GENETIC VARIABILITY, HERITABILITY AND RELATIONSHIPS AMONG DROUGHT TOLERANT-TRAITS IN COWPEA (*VIGNAUNGUICULATA*[L.]WALP.)MAGIC - RILS POPULATION

BY

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(P16LSBT8003)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN BOTANY

DEPARTMENT OF BOTANY

FACULTY OF LIFE SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NOVEMBER, 2019

DECLARATION

Ideclarethattheworkinthis dissertation entitledGENETIC VARIABILITY, HERITABILITY AND RELATIONSHIPS AMONG DROUGHT TOLERANT TRAITS IN COWPEA (*VIGNAUNGUICULATA* [L.] WALP.) MAGIC - RILS POPULATIONhasbeen performed by me in the Department of Botanyunder the supervision of Professor M. A. Adelanwa, Professor A. K. Adamu and Dr. N. Belko. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work was previously presented for another degree or diploma in any university.

ABIOLA, AbdurRazaaq ---------- ----------

Signature Date

CERTIFICATION

This dissertation entitledGENETIC VARIABILITY, HERITABILITY AND RELATIONSHIPS AMONG DROUGHT TOLERANT TRAITS IN COWPEA (*VIGNAUNGUICULATA* [L.] WALP.) MAGIC - RILS POPULATION by ABIOLA, AbdurRazaaqmeetsthe regulations governing the award of the degreeofMasterofScience of the AhmaduBelloUniversity,Zariaandisapprovedforits contribution to scientific knowledge and literary presentation.

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DEDICATION

To my family.

To all cowpea researchers.

To the loving memory of my friends who are now deceased – OluwaseyiAledare and Joseph OnojaOjonugwa.

ACKNOWLEDGEMENTS

My firstgratitude is to God, who taught man the use of the pen.

I am grateful to my ABU supervisors – Prof. M. A. Adelanwa and Prof. A. K. Adamu for taking theirtimetoreviewallthechaptersofthisthesis.I appreciatemy IITA supervisors, Dr. O.Boukar(Head, Cowpea Breeding Unit), Dr. N. Belko, Dr. S. Chamarthiand Dr.A. Togola for theirsupervision and support. I cannot forget the tremendous assistance I received from entire workforce of the cowpea breeding unit, IITA,Kano station. They worked with me as part of the cowpea team. It has been amazing working with you all.

I am indebted to the International Instituteof Tropical Agriculture (IITA, Kano) for providing the sponsorship for this research. I appreciate the management and staff of Botany Department, Ahmadu Bello University, Zaria. You have all been awesome. Worthy of mention is Prof.(Mrs.) D. N. Iortsuun (HOD Botany Department), a great leader worthy of emulation you are, Prof. W. S. Japhet (the P. G. Coordinator), Dr. (Mrs.) E. B. Adelanwa, Dr. (Mrs.) R. E. Aliyu, Mr. S. K. Sakariyahu and Mr. G. Godfrey. Indeed, my contact with you has left an indelible mark in my life.

I appreciate my parents, AlhajiTijaniAgboolaAbiola and AlhajaRabiatBalogun-Abiola for bringing me up to believe in my dreams and supportingmewithprayers– youarethebest.Tomysiblings(Mr. Jimoh, Pharmacist (Mrs.) Shakirah, Dr. Tajudeen, Dr. Muhydeen and Prince Sa'eed), their wives and husband; my nieces and nephews,thank you all for believing in my dreams and supporting me in all waystill they are achieved. Thank you for your understanding, patienceand love.

I cannot forget "My Coterie," youallhavebeenwonderful.To all my friends and colleagues, Ithankyouforyourprayersandwordsofencouragement throughout this journey. Remain blessed.

Finally, to everyone who has contributed in many ways to my success, names are just too numerous to mention, I say a very big thank you. Worthy ofmentionistheassistanceIreceivedfrom Mr. S. K. Sakariyahuon data analysis, Mr. Y. Wada, Mr. S. Ndabo, Mr. A. Aliyu, Mr. A. Ladan, Mr. M. Kazir and Mr. S. Talle for their various roles in pre-defense presentation and editing. To you all I owe a debt. All your help, I shall not forget. Indeed, a thousand words cannot express the gratitude I wish to extend.

ABSTRACT

Erratic rainfall pattern exposes cowpea to drought at critical stages of growth of the crop. This is inspite of the inherent capacity of cowpea to withstand drought. There is no information on drought adaptability of cowpea MAGIC-RILs germplasm. This research was therefore, conducted to evaluate genetic variability, heritability and relationships among seedling stage and post-flowering stage drought tolerant-traits in Cowpea MAGIC - RILs population and select parents for further breeding activities. Screenhouse evaluation was carried out using a simple Wooden Box technique to investigate the responses of the lines to drought stress at the seedling stage. Field trial was conducted to screen the lines for their responses to post flowering stage drought stress. Results obtained from the Wooden Box Experiment showed high genetic variability among the lines fordrought tolerance at the seedling stage. New drought tolerant genotypes were identified, for instance MAGIC 162 and MAGIC 012 which both had a recovery rate of 100% and both maintained low leaf senescence scores of 2 were found to be highly tolerant to drought stress at the seedling stage. They also had a Type 1 Drought Tolerant Type which was the same as DanIla which was the drought-tolerant check. Field trials results demonstrated that cowpea MAGIC-RILs is a promising genetic resource to address drought stress in sub-Saharan Africa. The quantitative indices of drought tolerance STI and GMP were used to classify the genotypes. MAGIC 061, MAGIC 063, MAGIC 144 and MAGIC 032 were found to be tolerant to drought at post-flowering stage and classified as Class A due to their uniform superiority in both water-stressed and well-watered environments. Genotypic variability for drought tolerance existed amongst the tested genotypes. There exist relationships among drought-tolerant traits at both the seedling and the terminal stages. Leaf senescence is the most important predictor of recovery at the seedling stage and it is negatively correlated with recovery rate. The negative correlation between leaf senescence and recovery rate could

serve as an early indicator of the ability of cowpea MAGIC RILs to recovery from drought stress after rehydration. For terminal drought tolerance, stress tolerance index (STI) and geometric mean productivity (GMP) were highly positively correlated at $p < 0.001$. The seven (7)MAGIC RILs (MAGIC 162, MAGIC 012, MAGIC 336, MAGIC 301, MAGIC 213, MAGIC 204, MAGIC 129) selected at the seedling stage are recommended for use as parents for the development of new germplasms for further breeding pipelines. Ten (10)MAGIC RILs (MAGIC 061, MAGIC 063, MAGIC 144, MAGIC 032, MAGIC 115, MAGIC 305, MAGIC 004, MAGIC 005, MAGIC 015, MAGIC 257) selected as terminal drought tolerant lines are recommended for preliminary and multi-locational trials.

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CHAPTER ONE

1.0 INTRODUCTION

Cowpea (*Vignaunguiculata* [L.]Walp.;Fabaceae) is an important grain legume grown mostly in the dry savannah agro- ecologies of sub- Saharan Africa (SSA) (Togola*et al.*, 2019). In Africa, cowpea is part of thetraditional cropping systems and it is considered as a strategic crop because of its multiple uses as human food, animal feed, source of nitrogen for soil restoration and income for resource- poor farmers as well as small- scale processors (Isubikalu*et al.*, 2000; Abtew*et al.*, 2016; Boukar*et al.*, 2016).

Cowpea grains represent a major source of protein (20%–32%), minerals and vitamins in the diet of majority of rural and semi- urban communities (Egho, 2010; Boukar*et al.*, 2013;Singh, 2014; Palanga*et al.*, 2016). The tender leaves, soft stems and green pods are also eaten as vegetables in Asian and East African communities (Abudulai*et al.*, 2006). About 85% of the world cowpea production comes from the West Africa sub- region (FAOSTAT, 2017). The International Institute of Tropical Agriculture (IITA) is conserving about 15,000 cultivated cowpea germplasm accessions from many countries in its Genetic Resources Centre (Togola*et al.*, 2019).

Out of the 12.5 million tonnes of grain produced worldwide on a total area of about 14 million hectares, the Sahel region of Africa accounts for 64% of the global production (Singh *et al*., 2003; Mucher*oet al*., 2008). In Asia, Africa, and Latin America more than 10 million hectares are planted with cowpea (Nedumaran*et al.*, 2013), with a global yield of over 5 million tonnes per year, but still a low productivity of around 472.66 kg/ha (Sousa *et al.*, 2015) due to abiotic and biotic stresses, which affect plant growth and consequently grain yield (Kareem and Taiwo, 2007; Farooq*et al*., 2012). The abiotic stress factors comprise

drought, low soil fertility, and heat while biotic constraints include insects, diseases, parasitic weeds, and nematodes (Boukar*et al.*, 2016)

Drought stress and virus infection, alone or in combination, in cultivated fields, are amongst the several biotic and abiotic stresses that affect cowpea (Singh *et al.*, 2002).Nigeria, with an estimated 45% of the world cowpea production and over 55% of the production in Africa, is the world's largest producer and consumer of cowpea, followed by Niger (15%), Brazil (12%), and Burkina Faso (5%) (Boukar*et al.*, 2016). Over the last three decades, global cowpea production grew at an average rate of 5%, with 3.5% annual growth in area and 1.5% growth in yield, and the area expansion accounting for 70% of the total growth during this period (Fatokun*et al.*, 2012b). Globally, the share of cowpea in total area under pulses grew from < 10% in 1990 to nearly 20% in 2007 (Boukar*et al.*, 2016).

In some tropical areas of developing countries, such as the African Sahelian zones and the Brazilian Northeast region, grain legumes are grown only during the rainy season and drought stress represents the most important threat to biomass production for many small farmers (Donohue *et al.*, 2013). For the people of these regions, cowpea grains are an important protein source (Rivas *et al.*, 2016).Although studies with cowpea are numerous and several important agronomic traits have been revealed (Bastos*et al.*, 2011; Nascimento*et al.*, 2011; Oliveira *et al.*, 2012),available drought-tolerant cowpeas are few, and identification of additional genotypes with even greater tolerance to drought would enable breeders to develop cultivars with higher and more stable yields across the semiarid ecologies where this crop is grown (Belko*et al.*, 2014).

Multi-parent Advanced Generation Inter-Crosses (MAGIC) was firstintroduced by Mott *et al.* (2000). It was coined by Mackay and Powell (2007) and advocated by them and Cavanagh *et*

al. (2008) for crops. Recombinant Inbred Lines (RILs) are stable cultivars derived from MAGIC population which no longer segregate after the eighth generation (Mansur, 2017).

Multi-parent Advanced Generation Inter-Cross (MAGIC) populations are an emerging type of resource to dissect the genetic structure of traits and improve breeding populations (Huynh *et al.*, 2018). MAGIC population for cowpea were developed from eight founder parents which are genetically diverse and carry many abiotic and biotic stress resistance, seed quality and agronomic traits relevant to cowpea improvement in the USA and sub-Saharan Africa, where cowpea is vitally important in the human diet and local economies (Huynh *et al.*, 2018). The eight parents were inter-crossed using structured matings to ensure the population would have balanced representation from each parent, followed by single-seed descent, resulting in 305 F8 recombinant inbred lines (RILs) each carrying a mosaic of genome blocks contributed from all founders (Huynh *et al.*, 2018)

The predicted changes in climate may lead to extreme events, such as increased precipitation and long drought periods (IPCC, 2007).Drought is a potential major constraint to crop production, it can strike at anytime, anywhere (Batieno*et al.*, 2016). Plants are most prone to damage due to limited water during flowering and pod setting stages (Bahar and Yildirim, 2010). Despite the inherent capacity of cowpea to withstand drought, the erratic pattern of rainfall exposes the crop to drought at the onset and at the end of rainy season (Singh and Matsui, 2002).

1.1 Statement of the Research Problem

Drought is a frequently observed event and recurrent in areas with agricultural potential, mainly in tropical regions, causing an increase in temperature and decrease in the relative humidity (Muñoz-Perea*et al.*, 2006).This stress can be caused by irregularities in the distribution of rainfall (Asfaw*et al.*, 2012; Blair *et al.*, 2012) and/or inadequate supplement

irrigation for crops.Thus, the water deficit acts as a limiting factor for both quality and production in several species with agronomic potential (Acosta-Díaz*et al*., 2009; Andrezej, 2012), especially cowpea because available drought-tolerant cowpeas are few (Belko*et al*., 2014).Cowpeas are mostly grown in the semi-arid regions, and often experience droughts ranging from being severe in the Sahelian zone to being moderate in the Sudanian Savanna zone. Drought stress forces cowpeas into early senescence thereby resulting in low yield which poses a real threat to food security.

1.2 Justification

Drought is the most economically important abiotic stress affecting cowpeaproduction, because it is typically grown in the Savanna and Sahel regions where rainfall amount and distribution are unreliable. There is therefore an increasing need to identify and develop additional drought-tolerant and high-yielding cultivars adapted to the changing agro-climatic environments (Van Duivenboode*et al.*, 2002; Fatokun*et al.*, 2009; Vadez*et al.*, 2012).Identification of additional genotypes with even greater tolerance to drought would enable breeders to develop cultivars with higher and more stable yields across the semiarid ecologies where this crop is grown (Belko*et al.*, 2014).To assess the performance of genotypes under stresses of drought with a view to identify lines that are tolerant as well as to identify selection criteria for easy identification of drought tolerance under moisture stressed and optimum field conditions (Ishiyaku and Aliyu, 2013). The result of this research will assist in selecting the best cowpea accessions for breeding drought tolerant varieties that will ensure national and regional food security by producing resilient cowpea varieties of dual purpose.

