UNIVERSITY OF BOTSWANA

BOTSWANA COLLEGE OF AGRICULTURE

IDENTIFICATION OF DROUGHT TOLERANT COWPEA [*VIGNA UNGUICULATA* (L). WALP)] GENOTYPES BASED ON MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES TO WATER DEFICIT.

A Dissertation presented to the Department of Crop Science and Production in Partial fulfilment of the Requirements for the Degree of Masters of Science (MSc) in Crop Science (Agronomy).

By

CHARLES F. KING, Jr.

ID: 201300265

AUGUST, 2015

supervisor : Dr Utlwang Batlang

Co-Supervisor : Prof. Samodimo Ngwako

DEPARTMENT OF CROP SCIENCE AND PRODUCTION

BOTSWANA COLLEGE OF AGRICULTURE

CERTIFICATE

Main Supervisor's Name and Signature Date

Co-Supervisor's Name and Signature Date

Head of Department's Name and Signature Date

APPROVAL

Main Supervisor's Name and Signature

Date

Co-Supervisor's Name and Signature

Date

Head of Department's Name and Signature

Date

STATEMENT OF ORIGINALITY

Every bit of work within this dissertation was completely put together by the author's at the University of Botswana, Botswana College of Agriculture from 2014 to 2015. This is the original except particularly where references are made and will not be submitted additionally for any level of degree or diplomat in any university around the world.

ACKNOWLEDGMENTS

With God all above, I am humble and unpresuming to thank the Botswana government and the Liberian government for the opportunity accorded me to study in the University of Botswana. My gratitudes also go to my Supervisors, Dr. Utlwang Batlang and Prof. Samdimo Ngwako for thier extraordinary supervision during my course of the study. I am indeed gratified in thanking the Botswana College of Agriculture staff for their mentorship in completing my dissertation. To my boss Mr. Aaron G. Marshall, Head of Central Agriculture Research Institute (CARI) Liberia for this confidence by granting the a scholarship. Part of my research was financed by the Southern African Science Service Centre for Climate Change and Adaptive Land Management (SASSCAL) Task 308 awarded to Botswana College of Agriculture and I would like to acknowledge the Centre for their contribution.

DEDICATION

I am happy and humble to dedicate my work to my Father, Mr. Charles F. King Sr. and my Mother (Madam Mary Lateh) for their unending and loving support throughout my educational sojourn. I am glad also to dedicate this to my selfless and loving brother Jeremiah D. King, whose love and kindness have overwhelmed me. To my dearest and darling friend Siah Tengbeh for her loving and caring behavior throughout my studies.

ABSTRACT

Drought stress poses a major threat to food security due to the devastating effect during growth and development of plants and leads to yield loses in Africa, especially Botswana. Therefore, there is an increasing need in providing part of the solutions, and for crops like cowpea through drought tolerance identification and improvement programs. Therefore, identification of drought tolerant cowpea [*Vigna unguiculata* (L). Walp)] genotypes based on morphological and physiological responses to water deficit was researched during 8-12 days imposition of drought stressed at vegetative stage using twenty cowpea genotypes under green house conditions. On these basis, two preliminary experiments followed by two major experiments were conducted at the Botswana College of Agriculture in 2014/2015 summer period. The preliminary experiments aimed at determining the suitable soil mixture for the entire experiment and the days required to reduce cowpea biomass yield to 50%, while the two major experiments intended to identify drought tolerance cowpea genotypes based on morphological (index) and physiological traits (gaseous exchange and chlorophyll content). The experiments were layout in a Randomized Complete Block Design with four replications and two treatments (well-watered and drought stressed) for the major experiments.

Drought stress significantly ($P > 0.05$) reduced growth parameters: plant height, leaf area, and biomass yield, Shoot dry weight, root dry weight and shoot dry weight. Physiologically, water stress also reduced relative water content (RWC) ($P > 0.05$), chlorophyll content ($P < 0.05$) and gaseous exchange ($P < 0.05$). The biomass mean productivity (BMP) was significant ($P < 0.05$) based on biomass yield under well-watered and drought stressed, and used to indentify tolerant cowpea genotypes respectively.

Overall, the BMP index showed that BCA001 and BCA003 were highly tolerant; BCA002, BCA006, BCA009, BCA016, BCA011 and BCA019 were drought tolerant; BCA004, BCA015 and BCA017 were moderately tolerance ; BCA020, BCA014, BCA013, BCA012, BCA007, BCA008, BCA010, BCA005 and BCA013 were sensitive &highly sensitive.

The poor relationship between BMP and gaseous exchanges [net photosynthesis (R^2 = 0.0345), stomata Conductance (R^2 = 0.040), transpiration (R^2 = 0.006)] and chlorophyll content results indicated that these were parameters to use for identification of cowpea drought tolerant rather the BMP. The BMP results can be wholly used in crop drought tolerance improvement program and breeding in Botswanna especially under green house condition.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

AQPs: Acquaporins

ATP: Adenosine triphosphate

BCA: Botswana College of Agriculture

BMP: Biomass mean productivity

BRDI: Biomass relative drought index

BSSI: Biomass stress susceptbility

BSTI: Biomass stress tolerance index

BYp : Biomass yield under well-water

BYR%: Biomass yield reduction percent

Bys: Biomass yield under water stress

cm: Centimeters

CO_2: carbon dioxide

DA: Drought avoidance

DL: Drought level

DLS: Delay leaf senescence

DT: Drought stress pure

DT: Drought tolerance

DTc: Drought tolerance categories

FAO: Food Agriculture Organization

FW: Fresh weight

HDT: Highly drought tolerant

IITA: International Institute of tropical Agriculture

LD: Lethal drought

LEA: Late embryonic abundant

LSD: Least significant difference

MOA: Ministry of Agriculture

MP: mean productivity

MT: Moderate tolerant

NIPs: Intrinsic proteins

OA: Osmotic adjustment

OP: Osmotic potential

PSII: Photosystem two

RCBD: Randomized complete block design

ROS: Reactive oxygen species

RWC: Relative water content

SAS: Statistical analysis system

ST: Susceptible

TIPs: Tonoplast intrinsic proteins

TOL: Tolerance

TW: Turgid weight

WP: Well-water pure

WUE: Water use efficiency

CHAPTER ONE

INTRODUCTION

1.1. General introduction

Cowpea [*Vigna unguiculata*(L.) Walp.] is a major economic important crop in tropical and subtropical regions of sub-Saharan Africa, where it is grown for its foliage and fresh and dry grain. Outside Africa, cowpea is grown in parts of Asia, Latin America, the south-eastern United States, and California (FAO, 2012). Cowpea is one of the ancient grain legume crop cultivated in semiarid region where rainfall resources are characteristically low (300-600mm) (Fussell *et al.*, 1991). Crops such as cowpea and many others are exposed to the ravages of drought in various ways and to different extents. Regrettably, global climate change in many parts of the developing world brings about shortage of water as a result of changes in rainfall patterns and the demand for water for cowpea productivity which is created due to the rising temperatures, which exacerbates the problem. Despite its inherent capacity to survive drought, significant differences exist among cowpea genotypes in drought tolerance (Mai-Kodomi *et al.*, 1999a). It suggests that there are both tolerant and susceptible varieties among collections of cowpea.

