EXPLORING THE GENETIC POTENTIAL OF LOCALLY ADAPTED GERMPLASM FOR DROUGHT TOLERANCE: A CASE FOR COWPEA (*VIGNA UNGUICULATA (L.)* Walp) FROM MALAWI

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Abstract

The shortage of improved cowpea (Vigna unguiculata (L.) Walp) varieties and increased frequency of droughts in Malawi have created a need to identify drought tolerant genotypes with desirable agronomic and utility characteristics. This research evaluated local germplasm maintained by the Malawi Plant Genetic Resources Centre (MPGRC), as an initial step towards the identification of genotypes with drought tolerance. Eco-geographic characterisation revealed diverse ecologies among the different germplasm collected. These genotypes were subsequently assessed for drought tolerance in a glasshouse study. All genotypes which tolerated low moisture conditions in the glasshouse originated from areas with high rainfall and low temperatures suggesting that extreme environmental conditions and/or human mediated actions interfered with adaptation processes. Furthermore, the eco-geographic characterisation identified germplasm gaps which need to be filled by either collection or repatriation of germplasm from international genebanks. The establishment of on-farm conservation in areas with low rainfall and high temperature such as Chikwawa and Nsanje districts may enhance adaptation of cowpea to drought conditions. Genotypes 479, 601, 645, 2226 and 3254 fully recovered from moisture stress, while 2232 started wilting within one week of drought stress initiation in the first glasshouse experiment. The genotypes which recovered from moisture stress showed low scores for wilting scales, low leaf wilting index (LWI), high relative water content, high scores for stem greenness and high levels for re-growth. In addition, the first glasshouse experiment resulted in the development of a leaf wilting index, which has been identified as an easily used method for scoring wilting, compared to common wilting scales. In a subsequent glasshouse experiment, all the genotypes which fully recovered from moisture stress showed high relative water content during the period of stress, but showed differences in other physiological traits. For example, genotypes 479, 601, 645 and 2226 had reduced stomatal conductance, transpiration rate and net photosynthesis, while 3254 maintained high scores for the three traits from the initial stage of moisture stress. Although 2232 showed a high transpiration rate and stomatal conductance, its net photosynthesis was significantly reduced, compared to all the other genotypes, after the third week of stress. The differences in physiological traits among genotypes indicated that 3254 has drought tolerance; 479, 601, 645 and 2226 avoid drought while 2232 is drought susceptible. The field performance of these six genotypes and two released varieties (Sudan 1 and IT82E16) was assessed in field trials in Malawi at Baka, Bvumbwe, Chitala, Chitedze and Kasinthula. Results from field trials revealed significant *variation for reproductive, yield and seed characteristics. Sudan 1, IT82E16, 409 and 601 matured in less than 65 days after planting; 3254 took 70 days and 645, 2226 and 2232 took more than 85 days. Genotype 3254 consistently gave high yields at sites with low rainfall and high temperatures compared to 2232 which yielded poorly at the same sites. The eight genotypes showed variation in seed size with genotype 2226 producing large seeds (>20g/100 seeds) at all sites. The seed size of 2232 was significantly lower than 2226 at sites with low rainfall and high temperatures. The field performance of these genotypes reflects the physiological responses observed in the glasshouse, confirming the drought response categories of the genotypes. The agreement between glasshouse experiments and the field trial suggests there is intrinsic value in the locally adapted germplasm maintained by the Malawi Plant Genetic Resources Centre. Among the genotypes tested in the field, farmers selected 479 for early maturity; 2226 and 2232 for high leaf biomass; 3254 for high pod load; 2226 and 2232 for large seeds; Sudan 1 for small seeds; and 601, IT82E16 and Sudan 1 for smooth seed texture. Genotype 3254 was ranked poorly at all the sites due to rough seed texture. Genotypes for potential use in improving production of cowpea in drought prone areas were identified. In the absence of released drought tolerant varieties, it is recommended that genotypes with drought avoidance characteristics be promoted in areas with mild droughts, while 3254 with its typical drought tolerance may be suitable for areas with intense droughts. However, the rough seed texture of 3254 may limit its usefulness due to its poor ranking by farmers at all sites. Priorities for future cowpea in Malawi include investigating inheritance of drought tolerance in cowpea.*

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Dedication

I dedicate this work to my wife, son, dad and mum, for being instrumental throughout my academic journey.

I also dedicate this work to the children of Malawi who do not complete their education, often because their parents do not appreciate its value. It is my prayer that such parents begin to realise the importance of education in changing the world. I also dedicate this work to all the people and organisations that selflessly support the education of children in Malawi. May you gain more blessings for this noble cause.

To the parents: *"If you think education is expensive, try ignorance"* by Derek Bok.

To the goodwill ambassadors: "*Education is the most powerful weapon which you can use to change the world*" by Nelson Mandela.

Candidate's declaration

This is to certify that the research carried out for my Doctoral thesis entitled "*Exploring the genetic potential of locally adapted germplasm for drought tolerance: A case for cowpea* (*Vigna unguiculata* (L.) Walp) *from Malawi"* at the Institute of Agriculture and Environment, Massey University, New Zealand is my own work and that the thesis material has not been used in part or whole for any other qualification.

CANDIDATE'S NAME LAWRENT L.M. PUNGULANI

SIGNATURE ....

DATE 23/10/2014

Supervisor's declaration

Chief Supervisor's Name James Miller

Signature J P Miller

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Co-Supervisor's Name Warren Williams

Signature J P Miller on behalf.

Date 24/10/14

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Acronyms

ABA	Abscisic Acid
ANOVA	Analysis of Variance
asl	Above Sea Level
ASWAp	Agriculture Sector Wide Approach
CBD	Convention on Biological Diversity
CIAT	International Centre for Tropical Agriculture
DAES	Department of Agricultural Extension Services
DARS	Department of Agricultural Research Services
DLS	Delayed Leaf Senescence
E	Net Transpiration
EPA	Extension Planning Area
FAO	Food and Agriculture Organisation of the United Nations
FGD	Focus Group Discussion
GDP	Gross Domestic Product
GIS	Geographic Information System
GLM	General Linear Model
Gs	Stomatal conductance
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
IAE	Institute of Agriculture and Environment
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LSD	Least Significant Difference
LWI	Leaf Wilting Index

MC	Moisture Content
MGDS II	Malawi Growth and Development Strategy II
MLS	Multilateral System
MPGRC	Malawi Plant Genetic Resources Centre
NZAID	New Zealand Aid for International Development
PGU	Plant Growth Unit
Pn	Net photosynthesis
PRA	Participatory Rural Appraisal
PVS	Participatory Variety Selection
QTL	Quantitative Trait Loci
RCBD	Randomised Complete Block Design
RWC	Relative Water Content
SLA	Specific Leaf Area
STG	Stem Greenness
TDR	Time-Domain Reflectometer
TE	Transpiration Efficiency
UCR	University of California Riverside
WS	Water Stressed
WUE	Water Use Efficient
WW	Well Watered

Chapter 1 : Introduction

1.0 Background

Agriculture plays a critical role in the economic development of Malawi. The agricultural sector alone employs approximately 80% of the total workforce and contributes approximately 30% to the country's gross domestic product (GDP). Consequently, agriculture has been identified as one of the key thematic areas in the Malawi Growth and Development Strategy II (MGDS II) (Malawi Government, 2011). In order to align agriculture with the development priorities in the Strategy, the Ministry of Agriculture and Food Security has embarked on a coordinated approach to the implementation of its programmes, through an Agricultural Sector Wide Approach (ASWAp) (Malawi Government, 2010). This approach stipulates the need to develop and promote new crop varieties, which will improve the food security and income of farmers, contributing to the sustainable development of Malawi.

The ASWAp framework fully recognises the role of legumes in addressing the malnutrition that is prevalent in farming communities. Malawi has high rate of malnutrition with nearly half of children suffering reduced height growth due to lack of protein and other important nutrients (Sassi, 2012). Legumes offer a significant contribution to the availability of proteins consumed in most developing countries (Upadhyaya *et al*., 2011); the protein content of cowpea seed and young leaves is typically more than 25% (Singh *et al*. 2003).

FAO statistics (FAOSTAT, 2013) show that groundnut *(Arachis hypogea)*, pigeon pea *(Cajanus cajan)*, bean *(Phaseolus vulgaris)*, soya bean *(Glycine max)* and cowpea *(Vigna unguiculata)* are the five major priority legume crops produced and consumed in Malawi. Production trends for these crops from 2004-2013 generally ranked cowpea fifth (Fig 1.1). Among these five priority grain legumes, only ground nut, bean and soya bean are included in the ASWAp framework, principally due to the availability of a wide range of improved varieties for production by farmers (Mviha *et al.*, 2011). In general, cowpea is regarded as one of the neglected and under-utilised crops (Negri *et al.*, 2000). However, cowpea is more drought tolerant than other commonly grown grain legumes in Malawi (Hall, 2004b; Singh *et al.*, 1999a). The exclusion of cowpea from the list of priority grain legumes is contributing to the high risk of low legume production in areas with frequent and prolonged droughts in Malawi.

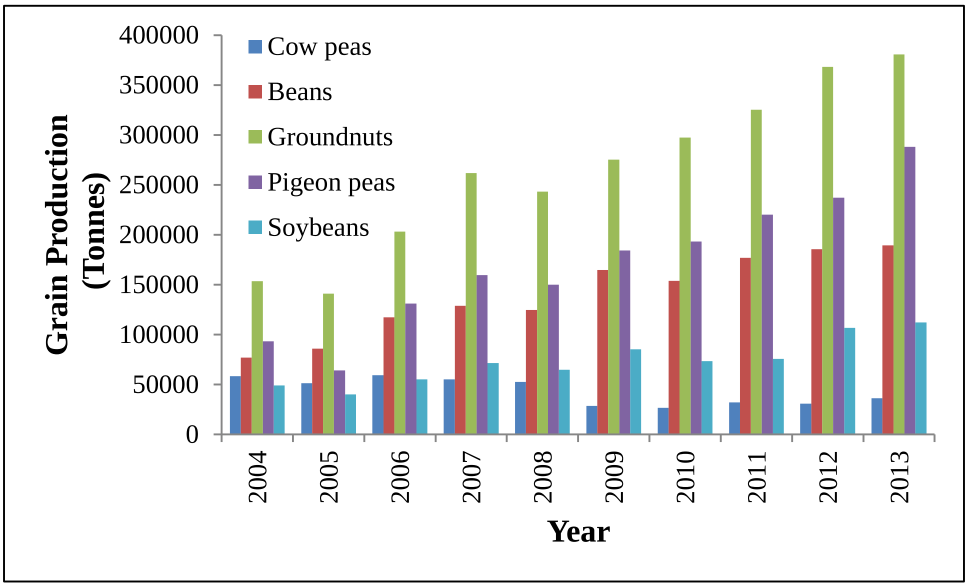

Figure 1.1. Production trends for five important grain legumes in Malawi over a period of 10 years.

Malawi has a sub-tropical climate with unimodal rainfall pattern which spans from November to April during which 95% of the annual precipitation takes place (Malawi Meteorological Services, 2015). Annual average rainfall varies from 725mm to 2,500mm with highlands receiving more rainfall than lowlands. The low lying areas are vulnerable to both droughts and floods. A hot, dry season spans from September to October with average temperatures between 25°C and 37°C. A cool, dry winter season starts from May to August with mean temperatures varying between 17°C and 27°C, with minimum temperatures falling to between 4°C and 10°C. In Malawi cowpea is primarily grown in warm areas with low rainfall (Nkongolo *et al*. 2008).

Farmers in Malawi use different cropping systems depending on the growth habit of cowpea; determinate types are usually grown in pure stands and indeterminate types intercropped with other crops including maize, sorghum, millets and cotton (Nkongolo, 2003). However, farmers mostly use landraces in their production systems due to lack of improved varieties. The use of landraces in Malawi contributes to low yields, often less than 500kg/ha compared to potential yields of more than 2000kg/ha (Government of Malawi, 2000; FAOSTAT, 2013). Besides the use of landraces, the low yields are also attributed to both biotic (pests, diseases and weeds) and abiotic (drought, high temperature, soil toxicity, soil pH etc.) factors (Mazuma et al., 2008; Hall, 2004a; Singh *et al.*, 1997).

Traditionally cowpea is regarded as a magnet for insect pests such as pod borers (*Maruca spp.*), flower thrips (*Megalurothrips sjostedti*) and pod sucking bugs (*Clavigralla spp.*) (Badiane *et al.* 2014). Ascochyta blight, mosaic virus disease, cercospora leafspot and scab are major diseases of cowpea (Mazuma *et al.*, 2008). Yield losses as high as 100% have been reported due to both diseases and pests. In addition to pests and diseases cowpea is affected by parasitic weeds; *Striga gesnerioides* and *Alectra vogelli.* In Malawi one variety resistant to parasitic weeds (*Alectra vogelii*) has been released for production by farmers (Kabambe *et al*., 2014; Mviha *et al.*,2011) and screening of local germplasm for resistance to major diseases and pests is currently underway (Mazuma et al., 2008).

Although cowpea is considered a relatively drought tolerant crop, its productivity is negatively affected by prolonged droughts and high temperatures (Hall, 2012) which recently is partly due to the effects of climate change (Tadross *et al*., 2009). The development and promotion of drought tolerant varieties of cowpea would significantly contribute to legume production in areas such as Malawi, where droughts are frequent and intense (Lobell *et al.*, 2008; Tadross *et al.*, 2009). Breeding drought tolerant crop varieties has been identified as one of the key options for risk management within the agricultural sector in Malawi (Malawi Government, 2010). Until recently, there has been no research on drought tolerance of the locally available cowpea germplasm in Malawi.

Drought tolerant cowpea varieties have been identified and released for use by both farmers and researchers in other parts of the world including Senegal, Nigeria, Niger and Burkina Faso (Hall, 2012; Singh, *et al*., 2003). The absence of genetic improvement targeting drought tolerance in Malawi may have contributed to low cowpea yields (less than 500kg/ha), compared to potential yields of more than 2000kg/ha (Government of Malawi, 2000; FAOSTAT, 2013). In order to contribute to the development of drought tolerance breeding in Malawi, this research sought to identify drought tolerant genotypes which could be used in future cowpea breeding programmes. Development of drought tolerant genotypes would help ensure sustainable cowpea production in drought prone areas, contributing to food security and consequently, economic growth.

1.1 Hypothesis and research questions

Drought tolerance has been identified in cowpea germplasm in other regions of the world. However, the genetic potential for drought tolerance of locally adapted cowpea genotypes from Malawi is not known. It is possible that genotypic variation for drought tolerance exists in locally adapted cowpea germplasm from Malawi, which could be directly released or used in breeding drought tolerant varieties. In order to assess this hypothesis, the following questions were formulated and subsequently explored.

- Does drought tolerance variation exist in locally collected and adapted Malawian cowpea landraces?

- If the variation exists, does it exist at morphological or physiological levels or both?
- How do tolerant genotypes interact with the environment in terms of reproductive, yield and seed characteristics?
- Do drought tolerant genotypes meet the criteria for selection by farmers in Malawi?

1.2 Objectives

This research is aimed at discovering drought tolerance in cowpea by a detailed evaluation of locally collected and adapted cowpea germplasm from Malawi. Specifically, the research is aimed at achieving the following objectives:

a. To understand the pattern of geographic distribution/origin of the cowpea germplasm conserved by the Malawi Plant Genetic Resources Centre;
b. To characterise cowpea germplasm for drought tolerance using both morphological and physiological parameters;
c. To test the reproductive and yield characteristics of cowpea genotypes over different environmental conditions;
d. To identify potential parental lines for drought tolerance breeding possessing traits of interest by local farmers.

1.3 Research outline

The research gaps, hypotheses and objectives outlined above led to the formulation of research components which have contributed to the identification of potentially drought tolerant genotypes with other desirable attributes. Chapter 2 lays out the theoretical framework of the research which expands into five technical chapters. Chapter 3 analyses the geographic distribution of cowpea germplasm, in order to give insights into possible adaptation of the local germplasm to environmental conditions, such as low rainfall and high temperature. Chapter 4 identifies potential drought tolerant genotypes through the maintenance of an active canopy under moisture stress conditions in a glasshouse experiment. Chapter 5 explores the physiological mechanisms in the drought tolerant and susceptible genotypes in a glasshouse as a follow up to the canopy maintenance experiment. Chapter 6 compares the performance of the drought tolerant and susceptible genotypes in Malawi alongside two released varieties for reproductive, yield and seed characteristics. Chapter 7 exposes nineteen genotypes including the eight genotypes tested in the field to local farmers' preference assessments. Finally, Chapter 8 provides an interpretation of the results with an emphasis on practical application, limitations and the future direction of cowpea research in Malawi.

1.4 Relevance of the research

This study explored a stepwise screening method based on the formulated hypothesis and research questions and objectives to identify drought tolerant genotypes from locally adapted cowpea germplasm. This is the first systematic research on cowpea from Malawi and it will potentially promote targeted conservation and utilisation of the available germplasm. Consequently, the research outputs may well contribute to the economic development of Malawi through the development of drought tolerant genotypes for production in drought prone areas.

Chapter 2 : Literature review: Available options and future direction for cowpea drought tolerance improvement in Malawi

2.0 Introduction

Cowpea is one of the oldest crops produced by man with its centre of origin and domestication in Africa (Padulosi & Ng, 1997). High diversity present in the cultivated cowpea in West Africa has led to a conclusion that cowpea was first domesticated in that region while high diversity of wild cowpeas is present in Southern Africa (Timko, 2007). The presence of high diversity of wild cowpea in Southern Africa where Malawi is located points to a need to explore the presence of important genotypes for future crop improvement

Limited information is available on the initiative to screen local cowpea germplasm for drought tolerance in Malawi. Therefore, identification of drought tolerant varieties will boost sustainable production systems in drought prone areas. This research has reviewed the available literature and suggests future direction for improved production of cowpea in drought prone areas in Malawi. Specifically the review examines:

a. Importance of cowpea in improving livelihoods of farmers in drought prone areas;
b. Impact of drought on agriculture production;
c. Global efforts to improve drought tolerance in cowpea;
d. Importance of integrating locally adapted germplasm in variety development;
e. Stepwise approach of characterising germplasm (eco-geographic, morphological and physiological characterisation) for identification of drought tolerant genotypes.

2.1 Importance and production trends of cowpeas

Cowpea (*Vigna unguiculata* (L) Walp.) is a leguminous crop produced in most dry regions of the world (Mai-Kodomi *et al*., 1999). It is estimated to be grown over 14 million hectares of land worldwide and with over 4.5 million metric tonnes of grain harvested per year (Singh *et al*., 2003). Sub-Saharan Africa alone accounts for 75% of the total land grown to cowpea and 66% of global grain yield (Ehlers & Hall, 1997; FAOSTAT, 2013). Malawi is part of Sub-Saharan Africa and contributes significantly to global cowpea production. According to FAOSTAT (2013), Malawi is ranked 12th on the global production scale of cowpea. Further analysis of leguminous grain crop production from FAO statistics indicates that cowpea ranks fifth among legumes in Malawi's agricultural production. FAOSTAT shows that production of cowpea in Malawi varied from 2004 to 2014. Land used for production of cowpea was significantly high between 2004 and 2006 (Fig. 2.1a) while annual grain production was high between 2004 and 2008 (Fig. 2.1b). However, yield per hectare was highest in 2007 and 2008 (Fig. 2.1c). Although the production trend varied among years the yields still remained below potential yield of 2000Kg/ha.

Cowpea is considered a multipurpose crop, since it provides a cheap source of protein, minerals and vitamins for farming families (Dugje *et al.*, 2009; Singh *et al.*, 2003). Cowpea is produced for consumption of its fresh leaves, fresh immature pods and seeds. Both fresh and dry seeds are marketed and provide a source of income for farming communities (de Ronde & Spreeth, 2007; Diouf, 2011). In addition to culinary functions, cowpea is used as a fodder crop due to its high biomass production and high forage value. As a legume crop, its roots contribute to soil fertility through biological nitrogen fixation (Ba *et al.*, 2004; Timko & Singh, 2008). Promoting production of cowpea is crucial in improving soil fertility in most African countries with poor soil fertility status. Nitrogen as high as 31kg/ha can be biologically fixed into the soil by growing cowpea (Adjei-Nsiah *et al.*, 2008). The high level of drought tolerance of cowpea, compared to other grain legume crops, makes it well adapted to drought prone areas (Hall, 2004b; Singh *et al.*, 1999a). However, despite its multiple uses and drought tolerance, the crop is neglected and underutilised in Malawi.

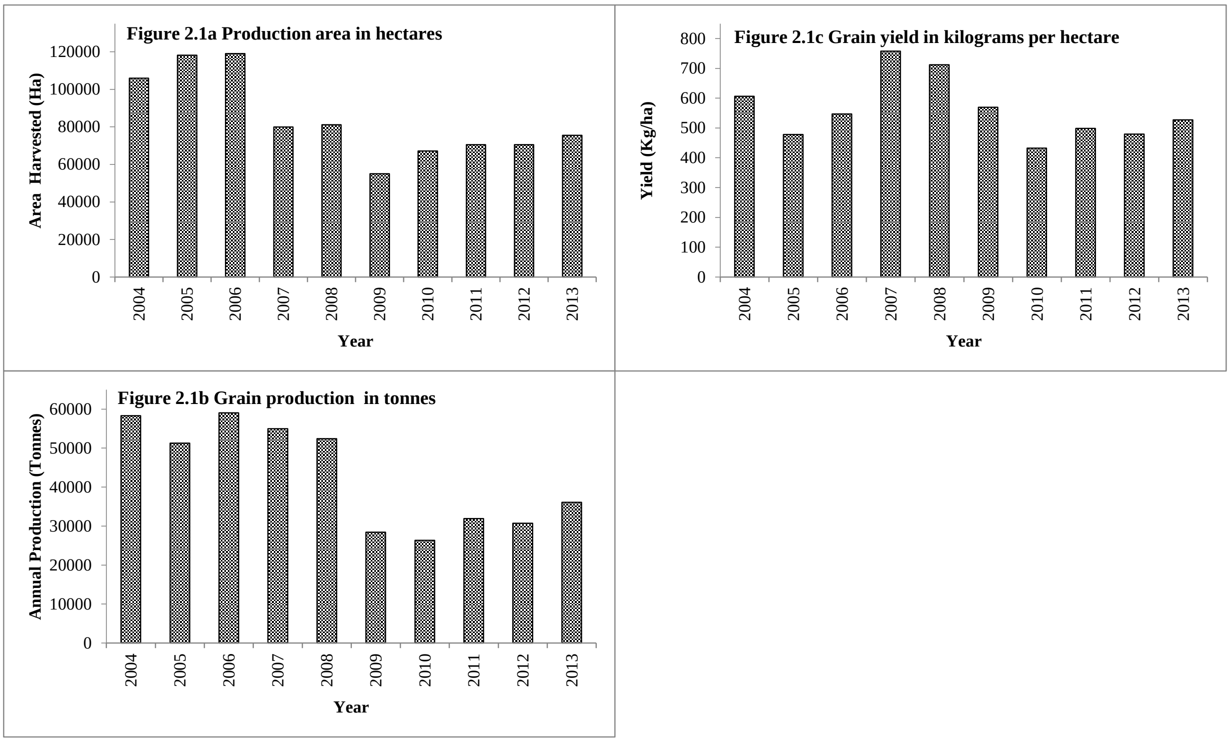

Figure 2.1 Production trends of cowpea in Malawi over a period of ten years in terms of a) production area in hectares, b) annual grain production in tonnes and c) yield per hectare (Source FAOSTAT, 2013).

The neglected status of cowpea research in Malawi is evident from the limited availability of released varieties. In the past fifteen years, only three varieties have been released in Malawi (Government of Malawi, 2000; Mviha *et al.*, 2011). A lack of well-established research on cowpea in Malawi is also evident from a report on the Tropical Legumes 2 Project (TL2) implemented by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), the International Centre for Tropical Agriculture (CIAT) and the International Institute of Tropical Agriculture (IITA) in various countries including Malawi (ICRISAT, 2011). The emphasis of this project was on seed production of introduced varieties through national programmes, including those in Malawi, while breeding efforts were being implemented by IITA and the University of California Riverside (UCR). However, the promotion of introduced varieties may face resistance from local farmers who have their own preferences and preferred traits, which may be lacking in the introduced materials.

2.2 Drought conditions in Sub-Saharan Africa

Drought is a major limiting environmental factor for agricultural productivity in Africa. In most dry parts of Africa, including Malawi, the rainfall pattern is not reliable. Rain may stop a few weeks after planting, during mid-season or during the flowering period (Fatokun, 2009). Specific studies on changes in seasonal patterns in Malawi, Zambia, Zimbabwe and Mozambique identified increases in dry spells and shorter rainfall seasons with times (Tadross *et al*., 2009). These climatic scenarios pose substantial challenges to agricultural production systems, more especially in areas where adaptation strategies have not been developed. Detailed studies have been conducted, to quantify the negative impact of droughts on agricultural production and possible solutions have been suggested, including breeding new varieties that will cope with drought conditions resulting partly from changing climates (Burke *et al.*, Lobell, & Guarino, 2009; Kumar, 2006; Lane & Jarvis, 2007; Lobell *et al.*, 2008). Nevertheless, implementation of the suggested solutions has not been fully explored in crops such as cowpea and other minor crops.

2.3 Drought tolerance research in cowpea

Although cowpea is drought tolerant compared to other grain legumes, water stress remains the most limiting abiotic factor affecting production, contributing to low production in drought prone areas (Ehlers & Hall, 1997; Timko & Singh, 2008). Singh *et al.* (2003) reported the existence of cowpea drought tolerance breeding programmes in Senegal, Nigeria, Niger, and Burkina Faso with a great deal of technical support from IITA and the

UCR. Other key studies on drought tolerance in cowpea have been conducted in Mozambique (Chiulele, 2010), South Africa (Nkouannessi, 2005) and the Netherlands (Agbicodo, 2009). In most of these studies, drought tolerant genotypes were identified, with most originating from IITA or the UCR and none from Malawi.

The promotion of drought tolerant genotypes originating from the same sources (IITA and UCR) may pose bottlenecks to the genetic base and more especially a lack of adequate variation for high performance in dry Malawi environments. In addition, the exotic germplasm could contribute to low adoption of new varieties due to lack traits of interest to farmers. Cowpea is one of the legumes with specific geographic farmer preference so that varieties bred for West Africa are not preferred in other regions such as Eastern and Southern Africa (Timko *et al.*, 2007). Such distinct geographic preference inhibits the promotion of varieties between regions. These two bottlenecks associated with the use of exotic germplasm could be overcome by decentralised breeding which requires local initiatives to explore the presence of desirable traits, such as drought tolerance, in locally adapted germplasm conserved by farmers and in national genebanks. The lack of research in cowpea drought tolerance in Malawi justifies the need to explore the presence of drought tolerance in the available germplasm. This direction in research would contribute to broadening the genetic base of the breeding populations. It would also accelerate the adoption of new drought tolerant varieties, as a result of satisfying local preferences for local germplasm.

2.4 Resistance of plants to drought conditions and implications on crop improvement

Plants adapt to drought conditions through three major mechanisms: drought escape, drought avoidance or dehydration postponement and drought tolerance or dehydration tolerance (Farooq *et al.*, 2009, Blum, 2005). Prior understanding of each mechanism in which plants respond to drought would significantly help plant breeders to develop relevant breeding programmes for drought adaptation. The understanding of resistance mechanisms would help breeders to target specific organs or times of flowering in assembling genetic material for cowpea breeding.

2.4.1 Drought escape

Drought escape is the ability of plants to flower, set pods and mature before severe terminal/late drought conditions occur (Ehlers & Goss, 2003, p. 4). The growth habits of cowpea genotypes provide guidance on the identification of drought escape varieties. Cowpea is classified into two major growth habits; determinate and indeterminate (IBPGR, 1983). The determinate genotypes flower and mature within two to three months, compared with indeterminate types which take more than three months to reach maturity (Nkongolo, 2003). All genotypes, which escape terminal drought, fall within the determinate class, due to their early maturing characteristics. In Malawi, the determinate genotypes are locally referred to as Nseula, and the indeterminate ones, Khobwe (Nkongolo *et al.*, 2009). Although most breeding programmes have developed early maturing varieties of cowpea as an adaptation strategy to escape terminal drought (Hall, 2004a), such genotypes usually succumb to early season drought, due to the absence of inherent drought tolerance in early stages of growth (Agbicodo *et al.,* 2009). Besides failure during the early season droughts, genotypes with drought escape mechanisms are deprived of lengthy growing periods, which limit the availability of both leafy vegetable and high value forage. The performance of genotypes, with drought escape under drought conditions, would be improved by crossing them with genotypes that show inherent drought tolerance.

2.4.2 Drought avoidance

Drought avoidance or dehydration postponement ensures an adequate water balance between uptake from the soil and loss into the atmosphere. Ehlers and Goss (2003) described genotypes with drought avoidance as either water savers, due to the reduction in water loss or water spenders, as a result of the efficient maintenance of water uptake from the deep layers of soil. Water savers have low stomatal conductance, leaf rolling and reduced radiation absorption, which contribute to water maintenance in the plant tissues (Mitra, 2001), while water spenders develop an efficient root system which taps water from deep layers of the soil (Fatokun *et al.*, 2009; Timko & Singh, 2008). Breeding for plants that manipulates both root and leaf traits, in order to improve water saving or water absorption, is appropriate for production in drought prone zones.

2.4.3 Drought tolerance

Drought or dehydration tolerance is described as the survival mechanism of plants under severe drought stress conditions (Ehlers & Goss, 2003) and is defined as the relative capacity to sustain or conserve plant function in dehydrated state (Blum, 2005).. Dehydration tolerance involves cellular activities, such as accumulation of metabolites for protection of cell membranes (osmoprotectants), osmotic adjustments to increase the ability for cells to take up water against an osmotic adjustment. The active adjustment of cellular activities ensures maintenance of stomatal conductance and photosynthesis under extreme moisture stress conditions (Manavalan *et al.*, 2009). Drought or dehydration tolerance is a second line of defence, when plants experience prolonged periods of stress. Crop varieties with drought or dehydration tolerance would be ideal for production in areas with prolonged droughts and lack of water in deep soil layers.

2.4.4 Breeding and crop management for drought adaptation

The time and intensity of drought is an important decision making factor in the selection of genotypes with particular aforementioned drought adaptation mechanisms. The ideal solution is to develop varieties with combined mechanisms of drought adaptation. However, developing a breeding programme that combines all the mechanisms will almost certainly be challenged by the nature of gene inheritance. In the absence of combined adaptation mechanisms, breeding for early maturity would be appropriate in areas with consistent terminal drought; breeding for drought avoidance would be more applicable in areas with mild droughts; and breeding for drought tolerance would be more relevant in areas with severe drought conditions. In areas where drought occurrence cannot be reliably predicted, farmers can practice variety intercropping, that is, planting varieties that escape drought, together with varieties that avoid or tolerate drought (Hall, 2004a). Farmers in Malawi practice variety intercropping where different landraces are mixed as tradition seed storage. Although cowpea is natural self-pollinating species with low chances (5%) of cross pollinating (Timko, 2007), the variety intercropping practiced by farmers may lead generation of varietal intercrosses. During variety intercropping, yield under terminal drought would be achieved from the early maturing varieties and yield during mild and prolonged droughts would be achieved from the drought avoiding and tolerant varieties. Subjecting cowpea germplasm from Malawi to systematic characterisation will help to classify the germplasm into appropriate drought adaptation mechanisms and consequently guide the establishment of an appropriate breeding programme for drought adaptation.

2.5 Systematic characterisation of germplasm for drought tolerance

Successful identification of drought tolerant germplasm depends on a step by step characterisation of the available germplasm. Four types of characterisation (eco-geographic, morphological, physiological and molecular) can improve the utilisation of germplasm. Molecular characterisation is beyond the scope of this study and thus only eco-geographic, morphological and physiological characterisation have been discussed in detail.

2.5.1 Eco-geographic characterisation of germplasm

Eco-geographic characterisation is the description of germplasm based on the characteristics of habitats at collection sites, in order to identify genotypes that are well adapted to particular habitats (Upadhyaya *et al.*, 2011). The importance of eco-geographic characterisation is based on the premise that environmental conditions, at the collection site dictate the evolution of plant populations (Allard, 1996). Eco-geographic characterisation is a quick and efficient method of identifying genotypes that are adapted to climates of particular interest to breeders, more especially with regards to genebank collections that have not been evaluated (Bennett *et al.*, 2011). The lack of eco-geographic characterisation clearly contributes to under-utilisation of conserved germplasm.

Several studies have demonstrated the importance of eco-geographic characterisation, as a first step in adding value to genebank collections. Typical eco-geographic characterisation, as part of managing plant genetic resources has been implemented in various crops such as wild chickpea (Ben-David *et al.*, 2006), lupin (Berger *et al.*, 2008), wheat and barley (Endresen *et al*, 2010; 2011), bladder clover (Ghamkhar *et al.*, 2007, 2008), *Biserrula spp* (Ghamkhar *et al.*, 2012), Subterranean clover (Ghamkhar *et al.*, 2014) *Cullen* spp. (Bennett *et al.*, 2011) and red clover (Greene *et al*. 2002). In all these studies, the distinctive roles of environmental factors influencing adaptation mechanisms were identified.

The success of eco-geographic characterisation is attributed to the availability of climatic data and Geographic Information Systems (GIS) software such as DIVA-GIS. Climatic data that could be used in characterising germplasm has been described by Hijmans *et al.* (2005) and the data sets are available online from www.worldclim.org. DIVA-GIS software, specifically designed for management of plant genetic resources, is also freely available for non-commercial uses (Hijmans *et al.*, 2001). In combination with this GIS software, several statistical packages have been used in identifying clusters of germplasm and environments using environmental variables. Despite the demonstrated importance of eco-geographic characterisation and the development of freely available tools, eco-geographic characterisation has not been fully explored in most genebanks due to their limited technical capacity (FAO, 2010). Most genebanks in developing countries have limited access to online resources, due to unreliable connectivity (Hazekamp, 2002) and consequently, this has hampered the successful implementation of eco-geographic characterisation.

The relevance of eco-geographic characterisation in the identification of unique populations adapted to particular niches and the development of freely available tools provides room for identifying genotypes adapted to specific climatic conditions of interest to the genebanks and breeders such as water stress or drought. Genotypes collected from areas characterized by low rainfall and high temperature would be potential sources for drought tolerance assuming minimum disturbance to the habitats of the plant populations. To date, no eco-geographic study has been conducted in cowpea germplasm from Malawi.

2.5.2 Morphological characterisation of germplasm.

The response of different genotypes of a species to drought stress is as diverse as their genetic diversity. The morphological screening of germplasm for drought tolerance evaluation is a cornerstone of the identification of drought tolerant genotypes (Fussell *et al.*, 1991; Szira *et al.*, 2008). Several morphological characters have been identified and recommended for use in selecting drought tolerant genotypes in many crops and in cowpea specifically. This study has dwelled on morphological characteristics associated with roots and canopy, as key features for adaptation to drought conditions. Morphological characteristics associated with an efficient root system used to extract water, together with a photo-synthetically active canopy under water stress are key measures for drought tolerance (Passioura, 1983).

2.5.2.1 Root characteristics

Roots contribute to the survival of plants in moisture stress environments. Both deep and shallow root systems have been reported to benefit plants under drought conditions (Blum, 2005). Some genotypes respond to drought by developing deep roots to capture moisture from deep layers of the soil while others develop roots on the top layer of the soil, in order to benefit from a brief water supply on the surface. Genotypes with a deep rooting system for adaptation to drought conditions have been identified in cowpea (Matsui & Singh, 2003; Onuh & Donald, 2009; Robertson *et al.*,1985), chickpea (Kashiwagi *et al.,* 2008) and beans (Sponchiado *et al.,* 1989). However, grain yield may be compensated in genotypes with deep root system when drought does not occur. A combination of both a deep and shallow rooting system to produce a dimorphic root system for the acquisition of moisture from multiple layers of the soil, has been reported to improve yield during drought stress conditions in common bean, (Ho *et al.,* 2005).

Different root measurement techniques have been developed and applied in selecting drought tolerant genotypes. A root measurement technique, referred to as pin-board root-box was used to identify seven drought tolerant genotypes of cowpea, which also showed increased root growth under water stress conditions (Matsui & Singh, 2003). Onuh and Donald (2009) measured root length at two week intervals in potted cowpea plants. The drought tolerant genotypes registered significantly longer roots under water stress conditions. The use of herbicide to track root growth has also been used in cowpea (Robertson *et al.,* 1985). Metribuzin was applied at different rooting zones, in order to monitor the growth rate of the taproot. All the root measurement methods highlighted here are either labour intensive, expensive or destructive in nature. For instance, the pin-board method requires frequent dismantling of the box to take measurements and this is labour intensive and disturbs plant growth. Similarly, the use of pots involves destructive sampling. The use of herbicides destroys the plants which prevents taking further measurements from the target plants. Complications associated with root measurements require equally complicated experimental arrangements to capture genotypic differences in a wide range of germplasm. The exploration of new and easy methods of taking root measurements would enhance the understanding of root mechanisms with regards to adaptation to drought.

2.5.2.2 Shoot root ratio

Reduced shoot/root ratio is associated with the adaptation mechanism of plants through an extension of their root system to capture more water and at the same time reduce canopy structures including leaves, in order to minimise water loss. The ratio of shoots and roots in water stressed environments has been used as an indicator of drought response in lentils (Sarker *et al.*, 2005), alfalfa (Erice *et al.*, 2010) and chickpea (Anbessa & Bejiga, 2002). A change in ratio signifies that drought tolerant genotypes partition more dry matter to roots than shoots as an adaptation mechanism during drought stress. Although shoot/ratio has been recommended as a good characteristic for selecting drought tolerant genotypes, care should be taken in breeding for an extended root system since increased dry matter in roots could be attained at the expense of an accumulation of dry matter in harvestable organs, such as grain and leaves in cowpea more especially when drought does not occur.

2.5.2.3 Leaf characteristics

Stay green or delayed leaf senescence (DLS) is a key leaf canopy characteristic for plants to cope with drought conditions due to its direct association with photosynthesis. Stay green is the ability of a plant to tolerate leaf drying during water stress by maintaining green leaf colour (Rosenow *et al.*, 1983). Stay green has been used as a selection criterion in breeding for drought tolerance in cowpea (Agbicodo, 2009; Muchero *et al.*, 2008; Singh *et al.*, 1999b) and other crops such as maize, rice and sorghum (Campos *et al.*, 2004). Stay green is associated with chlorophyll maintenance in leaves during moisture stress. Genotypes which maintain high chlorophyll perform better under drought conditions, due to high photosynthetic capacity and presumably smarter use of the available water (Fatokun *et al.*, 2009; Ismail *et al.*, 2000). The use of stay green as a selection criterion in cowpea at seedling stage could be useful in selecting drought tolerant genotypes. Cultivars with stay green in crops such as cowpea have the added advantage of maintaining leaf production for both vegetable and fodder production, even in times of moisture stress.

Despite the availability of several morphological traits for drought tolerance screening, leaf wilting still remains a fundamental morphological indicator for drought response as it simplifies complexities associated with drought evaluation in crops. The development of different wilting scales in cowpea confirms the importance of this trait. Bioversity International (formerly known as IBPGR) developed a scale for leaf wilting with 1 representing leaves with full turgor and 9 representing dry and dead plants under moisture stress conditions (IBPGR, 1983). Singh *et al*. (1999b) developed and used a 1-5 scale, with 1 representing completely unstressed and 5 representing dead plants. Watanabe *et al.* (1997) developed and used another 1-5 scale but in a reverse order to the Singh *et al*. scale. Leaf wilting scales for selecting drought tolerant varieties is a challenge to researchers (Xu *et al.*, 2000). The use of scales can be associated with biased scoring due to visual assessment and it requires experienced researchers to systematically and uniformly score for leaf wilting. Limitations associated with the use of wilting scales point to the need setting new standards to enhance the ease and reliability of drought tolerance assessment.

2.5.3 Physiological characterisation

Water stress affects many physiological processes in plants. Photosynthesis, transpiration rate, stomatal conductance, osmotic adjustment, accumulation of stress related proteins and accumulation of abscisic acid (ABA) are some of the physiological processes affected by water stress (Yordanov *et al.*, 2000). Changes to some of these key physiological processes may affect biomass accumulation, cell turgor, leaf water potential, reduced relative water content and consequently yield in crops (Reddy *et al.*, 2004). This section examines the main physiological processes affecting photosynthetic capacity of plants affected by water stress.

2.5.3.1 Stomatal conductance

Stomatal conductance is the most important mechanism regulating carbon and water exchange and consequently controls photosynthesis and transpiration in plants. Stomatal conductance is a function of density, size and opening of stomata and it acts as a plant's primary defence mechanism when exposed to drought conditions (Chaves *et al.*, 2003). Drought tolerant genotypes ensure that water loss is reduced through minimal stomatal opening and at the same time allowing carbon dioxide in for photosynthesis (Agbicodo *et al.*, 2009; Cruz de Carvalho *et al.*, 1998). Due to its critical role in regulating water and gas, stomatal conductance has been recommended as a reliable parameter in screening for drought tolerance. Stomatal conductance has been used in selecting drought tolerant genotypes in various crops. In cowpea and faba bean (Anyia & Herzog, 2004a; Hamidou *et al*, 2007; Khan *et al.*, 2007; 2010), significant genotypic variations were observed in stomatal conductance when exposed to drought conditions, providing room for the selection of genotypes adapted to drought conditions. In shorter term drought, it is better to select genotypes with high stomatal conductance for optimised yield. However, in an event of prolonged drought, low stomatal conductance would enhance survival of genotypes.

2.5.3.2 Transpiration

Transpiration under water stress varies between drought tolerant and susceptible genotypes. Some genotypes exhibit high transpiration, while others significantly reduce transpiration. In terms of drought adaptation, genotypes which reduce transpiration, when exposed to drought conditions, show their ability to tolerate drought (Hall & Schulze, 1980). This reduction in transpiration results from reduced leaf area, low stomatal frequency and orientation of leaves, to ensure low radiation loading and evaporative water loss to the environment (Farooq *et al*., 2009). However, reduction in transpiration, due to reduced stomatal conductance may reflect limited photosynthetic capacity, resulting in reduced carbon assimilation. Genotypes with reduced transpiration resulting from low stomatal conductance may only be important in areas with short periods of water stress, because such genotypes stop growing under prolonged drought conditions (Liu & Stützel, 2002).

2.5.3.3 Net photosynthesis

Generally, photosynthesis reduces with water stress due to several factors including stomatal conductance, carbon assimilation, and transpiration (Chaves *et al*., 2003). However, variation among genotypes may explain varying responses to drought conditions. Genotypes which maintain high net photosynthesis under water stress conditions generally indicate an ability to tolerate drought conditions (Farooq *et al*., 2009). High net photosynthesis is also associated with high chlorophyll maintenance under water stress conditions (Bertolli *et al.*, 2012). Therefore, the selection of genotypes with high net photosynthesis due to high chlorophyll concentration may contribute to an improvement in the yield performance of cowpea under water stress conditions.

2.5.3.4 Water use efficiency and transpiration efficiency

Water use efficiency (WUE) and transpiration efficiency (TE) have been used as indicators of drought tolerance. WUE is defined as the biomass accumulated per unit of water used and TE is the amount of biomass accumulated per unit of water transpired (Manavalan *et al.*, 2009). Both WUE and TE are affected by the key physiological processes in plants. For instance, WUE and TE were affected by changes in stomatal conductance and photosynthetic capacity in cowpea (Ahmed & Suliman, 2010). Genotypes with high transpiration efficiency and high water use efficiency under drought conditions reflect their ability to photosynthesise and accumulate more dry matter compared to genotypes with low WUE and low TE. High transpiration efficiency was positively correlated with yield under drought conditions in cowpea (Anyia & Herzog, 2004a) and groundnuts (Arunyanark *et al.*, 2008), making it a key selection criterion for drought tolerance. Consequently, the selection of genotypes with high efficient use of water and high TE would be beneficial for crop production in drought prone areas.