1.3 Aim

To evaluate genetic variability, heritability and relationship among seedling and post-flowering drought tolerant traits in Cowpea MAGIC - RILs population and select parents for further breeding activities.

1.4 Objectives

1. To screen cowpea MAGIC RILs germplasm for tolerance to drought at the seedling stage and select parents for use in developing droughttolerant improved cowpea lines.
2. To assess cowpea MAGIC RILs genotypes for terminal drought tolerance using some quantitative indices of stress tolerance to classify the genotypes into different yielding and drought tolerance group.
3. To examine the relationships between the traits for breeding of cowpea varieties with increased tolerance to drought at the seedling and terminal stages.

1.5 Hypotheses

1. Cowpea MAGIC RILs germplasms are not tolerant to drought at the seedling stage.
2. Cowpea MAGIC RILs genotypes are not tolerant to terminal drought tolerance.
3. There are no relationships among drought tolerant traits at the seedling and terminal stages.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background Information on Drought

In African countries, agriculture remains the most important sector of the economy given its multiple roles in food security, employment and contribution to national Gross Domestic Products (GDPs). Despite this, however, agriculture in Sub-Saharan Africa (SSA) is almost 80% dependent on rainfall which has become less reliable resulting in shorter growing seasons in many regions (Kandji*et al.*, 2006). Some of the most important impacts of global climate change including reduced or inconsistent rainfall are predicted to occur more frequently in the tropical regions and the impact will be more among "subsistence" or "smallholder" farmers in developing countries, thus threatening food security in the region (Morton 2007; Cavalieri*et al.*, 2011).

Drought is defined in agricultural terms as a condition of insufficient moisture caused by a deficit of precipitation over a given period. It is the most important environmental factor, which induces significant alterations in plant physiology and biochemistry leading to reduction in crop productivity (McClean*et al.*, 2011; Lauer *et al.*, 2012). Research directed at improving crop tolerance/resistance to production stresses, especially drought, is needed to complement other efforts under way to alleviate crop failure and malnutrition problems in the developing countries.

The cowpea crop plays a critical role in providing nutritional security to millions of people in Africa and other parts of the developing world, since it is one of the few available food sources that combines essential minerals and high quality protein (~ 26%) that nutritionally complements staple low-protein cereal crops and tuber crops predominant in the region (Santos and Boiteux, 2015; Yewande and Thomas, 2015). It is also a valuable component of cropping systems in the drier regions because of its ability to fix atmospheric nitrogen (Sinclair *et al.*, 2015). Although, cowpea is considered to be more drought tolerant compared

with other legume crops grown in the semi-arid tropics, its productivity is adversely affected by drought particularly because production areas are subject to water deficits causing substantial yield losses. This necessitates more effort to enhance its resilience to water deficits (Agbicodo*et al.*, 2009; Belko*et al.*, 2012a).

Availability and adoption of such cowpea varieties by farmers will contribute positively to ensuring food security and improving human nutrition in the SSA region. Efforts made in the past to develop drought-tolerant cowpea varieties have met with limited success. This limitation has been attributed to the complexity of factors at play in the determination of drought tolerance and high yield in the crops and limited information on the genetic basis for drought tolerance in cowpea (Fatokun*et al.*, 2012). Extensive research has been carried out on the screening for mid- and late-season drought tolerance in cowpea focusing on morphological, biochemical and physiological responses of cowpea to drought (Singh and Matsui 2002; Ogbonnaya*et al.*, 2003; Hamidou*et al.*, 2007; Belko*et al.*, 2012; Muchero*et al.*, 2013). Nevertheless, only very few studies have used the resultant indices from these studies to select parental genotypes for further genetic studies.

It has been reported that genes that could contribute to higher levels of drought tolerance exist in some of the under-exploited cowpea germplasm maintained in the genetic resources unit at the International Institute of Tropical Agriculture (IITA) (Watanabe *et al.*, 1997; Fatokun*et al.*, 2012). In order to be able to efficiently utilize these lines as parental lines to enhance the level of drought tolerance in the improved cowpea varieties, information on their performance in cross combinations to produce superior progenies that will combine desirable agronomic traits and the drought tolerance genes in the segregating generations is pertinent in addition to the study of nature of gene action controlling yield and other related traits under drought stress.

2.2 Origin, Domestication and Distribution of Cowpea

Cowpea [*Vignaunguiculata* (L.)Walp.] (2n = 2x = 22) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al.* 1974). Cowpea is commonly referred to as "niébé," "wake," and "ewa" in much of West African countries, and "caupi" in Brazil. In the United States, other names include "southern peas," "blackeyed peas," "field peas," "pinkeyes," and "crowders." These names reflect traditional seed and market classes that developed over time in the southern United States. The name cowpea probably originated from the fact that the plant was an important source of hay for cows in the southeastern United States and in other parts of the world (Timko*et al.*, 2007).

Cowpea most likely originates from Africa, as wild cowpeas only exist in Africa and Madagascar (Steele, 1976). The centre of diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah region of Nigeria, southern Niger, parts of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985). Carbon dating of cowpea (or wild cowpea remains from the Kimtampo rock shelter in central Ghana) has been carried out (Flight, 1976) and is the oldest archaeological evidence of cowpea found in Africa. Cowpea is considered to have been domesticated in Africa from its wild ancestral form, *V. unguiculata* subsp. dekindtiana (Harms) Verdc. (Ng and Marechal, 1985).

However, the precise location of origin where cowpea was first domesticated is still under speculation. Ba *et al.*, (2004) reported that the crop was probably domesticated by farmers in West Africa while Coulibaly*et al.*, (2002) presented some evidence that domestication occurred in northeastern Africa, based on studies of amplified fragment length polymorphism (AFLP) analysis. Cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago (Allen 1983).

Cowpeas had reached Europe from Asia and have been cultivated in southern Europe at least since the 8th century BC and perhaps since prehistoric times (Tosti and Negri, 2002). From the West Indies, cowpea was taken to the USA in about 1700 BC (Pursglove, 1968). The slave trade from West Africa resulted in the crop reaching the southern USA early in the 18th century however, many US cultivars appear more closely related to germplasm from Asia or southern Europe than West Africa (Fang *et al.*, 2007). Presently, cowpea is grown throughout the tropic and subtropic areas around the whole world.

2.3 Description and Classification of Cowpea

Cowpeas are generally more robust in appearance than common beans with better developed root systems and thicker stems and branches. Summerfield *et al.* (1974), Kay (1979) and Fox and Young (1982) described cowpea as an annual herb reaching heights of up to 80 cm with a strong taproot and many spreading lateral roots in the surface soil. Growth forms vary and include erect, semi-erect, semi-prostrate and prostrate. Fruits are pods containing seeds that vary in size, shape, colour and texture. Pods may be held erect, crescent-shaped or coiled.

They are usually yellow when ripe, but may also be brown or purple. The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are conspicuous, mostly self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale-blue or purple in colour. Flowers open in the early day and close at approximately midday.

Verdcourt (1970) and Marechal*et al.* (1978) classified cowpea as follows: ORDER: Fabales; FAMILY: Fabacea; SUBFAMILY: Faboideae; TRIBE: Phaseoleae; SUBTRIBE: Phaseolinae; GENUS: Vigna; SECTION: Catiang

Vigna has several species, but the exact number varies according to different authors. Cultivated cowpeas have been divided into five cultivar groups based mainly on pod and seed characteristics (Pursglove, 1968; Pasquet, 1999). Cultivar group Unguiculata is the largest

and includes most medium- and large-seeded African grain and forage-type cowpeas. Cultivar group Melanophthalmus includes “blackeye pea”-type cowpea with large, somewhat elongated seeds with wrinkled seed coats and fragile pods (Pasquet, 1998).

Members of cultivar group Biflora (also known as “catjang”) are common in India and characterized by their relatively small smooth seeds borne in short pods that are held erect until maturity. Cultivar group Textilis is a rather rare form of cowpea with very long peduncles that were used in Africa as a source of fiber. Cultivar group Sesquipedialis (known as “yardlong bean,” “long bean,” “Asparagus bean,” or “snake bean”) is widely grown in Asia for production of its very long (40 to 100 cm) green pods that are used as “snap” beans.

2.4 Importance of Cowpea

Members of the Phaseoleae (which cowpea belongs to) include many of the economically important warm season grain and oilseed legumes, such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vignaradiata*) (Timko*et al.*, 2007).

Cowpea is the most economically important indigenous African legume crop and has a wide variety of uses as a nutritious component in the human diet as well as nutritious livestock feed (Langyintuo*et al.*, 2003). It is usually the first crop harvested before the cereal crops are ready and therefore is referred to as "hungry-season crop". With more than 25% protein in dry seeds as well as in young leaves (dry weight basis), cowpea is a major source of protein, minerals and vitamins in daily diets and is equally important as nutritious fodder for livestock (Singh *et al.*, 2003). The high protein content of cowpea grain represents a major advantage for use in infant and children’s food (Lambot, 2002). The mature pods are harvested and the haulms are cut while still green and rolled into small bundles containing the leaves and vines. These bundles are stored on rooftops for uses as feed supplement in the dry season, making cowpea a key component of crop-livestock systems. Cowpea haulms fetch 50% or more of the grain price (dry weight basis).

Therefore, cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world, and is a valuable and dependable commodity that produces income for farmers and traders (Singh 2002; Langyintuo*et al.*, 2003). Additionally, cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky*et al.*, 2002; Tarawali*et al.*, 2002; Sanginga*et al.*, 2003). Figure 2 summarizes the potential contributions of cowpea described (Tarawali*et al.*, 2002).

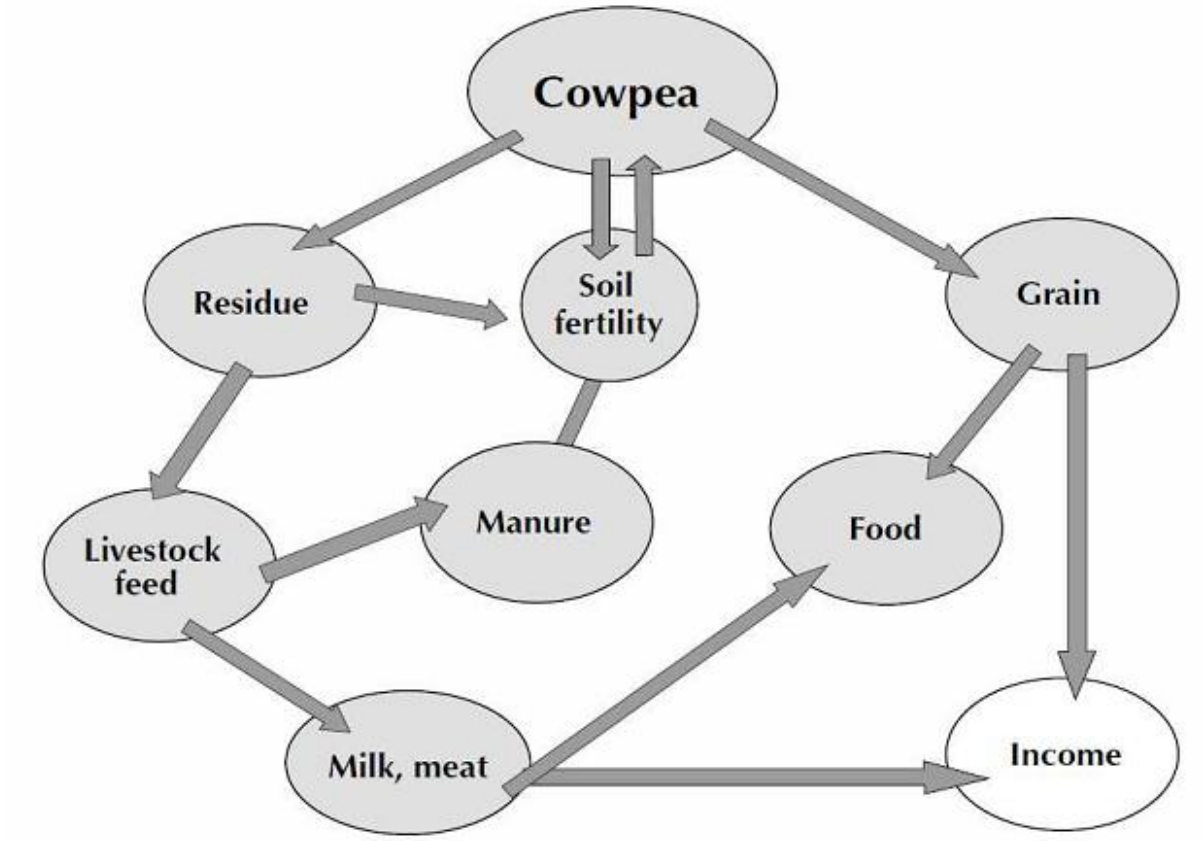


Fig. 2.1: Schematic representation of the potential contributions of cowpea in crop livestock systems in the dry savannas. Not all potential interactions are shown for simplicity (after Tarawali et al., 2002).

2.5 Dimension of Drought on Cowpea Production in Central and West Africa

Cowpea is one of the most ancient crops known to man. The crop originated and domesticated from Africa (Ng and Marechal, 1985) and is widely adapted and grown throughout the world. Based on information available from FAO and from scientists in several countries, cowpea researchers at IITA estimated that cowpea is now cultivated on at least 14 million hectares, with 3722 thousand metric tons worldwide in 2003 (FAO, 2004). Central and West Africa alone account for about 9.3 million hectares. A substantial part of cowpea production in the region comes from the drier areas of northern Nigeria (about 4 million ha, with 1.7 million tons), and southern Niger Republic (about 3 million ha, with 1 million tons) (Singh *et al.*, 1993). Millions of African farmers grow cowpea in small scale farming.