Drought, also known as water deficit, can result from insufficient moisture for a plant to grow adequately and complete its life cycle. Insufficient moisture can be the consequence of a shortage in rainfall, coarse textured soils that retain little water in the root zone, or drying winds (Swindale and Bidinger, 1981). Drought stress is one of the factors that most strongly limit the natural distribution of plant species, their growth and productivity worldwide (Tuberosa and Salvi, 2006). Water deficit affects all aspects related to the plant development, including anatomical, morphological, physiological and biochemical modification, and the losses directly related to its duration, severity and stage of crop development.

In plant stress physiology, drought tolerance is a constituent of drought resistance, whereby resistance refers to a combination of both avoidance and tolerance. According to (Ntombela, 2012; Watanable et al., 2012) drought tolerance (DT) is defined as the ability of a plant to live, grow, and reproduce satisfactorily with limited water supply or under periodic conditions of water deficit. Mechanisms of drought tolerance include: maintenance of turgor through osmotic adjustment, increased cell elasticity; decreased cell size; desiccation tolerance by protoplasmic resistance and increased antioxidant capacity. On the other hand, drought avoidance (DA) means the ability to complete their life cycle without severe water deficits developing (Ntombela, 2012; Tuberosa 2012). This is due to morphological development that enables them to access water or reduce loss. Reduced leaf area, deeper roots, and root: shoot ratio account for drought avoidance in most species (Hall, 1993). There is genetic basis for drought tolerance plant's response to drought stress, the activation of genes and transmission are involved in the genetic make-up of drought tolerant (Shinozaki and Yamaguchi-Shinozaki, 2007). These genes include those that governs the accumulation of compatible solutes; passive transport across membranes; energy-requiring water transport systems; protection and stabilization of cell structures from desiccation and reactive oxygen species (Shinozaki and Yamaguchi-Shinozaki, 2007). However, there also exist genotypic differences in crop varieties/genotypes in response to drought stress (Lenka *et al.*, 2011 ; Des Marais *et al.*, 2012) or crops like cowpea (Mai-Kodomi *et al.*, 1999a; Muchero *et al.*, 2008; Pungulani *et al.*, 2012).

In cowpea (pulses) and other plants, drought tolerant morpho-physiological traits, which are genetically controlled, have been determined. These traits include water use efficiency (WUE), water potential, relative turgidity, osmotic adjustment, leaf gas exchange, relative water content (RWC), diffusion pressure deficit, chlorophyll stability index and carbon isotope discrimination, (Ntombela, 2012; Morgan et al., 1991; Hall et al., 1990 ; Anyia and Herzog, 2004; Souza et al., 2003). However, the traits to be considered as potential selection targets for improving yield under water-limited conditions must be genetically correlated with yield, and should have a greater heritability than yield itself. Measurement of the target trait should be rapid, accurate, and in-expensive (Tuberosa, 2012). For traits such as osmotic adjustment, stomatal regulation, chlorophyll stability index and antioxidant systems that directly control drought tolerance, the deteriming approach is to study them and the ways they control avoidance separately and combine them in improved varieties during breeding.

In the past, 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 criteria, since yield is the integrated expression of the entire array of traits related to productivity under stress **(** Matsui and Singh, 2003; Cisse *et al.,* 1997). These empirical approaches are slow, laborious and expensive because of the need to assess large populations across many locations. Using a shallow soil layer in boxes a screening technique for drought tolerance in cowpea at the seedling stage has been developed (Singh *et al.,* 1999; Matsui and Singh, 2003). This technique identified significant number of drought tolerant genotypes in studies involving cowpea and other crops (Singh *et al.,* 1999; Agbicodo *et al.,* 2009; Hall *et al.,* 2004). The research aims to identify drought tolerant genotypes from a large population in cowpea and some mechanism of tolerance.

1.1.1. The cowpea crop: Its uses

Cowpea[*Vigna unguiculata* (L.) Walp.] belongs to the family Leguminasae.It is one of the members of the three Vigna genus in which both the freshly leaves and seeds are consumed (Madamba *et al.,* 2006). The other members are *Vigna subterranean* (L.) Verdc (Bambara groundnut) and *Vigna radiata* (L.) Wilczek (Mungbean) and they are consider as pulses. Cowpea was domesticated in Africa, where the richest genetic diversity of wild types occurs throughout Southern Africa. The largest genotypes of cultivated cowpea are found in West

Africa, in the savanna of Barkina Faso, Ghana, Togo, North-western part of Cameroon (Ng and Marechal, 1985). It was also suggested by Ogunkamni *et al,.* 2006 that cowpea might have originated from central Africa.

Cowpea growth types are determinate and indeterminate (Pandy *et al.,* 2006). The determinate types, grow vegetatively for an extended period of time before abruptly terminating growth of the main stem and initiating the flowering and reproductive stages. At this point, the vegetative stages become strongly repressed as physiological activity is directed towards reproduction (Pandy *et al.*, 2006). The determinate type is short, self supporting or bushy and of short growth duration. Cowpea seeds are an important source of affordable protein, vitamins and minerals in the predominantly carbohydrate diet of people mostly in Africa. Therefore, wider utilisation of cowpeas in the diet, presents a source of protein that is within the means of most rural households in southern Africa (Botswana, Malawi, South Africa, etc.) (FAO, 2012 and Pungulani *et al.*, 2012).

Cowpea provides approximately 20% crude protein, 64% carbohydrate, and 3% crude fiber (Ntombela, 2012). Cowpea can enhance the fertility of the soil with respect to nitrogen and phosphate, thereby benefiting crops. For example,cowpea can fix 73-354 kg N/ha per year of biological nitrogen (FAO, 2012). It may also be grown as a forage legume to provide fodder of higher quality than cereals or forage grasses. A major use of cowpeas in the Sahelian zone of Africa is as hay, after the pods have been harvested to feed draft animals, rams and goats (Ntombela, 2012).

1.1.2. The cowpea crop: Its responses and adaptation to drought stress

Among the pulses crops grown in Central and West Africa, cowpea belongs to the inherently more drought tolerant ones (Ntombela, 2012 ; Singh *et al.*, 1997; Ehlers and Hall , 1997). In a drought stress screening study, the overall ranking of crops in increasing order of drought tolerance crops were found to be cowpea and followed by: soya bean, black gram, ground nuts, maize, sorghum, Bambara groundnut and lablab (Matsui and Singh, 2003; Singh, 2005). However, cowpea still suffers considerable water deficit effects especially in Savanna and Sahel sub-regions. In fact, drought stress is regarded as major limitation to crop production in some developing countries and it periodically causes agricultural yield losses in crop like cowpea in developing countries like Botswana, South Africa, malawi, Zambia, (Bennie and Hensely, 2001; Ntombela, 2012; MOA, 2014). The pulses production level in Botswana could be an indicator of drought stress impact whereby in 2011 and 2012, the overall production was 4,700 - 2,285 metric tones and 63 – 133 Kg/ha (MOA, 2014) compared to to other countries like Malwai, Nigeria, Tanzania and Kenya (FAO, 2012).

