOVA) showed a significant variation within populations. Non-significant variation were detected among the populations (Table 3.8). The observed variance partitioned among the two groups is 0.029 percent of variance portioned among individual accessions and 6.542 percent within accessions. The lack of genetic variation between the populations was confirmed by the low pair-wise genetic differentiation ( $F_{st}$ ) values ranging between -0.006 and 0.004 and inbreeding coefficient ( $F_{is}$ ) of -0.351 to -0.362 (Table 3.9).

Table 3.8 Analysis of molecular variance involving 90 cowpea accession based on source of collection

<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Sum of squares</b>	<b>Mean squares</b>	<b>Estimated variance</b>	<b><i>P</i>-value</b>
Among Population	2	14.761	7.38	0.029	n.s
Within Population	86	562.58	6.542	6.542	<0.01
Total	88	577.34	13.92	6.571	

Table 3.9 Inbreeding coefficients (Fis) and genetic differentiation (Fst) among 90 cowpea genotypes collected from three different sources

Populations	Inbreeding coefficient (Fis)		
	G1	G2	G3
G1	-	-0.365	-0.362
G2	0.004	-	-0.351
G3	0.001	-0.006	-
	Genetic differentiation (Fst)		

G1 includes all genotypes sourced from Malawi, G2 is comprised of genotypes collected from the University of Zambia, G3 consists of genotypes collected from the National Gene Bank of Zambia

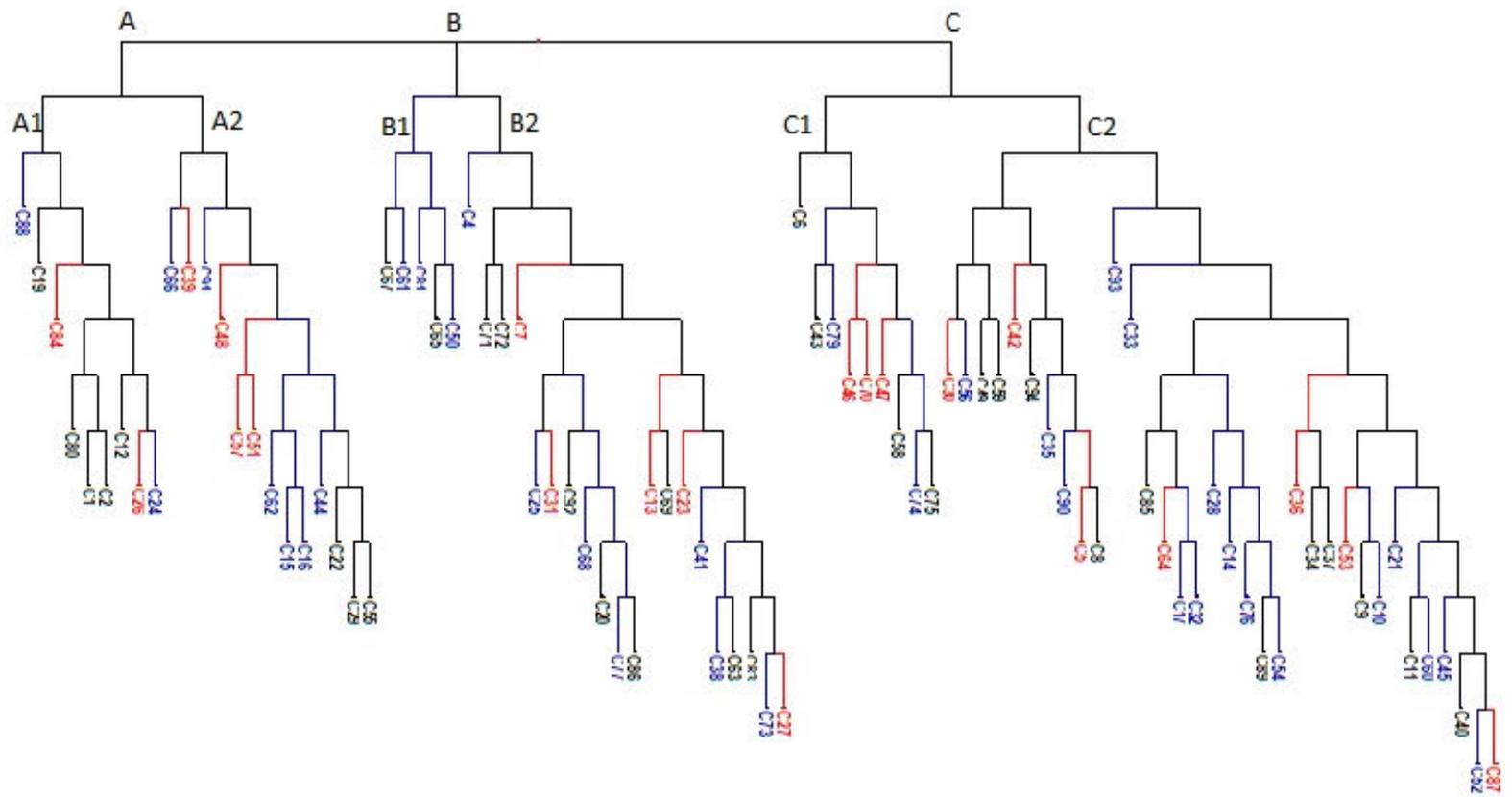


Figure 3.3 The neighbour-joining phylogenetic tree showing relatedness among the 100 cowpea genotypes based on 14 116 SNP markers

### 3.4 Discussion

#### 3.4.1 Genotypic variation and performance of test genotypes for key qualitative and quantitative traits

The present study evaluated the genetic diversity present among 100 diverse genotypes of cowpea germplasm collections from southern Africa using qualitative and quantitative phenotypic traits in two locations in Zambia. Further, high density SNP markers were used as a preliminary step to identify suitable and complementary parental lines for breeding. There were significant genotype  $\times$  location interaction (Table 3.2) effect signifying that the tested germplasm were genetically diverse for selection and cultivar development targeting the test locations. Also, the interaction effect shows that the genotypes responded differently in the test environments which can facilitate identification of cowpea lines with specific or broad adaptation. Specific and broad adaptation have been identified and exploited in the Brazilian cowpea breeding programs based on genotype  $\times$  location interaction analysis [10]. The interaction effect suggests that the test environments influence genotypic performance, which may confound genotype selection efforts by reducing the correlation between genotype and phenotypic expression [11].

In the present study, the assessed quantitative traits were affected by genotype  $\times$  location interaction effect. Hence, there is intrinsic genetic variation influenced by the test locations necessitating multi environment evaluation for selection. Differential genotype response to environmental conditions during germplasm evaluation is attributable to the differences in genetic constitution among test genotypes and micro-environmental conditions [12]. In this study, the SCCI (average yield 832kg ha<sup>-1</sup>) site is high yielding environment compared with the GART (average yield 764kg ha<sup>-1</sup>) site probably due to the prevailing favourable environmental conditions such as better soil fertility and higher moisture levels in the former. Genotype phenology and biomass production exhibit environmental plasticity due to variable soil and climatic factors [13; 14]. In the present study, some genotypes were high grain yielders (e.g. CP411 with 2197kg ha<sup>-1</sup>) and others were low yielders (e.g. ZM6680 with 87kg ha<sup>-1</sup>). Quantitative traits are under the influence of polygenes. Hence, it is pertinent for genotype selections in multiple test environments to minimise environmental variance and to enhance selection gains [16; 17]. Genotypes such as MS1-8-1-4, Msandile, BBXSC13, CP411, CP421, CP654, CP3413 and Bubebe that exhibited early to medium maturity are ideal candidates for

drought tolerance breeding to offset the incessant droughts experienced in southern Africa. Early maturity is associated with drought escape [18]. [52] reported that farmers in southern Africa prefer cultivars with a short flowering period and maturity, valuable traits to evade the “hunger period”. Highest number of seeds per pod (e.g. expressed by genotypes CP421 and Bubebe) is one of the factor affecting genotype responses based on their efficiency in growth resource utilisation and allocation. This could also be contributed to increased length of the pods by test genotypes [19]. Seed weight is directly associated with seed size and it is recommended to be used as an indirect selection criterion to maximise grain yield response in cowpea [54]. [52] reported a high yield potential of cowpea genotypes that can reach up to 3 t/ha. The yield level recorded in the present study by the landraces was relatively less. This could be the low yield potential of landraces grown by most farmers in SSA. In the region, landraces are continuously cultivated because they possess farmer-preferred quality traits and their ability to adapt under variable stress conditions due to their genetic diversity and plasticity [31; 32].

In the present study, qualitative traits such as seed coat, pod and leaf colour were more important traits for selection. These traits affect the market value of cowpea in Africa given that farmer and consumer preference are based on these attributes. Seed coat colour is often associated with processing quality (e.g. cooking time) and farmers deliberately select white seed types which have shorter cooking time [22]. The inheritance of seed coat colour is governed by few major genes that will enhance selection progress during cultivar development [23]. In this study, the genotypes Bubebe and Msandile, with predominantly light-green leaves exhibited determinate growth habit in comparison with BBXSC13 and Chimponongo that had dark green leaves and creeping growth habit. [24] reported that cowpea cultivars with a determinate growth type were more drought tolerant compared to the indeterminate types. [25] reported that indeterminate varieties of cowpea attained higher productivity due to their prolonged maturity and photosynthesis efficiency. Therefore, in order to promote sustainable production and productivity and enhanced adoption of improved cowpea cultivars, breeding programs should incorporate farmer- and market- preferred attributes in the newly developed cultivars.

### **3.4.2 Variance, heritability and genetic advance**

In this study, the heritability estimate for grain yield was high (64%), suggesting that the grain yield achieved by the accessions was highly repeatable ensuring genetic improvement through selection. The high heritability value for grain yield corroborates with the findings of [26] but lower than a heritability value of 97% reported by [19]. Genetic advance is directly related with yield gains achievable via selection. High estimates of genetic advance (e.g. for HSW and SDS) and high heritability indicate that selection would result in foreseeable genetic improvement [27; 28]. The higher values of PCV compared to GCV in this study, suggests that trait expression was also influenced by environment factors in addition to genetic effects, which was also confirmed by the significant location main effects in the ANOVA (Table 3.2).

### **3.4.3 Associations of quantitative traits**

The relationships among yield and yield components are critical in devising a selection strategy. Selection of one trait may amplify or negatively affect performance in the other traits. The high contribution and strong association of PDL, NSP, GYD, and NPP to PC1 as well as DTF and DTM with the PC2 indicated that these traits were highly discriminatory explaining the variation among the genotypes. [29] and [30] found that traits such as NPP and GYD in cowpea were associated with PC1 showing the importance of agronomic traits in cowpea evaluation corroborating with the findings of the present study. The genotype-trait biplot enables visual and simultaneous selection of genotypes for multiple traits. There was strong correlations between PDL, NPP, NSP and grain yield indicating their positive impact on genotype performance. Previous reports identified these traits being important yield-influencing attributes [19; 30]. Entries such as E10 (CP 411), E71 (LT16-7-2-5), E13 (CP421) and E20 (CP645) scored greater grain yield response and yield-influencing traits suggesting their utility in variety improvement for yield gains and breeding population development. Entries such as E10 (CP411), E20 (CP645), E13 (CP421) and E58 (Sundan1) are selected with desirable NSP, GYD, PDL and DTF, respectively.

#### **3.4.4 Population structure and genetic parameters**

Genetic analysis using SNP markers delineated the test populations into four genetic groups. This demarcation was irrespective of source of collection, suggesting that geographical sources of collection are not the sole factor for classification of cowpea genotypes. Nei's Neighbour joining algorithm was used in the study. This method provides the estimated number of individuals and populations to identifying the pattern of genetic differentiation. The landraces in the study were found in two different clusters (B1 and C2) while the elite cut across all the clusters. Malawi and Zambia have geographical proximity hence germplasm exchange between the two countries cannot be ruled out through cross border formal and informal marketing. The mutant lines were grouped together with the parental lines (elite lines) that were derived from. This shows that most of the traits remained the same in the mutants despite the genetic transformation that take place during mutation. Trait preference of farmer and the market in the region may not be significantly different leading to the overlap of cowpea genetic resources in these agro-ecologies. This has partly disallowed the population structure analysis without distinguishing the genotypes based on geographical sources agreeing to the report of [7]. Exchange of genetic resources is key for plant-breeding research and cultivar development that are dependent on wider genetic bases [54].

The PIC and GD values were essential for identification of genotypes with moderate genetic diversity within the populations from which parental lines could be selected for breeding. The elite lines together with the mutant lines that were derived from them displayed very different growth pattern compared with the landraces. This may be attributed to genetic differences in plant architecture in terms of growth habit and maturity period, among others. For example, in the study, elite and mutant lines sourced from University of Zambia such as Bubebe and MS1-8-1-4 respectively both have short maturity period and determinate growth type. Hence, selections from smallholder farmers from landraces with long duration to maturity and creeping growth habit such as Chimponongo and Kapita would be recommended to increase genetic variation and to enhance genetic gain through selection. This is consistent with the findings of other cowpea researches who indicated that architecture of the crop results in genetic diversification [53]. The mean  $F_{st}$  values recorded in the present study showed low genetic differentiation among the test populations ( $F_{st} = -0.362$ ). This could be attributed to possible genetic diversity resulted from gene combinations including through natural random mutation

events. The negative values could have been attributed to inbreeding because the crop is highly self-pollinated.

### 3.5 Conclusions

Phenotypic analysis using qualitative and quantitative traits and genotyping using high-density SNP markers revealed the presence of significant variation among 100 cowpea germplasm collections of southern Africa. Trait association analysis revealed significant correlation between NPP, NSP, PDL and GYD that could allow direct selection to improve grain yield. The SNP markers used in the study were able to deduce genetic variation among the tested cowpea populations. The largest proportion of variation was attributable to individual genotype differences that is essential for improving grain yield by crossing lines from different divergent populations. Test genotypes were classified in to four genetic groups irrespective of source of collection allowing selection for subsequent cross combinations to develop breeding populations for cultivar development. Genotypes Bubebe, CP411, CP421, CP645, Chimponogo and MS1-8-1-4 were identified being the most genetically divergent and high yielding making them ideal parental lines for breeding. This study provided a baseline genetic profile and identified promising cowpea genetic resources for effective breeding and systematic conservation.

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## CHAPTER 4. COMBINING ABILITY AND GENE ACTION IN COWPEA [*Vigna unguiculata* (L.) WALP.] GENOTYPES BASED ON GRAIN YIELD AND YIELD COMPONENTS

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### Abstract

Development of new breeding populations requires understanding of the combining abilities of complementary parents through their progenies based on economic traits. The objectives of this study were to determine the combining ability effects and deduce gene action controlling yield and yield components among selected cowpea (*Vigna unguiculata* [L.] Walp.) parental lines and their progenies for breeding. Eleven parental lines and 55 progenies generated using a half-diallel mating design were field evaluated using an 11 × 6 alpha lattice design with two replications in two representative sites in Zambia. The progenies and parents exhibited significant ( $P < 0.05$ ) variation for the assessed yield and yield components except for the number of pods per plant (NPP). Parental lines CP411, CP732, and BBXSC103 exhibited better grain yield (GYD), number of seeds per pod (NSP), pod length (PDL), and a hundred seed weight (HSW). The general combining ability (GCA) effects of parents and the specific combining ability (SCA) effects of progenies were significant ( $P < 0.05$ ) for all assessed traits except days to 50% flowering (DTF) and NSP. Parental lines CP411 and CP732 were the best combiners for NPP, HSW, and GYD. In addition, genotype CP411 exhibited desirable GCA effects for DTF and days to 90% maturity (DTM). Crosses such as CP732 × CP411, BBXSC103 × CP411 and Lutembwe × CP645 had higher and desirable SCA effects for grain yield. Traits such as HSW, NPP, and DTM were conditioned by mainly additive gene action, while DTF, PDL and NSP were under the control of dominant genes. The selected cowpea parents and progenies are useful genetic resources for breeding and genetic advancement to release improved cultivars in Zambia.