Some two hundred million Africans consume cowpea, and many possibly even a majority of the farmers are women. One of the most remarkable things about cowpea is that it thrives in dry environments and this makes it the crop of choice in the semi-arid/arid zones of West and Central Africa. Additionally, cowpea used to be the first crop harvested before the cereal crops are ready and therefore is referred to as "hungry-season crop". It is the most economically important indigenous African legume crop (Langyintuo*et al.*, 2003) and is of vital importance to the livelihood of several millions of people in West and Central Africa.

Cowpea is a most versatile African crop, it feeds people, their livestock and because of its ability in nitrogen-fixation, it improves soil fertility, and consequently helps to increase the yields of cereal crops when grown in rotation and contributes to the sustainability of cropping systems. Despite all its economic and cultural importance in Sub-saharan Africa, cowpea production is subjected to a wide range of biotic and abiotic constraints.

Hounam*et al.* (1975) and Glantz (1987) studied the effect of drought on hunger in Africa and reported that impact may range from slight personal inconvenience to endangered

nationhood. Drought is the major abiotic constraint of cowpea production. Since cowpea is grown mainly in the dry savanna and Sahel areas with no irrigation facilities, irregular rainfall especially early in the season have adverse effects on the growth of the crop. The drier zones of northern Nigeria and Niger harbour the largest area of cowpea production in the world but yields are only between 100 to 500 kg/ha, despite its 5 times higher biological potential (Karsky*et al.*, 2001).

Niger is the second largest producer of cowpea after Nigeria yet it has the lowest average grain yield of 110 kg/ha (Langyintou*et al.*, 2003). This is probably due to the fact that the whole country is located in the Sahel where rainfall is low. Moreover, drought conditions weaken the plants making them more vulnerable to disease infestations and insect pests attacks. As an African crop grown in resource-poor areas, few countries have cowpea improvement programs and the continent has very low average grain yield compared to for instance the United States (Langyintou*et al.*, 2003).

However, concerted multidisciplinary efforts including genetics, physiology and biochemistry are being developed to unravel drought mechanisms in cowpea and to develop varieties better adapted to the climate changes in Sub-saharan Africa.

2.6 Drought Tolerance Mechanisms

Several factors and mechanisms operate independently or jointly to enable plants to cope with drought stress. Therefore, drought tolerance is manifested as a complex trait (Krishnamurthy *et al.*, 1996). Traditionally, drought tolerance is defined as the ability of plants to live, grow, and yield satisfactorily with limited soil water supply or under periodic water deficiencies (Ashley 1993). According to Mitra (2001), the mechanisms that plants use to cope with drought stress can be grouped into three categories viz. drought escape, drought avoidance and drought tolerance. However, crop plants use more than one mechanism at a time to cope with drought.

Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits occur. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water deficit) and remobilization of pre-anthesis assimilates.

Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil-moisture. Plants develop strategies for maintaining turgor by increasing root depth or developing an efficient root system to maximize water uptake, and by reducing water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evapo-transpiration surface (leaf area) (Mitra, 2001).

Drought tolerance is the ability of plants to withstand water-deficit with low tissue water potential. The mechanisms of drought tolerance are maintenance of turgor through osmotic adjustment (accumulation of solutes in the cell), increased cell elasticity and decreased cell size and desiccation tolerance by protoplasmic resistance. However, all these adaptation mechanisms of the plant to cope with drought have some disadvantages with respect to yield potential. For instance, a genotype with a shortened life cycle usually yields less compared to a genotype with a normal life cycle.

The mechanisms that confer drought avoidance by reducing water loss (such as stomatal closure and reduced leaf area) decrease carbon assimilation due to reduction in physical transfer of carbon dioxide molecules and increase leaf temperature thus reducing biochemical processes, which negatively affects yield. Plants try to maintain water content by accumulating various solutes that are nontoxic (such as fructans, trehalose, polyols, glycine betaine, proline and polyamines) and do not interfere with plant processes and that are,

therefore, called compatible solutes (Yancey *et al.*, 1982). However, many ions concentrated in the cytoplasm due to water loss are toxic to plants at high concentrations leading to what is termed a glassy state. In this condition whatever liquid is left in the cell has a high viscosity, increasing the chances of molecular interactions that can cause proteins to denature and membranes to fuse (Hartung*et al.*, 1998).

Consequently, crop adaptation to water stress must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity. Drought escape, avoidance, and tolerance mechanisms have been described in cowpea. However, the drought response pathways associated with these mechanisms are not yet understood, and the degree to which these adaptations operate jointly or separately to allow the crop to cope with drought still needs to be established.

2.7 Drought Tolerance Mechanisms in Cowpea

2.7.1 Drought Escape in Cowpea

The increased incidence of drought in some cowpea growing areas has caused a shift to early maturing varieties (Mortimore*et al.*, 1997). Early maturity of cowpea cultivars is desirable and has proven to be useful in some dry environments and years because of their ability to escape drought (Hall and Patel, 1985; Singh, 1987; 1994). Such early cultivars can reach maturity in as few as 60 to 70 days in many of the cowpea production zones of Africa. Earliness is important in Africa as early cultivars can provide the first food and marketable product available from the current growing season, and they can be grown in a diverse array of cropping systems.

In addition to escaping drought, early maturing cultivars can escape some insect infestations (Ehlers and Hall, 1997). The International Institute of Tropical Agriculture (IITA) and the InstitutSenegalais de RecherchesAgricoles (ISRA) have been at the forefront in developing early maturing high yielding and pest resistant cultivars. Selection for early flowering and

maturity and yield testing of breeding lines under drought conditions has been used successfully in developing cowpea cultivars adapted to low rainfall areas (Hall and Patel, 1985; Cisse*et al.*, 1997). Early maturity cowpea varieties (i.e. IT84S-2246, Bambey 21) that escape terminal drought have been released and widely adopted by African farmers.

However, if exposed to intermittent drought during the vegetative or reproductive stages, these varieties performed very poorly. Efforts are therefore being made to breed cowpea varieties with enhanced drought tolerance for early, mid- and terminal season drought stresses. Different RIL populations are currently under evaluation for different traits including physiological, phenological and yields for drought tolerance at seedling and flowering/reproductive stages. These investigations aim at understanding which of the traits contribute importantly to yield under drought.

2.7.2 Mechanisms of Drought Avoidance and Tolerance in Cowpea

In cowpea, two types of drought tolerance have been described at the seedling stage using the wooden box technique (Mai-Kodomi*et al.*, 1999a). At 15 days after the termination of watering, all the seedlings of the two susceptible lines TVu 7778 and TVu8256, were completely dead. The “Type 1” drought tolerant lines like TVu 11986 and TVu 11979 stopped growth after the onset of drought stress and maintained uniformity, but displayed a declining turgidity in all tissues of the plants including the unifoliates and the emerging tiny trifoliates for over two weeks. All plant parts such as the growing tip, unifoliates and epicotyl gradually died almost at the same time. In contrast, the “Type 2” drought tolerant lines like Danila and Kanannado remained green for a longer time and continued slow growth of the trifoliates under drought stress. With continued moisture stress, the trifoliates of these varieties started wilting as well and died about 4 weeks after drought stress started.

The two types of tolerance responses by cowpea seedlings to drought stress indicate that cowpea genotypes evolved different mechanisms to cope with prolonged drought

encountered in the semi-arid regions of Africa where the crop is believed to have originated. Closure of stomata to reduce water loss through transpiration and cessation of growth (for Type 1 drought avoidance) and osmotic adjustment and continued slow growth (drought tolerance in Type 2) have been suggested as the possible mechanisms for drought tolerance in cowpea (Lawan, 1983; Boyer, 1996).

Cowpea is known as dehydration avoider with strong stomatal sensitivity and reduced growth rate (Lawan, 1983). This is the mechanism underlying the Type 1 reaction to drought of TVu 11986 and TVu 11979. The Type 2 reaction of Danila and Kanannado appears to be a combination of three mechanisms; stomatal regulation (partial opening), osmotic control and selective mobilization with distinct visible differences in the desiccation of lower leaves compared to the upper leaves and growing tips (Mai-Kodomi*et al.*, 1999a).

It is the Type 2 mechanism of drought tolerance is more effective in keeping the plants alive for a longer time and ensures better chances of recovery than Type 1 when the drought spell ends. Both drought tolerant lines Danila and Kanannado are local varieties commonly grown in the Sudano- Sahelian border areas of Nigeria and Niger Republic, indicating that in these areas, farmers have selected cowpea varieties with good adaptation to drought. Similarly, Muchero*et al.* (2008) studied 14 genotypes of cowpea at seedling stage and confirmed the existence of significant genetic variation in response to drought stress. Genotypes, IT93K-503-1 and IT98K-499-39 were consistently most tolerant whereas CB46 and Bambey 21 were most susceptible. However, the differences in phenotypic responses to seedling-stage drought among the 14 genotypes were not consistently associated to drought tolerance.

As for examples, genotypes IT82E-18(232) and Suvita 2 showed rapid loss of unifoliates but were found at opposite ends of the drought tolerance spectrum. While, genotypes CB27 and Bambey 21 preserved unifoliates but Bambey 21 was highly drought susceptible and CB27

moderately susceptible under similar stress conditions. Somehow, these clear phenotypic responses to drought stress provide an opportunity for detailed studies of specific drought responses and select genotypes to be used as parents to study the inheritance of these specific responses.

The association between crop performance and carbon isotope discrimination (Δ) has been reviewed for cowpea, common bean, and peanut (Condon and Hall, 1997). Genotypic differences in the potential grain yield of cowpea have been positively associated with Δ, indicating that more productive genotypes have a higher photosynthesis rate resulting in higher internal carbon dioxide concentration in their leaves (Hall *et al.*, 1997b; Condon and Hall 1997). Similar studies in other crops such as Pima cotton (*Gossypiumbarbadense*) and bread wheat (*Triticumaestivum*) have shown remarkable positive correlations between yield increases and increases in stomatal conductance (Lu *et al.*, 1998).

The authors argued that the higher Δ in more productive genotypes of cowpea, cotton (*G. barbadense*), and wheat (*Triticum sp.*) was probably due to their having more open stomata, which could have resulted in greater rates of photosynthesis due to diffusion effects (Condon and Hall, 1997), or beneficial effects on the plant resulting from greater evaporative cooling (Lu *et al.*, 1998). In favor to the more open stomata strategy under water stress, Cruz de Carvalho*et al.* (1998) compared physiological responses of cowpea and common bean genotypes and reported that the cowpea genotypes kept their stomata partially opened and had a lower decrease in their net photosynthetic rates than the common bean.

Further investigations on these cowpea genotypes are needed to demonstrate whether there are significant positive effects on grain yield related to the partial opening of stomata under drought conditions. Several other mechanisms may partially explain the extreme dehydration avoidance of cowpea. The mechanisms through which cowpea is able to resist vegetative-

stage drought may be related to the limited decrease of leaf water potential even under extreme drought. The lowest leaf water potential recorded for cowpea is -18 bar (-1.8 MPa) (e.g., Turk and Hall, 1980; Hall and Schulze, 1980), whereas peanut has developed leaf water potentials under drought as low as -82 bar (-8.2 MPa) (Turner *et al.*, 2000).

Cowpea also changes the position of leaflets under drought (a drought avoidance mechanism). They become paraheliotropic and orientated parallel to the sun's rays when subjected to soil drought, causing them to be cooler and thus transpire less (Shackel and Hall, 1979), which helps to minimize water loss and maintain water potential.

2.8 Screening Approaches for Drought Tolerance in Cowpea

Success in breeding for drought tolerance in cowpea has not been as pronounced as for many other traits (Singh *et al.*, 1997). This is partly due to the lack of simple, cheap, and reliable screening methods to select drought tolerant plants and progenies from the segregating populations. The complexity of factors involved in drought tolerance could also have contributed to this. Nevertheless, cowpea genotypes with contrasting response to drought have been identified.

Researchers have proposed two approaches for screening and breeding for drought tolerance in plants. The first is the empirical or performance approach that utilizes grain yield and its components as the main criteria, since yield is the integrated expression of the entire array of traits related to productivity under stress. The second is the analytical or physiological approach that identifies a specific physiological or morphological trait that will contribute significantly to growth and yield in the event of drought. Modest progress in cowpea breeding for dry environments has been achieved by selecting for yield in breeding lines over several locations and years (Turk *et al.*, 1980; Hall and Patel, 1985; Selvaraj*et al.*, 1986; Cisse*et al.*, 1997; Hall *et al.*, 1997b).

However, these empirical approaches are slow, laborious, and expensive because of the need to assess the yield of a large number of lines across several locations and years, and the substantial variation from the effects of environment, and genotype– environment interactions (Blum, 1985). As suggested by Blum (1983) and Fussell*et al.* (1991), the approach which combines selection for yield potential in favourable conditions with selection for the expression of physiological traits thought to be associated with drought tolerance under controlled, repeatable stress environments might be the most effective. This therefore requires the identification of specific traits associated with drought tolerance under adequate water management that are easy and reliable to measure (Fischer and Wood, 1979).