Therefore, drought-tolerant crop production and research is a priority to meet the growing demand for food and nutrition in the world for Such crop is cowpea, since early maturing varieties escape terminal drought (Bezzerra, 2003), but if exposed to intermittent moisture stress during the vegetative growth stage, they perform very poorly (Mai-Kodomi *et al.*, 1999a). Moreover, the early maturing cowpea cultivars tend to be very sensitive to drought that occurs during the early stages of the reproductive phase (Bezzerra, 2003). The effects of drought stress varies with crops and the level of tolerance they exhibit, the impacts of the water deficit and how long the plants experience this water deficit. Generally, it has been established that plants respond to drought stress, and the adaptive mechanism to deal with drought stress through maintance of tugor pressure and accummuation of osmolytes and protective molecules (Baier *et al.*, 2005). Additionally, drought responsive proteins such as dehydrins and heat shock proteins protect the cellular activiteies (tissue and cell of the plant) . Previous studies have indicated that proline (Hamidou *et al.,* 2007; Cheulile and Agenbag, 2004, sugars (Souza *et al.* 2004), antioxidants (D'Arcy-Lameta *et al.* 2006, Nair *et al.* 2008) are associated with drought tolerance in cowpea. While these are drought tolerant mechanisms, cowpea drought avoidance morpho-pysiological features have been studied too that includes; deep rooting, delayed leaf senescence (DLS), very sensitive stomata to soil drying (Tuberosa, 2012). Unfortunately, cowpea scientists are still identifying the ideal trait or traits to use in selection for drought tolerance. But, studies have shown that cowpea genotypes are more sensitive to drought stress at the vegetative stage than the reproductive stage (Ntombela, 2012).

1.2. Statement of the problem

As the world population increases, there will be a demand for food to meet population growth. Despite this, food production is on the trend of improvement in Africa but water shortage still remains a major constraint. In the past century, water use has increased worldwide at more than twice the rate of population expansion (FAO, 2007). For example, agriculture uses 66% of total water used; this can be as high as 90% in arid region (Shikomanov, 1991 and Ntombela, 2012) like South Africa and Botswana. However, drought stress or water stress poses a major threat to agriculture production by weakening the plants, making them more vulnerable to disease infections, insect and pest's infestation, thus, resulting in low yield (Belko *et al.*, 3014). Drought stress also poses negative impact on food security and the availability of food to meet the growing population of the world especially Africa. This can result in proverty, unhealthy human liveilhood and malnutrution, and degradation of ecosystem.

Therefore, there is an urgent need to identify and improve a crop like cowpea for drought tolerance in order to respond to this major threat to agriculture production. It will also aid in food security and human existance in Africa.

Botswana is still thus far in cowpea's drought tolerance improvement program, which is posing severe threat to cowpea production and utilization. Moreover, the identification of cowpea genotypes among cowpea's accessions in Botswana, with greater tolerance ability will enable breeders to develop suitable cultivars that will suit and respond to the drought prone region and the increasing climate change (drought) pattern.

The climate change in Africa especially in semiarid region thus serves as a need for the identification of drought tolerant crops and their improvement at all stages especially cowpea cultivation. With more drought stress reseached done at the seedling and reproductive stages of cowepea, the vegetative stage is paramount and it's use in this study; since it' has been noted to be most sensitive to drought stress (Ntombela, 2012) in South Africa.

Importantly, literature on cowpea's agronomy and water stress adaptation in Botswana is limited and this study will help to fill such gap . Additionally , researchers have been focused on established legumes such as dry bean (*Phaseolus vulgaris*) and Bamabara groundnut (Vurayai et la., 2011) over the years, neglecting cowpea and making it underutilized in Botswana.

1.3. Objectives

General objective:

I. The purpose of this research is to evalute the morphological and physiological traits of cowpea genotypes in response to drought stress thus resulting to the identification of drought tolerant cowpea genotypes at the vegetative stage.

II. Specific Objectives

I. To identify drought tolerant cowpea genotypes based on index selection under well-water and drought stress conditions.

II. To assess the effect of drought stress on the growth parameters of cowpea genotypes under green house condition

III. To evaluate the possibility of using physiological traits (chlorophyll and gaseous exchanges) to select cowpea genotypes drought tolerant.

CHAPTER TWO

LITERATURE REVIEW

2.1. Drought and its importance in crop production

In agriculture, the term "drought" refers to a condition in which the amount of water available through rainfall and/or irrigation is insufficient to meet the physiological needs of the plant, thus resulting in low productivity and crop losses accordingly (Tuberosa, 2012). Drought occurs around the world with complete devastating effects on crops production especially in regular limited rainfall areas like semi arid region (Singh *et al.*, 1997). On a global basis, drought is assumed to be soil and/ or atmospheric water deficit. This is accompanied with high temperature and high radiation that poses severe damage to the photosynthetic, respiration and biochemical activities. Shortage of water leads to drought with obvious agricultural and societal impacts. Furthermore, there is widespread agreement that increasing climate change will exacerbate the present shortages of water, and is likely to increase drought (IPCC, 2007). Essentially, drought affects aspect of food security; availability, stability and utilization (FAO 2012). It has been predicted that global warming associated drought will lead to dry areas becoming more drier, thus about 1.5% yields of crop will be reduced per decade (Lobell and Gpirdij, 2012). In Southern Africa, among other extremes there will be a decrease in rainfall variability with the region becoming generally dry (Van Jaarsveld *et al.,* 2005). These will render crop production agro-ecosytems water deficient and unfavourable for plants during periods of growth and development. To this end there is need to manage drought in crop production through; appropriate agronomy (production of best suited crops to the environment) and development of crops that produce sufficient yields in drought-under drought stressed. This can be done through understanding the physiological mechanisms that determine growth and water loss, and plant response to reduced water availability and ultimate resistance to drought (Morrison *et al.*, 2008).

2.2. Drought resistance and its mechanisms

In what is generally described as drought resistance, plants have developed a variety of strategies and mechanisms in response to changes in the environments. Among the several definitions of drought resistance that have been provided during the past decades, the original formulated by Levitt in 1972 retains its validity (Tuberosa, 2012). Accoding to this definition drought resistance is classified into two broad strategies: drought avoidance and drought tolerance. In this respect, morpho-physiological features such deep roots, early flowering, deposition of epicuticular waxes, osmotic adjustment (OA), and others that enable the plant, , to maintain hydration, and are classified under dehydration avoidance.Conversely, plants ability to maintain functionality in a severly dehydrated state is called drought tolerance. These include features such as remobilization of stem water-soluble carbohydrates (WSC), accumulation of molecular protectants. However, in their response to drought plants may engage both avoidance and tolerance strategies (Ntombela, 2012; Tuberosa, 2012).