**Keywords:** Combining ability, cowpea, cultivar development, diallel analysis, gene action, yield components, Zambia

## 4.1 Introduction

Cowpea is relatively productive in water-limited and poor soil fertility environments where other common legumes and cereals fail to produce (Carvalho, 2017). Cowpea yield levels remain low (< 500 kg/ha) and stagnant in sub-Saharan Africa (SSA) due to a multitude of production constraints and a lack of improved and adapted varieties (Horn et al. 2015). There is a need to develop high yielding cultivars and improved crop management practices to boost cowpea productivity. The development of improved varieties depends on the availability of adequate genetic variation for economic traits and identification of farmer-preferred, superior and complementary parents and progenies (Oseni, 1994; Asins, 2004; Pallavi et al. 2018). The response to selection is dependent on the magnitude of genetic variation and trait inheritance from parents to the offspring (Griffings 1956; Baker 1978; Falconer 1989).

Increased grain yield is one of the key goals in cowpea breeding programs. However, grain yield *per se* is a complex trait due to the involvement of several yield components, each controlled by many minor genes. Also, trait expression is subject to genotype, environment and genotype x environment interaction effects confounding selection progress. Hence, genotype selection based on yield related agronomic traits and multi-environment trials is pursued for genetic gains. Indirect selection for grain yield through associated component traits has been shown to be more effective in grain yield improvement (Olajide and Lori, 2016; Lopes et al. 2017; Freitas et al. 2019).

Development of new breeding populations requires understanding of the combining abilities of complementary parents through their progenies based on farmer-preferred and economic traits. Selection of high performing parental lines and progenies is dependent on parental performance *per se* and combining ability analysis or progeny tests (Fasahat et al. 2016; Owusu et al. 2018; Olunloyo et al. 2019). Combining ability estimates are key to establish the relative breeding value of parents or cross combinations based on a desirable mating scheme. Combining ability analysis enables to discern the gene action involved in conditioning the inheritance of traits (Allard 1960). Broadly, combining ability effects are divided into general combining ability (GCA) of parents and specific combining ability (SCA) of the progenies. Parents with desirable GCA effects are foundational in population development through recurrent, pedigree, or pure line selection methods. The progenies that exhibit good SCA effects are primed for genetic advancement to develop stable breeding pure line cultivars in

self-pollinating crops such as cowpea. The ratios of GCA and SCA effects derived through combining ability analysis are used to deduce the gene action involved in the expression of quantitative characters (Baker 1978; Dholariya et al. 2014). The GCA effects are associated with additive gene action, while the SCA effects relate to non-additive gene action. Both genetic effects are important in the development of breeding populations or in devising a suitable selection method (Viana and Matta. 2003). Hence assessing the combining ability effects among parental lines and new progenies is fundamental notably in a start-up cowpea improvement programs.

Various mating designs are available for crop breeding and quantitative genetic analysis. An ideal mating design assists in generating genetic parameters to estimate the magnitude of genetic variance, deduce the type of gene actions controlling yield and yield related traits, and develop breeding populations for cultivar development (Singh et al. 2004; Bernardo, 2010). The most common mating designs include biparental (Mather, 1949), North Carolina (Comstock and Robinson 1948), diallel (Griffing 1956), and the line  $\times$  tester (Kempthorne 1957). The diallel mating design was first proposed by Schmidt (1919) and became an important and widely used design. In the absence of a well characterized genotypes, the diallel mating design is useful to generate all possible cross combinations among the candidate parental lines. The diallel design is amenable to genetic analysis based on reciprocal crosses using the diallel method II to overcome fertility limitations (Tai, 1976) in self-pollinating species.

Cowpea is an important crop legume in Zambia after common beans (*Phaseolus vulgaris*) and groundnuts (*Arachis hypogaea*). Cowpea production is concentrated in the eastern, northern and southern provinces of the country (Mweetwa et al. 2014). The mean grain yield reported in Zambia and southern Africa is about 250kg/ha, which is very low compared to its potential yield of 5 t ha<sup>-1</sup> elsewhere (Rao and Shahid, 2011). In a preliminary cowpea genetic enhancement study, 100 diverse collections were phenotyped with key agronomic traits and genotyped with high density single nucleotide polymorphism (SNP) markers to examine the genetic variation and select potential parents for pre-breeding in Zambia (Nkhoma et al. 2020). The tested genotypes exhibited wide genetic variation important for grain yield improvement and cultivar development. The study selected and recommended genetically divergent parental lines with suitable agronomic performance and diverse genetic background for breeding. The

combining ability of the selected genotypes and their breeding value have not been evaluated to develop breeding populations adapted to Zambia agro-ecologies. Therefore, the objectives of this study were to determine the combining ability effects and deduce gene action controlling yield and yield components among the selected cowpea parental lines and their progenies for breeding.

## **4.2 Materials and Methods**

### **4.2.1 Plant materials**

The study used 11 cowpea parents selected from a preliminary evaluation trials based on agronomic performance and genetic analysis using SNP markers (Nkhoma et al. 2020). The descriptions of the parents are summarised in Table 4.1. The key selection criteria of the parents included farmer-preferred attributes such as high grain yield (850 to 2197 kg/ha), growth habit (determinate, indeterminate and creeper types), relatively early maturity (68 to 81 days), seed size (small, medium and large) as well as seed coat colour (brown, cream, purple, red, speckled brown and white).

### **4.2.2 Generation of crosses**

The above 11 lines were crossed using a half diallel mating design aiming 55 direct crosses. The F1 seeds were developed between June and November 2018 under greenhouse condition by stagger planting of the 11 parents in a 2-week interval to synchronize flowering and pollen supply. Crosses were done manually following the standard procedures outlined by Myers (1996). All the 55 F1 crosses were generated successfully.

Table 4.1 Descriptions of the characteristics of the 11 cowpea parents used in the study

Lines	Traits				
	Growth habit	Maturity (days)	Seed size	Seed coat colour	Yield (kg ha <sup>-1</sup> )
CP 411	Indeterminate	74	Medium	Cream	2197
CP 421	Indeterminate	73	Medium	Brown	1252
CP 645	Indeterminate	73	Medium	Purple	1899
CP 732	Indeterminate	81	Large	Cream	1672
Bubebe	Determinate	77	Medium	Red	1157
BBXSC103	Determinate	71	Medium	Red	1169
Chimponongo	Creeper	79	Large	Speckled brown	2093
Lutembwe	Indeterminate	78	Small	White	850
MSI-8-1-4	Determinate	68	Medium	White	1779
Msandile	Determinate	71	Medium	White	979
Namuseba	Determinate	76	Large	White	890

#### 4.2.3 Description of the study sites

The study was conducted at two selected sites belonging to the Zambia Agriculture Research Institute (ZARI). The first site is Chilanga situated at Mount Makulu, while the second site is Mazabuka which is found at the National Irrigation Research Station in Nanga. The Chilanga site is found at ZARI headquarters (15° 32'S 28°11'E, altitude 1206 m) in the central Zambia. This site receives an average annual rainfall of 1000mm. The site is found on a plateau that experiences higher temperatures of 36°C and is regarded as high potential crop production area. It has fertile soils (clay loam) that are classified as Haplustalf with a pH of 5.8. The Nanga site (15° 85'S 27°74'E, altitude 900 m) is found in the southern region of Zambia and receives average annual rainfall above 800mm with a maximum temperature of 38°C. The soil at this site is fertile with good water retention and classified as Haplustalf clay with an average pH of 6.8 (Sichinga, 2013).

#### 4.2.4 Experiment design and trial management

At each site the experiment was laid out using an 11 × 6 alpha lattice design with two replications due to limited genotype from the progeny of the crosses. Each genotype was sown in a plot with two rows of 5m long. The net plot area was 3.75m<sup>2</sup>. The inter-row and intra-row

spacing were 0.75 and 0.45m, respectively. Two seeds were sown per hole at a depth of 2cm and later thinned to one plant per hole two weeks after emergence. Basal fertilizer comprising 20% nitrogen, 10% phosphorus, and 20% potassium was applied at a rate of 200 kg ha<sup>-1</sup> prior to planting. All other agronomic practices for cowpea production were followed as recommended for Zambia (Muliokela, 1997).

#### **4.2.5 Data collection**

Data on seven quantitative traits were collected according to the International Board for Plant Genetic Resource (IBPGR, 1983) cowpea descriptors. The days to 50% flowering (DTF) were counted from the day of planting to the date when 50% of plants in a plot had flowers. The days to maturity (DTM) were determined from day of sowing to the date when 90% of plants in a plot had matured. The number of pods per plant (NPP) was counted and recorded from five sampled and tagged plants. The pod length (PDL) was the length of the pods from mature pods randomly selected from the five-tagged plants. The number of seeds per pod (NSP) was the average number of seeds from mature pods of the five-tagged plants in a plot. A hundred seed weight (HSW) was measured as the mean weight of 100 randomly selected grains harvested per plot. The grain yield (GYD) per hectare was calculated as:

$$\frac{\text{plot weight}}{\text{plot area}} \times \frac{100-14\%}{100-mc} \times 10\,000$$

where plot weight is the weight of grain in kilograms per plot, plot area is 3.75m<sup>2</sup>, and mc is grain moisture content measured at harvest, 14% is standard constant moisture content for legumes, and 10,000 is the conversion factor for hectare.

#### **4.2.6 Data analyses**

##### **4.2.6.1 Analysis of variance**

Data collected from each site was subjected to analyses using linear mixed model (LMM) to plot the residuals and identify outliers following the lattice procedure in GenStat® version 17 (Payne, 2014). The homogeneity of residual variance was determined using Bartlett's homogeneity test and a combined analysis of variance was conducted after detecting significant differences among tested genotypes in each location. The following linear model was used for

the combined analysis of variance:  $B_{ijk} = \mu + G_i + E_j + G_i * E_j + E_i(rk)(b) + \epsilon_{ijk}$ , where;  $\beta_{ijk}$ =observed response;  $\mu$ =grand mean  $G_i$  = the effect of  $i^{th}$  genotype;  $E_j$ = the effect of  $j^{th}$  location,  $G_i * E_j$ = the genotype x location interaction effect;  $E_j(rk)(b)$ = the effect of the  $i^{th}$  block in  $k^{th}$  replication in the  $j^{th}$  location and  $\epsilon_{ijk}$ = experimental error. The blocks within replications were considered as random factors, while genotypes and locations were fixed factors. Trait means were separated by the Tukey's honestly significant difference test the 5% probability level. Pearson coefficients of correlation (r) and their level of significance were calculated using the R package "corrplot" in R software (Fox, 2005; Wei et al., 2017).

#### 4.2.6.2 Combining ability analysis

The combining abilities were estimated per site based on Griffing's Method II and Model I (Griffings, 1956) using the AGD-R Version 4.0 software (Rodriguez et al., 2015). The ANOVA for combining ability effect was conducted based on the following linear model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + \epsilon_{ijk}$$

Where,  $Y_{ijk}$  = mean value of the cross derived from the cross of  $i^{th}$  female parent with  $j^{th}$  male parent in the  $k^{th}$  replication,  $\mu$  = overall mean,  $g_i$ = the GCA effects of the  $i^{th}$  female parent,  $g_j$  = the GCA effects of the  $j^{th}$  male parent,  $s_{ij}$  = the SCA effects for the cross between the  $i^{th}$  female parent and the  $j^{th}$  male parent and  $\epsilon_{ijk}$ = random error associated with  $ij^{th}$  genotype in the  $k^{th}$  replication. The GCA estimates were calculated as follows:

$$GCA = X_{ij} - \mu$$

Where  $X_{ij}$  is the mean of the  $i^{th}$  parent across  $j$  parents, and  $\mu$  is the overall mean.

The SCA estimates were calculated as follows:

$$SCA = X_{ij} - (GCA_i + GCA_j + \mu)$$

Where  $X_{ij}$  is the mean of the cross between  $i^{th}$  and  $j^{th}$  parents,  $GCA_i$  and  $GCA_j$  are the GCA estimates of the  $i^{th}$  and  $j^{th}$  parents, respectively, and  $\mu$  is the overall mean. The significance of GCA and SCA effects were tested as follows:  $t = \frac{GCA}{se}$  and  $t = \frac{SCA}{se}$ , respectively, where  $se$ =standard error of the GCA or SCA estimates.

#### 4.2.6.3 Contribution of the GCA and SCA effects

The relative contribution of GCA to the total sum of squares was estimated using the Baker's ratio (Baker 1978):

$$\text{Baker's ratio} = \frac{2\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2}$$

Where  $\delta^2\text{GCA}$  and  $\delta^2\text{SCA}$  are variance due to general and specific combining ability, respectively.

#### 4.2.6.4 Broad-sense heritability

The broad-sense heritability ( $H^2$ ) values were calculated from the GCA and SCA component analysis following Falconer et al., (1996):

$$H^2 = \frac{2\sigma_{GCA}^2 + \sigma_{SCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + \delta^2e}$$

Where  $2\delta^2\text{GCA} + \delta^2\text{SCA}$  is additive variance + dominance variance are genotypic variance and represents the genetic effect, while  $2\delta^2\text{GCA} + \delta^2\text{SCA} + \delta^2e$  is additive variance + dominance variance + environmental variance are phenotypic variance and represents the phenotypic effect.

### 4.3 Results

#### 4.3.1 Analysis of variance and mean response of test genotypes

Analysis of variance reveals that all assessed traits exhibited significant ( $P \leq 0.05$ ) difference due to the genotype main effect (Table 4.2). NPP was affected by genotype  $\times$  location interaction. The mean performance of the first top ten and bottom five test genotypes together with the eleven parents for seven quantitative traits is presented in Table 4.3. The DTF varied from 15 days (for family CP411  $\times$  Namuseba) to 50 days (parent CP732) with a mean of 42 days (Table 4.3). On average, the genotypes took 65 days to mature while the new family CP645  $\times$  MS1-8-1-4 matured after 54 days, which was relatively the earliest in the test populations. Other early maturing genotypes included CP645  $\times$  Lutembwe (65 days) and MS1-8-1-4 (67 days). The number of pods per plant varied from 4 to 33. Crosses CP645  $\times$  Lutembwe and CP411  $\times$  CP732 had the highest NPP of more than 20 pods plant<sup>-1</sup>. The longest PDL was recorded for CP645 and Chimponongo with a mean of 18 cm each. The genotypes that recorded

the higher number of seeds per pod with values of 26, 26, and 20 seeds per pod were Chimponongo, Chimponongo × CP411, and Namuseba, respectively (Table 4.3). The highest HSW was recorded for the crosses CP645 × Chimponongo (17.75 g/100 seed), CP421 × Chimponongo (17.57g), and CP645 × CP732 (16.67g). There existed significant genotype differences for GYD ranging from 100 kg ha<sup>-1</sup> for CP411 × Namuseba to 4232 kg ha<sup>-1</sup> (CP411 × CP732). The overall mean GYD of the test genotypes was 1140 kg ha<sup>-1</sup>. The crosses that performed better than their parents in a decreasing order included CP411 × CP732 (4232 kg ha<sup>-1</sup>), CP411 × BBXSC103 (3848 kg ha<sup>-1</sup>), CP421 × MS1-8-4-1 (2780 kg ha<sup>-1</sup>), CP654 × Lutembwe (2733 kg ha<sup>-1</sup>), and CP411 × Chimponongo (2054 kg ha<sup>-1</sup>) (Table 4.3). Figure 4.1 indicates the performance of F2 genotypes at Nanga.