2.9 Morphological, Biochemical and Physiological Traits for Drought Screening in Cowpea

Data on changes of morphological, biochemical and physiological traits in response to drought are available for some cultivars of *Vignaunguiculata* (Turk *et al.*, 1980; Ogbonnaya*et al.*, 2003; Matsui and Singh, 2003; Slabbert*et al.*, 2004). These traits include water-use efficiency (WUE), water potential, relative turgidity, leaf gas exchange, relative water content (RWC), diffusion pressure deficit, chlorophyll stability index, and carbon isotope discrimination (Bates *et al.*, 1981; Turk and Hall 1980; Morgan *et al.*, 1991; Hall *et al.*, 1990; 1997b; Anyia and Herzog, 2004; Souza *et al.*, 2004). Stomatal closure in the cowpea cultivar EPACE-1 was not related to any change in relative water content (RWC) indicating that early stomatal responses to substrate water depletion are not triggered by changes in leaf water content.

Therefore, RWC alone cannot be used as a drought indicator for cowpea. This also suggests the possible existence of a root to leaf communication, independent of the leaf water status that informs the shoot about changes in the root zone. Following exposure of six cowpea varieties to drought in the upper 20 cm rooting zone, Kulkarni*et al.* (2000) compared the rate

of abscisic acid (ABA) synthesis relative to total root mass and inherent variation per unit root mass.

The authors observed that the intrinsic ABA synthesizing capacity rather than the root mass is responsible for the total ABA produced in the roots of the dry soil zone. The relationship between stomatal conductance and total root ABA was assessed and found to be negative ($r = -0.90$, $n = 24$, $P = 0.05$) suggesting that the intrinsic capacity of cowpea varieties for ABA synthesis could play an important role in regulating stomatal conductance in a drying soil and provide useful selection criteria for tolerance to drought stress in cowpea. In support to these results, stomatal regulation was reported to be the common strategy used by the five different cowpea genotypes to avoid dehydration both under glasshouse and field conditions (Hamidou*et al.*, 2007).

These authors measured the physiological, biochemical and agronomic responses to water deficit at flowering stage in five cowpea genotypes, Gorom local, KVX61-1, Mouride, Bambey 21 and TN88-63, that were grown in the glasshouse and the field. The five cowpea genotypes are known to differ in their susceptibility to water stress. Water deficit significantly increased the canopy temperature and the proline content of the five genotypes while gaseous exchanges and starch content decreased significantly.

Yield components of the five genotypes, with the exception of seed number per pod, were also significantly affected. Number of pods and number of seeds per plant decreased after drought treatment by 57% in the glasshouse and by 64% in the field when compared to non-stressed plants. Genotypic differences were observed for both of the yield components. Genotype TN88-63 was more productive than the other four genotypes under glasshouse conditions, while under field conditions, Mouride and Gorom local proved to be more productive than KVX61-1, which in turn performed better than Bambey 21.

As an alternative to all the above investigations which focus on some specific physiological, biochemical and agronomic traits, an integrated approach which combines cellular water relations, rooting characteristics, leaf area and biochemical and morphological changes to screen cowpea for drought tolerance has been proposed by Slabbert*et al.* (2004). The different screening techniques that were tested included: the antioxidative response in the form of superoxide reductase (SOD), glutathione reductase (GR), ascorbate peroxidase (AP), proline accumulation, 2,3,5- triphenyltetrazolium chloride (TTC) assays, early drought screening at the seedling stage (wooden box technique), cell membrane stability (CMS), relative water content (RWC), leaf water potential (LWP), leaf area, chlorophyll a and b and carotenoid content and chlorophyll fluorescence (JIP test).

Contrary to the results of Cruz de Carvalho*et al.* (1998), RWC was a good parameter to discriminate genotypes under water stress in cowpea (Slabbert*et al.*, 2004). An important morphological trait that may contribute to drought adaptation is the delayed leaf senescence (DLS) trait (Gwathmey*et al.*, 1992). This trait enhances plant survival after a mid-season drought damages the first flush of pods, which enables a substantial second flush of pods to be produced. Cultivars with DLS also have enhanced production of forage because their leaves remain green and attached to the plant until harvest. The DLS trait allows the crop to stay alive through midseason drought and recover when rainfall resumes. Most importantly, DLS can be easily measured by visual observation using an appropriate scale.

In summary, based on the above findings from the different studies the following methods were most suitable for screening large number of cowpea lines for drought tolerance:

a) determination of chlorophyll fluorescence,

b) stomatal conductance measurements,

c) abscisic acid (ABA) measurements,

d) measuring free proline levels

e) wooden box screening for drought tolerance at the seedling stage,

f) delayed leaf senescence (DLS)

2.10 Screening Cowpea for Drought Tolerance at the Seedling Stage

Singh *et al.* (1999a) suggested that different cowpea plant organs (leaf, shoot and root) should be used to screen for drought tolerance. The authors argued that different tissues have different responses to abiotic stress and should therefore be studied individually. This may enable the identification of tissue-specific genetic factors underlying the drought responses and the elucidation of parts of the drought response pathways possibly making breeding for drought tolerance easier. A simple screening method using the ‘‘wooden box technique’’ has been found suitable for identifying seedling drought tolerance in cowpea.

This method eliminates the influences of the root system on drought tolerance, and permits nondestructive visual identification of shoot dehydration tolerance (Singh *et al.*, 1999a). The method has proven to be efficient in screening for drought tolerance in different crop species (Singh *et al.*, 1999b; Tomar and Kumar 2004; Slabbert*et al.*, 2004; Ewansiha and Singh, 2006). Field and pot testing of the plants of the different crop species demonstrated a close correspondence between drought tolerance in the seedling stage and reproductive stage.

The wooden box screening method has been used to identify cowpea genotypes with contrasting responses to drought (Danila, IT96D-602 and TVu 11986 which exhibit seedling drought tolerance and TVu 7778 which is susceptible). The RILs developed from the cross between Danila and TVu 7778 have been evaluated for seedling survival under severe drought stress using the wooden box technique (unpublished). Seeds of four RILs and the two parents were planted randomly in straight rows in each wooden box.

After emergence plants were thinned to one per stand. The boxes were watered daily with the same volume of water until the first trifoliate emerged and watering was completely stopped.

After 4 weeks of water stress, when all the plants of susceptible parent TVu7778 appeared dead, watering was resumed. Variable number of seedlings recovered in some RILs and the tolerant parent two weeks after watering resumed.

Similar to the wooden box technique, small plastic pots were tested to separate plant root systems and to eliminate competition among genotypes for a communal water source while still maintaining the low space requirement that is characteristic of wooden screening (Muchero*et al.*, 2008). The pot experiments in greenhouse were used to discriminate between 14 cowpea genotypes that exhibit significant genetic variation to drought stress at seedling.

These authors emphasized that stem greenness, survival and recovery dry weights in greenhouse were the useful traits to screen cowpea genotypes for their ability to withstand drought stress at the seedling stage.

2.11 Root Characteristics and Drought in Cowpea

Drought tolerance mechanisms in legume crops seem to be closely related to the root system or rooting pattern (Pandey*et al.*, 1984; 1986; Itani*et al.*, 1992; Silim and Saxena, 1993; Matsui and Singh, 2003). However, screening for root characteristics is difficult because of the underground distribution of root. The 'pin-board root-box' (Matsui and Singh 2003), herbicidal band screening (Robertson *et al.*, 1985) and polyethylene glycol (PEG) (Badiane*et al.*, 2004) methods were used to identify the role of cowpea root characteristics in drought tolerance.

Typically, the evaluation of rooting characteristics has only been performed in a few cultivars when choosing parents for crosses or with a few promising advanced lines. With the herbicide-band screening the authors succeeded in detecting significant genotypic differences in mean numbers of days to first herbicide symptoms among five cowpea genotypes. Cowpea genotypes CB5 and Grant developed symptom the earliest, 8006 and PI302457 developed symptoms the latest, and PI293579 was intermediate.

The ranking of genotypes was consistent with estimates of relative depth of effective rooting obtained from soil moisture extraction measurements. With pin-board root-box screening two-dimensional distribution of roots can be studied. Important varietal differences were observed in cowpea architecture and some varieties have a well-spread deep root system while others have concentrated roots only on the upper soil strata.

Although it has been reported that the results of this method are highly correlated with field observations (Matsui and Singh, 2003), the pin-board root-box technique received much less attention compared to wooden box technique as described previously. This is probably because it is not practical to screen large number of plants. As root characteristics are important traits involved in drought avoidance, cowpea physiologists at IITA (Kano Station) and researchers from different areas are working to establish simple methods for root screening in cowpea.

Root characteristics being a quantitatively inherited trait, an integrated screening approach as proposed by Slabbert*et al.* (2004) might be the most promising for phenotyping cowpea for drought tolerance. It is imperative that selected genotypes should always be tested in the field for confirmation of their yield performance under field drought. It would be helpful to identify traits that are associated with drought tolerance, but that are easier to measure and that have high heritability. Molecular markers closely linked to the loci with effects on these traits could be identified and later used in marker assisted selection (MAS) programs. However, any traits to be used in MAS programs for improving drought tolerance, must have a proven contribution to yield under drought conditions.

2.12 Breeding Options to Enhance Drought Tolerance in Cowpea

Attempts to improve drought tolerance of crops through conventional breeding programs have met with limited success because drought tolerance is physiologically and genetically a complex trait. The use of molecular markers to identify and locate different genes and

genomic regions possessing factors which influence drought tolerance in cowpea will help to gain insight into the complex trait of drought tolerance. In addition, these markers can be used to select for multiple traits and combine genes underlying these traits in cultivars with improved drought tolerance. These properties and prospects have initiated an increased interest in the application of Marker-Assisted Selection (MAS) for improving drought tolerance in many crops including cowpea. For better understanding of different biochemical and physiological pathways involved in drought tolerance in cowpea, three main approaches using molecular marker tools can be used.

The first approach assumes no prior knowledge about genes and is based on the so-called quantitative trait loci (QTL) method. On the most recent genetic map of cowpea (Ouedraogo*et al.*, 2002), consisting of 11 linkage groups (LGs) spanning a total of 2670 cM, with an average distance of approximately 6 cM between markers, no genes/QTLs related to drought tolerance were mapped. However, different RIL populations are being currently screened at IITA for mapping and identification of QTLs with effects on drought tolerance across populations. The development of a set of ESTs from drought-stressed and non-stressed drought-sensitive and tolerant cowpea lines will be helpful in genotyping.

The ESTs are utilized to develop other molecular markers such as simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and COS markers. The COS markers would facilitate cross-legume studies and allow better integration of cowpea into legume functional genomics. Currently cowpea genomics is receiving increased attention, which has resulted in projects that are producing large sets of ESTs and other genome sequences which has recently applied an IlluminaGoldengate SNP array with 1536 SNPs (UCR) to several RIL populations and diverse array of genotypes.

This is an opportunity for the cowpea community to use a common set of markers in a wide collection of crosses and germplasm for construction of a densely populated consensus genetic map and for connecting genetics and QTLs/genes in cowpea. All the efforts in improving genetic maps and increasing available sequence data are only useful for QTL analysis if drought tolerance parameters can be measured as heritable traits. For cowpea these include the traits mentioned earlier like stomatal conductance, chlorophyll fluorescence, abscisic acid (ABA) levels, free proline levels, wooden box screening for drought tolerance at the seedling stage, and delayed leaf senescence (DLS).

The second approach is to make an 'educated guess' from published data, i.e. select candidate genes (CG) that are known to be functionally relevant for drought tolerance and test in cowpea plants whether these genes can be linked to drought tolerance. Candidate genes refer either to cloned genes presumed to affect a given trait ('functional CGs') or to genes suggested by their close proximity on linkage maps to loci controlling the trait ('positional CGs') (Pflieger, 2001). The final validation of a CG will be provided through physiological analyses, and genetic transformation.

The most detailed studies relating candidate genes to drought QTLs have looked at genes that determine ABA levels, at genes involved in dehydrin production, at invertase activity and transcription factors (Pflieger, 2001). However, there has also been interest in mapping a wide range of regulatory and structural candidate genes to determine QTLs with effects on drought tolerance and this approach has been particularly effective in the case of rice (Nguyen *et al.*, 2004).

Cowpea orthologues of these genes that have been characterized in other species and crops as being involved in drought tolerance will be increasingly easy to discover, as the number of cowpea EST sequences as well as gene space sequences is increasing rapidly. An interesting

group of GCs are transcription factors that are involved in the drought response including Myb genes, WRKY genes, AP2 and ERF genes.

The third approach is comparative genomics. Earlier studies indicated that members of Papilionoideae subfamily to which cowpea belongs exhibit extensive genome conservation, based on comparative genome analysis between mungbean and cowpea (Menancio-Haute*a et al.*, 1993), between pea and lentil and orthologous seed weight genes in cowpea and mungbean (Fatokun*et al.*, 1992). Recent advances in comparative mapping among the legumes has clarified the genetic relationship of model and crop legumes and enabled linking of the genomes of the tropical and temperate legumes that represent the major clades of the legume family (Choi *et al.*, 2004a; Choi *et al.*, 2004b).

Drought tolerance is a highly appropriate target for comparative plant genomics because this information-rich approach has the potential to unveil the key genetic contributors to the complex physiological processes involved (Bennetzen, 2000). With the already extensive and rapidly increasing publicly available genomic data for cowpea, comparative genomics of cowpea with other legumes such as common bean (Blair *et al.*, 2002; Schneider *et al.*, 1997) and soybean (Mian*et al.*, 1996; Mian*et al.*, 1998; Specht*et al.*, 2001) could be applied.