2.2.1. Mechanism of drought avoidance

2.2.1.1. Root-related mechanisms

During drought avoidance plants exhibit a developmental trait, which enables them to maintain turgor by increasing root depth, efficient root system, to maximize water uptake. This is brought about by reduced shoot growth and increased root development during the time when drought is experienced (Tuberosa, 2012; Farooq et al., 2010; Kumar and Singh, 2003). Accumulated evidence has shown that inhibition of leaf growth and stomatal conductance are the first responses when root systems are exposed to stress conditions such as drought (Ogbonnaya *et al.,* 2003; Craz de carvalho, 2000). In this regard the roots are the drought sensory organs in plants during drought stress. Additionally, reduced shoot growth and increased root development could result in increased water absorption and reduced transpiration, there by maintaining plant tissue water status. In addition root length density and diameter help determine the ability of the plant to efficiently acquire soil water. The possession of a deep and thick root system which allows access to water deep in the soil profile is considered crucially important in determining drought avoidance in many crops species and substantial genetic variation exists for this. The importance of a deep and vigorous root system for drought resistance has been recoginized in rainfed rice (Nguyen *et al.,* 1997) beans (Mohamed *et al.*, 2002), barley (Forster *et al.,* 2005), soybean (Sadok and Sinclair, 2012) and chickpea (Varshney *et al.*, 2014)

2.2.1.2. Shoot-related mechanisms

When drought stress is sensed by plant roots, primary response to water deficit is the inhibition of shoot growth. This response can benefit drought survival by progressively limiting the leaf area available for evaporative loss of limited soil water reserves (Munne-Bosch and Alegre, 2004; Ahmed et al., 2010; Vurayai et al. 2011). The inhibition of leaf growth may then allow diversion of essential solutes from growth requirements to stress-related house-keeping functions, such as osmotic adjustment that improves cell water retention and turgor maintenance (Jaradat *et al.,* 2013). Shoot growth inhibition in response to water deficits may therefore extend the period of soil water availability and plant survival and can be considered as an adaptive response (Neumann, 2008). Under extreme condition plants may avoid drought by accelerated leaf senescence and leaf abscission as a means to decrease canopy size and the evapo-transpirative surface (Nguyen *et al.*, 1997). In perennial plants, this strategy contributes to the survival of the plant and the completion of the plant life cycle under drought stress. Senescence is an important aspect of drought responses. Accelerated leaf senescence followed by leaf abscission is triggered by prolonged stress to reduce water loss, remobilize nutrients to young leaves, fruits or flowers and to enable survival of the plant (Munne-Bosch and Alegre 2004, Jaradat *et al.*, 2013).

Plant stomata, the vital gate between plant and atmosphere may play a central role in plant/vegetation responses to environmental conditions, which have been and are being investigated from molecular and whole plant perspectives, as well as at ecosystem and global levels (Yoo *et al.,* 2010). Leaves growing under conditions of water deficit develop or alter their stomatal development and movement to regulate water loss. These leaves could develop smaller, but more densely distributed stomata, enabling the leaf to reduce transpiration by a quicker onset of stomatal regulation (Akinci and Losel, 2012). Reduction in transpiration and water conservation under drought stress can also be modulated through changes in stomatal morphology, development and movement under which have been found to confer dehydration avoidance in Arabidopsis (Masle *et al.,* 2005; Yoo *et al.,* 2010 and cowpea (Hall *et al.,* 2004). In these studies it has been invariably observed that under drought stress water is conserved through changes in stomatal density and size. Moreover, many studies have shown that water deficit leads to an increase in stomatal density and a decrease in stomatal size indicating this may enhance the adaptation of plant to drought (Hall *et al.* 2004).

In addition, leaves of genotypically adapted plants tend to have more densely cutinized epidermal surfaces, covered with thicker layers of wax. Increased wax deposition on the leaf surface, results in a thicker cuticle that reduces water loss at the epidermis (Hall et al., 2004). The positive correlations of wax deposits and drought resistance have been demonstrated in *Arabidopsis thaliana* (Aharoni *et al.,* 2004) rice (*Oryza sativa*) (Zhou *et al.,* 2012) *Camelina sativa* (Lee *et al.,* 2014). Leaf surfaces have been known to have trichomes function to protect plants against drought by reducing absorption of solar radiation, which in turn reduces heat load and the need for transpirational cooling. Studies involving natural population has demonstrated that trichome production conferred differential drought avoidance in *Encelia* species

(Ehleringer and Bjôrkman, 1978), *Piriqueta caroliniana* (Picotte *et al.,* 2007), *Arabidopsis lyrata* (Sletvold and Agren, 2012).

Drought avoidance can also involve rapid phenological development, here referred to as early vigor. Early vigor is the ability of annual plants to rapidly accumulate biomass and leaf area until canopy closure. It results from resource acquisition and conversion, organ and morphogenetic dynamics, plant and canopy architecture, which favors a rapid colonization of space and resources and contribution to improved yield stability in drought prone environments (Asch *et al.,* 1999; Dingkuhn *et al.,* 1999). Early vigor under conditions of low evapotranspiration may allow annual crops to optimize Water Use Efficiency (WUE) and limit the loss of water due to direct evaporation from the soil surface. This leaves more stored water available for later developmental stages when soil moisture becomes progressively exhausted and increasingly limiting for yield. By contributing to early canopy closure, it also reduces unproductive, non-transpirational water use and thus increases overall crop water use efficiency (WUE) (Condon *et al.,* 2004) . Early vigor has the characteristics of early maturity, early flowering, early leaves initiation, larger leaf area, and deeper root system and make good use of resources captured (Guar *et al.,* 2008). In related studies, early flowering was shown to be associated with high initial growth vigor in chickpea (Sabaghpour *et al.,* 2003) and cowpea (Maroufi *et al.,* 2011). Interestingly, early vigor was used as a selective criterion for drought adaptation in Common bean (Acosta-Daize, 1998). Conclusively, this trait is an essential trait for enabling high yield in short-duration variety and short duration directly translates into lower water consumption that makes plant to escape or avoid drought regimes.

2.2.2. Essential biochemical drought tolerance mechanisms

2.2.2.1. Accumulation of compatible solute, osmoprotection and osmotic adjustment

Drought tolerance is understood to be the ability of plant has to live, grow, and yield satisfactorily with limited soil water supply or under periodic water deficiencies timely, with duration and the intensity of the drought (Reyazul *et al.,* 2012). Plants growing under drought regimes are usually subjected to water deficits due to osmotic stress. This osmotic stress occurs when there is an imbalance in the plant water balance due to water scarcity in the environment. As drought prolong, soil dries, water potential becomes more inadequate to meet the plants demand (Reyazul *et al.,* 2012). Accumulation of compatible solute (osmolytes) by plant tissues reduces water potential during drought regime in order to making it possible for plants' to maintain turgor to lower water potentials, which facilitate extracting water from dry soils and maintaining cell turgor, gas exchange and growth in dry soil environments (Cheulile and Agenbag, 2004). This process is referred to as osmotic adjustment (OA) (Bohnert and Jenson, 1996). Metabolites which act as compatible solutes are different among various species of plants and include amino acids and their derivatives, water soluble carbohydrates (WSC), sugar alcohols, and quaternary ammonium compounds (Bohnert and Jensen, 1996). The contribution of compatible solutes in drought tolerance through osmotic adjustment (OA), helps to maintain cell turgor for cell enlargement and plant growth during water stress; and it can allow stomata to remain at least partially open and CO_2 assimilation to continue at water potentials that would be otherwise inhibitory (Impa *et al.,* 2012).

In addition to their function in OA, some of these compounds can protect enzymes and membranes against deleterious effects of destabilizing ions during water deficit. For example the amino acid, proline, is a compatible solute, which is involved in osmotic adjustment (OA) as well as protection of cell components during dehydration (Zhang *et al.,* 2009; Ghen and Jiang, 2010). Accumulation of proline during drought stress has been found to confer tolerance in tall fescue (*Festuca arundinacea)* (Clifford *et al.,* 1998), *Ziziphus mauritiana*; creeping bentgrass (*Agrostis stolonifera*) (DA *et al.,* 2011) *Pyracantha fortuneana* and *Rosa cymosa* (Liu *et al.,* 2011) and cowpea (Costa *et al.,* 2011; Farouk *et al.,* 2013).