### 4.3.2 Trait associations

Grain yield showed significant ( $P \leq 0.05$ ) correlations with DTF ( $r=0.44$ ), DTM ( $r=0.45$ ), PDL ( $r=0.32$ ), NPP ( $r=0.37$ ) and NSP ( $r=0.48$ ) (Table 4.4). There were significant ( $P \leq 0.05$ ) correlations among assessed agronomic traits. For instance, DTF exhibited significant correlations with DTM ( $r=0.53$ ), PDL ( $r=0.33$ ), NPP ( $r=0.45$ ) and HSW ( $r=0.41$ ). The strongest correlations were between NSP and DTM ( $r=0.76$ ), HSW and NSP ( $r=0.67$ ) and, NSP and NPP ( $r=0.66$ ).

Table 4.2 Mean squares and significance tests for seven quantitative traits among 11 parental cowpea lines and their 55 progenies evaluated in two locations in Zambia.

Source of variation	DF	DTF	DTM	PDL	NPP	NSP	HSW	GYD
Location (L)	1	45.94	244.21	192.75**	23.31	9.31**	8.42**	805680
Replication	1	280.24	658.67	43.37	905.80	4.64	0.17	206397
Block (Replication)	10	282.2	242	227.20	105.10	224.6	19.71	1452702
Genotype (G)	65	2479.33**	787.15***	37.81***	484.20***	32.03**	5.56**	3018868**
G × L	65	164.22	176.81	21.19	520.70*	18.31	6.19	1417453
Error	131	267.00	241.33	18.24	363.4	17.28	5.51	1597465
Total	272	3518.93	2350.17	540.56	2402.51	306.17	45.56	8498565

DF= degrees of freedom; DTF= days to 50% flowering; DTM= 90% days to maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively

Table 4.3 Mean values of the top 10 and bottom 5 new progenies and 11 parents of cowpea for seven quantitative traits evaluated in two locations in Zambia ranked based on grain yield.

Genotype	DTF	DTM	PDL	NPP	NSP	HSW	GYD
Top 10 progenies							
411 × 732	48.75	77.25	18.52	22.75	16.00	12.65	4232.00
411 × BBX	40.75	77.50	24.75	18.75	17.75	12.92	3848.00
421 × MS1	33.50	77.25	15.07	20.00	13.50	11.75	2780.00
645 × Lut	33.00	65.00	14.30	33.25	11.75	10.57	2733.00
411 × Chi	36.00	71.25	27.58	14.25	25.75	13.30	2054.00
421 × Chi	49.00	83.75	16.42	16.00	16.05	17.57	1644.00
645 × Chi	28.00	77.75	18.50	12.75	16.75	17.75	1579.00
645 × 732	30.50	77.00	22.32	15.25	19.75	16.67	1452.00
Lut × Nam	42.75	79.25	18.12	18.75	7.75	14.05	1419.00
Lut × MS1	42.75	79.25	18.12	9.00	7.00	14.05	1419.00
Bottom 5 progenies							
Chi x Msa	45.00	83.75	20.05	14.00	18.75	12.27	548.00
Chi x Nam	36.25	66.25	13.60	13.75	12.75	13.30	448.00
645 x MS1	18.75	54.25	12.90	11.25	13.50	9.72	415.00
411 x Msa	19.00	78.00	14.60	8.00	10.75	9.37	200.00
411 x Nam	14.75	68.75	13.40	4.00	11.25	8.85	100.00
Parents							
CP411	36.75	75.50	18.40	16.75	14.00	13.30	1669.00
CP421	22.00	68.50	15.10	11.00	14.25	14.47	1002.00
CP645	49.00	74.25	32.98	8.50	28.00	13.92	1069.00
CP732	50.00	84.25	6.65	18.00	5.75	13.00	1409.00
Bubebe	32.00	74.25	10.85	19.25	9.75	9.15	1023.00
BBXSC103	31.50	69.50	18.70	12.75	16.75	12.02	657.00
Chimponongo	49.25	84.50	30.60	11.25	27.75	8.88	1357.00
Lutembwe	20.25	75.50	13.85	19.25	12.75	13.60	742.00
MS1-8-1-4	24.75	67.00	12.40	17.75	11.00	15.52	905.00
Msandile	28.00	69.25	13.65	7.50	12.75	13.23	1221.00
Namuseba	42.50	67.25	26.20	14.25	20.00	12.67	445.00
Mean	42.22	65.31	18.07	17.34	14.52	13.01	1140.00
SE	12.19	10.46	10.36	9.24	3.14	4.36	740.00
THSDT (5%)	17.05	28.16	14.65	18.24	4.45	6.08	887.00
CV (%)	28.95	30.90	18.06	30.20	4.65	13.52	87.27

Note: CV: coefficient of variation; THSDT: Tukey's honestly significant difference test; SE: standard error;

DTF= days to 50% flowering; DTM= 90% days to maturity; PDL = pod length (centimetre); NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight (gram/100 seed); GYD= grain yield (kilogram/hectare)

Key for genotype designation: 411= CP411; 421= CP421; 645= CP645; 732= CP732; Bub= Bubebe; BBX= BBXSC103; Chi= Chimponogo; Lut= Lutembwe; MS1= MS1-8-1-4; Msa= Msandile; Nam= Namuseba

Table 4.4 Pearson correlation coefficients of grain yield and yield components among 11 cowpea parental lines and their 55 progenies evaluated at two locations in Zambia

Traits	Traits						
	DTF	DTM	PDL	NPP	NSP	HSW	GYD
DTF	1	0.53***	0.33**	0.45***	0.82	0.41***	0.44***
DTM		1	0.65***	0.91	0.76***	0.58***	0.45***
PDL			1	0.55***	0.54***	0.49***	0.32**
NPP				1	0.66***	0.48***	0.37**
NSP					1	0.67***	0.48***
HSW						1	0.2
GYD							1

DTF= days to 50% flowering; DTM= 90% days to maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively



Figure 4.1 Performance of F2 progenies at the Nanga site

### 4.3.3 Combining ability analysis

The analysis of variance showed that GCA mean squares for DTM, NPP, HSW, PDL and GYD were significant ( $P \leq 0.05$ ) (Table 4.5). The SCA mean squares for all assessed traits were significant ( $P \leq 0.05$ ). The lowest value of the Baker ratio was recorded for PDL (0.3), while the highest was recorded for DTF (0.64) (Table 4.5). The importance of additive and non-additive genes were indicated by the significant variance for GCA and SCA, respectively. However, the GCA variance for all the traits was lower than their corresponding SCA variance. The broad-sense heritability values varied from 0.05 (NSP) to 0.53 (DTM) (Table 4.5).

Table 4.5 Variance components and significant tests for general and specific combining ability effects among 11 cowpea parental lines and their 55 F1 crosses evaluated at two locations in Zambia

Parameter	Degree of freedom	Traits						
		DTF	DTM	NPP	PDL	NSP	HSW	GYD
GCA	10	231.26	597.66*	500.13**	374.63*	275.14	40.01*	3400109.00*
SCA	54	524.74**	821.60***	267.56*	325.17*	319.19*	24.89*	2949074.00**
GCA error	110	0.79	2.46	2.50	1.60	1.43	2.33	2.13
SCA error	110	1.81	3.38	1.37	1.40	1.66	1.45	1.84
$\delta^2$ GCA		159.65	61.76	41.31	15.32	13.18	6.46	202771.20
$\delta^2$ SCA		179.68	76.86	56.56	18.42	15.29	10.53	386647.30
Baker's ratio		0.64	0.61	0.59	0.36	0.63	0.55	0.56
H <sup>2</sup>		0.20	0.53	0.32	0.36	0.05	0.57	0.09

GCA= general combining ability; SCA= specific combining ability;  $\delta^2$ GCA= variance due to general combining ability;  $\delta^2$ SCA= variance due to specific combining ability; H<sup>2</sup>= broad sense heritability

DTF= days to 50% flowering; DTM= 90% days to maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively

### 4.3.4 GCA and SCA estimates

The GCA estimates varied among the eleven parental genotypes for the seven characters (Table 4.6). Two parental lines exhibited positive and significant GCA effects for grain yield in a desirable direction. The best general combiner for grain yield was CP411 with a positive and

significant ( $P < 0.001$ ) GCA effect of 545.70, followed by CP732 and BBXSC103, with respective GCA effects of 289.34 and 117.25. In addition, the genotypes CP411 and CP732 exhibited the highest positive and significant ( $P < 0.001$ ) GCA effects for NPP. Genotype Chimponongo was the highest general combiner for PDL and NSP. Negative GCA effects for DTF and DTM are desirable for developing breeding populations with early flowering and maturity. Genotypes CP411 and Namuseba exhibited desirable negative GCA effects for DTF and DTM. For HSW, Lutembwe followed by CP645, and CP732 had the highest positive and significant ( $P < 0.05$ ) GCA effects.

The SCA effects of the 55 crosses for the seven characters showed wide variation (Table 4.7). Three crosses, namely CP732  $\times$  CP411, BBXSC103  $\times$  CP411, and Lutembwe  $\times$  CP645, exhibited positive and significant ( $P \leq 0.01$ ) SCA effects for grain yield. Crosses MS1-8-1-4  $\times$  CP411, Namuseba  $\times$  CP411, Msandile  $\times$  CP645 and Chimponongo  $\times$  CP732 exhibited desirable negative and significant ( $P \leq 0.05$ ) SCA effects for DTF. Crosses with negative and significant ( $P \leq 0.05$ ) SCA effects for DTM included MS1-8-1-4  $\times$  CP411 and Msandile  $\times$  CP645. Good specific combiners for NPP were Namuseba  $\times$  CP411 and MS1-8-1-4  $\times$  CP411. Crosses CP421  $\times$  CP411, Namuseba  $\times$  CP732, and Bubebe  $\times$  CP411 showed positive and significant ( $P \leq 0.05$ ) SCA effects for PDL. For NSP, significant ( $P \leq 0.05$ ) and positive SCA effects were displayed by CP421  $\times$  CP411, Msandile  $\times$  BBXSC103 and Namuseba  $\times$  Bubebe while Chimponongo  $\times$  CP411, BBXSC103  $\times$  CP411, Namuseba  $\times$  Bubebe, Bubebe  $\times$  CP411 and Namusebe  $\times$  MS1 8-1-4 had desirable SCA effects for HSW.

Table 4.6 Estimates of general combining ability effects for seven traits in 11 cowpea parents evaluated at two locations in Zambia

Parents	Traits						
	DTF	DTM	NPP	PDL	NSP	HSW	GYD
CP411	-3.99*	-6.71***	11.58***	-1.02*	-1.04*	-0.45*	545.70***
CP421	1.26	1.21	-4.95*	-0.02	-0.13	0.20	21.52
CP645	-0.86	-0.87	-5.92**	-0.58	-0.65	0.62*	11.19
CP732	3.16*	3.77*	6.16*	0.11	0.35	0.45*	289.34*
Bubebe	-0.11	0.33	-1.99	0.36	0.44	0.09	-183.60
BBXSC103	-0.61	0.86	2.95	0.11	-0.19	0.18	117.25
Chimponongo	1.89	3.36*	-0.44	1.65*	1.06*	0.28	-46.12
Lutembwe	0.12	2.08	1.22	0.21	0.75	0.63*	-16.74
Msandile	4.34*	2.92	-2.65	0.48	0.41	-0.10	-94.88
MS1-8-1-4	-1.41	-2.00	-4.70**	-0.58	-0.52	-0.88**	-268.70*
Namuseba	-3.80*	-4.94**	-1.26	-0.70	-0.48	-1.01***	-374.96**
SE	0.06	0.02	0.05	0.03	0.03	0.06	4.25

DTF= days to 50% flowering; DTM= 90% days to maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively

Table 4.7 Estimates of specific combining ability effects of 55 cowpea crosses evaluated at two sites in Zambia

Crosses	Traits						
	DTF	DTM	NPP	PDL	NSP	HSW	GYD
CP421 × CP411	9.51	12.77***	12.09	6.06**	6.03**	1.60	-72.31
CP645 × CP411	0.38	4.35	-15.20*	1.37	1.79	-1.46	-715.82
CP732 × CP411	6.86	10.21	-4.16	2.43	3.79	1.63	4240.80***
Bubebe × CP411/*	9.63	13.91	-7.13	4.18*	0.70	2.71*	-314.82
BBXSC103 × CP411	14.38*	13.37***	-6.36	1.43	-0.17	2.80*	2028.86**
BBXSC104 × CP421	-4.37	-13.55	-6.82	-2.32	-3.07	-1.38	-551.94
BBXSC105 × CP645	-1.26	-4.48	7.64	0.49	-0.55	-3.42**	50.94
Chimponongo × CP411	1.38	4.62	-18.43*	-0.86	1.33	3.02**	398.44
Chimponongo × CP732	-14.53*	-18.86	-7.64	-2.49	-1.55	-1.16	-779.82
Lutembwe × CP645	-6.24	-6.19	-8.09	1.64	1.26	-1.05	1582.07**
Lutembwe × Chimponongo	-7.24	-6.92	-5.57	1.41	0.79	-2.76*	-92.16
Msandile × CP421	2.19	3.14	-5.18	-2.94	-3.67*	-0.30	1697.25
Msandile × CP645	-18.95*	-17.79*	-6.22	-4.38*	-3.15	-0.27	-657.29
Msandile × CP732	-6.72	-7.17	1.32	-3.82*	-3.65*	0.63	-559.41
Msandile × BBXSC103	-3.45	-1.52	2.88	0.18	4.14*	0.12	-74.54
MS1-8-1-4 × CP411	-41.56***	-61.27***	39.72***	-11.63***	-9.59***	-12.09***	-1433.26*
MS1-8-1-12 × Msandile	14.86	13.35	6.70	0.87	-1.53	2.21	200.53
Namuseba × CP411	-39.18***	-58.32	37.93***	-11.51***	-9.63***	-11.96***	-1327.00*
Namuseba × CP732	13.17*	15.20*	0.47	5.10*	3.74*	-0.74	149.97
Namuseba × Bubebe	4.69	6.89	2.26	1.35	1.89	3.22**	263.81
Namuseba × Lutembwe	13.46***	12.14	-2.70	2.51	3.08	-0.20	654.54
Namuseba × MS1-8-1-4	2.49	7.71	-10.39	-0.96	-0.90	2.17*	140.20
SE	1.20	1.54	2.75	0.62	3.53	0.27	41.05

DTF= days to 50% flowering; DTM= 90% days to maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively

#### 4.4 Discussion

Crop improvement depends on the availability of genetic variation for key farmer-preferred traits. The analysis of variance showed that the tested genotypes exhibited variation for all evaluated traits. This suggests that the test germplasm potentially are endowed with adequate genetic variation for cowpea improvement. The genetic variation among the parents and progenies is attributable to differences in the genetic constitution. The parents used in the study have diverse genetic background consisting of mutant lines, landraces, and introduced

varieties. Hybrids show variable performance compared with their parents due to favourable gene recombinations associated with hybrid vigour or heterosis (Dias et al. 2016). The existence of genetic variation among the parents is essential for the selection of promising progenies (Krause et al. 2012). Landraces are heterogeneous populations and express genetic variation due to segregation, which does not occur in pure line varieties. The testing sites for cultivar development play a vital role. Successful selection in genetically diverse populations is subject to the test environments. Testing in different locations enable identification of lines with specific or broad adaptation. The grain yield response of the top five crosses were higher than yield response of the corresponding parents. This suggests favourable recombination of additive or non-additive alleles derived from different parents (Marame et al. 2009).