This will allow aligning of drought QTLs between legume species including cowpea and determine the most important regions for saturated mapping. Moreover, the micro and macrosyntenic relationships detected between cowpea and other cultivated and model legumes (Timko*et al.*, 2008) would simplify the identification of informative markers for marker-assisted trait selection and map-based gene isolation necessary for cowpea improvement.

2.13 Approach to Breeding for Drought Tolerance in Cowpea

A multidisciplinary approach including breeding, physiology and biotechnology is required for efficient germplasm improvement for drought tolerance in cowpea. Concerted efforts are

being made worldwide to develop drought tolerant cowpea varieties. At IITA, RIL mapping populations are being used to identify markers associated with QTLs with effects on different traits with particular emphasis given to the genetic dissection of both yield component and physiological drought adaptive traits.

Important drought related cDNAs and genes have been isolated from cowpea. The advances that are currently being made in cowpea genomics will unlock even more candidate genes. The next step will be to select promising candidate genes and functionally characterize these genes. For candidate genes with well-known functions functional markers can be used for MAS. The molecular analysis of drought responses in plants has reached a stage where research can now build upon a large collection of well characterized genes. The use of novel approaches combining genetic, physiological, biochemical, and molecular techniques could provide exciting results in the development of drought tolerant cowpea varieties in the near future.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Sites

The study was carried out at IITA Kano Station and IITA Research Farms on latitude 12° 03'N and longitude 8° 34'E near Wase Dam in the Sudan Savanna of Minjibir Local Government Area, about 20 km Northeast of the state capital, Kano Nigeria.

3.2 Plant Materials

Cowpea MAGIC RILs consisting of 352 entries for screen house and 310 entries (293 MAGIC lines, their 8 parents and 9 checks) for field trial were used in this study.

3.3 Experimental Designs

3.3.1 Phenotyping Cowpea Genotypes for Shoot Drought Tolerance

A simple "wooden box screening technique" described by Singh *et al.* (1999) was used to screen the seedlings of the three hundred and thirty (330) test genotypes plus one susceptible (TVu 7778) and one tolerant (DanIla) check in the screen house in two splits because of availability of wooden boxes. The dimension of each wooden box was 100 cm length, 50 cm breadth and 10 cm depth. The planks at the bottom of the boxes were tight enough to retain the soil while allowing drainage of excess irrigation water. Boxes filled with soil were kept on raised benches in the screen house. Each wooden box was filled with mixture of sand and top soil in the ratio of 3:1 (Plate 3.1). A space of 2 cm was left at the top to allow irrigation of cowpea seedlings. In both splits one and two, each wooden box consisted of thirty (30) test plants plus a susceptible and tolerant check arranged in a randomized complete block design; a total of eleven boxes were used.

Table 3.1: MAGIC founder parents and their traits relevant to sub-Saharan Africa and other production areas

Parental lines	Source[a]	Agronomic trait	Resistance or tolerance trait
SuVita 2	INERA	High yielding under drought in Senegal, Burkina Faso and Mozambique; large dark-brown seed	Drought tolerant, resistant to Striga, foliar thrips and *Macrophomina* diseases
CB27	UCR	High yielding under drought in Mozambique; large black-eye seed; photoperiod insensitive; erect growth habit; early maturing.	Heart tolerant, resistant to root-knot nematode, fusarium wilt, and foliar thrips
IT93K-503-1	IITA	High yielding under drought in Senegal; brown-eye seed; stay-green under drought	Drought tolerant, resistant to nematode, fusarium wilt, and *Macrophomina*
IT89KD-288	IITA	High yielding under drought in Burkina Faso and Nigeria; brown–eye seed; photoperiod sensitive	Root-Knot nematode resistant
IT84S-2049	IITA	High yielding under drought in Burkina Faso; Brown–eye seed; erect growth habit	Resistant to aphid, bacterial blight, viruses, root-knot nematode
IT82E-18	IITA	High yielding under drought in Mozambique; early maturing, light-brown seed; photoperiod sensitive	Broadly adapted, resistant to root-knot nematode
IT00K-1263	IITA	High yielding under drought in Mozambique and Nigeria; dark–brown seed; stay-green under drought.	Resistant to Striga, aphid, fusarium wilt, root-knot nematode
IT84S-2246	IITA	High yielding under drought in Burkina Faso and Mozambique; Dark-brown seed	Resistant to aphid, bacterial blight, viruses, root-knot nematode

[a]INERA: Institut de l'Environment et des RecherchesAgricole, Burkina Faso; UCR: University of California – Riverside, United States; IITA: International Institute of Tropical Agriculture, Nigeria.

Each box was watered thoroughly to field capacity and allowed to drain for 24 hrs before planting. Four hills were later marked out for each genotype, 5.75 cm between rows and 4.75 cm between holes, 1.5 cm deep and two seeds per hill which were later thinned to one seedling per hill (Plate 3.2). After planting, boxes were watered to field capacity every two days for two weeks thereafter watering was completely stopped. Stress was measured by observing that all the plants for the susceptible check in each box were dead and thereafter watering was resumed every two days for two weeks. After resumption of watering, variable numbers of recovered seedlings were rated for recovery.

3.3.2 Field Screening of Cowpea Genotypes for Tolerance to Terminal Drought

The 310 entries of cowpea accessions (293 cowpea MAGIC RILs including their 8 parents and 9 checks) were randomized using Breeding Management System (BMS 3.9) into three replications and two treatments regimes (well-watered [WW] as control and water stressed [WS]). The layout was an alpha-lattice design. Each plot consisted of two rows of 2m each with hill spacing of 20cm. Adjacent plots were separated by 0.75m as walking space. Each replication had a total of 8 blocks with the first 7 blocks containing 40 plots while the 8th block had 30 plots. Both water regimes (WW and WS) were irrigated by rain till 42 DAP (days after planting) when the rainy season was over. Well-watered continued receiving water twice a week until physiological maturity with an overhead sprinkler irrigation system designed to dispense 17 mm of water (Ibitoye, 2015). Water stressed was separated from WW by an alley that was 20 m wide to restrict lateral movement of water from the fully irrigated treatment to the water stressed treatment. Water stresseddid not receive water from 42 DAP until harvesting while WW received water twice a week until physiological maturity. Except for the different irrigation treatments, all field management practices were uniform for both the well-watered and water-stressed blocks.



Plate 3. 1: Mixing sand and top soil in ratio



Plate 3. 2: Wooden Box layout

3.4 Land preparation

The fields were cleared, harrowed and ridged manually and mechanically using hand tools and tractor machine before planting.

3.5 Sowing

The cowpea accessions were planted towards the end of rainy season for terminal drought screening. Three seeds were planted per hill and later thinned to two plants per hill one week after germination.

3.6 Fertilizer and Pesticide Applications

One hundred kilogram of NPK (15:15:15)/ha as a basal dose was applied to both treatments (water-stressed and well-watered). Cypermethrin-dimetheote was sprayed as pesticide.

3.7 Weed Control

The fields were kept weed free, during and after early growth stage before attaining full cover at 3- and 6-weeks using hoe weeding and hand pulling.

3.8 Harvesting

The length of days to maturity varied among cowpea cultivars therefore, several hand pickings were employed to prevent shattering and damage to pods and seeds by insects.

3.9 Data Collection

3.9.1 Wooden Box

The following plant parameters were captured every 3 days from drought imposition (14 DAP) to re-watering to evaluate cowpea plants' recovery capacity.

i. Plant number = Number of plants (seedlings) that were alive per plot (0 to 4)
ii. Trifoliate number = Number of green trifoliate per plant (0 to 5 or more if any)
iii. Plant height = Plant height from the soil level to the youngest trifoliate insertion point on main branch or stem (cm)

iv. Leaf senescence score = Visual score of leaf senescence due to water deficit stress = (1 to 5) with 1: Green and fresh leaves; 2: Green and slightly wilted leaves; 3: Green-yellow and moderately wilted leaves; 4: Yellow-green and severely wilted leaves to 5: Yellow to brown and dead leaves

v. Stem green = Stem greenness (0 or 1) with 0: Not green and 1: Green

vi. Recovery = Number of plants that recovered (with development of new leaves) after re-watering. Seedlings of the most drought tolerant lines will recover and continue growing after resumption of water supply while those of the susceptible lines will not.

3.9.2 Field Trials

Canopy responses and other physiological and morphological data were carefully taken as follows:

i. Stand count: This was carried out by counting the number of plants per net plot after thinning.
ii. Number of days to first flowering:This was determined by recording the day when the first flower fully opened in an accession.
iii. Number of days to 50% flowering: This was determined by recording the day when half of the flowers fully opened in half of the accessions in a plot.
iv. Number of days to 95% maturity: This was achieved by recording the dates when about 95% of the pods matured in each plot.
v. Pod load score: This was determined by physical observation of pod number on a rated scale of 1-4 by considering the number of retained pods till maturity.
vi. Visual score 2 weeks after stress imposition: This was determined by physically observing the stay green ability of the cowpea accessions and then scoring on a scale of 1-5.
vii. Growth habits: This was achieved by careful observation of growth nature of the cowpea as erect, semi-erect, semi-prostrate and prostrate on a scale of 1-4 respectively.
viii. Development type: This was determined during harvesting as either maturing at the same time having only one picking (determinate) or different time having several pickings (indeterminate).
ix. Abortion rate: This involved careful observation to determine how many flowers aborted on the peduncle.
x. Number of pods per peduncle: Was achieved by counting the number of pods on each peduncle.

xi. Stand at harvest: This was done by counting the numbers of plants left on each plot during harvesting.

xii. Pod yield: Fully matured pods were sun dried and weighed after harvesting before threshing.

xiii. Seed yield: Pods were threshed with hand and then weighed.

xiv. 100 seed weight: This was done by counting 100 seeds from the grain after threshing of the pods.

xv. Fodder yield: After harvesting, the fodder was cut off, dried and weighed.

3.10 Drought Tolerance Indices

The selection indices of stress tolerance for the mean productivity (MP), the tolerance index (TOL), the stress susceptibility index (SSI), the stress intensity (SI), the geometric mean productivity (GMP), and the stress tolerance index (STI) were calculated based on yield data in the two contrasting environments using the following formulae (Fernandez, 1992):

Mean productivity (MP) $MP = (Ys+Yw)/2$

Tolerance index (TOL) $TOL = Yw-Ys$

Stress susceptibility index (SSI) $SSI = [1-(Ys/Yw)]/[1-(\bar{Y}s/\bar{Y}w)]$

Stress intensity (SI) $SI = 1-(\bar{Y}s/\bar{Y}w)$

Geometric mean productivity (GMP) $GMP= \sqrt{((Ys) \times Yw)}$

Stress tolerance index (STI) $STI= (Ys \times Yw)/\bar{Y}^2w$

Where Ys and Yw (known as Yp [Fernandez, 1992]) are the yields of each genotype under drought-stressed and non-stressed conditions. $\bar{Y}s$ and $\bar{Y}w$ are respectively the mean yields of all genotypes under drought-stressed and non-stressed conditions.

The stress intensity (SI) score was classified into mild, moderate and severe. Stress intensity was mild when yield reduction was between 0 and 25%, moderate when yield reduction was situated between 25 and 50% and severe when yield reduction was between 50 and 100% (Chiulele, 2010).

3.11 Statistical Analyses

Data on seedling drought traits, grain yield and yield components were analyzed using statistical software "R" (version 3.5.2). Analysis of variance for each character was done by using the statistical software "R" (version 3.5.2). Genotypes and replication were both considered random effects. The genotypic (σ^2_g) and error (σ^2_e) variances were computed, where genetic variance $\sigma^2_g = (MS_g\text{-}MS_e)/r$, variance due to errors $\sigma^2_e = MS_e/r$, r = number of replications. MS_g was considered as total phenotypic variation and MS_e was an estimate of non-genetic variation.

The phenotypic variance (σ^2_p) was manually calculated (Burton and Devane, 1953).

$$\sigma^2_p = (\sigma^2_g) + (\sigma^2_e)$$

$$H^2 = (\sigma^2_g / \sigma^2_p) \times 100$$

Heritability (H^2) in the broad sense was manually computed according to the formula given by Allard (1960). As suggested by Johnson *et al.* (1955), H^2 estimates were categorized as: Low (0-30%), medium (30-60%), High (above 60 %).

To investigate the inter-relationships among the variables and their direct and indirect contributions to yield performance under well-watered and water stressed conditions, entry means of variables that showed high genetic variation were subjected to correlation analyses. Correlation analyses were done on seedling drought traits, yield and yield components data and calculated quantitative indices for stress tolerance.

CHAPTER FOUR

4.0 RESULTS

4.1 Mean Squares, Heritability Estimates and Relationships Among Drought-Tolerant Traits of Cowpea MAGIC-RILs Evaluated in the Screen House

There were highly significant differences among the genotypes screened for tolerance to drought for the following parameters: PH1, PH2, PH3, SG2, TN1, TN2, TN3, TN4 and TN5 ($p < 0.001$); DTT was highly significant at $p < 0.01$; LS2 and RR were significant at $p < 0.05$ while LS1, LS3, SG1, SG2 and TN6 were not significant (Table 4.1).

Heritability varied among the traits. TN6 had the lowest heritability ($H^2 = 12.50\%$) while PH3 had the highest heritability ($H^2 = 51.88\%$) (Table 4.2).