Water soluble carbohydrates (glucose, fructose, sucrose, fructans) have been found to participate in OA by adjusting osmotic potential (OP), which leads to water flux into the cell thereby maintaining higher relative water content. Sugars act as OA compounds in protecting (osmoprotectant) plants against drought and they contribute to the stabilization of cell membrane structures. A strong correlation between sugar accumulation and osmotic stress tolerance has been reported (Streeter *et al.,* 2001; El-Tajeb, 2006). For example, sucrose accumulation was found to confer drought tolerance in wheat (Kameli and Losel, 1993) cocksfoot (*Dactylisis glomerata*) (Volaire and Thomas, 1995) and cowpea (Souza *et al.,* 2003).

A number of ‘‘sugar alcohols’’ or polyols (mannitol, trehalose, myo-inositol, ononitol, pinitol, sorbitol) have been shown to be drought induced and recognized as compatible solutes (Sheveleva *et al.,* 1997; Garg et al., 2002. Abebe *et al.,* 2003). According to Streeter, (2001) the sugar alcohol pinitol provide evidence that it accumulates in drought stressed soybean than either proline or sucrose indicated it was osmoprotectant (compatible solute that contributes to the stabilization of cell membrane druing drought regime) in crop. Suggestion of a genetic tendency for pinitol accumulation in plants adapted to dry climates is supported by the finding of much higher pinitol accumulation in a population of maritime pine (*Pinus pinaster*) adapted to a dry area than in a population adapted to a region with greater annual rainfall (Nguyen and Lamant 1988). In legumes, pinitol is a common sugar alcohol and it has been suggested as a common osmoprotectant (Silvente *et al.,* 2012). In drought stress experiments, drought tolerant soybean varieties were found to accumulate more pinitol that the sensitive genotype (Guo and Oosterhuis, 1997; Silvente *et al.* 2012). Under increasing drought stress intensity accumulation of pinitol increased in soy bean (Guo and Oosterhuis, 1997) white clover (*Trifolium repens*)

(Mcmanus *et al.,* 2000) alfalfa (*madicago sativa*) (Aranjuelo *et al.* 2010), compared to sucrose and other sugars, which indicated that it could be the preferred osmoticum in these species. Under water stress conditions, pinitol accumulated more in genotype that showed promising water stress tolerant than susceptible genotype in pegion pea (Keller and Ludlow, 1993) and in cowpea (Souza *et al.*, 2003).

2.2.2.2. Synthesis of protein chaperones and membrane channel proteins

The late embryonic abundant (LEA) proteins, which were first characterized in cotton, are a set of proteins that accumulate in embryos at the late stage of seed development (Xu *et al.*, 2014). Additionally, LEA proteins are thought to play an important role in seed maturation process. To this end, Veeranagamallaiah *et al.*, (2011) have suggested that LEA proteins could act as a special form of molecular chaperones that would prevent the aggregation and abrogation of other proteins induced by water stress. In addition to protein protection, their water soluble and hydrophilic properties allow them protect biological membranes desiccation damage or oxidative damage in leaves that happens during drought stress. Recently seven groups of LEA proteins were identified based on sequence similarity (Bhattarai and Fettig, 2005). The groups are group 1(D-19), Group 2(D-11), group 3(D7/D-29), group 4(D-113), Group 5(typical LEA proteins), group 6 (PVLEA18), group 7(ASR1) (Veeranagamallaiah *et al.*, 2011). Of these, group 2(D-11) commonly known as "dehydrins" are the most characterized, into seven groups (Bhattarai and Fettig, 2005). Several studies have confirmed that they accumulate during seed desiccation, and dehydration stress such as induced by drought, low temperature, or salinity (Alscher *et al.*, 2002). Transgenic plants expressing dehydrin proteins showed enhanced tolerance of water deficits, and these were in wheat (Cheng *et al.*, 2002) and rice (Babu *et al.*, 2004). The source of drought tolerance was associated with protection of cell membranes from injury under drought stress. Related studies have also indicated that accumulation of dehydrins under natural conditions confer drought tolerance in bermuda grass (*Cynodon spp*) (Hura *et al.*,

2009) barley (*Hordeum vulgare*), *Populus popularis*, durum wheat (*Triticum turgidum*) (Hanin *et al.,* 2011) cowpea (Hall *et al.,* 2002) and chickpea (Bahattarai and Fettig, 2005).

Another class of proteins involved in drought responses and tolerance are the water channel proteins called acquaporins (AQPs) in the membranes of plant cells.Biological activities related to drought and dehydration include stomatal movement, water and CO_2 transport. Based on amino acid sequence comparison, plant AQPs have been divided into four subfamilies: the tonoplast intrinsic proteins (TIPs), the plasma membrane intrinsic proteins (PIPs), the nodulin-like plasma membrane intrinsic proteins (NIPs) and the small intrinsic proteins (SIPs) (Maurel *et al.,* 2008; Johnson et al., 2000). Expression of aquaporins in plants have been found to be correlated with drought stress tolerance in tobacco *(Nicotiana tabacum*) (Mahdieh *et al.,* 2008) and cowpea (Simoe-Aranjo *et al.,* 2008). Other studies applying transgenic approaches have also indicated these proteins are involved in drought tolerance(Zhou *et al.,* 2012; Xu *et al.,* 2014).

2.2.2.3. The antioxidant systems and drought tolerance

During drought stress in chloroplasts, limitation of CO_2 fixation, overreduction of the electron transport chain electrons have a high-energy state are transferred to molecular oxygen (O_2) to form reactive oxygen species (ROS). Excess generation and accumulation of (ROS) (superoxide anion (O_2-), singlet oxygen ($^1O_2$), ozone (O_3), hydroxyl radical ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2)), cause oxidative damages to cell compoents, proteins and nucleic acids (Baier *et al.,* 2005). In addition to the chloroplast other sources may be the of these species are the apoplast, peroxisomes and mitochondira (Miller *et al.,* 2010). However, under optimal growth conditions, ROS are mainly produced at a low levels in these organelles, in which they play a key role in plants as signal transduction molecules involved in mediating responses to pathogen infection, environmental stresses, programmed cell death.

Plant cells and their components are protected against the detrimental effects of reactive oxygen species (ROS) by an antioxidant system that has been associated with stress tolerance in plants. The antioxidants include metabolites such as vitamin C (ascorbate), vitamin E (α-tocopherol), carotenoids, glutathione (GSH), and ROS detoxifying enzymes (superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) and glutathione reductase (GR) (Miller *et al.,* 2010).

Several studies involving different plants including soybean leaves, rosemary (*Rosmarinus officinalis*) and Mediterranean shrub (*Cistus creticus*) showed that drought stress resulted in an increase in α-tocopherol levels (Munné-Bosch and Alegre, 2004; Shao *et al.,* 2008; Munné-Bosch *et al.,* 2009). Additionally, over-expressing *Arabidopsis* tocopherol cyclase (VTE1), an enzyme required for vitamin E synthesis, in tobacco enhanced both vitamin E level and tolerance to drought stress (Ngugen *et al.,* 2004).