2.5.3.5 Relative water content

Leaf relative leaf water content (RWC) is the amount of water in leaf tissues expressed as a ratio in relation to the maximum amount of water the leaf can hold at the point of saturation (Suriya-arunroj *et al.* 2004). Relative water content is calculated as:

$$\mathbf{RWC} = \frac{\mathrm{FW}-\mathrm{DW}}{\mathrm{TW}-\mathrm{DW}}$$

where FW = Fresh weight of leaves; DW = Dry weight of leaves; TW = Turgid weight of leaves i.e weight of fresh leaves at saturation point.

And RWC has been widely used in evaluating genotypes for drought tolerance due to a high positive correlation with yield in crops. Generally, RWC decreases with an increase in moisture stress. However, some genotypes show relatively higher RWC than others indicating an ability to tolerate drought. High RWC indicates the ability of genotypes to retain plant tissue water under moisture stress. In cowpea (Kumar *et al.,* 2008) and wheat (Bayoumi *et al.*, 2008; Rampino *et al.*, 2006) wide variation in RWC among genotypes suggests that it is one of the traits that could be used in the identification of dehydration tolerant genotypes. Correlations of 0.8 and 0.87 between RWC and pod set ratio and number of pods, respectively, were observed in cowpeas evaluated under both moisture stress and non-stress conditions (Kumar *et al.,* 2008). The cowpea drought tolerant genotypes maintained minimal differences in RWC, between stress and non-stress conditions, resulting in high pod set ratio and number of pods per plant, which are directly related to yield. Bayoumi *et al.* (2008) also found a strong positive correlation (0.84) between RWC and yield under water stress in wheat. Kumar *et al.* (2008) indicated that cowpea plants are able to maintain high RWC either through efficient water uptake from the soil or reduced water loss through stomatal closure. Considering that RWC strongly correlated with yield in both cowpea and wheat, it can be used as a trait for germplasm selection under drought conditions. The RWC has the added advantage of simplicity in measurement because it does not require sophisticated equipment although laborious process.

2.6. Farmers' preference and adoption of improved varieties

Promotion and production of improved cowpea varieties is sometimes affected by lack of desirable attributes addressing farmers' needs due to breeder centred crop improvement (Kitch *et al.*, 1998). Crop improvement led by breeders sometimes considers farmers as field technicians for testing the already selected varieties. Consequently, in many occasions the system leads to the development of varieties not necessarily preferred by farmer due to early elimination of some germplasm of interest to farmers. Most varieties with high adoption rates are characterised by attributes of interest to both breeders' and farmers' attributes. For instance, attributes preferred by breeders (mostly yield and disease resistance) accounted for 46% of the selection criteria of cowpea varieties while preferred attributed by farmers such as seed characteristics, cooking quality and labour saving accounted for more than 50% in Cameroon for example (Kitch *et al*., 1998).

Although some improved varieties have been developed and released in Malawi (Government of Malawi, 2000; Mviha *et al.*, 2011), farmers predominantly grow cowpea landraces. Nkongolo *et al*., (2009) attributed the poor adoption of the improved varieties to limited involvement of farmers in the process of their development. In order to address the challenge of low adoption of improved varieties, it is important to involve farmers at an early stage of the breeding programme through participatory research (Sperling *et al.*, 1993). This participatory approach has been tested and proven to accelerate adoption of improved varieties as the farmers' desired attributes are included in the bred varieties (Pretty *et al.,* 2010). Involvement of farmers as collaborators through participatory research helps to develop demand driven research which enhances adoption of new technologies (Gyawali *et al*., 2007; Joshi *et al*., 2007). The early involvement of farmers should be encouraged for the development of readily acceptable and market competitive varieties. Therefore, successful development and promotion of drought tolerant cowpea varieties in Malawi should involve farmers in the process of selection and breeding at an early stage.

2.7 Conclusions

The increased occurrence of drought in Malawi requires concerted efforts to strengthen agricultural strategies including exploring the potential of local germplasm of cowpea which can adapt to such conditions. The local germplasm with desirable adaptation mechanisms would augment the international efforts of breeding drought tolerant genotypes by with meet needs and preferences of local farmers in Malawi. This literature review has identified key areas for consideration as a first step in the identification of drought tolerant genotypes of cowpea maintained at the Malawi Plant Genetic Resources Centre. Eco-geographic characterisation of the available germplasm would help to identify potential accessions with drought tolerance and identify potential sites for on farm conservation to enhance adaptation to drought. Classification of the available germplasm into appropriate response categories of drought avoiders or drought tolerators' adaptation mechanisms by using both morphological and physiological traits would help to establish drought tolerance breeding populations to complement the efforts by the international community. Leaf wilting remains a fundamental trait in screening for drought despite the associated scoring challenges. The revision of the scoring system for wilting would address some challenges associated with the current scoring systems. Once the drought tolerant genotypes are identified and confirmed, further tests on agronomic performance and farmers' preference would provide insights for direct benefits at farm level. Most breeding efforts in Malawi are thwarted by poor adoption, poor seed distribution and marketing of improved varieties. To accelerate adoption of future improved cowpea varieties, early involvement of farmers in the selection of breeding germplasm, efficient seed distribution and marketing are inevitable.

Chapter 3 : Eco-geographic characterisation of the locally adapted cowpea germplasm

Abstract

The availability of germplasm in genebanks is a rich resource for future agricultural development. However, utilisation of the conserved germplasm is limited by the lack of associated useful information, including eco-geographic information describing collection sites. This study characterised 66 cowpea accessions from Malawi using geographic and climatic variables, in order to identify potential genotypes adapted to drought conditions, identify gaps for future collection missions, and identify sites for on-farm conservation efforts. A distribution map of the 66 accessions was used to extract online eco-geographic variables of annual mean temperature; mean temperature of wettest quarter; mean temperature of warmest quarter; annual precipitation; precipitation of wettest month; precipitation of wettest quarter; and precipitation of warmest quarter from WORLDCLIM database (www.worldclim.org). The extracted variables were used in a cluster analysis to classify the accessions into distinct groups. The distribution map showed that, out of the total 27 possible districts in Malawi, the accessions were collected from 19 districts only, with Chikwawa registering the highest number (15). The eight other districts and areas with few accessions represent gaps, which require germplasm collection or repatriation of germplasm from international institutions holding cowpea germplasm from Malawi. The cluster analysis grouped the 66 accessions into Cluster 1 with 29 accessions, Clusters 2 and 3 with 16 accessions each and Cluster 4 with 5 accessions. Accessions in Clusters 3 and 4 may represent potential candidates for drought tolerance, as they were collected from dry and hot zones. On farm conservation of cowpea in hot dry zones, such as Chikwawa and Nsanje, denoted by Cluster 3, could enhance adaptation of germplasm to drought conditions. Further morphological and physiological studies are recommended, in order to identify the drought tolerance levels of the local germplasm.

3.1 Introduction

Landraces or traditional local varieties of different crops have been collected from a wide range of environments and conserved in genebanks. For instance, 50,000 accessions of cowpea germplasm are conserved at a global level (Bioversity International, 2011) and the Malawi Plant Genetic Resources Centre (MPGRC) has collected 66 accessions of the locally adapted cowpea landraces from local farmers around the country. Although a wide range of diversity is available in these genebanks, its utilisation is limited by the lack of detailed passport data including geographic and climatic variables (FAO, 2010; Hazekamp, 2002; Li *et al.*, 2013). Therefore, research which improves the availability and accessibility of detailed passport data would help to avail the potential of the currently collected germplasm.

Eco-geographic characterisation is one of the key steps which are often ignored in most genebanks, due to limited capacity. Eco-geographic characterisation involves a description of germplasm based on characteristics of collection site habitats. This is considered as a first step in identifying genotypes that are well adapted to particular habitats. The importance of eco-geographic characterisation is based on the premise that environmental conditions at the collection point impact the evolution of the population (Allard, 1996). Several studies have demonstrated the importance of eco-geographic characterisation when identifying plant eco-types. For instance, eco-geographic variables correlated with heading days, ripening days, height of plant, harvest index and volumetric weight in barley landraces (Endresen, 2010). In barley and wheat landraces eco-geographic parameters correlated with reaction to net blotch and stem rust diseases, respectively (Endresen *et al*., 2011). Horsegram landraces collected from varying altitudes in the Himalayan region showed significant genotypic variations, following altitudinal gradients (Gupta *et al.*, 2010). These variations of different traits, with the geographic and environmental patterns, indicate that crops adapt to specific environmental conditions for their survival; and this pattern could be further explored during identification of unique germplasm for crop improvement.

Although eco-geographic variables have a direct effect on crop adaptation, data availability is limited in most genebanks, including the Malawi Plant Genetic Resources Centre. The development of DIVA-GIS software for the management of germplasm (Hijmans *et al*., 2001; 1999) and the availability of climatic data from www.worldclim.org (Hijmans *et al*., 2005) have accelerated the extraction of both geographic and environmental data that are not readily available in the collections. Although such tools are available, their application in classifying germplasm has not been fully explored, due to limited capacity in most genebanks. This study characterised cowpea germplasm from Malawi, based on both geographic and environmental conditions at the collection points. The aims of the study were:

a) To identify potential germplasm adapted to drought conditions;
b) To identify gaps in the collection for future targeted collecting missions;
c) To identify potential sites for on-farm conservation of cowpea.

3.2 Materials and methods

3.2.1 Germplasm source and passport data

Sixty six accessions (Table 3.1) of cowpea germplasm collected from various parts of Malawi were used in this study. All the accessions were collected from farmers stores mostly located close to fields where they were grown. All the accessions had geographical coordinates of field locations as a pre-requisite for Geographical Information System (GIS) analysis.

Table 3.1: List of cowpea germplasm with district, latitude and longitude of origin.

Accession	District	Latitude (^{0}S)	Longitude (^{0}E)
3419	Balaka	14.80	35.13
3418	Balaka	14.90	35.14
3420	Balaka	14.90	35.14
438	Chikwawa	15.83	34.98
414	Chikwawa	16.40	34.92
2223	Chikwawa	16.25	34.87
421	Chikwawa	16.08	34.88
426	Chikwawa	16.08	34.88
3425	Chikwawa	15.96	34.77
3428	Chikwawa	15.96	34.77
2218	Chikwawa	16.02	34.78
2219	Chikwawa	16.02	34.78
2220	Chikwawa	16.02	34.78
411	Chikwawa	16.37	34.68
418	Chikwawa	16.45	34.78
436	Chikwawa	16.03	34.53
3422	Chikwawa	15.99	34.48
3423	Chikwawa	15.99	34.48
570	Chiradzulu	15.95	35.30
1861	Dowa	13.42	33.58
1865	Dowa	13.62	33.57
1867	Dowa	13.72	33.83
2863	Likoma	12.04	34.75
2883	Likoma	12.09	34.73
2869	Likoma	12.04	34.74
2876	Likoma	12.04	34.74
3254	Lilongwe	14.23	33.67
2234	Lilongwe	14.23	33.77
753	Machinga	15.05	34.92
755	Machinga	15.05	34.92
724	Machinga	14.92	35.00
727	Machinga	14.90	35.00
2232	Mchinji	13.62	33.07
2231	Mchinji	13.67	33.02
479	Mulanje	16.15	35.33
471	Mulanje	16.10	35.43
468	Mulanje	16.03	35.43
544	Mulanje	15.73	35.60
535	Mulanje	16.00	35.78
502	Mwanza	15.73	34.40
517	Mwanza	15.48	34.65
309	Mzimba	11.28	33.93
3215	Mzimba	12.10	33.43
168	Nkhatabay	11.63	34.12
169	Nkhatabay	11.63	34.05
391	Nsanje	16.73	35.28
399	Nsanje	16.43	35.18
3442	Phalombe	15.61	35.67
3443	Phalombe	15.61	35.67
320	Rumphi	10.95	33.68
305	Rumphi	10.87	33.63
1805	Salima	13.72	34.47
601	Thyolo	16.13	35.25
633	Thyolo	16.17	35.07
645	Thyolo	16.25	35.15
3412	Zomba	15.40	35.17
3413	Zomba	15.40	35.18
3416	Zomba	15.40	35.18
3417	Zomba	15.40	35.18
698	Zomba	15.67	35.43
710	Zomba	15.43	35.42
2226	Zomba	15.48	35.23
2227	Zomba	15.48	35.23
2229	Zomba	15.48	35.23
2230	Zomba	15.48	35.23
823	Mangochi	14.35	35.45

3.2.2 Distribution map and extraction of eco-geographic variables

The 66 cowpea accessions were mapped using DIVA-GIS software. Subsequently, the distribution map was used to extract eco-geographic and climatic variables for each accession from WORLDCLIM database (www.worldclim.org). In total, eight variables were extracted at a spatial resolution of 30 seconds, which is equivalent to a ~1km x 1km grid (Hijmans *et al*., 2005). Selection of variables was based on the importance to crop production with reference to cropping season. This resolution was selected to ensure the accuracy of the variables compared to lower resolutions of 2.5 minutes, 5 minutes, 10 minutes and 1 degree

(Scheldeman & van Zonneveld, 2011). The variables extracted and used in the analysis are provided in Table 3.2.

Table 3.2: List of variables extracted from the WORLDCLIM database.

Name	**Description**
Altitude (m)	Height above sea level
Bio 1(°C)	Annual mean temperature
BIO 8 (°C)	Mean temperature of wettest quarter
BIO 10 (°C)	Mean temperature of warmest quarter
BIO 12 (mm)	Annual precipitation
BIO 13 (mm)	Precipitation of wettest month
BIO 16 (mm)	Precipitation of wettest quarter
BIO 18 (mm)	Precipitation of warmest quarter

Source: Scheldeman & van Zonneveld (2011)

3.2.3 Statistical analysis

The eight eco-geographic variables were used in a cluster analysis, to classify the 66 collection sites into distinct groups using the Minitab 16 Statistical package (Minitab Inc. Pennsylvania, USA). Prior to cluster analysis, data were standardised, to minimise the dominance of variables with higher numerical values (Endresen, 2010). The standardisation was done by subtracting the mean of each variable from each individual accession followed by dividing each value by standard deviation so that variance is zero subsequent standard deviation is one. Cluster analysis was done using the Euclidean Similarity Index and the Ward linkage method. Cluster means for individual variables were further analysed using unbalanced one-way analysis of variance, due to variations in the number of accessions per cluster.

3.3 Results

3.3.1 Germplasm distribution

The distribution map of the 66 collection sites shows that cowpea germplasm has been collected from different parts of Malawi (Fig. 3.1 and Table 3.1). Germplasm samples were collected from 19 districts out of 27 districts of Malawi. Most accessions per district was from Chikwawa with 15 accessions. The three districts of Chiradzulu, Salima and Mangochi were only represented by one accession each.. No germplasm was collected from Neno, Dedza, Ntcheu, Blantyre, Chitipa, Karonga, Ntchisi and Kasungu districts.

Forty three sites were only represented by one accession each while the other twenty three sites had more than one accession in the collection (Table 3.1). Accessions 3412, 3413, 3416 and 3417 were collected from one site in Zomba; 2226, 2227, 2229 and 2230 were collected from another site in Zomba; 735 and 755 were collected from one site in Machinga: 3442 and 3443 were collected from one site in Phalombe; and accessions 2869 and 2876 were collected from one site at Likoma Island. The following sets of accessions were collected from different sites in the Chikawa district: (3422, 3423); (421, 426); (2218, 2219, 2220); and (3425, 3428).

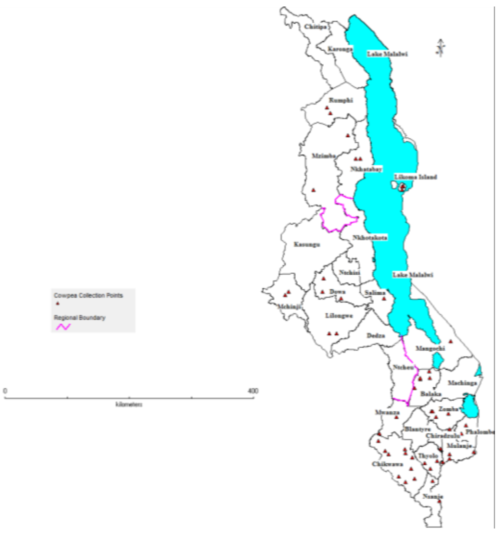

Figure 3.1: Map of Malawi showing collection sites of cowpea germplasm.

3.3.2 Characteristics of collection sites

The collection sites of cowpea germplasm show a wide variation in terms of altitude, precipitation and temperature (Table 3.3). The accessions were collected from areas with altitudes between 53 m and 1507 m above sea level (asl). Annual mean temperature (Bio 1) at the collection points ranged between 21°C and 28°C. The temperatures of the wettest quarter ranged between 22°C and 30°C, while the temperatures of the wettest month ranged between 24°C and 31°C. Annual precipitation (Bio12) at the collection sites ranged between 812 mm and 1930 mm. Precipitation during the wettest month (Bio 13) ranged between 236 mm and 504 mm, while precipitation during both the wettest quarter (Bio 16) and warmest quarter (Bio 18) ranged from 589 mm to 1153 mm and from 154 mm to 432 mm, respectively.

Table 3.3: Minimum, maximum and means with standard errors for the variables used in cluster analysis.

Variable	Minimum	Maximum	Mean
Altitude (m)	53	1507	638±6
Bio 1 (°C)	21	28	25±0.2
Bio 8 (°C)	22	30	26±0.3
Bio 10 (°C)	24	31	28±0.3
Bio 12 (mm)	812	1930	1185±31
Bio 13 (mm)	236	504	340±8
Bio 16 (mm)	589	1153	811±16
Bio 18 (mm)	154	432	274±8

3.3.3 Cluster analysis

Cluster analysis, using all eight variables, grouped the 66 accessions into four distinct clusters (Fig. 3.2, Table 3.4). Cluster 1 comprised 29 accessions, followed by Clusters 2 and 3 with 16 accessions each, while Cluster 4 comprised five accessions, thus representing the smallest cluster. Accessions in Cluster 1 were collected from areas with an average altitude of 657±15m. Precipitation of warmest quarter, annual precipitation and precipitation of wettest quarter contributed strongly to the grouping of accessions in Cluster 1 (Table 3.5). The positive association of this cluster with the three variables indicates that the accessions were collected from areas with high rainfall. Accessions in Cluster 2 were collected from areas with an average altitude of 1158±30 m. This cluster was positively associated with altitude, mean temperature of the wettest quarter, annual mean temperature and mean temperature of the warmest quarter. The negative association with variables associated with temperature (Bio 1, Bio 8 and Bio 10) shows that the accessions in this cluster were collected from areas with low temperatures, while the positive association with altitude indicates that the accessions were collected from high altitude areas (Table 3.5).

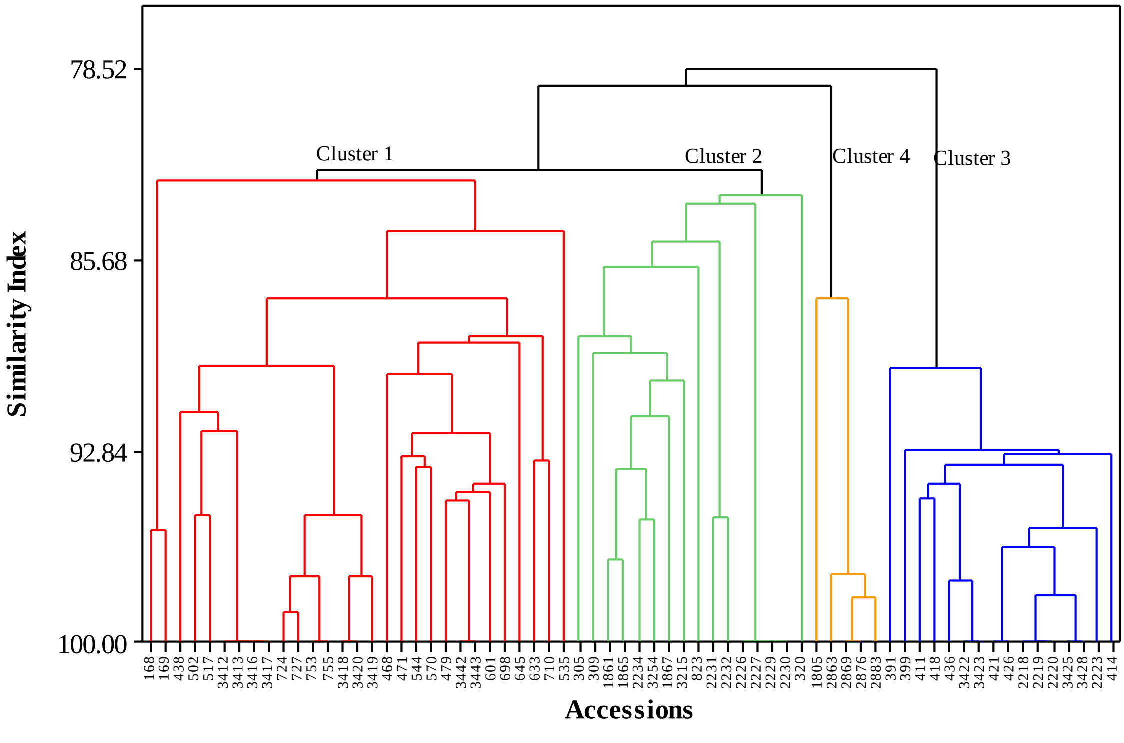

Figure 3.2: Dendrogram showing grouping pattern of 66 cowpeas accessions.

Accessions in Cluster 3 were collected from areas with an average altitude of 130±15m, thus representing the lowest altitude among all the clusters. This cluster was associated with mean temperature of the wettest quarter, mean temperature of the warmest quarter, altitude, annual mean temperature and precipitation of wettest quarter. The negative association with altitude and precipitation of the wettest quarter indicates that accessions in this cluster were collected from low lying areas and the lowest precipitation in the wettest quarter, while the positive association with all temperature related variables shows that accessions in this cluster were collected from areas with relatively high mean temperature (Table 3.5). Cluster 4 was comprised of accessions collected from areas with an average altitude of 493±6m. This cluster is associated with precipitation of the wettest month, precipitation of the warmest quarter and annual mean temperature. The positive association with annual temperature and precipitation of wettest month indicates that the accessions in this cluster were collected from areas with a high annual temperature and high precipitation in the wettest month. The negative association with precipitation in the warmest quarter shows that the accessions were collected from sites with low precipitation in the warmest quarter.

Table 3.4: Cluster means with standard errors and ranges for all eight variables measured.

Variable	**Statistic**	**Cluster**			
		1 (29)	**2 (16)**	**3 (16)**	**4 (5)**
Altitude (m)	Mean	657±15	1158±30	130±15	493±6
	Range	552-954	1021-1507	53-236	474-505
Bio 1 (°C)	Mean	24±0.1	22±0.2	27±0.1	27±0.2
	Range	22-25	21-24	27-28	26-27
Bio 8 (°C)	Mean	26±0.1	23±0.2	29±0.1	27±0.1
	Range	24-27	22-25	29-30	27-28
Bio 10 (°C)	Mean	27±0.2	25±0.2	31±0.1	29±0.1
	Range	25-29	24-26	30-31	29-30
Bio 12 (mm)	Mean	1349±53	1081±32	985±16	1209±2
	Range	978-1930	812-1255	930-1188	1203-1215
Bio 13 (mm)	Mean	363±11	326±10	287±8	435±13
	Range	287-504	236-361	254-346	383-456
Bio 16 (mm)	Mean	874±22	796±22	674±10	932±7
	Range	715-1153	589-892	632-769	915-955
Bio 18 (mm)	Mean	319±11	240±16	250±4	198±3
	Range	238-432	154-328	237-293	192-211

*Numbers in parenthesis represent total number of accessions per cluster

Table 3.5: Factor loadings for the four clusters.

Variable	**Cluster**			
	1	**2**	**3**	**4**
Altitude	0.05	1.39	-1.36	-0.39
Bio1	-0.20	-1.25	1.31	1.01
Bio8	-0.15	-1.27	1.42	0.38
Bio10	-0.21	-1.22	1.40	0.67
Bio12	0.64	-0.41	-0.79	0.09
Bio13	0.36	-0.25	-0.87	1.54
Bio16	0.50	-0.12	-1.08	0.96
Bio18	0.69	-0.52	-0.37	-1.16

3.4 Discussion

The distribution pattern of the conserved germplasm (Table 3.1 & Fig. 3.1) unveiled areas with no or scarce collection which might be gaps in the germplasm collection. Initiatives to further collect from these areas will enrich the diversity of cowpeas conserved in the genebank. Besides enriching the diversity, further collection will accelerate the safeguarding of germplasm that might be under threat, due to several factors, including climate change. Climate change poses a substantial challenge to the survival of biodiversity including crop landraces in natural environments. This challenge requires immediate attention, in order to safeguard the threatened biodiversity and more importantly from areas that have no representation of germplasm in genebanks (Burke *et al*., 2009). In addition to further collection, the gaps could be filled by the repatriation of materials collected by various research institutes prior to the establishment of the Malawi Plant Genetic Resources Centre (MPGRC). In total, 570 cowpea accessions from Malawi are being conserved in international genebanks and the International Institute of Tropical Agriculture (IITA) alone has 422 accessions (Bioversity International, 2011). The accessions collected before the establishment of the MPGRC may represent more diverse germplasm, due to the presence of unique genotypes which are no longer present within the farming communities and MPGRC.

Additionally, materials from West Africa which represent the centre of diversity of cowpea (Badiane *et al.*, 2014) would enrich diversity of cowpea conserved by MPGRC.

Another approach to the germplasm conservation in cowpea could be on-farm conservation of cowpea landraces. The maintenance of local varieties on-farm contributes to evolutionary processes and it improves the adaptation of genotypes to a changing environment as long as there is minimum disturbance to the natural environment of the populations (Allard, 1996). A study on the genetic diversity of cowpea germplasm in Malawi identified several populations which were genetically unique and a recommendation was made to safeguard such genetically diverse populations through *ex-situ* and on-farm conservation strategies (Nkongolo, 2003). However, only *ex-situ* conservation has been achieved through systematic collection and storage of cowpea germplasm from various sites. On-farm conservation in areas with low altitude, high temperatures and low rainfall would enhance adaptation of local germplasm to harsh environments, which would then contribute to breeding for drought tolerance and other traits in the near future. Based on eco-geographic variables (Table 3.5), the sites represented by Cluster 3 (Figure 3.2) could be better for on-farm conservation of cowpeas for drought adaptation. Accessions in this cluster were collected from Chikwawa and Nsanje districts. The establishment of on-farm conservation sites in these two districts would enhance evolutionary processes for adaptation to low rainfall and high temperatures. The suitability of on-farm conservation sites in these two districts is also suggested by Timko *et al*. (2007). Their study showed that the low lying zones of both Eastern and Southern Africa, such as Chikwawa and Nsanje, are suitable for hardy crops such as cowpea and sorghum. Therefore, this study confirms the need for the establishing on-farm conservation sites for cowpea in these two districts.

Understanding the variation of eco-geographic parameters of germplasm collection is the very first step in both conservation and utilisation of germplasm (Guarino *et al*., 2002; Peeters *et al.*, 1990). However, one of the major challenges associated with the implementation of eco-geographic characterisation is the limited availability of eco-geographic variables as part of passport information (FAO, 2010; Hazekamp, 2002). These results demonstrate the power of DIVA-GIS and its associated climate database in improving the quality of data in genebanks (Hijmans *et al*., 2001). It is recommended that such studies should form part of core genebank activities, which would improve the quality of data for the conserved germplasm.

Hierarchical Cluster Analysis is an important analytical tool, since it provides an understanding of variation among objects through branching and interconnected clusters. Furthermore, it provides factors that contribute to variations among different clusters (Mohammadi & Prasanna, 2003). The magnitude and sign of the particular variable for each cluster (factor /variable loadings) express the significance of each variable towards clusters (Table 3.5). The higher the absolute value, the more important a particular variable is towards a cluster. Positive and negative signs indicate whether the value of a particular variable is above or below average, respectively (Hopke *et al,* 1976).

Plant genetic resources collected from dry and hot environments may have developed adaptation mechanisms for efficient utilisation of moisture during water deficit (Read & Farquhar, 1991). Consequently, germplasm collection site data may be used as indicators of adaptation. For example, sorghum and millet landraces from dry arid environments exhibited high osmotic adjustment under moisture stress conditions compared with landraces collected from moist environments (Blum & Sullivan, 1986). Similarly, drought tolerance was found in wild wheat from hot dry locations in contrast with populations from wet locations (Peleg *et* *al.*, 2005). Contrasting physiological responses were observed in faba bean (*Vicia faba* L.) germplasm collected from dry and wet environments (Khazaei *et al.*, 2013). Germplasm from dry environments displayed an increase in stomatal density, and maintained higher relative water content leading to high water use efficiency than germplasm from wet conditions evaluated under moisture stress conditions. The identification of drought tolerant genotypes collected from dry and hot areas in the highlighted studies is an indicator of adaptation mechanisms under moisture stress conditions. Based on the premise that dry conditions contribute to adaptation to drought conditions, it would be rational to suggest that cowpea landraces from dry and hot areas have high probability of being drought tolerant. It is therefore expected that accessions in Cluster 3 could represent genotypes adapted to low rainfall and high temperature conditions, due to a strong and positive association with temperature and a strong and negative association with precipitation (Table 3.5).

The results also show that areas represented by Cluster 4 receive the lowest precipitation during the warmest quarter of the year (Bio 18), which coincides with the planting and crop establishment season. In addition, the areas represented by Cluster 4 receive most of the precipitation within one month of the wettest quarter (Bio 13). Therefore, the low precipitation during plant establishment and the biased rainfall distribution towards one month of the wettest quarter may create moisture stress for growing crops. Genotypes adapted to such environments may have developed seedling stage drought tolerance, due to the low precipitation received in the warmest quarter of the season, which coincides with the crop establishment stage. In contrast to Clusters 3 and 4, the accessions in Cluster 1 could be adapted to areas with high precipitation, due to high and positive association with variables associated with high precipitation.

Although accessions in clusters 3 and 4 may represent drought tolerant genotypes, possibilities of new germplasm with drought susceptibility introduced from wet regions should not be ruled out. Extreme natural events such as drought, may lead to extinction of plant populations including crop landraces maintained by farmers (Davis *et al.*, 2005). The extinction of landraces may result in introduction of new populations from other geographic areas. The repeated introduction of alleles from landraces adapted to moist conditions may contribute to the presence of drought susceptible genotypes in dry areas (Mercer & Perales, 2010). For example, consecutive years of intense droughts led to the loss of local wheat landraces and local farmers introduced seed from wet areas resulting in the presence of drought susceptible genotypes in the dry region (Blum *et al*., 1989). The presence of drought susceptible genotypes in dry areas suggests that not all landraces originating from dry regions are necessarily drought tolerant. This could be specifically the case for cowpea as targeted regions in this study are not categorised as dry areas based on their average annual precipitation, however, they are the driest areas where the germplasm has been collected. Therefore, eco-geographic characterisation should be complementary to morphological, physiological and molecular characterisation which would contribute to a greater degree of accuracy on the sources of drought tolerance in the cowpea germplasm from Malawi.

3.5 Conclusion

This study has identified a considerable level of variation in geographic locations for the collection points of cowpea germplasm in Malawi. Further collection and possible repatriation of germplasm from international genebanks and other geographic regions with high diversity of cowpea should be initiated. Clusters 3 and 4 are identified as the initial candidates for exploring drought tolerance, since they were collected from low rainfall and high temperature areas. However, the two clusters may also represent susceptible genotypes due to both natural disasters and introduction of genotypes from wet environments. For continued maintenance of evolutionary processes in locally adapted germplasm, we suggest on-farm conservation of cowpeas targeting areas with high temperatures and low rainfall. These results should be considered as an indicator that points towards targeted germplasm use and conservation. Further work on morphological, physiological and molecular characterisation and evaluation is recommended, in order to explore and confirm the drought tolerance attributes of the proposed clusters.

Chapter 4 : Screening germplasm for canopy maintenance under water stress conditions[1]

Abstract

Drought tolerant cowpea germplasm would offer potential solutions to challenges associated with low production in drought prone areas. This research characterised 36 accessions for canopy maintenance in a glasshouse, as a first step towards the identification of drought tolerant cowpea genotypes in Malawi. Canopy responses were scored using the International Board for Plant Genetic Resources (IBPGR) and Mai-Kodomi leaf wilting scales, relative water content (RWC) and leaf wilting index (LWI) after withdrawing water for four weeks. Re-growth and stem greenness were scored after the second week of re-watering. The reduction of soil moisture content from 26.2% to 2.9% provided sufficient stress over a period of four weeks to identify drought tolerant genotypes. The accessions showed highly significant variations (P=0.0001) for all the variables indicating that some accessions survived the low soil moisture level better than others. Accessions 479, 601, 645, 2226 and 3254 showed high RWC, low values on wilting scales and wilting index, high scores of stem greenness and apical re-growth in contrast with accessions 517, 2231, 2232, 2883 and 3215. Cluster analysis grouped the accessions into five distinct clusters with accessions 479, 601, 645, 2226 and 3254 in Cluster 4 and accession 2232 in its own Cluster 5. Clusters 1, 2 and 3 comprised 14, 12 and 4 accessions, respectively. Cluster 4 was strongly associated with apical re-growth, high RWC, high scores of stem greenness, and low scores for both leaf wilting scales and LWI as opposed to Clusters 5 and 1. The correlation analysis revealed highly significant positive and negative relationships between LWI and the commonly used traits in screening for drought tolerance, thus indicating the prospects of using LWI in screening cowpea germplasm to overcome limitations associated with leaf wilting scales. The genotypes in Clusters 4 and 5 require further study in order to understand the physiological mechanisms governing their responses.

[1] Some parts of this chapter have been published as:
a) Pungulani, L. L. M., Millner, J. P and Williams, W. M (2012). Screening cowpea (*Vigna unguiculata* (L.) Walp) germplasm for canopy maintenance under water stress: Agronomy New Zealand 42:23–32
b) Pungulani, L. L. M., Millner, J. P., Williams, W. M and Banda, M (2013). Improvement of leaf wilting scoring system in cowpea (*Vigna unguiculata* (L) Walp.): From qualitative scale to quantitative index: Australian Journal of Crop Science 7(9):1262–1269

4.1 Introduction

Germplasm present in genebanks may provide key solutions to the extreme climatic challenges, such as drought and high temperatures that are partly caused by climate change. Malawi Plant Genetic Resources Centre holds a total of 66 accessions of cowpea landraces. However, effective utilisation of the conserved cowpea germplasm in Malawi has been hampered by a lack of useful information associated with desirable attributes, such as drought tolerance and yield potential as in other genebanks (FAO, 2010). Frequent occurrences of drought in Malawi (Tadross *et al*., 2009) point towards the need for development of a resilient farming system, which would boost crop production. Therefore, a systematic evaluation of available cowpea germplasm for drought tolerance would enhance utilisation of the conserved germplasm in addition to enhancing crop production in drought prone areas in Malawi.

Agbicodo *et al*. (2009) and Hall (2004a, 2012) have provided detailed reviews on drought tolerance research in cowpeas, which has resulted in the identification of drought tolerant genotypes for use by both farmers and researchers. These genotypes have been identified by screening for traits such as yield and biomass (Singh & Matsui 2002), root characteristics (Matsui & Singh, 2003), stomatal conductance (Agbicodo, 2009; Labuschagne *et al*., 2008), leaf membrane stability (Labuschagne *et al*., 2008) and leaf wilting scales (Mai-Kodomi *et al*., 1999; Nkouannessi, 2005; Singh *et al*., 1999b; Watanabe *et al*., 1997). Despite the availability of several traits for drought tolerance evaluation, leaf wilting still remains a fundamental indicator for drought response in plants. However, scoring of wilting in drought tolerance evaluation is associated with the use of qualitative scales, which can contribute to biased-scoring and also requires experience to systematically and uniformly score for leaf wilting (Xu *et al*., 2000). These limitations point to a need for further research to enhance the ease and reliability of drought tolerance assessment.

Although research work on drought tolerance has been conducted elsewhere by using different methods, limited information exists on drought tolerance mechanisms in locally adapted cowpea germplasm from Malawi. In this research, cowpea germplasm from Malawi was evaluated for drought tolerance using wilting scales, wilting index, relative water content, stem greenness and re-growth. The specific aims of the study were:

a) To identify potential drought tolerant genotypes within the cowpea germplasm present at the Malawi Plant Genetic Resources Centre;
b) To improve the scoring system of wilting from a qualitative scale to a quantitative index which would ease the challenges associated with wilting scales encountered by non-experienced researchers.

Both drought tolerant genotypes and an improved scoring system for wilting would be useful for researchers working on drought tolerance in cowpeas.

4.2 Methodology

4.2.1 Plant material and experimental design

Thirty six cowpea accessions (Table 4.1) randomly selected from the Malawi Plant Genetic Resources Centre were evaluated in the glasshouse at the Plant Growth Unit (PGU) (40.38S; 175.61E) Massey University, Palmerston North New Zealand. The glasshouse experiment preceded an eco-geographic analysis (Chapter 3) which led to non-systematic selection of genotypes resulting in unbalanced number of genotypes from each of the clusters established in Chapter 3. For example, accessions 13, 10, 11 and 2 used in this experiment came from clusters 1, 2, 3 and 4 respectively. The experiment was laid out in a randomised complete block design (RCBD) with accessions, as treatments, replicated four times. Four healthy looking seeds from each accession were planted in 10 litre pots filled with growth media, which was prepared by mixing 100 litres of pot mix, 150g of short term release fertilizer and 150g of Dolomite. The seedlings were thinned to two plants per pot eight days after emergence.

Table 4.1: List of accessions evaluated for drought tolerance showing district of origin, latitude, longitude and cluster from eco-geographic characterisation.

Accession	**District**	**Latitude (^{0}S)**	**Longitude (^{0}E)**	***Cluster number from eco-geographic characterisation**
169	Nkhatabay	11.63	34.05	1
320	Rumphi	10.95	33.68	2
391	Nsanje	16.73	35.28	3
399	Nsanje	16.43	35.18	3
411	Chikwawa	16.37	34.68	3
414	Chikwawa	16.40	34.92	3
421	Chikwawa	16.08	34.88	3
426	Chikwawa	16.08	34.88	3
436	Chikwawa	16.03	34.53	3
471	Mulanje	16.10	35.43	1
479	Mulanje	16.15	35.33	1
517	Mwanza	15.48	34.65	1
535	Mulanje	16.00	35.78	1
544	Mulanje	15.73	35.60	1
570	Chiradzulu	15.95	35.30	1
601	Thyolo	16.13	35.25	1
645	Thyolo	16.25	35.15	1
753	Machinga	15.05	34.92	1
823	Mangochi	14.35	35.45	2
1805	Salima	13.72	34.47	4
2218	Chikwawa	16.02	34.78	3
2223	Chikwawa	16.25	34.87	3
2226	Zomba	15.48	35.23	2
2227	Zomba	15.48	35.23	2
2229	Zomba	15.48	35.23	2
2231	Mchinji	13.67	33.02	2
2232	Mchinji	13.62	33.07	2
2234	Lilongwe	14.23	33.77	2
2883	Likoma	12.09	34.73	4
3215	Mzimba	12.10	33.43	2
3254	Lilongwe	14.23	33.67	2
3419	Balaka	14.80	35.13	1
3420	Balaka	14.90	35.14	1
3422	Chikwawa	15.99	34.48	3
3425	Chikwawa	15.96	34.77	3
3442	Phalombe	15.61	35.67	1

*From cluster analysis in chapter 3

4.2.2 Moisture stress treatment

Moisture stress was applied according to Muchero *et al*. (2008). The plants were watered to field capacity (moisture content 30%) until full expansion of the first trifoliate leaves (three weeks after emergence) and then water was completely withdrawn for four weeks for drought response measurements. After this period of stress, the plants were re-watered twice a week for two weeks. During the period of stress, day and night temperatures were maintained at 22-27°C and 15-19°C, respectively. The soil moisture content for each pot was measured using 20cm long probes of Time Domain Reflectometer (TDR, model 1502C, Tectronix Inc. Beaverton, OR, USA) twice weekly during the water stress period.

4.2.3 Scoring procedure

The wilting scales, leaf wilting index (LWI) and relative water content (RWC) were repeatedly recorded during the period of water stress, while regrowth and stem green were recorded once after the period of re-watering. Two different wilting scales were used to assess the wilting of plants after the second, third and fourth weeks of stress. The first scale herein referred to as IB scale, was developed by the International Board on Plant Genetic Resources (IBPGR, 1983). This scale uses a 1-9 scoring system, where 1 represents normal and 9 represents dead and dry plants under moisture stress. The second scale, herein referred to as MAIK scale, was used by Mai-Kodomi *et al*. (1999). This scale uses a 1-5 scoring system with 1 representing green turgid leaves and 5 representing completely dead plants. The LWI was calculated weekly, from the first week to the final week of stress, as the ratio between leaves showing wilting signs and the total number of leaves per plant. The RWC was calculated on four new fully expanded leaflets per pot after the second and fourth weeks of stress, as outlined by Bogale *et al*. (2011). The leaves for RWC were detached from the plant between 10am and 2pm during bright days, in order to avoid the effects of weather conditions on water loss from the detached leaves. Immediately after cutting at the base of the lamina, the leaves were weighed to obtain the fresh weight (FW). After weighing, the leaves were soaked in deionised water for 24 hours at room temperature for rehydration and then re-weighed for turgid weight (TW). The leaves were then dried in an oven at 70^{0}C for 72 hours before dry weight (DW) measurements were taken. The RWC was calculated as follows:

$$\mathbf{RWC} = \frac{FW - DW}{TW - DW}$$

Stem greenness and re-growth were scored as recovery parameters, after the two weeks of re-watering, as implemented by Muchero *et al*. (2008). Stem greenness was scored using a scale of 1-5, where 1 was yellow and 5 was completely green. Regrowth was scored using three categories: i.e. 1 representing no re-growth; 3 showing re-growth from axillary buds; and 5 showing re-growth from the apical bud. In total, fourteen variables were recorded after stressing the plants, in order to assess canopy characteristics of the 36 accessions (Table 4.2).

Table 4.2: Variables used to categorise drought tolerance of the 36 cowpea accessions assessed in pots in a glasshouse.

Variable	**Description**
LWI 1	Leaf wilting index after the first week of stress
LWI 2	Leaf wilting index after the second week of stress
LWI 3	Leaf wilting index after the third week of stress
LWI 4	Leaf wilting index after the fourth week of stress
IB 2	IBPGR scale after the second week
IB 3	IBPGR scale after the third week
IB 4	IBPGR scale after the fourth week
MAIK 2	Mai-Kodomi scale after the second week
MAIK 3	Mai-Kodomi scale after the third week
MAIK 4	Mai-Kodomi scale after the fourth week
RWC 2	Relative water content after the second week
RWC 4	Relative water content after the fourth week
STG	Stem greenness after re-watering
Re-growth	Resumption of growth after re-watering

4.2.4 Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure in SAS package (SAS Inc. version 9.2, USA). Separation of means was undertaken using the least significant difference at 5% alpha level ($LSD_{0.05}$). Means for all variables were calculated and standardised for cluster analysis in a Minitab 16 statistical package (Minitab Inc., USA). Standardisation was undertaken, in order to minimise the dominance of variables with higher numerical values (Endresen, 2010). The standardisation was done by subtracting the mean of each variable from each individual accession followed by dividing each value by standard deviation so that variance is zero subsequent standard deviation is one. Euclidean Similarity Index and Ward linkage were used in the cluster analysis (Jeffers, 1967).