Leaf-senescence increased over time for the period of the drought stress. Average leaf-senescence scores were 2.81 and 3.59 at 21 and 28 DAP, respectively. The leaf-senescence scores ranged from 2.88 – 4.00 in the drought tolerant lines for MAGIC 204 and MAGIC 207, respectively. Among the susceptible lines, the range was 3.54 – 4.42 for MAGIC 004 and IT84S2-049 respectively. The positive and negative checks (DanIla and TVu 7778) had 3.44 and 3.98 respectively (Table 4.3).

Plant height measured at 14 DAP was positively correlated with plant height measured at 21 DAP ($r = 0.89$). Plant height measured at 21 DAP was positively correlated with plant height measured at 28 DAP ($r = 0.98$). Plant height had a negative correlation with leaf-senescence ($r = -0.03$), stem green ($r = -0.07$) and drought tolerant type ($r = -0.02$) while it had a positive relationship with trifoliate number and recovery rate ($r = 0.78$, $r = 0.31$) respectively. Leaf-senescence showed a negative correlation with recovery rate ($r = -0.42$). Stem green at 28 DAP showed a negative correlation with leaf-senescence at 21 and 28 DAP ($r = -0.37$, -0.18 respectively) but positively correlated with recovery rate ($r = 0.10$) (Fig. 4.1).

Table 4.1: Mean squares of measured traits for cowpea MAGIC genotypes evaluated in the screenhouse for tolerance to drought at the seedling stage in Kano in 2018

Traits	Rep	Box	Genotype	Residual
Df	1	20	331	311
PH1	22.85ns	77.56***	38.35***	19.56
PH2	4335.20***	91.50***	45.10***	22.70
PH3	3866.50***	86.10***	45.30***	21.80
LS1	0.00ns	0.00ns	0.00ns	0.00
LS2	2.92**	1.79***	0.41*	0.34
LS3	13.39***	1.25***	0.54ns	0.46
SG1	0.00ns	0.00ns	0.00ns	0.00
SG2	0.09**	0.03***	0.01ns	0.01
SG3	2.17***	0.16***	0.07***	0.05
TN1	2.98***	0.48***	0.31***	0.16
TN2	64.41***	0.71***	0.44***	0.26
TN3	66.07***	0.68***	0.48***	0.27
TN4	98.66***	0.41*	0.46***	0.25
TN5	71.34***	1.03***	0.37***	0.23
TN6	1.54**	0.44***	0.16ns	0.14
RR	272198.00***	4971.00***	991.00*	819.00
DTT	10.58***	0.57***	0.26**	0.19

, **, *, Significant at P < 0.05, 0.01 & 0.001 respectively; ns = non-significant; PH1, PH2 & PH3 = plant height at 12, 18 & 25 DAP respectively; LS1, LS2 & LS3 = leaf senescence at 12, 18 & 25 DAP respectively; SG1, SG2 & SG3 = stem green at 12, 18 & 25 DAP respectively, TN1, TN2, TN3, TN4, TN5 & TN6 = trifoliate number at 12, 15, 18, 21, 25 & 28 DAP respectively; RR = recovery rate; DTT = drought tolerant type*

Table 4.2: Estimate of genetic parameters, broad sense heritability of traits for cowpea genotypes evaluated in the screenhouse in Kano for tolerance to drought at the seedling stage in Kano in 2018

Traits	σ^2_g	σ^2_e	σ^2_p	H^2 (%)
PH1	9.40	9.78	19.17	49.03
PH2	11.20	11.35	22.55	49.67
PH3	11.75	10.90	22.65	51.88
LS2	0.04	0.17	0.21	19.05
LS3	0.04	0.23	0.27	14.81
SG3	0.01	0.03	0.04	25.00
TN1	0.08	0.08	0.15	53.33
TN2	0.09	0.13	0.22	40.91
TN3	0.10	0.14	0.24	41.67
TN4	0.10	0.13	0.23	43.48
TN5	0.07	0.12	0.18	38.89
TN6	0.01	0.07	0.08	12.50
RR	86.00	409.50	495.50	17.36
DTT	0.04	0.09	0.13	30.77

σ^2_g, σ^2_e&σ^2_p = genotypic, error and phenotypic variances respectively; H^2 = Heritability in the broad sense; PH1, PH2 & PH3 = plant height at 12, 18 & 25 DAP respectively; LS1, LS2 & LS3 = leaf senescence at 12, 18 & 25 DAP respectively; SG1, SG2 & SG3 = stem green at 12, 18 & 25 DAP respectively, TN1, TN2, TN3, TN4, TN5 & TN6 = trifoliate number at 12, 15, 18, 21, 25 & 28 DAP respectively; RR = recovery rate; DTT = drought tolerant type

Table 4.3: Mean performance of the best seven and worst three cowpea MAGIC RILs including the checks screened for drought tolerance at the seedling stage sorted by percentage rate of recovery in Kano in 2018

SN	Line	PH1	PH2	PH3	LS1	LS2	LS3	SG1	SG2	SG3	TN1	TN2	TN3	TN4	TN5	TN6	RR	DTT
1	**MAGIC 162**	19.19	21.38	21.50	1.00	2.38	3.63	1.00	1.00	1.00	1.25	1.75	1.88	1.88	1.88	1.13	100.0	1.00
2	**MAGIC 012**	15.19	18.19	18.50	1.00	2.38	3.13	1.00	1.00	1.00	0.63	1.25	1.25	1.25	1.25	0.88	100.0	1.00
3	**MAGIC 336**	16.13	18.00	17.69	1.00	1.63	3.25	1.00	1.00	1.00	1.25	1.75	1.75	1.75	1.63	1.25	87.50	1.00
4	**MAGIC 301**	13.75	13.56	18.69	1.00	2.25	3.13	1.00	1.00	1.00	0.38	1.13	0.88	0.75	1.13	0.88	87.50	2.00
5	**MAGIC 213**	16.00	18.13	17.75	1.00	2.50	3.38	1.00	1.00	1.00	0.88	1.50	1.50	1.38	1.38	0.88	87.50	2.00
6	**MAGIC 204**	9.44	10.75	9.88	1.00	2.50	2.88	1.00	1.00	1.00	0.25	0.50	0.38	0.50	0.38	0.50	87.50	2.00
7	**MAGIC 129**	13.81	18.56	18.13	1.00	3.00	3.50	1.00	1.00	1.00	0.75	1.38	1.25	1.38	1.25	0.63	87.50	1.00
8	DANILA	15.93	18.05	17.95	1.00	2.56	3.44	1.00	0.99	0.95	1.14	1.69	1.78	1.74	1.66	1.17	45.45	1.00
9	TVu 7778	13.77	16.74	16.72	1.00	2.93	3.98	1.00	0.99	0.88	1.13	1.66	1.88	1.84	1.78	1.11	11.36	2.00
10	MAGIC 007	16.88	18.63	18.69	1.00	2.75	3.75	1.00	1.00	1.00	1.13	1.63	1.63	1.50	1.50	1.38	0.00	1.50
11	MAGIC 004	24.73	28.75	28.71	1.00	3.33	3.54	1.00	1.00	1.00	1.00	1.75	2.00	2.04	1.71	1.13	0.00	1.50
12	MAGIC 001	7.58	9.00	9.17	1.00	4.00	4.17	1.00	0.83	0.83	0.33	0.83	0.67	0.67	0.83	0.33	0.00	2.00
	Mean	15.40	18.06	17.93	1.00	2.81	3.59	1.00	0.99	0.90	0.91	1.47	1.56	1.54	1.46	1.03	30.09	1.55
	Min	1.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Max	32.00	36.33	35.67	1.00	6.00	8.00	1.00	1.50	4.00	2.00	3.50	3.50	3.67	3.33	2.00	100.00	2.00
	CV	35.99	36.10	35.84	0.00	23.07	20.51	0.00	9.77	29.13	54.34	45.98	44.62	46.19	45.08	39.45	126.07	32.34
	SE	0.22	0.25	0.25	0.00	0.03	0.03	0.00	0.00	0.01	0.02	0.03	0.03	0.03	0.03	0.02	1.47	0.02

PH1, PH2 & PH3 = plant height at 12, 18 & 25 DAP respectively; LS1, LS2 & LS3 = leaf senescence at 12, 18 & 25 DAP respectively; SG1, SG2 & SG3 = stem green at 12, 18 & 25 DAP respectively, TN1, TN2, TN3, TN4, TN5 & TN6 = trifoliate number at 12, 15, 18, 21, 25 & 28 DAP respectively; RR = recovery rate; DTT = drought tolerant type

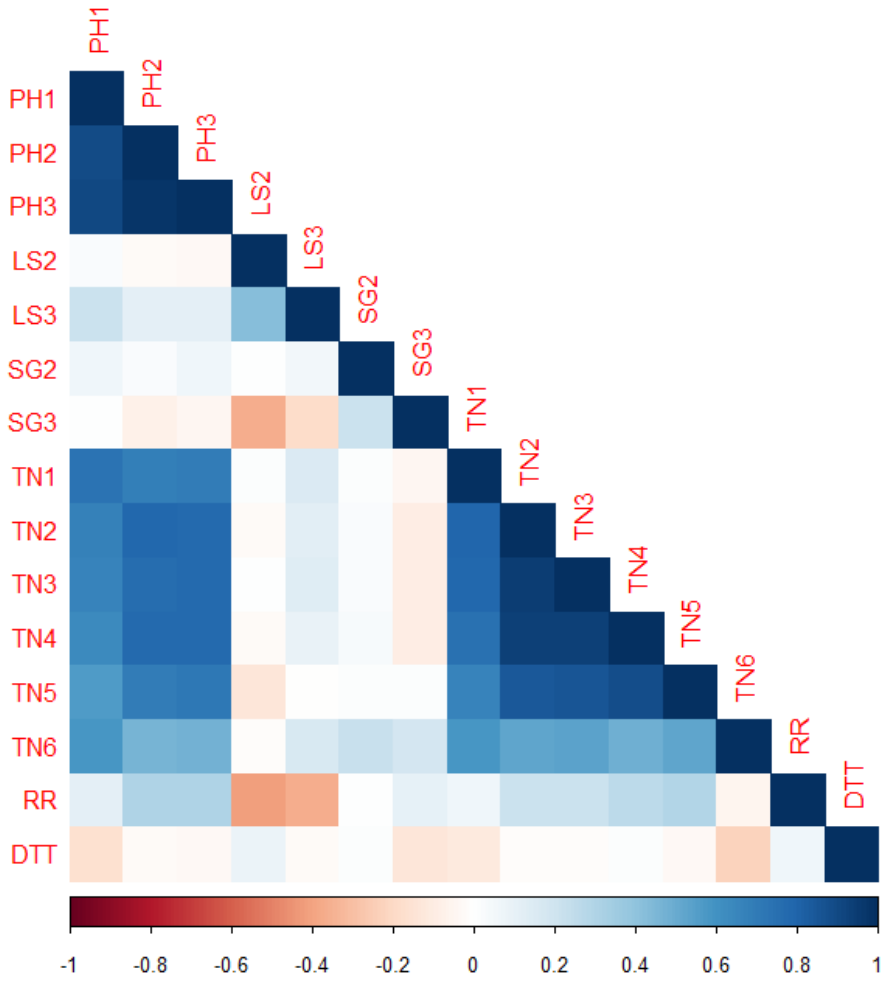


Fig. 4.1: Correlation matrix among traits of 330 cowpea MAGIC RILs evaluated in the screenhouse for tolerance to drought at the seedling stage in Kano in 2018

PH1, PH2 & PH3 = plant height at 12, 18 & 25 DAP respectively; LS1, LS2 & LS3 = leaf senescence at 12, 18 & 25 DAP respectively; SG1, SG2 & SG3 = stem green at 12, 18 & 25 DAP respectively, TN1, TN2, TN3, TN4, TN5 & TN6 = trifoliate number at 12, 15, 18, 21, 25 & 28 DAP respectively; RR = recovery rate; DTT = drought tolerant type

4.2 Mean Squares, Heritability Estimates and Relationships Among Drought-Tolerant Traits of Cowpea MAGIC-RILs Evaluated on the Field

There were highly significant ($p < 0.001$) differences among the RILs and interactions between RILs and treatmentsfor all the traits except for Pd_pdcl and AbrtRatewhile Grwthabt and PdLdScor were significant at $p < 0.01$, sdwt was significant at $p < 0.05$ (Table 4.4). The mean sums of squares (MS) and experimental errors (residuals) of analysis of variance for randomized block design with three replicates and two treatments are presented in Table 4.4. Result from the ANOVA indicate highly significant G x E interactions and also show that the RILs performed differently under water-stressed and well-watered conditions. The interactions of RILs and traits were highly significant ($p < 0.001$) for all the traits (Table 4.4).

Broad Sense Heritability (H^2) was very high in Pddrywt(94.34%), Sddrywt(93.93%),DyMat (93.92%), DFPFlw(93.26%) and DFFlw(92.26%), while it was very low in VS2WASI (25.00%) (Table 4.5).

The correlation matrix among traits of cowpea MAGIC RILs evaluated on the field for tolerance to post flowering drought across Water-Stressed and Well-Watered environments in Minjibir in 2017 are presented in Fig. 4.2.

The best seven (7) and worst three (3) RILs in terms of yield estimated in tonnes per hectare are presented in Table 4.6. MAGIC 061 had the highest yield (1.95t/ha) while MAGIC 147 had the lowest yield (0.18t/ha).

Stress tolerance indices of the best ten and the worst five cowpea MAGIC RILs sorted by STI are presented in Table 4.7. MAGIC 061 had the highest STI value of 5.10 while MAGIC 208 had the lowest STI value of 0.03.