In addition to the above established antioxidant system in plants, recent studies have indicated that metabolites classified as phenolic compounds (phenolic acids and flavonoids) have indicated that they are induced by drought stress. Among the various compounds present in plant tissues, phenolic compounds have antioxidative properties, the extent of which depends on the number and distribution of the hydroxyl groups (-OH), which they readily release during antioxidative action (Weidner *et al.,* 2009). The compunds were found to accumulate under drought stress in grape vine (*Vitis vinifera*) (Weidner *et al.,* 2009), *Achellia tenuifolia* (Gharibi *et al.* 2012) and soybean (Mohammed and Akladious, 2014). Drought tolerance have been associated with phenolic compounds in wheat (Hura *et al*., 2009), alfalfa (Kang *et al.,* 2011 (eg. flavonols, (iso) flavones, flavanones, flavan-3-ols proanthocyanidins, and anthocyanin). In cowpea genotypes water deficit selection studied, anthocyanin was assoicated with recovery from drought stressed condition (Muchero *et al.,* 2008). According to Nair *et al.,* (2008), drought tolerant cowpea variety showed significant increase in the activities of peroxidase and catalase on exposure to drought stressed treatment or conditioned. These enzymes form part of the enzymatic antioxidant system in plants.

CHAPTER THREE

MATERIALS AND METHODS

3.0. Experimental site

The Research was conducted at the Botswana College of Agriculture (BCA) Content Farm, Gaborone. Botswana College of Agriculture is located at Sebele Content farm (latitude 24^o 34^o'S and latitude 25^o 57' at altitude of 994 m above sea level). Two major experiments were conducted in the green house during the vegetative phase of cowpea genotypes, along with two preliminary experiments meant to determine suitable soil mix and stress treatment duration:

3.1. Establishment of the dry-down curve and plant performance in polythene bags.

3.1.1 Experimental set-up

The experiment was conducted from 2 to 28 , 2014 to establish a soil (sand, loamy top soil: compost) mixture; which was suitable for a smooth dry down curve that showed optimum plant growth throughout the experiment. The polythene bags were filled with the various soil mixes up to a depth of 11.5 cm and 10 seeds of cowpea (black-eye genotype) were planted and thinned to eight plants per polythene bag after one week. The eight plants were further grown to one (1) fully expanded trifoliate leaf after irrigation was withdrawn and followed by drought treatment. For this preliminary experiment, various volumetric soils were mixed as shown in the table below:

Table 1: Percent composition of soil mixtures

	% composition		
Soil Mix	River sand	Sand loamy	Compost
A	40	40	20
B	50	40	10
C	60	30	10
D	33	33	33

E	70	20	10

From A to E represents treatments

3.1.2 Experimental Design

The experiment layout was in a Complete Randomized Design, four replications for each treatment in the green house. The plant was under drought treatment period for 12 days. A cowpea genotype (Blackeye) was used as a proxy to determine the suitable soil mixture for the entire experiment due to time and resources limitation. During the 12 days water stress treatment, the following variables were measured:

3.1.3 Soil moisture content measurement

Volumetric soil moisture content was monitored with the MP 406 kit (ICT International, Armidale, New South Wales, Australia). Soil moisture content was measured 5.5 cm depth of the soil in polythene bag at 10 AM for 12 days. Data was used to plot volumetric soil content as a function of time in days to establish the dry down curve per treatment.

3.1.4 Chlorophyll content

During the dry down period, chlorophyll content was monitored with the hand held SPAD 502 Plus spectrophotometer (Spectrum Technologies INC, Aurora, IL) on the fully expanded terminal leaflet. The chlorophyll content was also monitored on a daily basis immediately after soil moisture content has been measured to establish chlorophyll loss as a function of time.

3.1.5. Plant height

The plant height was measured using a 30 cm ruler at the end of drought stress treatment on the plant. The measurement was taken on the last day of drought stress termination.

3.1.6. Plant Biomass Yield

The plant biomass yield was determined by harvesting the plant on the last day of drought stress period, and the plant was oven dried at 105.8 ^{o}C for 24 hours. The biomass was measured (weight) in grams (g).

3.1.7. Data Analyses

Means for the three replications were subjected to regression analysis in excel 2007 and the general linear models (Pro GLM) procedure of the Statistical Analysis System (SAS) program package analyses. Multiple comparisons among means were done using least significant difference (LSD) at $P = 0.05$. The dry down curve was determined by using a logarithmic decay function according to the equation:

$$Y = a\mathrm{Ln}(X) + C$$

Where;

Y = Soil moisture content

a = Slope of the curve

X = Number days after irrigation withdrawal

C = Y intercept (soil moisture content at or above field capacity)

Chlorophyll loss as a function of time of irrigation withdrawal was determined using a linear function equation:

$$\boldsymbol{Y = aX + C}$$

Where;

a = Slope of the curve

X = Number days after irrigation withdrawal

C = Y intercept (chlorophyll content when soil moisture is at or above field capacity)

Components of regression analysis equations of soil moisture and chlorophyll loss was ranked to determine the best soil which gives a suitable dry-down curve that supports optimum plant growth.

3.2. Determination of the lethal drought-50 (LD_{50}).

The aim of this preliminary experiment was to establish the extent to which plant needs to be exposed to drought stress to reduce plant biomass yield under drought stress treatment by half (50%).

3.2.1. Experimental Design/ Set-up

Complete Randomized Block Design was used in this green house experiment. The treatment was replicated four times. The trial was conducted from November to December 2014. Thinning was done a week after planting. Drought stress was applied for 6 days, 8 days and 11 days. Plants were grown according to the same polyethene protocol in 3.1 trial above, with the following treatments:

Drought Level 0 (maintained irrigation for the experimental period) (DL-0)

Drought Level 1 (irrigation withdrawal to 50% below field capacity for 6 days) (DL-1)

Drought Level 2 (irrigation withdrawal to 50% below field capacity for 8 days) (DL-2)

Drought Level 3 (irrigation withdrawal to 50% below field capacity for 11 days) (DL-3

3.2.2. Variables Measured

During the dry-down period, soil moisture content and chlorophyll content were monitored according to the same protocol in 3.1 above. At the end of experiment, the plants were harvested to determine biomass yield for well watered controls (BYp) and drought stressed treatment (BYs) in order to establish the lethal drought 50 (LD_{50}). The LD_{50} is defined as a stress level that will reduce biomass yield by 50%, and was determined according to the formula below:

$$LD_{50} = [BYp – Bys]/ [BYp)] \times 100.$$

Where;
BYp = Biomass yield under well watered conditions
BYs = Biomass yield under drought stressed conditions

3.2.3. Plant height

The plant height was measured using 30 cm rulers at the end of each irrigation withdrawal days for control and treatment.

3.2.4. Plant Biomass yield

Biomass yield was determined by harvesting the plant at the end of each irrigation withdrawal period and it was oven dried for 24 hours at 105.8 ^{o}C. The measurement (weight) was in grams (g).

3.2.5. Data analysis

The general linear models (Pro GLM) procedure of the Statistical Analysis System (SAS) program package analyses was used. Multiple comparisons among means were done using least significant difference (LSD) at $P = 0.05$.