4.3 Results

4.3.1 Changes in soil moisture content

The volumetric soil water content, at a depth of 20 cm showed no significant differences between genotypes and replicates for each day of measurement. As such, the daily measurements were averaged and plotted to visualise variations in moisture over the stress period (Fig. 4.1). The soil moisture content (MC) decreased from 26.2% to 2.9% between fully wet and the most stressful period respectively. During the first eight days the soil moisture content dropped drastically from 26.2 to 8.7% after which a gradual reduction in MC was observed from the 8^{th} day to the final day of stress.

4.3.2 Genotypic responses to water stress.

An analysis of variance showed a highly significant variation (P = 0.0001) for all variables measured (Table 4.3). The variations among genotypes were more noticeable in the advanced stages of moisture stress.

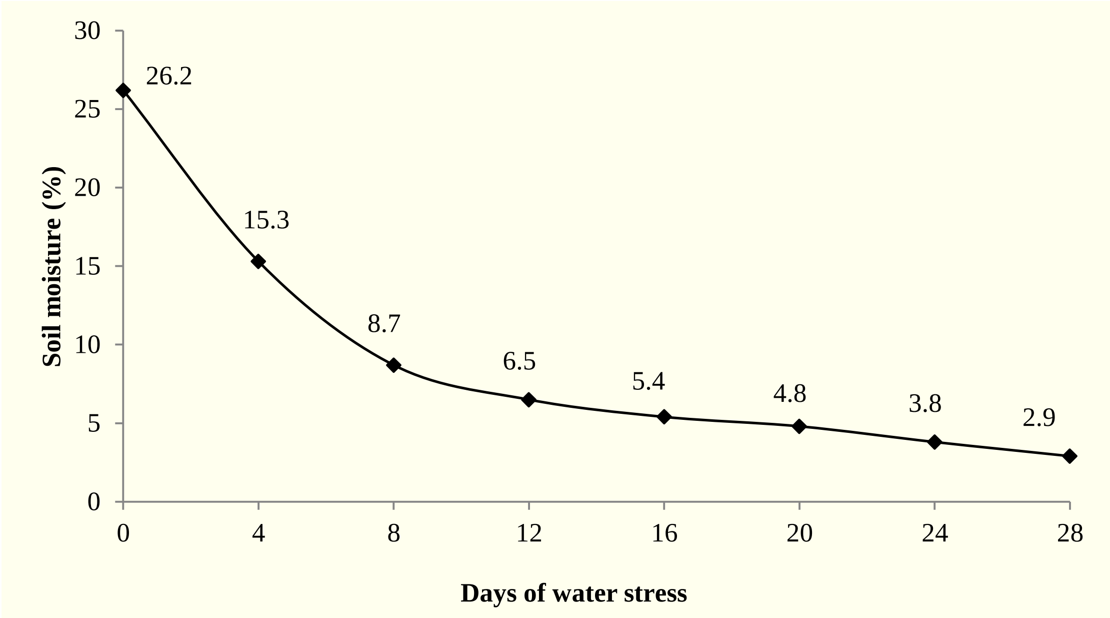

Figure 4.1: Changes in volumetric soil moisture content during the water stress period.

4.3.2.1 Relative water content

Relative water content (RWC) showed highly significant variations among genotypes after both the second and fourth weeks of stress. The values of RWC were significantly higher after the second week than after the fourth week. After the second week of stress RWC ranged between 0.55 and 0.81 with accessions 320, 479, 2226, 3254 and 3422 showing significantly higher values while 2232 showed the lowest value. After the fourth week of stress, RWC ranged between 0.20 and 0.57. Accessions 479, 601, 645, 2226 and 3254 showed RWC>0.50, while 517, 535, 2232, 2234, 3215, and showed RWC≤0.31.

4.3.2.2 Leaf wilting scales

The IB scale showed highly significant variation among genotypes. Different levels of wilting in selected genotypes are shown in Plate 4.1 & 4.2. After the second week, the scores ranged between 1.00 and 6.00. The maximum IB 2 value was scored for 2232 and the lowest for 601, 645, 3425 and 3254. After the third week, the scores ranged between 1.00 and 7.00. The maximum and minimum value was scored for 2232 and 3254, respectively. After the fourth week, the scores ranged between 1.25 and 7.50. Similarly, maximum and minimum values were observed in accessions 2232 and 3254, respectively. The MAIK scale showed trends similar to the IB scale. After the second week, the scores ranged between 1.00 and 3.75. Significantly low scores were observed in accessions 645, 601 and 3254, while the maximum score was observed in accession 2232. After the third week, the score of scale ranged between 1.00 and 4.00 with low scores observed on accessions 601, 645 and 3254 and a high score in 2232. Similarly, after the fourth week, accessions 601 and 3254 showed a minimum value of 1.25, while 2232 showed a significantly high score of 4.5 indicating low tolerance to water stress.

4.3.2.3 Leaf wilting index

Leaf wilting index (LWI) showed significant variation among genotypes. Accessions 471, 1805, 2229, 2232, 2883, 3215 and 3419 showed signs of wilting in the first week as indicated by relatively higher values of LWI after the first week of stress. Accession 2232 showed the highest value of 0.72. No wilting signs were observed in 17 accessions which showed a LWI 1 of 0. An increase in LWI was observed after the second, third and fourth weeks of stress. The maximum values after the second, third and fourth weeks were 0.77, 0.88, and 1.00, respectively, compared to the minimum of 0, 0 and 0.23. Accession 2232 consistently showed high LWI during all the four weeks while the minimum values were scored in 601, 645 and 3254 after the second week and 3254 after both the third and fourth weeks.

Table 4.3: Relative water content (RWC), leaf wilting scales (IB, MAIK), leaf wilting index (LWI), re-growth and stem greenness (STG) for the 36 cowpea accessions.

Accession	RWC 2	RWC 4	IB 2	IB 3	IB 4	MAIK 2	MAIK 3
169	0.67	0.36	2.50	3.00	5.75	1.25	1.75
320	0.72	0.41	3.50	3.50	4.75	4.75	1.88
391	0.66	0.37	2.75	2.50	5.50	1.38	1.75
399	0.72	0.35	2.50	2.50	5.00	1.13	1.75
411	0.71	0.37	2.50	2.50	4.50	1.50	1.75
414	0.72	0.49	2.50	2.00	5.25	1.25	1.50
421	0.65	0.43	2.75	2.50	5.50	1.38	1.75
426	0.64	0.36	2.75	3.00	1.38	1.75	2.75
436	0.68	0.38	3.00	3.00	4.75	1.50	1.75
471	0.60	0.32	3.75	4.50	5.25	2.38	2.50
479	0.79	0.52	1.00	1.75	2.00	1.25	1.50
517	0.61	0.29	3.50	4.50	5.75	2.13	2.50
535	0.70	0.31	2.75	6.00	6.00	1.50	2.50
544	0.67	0.34	2.75	3.00	5.75	1.63	1.75
570	0.64	0.43	3.00	4.00	3.75	1.75	2.50
601	0.75	0.56	1.00	1.00	1.75	1.00	1.00
645	0.74	0.51	1.00	1.00	2.00	1.00	1.00
753	0.69	0.41	3.25	4.00	5.00	2.00	2.25
823	0.66	0.32	3.00	3.50	5.75	1.50	2.00
1805	0.71	0.48	3.63	4.50	4.75	2.25	2.50
2218	0.69	0.39	3.50	5.00	4.50	1.88	2.75
2223	0.67	0.36	2.50	3.00	5.75	1.25	1.75
2226	0.76	0.54	2.50	2.00	2.50	1.25	1.50
2227	0.67	0.54	2.75	3.50	2.50	1.63	2.25
2229	0.68	0.35	3.00	3.50	5.50	1.88	2.50
2231	0.71	0.33	3.75	4.50	7.00	1.88	2.25
2232	0.55	0.20	6.00	7.00	7.50	3.75	4.00
2234	0.71	0.31	3.00	2.50	5.50	1.75	1.75
2883	0.62	0.32	3.25	4.00	6.00	2.25	2.50
3215	0.69	0.22	3.50	4.00	7.25	2.13	2.75
3254	0.81	0.58	1.00	1.00	1.25	1.00	1.00
3419	0.64	0.39	3.50	4.00	6.00	1.88	2.50
3420	0.77	0.46	2.25	2.00	4.50	1.13	1.25
3422	0.75	0.43	2.00	2.50	5.00	1.13	1.25
3425	0.67	0.32	1.75	2.25	6.00	1.25	2.00
3442	0.68	0.38	3.25	4.00	4.50	1.88	2.25
Mean	0.69	0.39	2.82	3.18	4.81	1.65	2.01
Minimum	0.55	0.20	1.00	1.00	1.25	1.00	1.00
Maximum	0.81	0.57	6.00	7.00	7.50	3.75	4.00
$LSD_{0.05}$	0.11	0.12	0.70	1.59	1.59	0.59	0.81
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 4.3: continued

Accession	MAIK 4	LWI 1	LWI 2	LWI 3	LWI 4	REGROWTH	STG
169	3.00	0.09	0.13	0.44	0.85	1.50	2.75
320	2.00	0.13	0.22	0.46	0.81	1.50	2.75
391	2.75	0.00	0.18	0.58	0.85	2.00	2.38
399	2.75	0.03	0.13	0.55	0.87	1.50	2.88
411	3.00	0.00	0.32	0.49	0.78	2.00	2.50
414	2.75	0.00	0.10	0.42	0.77	1.75	2.63
421	3.00	0.00	0.15	0.46	0.85	2.00	2.63
426	2.25	0.06	0.27	0.65	0.78	0.06	2.88
436	3.00	0.00	0.27	0.66	0.93	1.50	2.88
471	2.50	0.22	0.43	0.75	0.87	1.75	2.75
479	1.50	0.00	0.14	0.18	0.53	4.50	4.75
517	3.50	0.09	0.36	0.75	0.93	1.25	1.50
535	3.50	0.06	0.19	0.50	0.90	2.25	2.38
544	3.25	0.03	0.18	0.56	0.78	2.50	3.00
570	2.25	0.14	0.33	0.58	0.81	2.50	3.00
601	1.00	0.00	0.00	0.16	0.43	4.50	4.63
645	1.00	0.00	0.00	0.13	0.41	5.00	4.63
753	3.00	0.16	0.29	0.56	0.63	3.25	2.75
823	2.75	0.00	0.29	0.76	0.92	2.00	2.63
1805	3.00	0.25	0.34	0.55	0.83	1.25	2.38
2218	2.50	0.03	0.31	0.53	0.85	1.25	3.00
2223	3.00	0.00	0.13	0.44	0.85	1.50	2.75
2226	1.75	0.00	0.12	0.39	0.56	4.75	4.75
2227	2.00	0.13	0.32	0.35	0.59	4.00	4.00
2229	3.00	0.25	0.39	0.53	0.89	1.50	3.00
2231	3.25	0.00	0.22	0.53	0.96	1.00	1.75
2232	4.00	0.72	0.77	0.88	1.00	1.00	1.88
2234	3.25	0.00	0.22	0.52	0.82	1.50	2.38
2883	3.00	0.22	0.29	0.48	0.93	1.00	1.63
3215	3.50	0.19	0.44	0.59	0.93	1.00	1.00
3254	1.25	0.00	0.00	0.00	0.23	4.50	4.88
3419	3.00	0.25	0.47	0.63	0.84	1.75	2.75
3420	2.75	0.00	0.08	0.28	0.72	3.25	3.25
3422	2.50	0.00	0.05	0.28	0.73	2.50	3.13
3425	3.25	0.00	0.26	0.64	0.88	2.25	2.25
3442	2.75	0.16	0.38	0.71	0.89	1.75	2.88
Mean	2.73	0.09	0.25	0.50	0.78	2.25	2.87
Minimum	1.25	0.00	0.00	0.00	0.23	1.00	1.00
Maximum	4.50	0.72	0.77	0.88	1.00	5.00	5.00
$LSD_{0.05}$	0.84	0.19	0.20	0.24	0.20	1.59	0.60
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

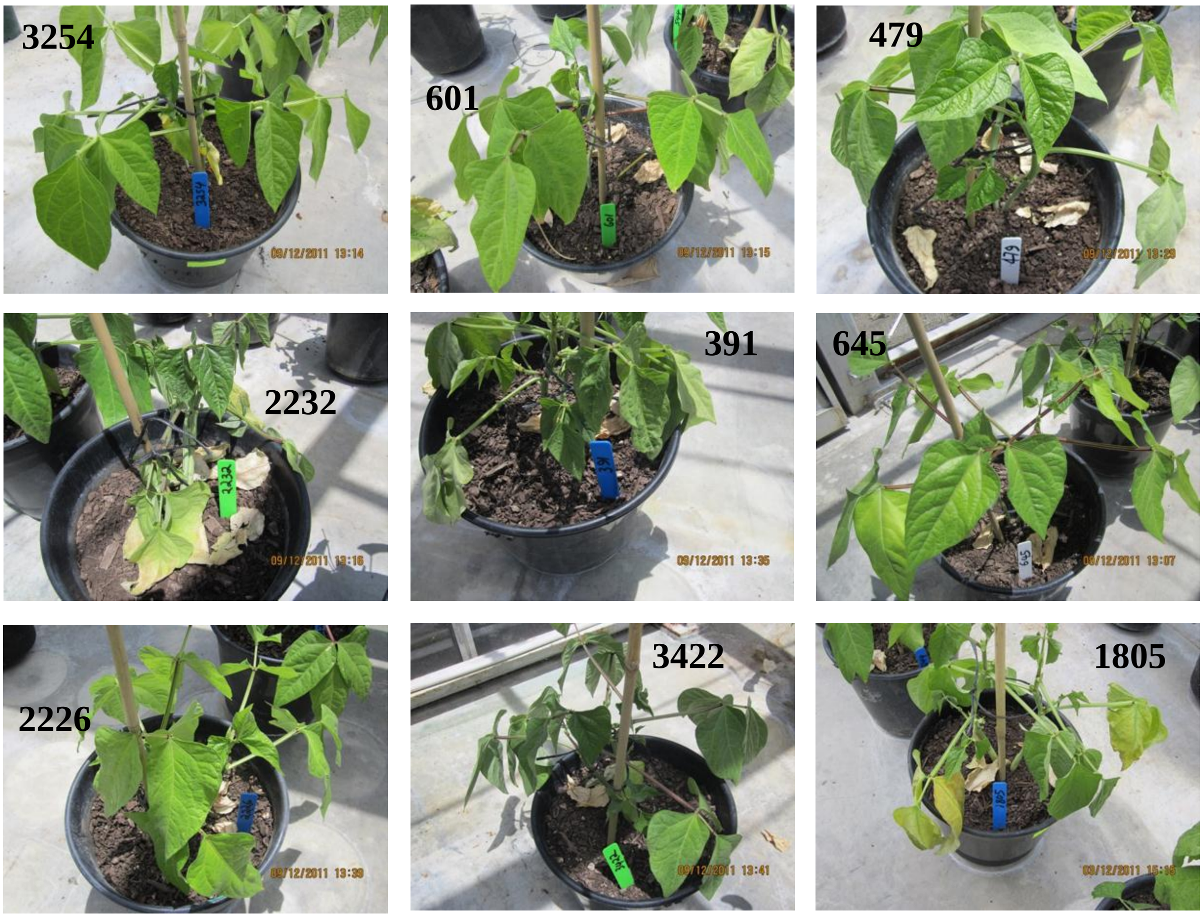

Plate 4.1: Variation in leaf wilting in second week of stress for selected genotypes.

4.3.2.4 Re-growth and stem greenness

Regrowth and stem greenness were scored to rate the recuperative ability of the genotypes after the re-watering period (Plate 4.3). Both traits showed highly significant differences among genotypes indicating that that some genotypes recuperated better than others after being re-watered. Genotypes 479, 601, 645, 2226 and 3254 showed higher scores for regrowth, thus indicating their ability to regrow from apical buds. However, accessions 2231, 2232, 2883 and 3215 showed a score of 1, thus indicating a complete lack of regrowth. Similar to re-growth, accessions, 479, 601, 645, 2226, 2227, 3254 and 3420 showed high levels of stem greenness, compared to accessions 517, 2231, 2232, 2883, and 3215 which showed completely dry stems even after being re-watered.

Plate 4.2: Variation in leaf wilting levels in the third week of stress.

Plate 4.3: Recovery responses of cowpea after two weeks of re-watering

4.3.3 Correlations between leaf wilting index (LWI) and leaf wilting scales, relative water content, stem greenness and re-growth

A Pearson correlation analysis showed strong correlations among all the variables measured (Table 4.4). The LWI showed a strong association with corresponding characters measured at the same period. For instance LWI 2 correlated strongly with IB 2 (0.873), MAIK 2 (0.894) and RWC 2 (-0.763), while LWI 4 strongly correlated with IB 4 (0.891), MAIK 4 (0.846), RWC 4 (-0.838). Also LWI 4 strongly correlated with re-growth (-0.906) and stem greenness (-0.874). Generally LWI correlated positively with IB and MAIK scales but correlated negatively with RWC, re-growth and stem greenness.

4.3.4 Cluster analysis

Cluster analysis grouped the 36 accessions into five distinct clusters (Fig. 4.2, Table 4.5 & Table 4.6). Cluster 1 was the largest with 14 accessions and it was associated with IB 3, MAIK 3, LWI 2, IB 2 and MAIK 2. Cluster 2 with 12 accessions was associated with LWI 1, MAIK 4, RWC 4 and IB 4. Cluster 4 with five accessions completely recovered from moisture stress. Cluster 3 comprised 4 accessions which were associated with RWC 4, LWI 3, MAIK 3 and RWC 2. The factors defining Cluster 4 with 5 accessions, in order of importance were LWI 4, re-growth, stem greenness, IB 4 and LWI 3 with LWI 4 (Table 4.6). Cluster 5 had only one accession and completely wilted after the period of stress. Factors describing cluster 5, in order of importance were LWI 1, MAIK 2, IB 2, LWI 2 and MAIK 3. Similarities in the pattern of factor loadings (signs) and differences in magnitude between Clusters 1 and 5 show that the accession in Cluster 5 is more susceptible to drought than those in Cluster 1. Factor loadings (signs) for Clusters 3 and 4 followed a similar pattern. However the two clusters differed in the magnitudes of the loadings with cluster 4 showing significantly higher scores than cluster 3. Similarity in signs and differences in magnitude for the two clusters show that accessions in both clusters may have drought tolerance, with cluster 4 showing a high level of tolerance. Cluster 2 with 12 accessions showed its own pattern of factor loadings. This cluster is positively loaded with LWI 3, LWI 4, IB 4 and MAIK 4 but negatively loaded with LWI 1, LWI 2, IB 2, IB 3, MAIK 2, MAIK 3, regrowth, RWC 2, RWC 4 and stem greenness.

Table 4.4: Pearson correlation coefficients for variables measured during water stress and after re-watering periods.

	LWI1	LWI2	LWI3	LWI4	IB2	IB3	IB4	MAIK3	MAIK4	MAIK4	REGROWTH	RWC2	RWC4	STG
LWI 1														
LWI 2	0.834													
LWI 3	0.479**	0.770												
LWI 4	0.350	0.625	0.850											
IB 2	0.805	0.873	0.739	0.663										
IB 3	0.752	0.846	0.708	0.646	0.911									
IB 4	0.364	0.540	0.755	0.891	0.612	0.580								
MAIK 2	0.905	0.894	0.634	0.536	0.937	0.890	0.540							
MAIK 3	0.813	0.924	0.738	0.674	0.893	0.906	0.607	0.899						
MAIK 4	0.363	0.561	0.761	0.846	0.586	0.550	0.926	0.532	0.607					
REGROWTH	-0.356*	-0.577	-0.744	-0.906	-0.626	-0.659	-0.843	-0.543	-0.636	-0.794				
RWC 2	-0.617	-0.763	-0.798	-0.684	-0.710	-0.704	-0.620	-0.697	-0.746	-0.606	0.602			
RWC 4	-0.417	-0.644	-0.795	-0.838	-0.638	-0.589	-0.891	-0.598	-0.667	-0.855	0.770	0.675		
STG	-0.347	-0.576	-0.748	-0.874	-0.605	-0.607	-0.915	-0.553	-0.641	-0.894	0.897	0.636	0.843	

*, ** Correlation significant at 0.05 and 0.01 level, respectively otherwise significant at 0.001

Table 4.5: Mean cluster scores for all 14 variables.

Variable	**Cluster**				
	1 (14)	**2 (12)**	**3 (4)**	**4 (5)**	**5 (1)**
LWI 1	0.16	0.02	0.03	0.00	0.72
LWI 2	0.34	0.21	0.14	0.05	0.77
LWI 3	0.57	0.57	0.33	0.17	0.89
LWI 4	0.86	0.85	0.70	0.43	1.00
IB 2	3.40	2.71	2.38	2.00	6.00
IB 3	4.11	2.67	2.50	1.30	7.00
IB 4	5.25	5.46	4.31	1.90	7.50
MAIK 2	1.98	1.43	1.28	1.10	3.75
MAIK 3	2.42	1.85	1.56	1.20	4.00
MAIK 4	2.89	3.02	2.50	1.55	4.00
REGROWTH	1.57	1.94	2.88	4.65	1.00
RWC 2	0.67	0.68	0.72	0.77	0.55
RWC 4	0.36	0.35	0.48	0.53	0.20
STG	2.38	2.64	3.25	4.73	1.88

*Numbers in parenthesis represent total number of accessions per cluster

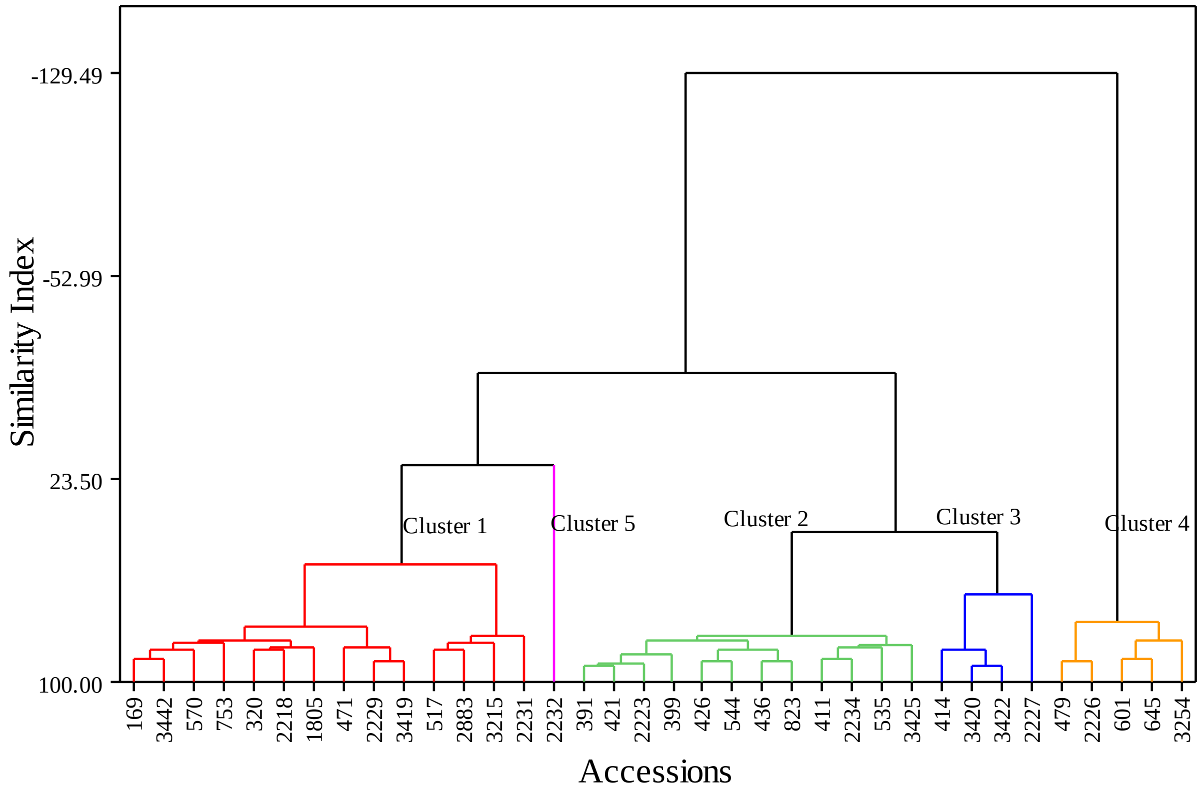

Figure 4.2: Dendrogram showing clusters of 36 accessions based on LWI, wilting scales, RWC, regrowth and stem greenness.

Table 4.6: Factor loadings for the five clusters.

Variable	Cluster				
	1	2	3	4	5
LWI 1	0.47	-0.53	-0.39	-0.64	4.51
LWI 2	0.61	-0.22	-0.71	-1.26	3.39
LWI 3	0.41	0.37	-0.88	-1.76	2.09
LWI 4	0.43	0.41	-0.48	-2.04	1.26
IB 2	0.59	-0.29	-0.71	-1.18	3.88
IB 3	0.75	-0.37	-0.49	-1.42	2.99
IB 4	0.29	0.43	-0.33	-1.94	1.79
MAIK 2	0.59	-0.41	-0.67	-0.99	4.21
MAIK 3	0.68	-0.26	-0.74	-1.34	3.28
MAIK 4	0.27	0.48	-0.38	-1.94	1.68
REGROWTH	-0.57	-0.26	0.53	2.03	-1.06
RWC 2	-0.39	-0.19	0.72	1.52	-2.56
RWC 4	-0.31	-0.45	0.97	1.59	-2.13
STG	-0.53	-0.27	0.41	1.99	-1.07

4.4 Discussion

4.4.1 Moisture stress

The soil moisture content of 2.9%, attained after a period of four weeks of no water (Fig. 4.1), created sufficiently severe stress for the identification of tolerant and susceptible genotypes. During the water stress period accessions 479, 601, 645, 2226 and 3254 maintained an active canopy, compared to accessions 517, 2231, 2232, 2883 and 3215, which completely wilted at the same level of soil moisture content (Table 4.3). The 2.9% moisture content level of the pot mix rooting medium in this study compares very well with findings from similar studies. In a cowpea study, Watanabe *et al.*, (1997) identified drought tolerant genotypes at soil moisture contents of between 2% and 5% and recommended 3% as an optimum moisture content level for screening cowpea germplasm. Similarly, Abraham *et al.* (2004) identified drought tolerant genotypes of bluegrass at a moisture content level of less than 3% after 35 days of water stress. The results in this study suggest that accessions which maintained active canopy at a soil moisture level of 2.9% can grow favourably under low moisture conditions.

4.4.2 Relative water content

Survival of plants under water stress conditions depends on the availability of water in the plant tissues including leaves. RWC presents a simplified way of expressing the amount of water in relation to dry matter in plants. In most plants, a decrease of RWC below 0.50 causes disruption of physiological processes and usually eliminates chances of recovery when it falls below 0.30 (Taiz & Zeiger, 1998). Photosynthesis is the main physiological process affected when RWC falls below 0.50 (Kaiser, 1987). The range of 0.55 – 0.77 (Table 4.3) after two weeks of stress, was above the critical RWC value, which suggests that, within the first two weeks, the water stress was not sufficient to disrupt the physiological processes and effective in separating germplasm into drought tolerant and susceptible groups. However, after the fourth week of stress, RWC ranged between 0.20 and 0.57, and some genotypes maintained RWC above the critical value of 0.50 while others did not. The results after the fourth week show that 479, 601, 645, 2226 and 3254 maintained RWC well above the critical value, indicating their ability to tolerate low moisture levels. Therefore, the findings on relative water content suggest the presence of drought tolerant genotypes and also support that the 2.9% volumetric soil moisture content is an appropriate to screen for drought tolerance in cowpea.

Maintenance of high RWC in drought tolerant genotypes is attributed to their ability to minimise water loss from the leaves and/or achieve better extraction of water from the deep layers of dry soils (Oliver *et al*., 2010; Taiz & Zeiger, 2010). Also reduced leaf areas may also have contributed to high RWC due to slow use of water in the reduced leaves. The effect of leaf area on RWC in cowpea was demonstrated by negative correlation (-0.77) between the two variables under water stress (Anyia & Herzog, 2004b). In this study, the minimised water loss from the leaves may explain the ability of the drought tolerant genotypes to maintain high

RWC under low soil moisture content. However, extraction of water from deep layers of soil was not possible as the plants were grown in confined 10 litre pots.

4.4.3 Leaf wilting

Wilting is the most common visible sign of drought stress in plants. Wilting is defined as the loss of rigidity, leading to a flaccid state, due to the turgor pressure falling to zero (Taiz & Zeiger, 2010). High values on both the IB and MAIK wilting scales observed after the second week of stress (Table 4.3) show that some accessions started wilting in the initial stages of water stress. Accessions in Clusters 1 and 5 (Fig. 4.2 and Table 4.5) showed the highest scores of the two wilting scales throughout the period of stress, in contrast to accessions in Clusters 3 and 4. Similar studies have identified susceptible genotypes, which also wilted within the first week of stress (Fatokun *et al*., 2009; Mai-Kodomi *et al*., 1999; Muchero *et al*., 2008). The current findings on wilting after the second week of stress, demonstrate that susceptible genotypes could be identified during the initial stages of stress, as was especially the case for cluster 5 (accession 2232).

The early wilting accessions, such as 2232, suggest the presence of physiological characteristics that accelerate water loss from the leaf tissues or defective rooting system. The early wilting genotypes keep their stomata open after the initiation of drought, whereas late wilting genotypes close their stomata during the initial phase of stress (Agbicodo *et al*., 2009). As stress advances, early wilting genotypes dry and drought tolerant genotypes survive through stomatal closure, in addition to osmotic adjustment, which involves accumulation of osmolytes such as proline (Jaleel *et al*., 2009; Singh & Raja, 2011). Stomata closure is regarded as the first defence mechanism followed by accumulation of osmolytes under water stress conditions. (Sharma & Kumar, 2008). Therefore, a lack of such desirable physiological traits in the susceptible genotypes, such as 2232, may have contributed to significant wilting in the early stages of stress. The genotypes which tolerated water stress conditions, until the late stages in this study, may have desirable physiological characteristics, such as stomata closure and the accumulation of osmolytes, thus leading to survival at the low moisture content of 2.9%. Therefore, follow up studies on the two contrasting groups of accessions should help to gain an understanding of the actual physiological mechanisms behind the variation in responses to water stress.

Significant correlations point towards the existence of underlying relationship between measured parameters. In this study, LWI correlated significantly with traits previously used in identifying drought tolerant cowpeas genotypes (Table 4.4). The LWI correlated negatively with RWC, stem greenness and re-growth, and correlated positively with the two leaf wilting scales. The strong correlations between LWI and other drought tolerance traits suggest the possibility of using the index as an easy to measure indicator for the more difficult or slower to measure traits associated with drought tolerance in cowpea and other related crops. Although LWI correlated strongly with reliable measures of drought tolerance, its application may be challenged by the lack of a clear cut-off point for determining tolerant.

The values of LWI ranged between 0 and 1, where 0 meant absence of wilting leaves and 1 meant all the leaves showing wilting signs. The index, after the fourth week of stress, quantitatively classified all the accessions between 0.23 and 1.00 for the most tolerant and susceptible genotypes respectively. Determining the cut-off point for the drought tolerant and susceptible genotypes may provide a substantial challenge, in the absence of other evidence. In this study, an index$\leq$0.6 is proposed as the cut-off point for determining tolerant genotypes. A comparison of means for the 36 accessions on RWC and LWI after the fourth week of stress (Figs. 4.3 & 4.4), shows that 479, 601, 645, 2226 and 3254 exhibited both RWC>0.5 and LWI <0.6. In addition to the evidence of high RWC after four weeks of stress, these five accessions fully recovered from water stress (Table 4.3).

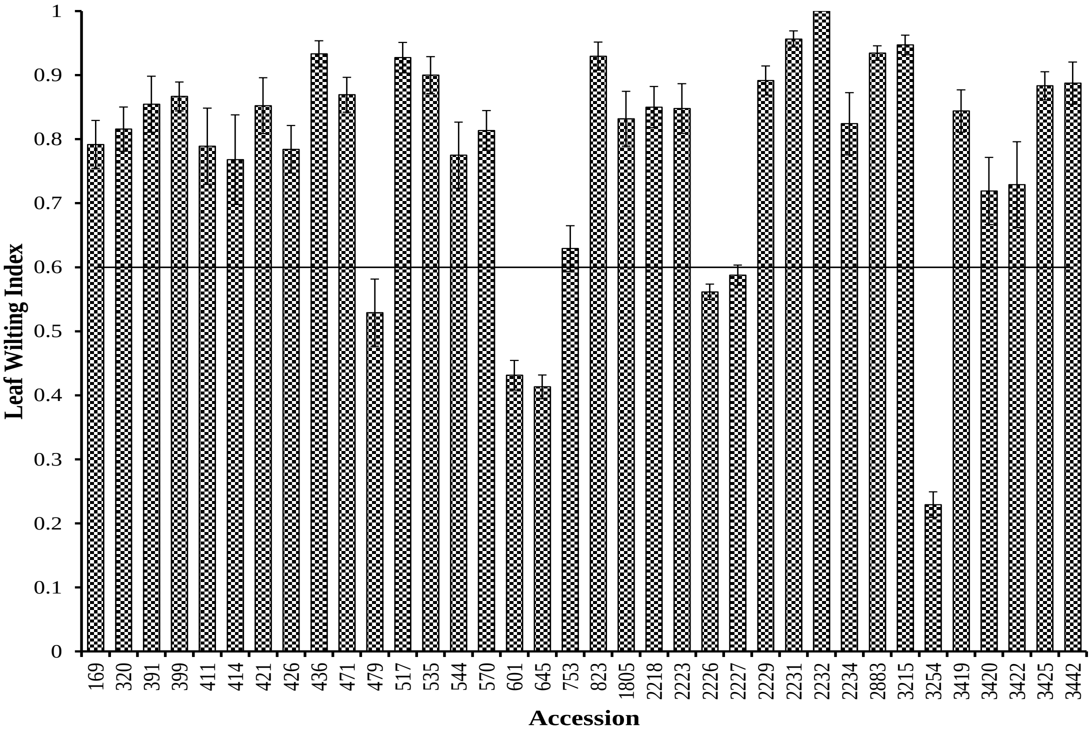

Figure 4.3: Means of 36 accessions for the leaf wilting index after four weeks of moisture stress (Error bars represent standard error for each accession).

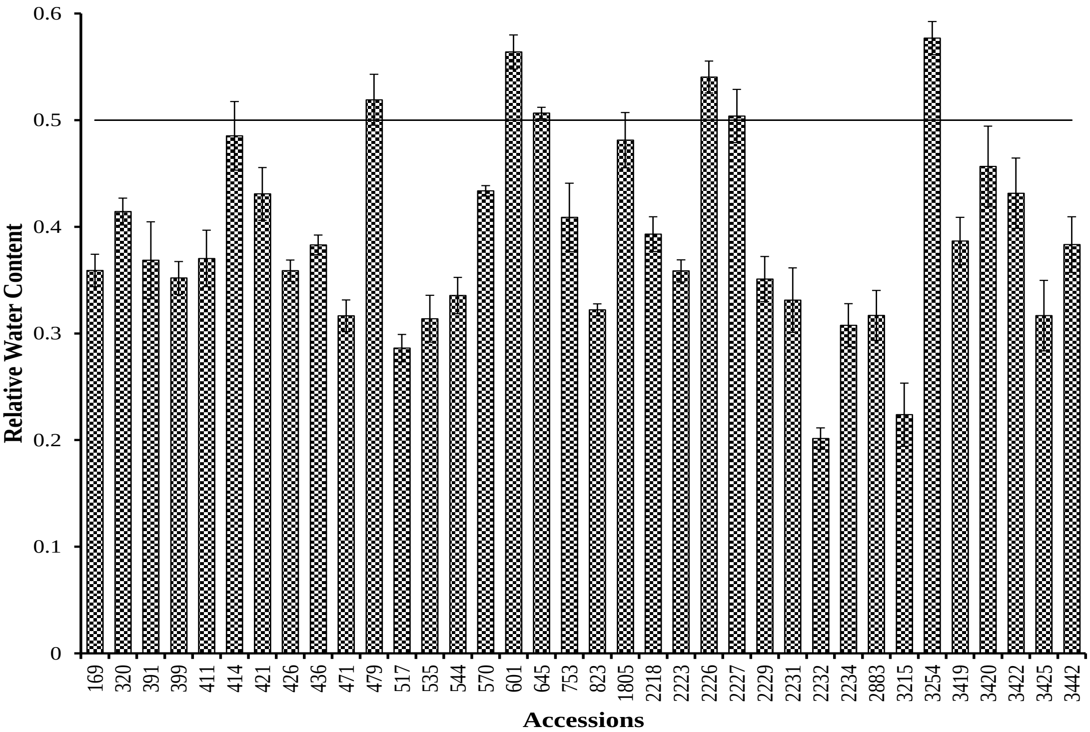

Figure 4.4: Means of 36 accessions for the relative water content after four weeks of moisture stress (Error bars represent standard error for each accession).

Conclusions on an association between RWC>0.5 and recovery ability are supported by Taiz and Zeiger (1998), who showed that genotypes with RWC>0.5 had high chances of recovery from water stress. In conclusion, an index<0.6 is a good threshold for identifying drought tolerant cowpea genotypes, after complete withdrawal of water for a period of four weeks.

The two commonly used wilting scales of IB and MAIK are associated with the limitations of qualitative scoring since they require specialised expertise when scoring. In contrast, the LWI, which involves counting the total number of leaves showing wilting signs and the total number of leaves per plant, is easy for non-experts, if drought-wilting signs are well understood. Therefore, the application of a LWI would be advantageous for breeders, since it easily classifies genotypes through a quantitative index, compared to the qualitative scales, which classify genotypes based on visual assessment and predefined classes.

Although LWI is a better measure for leaf wilting, it should only be applied to crops in which wilting is a good indicator of drought response. In cases where genotypes maintain active growth of apical meristems, by deriving water from lower leaves, the index may mis-classify such genotypes as being susceptible. This could apply to genotypes of cowpea with a Type 2 drought tolerance mechanism (Mai-Kodomi *et al*., 1999). Genotypes with Type 2 drought tolerance derive water from lower canopies, in order to support apical growth during water stress. Such genotypes may show relatively high values of LWI and yet fully recover after re-watering. Therefore, the use of a LWI in genotypes with Type 2 mechanism could be complemented by other traits, such as regrowth and stem greenness, in order to properly group genotypes into either tolerant or susceptible classes. However, genotypes with a Type 2 mechanism were not encountered in this study. Symptoms of diseases and pests may also limit the application of the LWI. In this case, a prior understanding of some diseases and pest symptoms, which could be mistaken for drought wilting, should be given due consideration. This scale is not applicable to aged plants, since old leaves will naturally senesce with age.

Therefore, it is being proposed that the application of this scale should be confined to the early vegetative stage of drought evaluation.

4.4.4 Stem greenness and re-growth

The role of stem greenness in the recovery process after drought is linked with the maintenance of chlorophyll, which contributes to photosynthesis (Xu *et al*., 2000). In this study, all the accessions with high scores for stem greenness (>4.00) fully recovered from moisture stress (Table 4.3). The co-relationship of stem greenness and regrowth in the drought tolerance is not by chance. A genetic linkage study identified that cowpea QTL for both stem greenness and recovery are co-located on the same chromosome (Muchero *et al*., 2009). Therefore, the accessions, which scored highly on stem greenness in this study, may have maintained greenness for active photosynthesis, which enabled them to fully recover from stress, compared with accessions which lacked this attribute.

4.4.5 Cluster analysis

Cluster analysis provides useful information to visualise similarities and differences among objects and it also explains the contribution of factors/variables towards the clustering of objects (Mohammadi & Prasanna, 2003). The five accessions in Cluster 4 showed desirable attributes for maintenance of canopy at soil moisture levels down to 2.9%. The strong relationship of Cluster 4 with LWI 4 and regrowth (Table 4.6) indicates the ability of the accessions in the Cluster to withstand water stress up to the end of the stress period; and recover after re-watering. However, Cluster 5 can be described as early wilting, since factors measured during the early weeks of stress contributed significantly towards the cluster. The only accession in Cluster 5 (2232) showed wilting signs as early as the first week at soil MC>8.7%, thus suggesting that the accession is highly susceptible to drought. The most important factor describing Cluster 5 is LWI 1, which confirms that wilting started in the first week of stress.

Clusters 3 and 4 showed characteristic of drought tolerance, while Clusters 1 and 5 showed drought susceptibility. However, Cluster 2 did not show a very specific response to water stress. One of the possible reasons for this unique response of accessions in Cluster 2 could be the presence of genotypic mixtures within accessions. Landraces are characterised by within-accession variability, due to local seed exchanges and intercropping of crops by subsistence farmers (Thomas *et al.*, 2011). Accessions in this group may have been collected as mixtures. The other possibility could be the presence of hybrids in the mixture, due to natural crossing in the field before collection, although cowpea is a predominantly self-pollinated crop (Timko *et al*., 2007).

4.5 Conclusion

This study has identified potential genotypes of cowpea for drought tolerance, which could be included in the National Cowpea Improvement Programme. These results will also help to strengthen on-farm conservation of drought tolerant genotypes in the areas where they were collected. Accessions 479, 601, 645, 2226 and 3254 have shown desirable attributes for the maintenance of canopy down to a soil moisture level of 2.9%, in contrast to accessions 517, 2231, 2232, 2883 and 3215, which completely dried at the same level of moisture. The accessions in clusters with contrasting drought response could be potential candidates for further genetic studies on drought tolerance in cowpeas.

The existing wilting scales for drought tolerance in cowpea provide challenges that need to be overcome by an improvement in the scoring system. A LWI has been identified as a reliable and easy method that will overcome some of the challenges associated with previous methods of scoring for wilting in cowpea and related crops. By counting individual leaves with wilting signs, the challenges associated with visual assessment would be reduced and it would require non-specific expertise in scoring for wilting. Such objective scoring, which generates a quantitative wilting index, would reduce bias when assigning genotypes into different wilting groups, as opposed to the qualitative scale. Its strong correlation with the key traits associated with drought tolerance, such as stem greenness, high relative water content, re-growth and wilting scales indicates the potential value of LWI in drought tolerance evaluation. In the present case, a cut-off point of LWI 4<0.6 identified the same drought tolerant genotypes as the much more laborious relative water content method.

Further work needs to be conducted, in order to explore the physiological mechanisms controlling canopy maintenance of the tolerant genotypes and yield potentials prior to genetic and crop improvement studies. LWI should be further explored in the field with cowpeas and other crops, in order to ascertain its application as a reliable method of scoring leaf wilting, despite the anticipated limitations due to Type 2 drought tolerance, symptoms of diseases and pests and leaf senescence due to age.

Chapter 5 : Physiological characterisation of cowpea genotypes with canopy maintenance attributes under moisture stress conditions

Abstract

Canopy maintenance under water stress is an indication of drought adaptation in plants, although it may not distinctly classify genotypes into drought avoidance and drought tolerance categories. This study used physiological responses to classify five cowpea genotypes (479, 601, 645, 2226 and 3254) with canopy maintenance characteristics, together with 2232 which senesced completely, into drought response categories. The experiment was conducted in a glasshouse under well-watered and moisture stress conditions. Plants were stressed after three weeks from emergence by completely withdrawing water. Stomatal conductance (Gs), transpiration rate (E) and net photosynthesis (Pn) were measured after the first and third weeks of stress, while specific leaf area (SLA) and relative water content (RWC) were measured after each week during the period of stress. Moisture stress reduced RWC, Gs, E and Pn but increased SLA in all the genotypes. Under moisture stress, genotypes 479, 601, 645, 2226 and 3254 maintained higher RWC and lower SLA compared to 2232. During the period of moisture stress genotypes 479, 601, 645 and 2226 reduced Gs, E and Pn compared with 3254. Both 3254 and 2232 showed higher Gs, E and Pn after the first week of stress, however, the drought susceptible 2232 reduced Pn after the third week of stress. In conclusion, genotype 3254 belongs to a drought tolerant group, due to high photosynthetic capacity, while 479, 601, 645 and 2226 belong to a drought avoidance group, due to a rapid reduction in Gs, E and Pn under low moisture conditions. Genotype 2232 belongs to a susceptible group, due to low Pn as well as high Gs and E under moisture stress. In addition to high photosynthetic capacity, the drought tolerant genotypes showed relatively low SLA, compared to the drought susceptible genotype 2232 under moisture stress. However, application of SLA in drought tolerance evaluation is challenged by an inability to distinguish between genotypes with drought avoidance and those with drought tolerance.