Table 4.4: Mean squares of measured traits for cowpea MAGIC RILs evaluated on the field for tolerance to terminal drought across Water-Stressed and Well-Watered environments in Minjibir in 2017

	Rep	**Block**	**Line**	**Genotype × Environment**	**Residuals**
df	5	18	309	309	1218
DFFlw	153.69***	44.19***	42.41***	6.31***	3.29
DFPFlw	166.81***	52.18***	52.82***	7.18***	3.56
DyMat	1445.78***	72.12***	62.79***	13.68***	3.82
Grwthabt	3.18***	0.70***	1.69***	0.33**	0.26
VS2WASI	206.29***	3.57***	0.31***	1.08***	0.23
PdLdScor	11.32***	1.17ns	2.08***	0.99**	0.79
Pd_pdcl	1.95***	0.20ns	0.36***	0.16ns	0.15
AbrtRate	34.69***	1.45***	0.70***	0.38ns	0.33
Fddrywt	1.30***	0.55**	0.54***	0.39***	0.28
Pddrywt	0.48***	0.40***	1.06***	0.20***	0.07
Sddrywt	0.32***	0.25***	0.67***	0.14***	0.04
sdwt	78.80***	16.28***	45.49***	6.47*	5.58

, **, *, Significant at P < 0.05, 0.01 & 0.001 respectively; DFFlw = days to first flowering; DFPFlw = days to 50% flowering; DyMat = days to 95% maturity, Grwthabt = growth habit; VS2WASI = visual score two weeks after stress imposition; PdLdScor = pod load score; Pd_pdcl = pod per peduncle; AbrtRate= abortion rate; Fddrywt = fodder dry weight; Pddrywt = pod dry weight; Sddrywt = seed dry weight; sdwt = 100 seed weight*

Table 4.5: Estimate of genetic parameters, broad sense heritability of traits for cowpea MAGIC genotypes evaluated on the field for tolerance to terminal drought across Water-Stressed and Well-Watered environments in Minjibir in 2017

Traits	σ^2_g	σ^2_e	σ^2_p	H^2(%)
DFFlw	19.56	1.65	21.20	92.26
DFPFlw	24.63	1.78	26.41	93.26
DyMat	29.49	1.91	31.40	93.92
Grwthabt	0.72	0.13	0.84	85.71
VS2WASI	0.04	0.12	0.16	25.00
PdLdScor	0.65	0.39	1.04	62.50
Pd_pdcl	0.10	0.08	0.18	55.56
AbrtRate	0.19	0.16	0.35	54.29
Fddrywt	0.13	0.14	0.27	48.15
Pddrywt	0.50	0.03	0.53	94.34
Sddrywt	0.31	0.02	0.33	93.93
Sdwt	19.96	2.79	22.75	87.74

σ^2_g, σ^2_e&σ^2_p = genotypic, error and phenotypic variances respectively; H^2 = Heritability in the broad sense;DFFlw = days to first flowering; DFPFlw = days to 50% flowering; DyMat = days to 95% maturity, Grwthabt = growth habit; VS2WASI = visual score two weeks after stress imposition; PdLdScor = pod load score; Pd_pdcl = pod per peduncle; AbrtRate = abortion rate; Fddrywt = fodder dry weight; Pddrywt = pod dry weight; Sddrywt = seed dry weight; sdwt = 100 seed weight

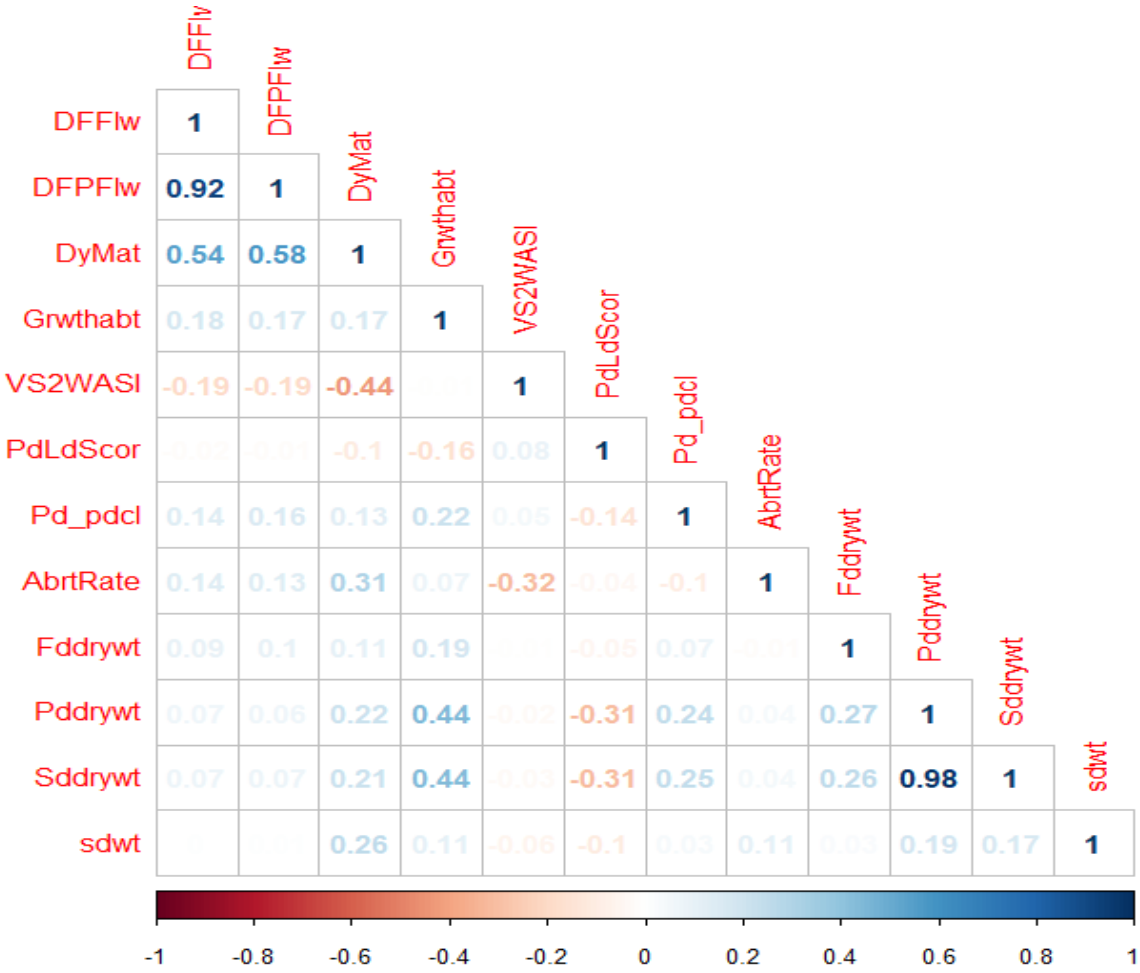


Fig. 4.2: Correlation matrix among traits of cowpea MAGIC RILs evaluated on the field for tolerance to terminal drought across Water-Stressed and Well-Watered environments in Minjibir in 2017

DFFlw = days to first flowering; DFPFlw = days to 50% flowering; DyMat = days to 95% maturity, Grwthabt = growth habit; VS2WASI = visual score two weeks after stress imposition; PdLdScor = pod load score; Pd_pdcl = pod per peduncle; AbrtRate= abortion rate; Fddrywt = fodder dry weight; Pddrywt = pod dry weight; Sddrywt = seed dry weight; sdwt = 100 seed weight

Table 4.6: Mean performance of the best seven and worst three cowpea MAGIC RILs screened for terminal drought tolerance on the field sorted by grain yield across Water-Stressed and Well-Watered environments in Minjibir in 2017

SN	Line	DFFlw	DFPFlw	DyMat	Grwthabt	VS2WASI	PdLdScor	Pd_pdcl	AbrtRate	Fddrywt	Pddrywt	Sddrywt	sdwt
1	**MAGIC 061**	40.33	44.83	74.33	2.83	1.17	1.17	2.83	1.50	1.46	2.48	1.93	16.83
2	**MAGIC 063**	41.83	45.17	74.83	2.67	1.00	1.00	2.00	2.33	1.99	2.20	1.67	18.67
3	**MAGIC 032**	41.00	44.67	72.00	3.00	1.50	1.00	2.50	2.33	1.09	2.04	1.61	15.67
4	**MAGIC 144**	38.33	44.17	70.50	2.17	1.50	1.33	2.00	1.83	0.96	2.04	1.61	15.83
5	**MAGIC 267**	42.17	45.17	67.67	1.50	1.33	1.17	2.17	1.83	1.32	2.10	1.60	12.67
6	**MAGIC 115**	38.50	43.50	72.67	2.00	1.33	1.33	2.17	2.17	1.01	1.87	1.55	16.00
7	**MAGIC 004**	42.83	45.83	77.33	2.67	1.17	1.17	2.17	2.00	2.04	2.11	1.55	22.33
8	MAGIC 208	43.50	49.17	75.00	1.33	1.67	2.50	1.83	2.00	0.60	0.28	0.19	11.83
9	MAGIC 194	39.00	44.50	67.33	1.17	1.50	3.00	1.67	1.83	0.83	0.25	0.19	12.67
10	MAGIC 147	36.67	40.00	64.00	1.00	2.00	3.00	1.67	1.17	0.72	0.26	0.18	12.50
	Mean	39.88	43.74	71.04	1.84	1.31	1.78	2.14	1.92	0.88	1.12	0.85	16.05
	Min	33.67	37.00	63.00	1.00	0.50	1.00	1.50	1.00	0.41	0.25	0.18	10.33
	Max	49.50	54.67	78.00	4.00	2.00	7.33	3.00	2.83	3.58	2.48	1.95	25.50
	CV	6.77	6.90	4.63	28.94	17.66	33.15	11.51	17.97	34.22	37.84	39.71	17.22
	SE	0.15	0.17	0.19	0.03	0.01	0.03	0.01	0.02	0.02	0.02	0.02	0.16

DFFlw = days to first flowering; DFPFlw = days to 50% flowering; DyMat = days to 95% maturity, Grwthabt = growth habit; VS2WASI = visual score two weeks after stress imposition; PdLdScor = pod load score; Pd_pdcl = pod per peduncle; AbrtRate= abortion rate; Fddrywt = fodder dry weight; Pddrywt = pod dry weight; Sddrywt = seed dry weight; sdwt = 100 seed weight

Table 4.7: Stress tolerance indices of best ten and worst five cowpea MAGIC RILs sorted by STI in Minjibir in 2017

SN	Line	Yww	Yws	MP	TOL	GMP	STI	SSI	YSI	YR
1	MAGIC 061	1.97	1.89	1.93	0.07	1.93	5.10	2.64	0.96	-3.70
2	MAGIC 063	1.98	1.35	1.67	0.63	1.64	3.68	22.64	0.68	-31.74
3	MAGIC 144	1.58	1.64	1.61	-0.06	1.61	3.55	-2.91	1.04	4.08
4	MAGIC 032	1.43	1.79	1.61	-0.35	1.60	3.51	-17.66	1.25	24.75
5	MAGIC 115	1.50	1.60	1.55	-0.11	1.55	3.29	-5.20	1.07	7.28
6	MAGIC 305	1.57	1.48	1.53	0.10	1.53	3.19	4.35	0.94	-6.09
7	MAGIC 004	1.95	1.14	1.55	0.81	1.50	3.07	29.54	0.59	-41.40
8	MAGIC 005	1.84	1.19	1.52	0.65	1.48	3.01	25.15	0.65	-35.25
9	MAGIC 015	1.77	1.21	1.49	0.55	1.47	2.94	22.34	0.69	-31.32
10	MAGIC 257	1.50	1.42	1.46	0.08	1.46	2.92	3.60	0.95	-5.05
11	MAGIC 272	0.37	0.12	0.25	0.25	0.21	0.06	48.45	0.32	-67.90
12	MAGIC 147	0.20	0.17	0.18	0.03	0.18	0.05	12.08	0.83	-16.93
13	MAGIC 194	0.24	0.15	0.19	0.09	0.19	0.05	27.46	0.62	-38.48
14	MAGIC 290	0.11	0.32	0.22	-0.21	0.19	0.05	-131.14	2.84	183.80
15	MAGIC 208	0.33	0.06	0.19	0.28	0.14	0.03	59.2	0.17	-82.98

Yww = yield under well-watered condition; Yws = yield under water stressed condition; MP = mean productivity, TOL = tolerance index; GMP = geometric mean productivity; STI = stress tolerance index; SSI = stress susceptibility index; YSI = yield stability index; YR = yield reduction

The correlation among the stress tolerances indices of cowpea MAGIC-RILs evaluated on the field for tolerance drought at the post flowering stage across Water stressed and Well-watered environments are presented in Table 4.8. STI was positively associated with GMP and had an r value of 0.97 at $p < 0.001$.