The results of the analysis of variance showed significant GCA and SCA effects for assessed agronomic traits indicating that both additive and non-additive gene effects were important in the inheritance of the traits. Kheradnam and Niknejad (1971) reported traits that exhibits significant variation for GCA and SCA is controlled by both additive and non-additive genes, in that order. The involvement of additive and non-additive genes shows that there is a probability of obtaining new recombinants with improved agronomic performance (Silva et al. 2004). The most appropriate and effective breeding approach to improve agronomic traits controlled by additive genes is through pedigree, pure line, and single seed descent selections methods (Raut et al. 2017). On the other hand, the family selection at advanced generations will be useful to exploit non-additive gene effects (Owusu et al. 2018). The results indicated the preponderance of non-additive genetic components for DTF, DTM, PDL, NPP, NSP, HSW, and GYD. The predominance of SCA effects over GCA could be attributed to dominant gene action, the repulsion phase linkage and linkage disequilibrium (Sokol and Baker, 1977). These results agreed with Anitha et al. (2017) and Olunloyo et al. (2019), who also found higher SCA variance compared to GCA variances for the same traits in cowpea. Singh et al. (2006) postulated that non-additive gene action could be exploited by multiple crosses followed by intermating among desirable segregants.

Considering the GCA effect (Table 4.6), parental lines with high and significant GCA effects had higher trait performance for certain characters (e.g. DTM, NPP, HSW and GYD). The presence of additive gene effects indicates a high chance of predicting the performance of progenies based on the phenotypic performance of the parents. Parents such as CP411 and

CP732, which are good general combiners for grain yield and number of pods per plant, are ideal in hybridization programs to exploit heterosis that can result in higher yield productivity. Parent CP411 also exhibited a negative and significant GCA effect for days to flowering and maturity making them suitable parents for breeding for early maturity. Ayo-vaughan et al. (2013) and Owusu et al. (2018) reported that lines Kananado, IT86D-610 and Padi-tuya were the potential parental lines for breeding in West Africa. In the present study, Lutembwe and CP732 are suitable parents for the development of large-seeded cultivars preferred by farmers. A cross between BBXSC103 and CP411 showed high SCA effects for grain yield and a hundred seed weight. It was observed that a cross between MS1-8-1-4 and CP411 exhibited low SCA effect for DTM. Horn et al. (2015) reported that farmers in southern Africa prefer cultivars with a short flowering period and maturity. Hence, early maturing genotypes are ideal in the region to evade the “hunger period”. Crosses Namuseba × CP411 and MS1-8-1-4 × CP411 that recorded high SCA effect for the number of seeds per pod are important in yield gains. High positive SCA effects obtained in crosses involving CP421 × CP411 for pod length and number of seeds per pod could be vital for improving total grain yield. Crosses with high SCA effects produced from parents with low or poor GCA effects for a particular trait show that there was favourable interaction of alleles. The superiority of these crosses could be attributed to complementary and duplicate gene actions (Romanus et al. 2008; Anitha et al., 2017; Boukar et al. 2019). Therefore, the crosses are expected to produce desirable segregants and could be selected successfully in cowpea varietal development programs.

The low values for Baker’s ratios reveal the relative importance of SCA effects in the inheritance of traits suggesting that progeny performance may not be predicted based on GCA effects of the parents (Bernando, 2017). In this study, all Baker’s ratios were less than unity, implying that either dominance, overdominance or epistasis genetic effects influenced the traits. The low Baker’s ratio in the study reveals that SCA effects were more important than GCA effects in the genetic determination of yield and its components on the present cowpea population. This suggests that the genes controlling the yield components can only be fixed in later generations by selecting transgressive segregants to exploit non-additive gene effects. The significance of the SCA effects emphasizes the existence of the non-fixable components of the genetic variation related to heterosis (Pandey and Singh, 2010). Owusu et al. (2020) reported

that yield components exhibited Baker's ratio that were less than a unit in Ghanian cowpea. Non-additive genes are non-fixable leading to low heritability estimates. This result agrees with the findings of Mwale et al. (2017) who documented low heritability of yield and related traits controlled by non-additive gene effects from parents to progeny.

The significant and positive correlations between grain yield and days to flowering, days to maturity, pod length, number of pods plant<sup>-1</sup>, and number of seed pod<sup>-1</sup> is important for (in)direct selection and multi-trait improvement. Hence, selection for DTF, DTM, PDL, NPP, and NSP has the potential to improve grain yield in this population. Adetiloye et al. (2017) reported that cowpea genotypes with a higher number of pods and seeds and early flowering produced relatively higher yields. Similarly, Anitha et al. (2017) and Olunloyo et al. (2019) reported that pod length to be significantly and positively correlated with grain yield. Longer pods generally contain more seeds compared to shorter pods. The PDL was found to be the best target trait for (in)direct selection to improve grain yield in this germplasm. However, association patterns determined through simple correlation analysis should be used cautiously during (in)direct selection (Mashilo et al. 2016) due to complex direct and indirect effects among the traits.

#### **4.5 Conclusions**

In the study both additive and non-additive gene effects were conditioned with the inheritance of the key characters evaluated. However, non-additive genetic effects were more important than additive gene effects for all assessed traits. Lines CP411 and CP732 were the best general combiners for NPP, HSW, and GYD and were selected as candidate parents. The best specific combiners for grain yield were CP732 × CP411, BBXSC103 × CP411, and Lutembwe × CP645. These crosses will potentially produce transgressive segregants for selection and genetic advancement to exploit non-additive gene effects. The selected cowpea parents and progenies are useful genetic resources for breeding and genetic advancement to release improved cultivars in Zambia.

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## CHAPTER 5. GENOTYPE × ENVIRONMENT INTERACTION EFFECTS OF COWPEA [*Vigna Unguiculata* (L.) Walp.] FOR GRAIN YIELD AND RELATED TRAITS IN ZAMBIA

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### Abstract

Yield and yield components in cowpea [*Vigna unguiculata* (L.) Walp.] are highly variable across diverse growing environments due to genotype by environment interaction (GEI) effects. Hence, evaluating and quantifying the GEI is vital to improve selection efficiency and genetic gains. The objectives of the study were to quantify the GEI effect and select cowpea genotypes with high grain yield and average adaptation across selected cowpea growing environments in Zambia. Thirty selected cowpea genotypes were evaluated using a 10×3 alpha lattice design with three replications in four selected cowpea-growing environments (Lusitu, Mansa, Nanga and Seed Control Certification Institute (SCCI)) in Zambia. Grain yield and yield components were significantly ( $P < 0.001$ ) affected by the genotype (G), environment (E), and GEI effects. The environment, genotype and GEI effects were highly significant ( $P < 0.001$ ) and accounted for 66.97, 12.4 and 20.63% of the total variation, respectively. The test environments were delineated into three mega- environments, with Lusitu and Mansa clustered together while Nanga and SCCI were distinct mega-environments. The ideal environment for discriminating the tested genotypes was the Lusitu location. Genotypes LT11-3-3-12, Bubebe and Msandile were best adapted to the Nanga, Lusitu-Mansa and SCCI mega-environments, respectively. The new families such as CP421 × CP732, CP645 × MS1-8-1-4, MS1-8-1-4 × BBXSC103 and a mutant line BB10-4-2-3 were identified as high yielding with average stability across the four sites making them ideal selections for future cowpea breeding programs in Zambia.

**Key words:** agronomic traits, additive main effects and multiplicative interaction (AMMI) model, cultivar superiority, genotype and genotype by environment interaction (GGE), yield components

## 5.1 Introduction

Statistical parameters such as additive main effects and multiplicative interaction (AMMI) (Gauch 1992), and the genotype main effects and genotype by environment interaction (GGE) (Yan et al. 2000; Yan and Tinker 2006) are available to investigate the GEI effect. These parameters can be used along with cultivar superiority and stability indices proposed by Lin and Binns (1988) and Huehn (1990), respectively. The AMMI and GGE-biplot analyses are complementary and have been used extensively due to their ability to identify genotypes with broad or specific adaptation (Kaya et al. 2006). The two models combine univariate methods for the environment and genotype additive main effects with a multivariate method for the multiplicative effect of GEI (Zobel et al. 1988). The AMMI model is effective in assessing the adaptability and stability of genotypes (Pacheco et al. 2005). It is a combination of the standard ANOVA and principal component analysis that quantify genotype and environment main effects with genotype  $\times$  environment interactions. AMMI provides a synopsis of the link between environments and genotypes (Zobel et al. 1988; Crossa 1990). In addition, it gives a detailed interpretation of data compared to the general ANOVA, thereby increasing its use in breeding programs (Zobel et al. 1988; Crossa, 1990). AMMI uses the environment and genotype scores that are used to construct biplots for depiction of genotype performance relative to environments (Gabriel, 1978). The AMMI analyses can be complemented with the AMMI stability values (ASV). The ASV is a metric of stability computed from mean performance variation over a number of environments as proposed by Purchase et al (2000). The ASV are derived from the relative distance of a genotype from the origin of an interaction principal component axes (IPCA) biplot. The ASV is used to identify stable genotypes across environments. Lower ASV values are indicative of higher stability of genotypes (Purchase 1997; Anley et al. 2013).

The GGE biplot is responsible for identification of genotype performance as well as genotype  $\times$  environment interaction. The technique facilitates clear visualisation of the complex GEI on a graph. The GGE model divides the environment effects and combine the genotype main effects with the genotype  $\times$  environment interaction (Yan et al. 2000). This results in identification of genotype similarities in different test environments based on their performance. In addition, cultivar superiority indices combine the average performance and consistence of a genotype in different environments (Linn and Binns 1988). The cultivar

superiority index complements the AMMI and GGE biplot to identify genotypes that perform well in the highest possible number of environments (Scapim et al. 2000). The stability index provides a method to identify genotypes whose performance remains relatively consistent over a number of environments (Huehn 1990). Thus, the cultivar superiority and stability indices complement the AMMI and GGE biplot analyses to identify broad and specific adaptation of candidate genotypes (Kaya et al. 2006). The complementarity of the stability and superiority indices, AMMI and the GGE biplot analyses enables the identification of superior genotypes evaluated in multiple test environments, which is vital for cultivar development and deployment in cowpea breeding. The objectives of the study were to quantify the GEI effect and select cowpea genotypes with high grain yield and average adaptation across selected cowpea growing environments in Zambia.

## **5.2 Materials and Methods**

### **5.2.1 Germplasm**

Thirty cowpea genotypes comprising of 16 families representing breeding lines (which are at the F4) derived from targeted crosses and selections (Nkhoma et al. 2020), 10 mutant lines obtained from the University of Zambia and four released varieties were used in this study (Table 5.1). The four released checks included Bubebe, Lutembwe, Msandile and Namuseba that were used as comparative controls. The families were developed based on parental selections for early maturity, desirable growth type and better grain yield potential. The test genotypes have three growth patterns: determinate, indeterminate and creeper types.

Table 5.1 List of the 30 cowpea germplasm used in the study.

Genotype	Type	Maturity (days)	Growth habit	Grain yield (kg/ha)
3 × 6	Advanced line	74	Indeterminate	1407
3 × 7	Advanced line	73	Indeterminate	1399
3 × 9	Advanced line	89	Creeper	1416
4 × 6	Advanced line	75	Indeterminate	1404
5 × 8	Advanced line	76	Indeterminate	1340
6 × 7	Advanced line	91	Creeper	1370
8 × 3	Advanced line	63	Determinate	1411
8 × 4	Advanced line	72	Indeterminate	1333
8 × 6	Advanced line	61	Determinate	1452
8 × 7	Advanced line	71	Indeterminate	1414
9 × 6	Advanced line	91	Creeper	3848
9 × 7	Advanced line	65	Determinate	4232
9 × 8	Advanced line	60	Determinate	1320
9 × 10	Advanced line	62	Determinate	1354
10 × 11	Advanced line	79	Indeterminate	1401
11 × 9	Advanced line	89	Creeper	1399
BB3-9-7-5	Mutant	59	Determinate	1224
BB10-4-2-3	Mutant	75	Indeterminate	1203
BB14-16-2-2	Mutant	73	Indeterminate	1501
LT3-8-4-1	Mutant	64	Determinate	1199
LT4-2-4-1	Mutant	93	Creeper	1200
LT11-3-3-12	Mutant	96	Creeper	1220
LT11-5-2-2	Mutant	91	Creeper	1203
MS1-8-1-4	Mutant	74	Indeterminate	1779
Bubebe	Variety (Check)	70	Indeterminate	1023
Lunkwakwa	Mutant	76	Indeterminate	1189
Lukusuzi	Mutant	63	Determinate	1210
Lutembwe	Variety (Check)	74	Indeterminate	742
Namuseba	Variety (Check)	61	Determinate	445
Msandile	Variety (Check)	72	Indeterminate	1221

Key for genotype designation: 3= CP421; 4=MS1-8-1-4; 5= Chimponongo; 6= BBXSC103; 7= CP732; 8= CP645; 9= CP411; 10= Lutembwe; 11= Namuseba

### **5.2.2 Description of the study sites**

The field experiments were set up in the 2018/2019 main cropping season at four selected sites representing different agro-ecological regions of Zambia (Table 5.2). The sites included Lusitu, Mansa, Nanga and Seed Control and Certification Institute (SCCI). The sites were selected for being the major belts of cowpea production and marketing. Lusitu (16° 8'S 28°50'E, altitude 1206 m) is situated in Chirundu district in the lower Zambezi valley, southeast of Zambia. This site receives an average annual rainfall of 400 mm with a mean temperature of 39°C with high temperatures reaching up to 43°C. The soils at this site are sandy loam with a pH of 5.9. The Mansa site (11° 12'S 28°53'E, altitude 1190 m) is found in Mansa, in the northern region of Zambia and receives average annual rainfall above 1800mm with mean and maximum temperatures of 32°C and 36°C, respectively. The soils at this site are fertile with good water retention capacity and classified as clay loam with an average pH of 5.5 (Sichinga, 2013). The Nanga site (15° 85'S 27°74'E, altitude 900 m) is found in Mazabuka in the southern region of Zambia and receives above 800mm of rainfall while the average and maximum temperatures are 34 and 38°C, respectively. The soils at this site are fertile with good soil moisture retention and classified as sandy loam with an average pH of 6.8 (Sichinga, 2013). The SCCI site is found at Mount Makulu (15° 32'S 28°11'E, altitude 1206 m) in Chilanga district in central Zambia. This site receives an average annual rainfall of 1000mm. The site is found on a plateau that experiences high temperatures with an average of 32°C and maximum temperatures reach 36°C. The area is regarded as a high potential crop production area with fertile soils (clay loam) with a pH of 5.8 (Chapota et al. 2016).

### **5.2.3 Trial design and management**

The experiment in each site was laid out in a 10×3 alpha lattice design replicated three times. Basal fertiliser comprising of 20% nitrogen, 10% phosphorus and 20% potassium was applied at a rate of 200 kg ha<sup>-1</sup> prior to planting at each site. Each genotype was sown in a plot with two rows of 5m length each. The inter-row and intra-row spacings were 0.75 and 0.45 m, respectively. The plot area was 3.75m<sup>2</sup>. Two seeds were sown per hole at a depth of 2cm. The seedlings were thinned two weeks after emergence to one plant per hole. All the other

agronomic practices including crop protection were followed as recommended for cowpea production in Zambia (Muliokela, 1997). The crops were grown under rain-fed conditions.