5.1 Introduction

In an effort to identify cowpea genotypes with drought tolerance, 36 genotypes from Malawi were evaluated in a glasshouse at the Plant Growth Unit, Massey University Palmerston North in New Zealand. Five genotypes (479, 601, 645, 2226 and 3254) maintained an active leaf canopy for a period of four weeks of water stress and fully recovered after re-watering (Pungulani *et al.*, 2012). Canopy maintenance has been used as a selection criterion for selecting drought tolerant genotypes (Agbicodo *et al*., 2009; Muchero *et al*., 2008; Singh *et al*., 1999b). However, the use of leaf canopy maintenance alone may not provide an in-depth assessment of the actual mechanisms of survival under drought conditions. Therefore, a physiological assessment of the mechanisms controlling canopy maintenance would help to appropriately classify genotypes into either drought avoidance, or drought tolerance categories.

Maintenance of canopy photosynthesis in cowpea is associated with several physiological parameters: photosynthesis, which is partly controlled by stomatal conductance; carbon dioxide assimilation and transpiration rate (Belko *et al*., 2012); water use efficiency (WUE) (Singh & Raja, 2011); membrane stability (Labuschagne *et al*., 2008); and relative water content (RWC) (Kumar *et al*., 2008). Changes to physiological parameters lead to reduced growth rate under water stress. One of the key growth traits affected by water stress is Specific Leaf Area (SLA), which is the ratio between leaf area and leaf dry matter. Genotypes with lower SLA under stress conditions have thicker leaves as an adaptation mechanism (Thumma *et al*., 2001). Simplicity in measurements under water stress conditions make SLA a good trait for selecting drought tolerant genotypes.

Although several physiological factors control drought adaptation in cowpea, drought tolerant genotypes exhibit different physiological responses. This means that physiological mechanisms controlling drought tolerance may vary by genotypes. The aim of this research was to understand the specific physiological mechanisms for drought tolerance of the genotypes demonstrating canopy maintenance under water stress, compared with a susceptible genotype.

5.2 Methodology

5.2.1 Experimental procedure

A glasshouse experiment was conducted at Plant Growth Unit (PGU) (40.38^{0}S and 175.61^{0}E) at Massey University, Palmerston North, New Zealand. Four healthy seeds of each genotype were planted in 10 litre pots and seedlings were thinned to two per pot eight days after emergence. Each pot was filled with growth medium, which was prepared by mixing 100 l of pot mix with 150 g of short term release fertilizer (3-4 months) and 150 g of Dolomite. The experiment was a 2x6 factorial arranged as a split plot design and replicated four times. The main plots were water regime (stress and unstressed) and subplots were genotype (479, 601, 645, 2226 and 3254). The plants were watered to field capacity using an automated irrigation system until the first trifoliate leaves were fully expanded (three weeks from emergence). At this time, the pots were separated into two sets. The first set received full irrigation, where plants were watered to field capacity three times a day using an automated irrigation system, while the second set was exposed to water stress by complete withdrawal of irrigation for four weeks. During the period of stress, day and night temperatures were maintained at 22-27°C and 15-19°C, respectively.

Volumetric soil moisture content for each pot was monitored twice a week for the whole period of water stress using 20cm long probes of Time-Domain Reflectometer (TDR model 1502C, Tectronix Inc. Beaverton, OR, USA). Stomatal conductance (Gs) (mmol $m^{-2}s^{-1}$); Transpiration rate (E) (mmol $m^{-2}s^{-1}$) and Net Photosynthesis (Pn) (µmol$CO_2m^{-2}s^{-1}$) were measured on four leaves per pot with CIRAS-2 V2.01 (a portable photosynthesis system).

Photosynthetic active radiation and reference CO_2 were maintained at 1400µmol photons $m^{-2}s^{-1}$ and 400ppm respectively. The leaves used for Gs, E, and Pn were detached from the plant for RWC measurements. Immediately after cutting at the base of the lamina, the leaves were weighed to obtain the fresh weight (FW). After weighing, the leaves were soaked in deionised water for 24 hours at room temperature for rehydration and then re-weighed for turgid weight (TW). The leaves were then dried in an oven at 70°C for 72 hours before dry weight (DW) measurements were taken. Relative water content was calculated on new fully expanded leaves using the formula below

$$RWC = \frac{FW - DW}{TW - DW}$$

where; FW = Fresh weight of leaves; DW = Dry weight of leaves; TW = Turgid weight of leaves.

The remaining fresh leaves per pot were used for Specific leaf area (SLA) (cm^2g^{-1}) measurements. SLA was calculated as the ratio between leaf area (cm^2) and leaf dry matter (g). Leaves were plucked from the plant and taken for the leaf area measurement using a LI-COR Portable Leaf Area meter (Model LI 3000, USA). After leaf area measurement the leaves were oven dried at 70°C for 72 hours and weighed.

Table 5.1: Description of parameters measured and used in the statistical analyses

Parameter	**Time of measurement (Week)**			
	1	**2**	**3**	**4**
Relative Water Content (RWC)	RWC 1	RWC 2	RWC 3	RWC 4
Specific Leaf Area (SLA)	SLA 1	SLA 2	SLA 3	SLA 4
Stomatal conductance (Gs)	Gs 1	-	Gs 3	-
Transpiration rate (E)	E 1	-	E 3	-
Net photosynthesis (Pn)	Pn 1	-	Pn 3	-

Plants were measured at either one week or two week intervals, during the period of stress. Stomatal conductance, transpiration rate and net photosynthesis were measured after the first and third weeks, while both SLA and RWC were measured at one week intervals during the period of stress. The naming of the variables was based on the time of measurement (Table

5.1). Plate 5.1 shows selected pictures for measurements taken during implementation of the experiment.

Plate 5.1: **A:** Setting automated irrigation in the glasshouse; **B & C:** Measuring soil moisture content using TDR equipment; **D:** Sampling leaves for RWC after third week of water stress; **E:** Taking leaf area measurements for SLA; **F:** Leaves soaked in deionised water for RWC; **G:** Taking dry weight for leaf samples for RWC and SLA; **H & I:** Measuring stomatal conductance, transpiration rate and net photosynthesis.

5.2.2 Statistical analysis

Data were subjected to a normality test for all parameters measured, prior to an analysis of variance using the General Linear Model (GLM) in the SAS 9.3 programme (SAS Inc., North Carolina, USA). Means for significantly different parameters were compared using the Least Significant Difference at 5% alpha level ($LSD_{0.05}$). Data for relative water content, stomatal conductance, transpiration rate and net photosynthesis under water stress conditions were further presented in graphical form as repeated measurements, to visualise the interaction of genotypes with the period of stress (Figs. 5.1a – 1d).

5.3 Results

5.3.1 Relative water content

The two way analysis of variance showed significant RWC differences among genotypes and moisture treatments, and an interaction between genotype and moisture (Table 5.2). Moisture stress significantly reduced RWC in all the genotypes, with a significant variation among genotypes under moisture stress. The one-way ANOVA (which was used to test genotypic variation under moisture stress and well-watered conditions, separately) revealed significant differences among genotypes under moisture stress conditions for the whole period of stress.

Table 5.2: Genotypic variation for RWC measured at one week interval for a period of four weeks of water stress.

Genotype	**RWC 1**		**RWC 2**		**RWC 3**		**RWC 4**	
	WW	**WS**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**
479	0.88	0.84a	0.89	0.74a	0.90	0.67bc	0.93ab	0.52a
601	0.84	0.84a	0.87	0.77a	0.88	0.66bc	0.88c	0.54a
645	0.83	0.81a	0.84	0.76a	0.86	0.64c	0.90bc	0.51a
2226	0.91	0.83a	0.89	0.78a	0.90	0.68ab	0.94a	0.54a
2232	0.86	0.73b	0.88	0.55b	0.89	0.37d	0.93ab	0.20b
3254	0.85	0.84a	0.86	0.79a	0.89	0.72a	0.94a	0.55a
Mean	0.86	0.81	0.87	0.73	0.88	0.62	0.92	0.48
$LSD_{0.05}$		0.06		0.05		0.04	0.03	0.05
P-value	ns	0.003	ns	<0.0001	ns	<0.0001	0.01	<0.0001
Two-Way ANOVA P-values								
Genotype	0.03		<0.0001		<0.0001		<0.0001	
Moisture	0.001		<0.0001		<0.0001		<0.0001	
Interaction	0.03		<0.0001		<0.0001		<0.0001	

WW = well watered, WS= water stressed, ns = non-significant difference; Different letters within same column shows significant differences

However, no differences were observed under well-watered conditions until the fourth week of stress. Under moisture stress conditions, genotypes 479, 601, 645, 2226 and 3254 consistently exhibited significantly higher RWC, compared to genotype 2232 which showed low values of RWC for the whole period (Fig.5.1a).

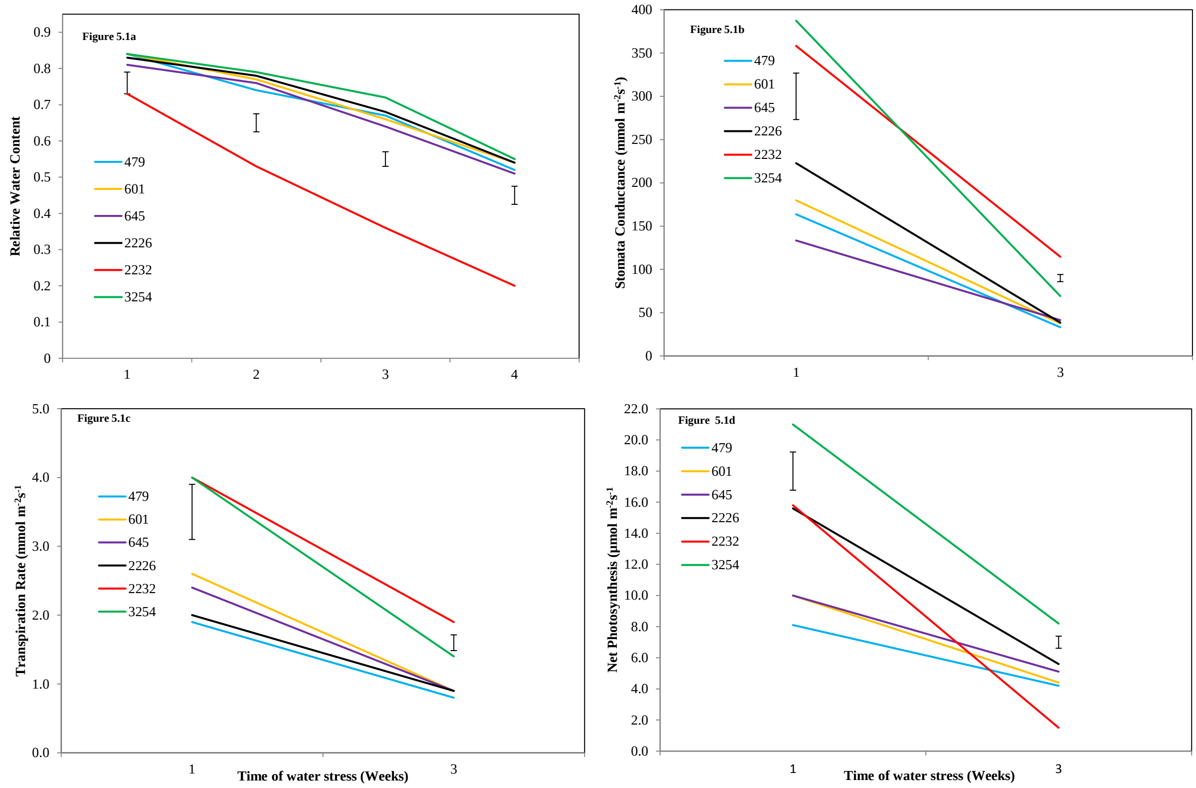

Figure 5.1: Change in relative water content at weeks 1, 2, 3 and 4 (a), stomatal conductance at weeks 1 and 3 (b), transpiration rate at weeks 1 and 3 (c) and net photosynthesis at weeks 1 and 3 (d) for each genotype over the period of water stress (Bars represent $LSD_{0.05}$).

5.3.2 Stomatal conductance (mmol $m^{-2}s^{-1}$)

Stomatal conductance differed significantly among genotypes and moisture after the first and third weeks of stress. After both periods of stress there was a significant interaction between genotype and moisture (Table 5.3). Moisture stress reduced stomatal conductance. After the first week under well-watered conditions, genotypes 2226, 2232 and 3254 showed higher stomatal conductance, than genotypes 479, 601 and 645. After the first week of stress, genotypes 2232 and 3254 had higher stomatal conductance, than other genotypes. After three weeks, genotype 2232 exhibited very high stomatal conductance under moisture stress conditions, while genotypes 479, 601, 645 and 2226 exhibited low stomatal conductance of between 33 – 42 mmol $m^{-2}s^{-1}$. Genotype 3254 showed stomatal conductance of 69.25 mmol $m^{-2}s^{-1}$. Stomatal conductance showed significant reduction between week 1 and week 3 (Fig. 5.1b).

Table 5.3: Genotypic variation for stomatal conductance (mmol $m^{-2}s^{-1}$) after one and three weeks of water stress.

Genotype	**Gs 1**		**Gs 3**	
	WW	**WS**	**WW**	**WS**
479	266.75cd	163.75c	439.25cd	33.25d
601	280.25c	179.75bc	500.75bc	37.75cd
645	237.00d	133.50c	554.50ab	41.50c
2226	494.00b	222.75b	376.50d	38.50cd
2232	556.00a	358.25a	596.50a	114.75a
3254	559.75a	387.25a	413.25d	69.25b
Mean	398.96	240.88	480.13	55.83
$LSD_{0.05}$	33.15	53.73	71.83	8.22
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Two-Way ANOVA P-values				
Genotype	<0.0001		<0.0001	
Moisture	<0.0001		<0.0001	
Interaction	<0.0001		<0.0001	

WW = well watered, WS= water stressed, ns = non-significant difference; Different letters within same column shows significant differences

5.3.3 Transpiration rate (mmol $m^{-2}s^{-1}$)

Significant differences among genotypes and moisture regimes were observed for transpiration rates after the first week (Table 5.4). One way ANOVA (which was used to test genotypic variation under specific moisture treatment) showed highly significant genotypic variation for E in both well-watered and moisture stress conditions. Genotypes 479, 601, 645 and 2226 showed lower E, compared to 2232 and 3254, which showed high values of E under both well-watered and moisture stressed conditions. Significant differences between genotypes and moisture were also found after the third week and there was significant genotype and moisture interaction. Under moisture stress, genotypes 479, 601, 645 and 2226 maintained relatively lower values of E, compared to 2232 and 3254 (Fig. 5.1c). However, genotype 2232 recorded significantly higher E than all others under moisture stressed conditions.

Table 5.4: Genotypic variation for transpiration rate (mmol $m^{-2}s^{-1}$) after one and three weeks of water stress.

Genotype	**E 1**		**E 3**	
	WW	**WS**	**WW**	**WS**
479	3.35b	1.88b	3.98b	0.83c
601	3.50b	2.63b	5.23a	0.90c
645	3.25b	2.35b	4.03b	0.93c
2226	3.45b	1.98b	4.43b	0.86c
2232	5.80a	4.05a	5.10a	1.95a
3254	6.45a	4.00a	4.18b	1.38b
Mean	4.30	2.81	4.49	1.14
$LSD_{0.05}$	0.89	0.80	0.56	0.23
P-value	<0.0001	0.0002	0.0003	<0.0001
Two-Way ANOVA P-values				
Genotype	<0.0001		<0.0001	
Moisture	<0.0001		<0.0001	
Interaction	ns		0.0001	

WW = well watered, WS= water stressed, ns = non-significant difference; Different letters within same column shows significant differences

5.3.4 Net photosynthesis (µmol$CO_2m^{-2}s^{-1}$)

After both the first and third weeks, net photosynthesis differed significantly among genotypes and between moisture treatments and there was significant interaction between the two factors (Table 5.5). Net photosynthesis under both well-watered and stressed conditions showed highly significant genotypic variation. Both genotype 2232 and 3254 exhibited high net photosynthesis under well-watered conditions, after the first and third weeks. After the first week of moisture stress, all genotypes showed lower Pn under moisture stressed conditions, compared to well-watered conditions, with genotype 3254 registering the highest Pn (Fig. 5.1d). Genotype 2226 compared very well to 2232, while genotypes 479, 601 and 645 recorded low values of Pn under moisture stressed conditions, after the first week. However, after the third week of moisture stress, genotype 2232 showed the lowest value of Pn among all genotypes. Genotypes 479, 601, 645 and 2226 recorded lower values of Pn than

3254, but higher than 2232. Genotype 3254 consistently maintained a high net photosynthesis during the period of stress.

Table 5.5: Genotypic variation for net photosynthesis ($\mu molCO_2m^{-2}s^{-1}$) after one and three weeks of water stress.

Genotype	**Pn 1**		**Pn 3**	
	WW	**WS**	**WW**	**WS**
479	15.15c	8.13c	11.33cd	4.20d
601	16.45c	10.03c	10.65d	4.43cd
645	15.20c	10.00c	12.75c	5.13bc
2226	21.93b	15.63b	16.03b	5.58b
2232	28.00a	15.75b	21.30a	1.48e
3254	28.88a	20.95a	20.63a	8.20a
Mean	20.93	13.41	15.44	4.83
$LSD_{0.05}$	2.06	2.45	1.85	0.78
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Two-Way ANOVA P-values				
Genotype	<0.0001		<0.0001	
Moisture	0.0001		<0.0001	
Interaction	0.0001		<0.0001	

WW = well watered, WS= water stressed, ns = non-significant difference; Different letters within same column shows significant differences

5.3.5 Specific leaf area (cm^2g^{-1})

Specific Leaf Area (SLA) was influenced by moisture and genotype for the whole period of stress (Table 5.6). After the first and fourth weeks of stress, significant differences were observed between moisture treatments, but there were no differences among genotypes. After the second and third weeks of stress, significant genotype and moisture differences were observed. Furthermore, a highly significant interaction between genotype and moisture was observed for SLA, after the second and third week of stress. Overall, moisture stress caused a significant increase in SLA, with a remarkable increase observed in genotype 2232. However, genotypes did not differ in SLA under well-watered conditions.

Table 5.6: Genotypic variation for specific leaf area (**cm^2g^{-1}**) measured at one week intervals for a period of four weeks of water stress.

Genotype	SLA 1		SLA 2		SLA 3		SLA 4	
	WW	WS	WW	WS	WW	WS	WW	WS
479	181.63	190.55	174.76	208.78bc	201.74	244.07b	246.97	374.06
601	183.25	185.53	190.21	217.51b	204.88	255.98b	246.97	374.06
645	174.78	180.09	176.10	187.39c	199.05	260.30b	257.67	365.03
2226	192.31	215.27	189.23	216.39b	184.40	252.45b	301.51	370.34
2232	179.43	193.89	167.31	243.08a	192.12	311.64a	255.40	443.55
3254	178.18	199.11	173.21	199.41bc	196.66	251.91b	254.18	347.56
Mean	181.59	194.07	178.47	212.09	196.47	262.73	267.51	377.46
$LSD_{0.05}$				24.70		24.42		
P-Value	ns	ns	ns	0.004	ns	0.0002	ns	ns
Two-Way ANOVA P-values								
Genotype	ns		0.03		0.0003		ns	
Moisture	0.03		0.03		<0.0001		0.0001	
Interaction	ns		<0.0001		<0.0001		ns	

WW = well watered, WS= water stressed, ns = non-significant difference; Different letters within same column shows significant differences

5.4 Discussion

5.4.1 Relative water content

Water stress reduces relative water content under moisture stress conditions and genotypes with RWC>0.50 have a high chance of recovery but, below this level, physiological injury occurs followed by death (Taiz & Zeiger, 1998). In this study, water stress caused significant variation among genotypes ranging between 0.20 and 0.55 after the fourth week of stress (Table 5.2, Fig. 5.1a). Genotypes 479, 601, 645 2226 and 3254 showed RWC>0.50 compared with 0.020 for 2232. The current results confirm previous results (Pungulani *et al*., 2012) that genotypes 479, 601, 645, 2226 and 3254 are drought tolerant due to maintenance of high RWC under moisture stress conditions. The different responses of RWC under water stress in cowpea have been attributed to water balance i.e transpiration vs water uptake (Hamidou *et al*., 2007), in addition to biochemical adjustments in plant cells (Lobato *et al*., 2008). The variation among genotypes in this study could be attributed to changes in physiological processes, including photosynthesis, thus indicating that genotypes with high RWC have a more desirable physiological response to drought.

5.4.2 Stomatal conductance

Under water stress, plants partly regulate water through reduced stomatal conductance. In this study, genotypic differences in stomatal conductance were observed under both stress and non-stressed conditions (Table 5.3, Fig. 5.1b). Genotypes 479, 601, 645 and 2226 responded to water stress by quickly closing their stomata (low stomatal conductance), for the maintenance of plant tissue water. Some drought tolerant cowpea genotypes exhibit prompt stomata closure in response to water stress, while drought susceptible ones keep their stomata open during water stress (Bertolli *et al*., 2012). Stomata closure during water stress results in improved water use efficiency in drought tolerant genotypes (Hall *et al.*, 1997), which may explain the response of genotypes to water stress in this study. In similar cowpea studies, (Anyia & Herzog, 2004a, 2004b; Souza *et al.*, 2004), low stomatal conductance reduced dehydration, as a drought avoidance mechanism. Genotypes 479, 601, 645 and 2226 may avoid drought by quickly closing stomata after sensing water stress.

Genotypes 2232 and 3254 maintained high stomatal conductance, compared with genotypes 479, 601, 645 and 2226 (Fig. 5.1b). The high stomatal conductance in 2232 and 3254 may indicate either a high transpiration of water from the leaves, or a high assimilation of carbon dioxide for increased photosynthesis or both. The high stomatal conductance in drought tolerant genotypes, such as 3254, contribute to high net photosynthesis (Fig. 5.1d), while in susceptible genotypes, such as 2232, high stomatal conductance contributes to increased water loss, which may be manifested by low RWC (Fig. 5.1a). In a similar study, Souza *et al*. (2004) identified a positive association between stomatal conductance and dehydration in drought susceptible cowpea genotypes, similar to genotype 2232. High stomatal conductance in 3254 is beneficial, through continued carbon assimilation for high net photosynthesis, while high stomatal conductance in 2232 contributes to a high transpiration rate, which consequently accelerates dehydration. In the previous study (Pungulani *et al.*, 2012), these two genotypes belonged to contrasting groups of wilting with 3254 showing tolerance to wilting and 2232 completely wilted after a water stress period of four weeks. Comparison of the stomata responses in the current study and wilting categories of the two genotypes in the previous study suggests that stomatal conductance alone may not be reliably used to classify genotypes into tolerant and susceptible categories.

5.4.3 Net photosynthesis

Net photosynthesis differed significantly among genotypes and between moisture conditions and there were significant interactions between genotype and moisture stress levels (Table 5.5, Fig. 5.1d). Generally, moisture stress reduced photosynthesis in all genotypes. However, significant differences were observed among genotypes in water stressed conditions, thus indicating the existence of genotypic differences in response to reduced availability of water. After the third week of stress, the genotypes could be grouped into three categories: a) genotype 3254 with high photosynthetic capacity; b) genotypes 479, 601, 645 and 2226 with moderate photosynthetic capacity; and c) genotype 2232 with the lowest photosynthetic capacity. The high photosynthetic capacity for genotype 3254 could be explained by high stomatal conductance, which enabled continued assimilation of carbon dioxide. By contrast, genotypes 479, 601, 645 and 2226 maintained relatively lower values of stomatal conductance and consequently low net photosynthesis under water stress. Genotypes with extremely low or no net photosynthesis under moisture stress are associated with a high metabolic impairment, which leads to disruption of cellular activities (Reddy *et al.*, 2004). The low net photosynthesis values for 2232 may be due to an early disruption of cellular processes, resulting from continued water loss from the onset of water stress resulting in loss of turgor and low relative water content (Table 5.2 & Fig. 5.1a).

Although genotypes 479, 601, 645, 2226 and 3254 maintained an active leaf canopy under water stress (Pungulani *et al*., 2012), the variation in net photosynthesis in the current study may indicate the presence of different drought response mechanisms among the genotypes. Genotypes with drought avoidance mechanisms exhibit reduced net photosynthesis, due to early stomata closure (Bertolli *et al*., 2012), while genotypes with drought tolerance exhibit high net photosynthesis, due to continued carbon dioxide assimilation under severe moisture stress (Costa França *et al*., 2000). Consequently, genotypes 479, 601, 645 and 2226, with relatively lower values of net photosynthesis, may have a drought avoidance mechanism, while genotype 3254 may have a true drought tolerance mechanism. Genotype 3254 maintained high stomatal conductance and high net photosynthesis, which indicates its ability to assimilate carbon dioxide for photosynthesis, even under low soil moisture.

5.4.4 Relationships among physiological parameters

Under water stressed conditions, drought response patterns for the six genotypes were explored using interaction plots from the repeated-measurements (Figs. 5.1a–5.1d). Genotypes 479, 601, 645 and 2226 maintained low stomatal conductance (Fig. 5.1b) and low transpiration rate (Fig. 5.1c) after the first week of stress, compared to 2232 and 3254. After the third week of stress, genotype 2232 maintained high stomatal conductance and high transpiration rate, while genotype 3254 had a somewhat reduced transpiration rate. Separation of genotypes 2232 and 3254, after the third week of stress, was consistent with a drought tolerance mechanism in 3254 (Pungulani *et al*., 2012). Comparison of Figures 5.1b and 5.1c indicates three categories of response to drought among the six genotypes. The first mechanism was the reduction of stomatal conductance and transpiration rate at the onset of water stress (after the first week), as shown by genotypes 479, 601, 645 and 2226. However, genotypes 2232 and 3254 still maintained high stomatal conductance and transpiration rate in the early stages of water stress. The second response type was the maintenance of high stomatal conductance and high transpiration after prolonged water stress, as exhibited by genotype 2232. The response of 2232 indicates continued loss of water through its high stomatal conductance, despite water stress conditions. The continued water loss by 2232 is supported by the consistently low RWC observed during the water stress period (Fig. 5.1a). The third response category was shown by genotype 3254, which reduced both stomatal conductance and transpiration rate after a more prolonged period of stress. Therefore, based on the stomatal conductance and transpiration rate, the six genotypes can be categorically classified into drought avoidance (479, 601, 645 and 2226), drought tolerance (3254) and drought susceptible (2232) responses.

Stomatal conductance has a more direct association with transpiration than it does with net photosynthesis. Figures 5.1b and 5.1c show a positive association between stomatal conductance and transpiration rate. The drought susceptible genotype exhibited high stomatal conductance, which contributed to high transpiration rate. However, Figures 5.1b and 5.1d showed no clear positive association between stomatal conductance and net photosynthesis: i.e. increase in stomatal conductance did not result in improved net photosynthesis. A strong relationship between stomatal conductance and transpiration was previously observed in cowpea, leading to a conclusion that stomatal conductance controls transpiration rate more than net photosynthesis (Singh & Raja, 2011). The limited association of stomatal conductance with net photosynthesis suggests the presence of additional non-stomata mechanisms controlling photosynthesis in drought tolerant genotypes.

Net photosynthesis under moisture stress conditions depends on water content in the leaf photosynthetic machinery. In this study, the comparison of genotypes 3254 and 2232 shows the importance of water potential and cell turgor in regulating photosynthesis. The drought tolerant genotype exhibited high net photosynthesis (Fig. 5.1d) and high relative water content (Fig. 5.1a), while the susceptible genotype (2232) showed low photosynthesis and low relative water content after the third week of stress. In drought tolerant genotypes, both high net photosynthesis and RWC are known to be ensured by both stomatal and non-stomata mechanisms. The non-stomata mechanisms, which control net photosynthesis and RWC in drought tolerant genotypes, include cell membrane stability, high proline accumulation and high soluble carbohydrates (Khan *et al*., 2007). The non-stomatal mechanisms may explain why drought tolerant genotypes were able to maintain high RWC and continue photosynthesising even under very low moisture content.

5.4.5 Specific leaf area

Water stress significantly increased specific leaf area in all the genotypes, with more increase observed in genotype 2232 (Table 5.6). The increase in SLA, due to water stress, was previously also observed in cowpea (Anyia & Herzog, 2004b) and wheat (Bogale *et al*., 2011). In the previous study (Pungulani *et al*., 2012), genotypes 479, 601, 645, 2226 and 3254 maintained an active canopy after four weeks of moisture stress, unlike 2232, which showed drought susceptible characteristics in the early stages of stress. Drought tolerant genotypes, which maintain low SLA under drought conditions, are associated with high water use efficiency (Songsri *et al*., 2009). Girdthai *et al*. (2012) recommended selecting genotypes with low SLA under water stress for drought tolerance breeding. The comparatively small SLA under water stress of the genotypes that previously maintained an active canopy further supports the importance of SLA in selecting genotypes with drought tolerance.

Although water stress increased SLA in this study and others, water stress generally reduces SLA (Liu & Stützel, 2004; Stutzel & Liu, 2004). These differences may be due to a modification in the leaf sampling method for leaf area and dry matter measurements. As a rule of thumb, SLA should be calculated from the fully expanded young and photosynthetically active leaves, which is the case in studies that have shown a reduction in SLA (Pérez-Harguindeguy *et al*., 2013). This study measured leaf area from all fresh leaves on the plant, which meant that most genotypes had an increased leaf area. It is proposed that the inclusion of all fresh leaves allows a better understanding of genotypic variation of SLA under water stress. The use of all the fresh leaves should be applied in species such as cowpea, in which most genotypes maintain active leaves and derive assimilates from the lower leaves to support photosynthesis in younger leaves under water stress (Mai-Kodomi *et al*., 1999).

Cowpea is evidently a very sensitive crop to water stress, as it completely ceases generation of new leaves (terminal bud dormancy). A comparison between cowpea and soybean showed that cowpea maintained a 40% larger leaf area under water stress (Bertolli *et al*., 2012). In our study, the leaf area remained almost unchanged, but significant variation in dry matter among genotypes under water stress was observed (data not shown). This means that differences in SLA between the current and other studies could be attributed mainly to changes in leaf dry mass, rather than changes in leaf area (Bogale *et al*., 2011). The terminal bud dormancy and prolonged maintenance of fresh leaves probably contributed to an increase in SLA, since leaf area remained almost constant (despite water stress), while dry matter changed.

The drought tolerant genotypes may have the capacity to photosynthesise and derive less assimilates from the old leaves, hence maintaining relatively high leaf dry matter and reduced SLA. Drought susceptible genotypes, instead of photosynthesising more, may completely rely on the available assimilates from the old leaves. This could be one of the factors which led to a large increase in SLA in genotype 2232, compared to other genotypes under moisture stress. Drought tolerant genotypes, with lower SLA under water stress, showed an ability to photosynthesise and accumulate more dry matter, compared to susceptible genotypes. Plants with low SLA are associated with high nitrogen content and more mesophyll cells per unit area which lead to high biomass production due to active photosynthesis (Thumma *et al*., 2001). Our results show that the five genotypes (479, 601, 645, 2226 and 3254) continued photosynthesising (Fig. 5.1d) regardless of moisture stress and hence this reduced SLA, as a result of increased leaf dry matter.

The similarities among genotypes under water stressed conditions, after both the first and fourth weeks of stress (Table 5.6), may indicate that variation in SLA is not very informative under adequate moisture, or severe drought stress. Although this requires further validation, it is suggested that the use of SLA for drought tolerance assessment should be limited to moderate moisture stress that excludes the initial phase of moisture stress and under severe water stress, as in weeks two and three of the present study. Comparison of RWC (Table 5.2) and SLA (Table 5.6) suggests that SLA may be applicable when RWC falls between 0.80 and 0.50.

5.5 Conclusion

Genotypes 479, 601, 645, 2226 and 3254 maintained higher photosynthetic capacity under water stress, compared to genotype 2232. The genotypes which maintained a high photosynthetic capacity showed variation in both stomatal conductance and net photosynthesis, thus indicating the presence of different physiological mechanisms governing the responses to moisture stress. In summary, genotype 3254 possesses drought tolerance, due to high stomatal conductance and net photosynthesis under water stress; genotypes 479, 601, 645 and 2226 possess drought avoidance characteristics, due to low stomatal conductance at the onset of water stress; and genotype 2232 possesses drought susceptible characteristics, due to high stomatal conductance, high transpiration rate and low net photosynthesis under water stressed conditions. Therefore, based on these physiological responses, genotype 3254 is being recommended as a good parental line for drought tolerance breeding that targets areas with severe rainfall shortage; genotypes 479, 601, 645 and 2226 could be used for breeding varieties that target areas with mild drought; and 2232 could be improved by crossing it with drought tolerant genotypes. However, desirable parentage would be appropriately determined after testing the field performance of all genotypes and exposing them to farmers' preference tests. Although genotype 2232 showed negative performance under severe drought conditions, its high photosynthetic rate and presumably high biomass production under well watered conditions render it useful in areas where drought is not a big problem. Therefore, this genotype could be considered for production in areas with good rainfall.

Besides the maintenance of relatively high photosynthetic capacity, genotypes 479, 601, 645, 2226 and 3254 exhibited low SLA, while genotype 2232 maintained exceptionally high SLA under moisture stress. However, SLA did not identify the genotypes in the drought avoidance and drought tolerance groups, which suggests that it is no better than using canopy maintenance alone. In addition to field testing of these genotypes, the application of SLA in drought tolerance evaluation needs further research. Attention should be focused on the comparison between SLA calculated from all the leaves on the plant and SLA from newly expanded leaves. Such studies would help to test the hypothesis that the use of all fresh leaves on a plant in cowpea would improve the use of SLA in drought tolerance evaluation, in contrast to the use of only newly expanded leaves.

Chapter 6 : Evaluation of eight cowpea genotypes for variability in reproductive characteristics, yield and seed size at five experimental sites in Malawi

Abstract

Five drought tolerant genotypes (479, 601, 645, 2226 and 3254), one drought susceptible genotype (2232) and two released varieties (Sudan 1 and IT82E16) were evaluated in a field experiment at five research stations during the 2012/13 season to identify potential genotypes with superior agronomic performance. Measurements were taken on days to first flowering; days to 50% flowering; days to first pods; days to 50% pods; days to first mature pods; number of pods per plant; number of seeds per pod; weight of pods per plant (g); weight of grain per square metre (g/m^2); and 100 seed weight (g). Sudan 1, IT82E16, 479 and 601 flowered and matured early, compared with 645, 2226 and 2232. Sudan 1, 601 and 2232 at Chitedze and 3254 at Kasinthula had higher yields than the remaining genotypes. Genotype 3254 consistently showed high yields at sites with low rainfall and high temperature. Although 2232 was among the high yielding genotypes at Chitedze, its yield was reduced significantly at Baka, Chitala and Kasinthula sites with low rainfall. Genotype 2226 showed 100 seed weight >20g at all sites compared with 3254 which showed 100 seed weight of between 17-19g and 601 and Sudan1 which showed 100 seed weigh 14g across the sites. However, 100 seed weight for 2232 reduced at the sites with low rainfall and high temperature. The early maturing and drought tolerant genotypes, late maturing and drought tolerant genotypes, high yielding and large seeded genotypes will form the basis for improved research and production of cowpea in Malawi. The genotypes with large seeds provide an opportunity for breeding varieties that can be used for the commercialising cowpea in Malawi, compared to the existing released small seeded varieties which are preferred for domestic consumption.

6.1 Introduction

Drought tolerance screening of cowpea germplasm from the Malawi Plant Genetic Resources Centre (MPGRC) showed that five genotypes (479, 601, 645, 2226 and 3254) survived low moisture stress under glasshouse conditions (Pungulani *et al*., 2012). However, because screening was undertaken at the vegetative stage in a glasshouse, utilisation of these genotypes for crop improvement requires information on their adaptation to different field environments. Studies undertaken elsewhere show that cowpea is very sensitive to environmental and geographical conditions, such as temperature, photoperiod, altitude and latitude (Adeigbe *et al.,* 2011; Adewale *et al*., 2010; Patel & Jain, 2012; Shiringani & Shimelis, 2011). Therefore, the sensitivity of cowpea to environmental conditions compels researchers to test the performance of new genotypes with desirable attributes such as drought tolerance under field conditions.

This study evaluated five drought tolerant genotypes alongside one susceptible genotype and two released varieties, for adaptation to different environments in Malawi. Specific objectives of this research were:

a) To identify the variability of reproductive and yield traits as well as the seed size of the eight cowpea genotypes;
b) To identify cowpea landraces with potentially desirable agronomic attributes, in comparison with the two released varieties.

It is anticipated that the results will improve cowpea production, through the identification of genotypes with desirable characteristics for entry into the national cowpea breeding programmes.

6.2 Materials and methods

6.2.1 Site characteristics

The experiment was conducted at five sites (Baka, Bvumbwe, Chitala, Chitedze and Kasinthula) during the 2012/2013 cropping season. The trial at Bvumbwe, Chitala and Chitedze were planted at the end of November while at Kasinthula and Baka the trial was planted in the second week of December. Locations of the experimental sites are presented in Appendix I. The general characteristics of the sites are provided in Table 6.1 and a detailed weather pattern for the 2012/2013 cropping season is provided in Figures 6.1a–6.1e.

Table 6.1: Characteristics of the five experimental sites.

Site	**Altitude (m)**	**Annual Rainfall (mm)**	**Mean Minimum Temperature (˚C)**	**Mean Maximum Temperature (˚C)**	**Longitude (Degrees East)**	**Latitude (Degrees South)**
Chitedze	1146	855	18	29	33.63	13.97
Chitala	606	676	23	32	34.25	13.67
Bvumbwe	1201	1155	16	26	35.07	15.92
Kasinthula	60	477	24	34	34.82	16.08
Baka	497	695	22	32	33.98	10.00

6.2.2 Genetic material

The experiment used eight genotypes (479, 601, 645, 2226, 3254, 2232, Sudan 1 and IT82E16). A description of each genotype is outlined in Table 6.2 below.

Table 6.2: Description of cowpea genotypes evaluated in the five sites.

Genotypes	**Status**	**Reference**
479	Drought tolerant landrace	(Pungulani *et al*., 2012)
601	Drought tolerant landrace	(Pungulani *et al*., 2012)
645	Drought tolerant landrace	(Pungulani *et al*., 2012)
2226	Drought tolerant landrace	(Pungulani *et al*., 2012)
2232	Drought susceptible landrace	(Pungulani *et al*., 2012)
3254	Drought tolerant landrace	(Pungulani *et al*., 2012)
Sudan 1	Released variety	(Government of Malawi, 2000)
IT82E16	Released variety	(Government of Malawi, 2000)

6.2.3 Evaluation procedure

The experiment was laid in a randomised complete block design (RCBD) with genotypes, as treatments, replicated three times per site. Each genotype was planted on a plot consisting of three rows of 4m each spaced at 75cm apart, with 30cm between planting stations. Four seeds were planted which were later thinned to three per station. Plots were manually weeded to control weeds for four times during the period of experiment. Dimethoate (Rogor 40EC) and Cypermethrin were applied at three week interval to control major insect pests (Pod borers, thrips, pod sucking buds and aphids). Both pesticides were applied at the rate of 200g a.i /ha using a Knapsack sprayer.

The following traits were measured on a plot basis: number of days to first flowering; number of days to 50% flowering; number of days to first pod; number of days to 50% podding; and number of days to first mature pod. The number of days was calculated with reference to the date the experiment was planted. A sample of twenty plants randomly selected from the middle ridge in each replicate was used to measure the number of pods per plant, number of seeds per pod 100 seed weight (g) and grain weight per square metre (g/m^2). Both 100 seed weight and grain weight were measured at 12% moisture content. At maturity pods were hand harvested and sundried in net bags for one week. After drying the pods were hand shelled and further dried to 12% moisture content.

6.2.4 Statistical analysis

All the measured characters were subjected to an analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in the SAS 9.3 statistical package (SAS Inc. North Carolina, USA). Genotypes were considered fixed effects while sites were considered random effects. Means for genotypes, sites and interaction between genotypes and sites were compared using the Least Significant Differences at 5% alpha level ($LSD_{0.05}$). Pearson's correlation coefficients were calculated to understand the relationship between different variables.

Data for days to first mature pod, grain weight per square metre (g/m^2) and 100 seed weight (g) were further subjected to stability analysis using GGEBiplot model (Yan, 2001) in R Statistical package (Frutos *et al.*,2014; R Core Team, 2014). Use of GGEBiplot in evaluating genotypes across environments is relatively advantageous over other commonly used models (Setimela *et al.*, 2007) such as the regression model by Eberhart and Russell (1966). The most unique feature of GGEBiplot is the graphical presentation of the relationship between genotypes and environments in two dimensions. Specifically GGEBiplot shows: a) mean performance and stability of genotypes across a subset of environments; b) the discriminatory power of environments on performance of the genotypes and; c) mega-environments for efficient breeding by separating subsets of environments. Three biplot tools were used to assess the performance of the eight genotypes across five environments (sites) as follows:

- Which won where/what for identifying the best genotype in each environment
- Mean against stability for visualising the mean performance and stability of a genotype
- Discriminativeness against representativeness for identifying representativeness and discriminating power of environments based on the performance of genotypes.

Biplots were generated from first two principal components (PC 1 and PC 2) which were derived from model defined by no scaling, tester-centred (environment centred) and singular value decomposition (SVD). Biplots for “Which won where/what” and “Mean against stability” with focus on genotypes were based on row metric preserving (1 “JK”) SVD while biplot for “Discriminativeness against representativeness” with focus on environments was based on column metric preservation (2 “GH”) SVD (Frustos *et al.*, 2014). In the analysis sites only were coded for easy presentation in the biplots as follows Baka (BK), Bvumbwe (BV), Chitala (CT), Chitedze (CZ) and Kasinthula (KS).

6.3 Results

6.3.1 Weather patterns for experimental sites

Malawi is characterised by a unimodal rainfall season of approximately five months from November to April in most parts of the country. During the 2012/2013 cropping season, rainfall and temperature data were recorded from all five experimental sites (Figs. 6.1a – 6.1e). The sites received varying amounts of annual rainfall between October and April, with Bvumbwe receiving more rainfall (1155mm) followed by Chitedze (855mm), Baka (695mm), Chitala (676) and Kasinthula (477mm). The sites experienced uneven monthly rainfall distributions, with Baka receiving a maximum monthly rainfall in March, Bvumbwe in February, Chitedze in December and Chitala and Kasinthula in January.

The highest maximum average temperature for the season was recorded at Kasinthula (34°C), followed by Baka and Chitala (32°C), Chitedze (29°C) and Bvumbwe (26°C). The highest minimum average temperature was recorded at Kasinthula (24°C), followed by Chitala (23°C), Baka (22°C), Chitedze (18°C) and Bvumbwe (16°C). Generally, all sites experienced a similar pattern of temperature changes with higher temperatures at the beginning of the cowpea growing season and a decline towards the end of the season.

6.3.2 Descriptive statistics of measured characters

Summary statistics show that there was considerable variation among the eight genotypes for all the characters measured. Table 6.3 shows mean, minimum, and maximum values, coefficient of variation (CV) and standard deviations of all the characters measured. The most variable characters were the number of pods per plant, weight of pods per plant and weight of grain per square metre, which all showed high CVs of 39.3, 39.3 and 41.2 respectively. A narrow variation was observed in pod length, which showed the lowest CV of 14.9.