Table 4.8: Correlation among stress index scores of 310 cowpea MAGIC RILs evaluated on the field for tolerance to terminal drought across Water-Stressed and Well-Watered environments in Minjibir in 2017

	Yws	**MP**	**TOL**	**GMP**	**STI**	**SSI**	**YSI**	**YR**
Yww	0.66***	0.92***	0.50***	0.91***	0.90***	0.40***	-0.40***	-0.40***
Yws		0.90***	-0.32***	0.91***	0.86***	-0.23***	0.23***	0.23***
RP		-0.11	-0.77***	-0.12*	-0.12*	-1.00***	1.00***	1.00***
MP			0.12*	1.00***	0.97***	0.11	-0.11	-0.11
TOL				0.11*	0.14**	0.77***	-0.77***	-0.77***
GMP					0.97***	0.12*	-0.12*	-0.12*
STI						0.12*	-0.12*	-0.12*
SSI							-1.00***	-1.00***
YSI								1.00***

, **, *, Significant at P < 0.05, 0.01 & 0.001 respectively; Yww = yield under well-watered condition; Yws = yield under water stressed condition; MP = mean productivity, TOL = tolerance index; GMP = geometric mean productivity; STI = stress tolerance index; SSI = stress susceptibility index; YSI = yield stability index; YR = yield reduction*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Screen House Experiment of Cowpea MAGIC-RILs

The aim of this study was to identify parents that are tolerant to drought that can serve as parental sources of favourable alleles for development of pure cowpea lines tolerant to drought. The use of wooden boxes or pots for screening for drought tolerance in the screen house have been reported to help quickly in identifying cowpea plants that would show drought tolerance especially at the vegetative stage. Studies by Watanabe *et al.* (1997) and Ewansiha and Singh (2006) have shown positive and significant correlation between drought tolerance in the seedling stage as observed using wooden boxes in a screen house and drought tolerance screening on the field. They therefore concluded that cowpea lines found to be drought tolerant at the seedling stage using wooden box should also perform well under drought condition.

The results of this experiment confirmed that despite the inherent comparative tolerance to drought, cowpea exhibits significant genetic variation in response to drought stress. The drought-stress responses were manifested in a range of discrete physiological and morphological characteristics apparently related to the ability of the cowpea plants to tolerate and recover from seedling stage drought stress. It was observed that within genotypes, individual plants expressed varying levels of survival and ultimate recovery in response to drought stress. A similar result was reported by Watanabe *et al.* (1997).

In this study, MAGIC 162 and MAGIC 012 that wilted slowly and maintained green stems till the resumption of watering were able to conserve water in leaves and stem tissues, survive the dry period and resume growth had a recovery rate of 100%. These genotypes may have a

mechanism to slow their transpiration rate and not deplete their soil moisture reserve as quickly as genotypes that have high wilting score.

However, MAGIC 001 that wilted fast died. Similar findings were observed in soybean (Ries*et al.*, 2012) and common bean (*Phaseolus vulgaris*) (Mukeshimana*et al.*, 2014). The slow wilting observed in this experiment was not associated with deep-water extraction by the root because the genotypes were planted in boxes with shallow soil profiles; it therefore meant that this trait is associated with water saving mechanism in the shoot. In grain legumes such as groundnut (*Arachishypogaea*), chickpea (*Cicerarietinum*), soybean and cowpea, traits such as lower transpiration rate under lower vapour pressure deficits (VPD) has been recognized as soil water-saving strategy in tolerant genotypes (Sinclair *et al.*, 2008; Devi *et al.*, 2010; ZamanAllah*et al.*, 2011; Belko*et al.*, 2012a).

Using a shallow wooden box for drought stress tolerance assessment at the seedling stage served to eliminate the effect of the cowpea root system in up-taking water from deeper soil, allowing one to screen the role of leaves and stem for cowpea seedling survival under drought stress. All the traits examined in this study varied significantly between the RILs. The results indicated that leaf senescence appeared to be the best indicator of cowpea plants seedling survival as shown by the high negative correlation (r = -0.42) between the two traits. The results are in agreement with those of Mucher*oet al.* (2008) who studied 14 cowpea genotypes using small plastic pots to eliminate competition among genotypes for communal water source while testing leaves and stems characteristics for cowpea seedling survival. Drought tolerance type (Type 1 and Type 2) is a strategy exhibited by cowpea to survive drought. A Type 1 response involved plants mobilizing moisture from lower leaves to sustain growth of new trifoliates, with rapid senescence of unifoliates at the onset of drought conditions. In a Type 2 response, plants ceased

all growth and conserved moisture in all plant tissues, thereby allowing subsequent recovery of the entire shoot following rehydration. MAGIC 162 and MAGIC 012 that had a 100% survival rate showed a Type 1 response which was the same as Danila (drought tolerant check) while MAGIC 001 with 0% survival rate showed a Type 2 response which was the same asTVu7778 (drought susceptible check). Inheritance studies suggested single-gene control for both Type 1 and 2 tolerance mechanisms (Mai-Kodomi*et al.*, 1999). Johnson *et al.* (1955), categorized heritability (H^2) estimates as: Low (0-30%), medium (30-60%), High (above 60 %). The H^2 for DTT in this work was low (30.77), this did not confirm the single-gene control described by Mai-Kodomi*et al.* (1999). This might be due to the intensity of water stress and to the differences in scoring methods. This implies that DTT is not the best trait to consider for selection because MAGIC 301, MAGIC 213 and MAGIC 204 that had a recovery rate of 87.50% had a Type 2 DTT response.

In other crops, various shoot characteristics have been used to evaluate drought tolerance at the seedling stage. In cotton, seedling recovery after stress was used as a criterion to assess drought tolerance in cotton (*Gossypiumhirsutum*) (Longenberger*et al.*, 2006; Rauf, 2008). Tolerance to seedling leaf death and recovery was used in rice (De Datta*et al.*, 1988; Mitchell *et al.*, 1998). In soybean, slow wilting was reported to be associated with seedling drought tolerance (Sadok*et al.*, 2012). In cowpea, significant correlations were reported between stem greenness, survival and recovery dry weight (Thomas and Howart, 2000). The fundamental method to develop variation for selection is crossing genotypes where selecting the parents may be very important and existing genetic differences between the crossing parents be required. Grouping the genotypes can give breeders the opportunity to select appropriate parents for crossing.

5.2 Field Trials of Cowpea MAGIC-RILs

5.2.1 Phenology and Stay-green Character Under Drought Conditions

Flowering time was not affected by the water treatment because genotypes experienced the same water regime in the WS and WW environments until initiation of flower buds. Across the environments, the average days to flowering were 40 and 44 days for the DFFlw and DFPFlw respectively. Under terminal drought conditions, number of days to maturity is often shortened (Urrea*et al.*, 2009). Here, cowpea genotypes matured an average of 4 days earlier in the WS than in the WW treatment. Similar result was reported by Belko*et al.* (2014).

The leaf damage scores under drought conditions varied significantly among the genotypes and ranged from 1 (green and turgescent leaves) to 4 (yellow-green and severely wilted leaves). IT90K-277-2, IT99K-573-1-1, MAGIC 063 and IT89KD-288 kept their leaves greener than MAGIC 147, MAGIC 093 and MAGIC 069 which showed senescent and wilted leaves under drought. This ranking is supportive of Belko*et al.* (2012a). In short, the genotypes that showed the stay-green character in the field were the same that had a lower water use during the vegetative stage and the capacity to restrict their leaf transpiration in response to high vapour pressure deficit (Belko*et al.*, 2012b). These water-saving traits are hypothesized to contribute making more water available in the soil profile, allowing the genotypes to stay green and maintain plant functions that are crucial for terminal drought tolerance (Belko*et al.*, 2012a).

5.2.2 Variation in Fodder and Grain Yields Under WS and WW Conditions

For any given environmental condition, drought tolerance depends on the genotype and its potential yield, which varies from one plant to another (Shiri*et al.*, 2010). The use of yield-based selection of drought-tolerant cultivars under both WS and WW conditions has been suggested as a path leading to the identification of drought-tolerant and high-yielding cultivars because the

heritability of grain yield under optimal conditions is usually higher than under stress conditions, and for some crops there is a correlation between performance under drought and optimal growing conditions (Mitra, 2001). In this study, the heritability for grain yield in WW was 7% higher than in WS which agrees with Mitra (2001).

Fernandez (1992) categorized plant genotypes into four groups based on their grain yield performance under DS and NS environments: A = genotypes that express uniform superiority under both DS and NS conditions, B = genotypes that perform favorably only under NS conditions, C = genotypes that yield relatively higher only under DS conditions, and D = genotypes that perform poorly under both DS and NS conditions. According to Fernandez (1992), the most suitable index for selection for drought resistance is the one that could distinguish Class A from others because of the expected higher yield stability in this class. In this study, IT07K-292-10 and MAGIC 061 had the highest grain yields under both WS and WW treatments, and were therefore classified as Class A based on Fernandez's (1992) model. MAGIC 063 had higher grain yield only in the WW treatments and was classified as Class B. MAGIC 267, MAGIC 032 and MAGIC 044 had higher grain yield only in the WS treatments and were classified as Class C. MAGIC 147, MAGIC 194 and MAGIC 208 had the lowest grain yields under both WS and WW treatments and were classified as Class D.

The 100-seed weight varied significantly among the genotypes and across the water regimes, indicating a high genotypic variability and significant water treatment and genotype × treatment interaction effects for seed filling. Generally, drought stress reduced 100-seed weight by an average of 2%. As a result of this, the most promising genotypes for terminal drought tolerance had relatively low reductions in their 100-seed weight. This agrees with Belko*et al.* (2014) but is in contrast with dry bean that showed relatively greater 100-seed weight reduction (12.5 to

23.1%) under drought (Munoz-Perea*et al.*, 2006; Singh, 2007; Urrea*et al.*, 2009), these results suggest that seed filling may be less affected by post-flowering drought in cowpea as compared to bean. Direct comparative experimentation would be needed to confirm whether this is true as suggested by Belko*et al.* (2014).

5.2.3 Stress Tolerance Indices and Selection for Drought Tolerance

Across the environments, there were poor relationships between grain yield under WS and grain yield under WW conditions for the genotypes (r = 0.14), indicating that high potential yield does not necessarily result in improved yield under stress conditions. This is demonstrated with the genotypes of Class B that were among the top performers under WW environments and the low performers under WS environments. Similar results were reported in durum wheat by Sio-Se Mardeh*et al.* (2006) and Talebi*et al.* (2009).

Although there were some sorts of auto-correlations, yield being part of the indices, STI and GMP were closely related to grain yield across the genotypes and the environments. The genotypes of Class A which had the greatest average grain yields under both WS and WW environments, also had the highest STI and GMP values. These indices appear to be useful for breeding drought-tolerant cowpea as they have for selection of drought tolerance in various crop species (Moghaddam and Hadizadeh, 2002; Baheri*et al.*, 2003; Jafari*et al.*, 2009; Talebi*et al.*, 2009; Shirinzadeh*et al.*, 2010).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

- Cowpea is the major grain legume with widespread cultivation across Nigeria.Erratic rainfall pattern exposes cowpea to drought at critical stages of growth of the crop. This is inspite of the inherent capacity of cowpea to withstand drought.There is no information on drought adaptability of cowpea MAGIC-RILs germplasm in Nigeria.This study evaluatedthe genetic variability, heritability and relationships among drought tolerant-traits in Cowpea (*Vignauguiculata*) MAGIC - RILs population at both the seedling and post-flowering stages and selected parents for further breeding activities.
- Screenhouse evaluation of Cowpea MAGIC RILs was carried out using a simple Wooden Box technique to investigate the responses of the lines to drought stress at the seedling stage. Field trial was conducted to screen the lines for their responses to post flowering stage drought stress.
- Results obtained from the Wooden Box Experiment showed high genetic variability among the lines fordrought tolerance at the seedling stage. New genotypes of cowpea MAGIC-RILs tolerant to drought at the seedling stage were identified. Field trials results demonstrated that cowpea MAGIC-RILs are promising genetic resources to address drought stress in sub-Saharan Africa. The quantitative indices of drought tolerance STI and GMP were used to classify the genotypes.

6.2 Conclusions

- DanIla which is known to be drought tolerant confirmed its drought tolerant status by having a recovery rate of 45% and leaf senescence score of 3.44. New drought tolerant genotypes were phenotyped and selected as parental lines. Examples are MAGIC 162

and MAGIC 012 which had a recovery rate of 100% and maintained low leaf senescence scoresof 3.63 and 3.13 respectively.

- Amongst the quantitative indices of drought tolerance, STI and GMP were the best because they enabled the identification of Class A genotypes which had uniform superiority across both environments. Examples include MAGIC 061 (5.1; 1.93), MAGIC 063 (3.68; 1.64), MAGIC 144 (3.55; 1.61) and MAGIC 032 (3.51; 1.6). MAGIC 061 and MAGIC 063are the most resilient cowpea lines of dual purposes because they had the highest grain and fodder yield in both environments.
- Genotypic variability for drought tolerance existed amongst the tested genotypes. There exist relationships among drought-tolerant traits at both the seedling and the terminal stages. Leaf senescence is the most important predictor of recovery at the seedling stage and it is negatively correlated with recovery rate. The negative correlation between leaf senescence and recovery rate could serve as an early indicator of the ability of cowpea MAGIC RILs to recovery from drought stress after rehydration. For terminal drought tolerance, stress tolerance index (STI) and geometric mean productivity (GMP) were highly positively correlated at $p < 0.001$.

6.3 Recommendations

- The seven (7)MAGIC RILs (MAGIC 162, MAGIC 012, MAGIC 336, MAGIC 301, MAGIC 213, MAGIC 204, MAGIC 129) selected at the seedling stage are recommended for use as parents for the development of new germplasms for further breeding pipelines.
- Ten (10)MAGIC RILs (MAGIC 061, MAGIC 063, MAGIC 144, MAGIC 032, MAGIC 115, MAGIC 305, MAGIC 004, MAGIC 005, MAGIC 015, MAGIC 257) selected as

terminal drought tolerant lines are recommended for preliminary and multi-locational trials.

- A different scoring method should be employed.

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