Table 5.2 Characteristics of the four environments used to evaluate 30 cowpea genotypes

Environment	Code	Production region in Zambia	Latitude	Longitude	Elevation (masl)	Total rainfall (mm)	Minimum Temperature (°C)	Maximum Temperature (°C)	Soil type	pH	Reference
Lusitu	1	I	16°8'S	28°50'E	379	380	12.7	43.1	Sandy loam	5.9	Muliokela (1997); Sichinga (2013), Chapota et al. (2016)
Mansa	2	III	11°12S	28°53'E	1190	1860	16.8	36.5	Clay loam	5.5	
Nanga	3	IIa	15°85'S	27°74'E	900	720	14.5	38.3	Sandy loam	6.2	
Chilanga	4	IIb	15°32'S	28°11'E	1206	950	15.7	38.1	Clay loam	7.2	

SCCI= seed control and certification institute; Region I, IIa, IIb, and III denote agro- ecological regions I, IIa, IIb and III of Zambia; S= south; East; masl = meters above sea level; mm= millimetres; °C= degrees Celsius

#### 5.2.4 Data collection

Data on yield and yield components were collected. Data were collected according to cowpea descriptors of the International Board for Plant Genetic Resource (IBPGR, 1983). The days to flowering (DTF) were counted from the day of planting to the date when 50% of plants in a plot had flowers. The days to maturity (DTM) were determined from the day of sowing to when the 90% of plants in a plot had matured. The number of pods per plant (NPP) was recorded as a mean number of mature pods from five selected plants. The pod length (PDL) was the average length of the pods from mature pods randomly selected from the five-tagged plants. The number of seeds per pod (NSP) was the average number of seeds from mature pods randomly selected from the five-tagged plants in a plot. The hundred seed weight (HSW) was the mean weight of 100 randomly selected grains from the grains harvested per plot. The grain yield (GYD) per hectare was calculated as  $\frac{\text{plot weight}}{\text{plot area}} \times \frac{100-14\%}{100-mc} \times 10\,000$ , where plot weight is the weight of grain in kilograms per plot, plot area is 3.75m<sup>2</sup>, mc is grain moisture content measured at harvesting, 14% is standard constant moisture content for legumes (UPOV, 2008) and 10,000 is conversion factor for a hectare.

#### 5.2.5 Data analyses

##### 5.2.5.1 Analysis of variance

The data across the four sites were subjected to analysis using linear mixed effects model (LMM) after conducting Bartlett's test for homogeneity of variance in GenStat® version 17 (Payne, 2014). Means were separated by Tukey's honestly significant difference test. The replications and genotypes were considered as random factors while the environments were considered to be fixed factors. The following model was used:

$$Y_{ijkl} = \mu + G_i + E_j + R_k(j) + Bl(jk) + GE_{ij} + \varepsilon_{ijkl}$$

Where  $Y_{ijkl}$  is the response of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment and  $k^{\text{th}}$  replication within environment and  $l^{\text{th}}$  block within replication;  $\mu$  is the grand mean,  $G_i$  is the genotype effect  $i$ ;  $E_j$  is the environment effect  $j$ ;  $R_k(j)$  is the replication within environment effect  $k$ ;  $Bl(jk) +$  is the block within replication effect  $Bl$ ;  $GE_{ij}$  is the genotype  $\times$  environment interaction effect; and  $\varepsilon_{ijkl}$  is the random error.

### 5.2.5.2 Additive main effects and multiplicative interaction (AMMI) and AMMI stability value (ASV) analyses

The AMMI analysis was carried out in GenStat® version 17 (Payne, 2014). The AMMI model combines both ANOVA and PCA in assessing the stability and adaptability of genotypes. The genotype and environment main effects are considered to be additive using ANOVA, while the GEI was taken to have a multiplicative effect by principal component analysis (PCA). The following AMMI model was adopted following Zobel et al. (1988):

$$Y_{ij} = \mu + \alpha_i + \beta_j + \sum_n \lambda_n \delta_{in} \gamma_{jn} + P_{ij} + \varepsilon_{ij}$$

Where  $Y_{ij}$  is the mean yield of the  $i^{\text{th}}$  genotype effect in  $j^{\text{th}}$  environment in all replications; and the additive components are  $\mu$  (the grand mean),  $\alpha_i$  (the  $i^{\text{th}}$  genotype effect) and  $\beta_j$  (the  $j^{\text{th}}$  environment effect). The multiplicative effect consists of terms  $\sum_n \lambda_n \delta_{in} \gamma_{jn}$  and  $P_{ij}$  where,  $\sum_n$  is the singular value,  $\lambda_n$  is the interaction principal component,  $\delta_{in}$  is eigen vector for the genotype principal component,  $\gamma_{jn}$  is the environment principal component,  $P_{ij}$  is the AMMI residuals and  $\varepsilon_{ij}$  is the random error.

The AMMI stability values (ASV) were calculated using the formula proposed by Purchase (1997). Lower ASVs are associated with greater stability of genotypes. The ASV were calculated as follows:

$$AMMI \text{ Stability Value (ASV)} = \sqrt{\left[ \left( \frac{SSIPCA1}{SSIPCA2} (IPCA1) \right)^2 + [IPCA2]^2 \right]}$$

Where, SSIPCA1 and SSIPCA2 are the sum of squares for the first and second interaction principal component axes, respectively.

### 5.2.5.3 Genotype and genotype by environment interaction (GGE) biplot model

The GEI was further partitioned and analysed using the GGE model (Yan and Kang, 2003) in GenStat® version 18 (Payne, 2014). The GGE biplot depicts the results derived from the sites regression (SREG) linear –bilinear model in a two-dimensional biplot (Cornelius et al. 1996). The GGE biplots presented the first two principal components (PC1 and PC2) derived from subjecting locational yield data to the SREG model (Cornelius et al. 1996). The environment-focused scaling biplots enabled comparison among environments and selection of the most

discriminating and representative environment. The genotype-focused scaling biplots were used for genotype comparison to allow identification of stable and superior genotypes and adaptation to specific environments. Specific adaptation was indicated by acute angles between genotype and environment vectors. The model used is based on singular value decomposition of PC1 and PC2:

$$Y_{ij} - \mu + \beta_j = \lambda_1 \gamma_{i1} \delta_{j1} + \lambda_2 \gamma_{i2} \delta_{j2} + \varepsilon_{ij}$$

Where  $Y_{ij}$  is the mean of  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment,  $\mu$  is the grand mean,  $\beta_j$  is environment main effect in the  $j^{\text{th}}$  environment and  $\mu + \beta_j$  is the mean of all genotypes in  $j^{\text{th}}$  environment. The terms  $\lambda_1$  and  $\lambda_2$  are the singular values for PC1 and PC2, respectively;  $\gamma_{i1}$  and  $\gamma_{i2}$  are eigenvectors of the  $j^{\text{th}}$  environment for PC1 and PC2, respectively. The components  $\delta_{j1}$  and  $\delta_{j2}$  are eigenvectors of the  $j^{\text{th}}$  environment for PC1 and PC2, respectively.  $\varepsilon_{ij}$  is the the residual associated with the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment.

## 5.3 Results

### 5.3.1 ANOVA and mean performance

The Bartlett's test showed that the variance among the four sites were homogenous and subsequently a combined ANOVA was conducted. The combined ANOVA revealed that genotype, environment and genotype  $\times$  environment interaction (GEI) effects were highly significant ( $P < 0.001$ ) for all assessed traits (Table 5.3). The mean performances of the top 10 and bottom five genotypes are presented in Table 5.4. The DTF varied from 28 days recorded for BBXSC103  $\times$  CP732 to 46 days recorded for LT4-2-4-1, while the overall mean day to flower for the population was 38 days. . The family CP645  $\times$  MS1-8-1-4 matured early at 58 days, which was 11 days earlier than the overall mean. Mutant line LT11-3-3-12 and Bubebe (check) had the highest NPP of more than 28 pods plant<sup>-1</sup>. The longest PDL was recorded for LT11-3-3-12 (23cm) followed by families Chimponongo  $\times$  Namuseba and CP645  $\times$  CP421 with a mean PDL of 22 cm each. The genotypes that recorded the highest number of seeds per pod were CP411  $\times$  CP732 (15 seeds/pod) and BB10-4-2-3 (14 seeds/pod). The highest HSW was recorded for genotypes Msandile (15.65 g), MS1-8-1-4 (15.12g) and BB10-4-2-3 (13.90g). There existed significant genetic variation for GYD ranging from 293 kg ha<sup>-1</sup> for LT4-2-4-1 to 1360 kg ha<sup>-1</sup> for LT11-3-3-12. The overall mean GYD of the test genotypes was 720 kg ha<sup>-1</sup>.

The only genotype that performed better than the checks was LT11-3-3-12 (with grain yield of 1360 kg ha<sup>-1</sup>), while CP411 × CP645 (1098 kg ha<sup>-1</sup>) and CP645 × CP732 (1095kg ha<sup>-1</sup>) performed better than all the checks except Bubebe (Table 5.4).

Table 5.3 Mean squares and significant tests among 30 cowpea genotypes evaluated based on seven quantitative agronomic traits in four locations in Zambia

Source of variation	DF	DTF	DTM	PDL	NPP	NSP	HSW	GYD
Environment (E)	3	359.07***	1791.71***	9433.11***	12397.32***	143.43***	64.74***	48355038.00***
Rep	2	8.81*	9.98*	0.62	369.65***	4.6	19.16***	112475.00**
Block (Rep)	3	34.08***	389.05***	20.83*	295.34***	1300*	1.09	712463.00***
Genotype (G)	29	226.28***	287.81***	16.98***	93.75**	6.90**	10.97***	853830.00***
G×E	89	89.61***	162.80***	11.75***	70.70**	5.99**	6.27***	513733.00***
Error	235	3.27	11.66	6.62	46.11	3.72	4.4	17835
Total	359	721.12	2653.01	9489.55	13262.87	177.64	106.63	50565374

DF= degrees of freedom; DTF= days to 50% flowering; DTM= days to 90% maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively

Table 5.4 Mean values for grain yield and yield components of 30 cowpea genotypes showing the top 10 and bottom 5 ranked genotypes based on grain yield (kg ha<sup>-1</sup>) when assessed in four locations in Zambia

Genotype	DTF	DTM	PDL	NPP	NSP	HSW	GYD
Top 10 genotypes							
LT11-3-3-12	44.50	70.25	22.96	30.25	13.17	12.97	1360.30
Bubebe	39.17	71.08	20.87	28.08	13.25	12.72	1243.70
CP411 × CP645	34.33	70.92	20.42	23.33	13.67	13.31	1098.20
CP645 × CP732	34.58	62.92	20.35	25.25	13.08	13.87	1095.10
Msandile	35.67	60.92	19.33	26.50	12.58	15.65	992.00
MS1-8-1-4	35.08	69.08	18.73	22.75	13.42	15.12	958.70
CP645 × MS1-8-1-4	34.50	57.67	21.83	22.83	13.67	12.83	933.80
CP411 × CP732	41.42	75.58	18.99	18.50	14.92	12.18	920.90
BB10-4-2-3	40.25	68.92	20.62	20.46	14.42	13.90	883.50
BBXSC103 × CP732	28.42	65.00	19.58	23.42	12.83	13.48	868.40
Bottom 5 genotypes							
CP421 × BBXSC103	37.00	69.75	20.30	18.50	13.25	11.66	372.90
Chimponongo × Namuseba	39.67	71.17	21.50	11.42	13.67	13.37	361.10
CP645 × BBXSC103	41.33	66.00	19.92	12.08	12.50	12.86	348.80
CP645 × CP421	42.17	74.58	21.88	10.67	11.67	12.94	346.10
LT4-2-4-1	46.17	79.75	17.89	9.83	11.58	13.61	293.00
Mean	38.45	69.09	20.41	22.07	13.80	13.58	720.75
SE	1.79	3.39	2.57	6.48	1.94	1.99	19.91
THSHT (5%)	3.06	5.81	4.39	11.07	3.32	3.41	39.22
CV (%)	4.65	4.92	12.60	29.34	14.09	14.70	17.79

Note: CV: coefficient of variation; THSDT: Tukey's honestly significant difference test; SE: standard error; DTF= days to 50% flowering; DTM= days to 90% maturity; PDL = pod length (centimetre); NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight (gram/100 seed); GYD= grain yield (kilogram/hectare)

### 5.3.2 Trait associations

The Pearson's correlation coefficients showing pairwise associations between the assessed quantitative traits are presented in Table 5.5. Grain yield exhibited variable associations with all the traits ranging from weak to strong associations. The strongest correlations with GYD were exhibited by NPP ( $r=0.76$ ;  $P<0.001$ ) and PDL ( $r=0.46$ ;  $P<0.05$ ) (Table 5.5). Among the

yield components, significant and positive correlations were observed between DTM and DTF ( $r=0.41$ ), PDL and DTM ( $r=0.23$ ), NSP and NPP ( $r=0.20$ ) as well as HSW and PDL ( $r=0.25$ ).

Table 5.5 Pearson correlation coefficients of grain yield and yield components among 30 cowpea genotypes evaluated at four locations in Zambia

Traits	DTF	DTM	PDL	NPP	NSP	HSW	GYD
DTF	1	0.41***	0.07	0.12*	0.19***	0.01	0.16**
DTM		1	0.23***	-0.25***	-0.11*	0.02	-0.20***
PDL			1	0.02	-0.21***	0.25***	0.46*
NPP				1	0.20***	0.09*	0.76***
NSP					1	-0.11*	0.12*
HSW						1	0.09*
GYD							1

DTF= days to 50% flowering; DTM= days to 90% maturity; PDL = pod length; NPP = number of pods per plant; NSP= number of seeds per pod, HSW= hundred seed weight; GYD= grain yield

\*, \*\*, and \*\*\* denote significant differences at the 5%, 1%, and 0.1% probability levels, respectively

### 5.3.3 Additive main effect and multiplicative interaction

The AMMI analysis revealed highly significant ( $P<0.001$ ) genotype, environment, and genotype by environment interaction effects on grain yield (Table 5.6). The additive main effects due to environment and genotype accounted for 66.97 and 12.4 % of the total observed variation, respectively. The multiplicative effects due to GEI accounted for 20.63% of the variance in grain yield productivity. The first interaction principal component axis (IPCA1) and second interaction principal component axis (IPCA2) of the GEI were highly significant ( $P< 0.001$ ). The IPCA1 explained 90.69%, while the IPCA2 accounted for 6.05% of the interaction variance.

### 5.3.4 AMMI stability value (ASV)

The AMMI stability values (ASV) revealed variable yield stability among the 30 genotypes (Table 5.7). Purchase (1997) described a stable variety as one with ASV values closer to zero. Therefore, the family CP421  $\times$  CP732 (3  $\times$  7) with ASV value of 15.6 was the most stable

followed by BB10-4-2-3 (ASV 20.3), CP645 × MS1-8-1-4 (ASV24.1) and MS1-8-1-4 × BBXSC103 (ASV31.1). The least stable genotypes included LT11-3-3-12 (ASV 527.4), CP411 × CP732 (ASV 338.7) and BBXSC103 × CP732 (ASV 229.4).