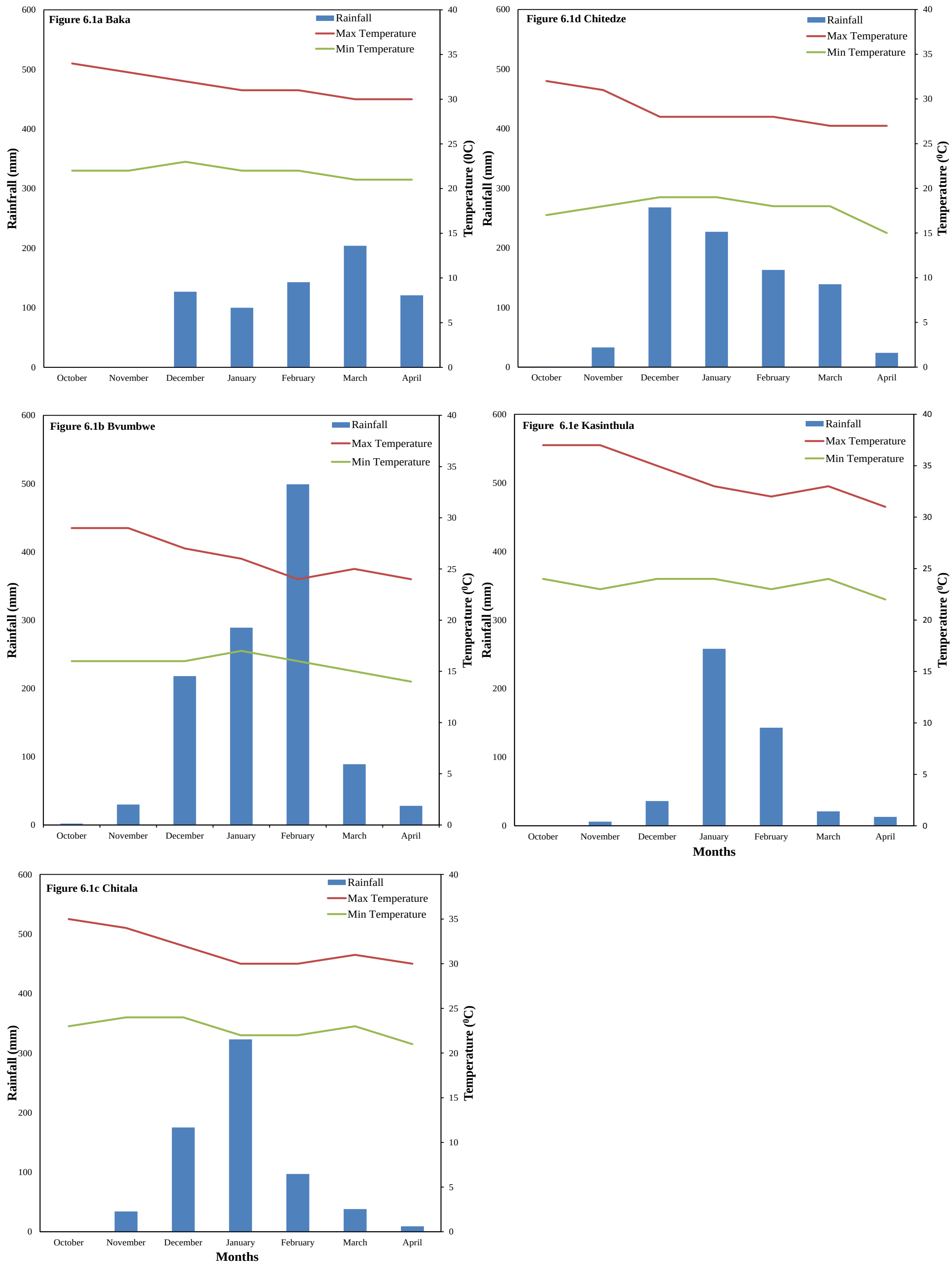

Figure 6.1: Monthly average rainfall, maximum and minimum temperatures for 2012/2013 growing season of the five experimental sites.

Table 6.3: Mean, minimum (min), maximum (max) values and coefficient of variations (CV) for all characters measured.

Variable	n	Mean	Min	Max	CV	StDev
Days to first flowering (DFF)	40	53.7	38.7	94.7	26.7	14.4
Days to 50% flowering (D0.5F)	40	61.5	42.3	109.0	30.1	18.5
Days to first pod (DFP)	40	58.3	41.3	100.3	27.5	16.0
Days to 50% podding (D0.5P)	40	68.1	48.7	117.7	31.0	21.1
Days to first mature pod (DFMP)	40	73.1	51.7	122.3	24.0	17.6
Number of pods per plant (PODS)	40	24.1	6.7	46.3	39.3	9.5
Seeds per pod (SEEDSPP)	40	14.4	6.7	17.7	18.2	2.6
Weight of pods per plant (PODWT) (g)	40	30.7	8.5	59.1	39.3	12.1
Weight of grain (GRAINWT) (g/m^2)	40	151.5	41.2	269.3	41.2	62.4
100 seed weight (SEEDSIZE) (g)	40	15.9	9.8	25.3	24.6	3.9

Pearson correlation coefficients (Table 6.4) show strong and significant relationships (r≥0.91) among reproductive characters (Days to first flowering (DFF), 50% to flowering (D0.5F), days to first pod (DFP), days to 50% podding (D0.5P) and days to first mature pod (DFMP).

Table 6.4: Pearson correlation coefficients for all characters measured

	DFF	D0.5F	DFP	D0.5P	DFMP	PODS	SEEDSPP	PODWT	GRAINWT
D0.5F	0.96**								
DFP	0.97**	0.93**							
D0.5P	0.95**	0.96**	0.95**						
DFMP	0.93**	0.92**	0.91**	0.94**					
PODS	-0.37**	-0.30**	-0.38**	-0.26**	-0.39**				
SEEDSPP	-0.18*	-0.17	-0.16	-0.13	-0.19*	0.02			
PODWT	-0.36**	-0.29**	-0.37**	-0.25**	-0.37**	0.97**	0.03		
GRAINWT	-0.37**	-0.30**	-0.38**	-0.26**	-0.38**	0.99**	0.03	0.98**	
SEEDSIZE	0.58**	0.56**	0.60**	0.58**	0.50**	-0.12	-0.24**	-0.11	-0.13

N = 40
**, * . Correlation is significant at the level 0.01 and 0.05, respectively.
DFF = number of days to first flowering; DO.5F = number of days to 50% flowering; DFP = days to first pod; DO.5P = days to 50% pod set; DFMP = days to first mature pod; 100SEEDWT = 100 seed weight; SEEDPP = number of seeds per pod; PODL = pod length ; WTPODS = weight of pods per plant; GRAINWT = grain weight per square meter; and PODS = number of pods per plant

Similarly, correlation coefficients among yield related characters (number of pods per plant (PODS), Weight of pods per plant (PODWT), and Weight of grain (GRAINWT)) showed significant and strong relationships ($r \geq 0.96$). Both seeds per pod and seed size showed significant but weak relationships with reproductive and yield related characters. Therefore, based on the high correlation coefficients, detailed statistical results will be presented only for days to first flowering and days to first mature pods for reproductive characters, together with grain weight per square metre, number of seeds per pod and seed size (100 seed weight) for yield related characters.

6.3.3 Reproductive characteristics

6.3.3.1 Number of days to first flowering

Days to flowering differed significantly among genotypes, sites and interaction between genotypes and sites (Table 6.5 and Fig. 6.2). In general, the results showed three categories of genotypes for flowering pattern. Sudan 1, 479, 601 and IT82E16 took shorter periods (39-52 days) to flower across the sites. In contrast, 645, 2226 and 2232 took more days (52 -95 days) to flower and 3254 was intermediate, taking between 40 and 63 days. In terms of site performance, all the genotypes took significantly more days to flower at Bvumbwe and Chitedze (the cooler sites), while at Kasinthula, Baka and Chitala (the warmer sites) flowering time was significantly reduced. Flowering at Bvumbwe ranged between 49 and 95 days, while at Chitedze it ranged between 50 and 84 days. Flowering among the early flowering sites ranged between 39 and 63 days, i.e. 41 to 61 days, 39 to 55 days and 42 to 63 for Baka, Chitala and Kasinthula, respectively.

The interaction between genotypes and environment (sites) shows that the flowering pattern of the genotypes was differentially affected by the environment (Fig. 6.2). The early flowering genotypes did not show significant variation within each site, but there was significant variation between the warmer and cooler sites. Genotype 3254 did not significantly differ from the early flowering genotypes at Chitala and Baka, but it flowered later than the early flowering genotypes at Bvumbwe, Chitedze and Kasinthula. At

Bvumbwe, 645 was the latest flowering genotype, but at Chitedze it flowered at the same time as 2226 and at Kasinthula and Baka it did not significantly differ from 2232. Among the late flowering genotypes, 2226 consistently took the most days to flower at Chitedze, Kasinthula, Baka and Chitala.

Table 6.5: Means of main effects (genotype and site) on flowering and maturity periods.

Factor	Days to first flowering	Days to first mature pod
Genotypes (n=15)		
Sudan 1	44.9	62.5
479	45.3	64.1
601	44.5	62.3
645	67.3	87.4
2226	69.9	84.9
2232	62.3	86.8
3254	50.3	70.3
IT 82E16	45.5	64.9
P- Value	**<0.0001**	**<0.0001**
$LSD_{0.05}$	3.9	4.1
Site (n=24)		
Baka	46.7	58.8
Bvumbwe	65.9	92.5
Chitala	45.4	65.3
Chitedze	62.0	83.8
Kasinthula	48.6	65.0
P- Value	**<0.0001**	**<0.0001**
$LSD_{0.05}$	3.1	3.3
Interaction		
(Genotype*Site) P- Value	**<0.0001**	**<0.0001**
Grand Mean	53.7	73.1

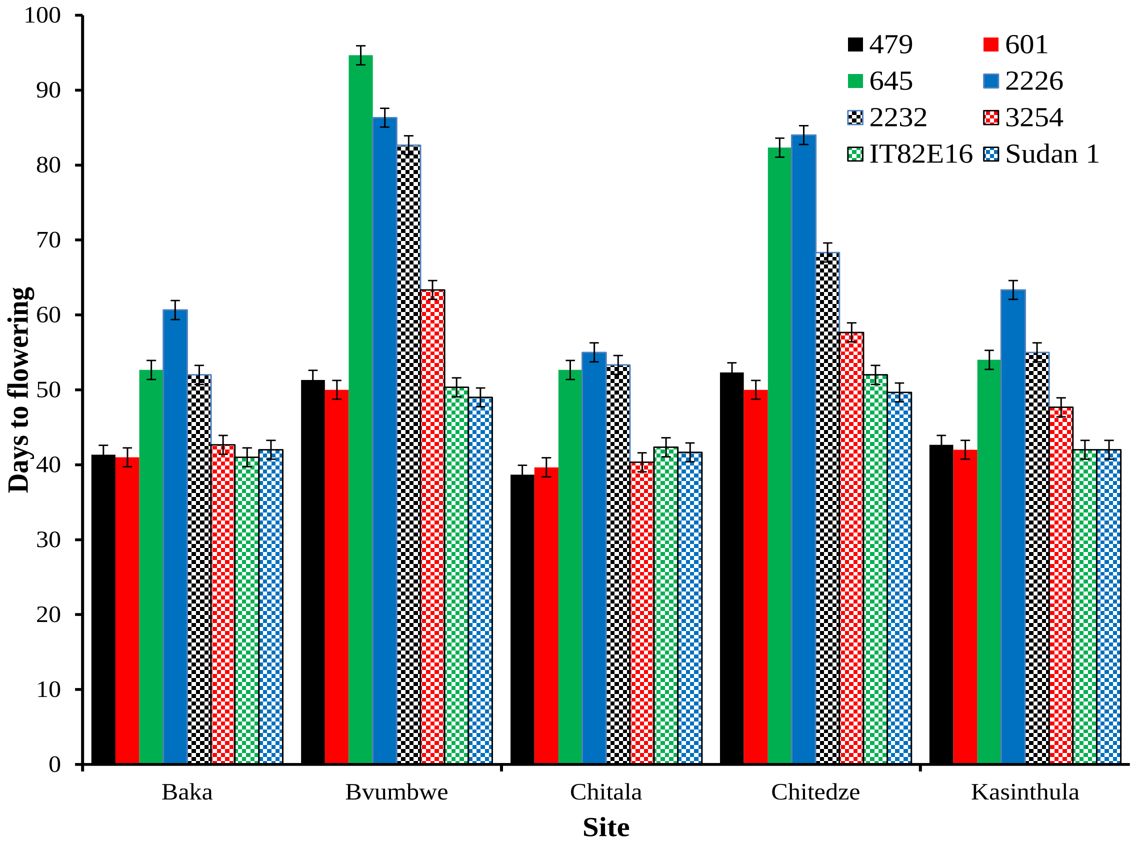

Figure 6.2: Number of days to first flowering (DFF) of the eight genotypes at five experimental sites (Bars represent LSD0.05)

6.3.3.2 Number of days to first mature pod (Maturity)

Days to maturity showed highly significant variation among genotypes, sites and interaction between genotype and site (Table 6.5 & Fig. 6.3). As with the flowering pattern, the maturity period showed the same three distinct groups among the eight genotypes. In general, Sudan 1, 479, 601 and IT82E16 consistently matured early, while 645, 2232 and 2226 showed late maturity and 3254 showed intermediate maturity across all the sites. Among the sites, Bvumbwe showed the longest average maturity period followed by Chitedze, Kasinthula, Chitala and Baka.

The variation in ranges for maturity period (DFMP) (Fig. 6.3) among sites confirmed that the maturity periods of the genotypes depended on environment (site). Sudan 1, 479, 601 and IT82E16 (the early maturing genotypes) showed ranges of 52 – 75 (23 days), 54 – 80 (26 days), 53 – 77 (24 days) and 55 – 78 (23 days), respectively, while the late maturing genotypes showed ranges of 64 – 122 (58 days), 68 – 107 (39 days) and 64 – 110 (46 days) for 645, 2226 and 2232, repectively. The intermediate maturing genotype showed a range of 60 – 89 (29 days). The small range for the early maturing genotypes showed the more limited response of these genotypes to the environment. However, the wide range among the late maturing genotypes showed that these genotypes were very responsive to the environment. Genotype 645 showed the largest range of 58 days, thus indicating that this genotype was the most responsive to changes in the environment. This genotype took markedly more days to mature at Bvumbwe, while its maturity at Chitala, Kasinthula and Baka was more smilar to the other late maturing genotypes. Among the late maturing genotypes, 2226 took the shortest period to mature at Bvumbwe, Chitedze and Chitala. The early maturing genotypes showed no significant differences within sites.

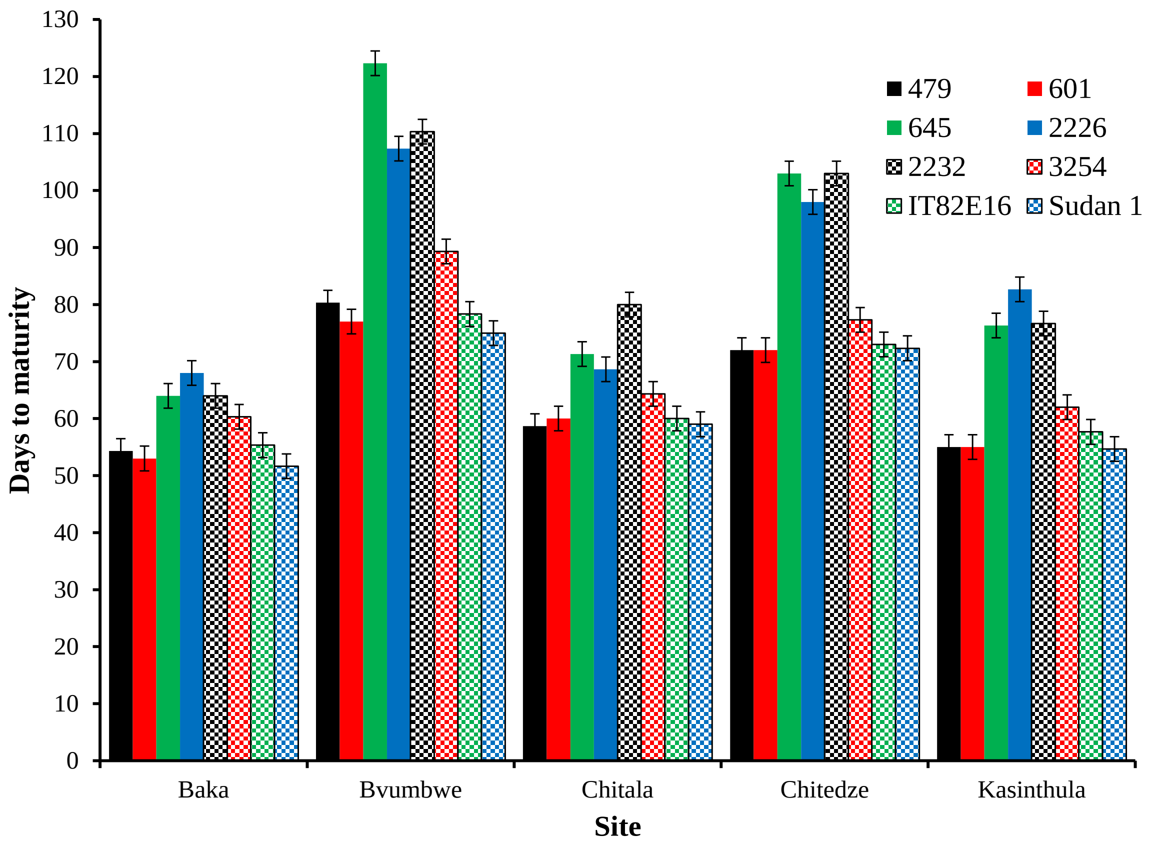

Figure 6.3: Number of days to first mature pods (DFMP) of eight genotypes at five experimental sites. (Bars represent $LSD_{0.05}$).

The GGE biplot showed both Axis 1 and Axis 2 accounted for 97.7% of the total variation for days to first mature pods with Axis 1 contributing to 95.6% (Fig. 6.4). Sudan 1, 2226 and 645 formed the vertex genotypes of the polygon for the which won where/what model (Fig. 6.4a).

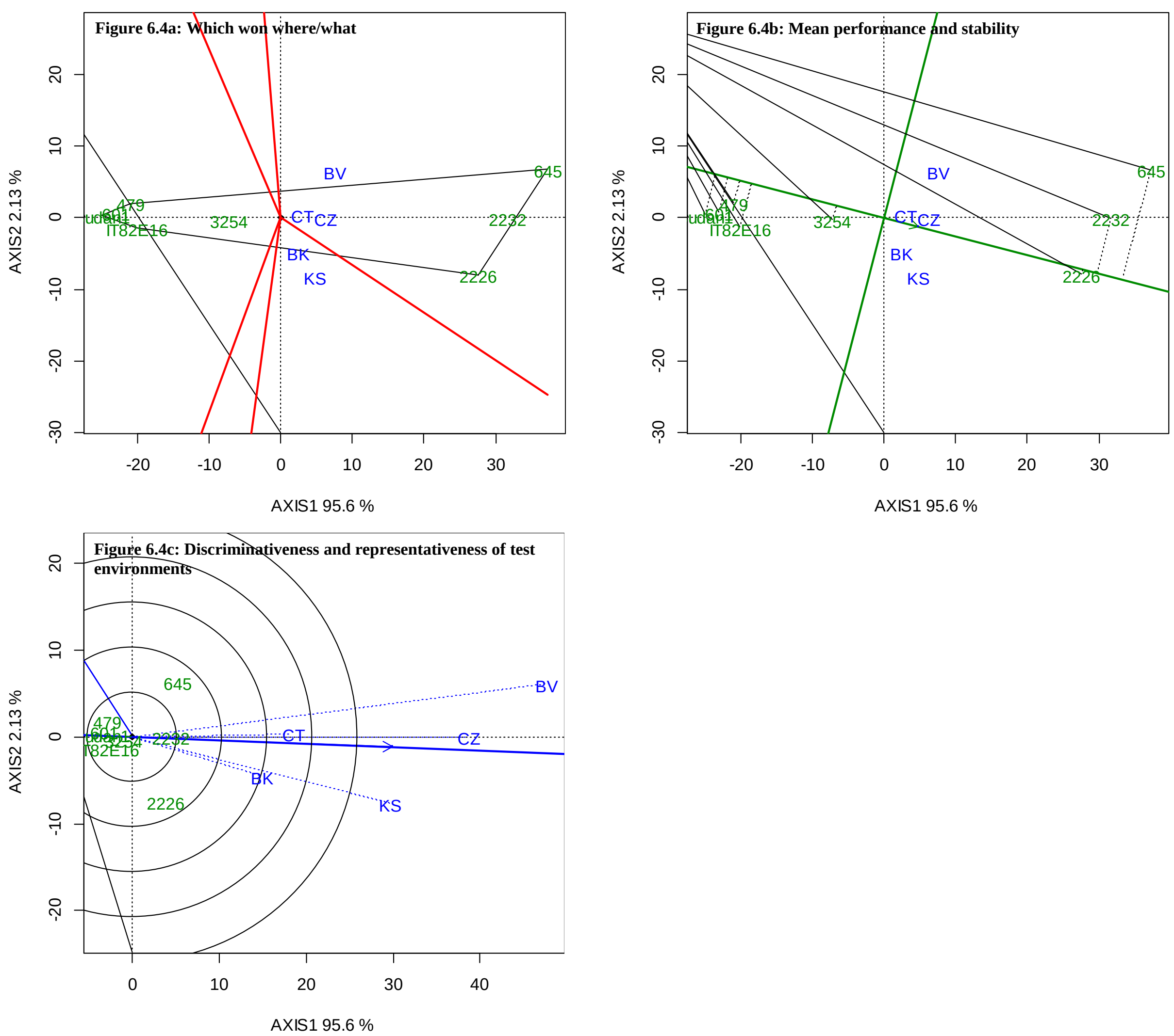

Figure 6.4: Three GGE biplots for maturity of the eight genotypes tested at Baka (BK), Bvumbwe (BV), Chitala (CT), Chitedze (CZ) and Kasinthula (KS). a) Polygon for which genotypes won in which environment; b) Mean performance and stability and; c) Representativeness and discriminating power of the test environments.

Bvumbwe, Chitedze and Chitala were located in a sector where 645 and 2226 were vertex genotypes. However, Sudan 1 fell in a sector where site is not represented. Genotype 645 was located at the highest point of abscissa (the arrow for the single-arrowed line) followed by 2232 and 2226 (Fig. 6.4b). Genotype 2226 showed the least projection from the abscissa while 645 showed the greatest projection. Sudan 1 was located near the base of the abscissa adjacent to 601, 479, IT82E16 and 3254. Among the genotypes on the negative side of the abscissa 3254 showed least projection. Bvumbwe represented the most discriminating sites due to the longest vector from the origin of the biplot while Baka showed the shortest vector (Fig. 6.4c). Both Chitedze and Chitala showed the least deviation from the single-arrowed line indicating that they were the most representative among the five tested sites. However, Chitedze was more discriminating than Chitala.

6.3.4 Yield characteristics

6.3.4.1 Number of seeds per pod

The number of seeds per pod varied significantly among genotypes, sites and the interaction between genotypes and sites was significant (Table 6.6 & Fig. 6.5). In general, Sudan 1 and IT82E16 showed a high number of seeds per pod at all sites, except for IT82E16 at Chitedze. On the contrary, across sites 3254 showed a significantly lower number of seeds per pod than all genotypes, with the lowest recorded at Bvumbwe. A wide variation was observed for 645, which recorded a high number of seeds per pod at Chitedze and the lowest number of seeds per pod at Bvumbwe.

Comparison of genotypes within sites (Fig. 6.5) showed that 3254 and 2226 had significantly fewer seeds per pod than other genotypes at Kasinthula; at Baka 2232 had significantly fewer seeds than IT82E16, 645 and 479; at Bvumbwe 645 and 3254 showed a significantly low numbers of seeds. At Chitedze, 479 was significantly lower than 645, 2226, Sudan 1 and 2232, while 645 and 2226 showed significantly higher numbers of seeds per pod than 601, 3254, IT82E16 and 479. At Chitala, 2226 and 3254 had significantly fewer seeds per pod than IT82E16. This resulted in a significant interaction term for this genotype and was manifested by very large changes in rank from site to site.

Table 6.6: Means of main effects (genotype and site) on number of seeds per pod, grain yield and 100 seed weight.

Factor	**Number of seeds per pod**	**Grain yield (g/m^2)**	**100 seed weight (g)**
Genotypes (n=15)			
Sudan 1	15.6	186.4	11.4
479	14.8	143.1	14.5
601	14.9	163.8	13.0
645	13.8	125.7	17.5
2226	14.5	147.3	22.1
2232	14.2	119.7	17.8
3254	11.5	178.2	18.0
IT 82E16	16.1	147.5	13.0
P- Value	**<0.0001**	**<0.0001**	**<0.0001**
$LSD_{0.05}$	1.9	33.2	1.5
Site (n=24)			
Baka	14.6	162.0	14.5
Bvumbwe	14.3	79.5	16.6
Chitala	13.6	153.1	17.6
Chitedze	13.9	193.9	15.6
Kasinthula	15.6	168.9	15.3
P- Value	**<0.0001**	**<0.0001**	**<0.0001**
$LSD_{0.05}$	1.5	26.3	1.2
Interaction			
(Genotype*Site) P- Value	**<0.0001**	**<0.0001**	**<0.0001**
Grand Mean	14.4	151.5	15.9

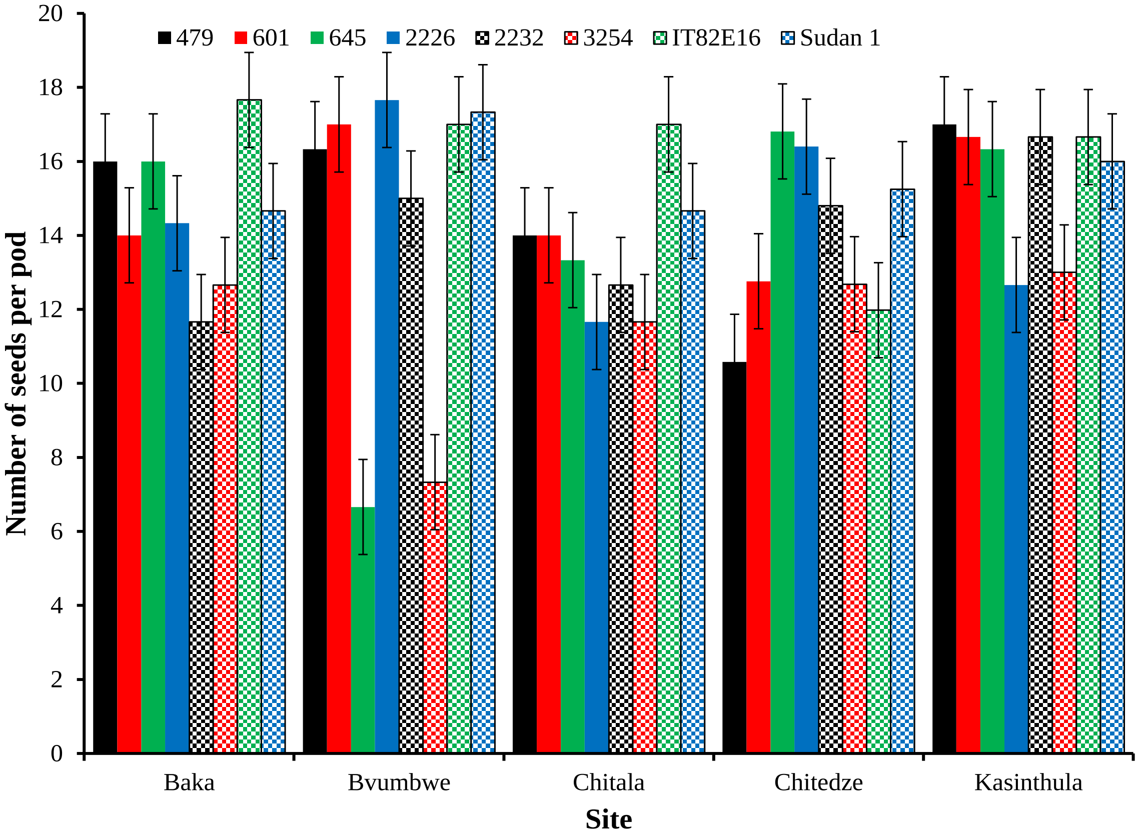

Figure 6.5: Average number of seeds per pod of eight genotypes at five experimental sites. (Bars represent $LSD_{0.05}$).

6.3.4.2 Grain weight per square metre (Yield) (g/m^2)

Dry grain yield per square metre significantly differed among genotypes, sites and interaction between genotype and site (Table 6.6 & Fig. 6.6). Overall, Sudan 1 gave the highest grain yield, followed by 601, IT82E16, 2226, 479, 645 and 2232. In terms of site effects on grain yield, Chitedze gave the highest average yield, followed by Kasinthula, Baka, Chitala and Bvumbwe. Significantly superior grain yields were observed for Sudan 1, 601 and 2232 at Chitedze and 3254 at Kasinthula. However, low grain yields were observed on all eight genotypes at Bvumbwe; 2232 and 645 at Chitala; 2232 at Baka and 601 and 2232 at Kasinthula.

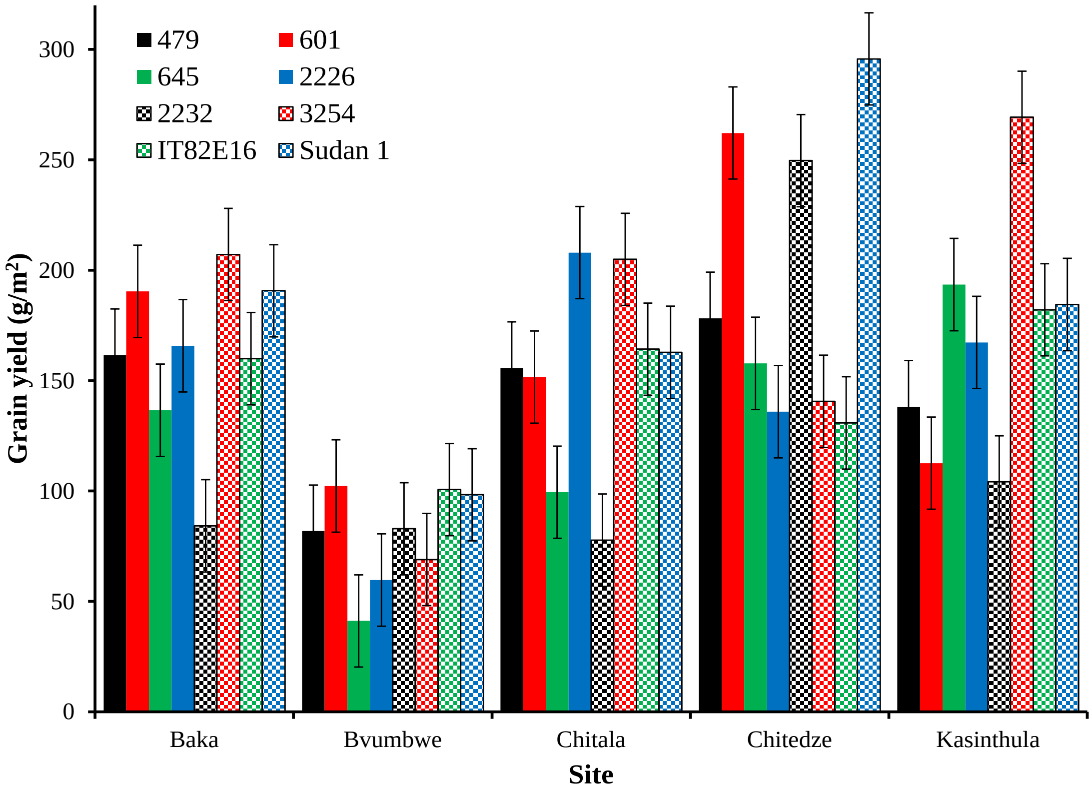

Figure 6.6: Yield performance (g/m^2) of eight genotypes at eight experimental sites. (Bars represent $LSD_{0.05}$).

The performance of the released variety, Sudan 1 at Baka, Chitala and Kasinthula compared very well with some drought tolerant genotypes. For example, at Baka, Sudan 1 did not differ from 601 and 3254; at Chitala it did not differ from 601 and 479; and at Kasinthula it did not differ from 2226 and 645. Genotype 645 at Chitedze and Kasinthula showed yields above average with the highest yield observed at Kasinthula. However, this genotype performed below average at Baka, Chitala and Bvumbwe. Despite the recorded high yield of 2232 at Chitedze, this genotype performed poorly at the other four sites. On the other hand, genotypes 3254, IT82E16 and 2226 showed below average yields at both Bvumbwe and Chitedze but the three genotypes yielded well at Baka, Kasinthula and Chitala with 3254 giving the highest yields at the three sites.

The GGE biplot showed that Axis 1 and Axis 2 accounted for 85.7% of the total variation for grain yield with Axis 1 contributing 59.2% (Fig. 6.7). 3254, Sudan1, 2232 and 645 formed the vertex genotypes of the polygon for the which won where/what model (Fig. 6.7a). Bvumbwe and Chitedze were located in a sector where Sudan1 was vertex genotype while Kasinthula, Chitala and Baka fell in a sector where 3254 was a vertex genotype. However, 645 occurred in a sector where site is not represented. Sudan 1 representing the highest yielding genotype, was located at the highest point of abscissa (the arrow of the single-arrowed line) followed by 3254 and 601 (Fig. 6.7b). All the three genotypes showed large projection from the abscissa indicating their varied responses to environment.

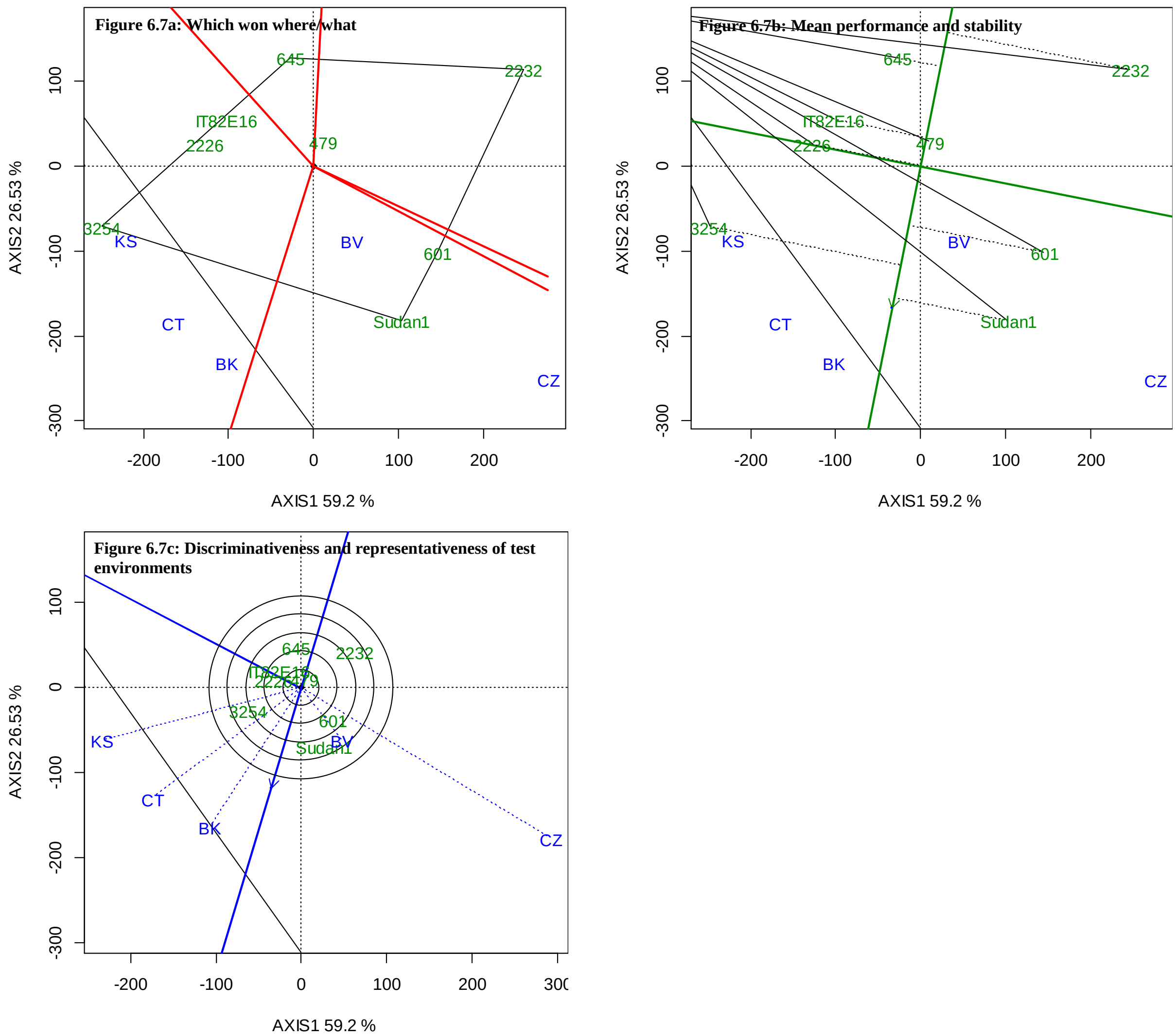

Figure 6.7: Three GGE biplots for grain yield of the eight genotypes tested at Baka (BK), Bvumbwe (BV), Chitala (CT), Chitedze (CZ) and Kasinthula (KS). a) Polygon for which genotypes won in which environment; b) Mean performance and stability and; c) Representativeness and discriminating power of the test environments.

In contrast 2232 was located near the base of the abscissa adjacent to 645, IT82E16, 479 and 2226. Genotype 479 showed least projection from the abscissa indicating that it was the most stable genotype across the five sites. Chitedze represented the most discriminating site due to the longest vector from the origin of the biplot while Bvumbwe showed the shortest vector (Fig. 6.7c). Baka showed the least deviation from the single-arrowed line indicating that it was the most representative among the five tested sites.

6.3.4.3 Seed size (100 seed weight in grams)

Seed size, which was determined as 100 seed weight (g/100seeds), varied significantly among genotypes, sites and the interaction between genotype and site was significant (Table 6.6 & Fig. 6.8). On average, the largest seed size was recorded on 2226, followed by 3254, 2232, 645, 479, 601, IT82E16 and Sudan 1. Genotype 2226 consistently showed 100 seed weights of greater than 20 g, suggesting that this genotype was large seeded, regardless of environmental conditions (Fig. 6.8). Genotypes 601 and Sudan 1 consistently showed low 100 seed weights (less than 14 g) across the sites, while 3254 showed medium 100 seed weights across the sites.

Seed weights of some genotypes varied from site to site, thus indicating the effects of environment on seed size of these genotypes. Genotype 2232 showed large seed size at both Chitedze and Bvumbwe, but a much reduced seed size at Baka, Chitala and Kasinthula. Genotypes 479 and IT82E16 produced intermediate seed sizes at Chitala, while elsewhere these genotypes produced small seeds. Genotype 645 showed a marked response to the environment by exhibiting three categories of seed size among sites. This genotype produced large seeds at Chitala (21 g), medium seeds at Bvumbwe (18 g), Chitedze (17 g) and Kasinthula (17.5 g) and small seeds at Baka (13 g).

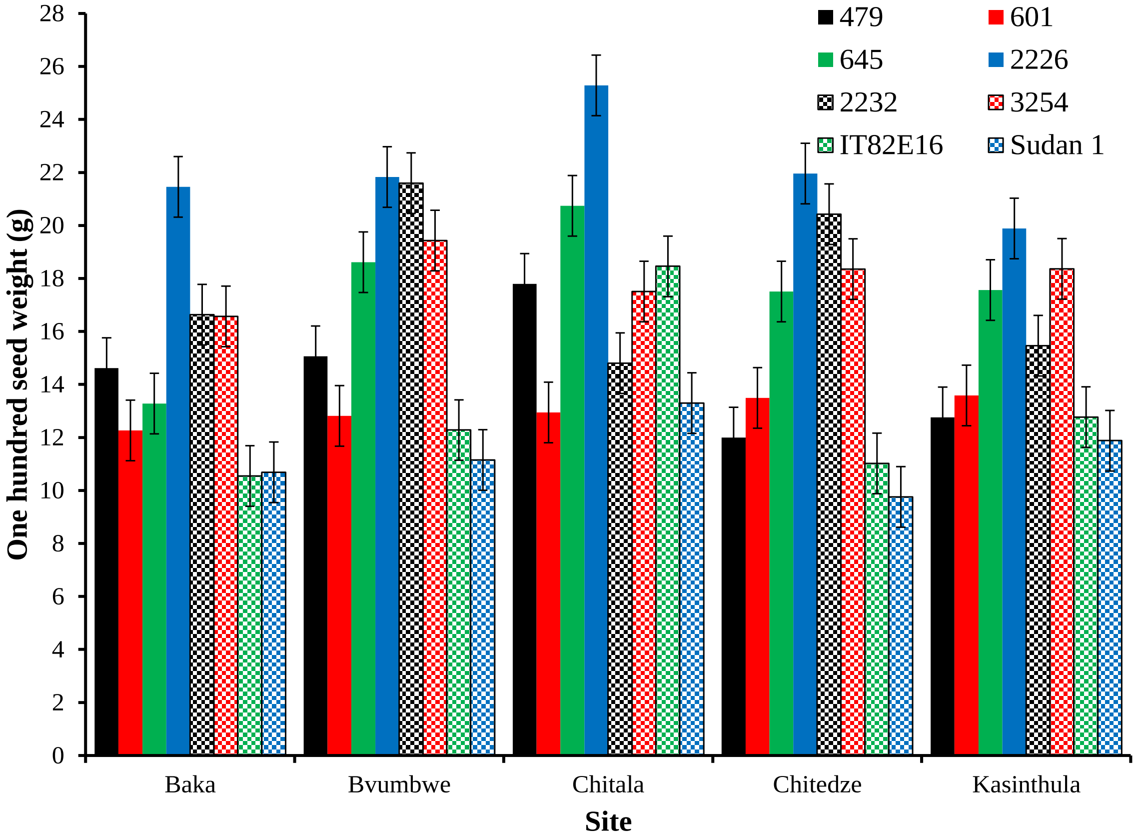

Figure 6.8: Hundred seed weight (g) of eight genotypes at five experimental sites. (Bars represent $LSD_{0.05}$).

The GGE biplot showed that Axis 1 and Axis 2 accounted for 95.1% of the total variation for grain yield with Axis 1 contributing 82.4% (Fig. 6.9). Sudan1, IT82E16, 2226 and 2232 formed the vertex genotypes of the polygon for the won where/what model (Fig. 6.9a). All the five sites were located in a sector where 2226 was vertex genotype. 2226 representing the genotype with largest seeds was located at the highest point of abscissa (the single-arrowed line) followed by 3254, 2232 and 645 (Fig. 6.9b). Both 2226 and 645 showed least projection from the abscissa compared with 2232 and 3254. On the contrary Sudan 1 was located near the base of the abscissa adjacent to 601, IT82E16 and 479 with Sudan 1 showing the least projection from the abscissa. Chitedze and Chitala represented the most discriminating sites due to the longest vectors from the origin of the biplot while Kasinthula showed the shortest vector (Fig. 6.9c). Kasinthula showed the least deviation from the single-arrowed line indicating that it was the most representative among the five tested sites.