3.3. Determination of drought tolerance in different genotypes of cowpea

3.3.1 Experimental set-up

Twenty (20) genotypes of cowpea seed was obtained from Farmers and Traders, Seed Multiplication Unit and National Plant Genetic Resources Centre (NPGRC) and The International Institute of Tropical Agriculture (IITA) in Botswana.

Table 2: Description of the twenty cowpea genotypes used in this study.

ID No	Genotypes	Source
BCA001	Blackeye	BCA See Bank
BcA002	Speckled Grey	Hukuntsi
BCA003	Makoro	Makoro
BCA004	Speckled brown	Tshane
BCA005	B 212	NPGRC-DAR
BCA006	B069 E	NPGRC-DAR
BCA007	B079-C	NPGRC-DAR
BCA008	B020-A	NPGRC-DAR
BCA009	Tswana brown	Hukuntsi
BCA010	B 505A	NPGRC-DAR
BCA011	B 500	NPGRC-DAR
BCA012	B111-B	NPGRC-DAR
BCA013	Tswana Red	Hukuntsi
BCA014	E 129	NPGRC-DAR
BCA015	E 129 (2)	NPGRC-DAR
BCA016	Speckled brown	Lecheng
BCA017	Tswana cream	Hukuntsi
BCA018	Bo11-A 7	NPGRC-DAR
BCA019	Speckled grey	Lecheng
BCA020	E7	NPGRC-DAR

3.3.2. Experimental Design

Complete Randomized block Design was used for the experiment in the green house from November, 2014 to January, 2015.

The 20 genotypes were planted in wooded boxes (block) with 5 cm in row and 10 cm between rows, each row carried 8 plants per genotype. The screening boxes had a depth of 12 cm width of 85cm and length of 117cm. Drought stress was applied after the first trifoliate leave had finally well expanded. The treatment that caused LD50 established in 3.2 above was applied according to the experimental procedures and protocols.

3.3.3. Variables Measured

At the end of the experiment, BYp and BYs were measured and used to calculate the following: Biomass stress susceptibility index (BSSI), Relative drought index (RDI), Stress tolerance index (STI), Tolerance (TOL), Mean production (MP), Drought resistance index (DI) and Biomass yield reduction percentage (%BYR) (Naghavi *et al.*, 2013), according to the formula in the table below. The indices were used to identify the highly drought tolerant genotype (HDT), drought tolerant genotype (DT), moderate drought tolerance (MDT) and drought sensitive genotype (ST) by means of the three dimensional plot (Naghavi *et al.* (2013).

Table 3: Drought stress tolerance indices and stress susceptibility index.

No.	Index	Calculation
1	Biomass Stress Susceptibility Index (BSSI)	$\frac{1-(BYs/BYp)}{1-(B\bar{Y}s/B\bar{Y}p}$
2	Biomass Relative Drought Index (BRDI)	$\frac{(BYs/BYp)}{(B\bar{Y}s/B\bar{Y}p)}$
3	Biomass Stress Tolerance Index (BSTI)	$\frac{(BYs \times BYp)}{(B\bar{Y}s^2)}$
4	Tolerance (TOL)	BYp – BYs
5	Biomass Mean Production (BMP)	(BYs+BYp)/2
6	Biomass Drought Resistance Index (BDRI)	(BYs×(Bys/BYp) $B\bar{Y}s$
7	Biomass Yield Reduction percent (BYR%)	*[BYp – Bys]/ [BYp)] x 100*

BYp = biomass yield under well watered conditions, BYs = biomass yield under drought stress Conditions, $B\bar{Y}p$ = biomas yield mean under well watered conditions, $B\bar{Y}s$ = biomass yield mean under drought stress conditions .

3.3.4. Plant height

The plant height was measured using 30 cm rulers at the end of each irrigation withdrawal days for control and treatment.

3.3.5. Plant Biomass yield

Biomass yield was determined by harvesting the plant at the end of each irrigation withdrawal period and it was oven dried for 24 hour at 105.8 ^{o}C. The measurement (weight) was in grams (g).

3.3.6. Data analysis

The significant index (BMP) was subjected to IBM SPSS statistics 21 analysis in order to construct a dimensional plot – which categorised drougt tolerant.

3.4. Morpho-physiological determination of drought tolerance using drought tolerant and sensitive genotypes.

3.4.1. Experimental set-up.

In this experiment, the eight (8) cowpea genotypes of interest were selected from the 20 genotypes result: 2 highly drought tolerant (HDT), 3 drought tolerant (DT), 2 moderately tolerant and 1 drought sensitive (DT) identified in 3.3. This experiement was meant for morpho-physiological screening in the green house. The same ploythene bag size used in 3.2 was used and filled with soil Mixed C. The experiement was conducted in the green house from January to March, 2015.

3.4.2. Experimental Design

The experimental layout was in a Complete Randomized Block Design, replicated four times, with two treatments (well-water and drought stress). The layout was as follows:

1-(Well watered pure planted drought tolerant) (WP-DT)

2-(Drought stressed pure planted drought tolerant) (DP-DT)

3-(Well watered pure planted moderate drought tolerant) (WP-MT)

4-(Drought stressed pure planted drought tolerant) (DP-MT)

5-(Well watered pure planted drought sensitive) (WP-ST)

6-(Drought stressed pure planted drought sensitive) (DP-ST)

7. Well-watered pure planted highly drought tolerant (WP-HDT)

8. Drought Stressed pure planted highly drought tolerant (WP-HDT).

3.4.3. Determination of plant response to drought stress (variable measured)

Plants were grown to the eight leaf fully expanded five triafoliate stages and was exposed to drought stress treatment for 8 days. At the end of the drought treatment period, the following variables were determined:

3.4.3.1. Soil moisture status

This was determined with the MpKit portable soil moisture sensor kit (ICT International, Armidale, New South Wales, Australia) following manufacturers protocols.

3.4.3.2. Plant water status

The terminal leaf from one of the most expanded and exposed leaf was excised, its fresh weight (FW) was measured, and immersed in distilled water for 24 hours after which its turgid weight (TW) was measured. The samples were then oven dried at 82 ^{O}C for 24 hours and weight also measured (DW). Relative water content was calculated according to the formula; and its RWC% determined according to formula;

$$RWC\ (\%) = [(FW-DW) / TW- DW)] \times 100.$$

Where;
FW = Fresh weigh
TW = Turgid weight
DW = Dry weight

3.4.3.3. Leaf gaseous exchange measurement.

The full leaflet of each cowpea genotypes was used for non-destructive gaseous exchange measurements with a portable LiCOR 6400 XT photosynthesis system (LICOR, Lincoln, NE), according to manufacturers protocols. Data output from gaseous exchange measurements were photosynthesis, transpiration, and stomatal conductance.

3.4.4. Analyses of differential plant dry matter response to drought stress.

After gaseous exchange measurement, whole plants were harvested (leaf separated from stem) and oven dried at 82 ^{O}C for 48 hours, which root, shoot and root:shoot ratio were calculated.

3.5 Statistical analyses.

Overall data collected for the two perliminary experiments and the last experiment (3.4) were subjected to the general linear models (Pro GLM) procedure of the Statistical Analysis System (SAS) program package analyses. Multiple comparisons among means were done using least significant difference (LSD) at P = 0.05.