### 5.3.5 AMMI biplots

The first two principal components, IPCA1 and IPCA2 explained 96.74% of the total GEI variation (Figure 5.1). According to the AMMI model, the length of the vector of an environment from the biplot origin is proportional to its contribution to the total GEI. This leaves the interrelations among the environments. Yang and Kang (2003) indicated that the length of a vector that approximates the standard deviation (SD) with each test environment is a measure of the environments' ability to discriminate the genotypes. In view of this study, Environment 1 (the Lusitu site) had the strongest interactive effect followed by 3 (Nanga) and 4 (SCCI). The environment 2 (Mansa) had the weakest interactive effect. Also, genotype and environment with vectors in the same direction and separated by acute angles are positively correlated. Conversely, genotypes and environments with vectors in opposite directions will be negatively correlated while genotypes and environments whose vectors are perpendicular have little interaction. Lusitu (environment 1) positively interacted with genotype Bubebe and Msandile, while genotypes BB10-4-3-2 and LT11-3-3-12 exhibited positive GEI with Mansa (environment 2) and Nanga (environment 3), respectively. Genotype MS1-8-4-1 had positive GEI with SCCI (Figure 5.1).

Table 5.6 AMMI analysis of variance for grain yield among 30 cowpea genotypes assessed across the four sites

Source of variation	DF	SS	MS	Total variation explained (%)	GxE explained (%)
Genotypes	29	26869170	926523***	12.4	
Environments	3	145065114	48355038***	66.97	
Block	8	571779	71472***	0.26	
Interactions	87	44691941	513700***	20.63	
IPCA 1	31	40532004	1307484***		90.69
IPCA 2	29	2703926	93239***		6.05
Residuals	27	1456011	53926***		
Error	232	3876631	16710		

DF=degree of freedom; SS=sum of squares; MS= mean squares; GxE= genotype by environment interaction; IPCA 1= interaction principal component axis 1; IPCA 2= interaction principal component axis 2

\*\*\* denote significant differences at the 0.1% probability levels.

Table 5.7 AMMI adjusted mean grain yield (kg/ha), IPCA scores and AMMI stability value (ASV) of 30 cowpea genotypes evaluated across four environments in Zambia

Genotype	Rank	Mean	IPCA1	IPCA2	ASV
3 × 7	1	629.40	1.04	-0.86	15.60
BB10-4-2-3	2	883.50	-1.36	-0.69	20.30
8 × 4	3	591.30	-1.60	2.84	24.10
4 × 6	4	666.10	2.06	3.63	31.10
Msandile	5	992.00	-2.50	-7.45	38.20
5 × 8	6	610.20	4.74	-2.72	71.10
Lunkwakwa	7	683.50	4.88	-11.60	74.00
9 × 10	8	824.30	-5.07	8.49	76.50
BB14-16-2-2	9	600.40	-5.24	3.99	78.70
Namuseba	10	756.60	5.75	-1.40	86.20
BB3-9-7-5	11	382.40	6.27	3.54	94.10
LT11/5-2-2	12	584.90	6.82	4.00	102.30
8 × 3	13	361.10	6.85	3.66	102.70
Bubebe	14	1243.70	-7.02	-4.15	105.40
8 × 4	15	933.80	-7.45	-7.80	112.00
LT3-8-4-1	16	618.80	7.60	-3.49	114.00
Lutembwe	17	863.20	-7.95	2.04	119.10
8 × 7	18	1095.10	-8.06	-3.79	120.90
3 × 9	19	633.30	8.72	-6.59	130.90
9 × 6	20	568.50	8.79	14.30	132.50
8 × 6	21	348.80	10.44	2.03	156.50
3 × 6	22	293.00	11.00	4.43	164.90
LT4-2-4-1	23	372.90	11.08	1.91	166.20
Lukusuzi	24	532.90	11.15	3.84	167.10
10 × 11	25	346.10	12.59	2.52	188.70
MS1-8-1-4	26	958.70	12.67	-12.63	190.30
9 × 8	27	1098.20	-13.11	-3.22	196.60
6 × 7	28	868.40	-15.30	-0.90	229.40
9 × 7	29	920.90	-22.59	4.25	338.70
LT11-3-3-12	30	1360.30	-35.19	1.81	527.40

Key for genotype designation of families: 3= CP421; 4=MS1-8-1-4; 5= Chimponongo; 6= BBXSC103; 7= CP732; 8= CP645; 9= CP411; 10= Lutembwe; 11= Namuseba

AMMI= additive main effects and multiplicative interaction; IPCA 1= interaction principal component axis 1; IPCA 2= interaction principal component axis 2; ASV= AMMI stability value

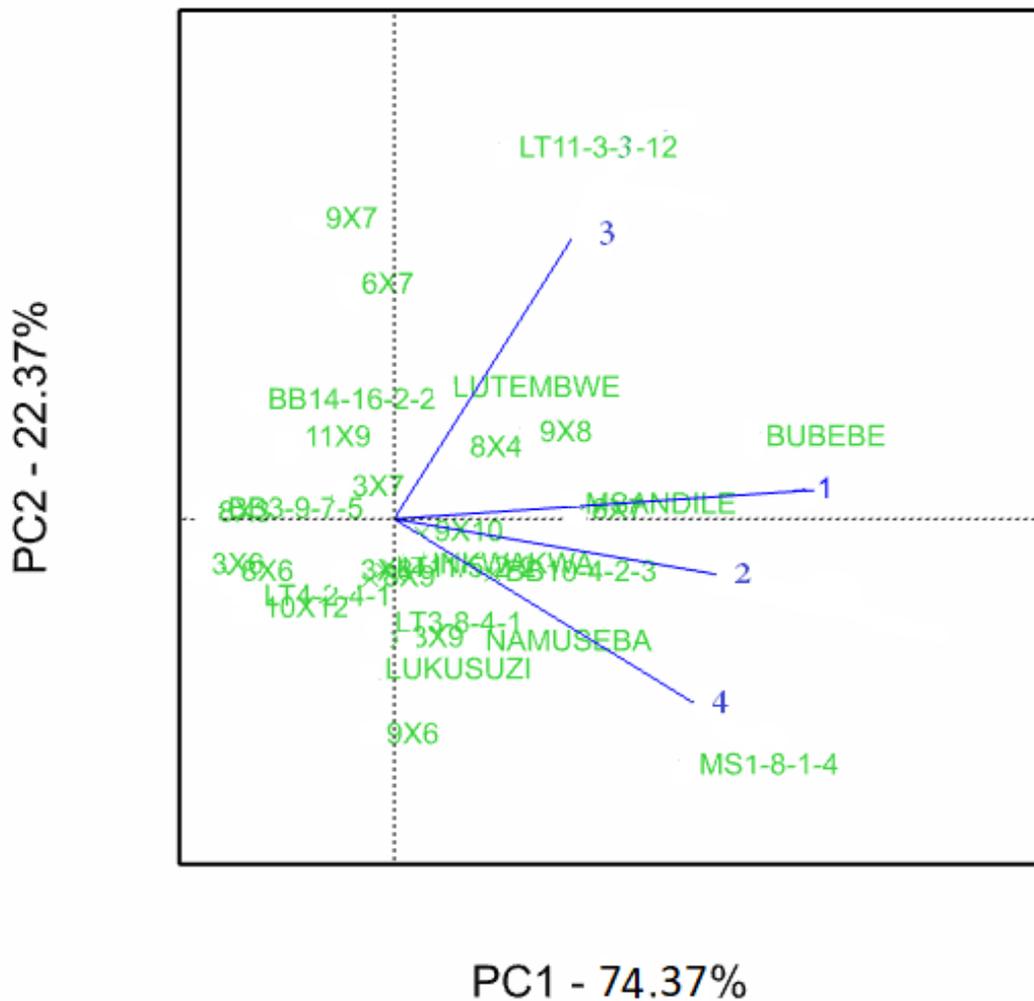


Figure 5.1 The vector view of GGE biplot based on environment-focused singular value showing the discriminating power and representativeness of the test environments. Numbers in blue denote environments: 1= Lusitu, 2= Mansa, 3= Nanga and 4= SCCI. See codes of genotype designation in Table 5.7

### **5.3.6 Genotype and genotype by environment (GGE) analysis**

#### **5.3.6.1 Delineation of mega environments and superior genotypes**

The “which-won-where” polygon delineated the sites into three mega-environments. The identified mega-environments were Nanga (mega environment 1), Lusitu and Mansa (mega environment 2) and SCCI (mega environment 3) (Figure 5.2). The biplot identified the genotypes with specific adaptation to each mega environment. Genotypes with specific adaptation were plotted on the vertices of the polygon. At Nanga, mega environment 1, mutant line LT11-3-3-12 and family CP411 × CP732 (9x7) were the vertex genotypes as they outperformed all the other genotypes (Figure 5.2). Bubebe (check) was the best performing genotype in mega environment 2 that encompassed the Lusitu and Mansa sites. The mutant line MS1-8-1-4 was the vertex genotype for the SCCI site, mega environment 3.

#### **5.3.6.2 Ideal genotype**

An ideal genotype is denoted by the longest IPCA1 vector from the origin and shortest IPCA2 distance. It should be close to the epicentre of the concentric circles of the biplot. Bubebe was the closest to the ideal genotype as it was located in the innermost concentric circle (Figure 5.3). Genotypes CP421 × CP732 (3×7), BB10-4-2-3, Msandile and CP411 × Lutembwe that were placed close to the horizontal axis with longer vectors from the origin showing that they had high grain yield and were stable. On the contrary, genotypes such as CP421 × BBXSC103 (3×6), CP645 × BBXSC103 (8×6), and LT4-2-4-1 performed below the overall mean with least stability and were placed furthest from the centre of the concentric circles.

#### **5.3.6.3 Discriminating ability and representativeness of test environments**

The arrow at the centre of the concentric circles represented the ideal environment (Figure 5.4). An ideal test environment, according to Yan and Kang (2003) should be both discriminating and representative. Thus, the ideal environment was used in this study, as the centre of a set of concentric lines to measure the distance between each environment and the ideal environment. Lusitu (environment 1) was the closest to the ideal environment. The next best environment

for cowpea evaluation was environment 2 (Mansa) followed by environment 4 (SCCI) while environment 3 (Nanga) was the least suitable environment.

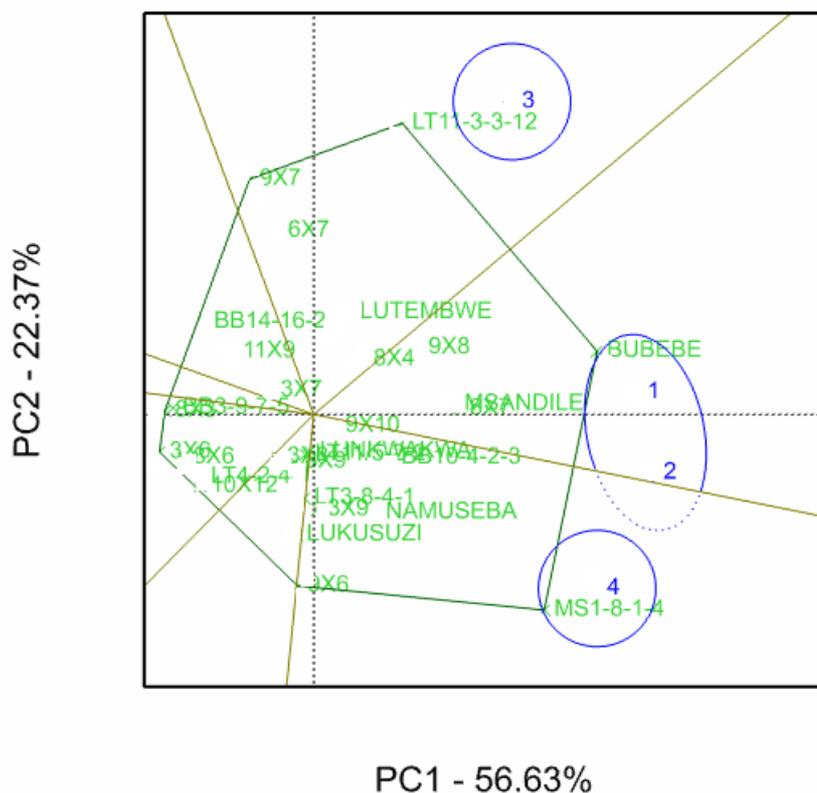


Figure 5.2 The “which- won- where” view of GGE biplot showing genotype performance under each mega- environment. Numbers in blue denote environments 1= Lusitu, 2= Mansa, 3= Nanga and 4= SCCI. The dotted vertical and horizontal lines indicate points where the PC1 and PC2 axes had respective values of zero. Genotypes at the vertices of the polygon were superior in each sector. See codes of genotype designation in Table 5.7.

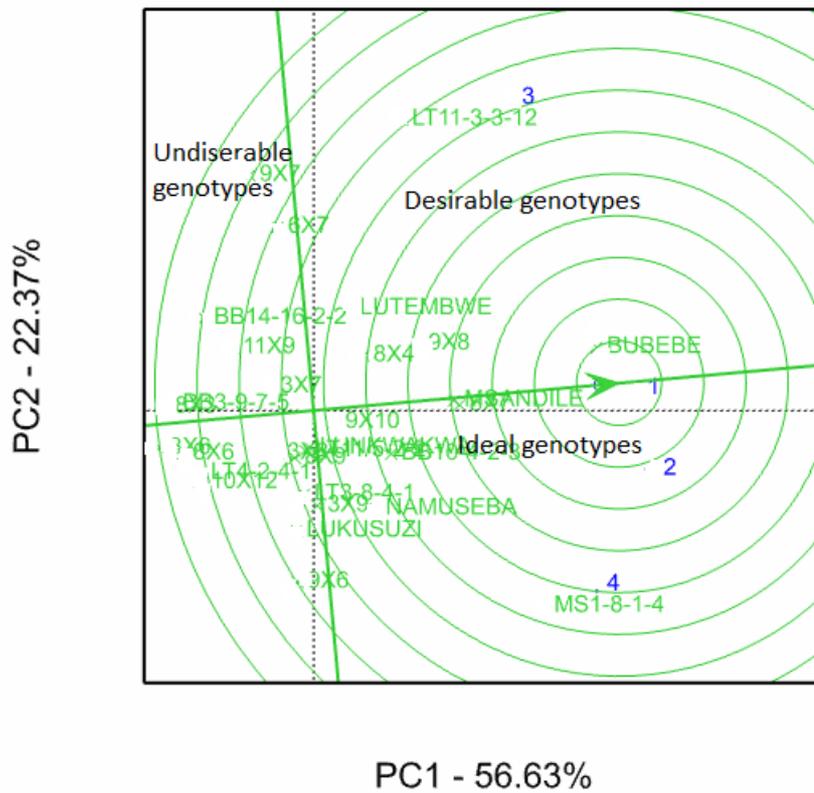


Figure 5.3 The average-environment coordination (AEC) view comparison biplot comparing genotypes relative to an ideal genotype (the center of the concentric circles). Numbers in blue denote environments: 1= Lusitu, 2= Mansa, 3= Nanga and 4= SCCI. The dotted vertical and horizontal lines indicate points where the PC1 and PC2 axes had respective values of zero. Green circle on the arrowed line represents the average environment and the green arrow represents ideal environment. See codes of genotype designation in Table 5.7.



CP732) were among the genotypes with high average grain yield across the four sites. The high performing families were best combiners developed from divergent parental lines by Nkhoma et al. (2020). These families are useful genetic resources for genetic advancement and variety release in Zambia. The other genotypes that performed well were the mutant lines (BB10-4-2-3, LT11-3-3-12 and MS1-8-1-4) that are currently in the preliminary stages of evaluation prior to release. Mutant lines are unique genetic resources, as they possess genetic variation making them useful resources for future cowpea breeding programs in SSA (Horn et al. 2018).