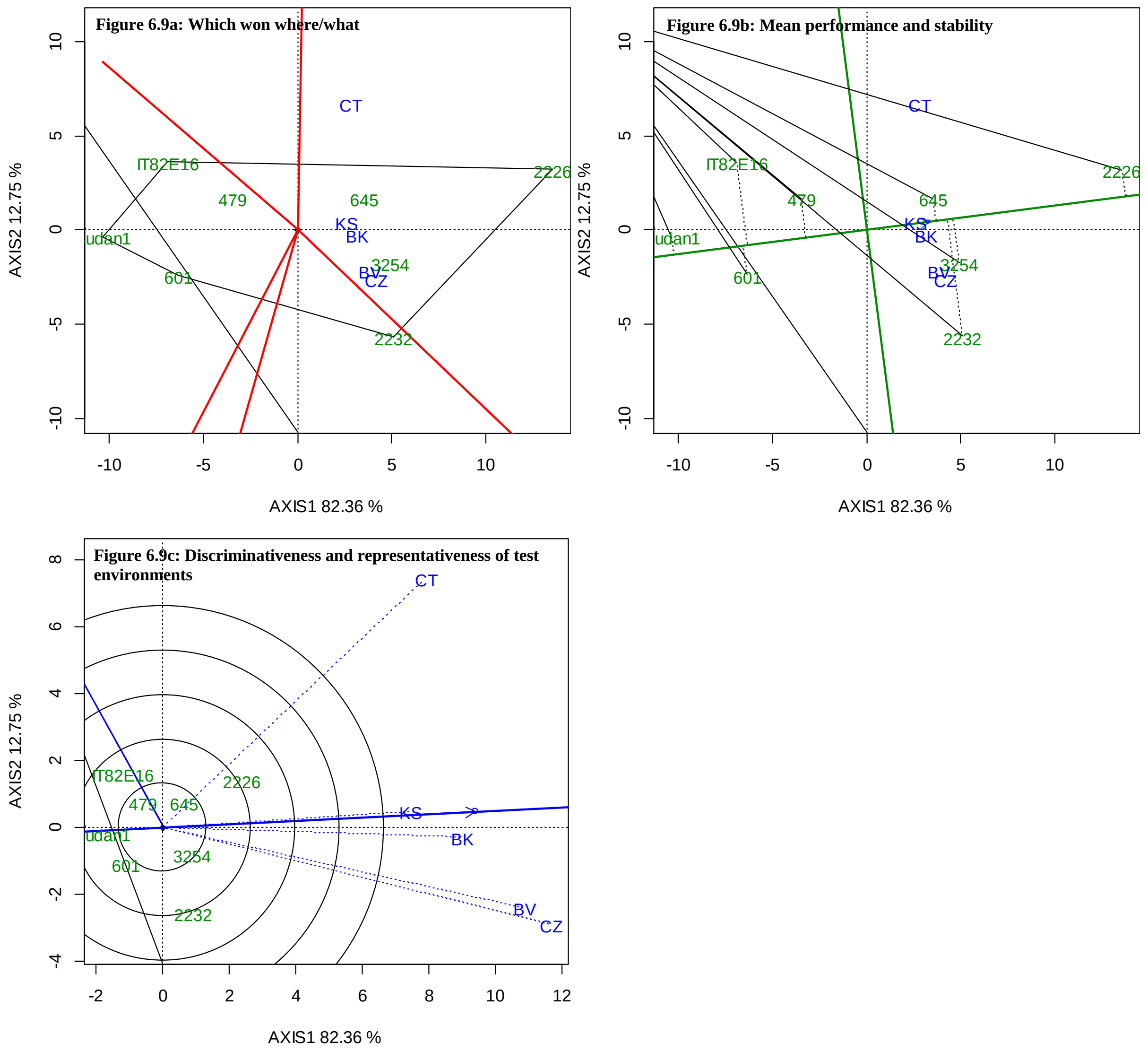

Figure 6.9: Three GGE biplots for 100 seed weight of the eight genotypes tested at Baka (BK), Bvumbwe (BV), Chitala (CT), Chitedze (CZ) and Kasinthula (KS). a) Polygon for which genotypes won in which environment; b) Mean performance and stability and; c) Representativeness and discriminating power of the test environments.

6.4 Discussion

6.4.1 Reproductive characteristics

The differences in flowering and maturity characteristics among genotypes observed between warm and cooler sites in this study (Figs. 6.2 & 6.3; Table 6.5) could be explained by the effects of temperature. Both flowering and maturity times were prolonged by cooler environmental conditions and reduced by warmer conditions. Physiologically, the effect of temperature on flowering is proportional to temperature, which consequently affects the rate of leaf production and initiation of flower bearing nodes (Craufurd *et al.*, 1997). High temperature accelerates the initiation of flowering and also increases the frequency of flower bearing nodes. Hall (2004b) reported that, under high air temperature, genotype CB5 took 65 days to mature, while in a cooler environment the same genotype took 100 days to reach physiological maturity. The higher temperatures at Baka, Chitala and Kasinthula (Figs. 6.1a, 6.1c and 6.1e) may have triggered production of nodes, which contributed to a significant reduction in the period to first flower buds while the lower temperatures at Bvumbwe and Chitedze (Figs. 6.1b and 6.1d) had the opposite effect, leading to prolonged periods to first flower buds and consequential late maturity.

Besides temperature, photoperiodism also plays a critical role in the reproductive characteristics of cowpea. Ishiyaku and Singh (2001) identified photoperiod insensitive (day neutral) genotypes which flowered between 39 and 49 days and photoperiod sensitive (short day) genotypes which flowered between 65 and 117 days. In the current study, the early maturing genotypes (Sudan 1, 479, 601 and IT82E16), which flowered in less than 46 days (Table 6.5, Fig. 6.2), may belong to the photoperiod insensitive group while the late maturing genotypes (645, 2226 and 2232), which flowered between 62 and 70 days, may belong to the photoperiod sensitive group. However, the intermediate maturing genotype (3254) which took 50 days to flower may belong to either slightly sensitive or moderately sensitive photoperiod categories, as suggested by Craufurd *et al.* (1996). The suggested groups for photoperiod sensitivity conform with results by Manggoel and Uguru (2011) that cowpea genotypes, which take 45 days or less to flower, belong to day neutral groups (photoperiod insensitive), while genotypes which take more than 45 days belong to short day groups but these workers did not encounter genotypes with an intermediate response such as 3254. The proposed photoperiod insensitive genotypes consistently took a shorter period to flower and mature at all sites, compared to the photoperiod sensitive ones, which flowered and matured towards the end of the rainy season. However, further research on photoperiod sensitivity should be conducted to confirm the proposed groups.

Reproductive characteristics of cowpea varieties determine the final end-use since most uses depend on the availability of grain and vegetative parts (leaves and haulms). For example, cowpea varieties, which mature early, contribute to increased grain yield, while varieties which mature late contribute to prolonged availability of leaf for vegetable and animal fodder (Hall, 2012; Timko & Singh, 2008). Consequently, varieties which combine early maturity and prolonged leaf production for vegetable and animal fodder (dual purpose) would be more beneficial to farming communities in areas where other grain legumes do not perform well.

Early maturity in cowpea benefits farmers in various ways. In Malawi, most farmers face an acute seasonal maize shortage mostly during the rainy season, which results in chronic food insecurity (Ellis & Manda, 2012). The use of early cowpea genotypes would help to alleviate hunger and poverty during the periods of acute food shortages. In addition, the use of early maturing varieties would enhance cowpea production, by providing the opportunity for increased grain production, as farmers realise multiple harvests within one season.

Furthermore, the double cropping system improves land use efficiency, as more grain is harvested from the same piece of land. Therefore, Sudan 1, 479, 601 and IT82E16, which flowered and matured early (Figs. 6.2 & 6.3), would fit very well into the double cropping system, considering that the rainy season lasts about 150 days in Malawi (Figs. 6.1a–6.1e). The double cropping systems of cowpea would be more appropriate for farmers located in sites with environmental conditions similar to Baka, Chitala and Kasinthula, where the genotypes matured between 52 and 60 days.

The late maturing genotypes such as 2226, 2232 and 645 may increase fodder production in areas where animal production is the main agricultural activity. Farmers in West Africa grow long duration varieties for fodder which is rich in protein (17-18%) and has a high dry matter digestibility of between 64% and 71%) (Singh *et al*., 2003). In Malawi, some areas depend on animal production which requires prolonged availability of fresh fodder. However, in drought prone areas such as the Lower Shire Valley, erratic rainfall poses a substantial challenge to the availability of high quality fodder throughout the year (Nkomwa *et al.*, 2014). Cowpea being a relatively drought tolerant crop, compared with other leguminous crops (Singh *et al*., 1999a; Timko & Singh, 2008) may significantly improve production of fodder in areas with harsh environments. Therefore, the promotion of late maturing and drought tolerant cowpea genotypes, such as 645 and 2226, would enhance fodder production in such harsh environments. However, the promotion of cowpea as a fodder crop has not been fully explored in Malawi. It is therefore suggested that integrated animal and cowpea production research should be initiated, in order to target communities which derive their livelihoods from livestock production and more especially in drought prone areas.

Late maturing genotypes may also contribute to the continuous availability of fresh green food for human consumption. Cowpea is a multipurpose crop, given that its leaves and immature pods are often consumed as a vegetable. Immature and tender pods are harvested and consumed like snap beans (Pandey *et al.,* 2006). Similarly, fresh and tender leaves are cooked and directly consumed or dried for future use (Saidi *et al.*, 2007). Therefore, the late maturing and drought tolerant genotypes, such as 645 and 2226, provide room for developing and promoting varieties specifically for vegetable production.

GGE biplot stability analysis helps to identify appropriate genotypes for production in either specific or wider environments. It is used to evaluate genotypes for their performance and stability in various environments. Furthermore it is helps to understand the discriminating ability and representativeness of the test environments. GGE biplot analysis of maturity period (Figs. 6.4a, b & c) shows variation in genotypes in the tested environments (sites). The "which won where/what model" divided the eight genotypes into early and late maturity groups (Fig. 6.4a). The late maturing genotypes fell in a sector where Bvumbwe, Chitedze and Chitala are located indicating that these genotypes took more time to rich maturity in these sites. However, presence other genotypes in a sector not associated with any site indicates that these genotypes took less time to mature Mean performance and stability for maturity (Fig. 6.4b) shows that genotype 2226 consistently took more days to mature due to least projection and such genotypes could be well adapted for increased fodder and vegetable production in various environments. However, genotypes with large projection from the single arrowed line could be specifically adapted to areas such as Bvumbwe and Chitedze for both fodder and vegetable production while the same varieties could be used for increased grain production in areas where the genotypes could mature early. Although Bvumbwe was the most discriminating site (Fig. 6.4c), the largest deviation from the single-arrowed line disqualifies it from being the ideal site for evaluating maturity characteristic of the tested genotypes. Chitala and Chitedze showed the least deviation from the line indicating that the two sites were representative. Therefore, among the tested sites, Chitedze represents the ideal conditions for evaluating maturity period of the tested genotypes due to the least deviation from the single-arrowed line and the longest vector from the origin of the biplot.

6.4.2 Yield characteristics

6.4.2.1 Number of seeds per pod

A high number of seeds per pod contribute to the high yield potential of varieties. The number of seeds per pod ranged between 5 and 20, with a mean of 14 seeds per pod (Table 6.6 & Fig. 6.5). The results show a wide range of number of seeds per pod, compared to similar studies. Uguru (1996) found a range of 9 – 17 with a mean of 13 seeds per pod; Manggoel and Uguru (2011) found a range of 10.5 – 16.7 with a mean of 13.5 seeds per pod; and Omoigui *et al*. (2006) found a range of 7.00 – 11.37 with a mean of 9.15. The wide range of number of seeds per pod in this study points towards the high diversity of materials and identification of genotypes with very high numbers of seeds per pod, which could be further explored for improved yields in Malawi. In particular, Sudan 1 and 601, which showed an average of more than 14 seeds per pod at all sites, should be explored further to understand the genetic control of this trait.

6.4.2.2 Grain yield

High rainfall during the growing period of cowpea is detrimental as it is associated with loss of flowers, which leads to poor pod setting and consequently low yields. In addition, extremely moist soil can lead to water logging conditions, which also contribute to poor yields in cowpea (Hadi *et al.*, 2012). Low yields under excess water are associated with rotting of roots (Timsina *et al.*, 1994) and a reduced capacity of cowpea to properly nodulate, which consequently contributes to low nitrogen fixation (Minchin *et al.*, 1978). The highest monthly rainfall of 499 mm in February at Bvumbwe (Fig. 6.1b) coincided with the flowering of most genotypes and this may have contributed to loss of flowers, which resulted in significantly low yields of all the genotypes at the site (Fig. 6.6). Similarly, the consistently wet conditions at Bvumbwe (highest rainfall) may have reduced the ability of genotypes to fix nitrogen, hence limiting expression of the full genetic yield potential of all the genotypes.

Amount of ultra violet (UV) radiation intercepted by growing plants affects flowering, maturity and consequently yield and the effect varies with altitude (Terfa *et al*., 2014). Generally, radiation often decreases with elevation since clouds stay around high ridges and blocks out sun. In this study, the low yields obtained at Bvumbwe (the highest altitude site) (Table 6.1) for almost all the genotypes could be attributed to amount of radiation intercepted at the site compared with other sites which are located at relatively lower altitude.

Bvumbwe showed very low yields for most genotypes, with 645 giving exceptionally low yields (Fig. 6.6). In addition to the rainfall related reasons, the exceptionally low yield for 645 at Bvumbwe may be explained by the additional attributes of this genotype. Cowpea has different pod setting characteristics in terms of position of pods (IBPGR, 1983). Some genotypes have pods above the canopy; others have their pods throughout the canopy; while others bear pods below the canopy close to the ground. During the field experiment, genotype 645 showed below canopy pod setting (data not shown), leading to rotting of the pods, which were in direct contact with the wet soil during the growing season. Similar low yields for 645 observed at Baka and Chitala could also be attributed to pod position as well. High incidences of termites were observed at Baka and Chitala and may have contributed to loss of pods which were in direct contact with the soil. Therefore, the below canopy pod setting of 645 may not be beneficial in areas which receive high rainfall and/or have high termites populations, as was the case for Bvumbwe, Baka and Chitala.

Although cowpea is considered to be a drought tolerant crop (Singh *et al*., 1999a), nevertheless, sensitive genotypes perform poorly under low moisture conditions. Genotype 2232 showed the lowest yields (Fig. 6.6) at Baka, Chitala and Kasinthula, sites, which received low rainfall between November and December (Figs. 6.1a, 6.1c and 6.1e) and thus unfavourable growing conditions during the initial vegetative growth of 2232. The different yield response of 2232, relative to the drought tolerant genotypes at these three sites was consistent with the drought susceptible characteristic of the genotype found in the glasshouse experiments (Chapters 4 & 5). The stability analysis (Fig. 6.7) also confirms that 2232 is susceptible to drought conditions. The highest regression coefficient (2.69) indicates that this genotype is best adapted to high yielding (high rainfall) environments. The poor field performance of 2232, in areas with low rainfall and high temperature demonstrated the importance of seedling stage drought tolerance in areas where rainfall is not reliable during the onset of the growing season.

High temperature also negatively affects yield in cowpea. Ehlers and Hall (1998) observed a reduction in pod and grain yield in drought susceptible cowpea genotypes when the minimum night temperatures rose above 20°C. Low night temperatures enhanced pod retention up to 70%, compared to 30% when night temperatures were high (Warrag & Hall, 1984). In a study by Nielsen and Hall (1985), an increase in night air temperature increased flower abortion in a heat sensitive genotype (CB5), while heat tolerant genotypes (Tvu4552 and Prima) maintained significantly higher numbers of flowers under the same conditions. Physiologically, high night temperatures cause heat-induced male sterility and premature anther drying, leading to high flower abortion (Ahmed *et al.* 1992; Patel & Hall, 1990). Comparison of the low yields of 2232 at Baka, Chitala, and Kasinthula and the high yield of the same genotype at Chitedze (Fig. 6.6) was consistent with the negative effect of high minimum temperatures on yield in the drought susceptible genotypes. Baka, Chitala and Kasinthula experienced average minimum temperatures of 22°C, 23°C and 24°C respectively (Figs. 6.1a, 6.1c and 6.1e), well above the threshold of 20°C, compared to Chitedze, which experienced an average minimum temperature of 18°C (Fig. 6.1d).

Comparison of yields for the drought tolerant genotypes (479, 601, 645, 2226 and 3254) at Baka, Chitala and Kasinthula (Fig. 6.6) showed that 3254 consistently outperformed the other genotypes and this pointed towards the presence of a unique drought tolerance mechanism. Results of the glasshouse study on physiological mechanisms controlling drought tolerance in the five genotypes (Chapter 5) showed that 3254 maintained high photosynthetic capacity and stomatal conductance, even under low soil moisture contents. The other four genotypes exhibited low stomatal conductance from the onset of drought stress conditions. Therefore, the presence of high photosynthetic capacity in 3254, compared with the other tolerant genotypes under low soil moisture content, explains the consistently high yield performance at the three sites with low rainfall.

Although 3254 performed well at Baka, Chitala and Kasinthula, its poor performance at Chitedze may indicate unfavourable conditions at the site, or negative physiological attributes of the genotype. Analysis of maturity periods in this study shows three categories of genotypes, i.e. early, intermediate and late maturity. Both early and late maturing genotypes yielded highly, compared with 3254, the intermediate genotype. High soil moisture content leading to waterlogging conditions, even for a short period, can reduce the yield of cowpea due to rotting of roots (Timsina *et al*., 1994) and yield reduction as high as 90% due to water logging conditions have been reported (Umaharan *et al.*, 1997. The yield reduction depends on the time of waterlogging conditions in relation to maturity time. Genotypes with enough recovery time before the reproductive stage have the ability to yield highly, compared to genotypes which do not have sufficient recovery time (Umaharan *et al*., 1997). Chitedze received high rainfall (268mm) in the month of December (Fig. 6.1d), which may have increased soil moisture and caused waterlogging. This month of high rainfall may not have had negative effects on either the early or late maturing genotypes. During this time, the early maturing genotypes may have already formed flower primordia, while the late maturing genotypes may have had enough recovery time before the on-set of the reproductive stage. However, the water logging conditions may have coincided with the initiation of reproductive processes in 3254, the genotype with intermediate maturity period, thus leading to low yields. The poor performance of 3254 at Chitedze indicates a need for a systematic study on the effects of waterlogging conditions on the reproduction of cowpea.

GGE biplot shows that Sudan1 was specifically adapted to Bvumbwe and Chitedze the high yielding environments since it was a vertex genotype in a sector where the two sites were located (Fig. 6.7a). Similarly 3254 was best adapted to Kasinthula, Chitala and Baka the low yielding environments. However, 2232 and 645 showed no specific adaptation since they were vertex genotypes in sectors with no sites at all. Sudan 1, 601 and 3254 showed above average yield performance across the sites but none of these three genotypes showed a stable response to the environment (Fig. 6 7b). The lack of stable performance for yield confirm that these genotypes to specific environments (Fig 6.7a). Therefore, Sudan 1 and 601 should be promoted in high yielding environments, while 3254 should be specifically recommended for production in poor environments (low rainfall and high temperature). The other five genotypes showed below average yield performance with 2232 ranked poorly. Genotype 479 showed the least projection from the single arrowed line indicating that it was the most stable genotype but its low yield renders it unsuitable for production in various or specific environments. Among all the test environments, Chitedze showed the highest discriminating power while Bvumbwe showed the least discriminating power for evaluating the eight genotypes because of the lengths of their vectors from the origin of the biplot. Although Chitedze had the highest discriminating power, the largest deviation from the single-arrowed line renders it unsuitable for testing yield of the eight genotypes. Baka was closer to ideal environment since it was the most representative among all the tested sites due to small deviation from the single-arrowed line and also had a relatively longer vector from the origin of the biplot.

The experiment used two released varieties (Sudan 1 and IT82E16) alongside five drought tolerant landraces and one susceptible landrace. Comparable yield performances of the two released varieties with some drought tolerant genotypes (Fig. 6.6) at Kasinthula, Baka and Chitala, suggest that the released varieties may have drought tolerance attributes. The two varieties Sudan 1 and IT82E16 are introductions from IITA and possibly originate from the global cowpea breeding programme, which has drought tolerance as a key breeding objective (Hall, 2012). Therefore, the two released varieties should be tested in a drought tolerance experiment under glasshouse conditions in order to validate the comparable yield performance under low moisture and high temperature conditions.

6.4.2.3 Seed size

Cowpea exhibits three categories of seed size, i.e. large, medium and small (Omoigui *et al*., 2006). In this study, all categories of seed size were observed. Some genotypes maintained the same seed size across sites while others showed a significant variation of seed size across sites (Fig. 6.8). Both 2226 and 2232 showed significantly large 100 seed weight (>20g) at Bvumbwe and Chitedze, but seed size for 2232 was significantly reduced at Baka, Chitala and Kasinthula. The large seed size of these two genotypes at Bvumbwe and Chitedze may indicate the genetic potential of the two genotypes under adequate moisture conditions.

However, the significant reduction in seed size for 2232 at Baka, Chitala and Kasinthula may be due to low rainfall and high temperature. Genotypes 2226 and 2232 belonged to different drought response categories (Chapters 4 & 5), with 2232 exhibiting drought susceptibility. The reduction of seed size in drought susceptible genotypes is partly caused by disruption of the photosynthetic machinery under drought and high temperature conditions (Hall, 2004b). The disruption of the photosynthetic machinery limits conversion of photons into carbohydrates, which form a large component of seed. Therefore, the different responses of the two genotypes reflect the presence of drought tolerance in genotype 2226, which consistently exhibited large seed size regardless of the site and the drought susceptibility of 2232, which showed large seed size at Chitedze and Bvumbwe only.

Genotype 2226 was the vertex genotype in a sector where all the sites were located indicating that it exhibited largest seed size in all the tested sites. In contrast, Sudan 1, IT82E16 and 601 were vertex genotypes in a sector not represented by site (Fig. 6.9a). Among the four vertex genotypes, Sudan 1, 601 and 2226 showed small projections from the single-arrowed line indicating their stable seed sizes regardless of environment (Fig. 6.9b). For example, Sudan 1 and 2226 exhibited smallest and largest seed size respectively at all sites. The high projection for 2232 from the single arrowed line indicates that this genotype was responsive to the environment and produced larger seeds in good environments. This was consistent with the known drought susceptibility of this genotype. Among the tested environments Chitala was the most discriminating with the longest vector from the origin of the biplot (Fig. 6.9c). However, Baka was the least discriminating with the shortest vector but the most representative due to smallest deviation from the single arrowed line. Comparison of representativeness and discriminating power of the sites shows that Baka is close to the ideal site.

6.5 Conclusion

The eight genotypes showed significant variation for reproductive, yield and seed characters tested at the five sites indicating the availability of genotypes with desirable attributes which could be included in the National Cowpea Improvement Programme. Sudan 1, 479, 601 and IT82E16 flowered and matured earlier compared to 645, 2226 and 2232, while 3254 showed intermediate responses. The variation in reproductive characteristics among the eight genotypes implies that 479, 601, Sudan 1, IT82E16 and 3254 could be grown for grain across all sites, while the late maturing genotypes could be grown for both grain and fodder in warmer environments and for fodder in cooler environments. Drought tolerant genotypes yielded better in areas with low rainfall and high temperatures than the susceptible genotype. Consistently high yields of 3254, among the drought tolerant group in drought prone areas, indicated the presence of an additional desirable physiological attribute for high grain yield under drought conditions. The eight genotypes exhibited different seed size categories. Genotype 2226 consistently showed large seeds at all sites compared with 3254 which showed medium seed size and; Sudan 1 and 601 which showed small seed size in all the sites. However, further research on farmers' preference of the identified genotypes needs to be initiated in regards to the development of farmer-oriented varieties.

Chapter 7 : Towards the development of a farmer-oriented cowpea improvement programme in Malawi

Abstract

This research was conducted with local communities at Baka, Chitala, Chitedze and Kasinthula research stations, to streamline farmers' preference in regards to the cowpea improvement programme through focus group discussions (FGD) and participatory variety selection (PVS). The study identified eight major challenges affecting production and promotion of cowpea, including drought; low yielding varieties; poor extension services; limited research on production and utilisation; and poor markets. Farmers identified 12 desirable attributes including high yields; high leaf biomass; early maturity; drought tolerance; large and small seed size; fast cooking; smooth seed testa; high pod load; and resistance to pests and diseases. Selection of preferred genotypes differed from attribute to attribute. For instance, 305, 309 and 479 were selected for early maturity; 2226, 2227 and 3422 were selected for high leaf biomass; 305, 309, 3254 and Sudan 1 were selected for high pod load; 544, 2226 and 2227 were selected for large seeds; 305, 309 and 421 were selected for small seeds; and 305, 309, 479, 601, IT82E16 and Sudan 1 were selected for smooth seed testa. Genotype 3254 showed rough seed testa, a characteristic not liked by farmers. In summary, future cowpea improvement programmes require a policy environment conducive to the involvement of all key stakeholders with more emphasis on improving research, extension, seed systems, marketing and processing. The genotypes with desirable attributes form a good foundation for breeding varieties with combined attributes. Commercialisation of cowpea would rely on the development and promotion of varieties with large seeds, such as 2226 and 2227, in addition to varieties with rough seed testa, such as 3254, which are easy to process. Although 3254 (the only genotype with rough seed testa) was poorly ranked, its potential within the processing industry needs further pursuance.

7.1 Introduction

The involvement of farmers in variety selection can influence the adoption rate of new varieties, especially by small scale farmers. Non- or limited involvement of farmers in the breeding chain may continue to frustrate breeders' efforts, due to an inability to address farmers' needs (Wale & Yalew, 2007). Breeders usually place great emphasis on yield and other agronomic parameters, as opposed to other equally important traits needed or preferred by farmers (Sperling *et al.*, 1993). Generally, farmers would select varieties with desirable utility and adaptation benefits and not just high yields. Consequently, the adoption of varieties which do not address the immediate needs of farmers is often poor.

Production of cowpea in Malawi is largely characterised by the use of landraces (Nkongolo *et al.*, 2009), even though three improved varieties have been released (Government of Malawi, 2000; Mviha *et al.*, 2011). The poor adoption of these three varieties and the dominance of landraces demonstrate a lack of desirable attributes in the new varieties, due to the limited involvement of farmers in the development process of these varieties. Future breeding programmes should maximise the involvement of farmers to improve adoption of new varieties.

Despite the poor adoption of improved varieties, cowpea still holds the potential of improving legume production in Malawi, due to comparative advantages the crop has over other grain legumes. Firstly, cowpea is naturally considered a drought tolerant crop in comparison with other grain legumes (Hall, 2004b; Singh *et al.*, 1999a). Secondly, cowpea is a multi-purpose crop which covers both human to animal consumption and most parts of the crop are used, compared to other legumes (Timko & Singh, 2008). The comparative advantages of cowpea over other grain legumes may reduce competition for production, particularly in drought prone areas where crops like beans, groundnuts and peas perform poorly. However, the successful promotion of the crop relies on the availability of improved varieties which are also readily accepted by farmers in the drought prone areas.

Promotion of the five potential drought tolerant genotypes identified in a glasshouse experiment (Pungulani *et al*., 2012) may also be challenged by a lack of consideration of farmers' attributes. Therefore, this research aimed at establishing a farmer-oriented cowpea improvement programme through:

a) Identification of production challenges;
b) Defining the desirable characteristics of cowpea varieties and;
c) Selecting potential genotypes from available germplasm including drought tolerant genotypes for future cowpea improvement.

7.2 Methodology

7.2.1 Experimental sites

The study was conducted in collaboration with four rural communities at Baka, Chitala, Chitedze and Kasinthula research stations. The communities involved in the study were selected by the agricultural extension staff from Extension Planning Areas (EPAs), which were located close to the respective research stations. Specifically, the communities were drawn from Lupembe EPA, Chingulube EPA, Mpingu EPA and Mikalango EPA for Baka, Chitala, Chitedze and Kasinthula Research Stations, respectively.

7.2.2 Research design

The study involved focus group discussions (FGD) which were aimed at soliciting farmers' perceptions of production challenges and the desirable attributes of cowpea varieties for future production and participatory variety selection (PVS). The PVS was applied to identify cowpea genotypes with farmers' preferred attributes.

7.2.2.1 Focus group discussions

Focus group discussion was used as a tool for participatory rural appraisal (PRA) (King, 2000), to identify key production challenges and desirable traits of cowpea varieties. Forty farmers at each site were selected for participation in the FGDs. The selection of these farmers was based on their knowledge of cowpea production. Farmers were briefed by the agricultural extension staff, in order to set a common understanding among members of the group. After this briefing, a research scientist led the farmers' discussion, by probing farmers on key challenges affecting the production of cowpea and the desirable attributes of cowpea varieties, which could be considered in the crop improvement programme. The listed attributes were then scored by each farmer on a scale of 1-5, where 1 represented the most important and 5 the least important attribute.

7.2.2.2 Participatory variety selection

A total of 19 genotypes (two released varieties, five drought tolerant genotypes, one drought susceptible genotype and 11 other landraces) (Table 7.1) were included in the PVS conducted at the four research stations during the 2012/2013 cropping season. Farmers were not informed about the presence of released varieties and drought tolerant genotypes to avoid biased scoring. The experiment was laid in a randomised complete block design (RCBD) with genotypes, as treatments, replicated three times per site. Each genotype was planted on a plot consisting of three rows of 4m each spaced at 75cm apart, with 30cm between planting stations. Four seeds were planted which were later thinned to three per station. Plots were manually weeded to control weeds for four times during the period of experiment. Dimethoate (Rogor 40EC) and Cypermethrin were applied at three week interval to control major insect pests (Pod borers, thrips, pod sucking buds and aphids). Both pesticides were applied at the rate of 200g a.i /ha using a Knapsack sprayer.

The farmers involved in the FGDs were also involved in scoring the varieties in PVS. A scale of 1 5 was used to score all varieties for maturity, leaf biomass yield, pod load, seed size and seed testa texture. Selection of the five attributes was based on farmers' preference and also on the premise that farmers tend to pay more attention to preference and quality related traits (Kitch *et al*. 1998). A description of the scoring scale is provided in Table 7.2. The scoring was undertaken in two stages: during the growing season and at maturity. The scoring during the growing season was aimed at identifying genotypes with early maturity, high leaf biomass production and high pod load. The assessment at maturity was aimed at identifying genotypes with desirable seed characteristics (seed size and seed testa texture). Selected pictures for participatory variety selection are presented in Appendix II.

Table 7.1: Description of biological materials used in participatory variety selection.

Genotype	**Description**	**Source**	**Reference**
IT82E16	Released variety	Breeder	(Government of Malawi, 2000)
Sudan 1	Released variety	Breeder	(Government of Malawi, 2000)
305	Landrace	Genebank	(Nkongolo *et al*., 2009)
309	Landrace	Genebank	(Nkongolo, 2003)
399	Landrace	Genebank	
421	Landrace	Genebank	
479	Drought tolerant landrace	Genebank	(Pungulani *et al*., 2012)
535	Landrace	Genebank	
544	Landrace	Genebank	(Nkongolo, 2003)
570	Landrace	Genebank	(Nkongolo, 2003)
601	Drought tolerant landrace	Genebank	(Pungulani *et al*., 2012)
645	Drought tolerant landrace	Genebank	(Pungulani *et al*., 2012)
698	Landrace	Genebank	
727	Landrace	Genebank	(Nkongolo *et al*., 2009)
2226	Drought tolerant landrace	Genebank	(Pungulani *et al*., 2012)
2227	Landrace	Genebank	(Nkongolo, 2003)
2232	Drought susceptible landrace	Genebank	(Pungulani *et al*., 2012)
3254	Drought tolerant landrace	Genebank	(Pungulani *et al*., 2012)
3422	Landrace	Genebank	

Table 7.2: Description of scoring scale used by farmers for the selection of cowpea genotypes.

Character/Scale	**1**	**5**
Maturity	Early maturing	Late maturing
Leaf biomass	Low yield	High yield
Seed size	Small seeds	Large seeds
Pod load	Few pods	Many pods
Texture of seed testa	Smooth	Rough

7.2.3 Statistical analysis

Statistical analyses were performed using SPSS 20.0 and SAS 9.4 statistical packages. Initial exploratory data analysis using box plots (Morgenthaler, 2009) showed that the data did not meet the conditions for normal distribution (Appendix III). Consequently the data were subjected to the nonparametric Kruskal-Wallis test also known as rank transformation test (Montgomery, 2013) using SPSS 20 to compare differences among genotypes. The Kruskal-Wallis test corresponds to a one way ANOVA of parametric data (Corder & Foreman, 2014, p. 117; Steel *et al.,* 1997, p. 577). The Kruskal-Wallis test was followed by stepwise step-down multiple comparison of mean ranks at each site at the 5% alpha level (Campbell & Skillings, 1985). The stepwise step-down multiple comparison returns a sequence of subsets of groups with homogenous characteristics. However, considering that Kruskal-Wallis is a rank based analysis and only analyses ranks for different groups, Montgomery (2013, p. 130) recommended comparing results from the Kruskal-Wallis test and means of scores from a standard ANOVA; if similar results are obtained then a standard ANOVA is satisfactory. Consequently, the results in this study were analysed using one way ANOVA in SAS 9.4 (SAS Instititute, 2013) and the results from the Kruskal-Wallis analysis are presented in Appendices IV – VIII for comparison. The one way ANOVA used Least Significant Difference at 5% alpha level ($LSD_{0.05}$) to compare scores for the 19 genotypes.

Pattern of selection of genotypes across sites was analysed using Spearman’s rank correlation and interpreted according to Taylor (1990) who classified correlation coefficients into three major groups of low or weak association (r<0.35), modest or moderate (0.36 – 0.67) and strong or high (0.67 – 1). In order to identify a relatively universally accepted priority list of genotypes, scatter plots for genotype means across the sites and standard errors of means were produced. Standard error of means is a measure of spread of means about the overall mean (Steel *et al*., 1997).

7.3 Results

7.3.1 Production challenges

Farmers listed eight challenges associated with the production and promotion of cowpea within the selected areas for consideration in the cowpea development programmes. The challenges, which ranged from agronomic to socio-economic, were as follows:

a. Lack of improved and high yielding varieties;
b. Poor availability of seed for improved varieties;
c. Frequent occurrence of drought;
d. Yield losses caused by field pests and diseases;
e. Low market premiums due to a lack of well-structured markets for cowpea;
f. Poor extension services for cowpea production;
g. Post-harvest losses due to heavy infestation of storage pests;
h. Limited options for the utilisation of cowpea.

7.3.2 Farmers' desirable attributes

Farmers initially identified ten attributes of cowpea varieties, which influence their choice of varieties to grow (Table 7.3). However, maturity was split into early and late maturity, while seed size was split into large and small seed sizes, as individual characteristics, making a total of 12 attributes (Table 7.4). post-harvest losses and resistance to field pests and diseases. At Chitala, high priority was accorded to early maturity, drought tolerance and high grain yield.

Table 7.3: Farmers' criteria for selecting desirable cowpea genotypes.

Variety Character	**Desirable attribute**	**Reason for selection**
Maturity period	Early	• Terminal drought adaptation • Safeguard for food availability in times of food shortages (February –March) • Double cropping within one season (increased food and income)
	Late	• Leaf biomass; for use as a leaf vegetable • Labour saving as harvesting is done after other crops have been harvested
Seed Colour	White/cream	• Short cooking time
	Red /brown	• Storage pests resistance
Seed texture	Smooth	• Appealing when cooked
Grain yield	High	• Increased income and food security
Biomass yield	High	• Vegetable production • Haulms as animal fodder
Seed size	Large	• Good as a snack (boiled fresh pods)
	Small	• Good for cooking in stews
Pod load	High	• Increased grain yield • Immature fresh pods for use as snap bean.
Cooking characteristics	Short cooking time	• Little energy requirement for cooking • Saves time for other tasks more especially for women involved in cooking
Postharvest losses	Resistant to weevils (bruchids)	• Safe prolonged storage of grain which enables farmers to sell grain when demand is high
Field diseases and pest resistance	Resistance to viral diseases	• Most diseases destroy leaves and pods

Comparison of these 12 attributes showed variation within sites. Farmers at Baka prioritised high grain yield, drought tolerance, early maturity, resistance to Farmers at Chitedze prioritised high pod load, resistance to field pests and diseases and high yield. Farmers at Kasinthula showed more interest in varieties with high leaf biomass, resistance to post-harvest losses, early maturity, drought tolerance and high yield.

Although drought tolerance and early maturity featured highly at Baka, Chitala and Kasinthula, the two traits were poorly ranked at Chitedze. Analysis across the sites shows that grain yield, drought tolerance, early maturity, resistance to post harvest losses and resistance to field pests and diseases were ranked highly. Generally, low priority was given to attributes associated with seed characteristics, such as seed colour, seed size, cooking time and texture of seed testa within and across sites.

Table 7.4: Farmers' priority scores of desirable traits using a scale of 1-5 during Focus Group Discussion (FGD) by 40 farmers per site.

Trait	**Baka**	**Chitala**	**Chitedze**	**Kasinthula**	**Mean**
Grain yield	1.00	1.10	1.08	1.08	1.06
Drought tolerance	1.00	1.00	3.00	1.00	1.50
Early maturity	1.00	1.00	3.00	1.00	1.50
Postharvest pest resistance	1.00	2.00	2.00	1.00	1.50
Field disease and pest resistance	1.00	2.00	1.05	2.00	1.51
Pod load	2.00	2.00	1.00	2.00	1.75
Leaf biomass	2.15	2.00	2.00	1.00	1.79
Cooking time	3.00	2.00	2.00	2.00	2.23
Late maturity	3.00	4.00	2.00	2.00	2.75
Seed size	3.00	3.00	5.00	3.00	3.00
Seed colour	5.00	4.00	5.00	5.00	4.75
Texture of seed testa	5.00	5.00	5.00	5.00	5.00

*1 represents high priority and 5 represents low priority

7.3.3 Variation among genotypes based on farmers' preference

7.3.3.1 Rank correlations

Spearman's rank correlations of farmers' preferences were significant among all sites for maturity, leaf biomass, seed size and seed texture (Table 7.5). However, pod load was typically not correlated among different sites except between Baka and Kasinthula (r=0.486, P=0.05). According to the predefined categories (Taylor, 1990), both maturity and seed texture, with correlation coefficients between 0.727 and 0.992, showed strong and significant correlation among all the sites, while leaf biomass showed strong and significant correlations among all sites, except for Baka and Kasinthula, which resulted in a moderate correlation (r = 0.570, P=0.05). Similarly, preferences for seed size showed strong correlations among all sites, except Baka and Chitala. Both moderate and strong Spearman's correlations indicate that the farmers' selection of genotypes across the sites were statistically consistent. Specifically, maturity, leaf biomass, seed size and seed texture showed a statistically consistent selection of genotypes, while weak and non-significant correlations for pod load pointed towards differences in preferences for genotypes across the sites.

Table 7.5: Spearman's rank correlation coefficients between sites for scores of leaf biomass, maturity, podload, seedsize and seedtexture.

	Baka	**Chitala**	**Chitedze**
		Maturity	
Chitala	0.754**		
Chitedze	0.867**	0.727**	
Kasinthula	0.813**	0.834**	0.807**
		Leafbiomass	
Chitala	0.810**		
Chitedze	0.792**	0.850**	
Kasinthula	0.570*	0.799**	0.778**
		Podload	
Chitala	0.395		
Chitedze	0.161	-0.168	
Kasinthula	0.486*	0.433	-0.133
		Seedsize	
Chitala	0.638**		
Chitedze	0.800**	0.667**	
Kasinthula	0.787**	0.697**	0.793**
		Seedtexture	
Chitala	0.979**		
Chitedze	0.992**	0.984**	
Kasinthula	0.985**	0.977**	0.991**

N = 57 i.e 19 genotypes x 3 replicates per site*, **Spearman's correlation significant at 0.05 and 0.01 alpha levels respectively

7.3.3.2 Farmers' scores for maturity

Farmers recognised different maturity characteristics among the nineteen genotypes. These varieties ranged from early to late maturing. Comparison of mean scores for genotypes across the four sites showed that genotypes 305, 309, 698, 601 and 479 were the early maturing genotypes (Table 7.6). Genotypes 305 and 309 were very early and showed no variation among sites (Fig 7.1). However, 2226, 2227, 2232, 645 and 3422 were least preferred, due to late maturity characteristics. The two released varieties (Sudan 1 and IT82E16) showed mean scores of 2.13 and 2.11 respectively, indicating that their duration was more appealing than some landraces. Although some genotypes were consistently scored for either early or late maturity across different sites, other genotypes showed wide variation between sites.

Table 7.6: Mean scores for 19 genotypes for maturity, leaf biomass, pod load, seed size and seed texture on a scale of 1 – 5.

Genotype	Maturity	Leaf biomass	Pod load	Seed size	Seed texture
305	1.00k	1.74±0.06j	4.56±0.04b	1.19±0.03m	1.00h
309	1.00k	1.81±0.06j	4.78±0.03a	1.16±0.03m	1.00h
399	3.99±0.06d	3.86±0.07d	3.38±0.08f	2.78±0.07fg	3.90±0.08cd
421	3.96±0.07d	3.95±0.07d	3.22±0.04g	1.49±0.04l	3.85±0.08d
479	1.86±0.06j	2.50±0.07h	2.44±0.08m	2.87±0.07f	1.00h
535	2.24±0.08gh	2.35±0.08i	2.21±0.08n	2.26±0.06jk	3.81±0.08d
544	2.64±0.08e	2.53±0.07h	2.69±0.09k	4.23±0.06b	3.94±0.08cd
570	2.27±0.08fg	2.69±0.08g	3.57±0.08e	3.01±0.05e	4.04±0.08bc
601	1.86±0.06j	2.49±0.07hi	3.64±0.10e	2.52±0.06i	1.00h
645	4.66±0.04b	3.90±0.06d	2.64±0.09kl	3.93±0.08c	2.60±0.06g
698	1.77±0.06j	1.52±0.05k	2.77±0.09jk	2.66±0.09gh	4.17±0.08b
727	2.08±0.07i	2.74±0.07g	2.65±0.08kl	2.60±0.08hi	4.14±0.077b
2226	4.86±0.03a	4.82±0.03a	2.92±0.10hij	4.88±0.03a	3.00f
2227	4.84±0.03a	4.79±0.03a	3.04±0.10h	4.88±0.03a	3.00f
2232	4.77±0.03ab	4.23±0.06c	2.54±0.11lm	3.58±0.08d	2.45±0.01g
3254	2.36±0.05f	3.31±0.07e	3.85±0.12d	3.97±0.06c	5.00a
3422	4.38±0.05c	4.48±0.04b	2.87±0.07ij	2.38±0.07j	3.42±0.07e
IT82E16	2.11±0.08i	3.03±0.06f	2.94±0.09hi	2.65±0.09h	1.00h
Sudan1	2.13±0.03hi	2.61±0.09gh	4.09±0.08c	2.19±0.08k	1.00h
Mean	**2.88±0.03**	**3.12**±0.02	**3.20**±0.02	**2.90**±0.02	**2.81**±0.03
Sig.	***	***	***	***	***

Maturity: 1 represents early maturity and 5 late maturity**; leaf biomass**: 1 represents low biomass and 5 high leaf biomass yield; **Pod load:** 1 represents low pod load and 5 high pod load; **Seed size**: 1 represents small seed size and 5 large seed size; **Seed texture:** 1 represents smooth seed and 5 rough seed

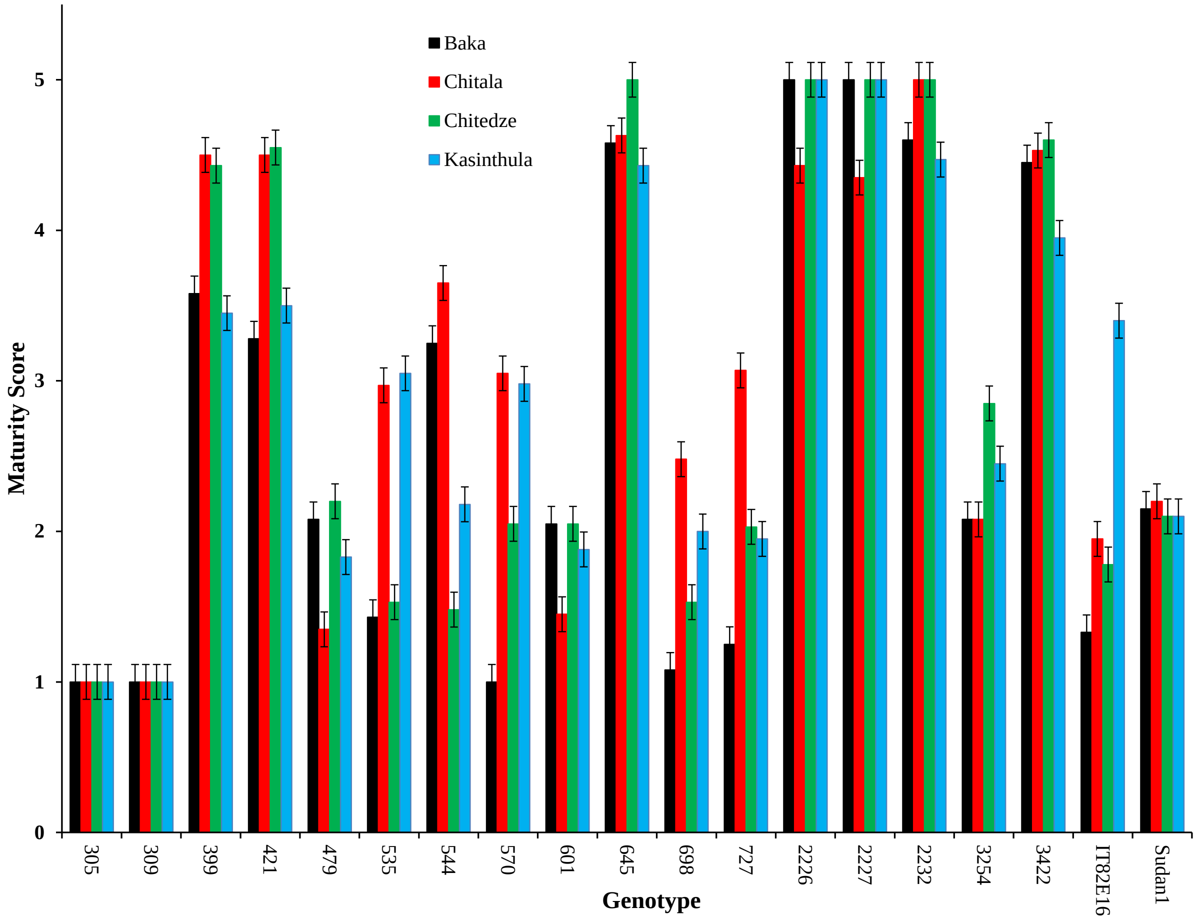

Figure 7.1: Average scores of 19 genotypes for maturity on a scale of 1–5 where 1 represents early maturity and 5 late maturity (Bars represent $LSD_{0.05}$).

Genotypes 535, 544, 570, 698, 727, and IT82E16 showed wide variation between sites. This variation indicated interaction between genotype and site scores suggesting site specific preference for maturity. Site specific preferred genotypes included 570, 698, 727, IT82E16 and 535 for Baka; 479, 601 and 479 for Chitala; 544, 535, 698 and IT82E16 for Chitedze and 479, 601; and 727 for Kasinthula.

7.3.3.2 Farmers' scores for leaf biomass yield

Farmers across the sites showed consistent scoring for leaf biomass by showing similar categories of genotypes with high and low scores. Genotypes 2226, 2227 and 3422 scored highly for high leaf biomass, based on pooled means across the sites (Table 7.6) showing that these three genotypes scored highly at all sites. In comparison, genotypes 698, 309, 305, 535, 601, 544 and 479 were ranked poorly for leaf biomass production across sites. Genotypes 399, 421, 645 and 2232 showed high mean scores but wide variation between sites indicating specific site preference. The site specific preferred genotypes include 2232, 399 and 421 for Baka; 2232, 421 and 399 for Chitala; 645, 399, 2232 and 421 for Chitedze and; 645 for Kasinthula.

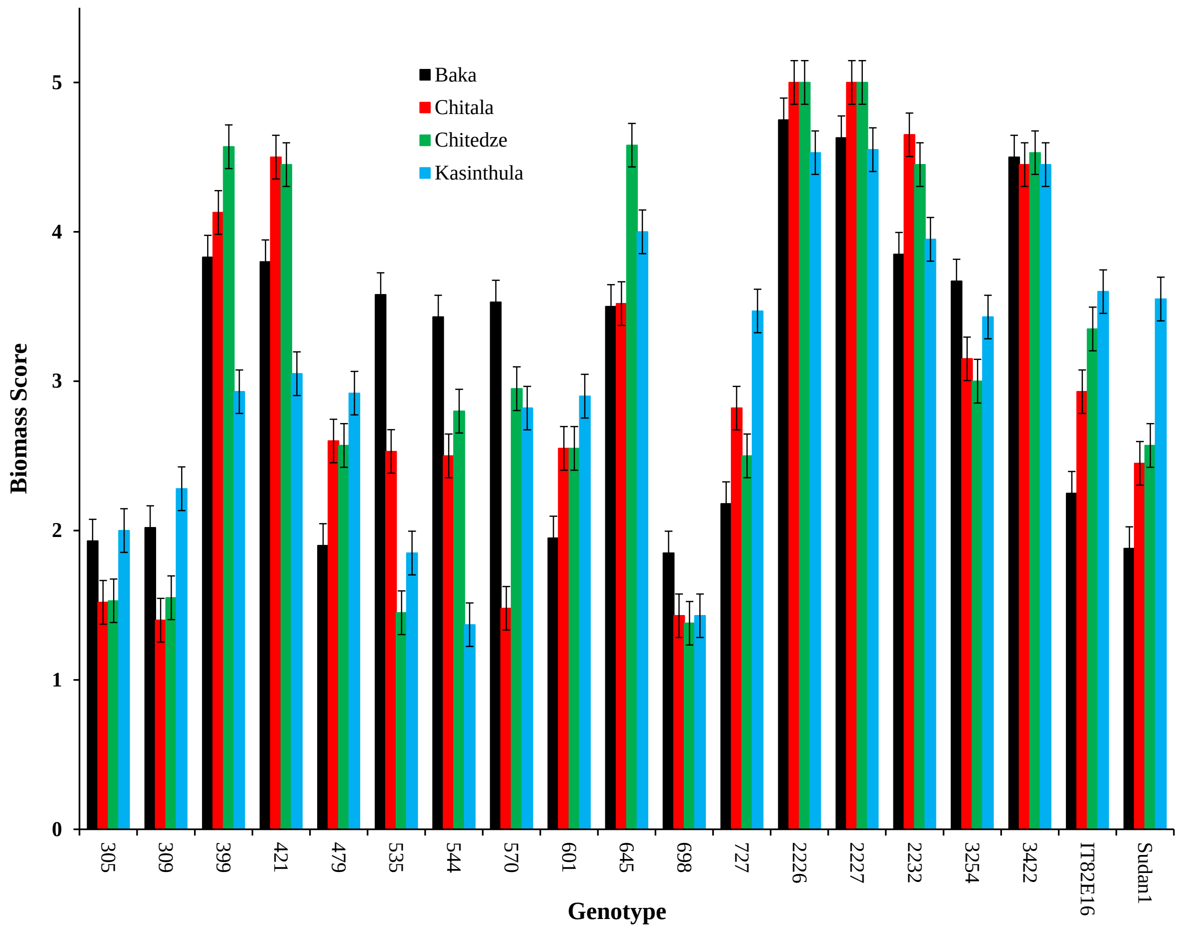

Figure 7.2: Mean scores for 19 genotypes for leaf biomass on a scale of 1–5 where 1 represents low biomass and 5 high leaf biomass yield (Bars represent $LSD_{0.05}$).

7.3.3.3 Farmers' scores for pod load

Farmers preferred genotypes with a high pod load, specifically for high grain yield and yields of immature pods, which are used as a vegetable. Results from pooled data on pod load showed a high preference for 309 and 305 at all sites (Table 7.6), while Sudan 1 and 3254 scored highly at all sites except Chitala and Chitedze respectively (Table 7.6 & Fig. 7.3).

Sudan 1 showed an average score of 2.95 at Chitala and 3254 scored an average of 1.35 at Chitedze. High scores for some genotypes at some sites and low at others indicate site specific preference (interaction between genotypes and sites). Genotypes which scored highly at specific sites include: 601 for Baka; 2226 and 2227 for Chitala; 698, 2232 and 570 for Chitedze and; 570, IT82E16 and 544 for Kasinthula (Fig. 7.3).

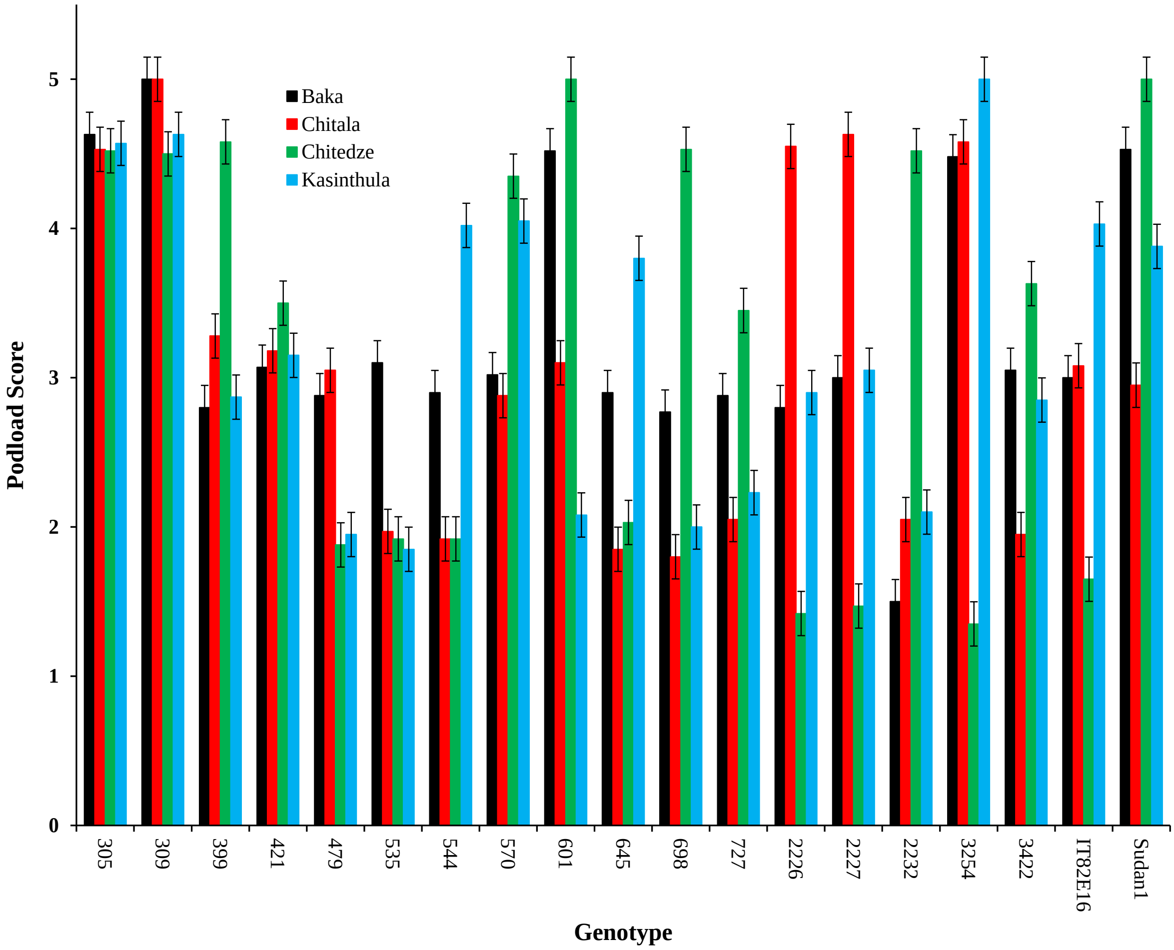

Figure 7.3: Mean scores for 19 genotypes for pod-load on a scale of 1-5 where 1 represents low pod load and 5 high pod load (Bars represent $LSD_{0.05}$).

7.3.3.4 Farmers' scores for seed size

Farmers' preference for cowpea genotypes, based on seed size, showed that both small and large seed sizes were preferred, depending on the use for the grain. Small seed size was preferred for making stew, which is consumed with rice or Nsima (hard porridge prepared from maize flour), while large seed size was preferred for use as a snack food where fresh mature pods are boiled and eaten. The pooled data analysis show that farmers preferred genotypes 2226, 2227 and 544 for large seeds, while 309, 305 and 421 were preferred for preparing stew due to their small seeds (Table 7.6 & Fig. 7.4).

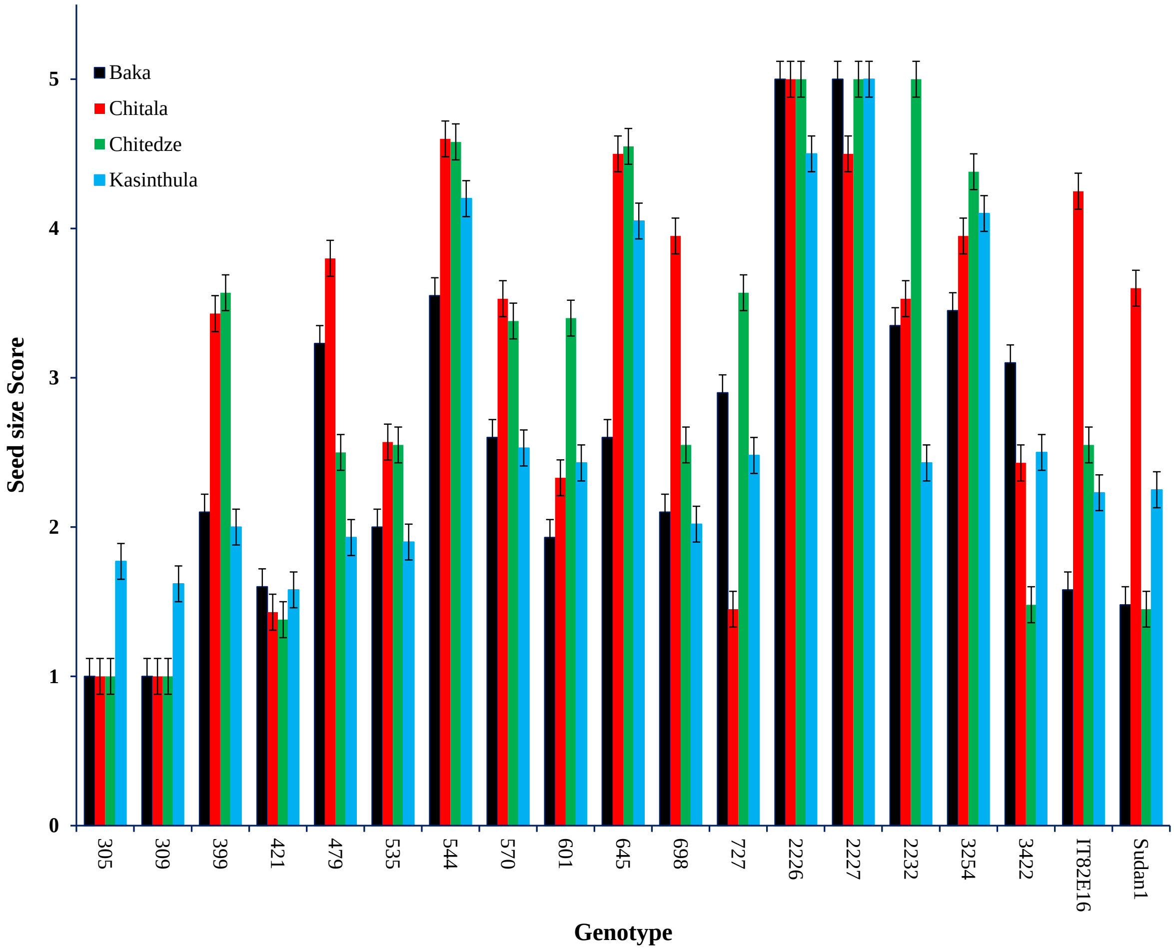

Figure 7.4: Mean scores for 19 genotypes for seed size on a scale of 1–5 where 1 represents small seed side and 5 large seed size (Bars represent $LSD_{0.05}$).

Genotypes 305, 309 and 421 achieved low scores (small seeds), while 2226, 2227 and 544 achieved high scores (large seeds) at all sites. Genotype 645 achieved high scores at all sites except Baka, where it scored an average of 2.10. Some genotypes showed significant differences within site suggesting site specific genotype preference. Site specific genotypes preferred for large seeds were 698, IT82E16 and Sudan1 for Chitala and 2232 for Chitedze. Genotypes preferred for small seeds at specific sites include: Sudan1 and IT82E16 for Baka; 727 for Chitala and; Sudan 1 and 3422 for Chitedze (Fig. 7.4).

7.3.3.5 Farmers' scores for texture of the seed testa

Farmers categorised the 19 genotypes into three categories, based on the texture of the seed testa, i.e. smooth, rough and intermediate. Farmers consistently showed a high preference for genotypes with a smooth testa, compared to genotypes with a rough testa. Both pooled and site specific analysis revealed a high preference for 305, 309, 479, 601, IT82E16 and Sudan 1, while 3254 was the least preferred because of a very rough seed testa (Table 7.6 & Fig. 7.5). Other genotypes which scored poorly for seed texture include 698, 727 and 570.

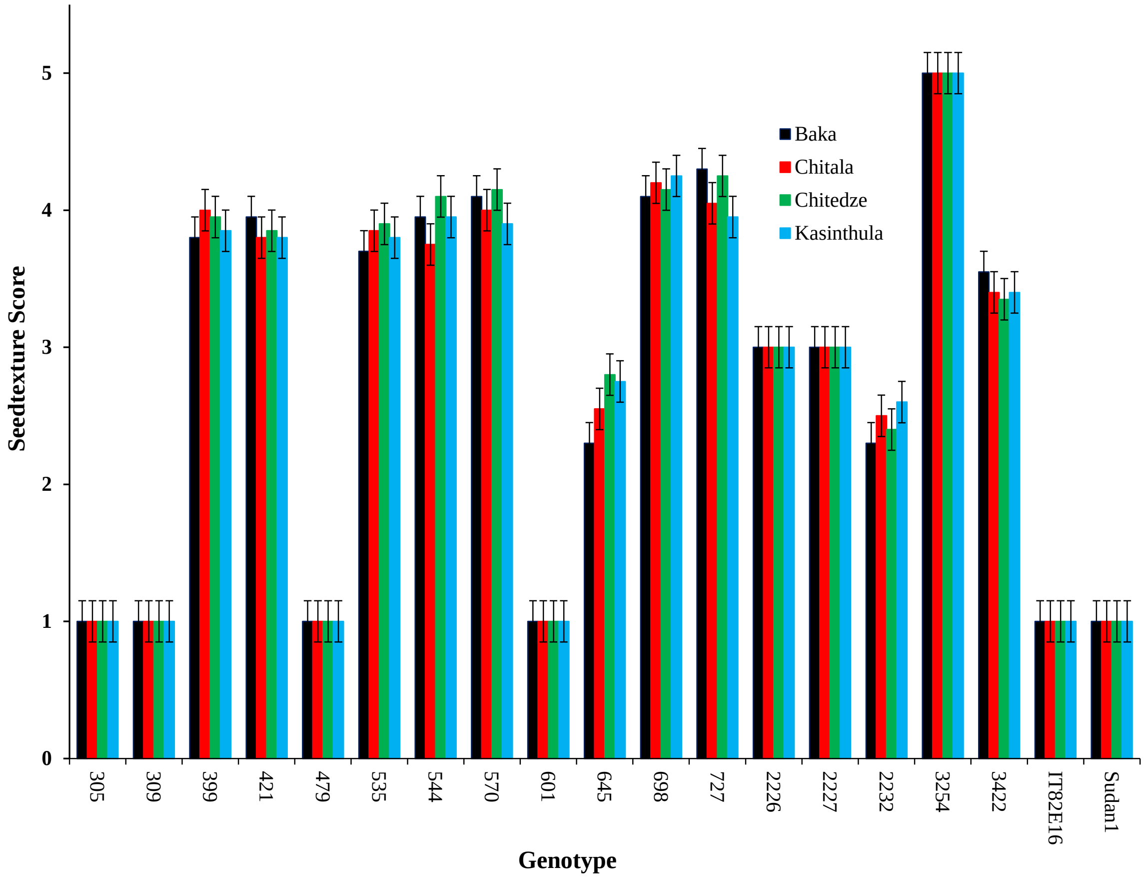

Figure 7.5: Mean scores for 19 genotypes on texture of seed testa on a scale of 1–5 where 1 represents smooth seed and 5 rough seed (Bars represent $LSD_{0.05}$).

7.3.3.6 Priority list of widely accepted genotypes

Scatter plots for pooled means and standard error of means (Figs. 7.6a – 7.6e) showed different groupings of genotypes. Genotypes 305 and 309 showed low scores for maturity and low standard errors of means (Fig. 7.6a), meaning that farmers uniformly scored them for early maturity across the sites.

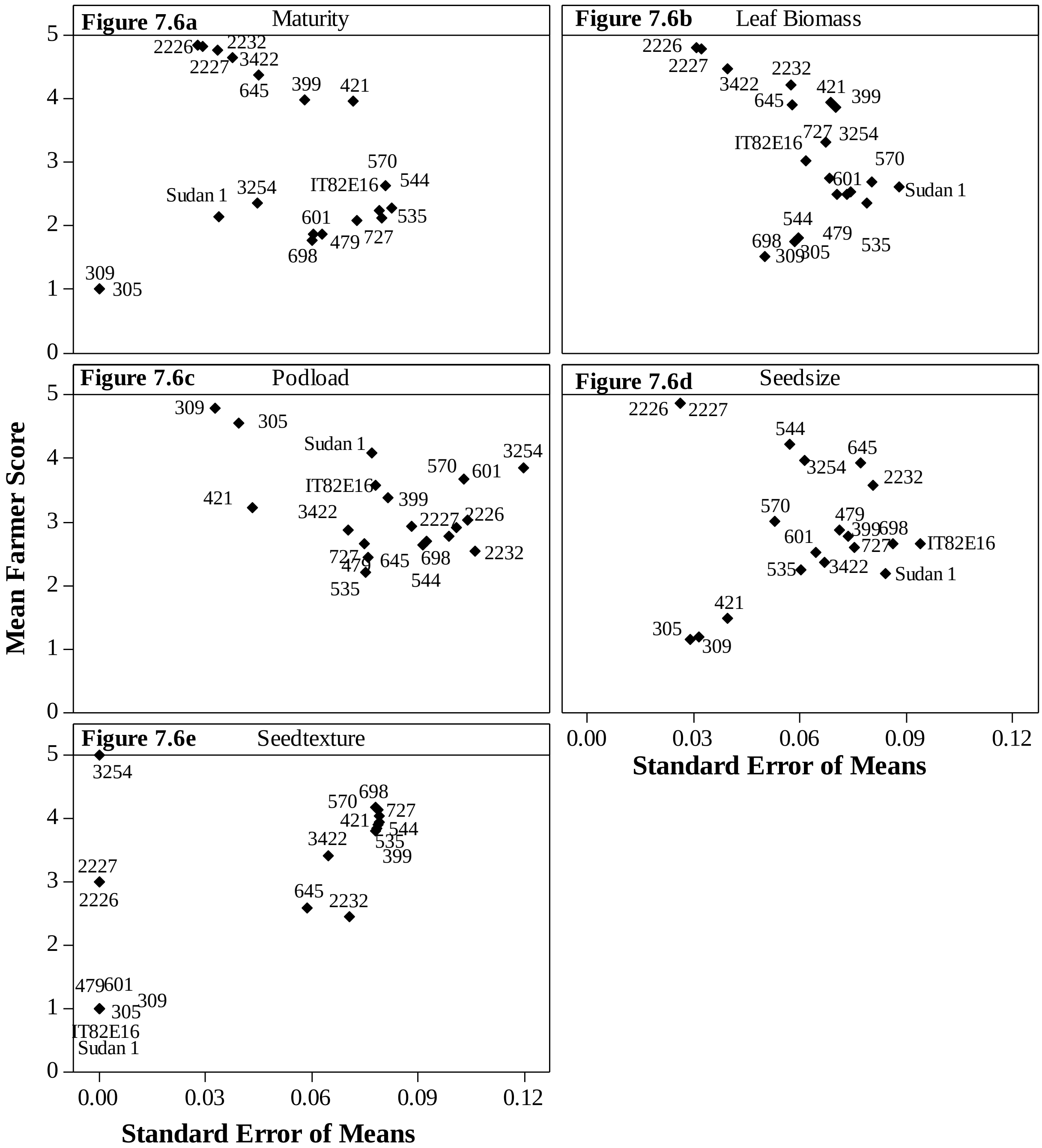

Figure 7.6 Scatterplots for pooled means and respective standard error of means for different genotypes.

Similarly, genotypes 2226, 2227 and 3422 showed high scores for leaf biomass and exhibited low standard errors of means (Fig. 7.6b). A scatter plot for pod-load showed high scores for 305 and 309 and low standard errors of means (Fig. 7.6c). Genotypes 2226 and 2227 scored highly for seed size and also showed low standard errors of means. Similarly, 305, 309 and 421 had low seed size scores and low standard errors of means (Fig. 7.6d). The scatter plot for seed texture shows that 3254 achieved a high score and low standard error of means, while 305, 309,479, 601, IT82E16 and Sudan 1 produced low scores and low standard error of means (Fig.7.6e).

7.4 Discussion

7.4.1 Production challenges

Farmers identified eight key challenges affecting the production and promotion of cowpea in the selected areas. These can be broadly grouped into agronomic and institutional challenges. The occurrence of frequent droughts, crop damage caused by pests and diseases in the field and in storage fall within agronomic challenges, while poor seed availability for improved varieties, low market premiums, poor extension services and limited options for the utilisation of cowpea fall within institutional challenges. The agronomic challenges would require more research on the crop by relevant institutions, in collaboration with farmers. Institutional challenges would require the creation of policies conducive to the involvement of key stakeholders in the value chain, in order to improve the production and utilisation of cowpea. Key options for addressing institutional challenges include strengthening extension services for cowpea production; establishing formal seed production systems, which would reduce dependence on landraces as planting material; creating good markets for cowpea by involving processing industries; developing an agricultural research policy framework which could include research on under-utilised crops such as cowpea; and creating public awareness of the importance of crops, such as cowpea, which could significantly contribute to improved food security.

7.4.2 Selection of desirable attributes for cowpea varieties

Although three improved varieties (Sudan 1, IT82E16, IT99K-494-6) have been developed and released for production in Malawi (Government of Malawi, 2000; Mviha *et al*., 2011), farmers still use landraces. The low adoption rate of these improved varieties may be attributed to lack of initiatives to promote the new varieties and/or that these varieties do not meet demands for growers and markets (Kamara *et al*., 2010). The latter explanation is well supported by the results of this research where landraces were rated more highly by farmers. The Department of Agricultural Research Services (DARS) and the Department of Agricultural Extension Services (DAES) conduct demonstrations of new varieties before releasing them to farming communities suggesting lack of promotion is not the key problem. All three varieties have been introduced by the International Institute of Tropical Agriculture (IITA), an international research institute mandated to conduct research on cowpea. Therefore, the low adoption of these varieties may reflect the non-involvement of Malawian farmers, who probably have different preferences to farmers from areas where these varieties were first selected.

The 12 preference criteria identified by the farmers in this study (Table 7.4) can be broadly categorised into two major groups: a production related group and a consumption related group. High grain yield, drought tolerance, maturity, field pests and disease, leaf biomass and high pod load fall within the production related group, while seed size, seed colour, seed testa texture, cooking characteristics and post-harvest pest resistance fall within the consumption related group. Similar to these groups, Kitch *et al*. (1998) identified three categories of farmers' preferences for cowpea. In their study, they identified yield related characteristics, preference related characteristics and labour related characteristics. Some of the labour related characteristics included erect growth habit for easy harvesting, spreading habit for suppressing growth of weeds, many pods per plant and many seeds per pod. In this study, no labour related characteristics were rated as important by farmers, which may indicate the importance of yield and consumption attributes in the studied areas. Although labour related characteristics were not rated in the current study, their importance should not be completely ruled out in future breeding programmes, in order to avoid further low adoption of improved varieties.

Farmers at Baka, Chitala and Kasinthula ranked drought tolerance and early maturity as the most desirable attributes of varieties for production in their areas, while farmers at Chitedze prioritised high pod load, grain yield and resistance to field pests and diseases. The preference for early maturity and drought tolerance at these three sites was consistent with the need for strategies by farmers, in order to cope with drought conditions. Growing early maturing varieties is an adaptation strategy in areas with terminal droughts, while drought tolerant varieties ensure grain yield in areas experiencing intermittent droughts (Hall, 2004a). These three sites lie within the Rift Valley, which is characterised by high temperatures and low rainfall and where farmers consider early maturity and drought tolerance as adaptation mechanisms.

Preference for high leaf biomass at Kasinthula (Table 7.4) was consistent with the importance of livestock in the daily livelihoods of these farmers. Kasinthula is located in an area where farmers keep livestock including goats and cattle. This area continues to experience a decrease in the quality and quantity of forage, and consequently new options, such as forage cowpea may improve the situation, particularly as the impact of climate change has become more pronounced in the area (Nkomwa *et al.*, 2014). Cowpea is generally drought tolerant (Singh *et al.*, 1999a) and it has the potential to significantly improve the availability of forage under drought conditions. The utilisation of cowpea as a fodder crop needs to be explored at

Kasinthula and other areas which rely on livestock production but which experience erratic rainfall.

All seed characteristics were given low preference at all sites (Table 7.4). This contrasts with other studies, for example in Cameroon where seed characteristics, such as white seed colour, large seed size and rough seed texture, were given high priority, due to high market premiums (Kitch *et al*., 1998). Seeds with wrinkled testa were specifically preferred by processing companies. The low preference for seed characteristics as is the case for poor ranking of 3254 in the current study suggests that most cowpea production in Malawi is aimed at meeting domestic demand, rather than responding to commercial market demands, as was the case for Cameroon.

7.4.3 Selection of genotypes

7.4.3.1 Maturity

Genotypes 305, 309, 479, 570 and 544 were preferred for early maturity (Figs. 7.1 & 7.6a), with 305 and 309 selected at all the sites. Farmers prefer early maturing genotypes for food availability in times of critical food shortages. Food in Malawi is always in short supply during the wet season when crops are growing (Ellis & Manda, 2012). In addition, such varieties ensure grain yield under terminal drought conditions. However, the selection of early maturing genotypes by farmers should be considered with caution, because early maturing genotypes are associated with low yields, mainly due to a reduced vegetative growth period (Agbicodo *et al*., 2009). Besides low yield characteristics, the early maturing genotypes are associated with low leaf biomass production. Therefore, the development of a cropping system which integrates genotypes with early maturity, high grain yield and a prolonged growth period would help ensure the high production of cowpea.

7.4.3.2 Leaf biomass

Genotypes 2226, 2227 and 3422 scored highly for leaf biomass (Figs. 7.2 & 7.6b). Genotypes with high leaf biomass were selected for fresh and dried leaf vegetables and animal fodder. In Malawi, no named varieties have been released for high leaf biomass, to cater for the demand for leaf vegetable and animal fodder. The genotypes identified in this study form a good starting point for research on high leaf biomass in cowpea. This may help ensure increased benefits from cowpea production, compared with the current situation where emphasis is laid only on increased grain yield, rather than other products, and would support the notion that cowpea is a multi-purpose crop (Timko & Singh, 2008).

7.4.3.3 Pod load

Cowpea is used as a vegetable in various forms including immature fresh pods, fresh seeds and tender young leaves (Timko & Singh, 2008). Farmers in this study selected genotypes with a high pod load, specifically for immature pods. Genotypes 309 and 305 were preferred for high pod load at all four sites, while 3254 and Sudan 1 were preferred at three sites each and some genotypes were selected at specific sites (Figs. 7.3 & 7.6c). The selection of genotypes with a high pod load by farmers supports the recommendation by Umaharan *et al*. (1997) that vegetable cowpea (immature pods) improvement should target genotypes with a large cluster of pods per plant and a high pod weight per plant. However, the use of a high pod load or large cluster of pods alone may not be a good selection criterion. Additional pod attributes such as green colour and tender fibreless pods, are also used in the selection of vegetable cowpea varieties (Pandey *et al*., 2006). The development of varieties with a high pod load and other desirable attributes, such as a green colour and tender fibreless pods, together with long large pods, would significantly improve the utilisation of cowpea as a vegetable crop in Malawi.

7.4.3.4 Seed size

Genotypes with large (2226 and 2227) and small (305, 309 and 421) seeds were preferred for domestic uses at all the sites (Figs. 7.4 & 7.6d). The genotypes with large seeds were specifically preferred for use as snacks, while genotypes with small seeds were preferred for making stew. In contrast, varieties with large seeds were preferred for high market premiums, rather than domestic use in Nigeria (Kamara *et al*., 2010), Ghana (Quaye *et al*., 2011) and Cameroon (Kitch *et al*., 1998). The different cowpea utilisation patterns between Malawi and West Africa (Nigeria, Ghana and Cameroon) indicates that production of cowpea in West Africa has moved towards industrial use and hence the demand for varieties with large seed size to meet market demand.

Based on the split preference for seed size in this study and the potential of high market premiums from genotypes with large seeds in West Africa, it is imperative to establish parallel breeding programmes that target varieties in each category. The varieties with small seeds will address the domestic demand for making stew, while varieties with large seeds will address domestic need for snack consumption and potential future commercial needs. In this study, genotypes 2227 and 2226 showed good potential for cowpea commercialisation, due to their large seeds. However, the commercialisation of cowpea requires the involvement of the private sector if markets are to be stable. A proper market analysis needs to be conducted in order to establish the commercial value of the crop in Malawi, prior to the establishment of a breeding programme for varieties with large seeds. The other option for commercialisation of cowpea is to encourage processing companies from within and outside Malawi to start developing products from cowpea.

7.4.3.5 Seed testa texture

Cowpea varieties differ in the texture of the seed testa, with some varieties exhibiting smooth seeds, while others exhibit rough (wrinkled) characteristics (Uguru, 1996). In this study, farmers preferred genotypes with smooth rather than rough characteristics (Figs 7.5 & 7.6e)). This is evident from the poor ranking of 3254, the only genotype with a rough seed testa. The poor ranking of genotype IT84s-2246, with seed characteristics similar to 3254, was also observed in Mozambique (Chiulele *et al.*, 2011). Similar results from farmers in Malawi and Mozambique indicate a common preference for varieties in the two countries located in Southern Africa. However, in West Africa, varieties with wrinkled seeds are preferred by farmers and cowpea processing industries, due to easy de-hulling (Zannou *et al*., 2004). The poor ranking of 3254 in Malawi and IT84s-2246 in Mozambique is a reflection of the fact that cowpea is currently mainly produced for domestic use in these countries, and that varieties suitable for processing may not be readily accepted. Viable commercialisation of cowpea varieties in these two countries would require the availability of varieties with easy processing characteristics, such as 3254 and IT84s-2246. Consequently, varieties with a rough seed testa should not be completely rejected by research for future commercialisation. Research institutions in the two countries should strive to increase acceptance of varieties with rough seeds, through public awareness on the importance of such varieties with an emphasis on the involvement of private companies in the marketing and processing of cowpea.

7.4.4 Scatterplots as a statistical method for selection of widely accepted varieties

The scatter plots of pooled means and standard error of means suggest the importance of two dimensional data exploration (means and standard error of means) in understanding scores by farmers in participatory variety selection. The clustering of genotypes with similar means and standard errors in a scatterplot has aided the visualisation pattern in the dataset. Statistically, genotype means indicate overall performance across the sites, while the standard error of means indicates the spread of site means around the pooled mean (Steel *et al*., 1997). Therefore, genotypes with a particular preferred category and small standard errors of means show consistency in farmers' scores across the sites, while large standard error of means may indicate variation among site means and point towards site specific preferences. In addition scatterplots help in identifying genotypes with intermediate and uniform performance such as Sudan 1 and 3254, for maturity (Fig. 7.6a), 421 for pod load (Fig. 7.6c), 570 for seedsize (Fig. 7.6d), 2226 and 2227 for seed texture (Fig. 7.6e). This is the first case where scatter plots have been used to help select widely accepted genotypes from farmers' scores. Most studies use variety rankings in selecting preferred genotypes in PVS (Chiulele *et al*., 2011; Sperling *et al.*, 2001). It is anticipated that the use of ranks alone may provide substantial challenges to identifying the widely accepted genotypes, by masking the component of variation of means is an indication of stability for the selected genotypes. Genotypes 421 and 645 are a good example of such a case. Based on variety ranks using the pooled data for leaf biomass, 421 may be ranked higher than 645 (Fig.7.6b). However, genotype 645 has a low standard error which shows that this genotype has scored more consistently across sites than 421. The use of scatterplots to select highly preferred and stable genotypes should be further explored.

7.4.5 Genotypes with contrasting characteristics

The widely accepted genotypes identified in this study have contrasting attributes. For example, all the early maturing genotypes were poorly ranked for high leaf biomass, a character desired for leaf vegetable and animal fodder (Figs 7.6a & 7.6b). To address contrasting preferences, farmers can practice varietal intercropping of early maturing genotypes and varieties with high leaf biomass. Varietal intercrops have the advantage of producing more leaf biomass and grain compared to any of the sole varieties (Hall, 2012). The other option for enhancing early maturity and high leaf biomass is to cross the contrasting genotypes, to produce dual purpose varieties. Varieties with dual purpose, i.e. high grain and high leaf biomass, have been produced by crossing early maturity with late maturing genotypes (Singh *et al*., 2003). However, to address contrasting preferences in the absence of dual purpose varieties, farmers should practice varietal intercropping while breeders attempt to produce dual varieties purpose.

7.4.6 Variation in farmers scores within and among sites

Differences in scores among sites were observed in some genotypes. Genotypes 535, 544, 570, 727 and IT82E16 showed variation for maturity (Fig. 7.1) while 535, 544, 570, IT82E16 and Sudan 1 showed variation for leaf biomass (Fig. 7.2). All genotypes except for 305 and 309 showed variation among sites (Fig. 7.3) for pod load. For seed size, genotypes 305, 309, 421, 2226 and 2227 showed uniform size across sites while others showed significant variation among sites (Fig. 7.4). The differences in scores among sites for particular genotypes may be explained by seed mixtures present in landraces, different perceptions by farmers and genotype x environment interaction. Mixtures in the landraces may be a possible cause of the differences in scores because landraces are characterised by mixtures due to local seed exchange among farming communities (Thomas, *et al.* 2011). Farmers may judge (score) the same genetic material differently due to different levels of understanding, economic status, market orientation and gender (Sperling *et al.*, 1993). Genotype x environment interaction also plays a critical role in the selection of varieties for a particular environment due to specific adaptation (Joshi *et al*., 2007).

This study has shown that genotypes with scores at the extreme ends of the scale have mostly been similarly scored across the sites. For example, 305 and 309 were scored for early maturity; 2226, 2227, 3422 and 2232 were scored for late maturity; 2226 and 2227 were scored for high leaf biomass; 305 and 309 were scored for high pod load; 2226 and 2227 were scored for large seeds and 305, 309 and 421 were scored for small seeds. However, genotypes falling in the middle of the scale showed significant variation among sites.

Sperling *et al.* (2001) pointed out that genotypes which consistently appear at end extremes of scoring scale correspond to high degree of homogeneity leading to uniform scores across site. Therefore, the similar scoring of genotypes across the sites suggests deliberate selection by farmers.

Among all the scored traits, seed texture showed no significant differences among sites (Fig. 7.5). The uniform scores among sites indicate that seed texture is a stable trait not easily affected by environment compared to maturity, leaf biomass, pod load and seed size. These results are in agreement with a cowpea characterisation study (Stoilova & Pereira, 2013), where maturity, yield and seed size showed significant interaction with environment but seed texture did not change with environment.

7.5 Conclusion

Farmers have identified key challenges associated with the production of cowpea in the studied areas. The identified challenges require a holistic approach, to cater for all stakeholders involved in the value chain (production to consumption). The improved production of cowpea would depend on creating a policy environment conducive to strengthening research, extension services, seed production, marketing, processing and utilisation of cowpea. To develop readily accepted varieties, farmers have identified desirable attributes, such as high grain yield; early maturity; high leaf biomass; high pod load; seed size; texture of seed testa; cooking time; seed colour; resistance to field pests and diseases; and resistance to storage pests. Considering the complexities associated with breeding to integrate these desirable attributes into a single variety, streamlined breeding objectives which target easily combined attributes is crucial. Genotypes 305 and 309 were specifically identified for early maturity and smooth seed testa at all the sites. For high leaf biomass and large seed size, genotypes 2226 and 2227 were ranked highly across all sites. Although 3254 was poorly ranked for texture of the seed testa, it has the potential to revolutionise the processing of cowpea into other commercial products and therefore may be useful in the future. In addition to 3254, genotypes 2226 and 2227 have the potential for commercialising cowpea in Malawi, due to their large seeds, which may achieve high market premiums in export markets.

This research suggests that the inclusion of 305, 309, 2226 and 2227, would help integrate farmers' preferences into the national breeding programme while including 3254 would be useful for the processing industry. In the absence of improved varieties with desirable attributes, such as early maturity, large seed size, high pod load, high leaf biomass and rough seed texture, it is recommended that all genotypes with these attributes should be further tested prior to release for large scale production. Releasing of 2226 and 3254 for large scale production has the added advantage of drought tolerance suitable for production in drought prone areas. Further research needs to be undertaken on the selected genotypes, more particularly field disease and pest resistance, post-harvest pests' resistance, cooking traits, palatability and processing characteristics, which have not been addressed in the current study.

Chapter 8 : General discussion: Application of key findings and opportunities for commercialisation and future directions for cowpea research in Malawi

8.0 Introduction

Several factors contribute to the low production of cowpea in Malawi; yields can be as low as 400kg/ha, compared to potential yields of 2000kg/ha (Government of Malawi, 2000). A lack of improved and well-adapted varieties has been singled out as a major contributor to these low yields. To date, three cowpea varieties have been released to the farming community, but acceptance has been poor (Mviha *et al*., 2011). The low adoption rate of these varieties suggests low farmer preference compared to the landraces, which are commonly used as seed. Currently, the occurrence of frequent and intense droughts, partly due to climate change, has aggravated the problem of low production of crops, including cowpea (Pangapanga *et al.*, 2012). A lack of improved varieties and the occurrence of frequent and intense droughts have created a need for the development of improved varieties that are adapted to drought conditions and can satisfy farmers' needs.

This study was formulated as a first step towards the development of a drought tolerance breeding programme in Malawi. A systematic approach was utilised starting with an understanding of the geographic distribution of the available germplasm, which has pointed towards the identification of germplasm that is adapted to low rainfall and high temperature conditions (Chapter 3). Germplasm was screened for canopy maintenance under moisture stress in a glasshouse (Chapter 4). Those genotypes which maintained an active canopy under drought were further screened for physiological mechanisms governing their responses to moisture stress (Chapter 5). The drought tolerant genotypes, one drought susceptible genotype and two released varieties were tested in the field for reproductive, yield and seed characteristics (Chapter 6). Finally, the drought tolerant genotypes were included in the participatory variety selection, together with two released varieties and other landraces, to identify genotypes preferred by farmers (Chapter 7). Experiments investigating canopy maintenance and physiological mechanisms governing drought responses were conducted at Massey University, Palmerston North New Zealand, while the field experiments and farmers' preference studies were implemented in Malawi.