The significant and positive correlation between grain yield and characters such as number of pods plant<sup>-1</sup> and pod length are important for devising a breeding strategy. These are important secondary traits for improving cowpea grain yield. Higher number of pods per plant ensures improved grain yield production in this germplasm. Adetiloye et al. (2017) and Olunloyo et al. (2019) reported that cowpea genotypes with a higher number of pods and seeds, and long pods produced relatively higher yields.

The AMMI model partitioned the variance components and deduced that the environment accounted for the largest proportion of the total variation suggesting that much of the phenotypic observed was conditioned by environmental conditions (Table 5.6). Environmental variance has been identified as an impediment during cultivar evaluation as it reduces the correlation between genotype and phenotypic expression (Bustos-Korts et al. 2019). Similarly, Gerrano et al. (2020) and Mukendi et al. (2019) found that the environment accounted for the highest proportion of the total variation among cowpea genotypes evaluated in different environments in South Africa and the Democratic Republic of Congo, respectively. Contrasting test environments are used in plant breeding programs to select genotypes for adaptability to various environments (Zobel et al. 1988; Akter et al. 2015). The magnitude of variation among test genotypes indicate significant differences in genotype response across environments leading to crossover interaction.

The presence of crossover GEI is a result of variable ranking of genotypes in each environment. Crossover GEI complicates selection and recommendation of genotypes for production and justifying the need for stability analysis (Yan and Tinker 2006). Genotypes Bubebe, BB10-4-3-2, LT11-3-3-12 and MS1-8-1-4 situated further from the center of biplot (Figure 5.2) revealed positive interaction with environments Lusitu (environment 1), Mansa (environment 2), Nanga (environment 3) and SCCI (environment 4), respectively. This indicates specific

adaptation of these genotypes to the respective environments. For a genotype adaptation to an environment, Yan and Tinker (2006) suggested three different ways to exploit GEI. The first is by identifying best genotype that are suited to a specific environment, secondly; identifying best performing stable genotypes with wide adaptation and thirdly by portioning the environments into mega environments including identification to genotypes adapted to those mega- environments. The Lusitu site had the strongest interactive effect, which enabled most genotypes to express higher mean values for yield. A combination of high and evenly distributed rainfall at drought sensitive growth stages such as flowering and pod-filling stages, and suitable soils may have contributed to the high grain productivity of cowpea at the Lusitu site. Faloye et al. (2017) contend that high grain yield is conditioned by the availability of sufficient soil moisture during critical growth stages.

Based on the AMMI stability values, the stable genotypes were detected mainly comprising of families and mutant lines. Gurmu et al. (2009) and Annicchiarico (2020) defined a stable and widely adapted genotype as the one with the ability to perform consistently and produce above average mean performance in all test locations. The stable genotypes in this study were among the highest yielding genotypes across the four environments. These stable genotypes could be potential sources of genes for high yield stability that could be utilized in cowpea breeding programs. The most stable genotypes included CP421 × CP732, CP645 × MS1-8-1-4, MS1-8-1-4 × BBXSC103 and BB10-4-2-3 with indeterminate growth habit and early maturity making them suitable candidates for direct production or breeding.

The delineation of the sites into three mega-environments (Figure 5.3) indicate that some environments exhibited similar discriminatory power. The GGE clustered the mega-environments mainly based on genotype performance in that season. The Lusitu and Mansa sites (mega environment 2) were grouped together indicating that they were similar in terms of their environmental conditions supporting cowpea production in Zambia. The soils at both the Lusitu and Mansa sites are fertile with good water retention with an average pH of 5.7. The homogeneity of a mega environment signify that they have similar discriminatory power and could replace each other during multi- environmental trials to reduce breeding costs. A mega environment represents a group of homogeneous locations (Nzuve et al. 2013). Yan and Tinker (2006) and Nyombayire et al. (2018), proposed a reduction in the number of testing sites by substituting sites within identified mega environments during cultivar evaluation.

The GGE biplot analysis plays a vital role by identifying genotypes with specific adaptation to the different mega-environments. The placement of LT11-3-3-12, Bubebe and MS1-8-1-4 on the vertices of the GGE- biplot polygon (Figure 5.3) suggested that these genotypes were adapted to and performed well at the Nanga site (mega- environment 1), Lusitu and Mansa sites (mega- environment 2) and the SCCI site (mega- environment 3), respectively. The selection of genotypes adapted to specific environments is important to guide farmers to grow appropriate genotypes in their respective areas. The heterogeneity of test environments assists with evaluation and deployment of genotypes to specific environments despite their broad adaptation across many environments (Gauch and Zobel 1997). Sousa et al. (2018) found specific genotypes for the test environments that were delineated into three mega environments in Brazil, while Matova and Gasura (2018) found that all their study sites could be grouped into a single mega-environment after evaluating diverse cowpea genotypes in five environments in Zimbabwe.

During a GGE biplot analysis, a genotype is regarded as superior if it combines high grain yield and good yield stability (Yan and Tinker 2005; Yan et al. 2007). The study identified three genotypes (CP645 × MS1-8-1-4, BB10-4-2-3 and Msandile) with high grain yield and high stability (Table 5.7). These genotypes also exhibited desirable performance in other agronomic traits such as pod length, number of pods per plant and hundred seed weight. Similarly, the GGE biplot recognized the same high yielding genotypes with average stability found in the concentric circles for desirable genotypes (Figure 5.4). These superior genotypes out yielded all the other genotypes including the other two commercial checks, Lutembwe and Namuseba. The performance of these genotypes could be attributed to enhanced genetic gain and tolerance to biotic and abiotic stresses. Sousa et al. (2018) and Matova and Gasura (2018) identified new experimental genotypes that were both high yielding and stable in Brazil and Zimbabwe, respectively using the GGE biplot technique.

In the identification of optimal environments for genotype performance, the GGE- biplot technique can be used to prioritize available resources for testing new genotypes. Test locations that are both representative and discriminating are favorable sites for selecting generally adapted genotypes. According to Sousa et al. (2018), the ideal sites tend to easily discriminate the performance among different genotypes, hence it provides basis for selecting superior genotypes. In this study, Lusitu was regarded as the most discriminating environment (ideal

environment) and representative of other environments. An ideal environment allows all genotypes to express their full potential thus providing opportunities for the identification of the best performing genotypes (Sousa et al 2018).

## 5.5 Conclusion

Three mega-environments were identified. Genotypes LT11-3-3-12, Bubebe and MS1-8-1-4 exhibited specific adaptation to the Nanga, Lusitu and ansa and, SCCI sites, respectively. Lusitu was the ideal environment for cowpea variety discrimination in Zambia. This study selected high yielding and stable genotypes (CP645 × MS1-8-1-4, BB10-4-2-3 and Msandile)) and high yielding but with specific adaptation (e.g. LT11-3-3-12, Bubebe and MS1-8-1-4). These are recommended for direct production in the respective mega-environments or future cowpea breeding programs.

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## GENERAL OVERVIEW AND IMPLICATIONS OF THE STUDY

### Introduction and objectives of the research

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the key food security crops supporting millions of people in sub-Saharan Africa. In Zambia, cowpea is the third most important and largely produced legume after the common bean and groundnut. However, the actual cowpea yield is low (< 600 kg/ha) in the country compared with the potential yield reaching up to 3000 kg/ha. This is attributable to a lack of high yielding varieties and several abiotic and biotic stresses and socio-economic constraints. Hence breeding and deployment of superior cultivars with farmer preferred traits is the best strategy to bolster cowpea productivity in Zambia. Breeding for yield improvement is dependent on selection of complementary and best performing parents based on economic traits and high throughput molecular markers. Genetic materials including modern varieties, landraces, and breeding lines are key sources of genetic variation to improve yield potential in cowpea. This chapter outlines the research objectives, the core findings and implications of the study.

The objectives of this study were to:

- Identify farmers' preferences and production constraints and perception on cowpea production in Zambia to guide pre-breeding.
- Assess the genetic diversity among cowpea genotypes using phenotypic traits and single nucleotide polymorphism (SNP) markers and to select distinct and complementary genotypes to developing improved cultivars.
- Determine combining ability and gene action controlling yield and yield components among crosses derived from eleven-selected cowpea [*Vigna unguiculata* (L.) Walp.] parents to advance best performing families.
- Quantify the genotype by environment interaction effect and select cowpea genotypes with high grain yield and average adaptation across selected cowpea growing environments in Zambia

## **Summary of the research findings**

### **Diagnostic assessments of farmer preferences and production constraints of cowpea in Zambia: implications for pre-breeding**

Participatory rural appraisal (PRA) research tools such as transect walks, semi-structured questionnaires and focus group discussion were used for the study. Data were collected involving 187 participant farmers through interviews and focused group discussion with 43 farmers in Eastern, Southern and Northern provinces of Zambia. The following were the main outcomes:

- Both males and females participated in the cowpea production, with the former constituting the majority (56%).
- Unimproved varieties were dominant in the production systems with 93.6% of varieties under cultivation were unimproved with low yield potential and susceptible to insect pests and diseases.
- The farmer preferred traits across the three agro- ecological regions included high yields (46.7%), good eating quality (5%), insect pest and disease resistance (15%).
- The majority of farmers perceived low yielding varieties (45.1%) as the major constraint to cowpea production, while 18.1 and 14.8% considered insect pests and diseases and limited access to production inputs, respectively, as major constraints to cowpea production.

### **Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] germplasm collections using phenotypic traits and SNP markers**

One hundred cowpea genotypes were evaluated based on economic agronomic traits in two selected sites in Zambia during the 2017/18 cropping season. Ninety-four of the test genotypes were profiled with 14,116 SNP markers. Eight quantitative and six qualitative traits were recorded for phenotypic assessment. The following were the main findings from this study:

- Number of pods plant<sup>-1</sup> (NPP), pod length (PDL), and number of seeds pod<sup>-1</sup> (NSP), were significantly ( $p < 0.05$ ) affected by genotype  $\times$  environment interaction effects.
- Genotypes such as CP411, CP421, CP645, CP732, Chimponongo, and MS1-8-1-4 exhibited higher grain yield of  $> 1200 \text{ kg ha}^{-1}$  with excellent performance in yield components such as NSP, PDL, hundred seed weight and grain yield.

- Grain yield had significant ( $p < 0.05$ ) associations with NPP ( $r = 0.50$ ), NSP ( $r = 0.46$ ) and PDL ( $r = 0.42$ ).
- The SNP markers revealed gene diversity and polymorphic information content of 0.22 and 0.17, respectively.
- Test genotypes were classified into four genetic groups irrespective of source of collection.
- Genotypes Bubebe, CP411, CP421, CP645, Chimponogo and MS1-8-1-4 were identified to be the most genetically divergent and high yielding.

### **Gene action and combining ability studies for grain yield and yield components in cowpea [*Vigna unguiculata* (L.)Walp.] genotypes**

Eleven parental lines were crossed using a half-diallel mating design and 55 progenies were successfully generated. The parents and progenies were field evaluated using an  $11 \times 6$  alpha lattice design with two replications in two representative sites in Zambia. The quantitative traits measured included days to 50% flowering (DTF), days to maturity (DTM), number of pods per plant (NPP), pod length (PDL), number of seeds per pod (NSP), hundred seed weight (HSW) and grain yield (GYD). The core findings of the study included:

- The progenies and parents exhibited significant ( $p < 0.05$ ) variation for the assessed yield and yield components except for the number of pods per plant (NPP).
- Parental lines CP411, CP732, and BBXSC103 exhibited better grain yield (GYD), number of seeds per pod (NSP), pod length (PDL), and a hundred seed weight (HSW).
- The general combining ability (GCA) effects of parents and the specific combining ability (SCA) effects of progenies were significant ( $p < 0.05$ ) for all assessed traits except days to 50% flowering (DTF) and NSP.
- Parental lines CP411 and CP732 were the best combiners for NPP, HSW, and GYD.
- Genotype CP411 exhibited desirable GCA effects for DTF and days to 90% maturity (DTM).
- Crosses CP732  $\times$  CP411, BBXSC103  $\times$  CP411 and Lutembwe  $\times$  CP645 had higher and desirable SCA effects for grain yield.
- Traits such as HSW, NPP, and DTM were conditioned by mainly additive gene action, while DTF, PDL and NSP were under the control of dominant genes.

- Early generation selection would be effective for improving traits controlled by additive gene effects while traits controlled by non-additive gene effects would be selected in advanced pure line generations.

### **Genotype × environment interaction effects of cowpea (*Vigna unguiculata* L. Walp.) for grain yield and related traits in Zambia**

Thirty cowpea genotypes were evaluated for grain yield in four sites in Zambia. The experiments were laid out using a 10×3 alpha lattice design with three replications at each site. The yield components measured included days to 50% flowering (DTF), 90 % days to maturity (DTM), number of pods per plant (NPP), pod length (PDL), number of seeds per pod (NSP) and hundred seed weight (HSW). The main outcomes were as follows:

- Grain yield and yield components were significantly ( $P < 0.001$ ) affected by genotype (G), environment (E), and genotype × environment interaction (GEI) effects.
- The additive main effects and multiplicative interaction (AMMI) analysis indicated that environment, genotype and GEI effects were highly significant ( $P < 0.001$ ) and accounted for 66.97, 12.4 and 20.63% of the total variation, respectively.
- The test environments were delineated into three mega- environments, with the Lusitu and Mansa sites clustered together while the Nanga and SCCI sites were distinct mega-environments.
- The Lusitu location was the ideal environment for discriminating among the genotypes.
- Genotypes LT11-3-3-12, Bubebe and Msandile were specifically adapted to the Nanga, Lusitu-Mansa and SCCI mega-environments, respectively.
- New families CP421 × CP732, CP645 × MS1-8-1-4, MS1-8-1-4 × BBXSC103 and a mutant line BB10-4-2-3 were identified as high yielding with average stability across the four sites. These are recommended for further evaluation and release.

### **Implications of the findings of the study**

#### **Farmer preferences and production constraints**

The cultivation of crop cultivars that have high yield potential and resilient to multiple stresses ensure food and nutrition security. The successful development and adoption of cowpea cultivars among farmers require involvement of the farmers to understand their preferences and production challenges they encountered in their production systems. Engaging farmers during the initial phase of breeding is an important step to supporting pre-breeding initiatives through targeting specific traits and attributes that are required by farmers and clients. In this study, the views of the farmers who participated in the survey will be incorporated into breeding programs for cowpea varietal selection and setting of cowpea breeding priorities in Zambia.

### **Genetic diversity of cowpea**

Phenotypic characterisation in the target production environment enables identification and quantification of genetic variation for key qualitative and quantitative traits for ideotype breeding. Knowledge of phenotypic variation and multivariate relationships among traits assist crop breeders to develop the most adaptive and productive cultivars. The wide distribution of single nucleotide polymorphism (SNP) markers throughout the genome, their cost effectiveness and amenability to automation has increased their use in breeding programmes and helped to circumvent environmental variance that affects selection. The presently used SNP markers were useful and provided four distinct genetic groups enabling breeders to design targeted crosses for hybrid formation. Six genotypes that were identified through SNP markers became part of the eleven parental lines identified for further breeding purposes.

### **Gene action and combining ability studies**

Development of new breeding populations require understanding of the combining abilities of complementary parents and their progenies. Knowledge on the nature and magnitude of combining ability and gene action is vital for selection of good general combiners that would contribute good genes and good specific combiners from which superior lines can be selected. Therefore, progeny testing serves as a guide in selection of relevant breeding methodologies to attain possible genetic gains. Significant general and specific combining ability effects of parents and progenies observed in this study for different traits imply that some of the parents and related progenies evaluated can contribute additive and non additive genes towards grain

yield and yield components in cowpea. Pure line cultivars can be developed following selection of transgressive segregants for yield and yield traits.