This is the first systematic study on local cowpea germplasm from Malawi and its results have wide and practical applications in the development of a cowpea improvement programme in Malawi. For example, the identification of germplasm gaps and potential on-farm conservation sites will contribute to the improved conservation of a wide diversity of cowpea germplasm in Malawi; the identification of drought tolerant genotypes with other desirable attributes, such as early maturity, large seed size, smooth testa and high leaf biomass, will enhance breeding for improved production of cowpea; and the development of a leaf wilting index will reduce complexities associated with the scoring system for drought-induced wilting in cowpea. In addition, the presence of genotypes with large seeds and rough seed testa is an indication of possibility for developing cowpea varieties for commercial production. It has also opened up new research dimensions for cowpea, which would enhance the production of this crop in Malawi.

8.1 Application of key findings

The key findings are chapter specific and detailed discussions are provided within the respective chapters. However, this section provides an insight into the empirical findings in relation to the research questions and objectives of the study. More importantly, this section focuses on the technical application of the results for the improved management and utilisation of available cowpea germplasm and it also proposes the next course of action, which will lead to the improvement of cowpea production in Malawi.

8.1.1 Management of plant genetic resources

This study has demonstrated the importance of maintaining local germplasm at a national level. Researchers in most countries do not fully utilise their locally adapted germplasm, due to a lack of important information associated with conserved germplasm (FAO, 2010). As an alternative, scientists use exotic germplasm from international genebanks, such as the International Institute for Tropical Agriculture (IITA), as in the case for cowpea in Malawi. The evaluation of local cowpea and the identification of genotypes with drought tolerance and other desirable attributes in this study will contribute to an improved utilisation of the conserved germplasm in Malawi.

Eco-geographic characterisation can assist in focussing the search for genotypes with stress tolerance from large collection of genetic resources from a diversity of ecologies (Redden, 2013). A study of peas from 804 accessions collected from different ecological sites in China (Li *et al*., 2013) identified drought tolerant genotypes from areas characterised by low rainfall and high temperatures. In addition, a geographic pattern of genetic variation was observed in 146 chickpea accessions, which indicated the adaptation of genotypes to specific geographic and environmental conditions (Shan *et al.*, 2005). In this study, none of the five drought tolerant genotypes (Chapter 4) came from areas characterised by low rainfall and high temperature, represented by Cluster 4 (Chapter 3). The failure of the eco-geographic characterisation results to agree with the morphological and physiological characterisation of cowpea may be due to unbalanced sampling of genotypes from both wet and dry environments; and/or role of other factors contributing to the presence of drought tolerant genotypes in wet environments.

Comparison of results from chapters 3 and 4 shows that all the drought tolerant genotypes came from clusters 1 and 2 of chapter 3. These results suggest that the wetter and cooler environments may be better sources of drought tolerance. The cooler sites may not have provided selection pressure against the trait or drought tolerance was maintained in wet environments because of occasional droughts which gave selective advantage in dry years to the available populations. Consequently, such environments may lead to retention of maximum genetic diversity. The presence of drought tolerant genotypes from wet environments suggests that adaptations for extreme conditions such as drought can often be found in wet and cooler environments.

On the other hand, lack of drought tolerant genotypes from clusters 3 and 4 of chapter 3 may be explained by three factors. Firstly, farmer-mediated selection in the hot and dry areas may lead to selection of short season landraces as an adaptation mechanism without necessarily considering drought tolerance (Peleg *et al*., 2005). Secondly, the dry and hot areas may have serious bottlenecks because just one or two consecutive drought seasons may lead to extinction of most populations including drought tolerant ones (Blum, 2011; Penuelas *et al*., 2013). Thirdly, complete loss of locally adapted landraces in drought prone areas may have prompted farmers to acquire new germplasm adapted to wet conditions (Blum *et al.*, 1989). Therefore, to capture maximum diversity for drought tolerance, seed collectors and breeders should also target wet environments as potential sources of drought tolerant genotypes rather than concentrating in hot and dry environments only.

Despite the failure to identify drought tolerant genotypes from areas with low rainfall and high temperature, the eco-geographic characterisation (Chapter 3) has identified geographic gaps in cowpea germplasm (places with no or limited locally available germplasm). These identified gaps could be filled by either conducting fresh collection missions, or the repatriation of cowpea from international genebanks that collected cowpea samples prior to the establishment of the National Genebank of Malawi in 1992. The International Institute for Tropical Agriculture genebank alone holds more than 400 accessions of cowpea collected from Malawi (Bioversity International, 2011). Repatriation of this germplasm may cost less than conducting collection missions. In addition, materials collected many years back may represent true landraces, as they were collected before the introduction of improved varieties, which may have crossed with the landraces. However, these repatriated materials may be characterised by arrested evolutionary processes, compared to newly collected germplasm, which may have evolved within changing environments on farms (Hammer *et al.,* 2003). Therefore, the filling of gaps using both options is ideal for the conservation of a wide diversity of cowpea germplasm as long adequate storage facilities are available at the National Genebank to accommodate the new germplasm.

On-farm conservation of germplasm is an *in situ* conservation strategy, which recognises farming communities as custodians of local crop diversity (Maxted *et al*., 2002). The advantage of this strategy is that it enhances the adaptation of germplasm to local conditions. Eco-geographic characterisation identified sites which could enhance the adaptation of cowpea to drought conditions (Chapter 3). On-farm conservation initiatives in Nsanje and Chikwawa districts areas characterised by low rainfall and high temperature would enhance the adaptation of cowpea and other crops to drought conditions. Consequently, the Malawi Plant Genetic Resources Centre (MPGRC), in collaboration with other organisations, should consider the establishment of on-farm conservation in these areas. The establishment of such conservation sites now would ensure the future availability of germplasm that is adapted to low rainfall and high temperature.

Eco-geographic characterisation has improved the quality of passport data of the available germplasm. In most genebanks, details of environmental conditions describing collection points are scarce (Hijmans *et al*., 2001). In this study, rainfall, temperature and the altitude of the collection points of cowpea germplasm have been acquired through the use of DIVA-GIS, a tool specifically developed for the management of plant genetic resources and freely available climatic data (Hijmans *et al*., 2005). Taking advantage of the available tools, eco-geographic characterisation should be considered as a key genebank management operation, more especially in genebanks where germplasm is not properly described by environmental conditions. The acquisition of climatic variables of the available germplasm would assist in the search for genotypes adapted to specific environmental conditions.

8.1.2 Drought tolerance in local germplasm

Out of 36 genotypes screened for canopy maintenance, only 479, 601, 645, 2226 and 3254 maintained an active canopy under severe moisture stress (Chapter 4). The maintenance of an active canopy under low moisture indicated the presence of drought tolerance in the local germplasm. Further research on these potentially drought tolerant genotypes has shown different physiological mechanisms controlling drought tolerance (Chapter 5). Genotype 3254 maintained a high photosynthetic capacity during the period of water stress, while 479, 601, 645 and 2226 showed a low photosynthetic capacity after two weeks of water stress. The difference between 3254 and other drought tolerant genotypes conform to previously defined categories of drought responses in plants. Plants adapt to drought conditions through drought escape, drought avoidance and drought tolerance (Farooq *et al*., 2009). Drought escape is associated with early flowering and maturity. Drought avoidance is associated with reduced stomatal conductance, which further reduces net photosynthesis at an early stage of drought stress, as a water conservation mechanism, as in the case of 479, 601, 645 and 2226. Drought tolerance (e.g 3254) ensures the maintenance of stomatal conductance and high net photosynthesis, due to the adjustment of cellular activities during high moisture stress. The different drought adaptation mechanisms have implications on the choice of parental lines for drought tolerance breeding. Genotypes with drought avoidance, such as 479, 601, 645 and 2226, are suitable for breeding varieties for production in areas with mild drought conditions, while genotypes with drought tolerance, such as 3254, are suitable for breeding varieties for production in areas experiencing severe drought conditions.

Results from the field experiments conducted at Baka, Bvumbwe, Chitala, Chitedze and Kasinthula confirmed different drought tolerance levels in the five drought tolerant genotypes (Chapter 6). All the drought tolerant genotypes had yields greater than the susceptible genotype (2232) (Fig. 6.6) in the areas with low rainfall and high temperature: i.e. Baka, Chitala and Kasinthula (Fig.6.1). The high yield of 3254 among the drought tolerant genotypes at the three sites is an indication of a unique drought tolerance mechanism associated with high photosynthetic capacity under drought conditions, as seen in Chapter 5. Results from the field experiments also demonstrated the susceptibility of 2232, due to changes in seed size (Fig.6.8). Genotypes 2226 and 2232 showed large seed size at Bvumbwe and Chitedze. However, the seed size of 2232 reduced at Baka, Chitala and Kasinthula sites, which are characterised by low rainfall and high temperatures. The reduction in seed size in 2232, at the three sites, confirms that genotype 2232 was susceptible to drought compared to the drought tolerant genotypes, which did not change seed size.

Similar results from the glasshouse experiments (Chapters 4 & 5) and field experiments (Chapter 6) indicated the presence of drought tolerant genotypes in the local germplasm. Consequently, genetic studies should be pursued to gain an understanding of the inheritance of drought tolerance, which will help to identify good parental lines for this trait. The identification of good donors for drought tolerance would be in agreement with farmers' preferences, more especially in areas such as Baka, Chitala and Kasinthula, where farmers prioritised drought tolerance as a key attribute in variety development (Chapter 7).

Genotypes with both early maturity and drought tolerance have the advantage of giving substantial yields within a short period, even under drought conditions. In this study, genotypes 479, 601 and 3254 showed early maturity (Chapter 6) and drought tolerance (Chapters 4 & 5). Similar dual benefits and attributes of early maturity and drought tolerance have been identified and very early varieties with drought tolerance have been developed and promoted in the Sahel region (Hall, 2012). Therefore, genotypes 479, 601 and 3254 are being proposed as potential parental lines for improving cowpea production in areas with early season droughts in Malawi.

Drought tolerant and late maturing genotypes may provide a reliable supply of animal fodder in areas where rainfall is erratic. Farmers in West Africa grow late maturing varieties for high protein (17-18%), high dry matter digestibility (64 -71%) forage and yield as high as 6 tonnes/ha compared to other fodder crops (Singh *et al*., 2003). Genotypes 2226 and 645 exhibited drought tolerance (Chapters 4 & 5), late maturing characteristics (Chapter 6) and scored highly for leaf biomass (Chapter 7). These two genotypes may provide significant benefits in areas where animal production is the main agricultural activity. For example, the Shire Valley area is the main source of beef for the Southern region of Malawi, but is characterised by erratic rainfall. This erratic rainfall poses a substantial challenge to the availability of high quality fodder throughout the year, which has resulted in a change to livestock production patterns; farmers have resorted to goat production rather than cattle, due to a scarcity of fodder (Nkomwa *et al*., 2014). The promotion of drought tolerant and late maturing cowpea genotypes, such as 2226 and 645, in such areas could significantly improve the availability of animal fodder for improved livestock production. However, no specific varieties for the production of fodder have been developed in Malawi and farmers use cowpea haulms as fodder after harvesting the grain. A research programme aimed at integrating cowpea and livestock production would be beneficial to farmers in areas such as Baka, Chitala and Kasinthula, where rainfall provides a challenge to the production of crops.

8.1.3 Leaf wilting index as a measure for wilting due to moisture stress

Wilting, as an indicator for drought response in cowpea, is commonly assessed by using qualitative scales. The commonly used scoring systems involve scales of 1-5 (Singh *et al*., 1999b); 1 – 9 (IBPGR, 1983); and 5 – 1 (Watanabe *et al*., 1997). However, the use of these scales, which involves visual assessment, requires experience and non-experienced researchers face substantial challenges to properly identify appropriate wilting levels. This research has developed a quantitative index for scoring wilting in cowpea (Pungulani *et al.,* 2013). The cut-off point of 0.6 for the wilting index provided the same results as a cut-off point of 0.5 for relative water content. In addition, all the genotypes which showed an index of less than 0.6 maintained green stems during the period of stress and fully recovered after re-watering. The association between a leaf wilting index and relative water content, stem greenness and re-growth (recovering after re-watering), all traits which have been previously used in drought tolerance screening (Muchero *et al*., 2008), validate the application of leaf wilting in scoring for drought tolerance. Researchers working on cowpea should consider applying this index when evaluating cowpea genotypes for drought tolerance. However, due attention should be paid to the compounding factors of wilting, especially diseases.

8.1.4 Super early maturing genotypes

Sudan 1, IT82E16, 479 and 601 took fewer than 65 days to mature (Chapter 6). However, farmers selected 305 and 309 as early maturing genotypes (Chapter 7) because these two genotypes matured much earlier than Sudan 1, IT82E16, 479 and 601. Super early cowpea varieties, which start producing mature grain in less than 45 days, have been identified and recommended for production in areas with either short rainfall season or low rainfall (Hall, 2012). Those reported varieties that matured within a period of less than 45 days may have similar phenological characteristics as 305 and 309 in this study. Therefore, these two genotypes can be referred to as super early maturing genotypes. However, a systematic evaluation of these super early genotypes identified in this study needs to be initiated, in order to determine farmers' scores and yield before inclusion into the national breeding programme.

8.1.5 Seed characteristics and drought tolerance

Farmers preferred varieties with either large or small seeds (Chapter 7). Among the drought tolerant genotypes, 2226 produced very large seeds (>20 g) at all sites, while 601 produced small seeds (10-14 g) across all sites (Chapter 6). This suggests that 2226 may be a good parental line for breeding varieties with large seeds and drought tolerance, while 601 could be a good parental line for breeding varieties with small seeds and drought tolerance.

Genotype 3254 scored poorly at all the sites for its undesirable characteristic of wrinkled/rough seed testa (Chapter 7) and farmers indicated that this genotype resembled rotten grain, due to its brown and wrinkled appearance. The same genotype showed drought tolerance by exhibiting high photosynthetic capacity under water stress conditions (Chapter 5) and a high yield among the drought tolerant genotypes at sites with low rainfall (Chapter

6). The production of genotypes with drought tolerance similar to 3254 would enhance yields in drought prone areas. However, poor farmer ranking for domestic use due to wrinkled seeds poses a considerable challenge to the promotion of varieties with this attribute. It may be possible to improve the seed characteristics of this genotype through breeding without compromising its drought tolerance characteristics.

8.2 Opportunities for commercialisation of cowpea in Malawi

Studies conducted elsewhere have demonstrated that genotypes with large seeds and rough seed testa are associated with high market premiums, thus making cowpea a commercial crop. Varieties with large seeds are desirable for the canning industry, while varieties with rough seed testa are preferred by processing companies, due to easy de-hulling for production of other products (Henshaw, 2008). Comparison of genotypes with large and small seeds shows that large seed fetches high market premiums (Quaye *et al*., 2011). Genotypes, such as 2226 and 2227 with large seeds and 3254 with rough seed testa, have the potential to enhance the incomes of local farmers in Malawi. Promotion of cowpea varieties with large seeds and rough seed testa requires a shift from domestic to commercial production.

Production of cowpea as a vegetable (immature fresh pods) also has the potential to shift cowpea from domestic to commercial production. The gross income from fresh immature pods surpasses that from dry grain by several-fold. On average, a yield of fresh pods ranges between 4 – 10t/ha (Nwofia, 2012), while grain yield ranges between 1 – 3.6t/ha (Timko & Singh, 2008). Prices at the Lilongwe market showed that fresh pods sell at MK150/kg, while dry grain sells at MK250/kg. Based on these market prices, a farmer would expect to earn a gross income between MK250,000 and MK900,000/ha from grain, but between MK600,000 and MK1,500,000/ha from fresh pods. Therefore, the use of cowpea as immature pods has good potential to improve the income of poor farmers. With fresh harvest the crop season is reduced which provides more opportunities for other crops. Besides the economic benefits, the high content of protein, minerals and other trace elements present in fresh immature pods (Ano & Ubochi, 2008) demonstrate the nutritional benefits of vegetable cowpea. Further research on the identified genotypes with a high pod load, such as 305 and 309 (Chapter 7), may help improve economic, nutritional and health benefits for local farmers.

Integrated cowpea and livestock production may be highly profitable especially in drought prone areas, where livestock production is affected by a lack of adequate feed. The relative drought tolerance of cowpea (Singh *et al.*, 1999a) puts it at an added advantage in dry areas compared to other fodder crops. The use of cowpea as a fodder crop is well developed in West Africa, due to its drought tolerance and high forage value (Singh *et al.*, 2003). The interest of farmers to produce varieties with high leaf biomass, shown in Chapter 7, is a clear indication of the need for the potential development and promotion of fodder varieties. Farmers producing fodder would enhance the productivity of their livestock and they would also generate income through the sale of surplus fodder to other farmers.

8.3 Policy considerations for production and commercialisation of varieties derived from local germplasm

This research has identified some local germplasm with the potential to improve cowpea productivity in Malawi which may result in commercialisation of the crop. However, breeders and the private sector, who are interested in the commercialisation of the identified genotypes and their derivatives, should take full cognisance of policy regimes governing the utilisation of plant genetic resources for food and agriculture. Malawi is a contracting party to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), an international instrument governing the conservation and sustainable utilisation and the fair and equitable sharing of benefits arising out of their use, in harmony with the Convention on Biological diversity (CBD) (FAO, 2009, 2014). Articles 9 and 10 of the treaty are pivotal in sustainable utilisation of plant genetic resources for food and agriculture. Article 9 recognises farmers as custodians of crop diversity and stipulates the need for the benefits of utilising crop diversity to trickle down to them. Article 10 encourages all contracting parties to include genetic materials into the multi-lateral system (mls) which accelerates the sharing of genetic resources for non-commercial use and (at the same time) it ensures that benefits arising from the commercialisation of genetic material are shared fairly and equitably. All cowpea accessions included in this research were collected from farmers and they have also been included in the multi-lateral system. Therefore, any commercialisation of varieties derived from materials identified in this study should ensure that farmers gain appropriate benefits from their efforts in conserving these resources. Such benefits would be facilitated by signing material transfer agreements for easy tracking as provided for in the ITPGRFA.

Some of the key considerations to ensure that farmers benefit from their efforts to maintain cowpea diversity include but are not limited to non-restrictive seed recycling by farmers; involvement of farmers in seed multiplication as a business; involvement of farmers in breeding cowpea varieties through participatory variety selection; and the establishment of a gene fund, where private companies using varieties derived from local germplasm would deposit a percentage of the proceeds from the sales of these varieties. This fund will ensure the sustainable conservation of plant genetic resources for food and agriculture, as a provision within farmers' rights.

8.4 Limitations of the study

Although this research has brought into light some significant results, the interpretation and application of these results should take full cognisance of some potential shortfalls. The identification of drought tolerant genotypes was based on above-ground characteristics only, without examining the root characteristics, which also play a critical role in defining drought tolerance. The identification of root characteristics would strengthen the validity of tolerance levels of the drought tolerant genotypes. Results from the field experiments conducted over one season provide insights into the existence of high yielding and well-adapted genotypes. However, repeated experiments over several seasons and sites would strengthen the validity of the results, more especially on yield related characteristics, which are easily affected by the environment. Farmers’ selection of genotypes was applicable to the characteristics used in this study only. Other important attributes, such as cooking time and palatability characteristics, which were not considered in this study, also play a pivotal role in determining preferred varieties. Consequently, more work needs to be undertaken with farmers, with respect to cooking and palatability tests. Disease and pests resistance of the genotypes with desirable attributes is not well understood. Therefore, more research is required to comprehend the pests and disease resistance of these genotypes. In the absence of knowledge on pests and disease resistance of these genotypes, their production should be accompanied with appropriate plant protection practices that target the most important pests and diseases.

The assessment of drought tolerance at vegetative stage only in this study is a great drawback to application of results for future cowpea improvement. Cowpea suffers significant yield reduction when stressed at reproductive stage (Hall *et al.*, 2003; Belko *et al*., 2014). Therefore, assessment of drought tolerance at vegetative stage only rather than reproductive stage (flowering and pod set) provided limited understanding of effect of drought on yield of the evaluated germplasm. Cowpea germplasm which showed drought tolerance at vegetative stage in this study should be further tested at both reproductive for the identification of genotypes with high yield potential under drought conditions.

This research takes full cognisance of inadequate germplasm (low numbers of genotypes) included in the study which may result in a breeding program being started using germplasm that is not best available. Several factors contributed to the low numbers of genotypes included in all the experiments. Prior to set up of the first glasshouse experiment all the sixty-six accessions from Malawi reported in Chapter 3 were quarantined for biosecurity assessment. Forty accessions passed the assessment and were recommended for inclusion into the first glasshouse experiment. However, due to limited space in the available glasshouses at the Plant Growth Unit (PGU), only thirty six accessions were included in the first experiment. Results from the first glasshouse experiment defined the number of genotypes in the subsequent two experiments except for two released varieties (Sudan 1 and IT82E16) which were included in the field experiment conducted in Malawi. This shortfall requires further research to survey available genetic variability in the elite backgrounds including those from IITA and other research institutions.

8.5 Conclusions

The goal of this study was to explore the presence of cowpea genotypes with drought tolerance, high yield and other desirable attributes among local germplasm conserved at the Malawi Plant Genetic Resources Centre (National Genebank of Malawi). One of the significant results emerging from the study is the identification of genotypes with different responses to drought; drought avoidance (479, 601, 645 and 2226) and drought tolerance (3254). The second major finding is the development of a leaf wilting index, which will contribute to an improved scoring system for wilting in cowpea and related crops. In addition to drought tolerant genotypes, the research has also identified genotypes with other desirable attributes. Firstly, Sudan 1 was preferred for high yields at various sites, while 3254 yielded highly in areas with low rainfall and high temperature. Secondly, genotypes 305 and 309 scored high for early maturity and high pod load, a characteristic liked specifically by farmers for the production of vegetable (immature fresh pods) cowpea. Thirdly, 2226 and 2227 showed large seeds. Finally, 2226, 645 and 2227 were preferred for late maturity and high leaf biomass, a characteristic suitable for the production of high value forage and leaves for human consumption. The presence of multiple desirable attributes provides support for the conceptual premise that well adapted and preferred cowpea varieties can be developed from the available germplasm.

Undesirable characteristics were identified in some genotypes. Genotype 2232 showed wilting signs within the first week of stress suggesting poor performance under low rainfall and high temperature conditions. Genotype 3254 was poorly ranked by farmers due to its wrinkled seeds potentially limiting its utilisation by small growers for domestic use. However, this genotype is potentially useful for commercial production targeting the processing industry. A comprehensive breeding programme is required, in order to improve these undesirable characteristics, without compromising the positive attributes of the two genotypes.

8.6 Future research direction

The results from this study, including the identification of some limitations, have opened up a new research agenda with the potential to significantly improve production and utilisation of cowpea in Malawi. For example, the drought tolerance of 3254 may be challenged by small growers due to its wrinkled seed testa. Therefore, an improvement in seed characteristics, without compromising the drought tolerance of this genotype is required. Future promotion of genotypes with wrinkled seed testa requires public awareness on the economic benefits farmers could gain from such varieties. The potential of 305 and 309 for high pod yield requires further research on the acceptability of fresh pods for vegetable use in Malawi.

Taking advantage of the drought tolerance of cowpea and the presence of genotypes with promising high leaf biomass, further research and experimentation on cowpea, as a forage crop is required. Diseases and pests cause significant reduction of yields in cowpea. Testing genotypes with disease and pest resistance should be a priority. Cowpea production and promotion will not expand without an appropriate policy environment. The lack of a well-structured market is impeding the production and promotion of cowpea in Malawi. A detailed market research project would help provide policy direction for the commercialisation of cowpea in Malawi. Future research should complement the existing international efforts to develop improved cowpea varieties led by IITA.

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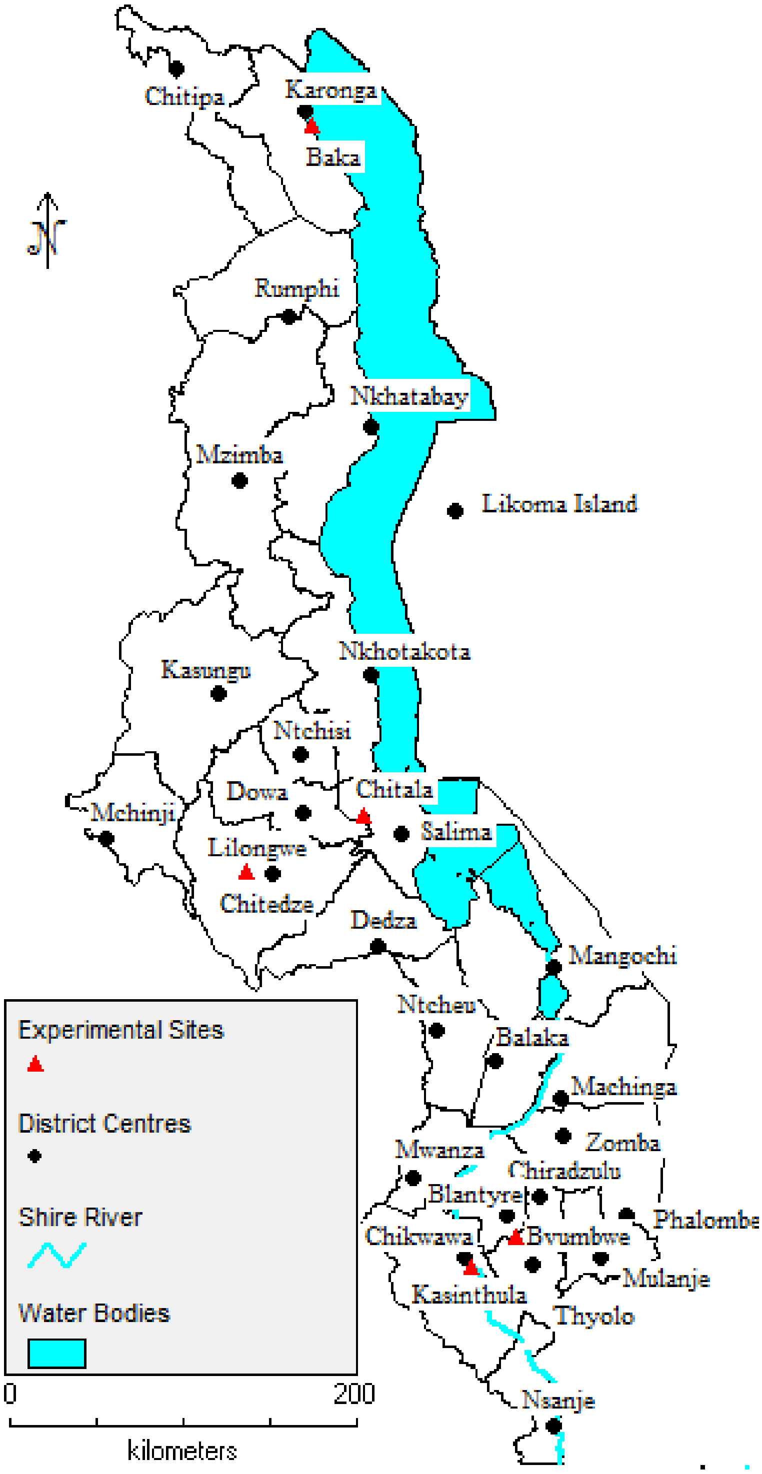
Appendices

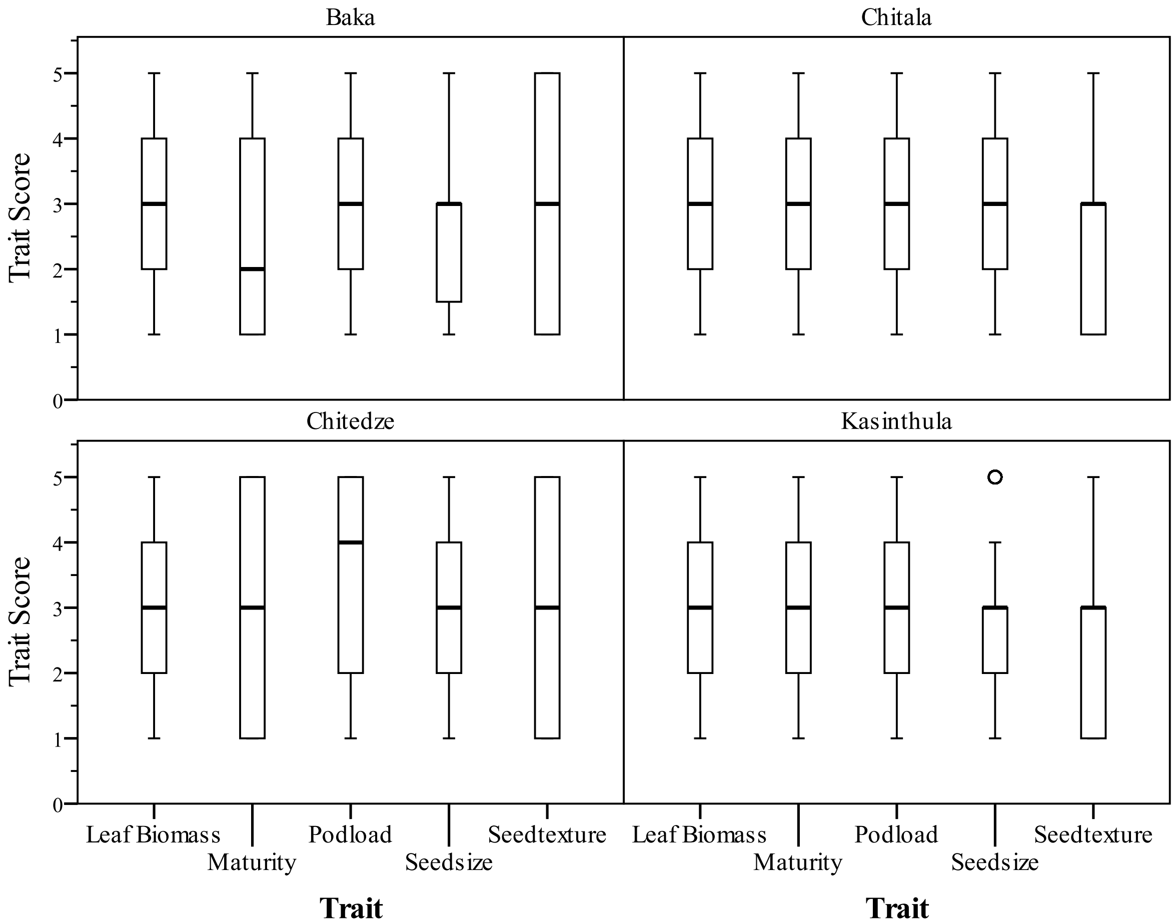
Appendix I: Map of Malawi showing field experiment sites.



Appendix II: Photographs of participatory variety selection (PVS) trials.

A: Laying out PVS trial; **B:** Seedlings of cowpea in the PVS trial; **C:** Vegetative performance of cowpea; **D:** One of the late maturing genotypes; **E:** Early maturing genotypes; **F:** Variation in maturity characteristics; **G:** Farmers scoring for seed size; **H:** Supervisory visit during one of the PVS exercise; **I:** Farmers scoring for seed texture

Appendix III: Box plots for exploring distribution pattern of the data set of different variables at each site.



Appendix IV: Kruskal-Wallis mean ranks of 19 genotypes for maturity scores at each site and across four sites.

Genotype	Baka	Chitala	Chitedze	Kasinthula	All sites
305	130.5 [1]	73.5 [1]	103.0 [1]	72.5 [1]	378.0 [1]
309	130.5 [1]	73.5 [1]	103.0 [1]	72.5 [1]	378.0 [1]
399	522.0 [5]	601.0 [7]	568.5 [5]	471.2 [5]	2157.9 [8]
421	484.2 [5]	601.0 [7]	586.0 [5]	479.5 [5]	2138.0 [8]
479	324.8 [4]	123.9 [2]	306.8 [3]	203.8 [2]	947.5 [2,3,4]
535	220.3 [3]	361.1 [5]	200.6 [2]	403.5 [4,5]	1171.3 [5,6]
544	483.1 [5]	464.8 [6]	191.3 [2]	257.5 [2]	1402.8 [7]
570	130.5 [1]	372.2 [5]	281.3 [3]	391.3 [4]	1180.2 [5,6]
601	323.9 [4]	138.3 [2]	282.5 [3]	211.9 [2]	946.2 [2,3]
645	628.7 [6]	621.2 [7]	649.0 [6]	620.6 [7]	2517.2 [10]
698	146.3 [2]	286.3 [4]	200.6 [2]	232.0 [2]	889.0 [2]
727	183.3 [3]	374.3 [5]	276.6 [3]	224.7 [2]	1071.4 [3,4,5]
2226	682.5 [7]	588.8 [7]	649.0 [6]	700.0 [8]	2630.5 [11]
2227	682.5 [7]	576.7 [7]	649.0 [6]	700.0 [8]	2619.9 [11]
2232	631.9 [6]	682.0 [8]	649.0 [6]	627.5 [7]	2580.9 [11]
3254	351.2 [4]	228.5 [3]	405.8 [4]	303.5 [3]	1277.5 [6]
3422	612.9 [6]	605.1 [7]	593.0 [5]	550.3 [6]	2364.5 [9]
IT82E16	199.2 [3]	210.5 [3]	236.2 [2,3]	462.8 [5]	1088.2 [4,5]
Sudan 1	360.5 [4]	246.5 [3]	297.9 [3]	243.8 [2]	1149.6 [5]
Kruskal-Wallis H Test	663.4	672.7	643.3	617.2	2293.2
df	18	18	18	18	18
Sig.	***	***	***	***	***

*Superscripts represent homogenous rank groups at 5% alpha level

Appendix V: Kruskal-Wallis mean ranks of 19 genotypes for leaf biomass scores at each site and across four sites.

Genotype	Baka		Chitala		Chitedze		Kasinthula		All sites	
305	173.9	1	136.4	1	114.9	1	189.2	2	611.7	2
309	186.4	1	117.8	1	118.4	1	228.2	2	652.7	2
399	504.9	2	543.5	5	599.3	5	334.4	3	2010.0	7
421	501.2	2	601.2	5,6	578.8	5	363.3	3,4	2075.6	7
479	167.7	1	305.3	2,3	280.5	2,3	339.2	3	1090.5	3,4
535	465.5	2	293.1	2	104.6	1	161.9	2	1003.7	3
544	432.4	2	289.0	2	315.0	2,3	89.6	1	1111.3	3,4
570	454.5	2	129.0	1	337.5	2,3,4	320.3	3	1228.5	3,4
601	183.4	1	297.4	2,3	276.2	2	336.1	3	1086.5	3,4
645	449.0	2	449.7	4	599.3	5	538.7	6	2043.9	7
698	165.7	1	121.5	1	94.2	1	95.5	1	477.7	1
727	211.3	1	338.6	2,3	267.5	2	447.5	4,5	1249.5	4
2226	672.3	3	681.0	7	669.0	6	636.1	7	2653.7	10
2227	653.5	3	681.0	7	669.0	6	639.9	7	2638.0	10
2232	510.4	2	625.1	6	578.8	5	529.6	5,6	2262.9	8
3254	487.6	2	390.1	3	347.4	3,4	430.7	3,4,5	1638.7	6
3422	634.7	3	593.2	5,6	591.1	5	624.5	7	2441.9	9
IT82E16	205.7	1	354.6	2,3	407.1	4	470.3	5,6	1440.3	5
Sudan 1	168.4	1	281.2	2	280.5	2	454.1	4,5,6	1171.7	3,4
Kruskal-Wallis H Test	544.5		577.1		639.3		476.2		1855.3	
df	18		18		18		18		18	
Sig.	***		***		***		***		***	

*Superscripts represent homogenous rank groups at 5% alpha level

Appendix VI: Kruskal-Wallis mean ranks of 19 genotypes for pod load scores at each site and across four sites.

Genotype	Baka	Chitala	Chitedze	Kasinthula	All sites
305	632.4 [3]	624.9 [3]	569.5 [4]	620.2 [4]	2445.6 [12]
309	697.5 [4]	695.0 [4]	564.7 [4]	627.9 [4]	2591.3 [13]
399	280.0 [2]	418.7 [2]	579.1 [4]	317.2 [2]	1627.8 [7,8]
421	332.7 [2]	403.1 [2]	397.5 [3]	370.8 [2]	1520.4 [6,7]
479	295.5 [2]	378.6 [2]	186.2 [2]	166.6 [1]	1002.8 [1,2]
535	340.5 [2]	198.9 [1]	192.1 [2]	149.1 [1]	864.1 [1]
544	300.2 [2]	187.1 [1]	192.1 [2]	525.1 [3]	1178.9 [2,3,4,5]
570	325.8 [2]	346.8 [2]	536.0 [4]	529.0 [3]	1763.1 [8,9]
601	615.0 [3]	388.1 [2]	660.5 [5]	183.1 [1]	1838.4 [9,10]
645	300.7 [2]	178.8 [1]	203.9 [2]	484.4 [3]	1150.1 [2,3,4]
698	275.1 [2]	162.3 [1]	569.5 [4]	162.6 [1]	1225.3 [2,3,4,5]
727	295.3 [2]	211.9 [1]	390.3 [3]	212.1 [1]	1146.5 [2,3,4]
2226	279.5 [2]	628.6 [3]	128.7 [1]	322.3 [2]	1330.9 [4,5]
2227	321.4 [2]	639.6 [3]	135.5 [1]	350.9 [2]	1411.9 [5,6]
2232	65.5 [1]	208.3 [1]	569.5 [4]	185.9 [1]	1088.4 [1,2,3]
3254	606.4 [3]	632.3 [3]	118.4 [1]	685.5 [5]	1977.8 [10,11]
3422	330.2 [2]	181.2 [1]	415.3 [3]	312.4 [2]	1276.9 [3,4,5]
IT82E16	319.8 [2]	383.4 [2]	159.5 [1,2]	524.3 [3]	1334.3 [4,5]
Sudan 1	615.0 [3]	361.4 [2]	660.5 [5]	499.5 [3]	2114.0 [11]
Kruskal-Wallis H Test	442.8	532.2	647.5	495.0	924.6
df	18	18	18	18	18
Sig.	***	***	***	***	***

*Superscripts represent homogenous rank groups at 5% alpha level

Appendix VII: Kruskal-Wallis mean ranks of 19 genotypes for seed size scores at each site and across four sites.

Genotype	Baka		Chitala		Chitedze		Kasinthula		All sites	
305	92.0	1	63.0	1	74.5	1	202.9	1,2,3	401.6	1
309	92.0	1	63.0	1	74.5	1	173.5	1,2	377.9	1
399	300.0	4	397.6	4	446.9	4	259.5	2,3,4,5	1463.1	5,6,7
421	203.9	2,3	111.2	2	125.8	2	163.7	1	591.5	2
479	501.0	6,7	469.6	5,6	285.2	3	239.7	2,3,4	1517.8	6,7
535	283.3	3,4	256.7	3	292.6	3	242.4	2,3,4	1118.9	3
544	562.4	7	614.8	7	603.1	5	629.6	7	2370.9	10
570	398.2	5	416.8	4	416.3	4	376.1	6	1625.7	7
601	269.0	2,3,4	221.8	3	420.2	4	351.5	5,6	1292.2	4,5
645	398.2	5	597.0	7	599.1	5	615.9	7	2175.4	9
698	302.3	4	493.8	5,6	292.6	3	269.4	2,3,4,5,6	1373.9	4,5,6
727	448.8	5,6	114.1	2	446.9	4	363.8	6	1345.1	4,5
2226	720.5	8	686.0	8	670.5	6	665.2	7	2718.8	11
2227	720.5	8	597.0	7	670.5	6	707.0	8	2718.8	11
2232	531.7	6,7	416.8	4	670.5	6	351.5	6	1961.2	8
3254	547.0	7	494.2	5,6	571.4	5	615.4	7	2220.0	9
3422	478.4	6,7	235.7	3	139.5	2	370.0	6	1188.7	3,4
IT82E16	199.2	2,3	549.0	6,7	292.6	3	311.1	3,4,5,6	1355.7	4,5
Sudan 1	180.5	2	431.2	4,5	136.1	2	321.0	4,5,6	1071.3	3
Kruskal-Wallis H Test	569.4		623.8		693.1		503.6		1899.6	
df	18		18		18		18		18	
Sig.	***		***		***		***		***	

*Superscripts represent homogenous rank groups at 5% alpha level

Appendix VIII: Kruskal-Wallis mean ranks of 19 genotypes for seed texture scores at each site and across four sites.

Genotype	**Baka**	**Chitala**	**Chitedze**	**Kasinthula**	**All sites**
305	133.5 [1]	130.0 [1]	128.5 [1]	124.5 [1]	515.0 [1]
309	133.5 [1]	130.0 [1]	128.5 [1]	124.5 [1]	515.0 [1]
399	519.0 [4,5]	544.7 [4,5]	531.2 [6]	527.1 [4,5]	2120.3 [5,6,7]
421	537.3 [4,5]	519.7 [4,5]	518.6 [6]	520.9 [4,5]	2095.3 [5,6]
479	133.5 [1]	130.0 [1]	128.5 [1]	124.5 [1]	515.0 [1]
535	506.7 [4,5]	525.9 [4,5]	524.9 [6]	520.9 [4,5]	2076.6 [5]
544	537.3 [4,5]	513.4 [4,5]	550.1 [6]	539.7 [4,5]	2139.0 [5,6,7]
570	555.7 [4,5]	544.7 [4,5]	556.4 [6]	533.4 [4,5]	2188.9 [5,6,7]
601	133.5 [1]	130.0 [1]	128.5 [1]	124.5 [1]	515.0 [1]
645	319.5 [2]	354.3 [2]	383.2 [3]	378.7 [2]	1434.8 [2]
698	555.7 [4,5]	569.8 [5]	556.4 [6]	577.3 [5]	2257.6 [7]
727	580.2 [5]	551.0 [4,5]	569.0 [6]	539.7 [4,5]	2238.8 [6,7]
2226	421.0 [3]	419.5 [3]	411.5 [4]	420.5 [3]	1671.0 [3]
2227	421.0 [3]	419.5 [3]	411.5 [4]	420.5 [3]	1671.0 [3]
2232	320.3 [2]	347.1 [2]	326.6 [2]	361.3 [2]	1353.1 [2]
3254	666.0 [6]	670.0 [6]	663.5 [7]	671.5 [6]	2669.5 [8]
3422	488.3 [4]	469.6 [4]	455.6 [5]	470.7 [4]	1883.1 [4]
IT82E16	133.5 [1]	130.0 [1]	128.5 [1]	124.5 [1]	515.0 [1]
Sudan 1	133.5 [1]	130.0 [1]	128.5 [1]	124.5 [1]	515.0 [1]
Kruskal-Wallis H Test	618.6	618.6	624.8	629.3	2485.4
df	18	18	18	18	18
Sig.	***	***	***	***	***

*Superscripts represent homogenous rank groups at 5% alpha level

Appendix IX: Statement of contribution to Doctoral thesis containing publications.

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MASSEY UNIVERSITY
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STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Lawrent Lino Michael Pungulani

Name/Title of Principal Supervisor: James P. Millner

Name of Published Research Output and full reference:

1. Pungulani, L.L.M., Millner, J.P., & Williams, W.M. (2012). Screening cowpea (Vigna unguiculata) germplasm for canopy maintenance under water stress. Agronomy New Zealand, 42, 23-32.

2. Pungulani, L.L.M., Millner, J.P., Williams, W.M., & Banda, M. (2013). Improvement of leaf wilting scoring system in cowpea (Vigna unguiculata (L) Walp.): From qualitative scale to quantitative index. Australian Journal of Crop Science, 7(9), 1262-1269.

In which Chapter is the Published Work: Chapter 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Lawrent Pungulani, carried out all the experimental work and data analysis which resulted in publishing the two papers. The co-authors are his supervisors who provided usual guidance in the process of his PhD study.

Lawrent Pungulani (Digitally signed by Lawrent Pungulani)
Candidate's Signature

08/10/2014
Date

James Millner (Digitally signed by James Millner; Date: 2014.10.21 11:18:48 +13'00')
Principal Supervisor's signature

21/10/2014
Date

GRS Version 3– 16 September 2011