### **Genotype × environment interaction effects**

The influence of the environment on genotype expression has profound implications for cultivar evaluation and recommendation. Multi-environment trials are necessary to deduce genotype by environment interaction (GEI) effects on yield and agronomic traits in order to devise suitable breeding strategies and identify superior genotypes for recommendation or breeding population development. In this study, the ideal test environment, the Lusitu site, was identified for cowpea evaluation and selection. High yielding and stable genotypes derived from the families CP421 × CP732, CP645 × MS1-8-1-4, MS1-8-1-4 × BBXSC103 and BB10-4-2-3 were selected for multilocation evaluation, registration and release in Zambia.

## APPENDICES

### Appendix 1: House hold Participatory rural appraisal survey questionnaire for cowpea

#### Individual farmer's questionnaire

Date of interview.....

Name of enumerator.....

Gender ..... Code.....1) Male 2) Female

Province ..... Code.....1) Eastern, 2) Southern, 3) Western

District.....Code.....

Block..... Code .....

Camp..... Code .....

Village.....Code .....

GPS position: Latitude.....

Longitude.....

Elevation (m).....

#### Section 1a: Farmer profile

Name of respondent:									
Sex 1=male 2=female	Age (yrs)	Marital status (codes)	Household head (codes)	Education level (highest class completed)	Primary economic activity codes	Secondary economic activity codes	Use of hired labour codes	Received extension services on cowpea last year (1=Yes, 2=No)	Source of extension services codes

**Marital status codes:** 1=married, 2 =divorced/separated, 3=widow/widower, 4=single, 5= NA (below marriage age), others (specify.....)

**Household head codes:** 1=Male adult, 2=Female adult, 3= Male Child, 4=Female child 6= Grandchild, 8=other relatives (nephew, niece, cousins, uncle, aunt...)

**Education level codes:** 1= never attended formal education, 2= some primary, 3= completed primary, 4=some 'O' level, 5=completed 'O' level, 6=some 'A' level, 5=completed 'A' level, 7=tertiary institution, 8=University

**Economic activity codes:** 1= Farming (crop + livestock), 2= Salaried employment (specify), 3=Self-employed off-farm/business, retail business (specify), 4=Casual laborers on-farm, 5=studying (primary, secondary, tertiary), 6=Herds boy/girl, 7=Household chores, 8=brick making, 9=craftsmanship (metal fabrication, black smith), 10=cannot work due to old age or still young or sickness, 11=building/construction, 12=none, 13=not applicable (NA), others specify.....

**Labour codes:** 1= Contract labour, 2 = Wage labour, 3 = None

**Source of extension services Code:** 1= Government extension service, 2=NGO, 3=Church, 4=Seed company, 5=Fellow farmer,6= others (specify.....)

**Section 2: Access to land and crop production**

**Section 2a: Access to land since 2016 to date**

Access of household to agriculturally productive land (include fallow land, land rented/borrowed out and share-cropped)

Easiness to acquire land in the village: 1=easy, 2=difficulty, 3=not applicable.....

No.	Village	Total Land size owned (acre)	Distance to field from home (km)	Soil fertility (1=poor 2=average 3=good 4=very good)	Forms of land ownership								
					owned	rented	Renting cost (kwacha/year)	Borrowed	Borrowing cost (kwacha/year)	Sharecropped	Sharing cost (kwacha/year)	Other	
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

1ha~2.5acres, 1ha=10,000m<sup>2</sup>

**Section 2 b: Cowpea production in 2017**

**Part 2b 1: Seed input in the first season (cowpea only)**

No.	Village of planting	Variety codes	Area planted (size of field)	Seed source ( use codes)	Method of planting 1=ridge planting 2=row planting 3= broadcasting 4= Drilling	Amount of seed used	Unit ( cups, basins or bags)	Equivalence in kg	Unit value (kwacha.)	Total value (kwacha)
1										
2										
3										
4										

**Variety codes:** 1= Lutembwe , 2= Msandile , 3= Bubebet , 4=. Namuseba , Others (specify)

**Seed source codes:** 1=own farm saved seed, 2=bought, 3=given by NGO, 4= government agency, 5= others specify...

**Section 2b 2: Major crop production season 2017 (including cowpea)**

No.	Village planting	Crop name	Area (ac)	Production system 1=sole, 2=intercrop,	Amount produced ( bags, basins or cups)	Equivalence in Kg	Total (kg)	Amount Sold	Unit Cost	Total value sold (Ushs)	No. of times the crop has been planted in last 5 years	Labour source

**Crop codes:** 1=cowpea, 2=finger millet, 3= maize; 4=sorghum; 5=cassava; 6=sweet potato; 7=bambara 8=beans; 9=ground nuts; 10=pigeon peas; 11=field peas; 12=pearl millet 13=other (specify)

**Section 2b 3: Other crop production 2017 (including cowpea)**

No.	Village name	Crop codes	Area (field)	Production system 1=sole, 2=intercrop,	Amount produced (basins, bags or cups)	Equivalence in kg	Total (kg)	Amount Sold	Unit cost (kwacha/cup, basin or bag)	Total value sold (kwacha)	Times planted per year	Labour source codes
1												
2												
3												
4												
5												
6												

**Crop codes:** 1= cowpea, 2 = finger millet, 3 = maize, 4 = sorghum; 5 = cassava; 6 = sweet potato; 7= bambara; 8=beans; 9=ground nuts; 10=pigeon peas; 11=field peas;12=pearl millet 13 = other (specify)

**Labour codes:** 1= Contract labour, 2 = Wage labour, 3 = Family labour

**Section 2c: Challenges experienced by the household**

Type of challenge	Has your household experienced any of the following challenges in the past five years? 1 = Yes 2 = No
Illness of a household head (lost labor or capital)	
Death of a household head (lost labor or capital or resources)	
Prolonged dry spell	
Excessive rainfall	
Loss of crops in the field to pests and diseases (if yes answer section 2c 1)	
Loss of crops in storage (specify which crop was lost and quantity lost).....	
Major change in price of cowpea last year	
Loss of employment	
Theft (specify what was lost and value).....	
House damaged or destroyed by fire	
Loss of land (Specify cause of loss of land).....	

**Section 2c 1: What are the insect pests that affect your cowpea**

Important pests of cowpea	Rank (1=most important...) ( codes)
Aphids	
Flower thrips	
Pod sucking bugs	
Pod borers	
Caterpillars	
Other	

**Codes for pests** (1=Aphids, 2=Flower thrips, 3=Blister beetles, 4=Pod sucking bugs, 5= Pod borers, 6= caterpillars, 7= others)

**Section 2c 2: Do the following diseases affect your cowpea**

Important diseases of cowpea	Rank (1=most important...)
Cowpea aphid borne mosaic virus	
Ascohyta blight	
Cercospora leafspot	
Root and crown rot	
Anthracnose	
Other	

**Section 2d: Effects/control of insect pests and diseases**

Are there any varieties grown in your area resistant to the pests and diseases you mentioned?

Yes.....

Name the varieties and the insect pests to which they are resistant

.....  
 .....  
 .....  
 .....  
 .....

Name the varieties and the diseases to which they are resistant

.....  
 .....  
 .....

How do you control the insect pests and diseases you have mentioned? (Use codes).....

**Codes** (1= adopt crop rotation, 2= use clean seeds,3= dress seeds before planting, 4= use a resistant variety, 5= uproot and bury infested plants, 6= plow contaminated topsoil, 7= use insecticide, 8= others)

**Section 2e: Weeds and storage pests**

Are weeds a problems in your field?.....

How many times do you weed the field before you harvest the crop?.....

How do you control weeds?

Use herbicides.....

Use hoes.....

**Cowpea storage**

How do you store cowpea?.....

**Codes** (1= brick bin, 2= mud bin, 3= Polypropylene bag, 4= metal bin, 5= open surface, 6= other)

**Storage pests**

Are storage pests a problem?

Yes.....

No.....

How do you control the storage pests?.....

**Codes** (1= Spray with insecticide, 2= Coat seed with insecticide, 3= Dress seeds before planting, 4= Use a resistant variety, 5= other)

**Section 3: Cowpea-related aspects**

3.1 In which part of rainy season is cowpea normally grown and why? 1=first part (October to December), 2=second part (January to march), 3= both

Why?.( 1=Less rainfall, 2=much rainfall, 3=high pests, 4=high demand, 5= Price is high, 6= long season, 7= No diseases ,8= Less risk from prolonged dry spell at end of season ,9=Others, specify.....

**Part 3a: Cowpea types/varieties currently grown**

Variety (codes)	1=local 2=improved 3= Not sure	How long have you been growing this variety(yrs)	Rank type in terms of preference from most preferred to least	Reasons for growing the variety (Use codes)	Growth characteristics of type e.g. Erect, semi erect, spreading testa colour, kernel size, maturity duration	utilisation quality 1=good 2=moderate 3=poor
				1. 2. 3. 4. 5.		

				1. 2. 3. 4. 5.		
				1. 2. 3. 4. 5		
				1. 2. 3. 4. 5.		

**Variety codes:** 1= Lutembwe1, 2= Msandile 2, 3= Bubebe 3, 4= Namuseba 4, 5= local 5, 6= others, Specify.....

**Reason for growing variety codes:** 1 = High yielding, 2 = Early maturity, 3 = Drought tolerance, 4 = Pest and disease resistance, 5 = good leaf taste, 6 = Attractive seedcoat colour, 7 = medium seed size, 8 = smooth texture, 9 = easy to harvest, 10 = good pod taste for home consumption, 11 = good shelf life, 12 = sales at higher price, 13 = only variety available

**3.1 What types/varieties grown last season**

.....

3.2 Which types/varieties will you grow next season (use variety codes, multiple choices).....

.....

3.3 Which types/varieties will you never grow again (use variety codes, multiple responses required).....

and why? .....

**Section 3b: What are the uses/importance of cowpea?**




**Codes:** 1= High yielding, 2= Early maturity,3= Drought tolerance,4= Pest and disease resistance, 5= good taste, 6= medium seed size, 7=smooth texture, 8= short time cookability, 9=quick drying, 10=good leaf taste, 11=acceptable testa colour, 12=easy to remove husks, 13=easy to pluck, 14=good pod taste for home consumption, 15=good shelf life of seed (storability), 16= Spreading for type for higher yields

**Section 3d. What are characteristics/traits of bad cowpea varieties with respect to farmers?**

Characteristics/Traits (Codes)	Rank (1=worst....)

**Codes:** 1 = Bitterness, 2 long time cookability,3=Very small seed, 4= dull seed colour, 5 = Susceptibility to pests and diseases, 6= difficult to harvest,7= difficult to remove husks, 8= Long maturity duration, 9 = Seed has short shelf life,10= susceptible to droughts, 11= if not planted early, gives low yields, 12 = Does not tolerate high rainfall, 13= kernels are of mixed colour, 14=soil type-dependent performance, , 15 = sauce has a short shelf life, 16 = Others, specify.....



**Storage constraints (apart from pests)**

Constraint (codes)	Rank (1= worst)	Coping strategy/control

**Codes:** 1= No proper storage facility, 2= limited storage space, 3=thieves, 4= spoilage due to humid weather, excessive rainfall, 5= Others, specify....

**Taste constraints (both leaf and grain)**

Constraint (codes)	Rank (1=worst...)	Coping strategy/control

**Codes:** 1= Bitter leaves, 2= Lack of good thick sauce, 3= lack of good taste in the improved varieties, 4= short shelf life of cowpea sauce, 5= heart burn after consumption, 6= others, specify...

### Marketing constraints

Constraint (codes)	Rank (1=worst....)	Coping strategy/control

**Codes:** 1 = low price, 2 = lack of lucrative markets, 3 = Small size, 4=very large kernel size, 5 = unpleasant testa colour, 6= short shelf life of sauce, 7= Distant market places, 8= high transport costs, 9=No available market 10=high market charges, 11= Others, specify....

**Part 4g:** Qualities that may be introduced/improved in the most desired cowpea types/varieties with respect to the farmer

Types /varieties	Attributes to be introduced/improved
	<ol style="list-style-type: none"> <li>1.</li> <li>2.</li> <li>3.</li> <li>4.</li> </ol>
	<ol style="list-style-type: none"> <li>1.</li> <li>2.</li> <li>3.</li> <li>4.</li> </ol>

**Codes:** 1= High yielding, 2= Early maturity,3= Drought tolerance,4= Pest and disease resistance, 5= good taste, 6= medium seed size, 7 = smooth kernel texture, 8= easy to cook, 9=quick drying, 10=easy to process, 11 = acceptable seed colour, 12=easy to remove husks, 13=easy to harvest, 14=good taste for home consumption, 15=good shelf life, 16=less sensitivity to soil type)

**Section j: Agronomic practices in cowpea production**

	Practice	Yes	No	Reasons for doing or not doing the practice
1.	Crop rotation (how long between cowpea crops.....)			
2.	Planting When?..... Plant spacing.....			
3.	Weeding? how often?.....			
4.	Do you do scouting for diseases in the field, if Yes, how often/week.....			
5.	Gap filling			
6.	Rouging of disease plants during growing season			
7.	Removal of old plants parts (trash) after harvest			
8.	Do you intercrop? if yes, with which crop? Why?.....			
9.	Any soil fertility measure?.....			
10.	How do you harvest? When?			
	How do dry you dry the			
	1. Leaves			
	2. Grain			
	3. Seed			

**Section4j 1: Land preparation:**

How do you prepare land for cowpea cultivation? (Hand digging = 1, Ox-plough=2, Tractor plough = 3, 4= conservation tillage, 5 = others, specify.....)

.....

**Section 4k 2: Average yield**

Average yield of cowpea (estimate yields (bags) from an average field)

- 1) When season is very good .....
- 2) Worst season.....

**Section 5: Variety adoption**

5a) Are you aware of improved cowpea varieties ( Yes=1, No = 2).....  
 .....  
 .....

5b) Have you ever grown improved cowpea varieties (Yes= 1, No= 2, Not sure=3)  
 .....  
 .....

5d) What new varieties have you experimented within the last two years? Were they successful and why or why not? Codes A, B and C

Variety tested ( Code A)	Was it successful? Yes= 1, No=2	If yes, Why ( code B)	If No, Why not (Code C)
1.			
2.			
3.			
4.			

**Codes A:** 1= Lutembwe, 2= Msandile, 3= Bubebe, 4= Namuseba, 5 =Others, specify....

**Codes B:** 1= Seed not available, 2= No money to buy seed,3=Low yielding variety,4= Variety liked by pests, 5= Susceptible to disease,6=Late maturity, 7= Not good for intercropping,8= Lack of information on management, 9= No market for output, 10 = Poor price, 11 = Small kernel size, 12 = Unacceptable grain color, 13 = Poor taste, 14=Not enough land, 15= Short shelf life of sauce, 16=Too long to dry, 17= Hard to process, 18 = Lost seed due to drought, 19= Consumed all harvest, 20 Cookability, 23 = Other, specify.....

**Codes C:** 1 = Affordable seed, 2 = Seed availability, 3 = High yielding variety,4 = Good for intercropping, 5= Resistant to diseases), 6= Variety not liked by pests, 7= Enough land, 8=Good seed size, 9 = Good shelf life, 10 = Easy to dry, 11 = Easy to shell, ,12 = Acceptable colour, 13 = late maturing, 14 = Early maturity,15 = Sells at a higher price, 16 = Easy to sell, 17 = Good taste for home consumption, 18 = Easy to process, 19 = Seed given as gift, 20 = Others, specify.....

5e) Any information you would like to provide that is relevant to enhancing adoption of improved cowpea varieties production in your area.....  
.....  
.....  
.....  
.....  
.....

Thank you very much for your co-operation