CHAPTER FOUR

RESULTS

4.1. Determination of soil dry-down curve and plant mor-physiological responses

4.1.1. Selection of appropriate growth media

In order to determine an appropriate plant growth media for the experiments, sandy soil (from Metsimotlhabe river), loam soil (from Botswana College of Agriculture Gardens) and agricultural compost (from Botswana College of Agriculture Compost Sheds) were mixed on volume basis as indicated in Table 1. A cowpea variety, blackeye was planted in these soil mixtures, afterwhich half of the plants were exposed to drought by withdrawing irrigation for (12) twelve days. During this period, soil moisture and chlorophyll content losses were monitored on a daily basis. The results presented on Figure 1 and Table 4 show that soil mix C had the highest initial soil moisture content of 16.3 % (pre-dry down) and the lowest soil moisture content of 0.2% (post-dry down) compared to other mixes (A, B, D, E). The rate of chlorophyll content loss was also high as shown by the gradient of the the curve and the lowest number of days it was predicted to be at its lowest. At the end of twelve (12) days of experiment, plant perfomance was measured in the five soils. The results showed that plants grown in the soil mixed C media had the highest plant height and biomass yield, which indicated good perfomance compared with other soil mixes (Figure 2). Therefore, the soil mixture C (60% river sand, 30% loam soil, 10% compost) was chosen appropriate for subsequent experiments in this research project.

4.2. Determination of lethal drought-50 (LD_{50}).

The experiment was conducted to determine the number of days of drought stress for plant biomass to be reduced by 50%, herein refered to as lethal drought 50 (LD_{50}). To determine this, the genotype (blackeye) that was used as a proxy of the twenty (20) genotypes grown in the identified soil mixed in section 4.1 above. The results presented in Figure 3 and Table 5 shows the effect of drought stress duration was significant as early as eight (8) days when biomass was reduced by approximately 50% (Figure 3a). The reducdtion in biomass yield was also followed by reduction in plant height, which was also approximately 50% (Figure 3b). In summary, the withdrawal of irrigation for 8 days established the LD_{50} and was used in subsequent experiments.

4.3. Agro-morphological and physiological responses of genotypes in screening boxes.

In this experiment, twenty (20) genotypes (Table 2) were screened for drought tolerance according to protocols developed in 4.1 and 4.2 above. Plant were grown for two weeks after which irriggration was withdrawal for eight days. Soil moisture content was monitored each other day and the results are shown on Figure 4, the effect of irrigation withdrawal on soil moisture loss. At the end of the eight days, plant performance was evaluted by plant height, chlorophyll content and leaf area from which their percentage reduction due to drought were calculated. The results presented in this section, shows that drought stress affected plant performance in all the twenty genotypes in terms of plant height (Figure 5); and leaf area (Figure 6) chlorophyll content (Figure 7). Variations in these parameters were; 48.67- 59.20 % (for plant height), 11.3 – 51.4% (for leaf area), 1. 42 – 25.48% (for chlorophyll content). The result further showed that there were differences between the twenty (20) genotypes for the above parameters. However, the result of plant perfomance could not be used at this level to cleary identify differences in drought tolerance. For this reason plant biomass under well watered and drought stress conditions was used to identify a suitable index for idenfication of drought tolerant genotypes.

4.3.1. Determination of the most suitable index and its application in identifying drought tolerance.

The study was undertaken to determine the most suitable index (Table 3), which can be used to identify the most drought tolerant genotypes among the twenty that were screened. Biomass yield under both well watered (Byp) and drought stress (Bys) was measured (Table 6). Correlations analysis of these was done with each other and TOL, BMP, BSTI, BRDI, BSSI, DI, and %BYR determined. The highest positive correlation was observed between Byp and BMP (0.98) and TOL (0.97), while for Bys this correlation was between BMP (0.66) (Table 5). While TOL was positively correlated with Byp (0.97), its correlation with Bys (0.28) was not significant . A suitable index should have a significant correlation with biomass yield under both well watered condition (ByP) drought stress conditions (Bys). For the above reason, BMP was selected for further analysis of drought tolerance/resistance.The results further show that there is genetic diversity between the genotypes for Byp, Bys and BMP, which ranges from 1.891-8.098g (for Byp), 1.030-2.725g (for Bys) and 2.057 to 5.194g (for BMP) (Table 7).

Biomass mean productivity (BMP) for each genotype (Figure 8) was further used to generate a three dimesional plot show interelationships among BMP, Byp and Bys. The interelationship is presented as a cluster into the highest biomass yielding genotypes under both well watered and stress conditions (*highly drought tolerant/resistant*: BCA001 and BCA003), high biomas yielding genotypes under both well watered and stress conditions (*tolerant/resistant*: BCA002, BCA009, BCA016, BCA019, BCA018 and BCA006) and (moderate: BCA017, BCA004 and BCA015); high yielding only under well conditions (*drought sensitive* : BCA011, BCA010, BCA013, BCA007, BCA012 and BCA020) and low yielding under both well watered and drought stress conditions (*highly drought sensitive*: BCA008 and BCA005) (Figure 9).

4.4. Agro-morphological and physiological responses to drought stress

Above ground biomass yield under well watered conditions (Byp) and drought stress (Bys) was determined and the mean biomass yield (BMP) was also calculated (Table 8). The results were used for cluster analyis to determine drought tolerance. Cluster analysis showed that the genotypes: BCA001, BCA003 were highly drought tolerant (HDT), BCA002, BCA019, BCA006 were drought tolerant (DT), BCA004, BCA017 were moderately drought tolerant (DM), while BCA011 was drought sensitive (DS) (Figure 9 and 10). The results confirms the finding in section 4.3.1 and as the objective of this part of study was to determine the underlying physiological mechanisms responsible for drought resistance between the eight (8) genotypes.

The imposition of drought stress for tweleve (12) days caused reduction of soil moisture in drougtht stressed treatments, which ranged from 0.30-2.5%. There were significant differences between the genotypes, whereby BCA001 had the highest soil moisture and BCA002 had the lowest, while other genotypes also indcated significant soil moisture content (Figure 11). Reduction in soil moisture content resulted in RWC% reduction. However, there were no significant differences between the eight genotypes (Table 10). Under drought stress conditions, BCA002 and BCA019 had the highest chlorophyll content, while BCA001, BCA003, BCA006, BCA017, BCA007, BCA004 and BCA011 did not show significant differences in chlorophyll content (Table 11). An analysis for percent reduction in chlorophyll content was performed and it was found that BCA001, BCA002, BCA019 had increased chlorophyll content as a results of drought, while BCA003, BCA006, BCA017, BCA004 and BCA011 had reduced chlorophyll content, with BCA004 and BCA011 showing the highest reductions (Figure 12). Percent reduction in chlorophyll content results show that there are differences between the genotypyes and than those that had less reductions were more drought tolerant and also was significant ($P<0.05$) with biomass mean productivity (Table 13).

The biomass characteristics (shoot and root dry weight and shoot:shoot ratio) were also analysized. The result showed that there were significant differences between the eight genotypes under both well-watered and drought stressed conditions for shoot and root dry weight, which indicates genotypic differences in shoot and root characteristics. The shoot and root dry weight data were further used to determine the root:shoot ratio, which is relevant characteristic for drought stress phenotyping in crop plants. It was found that the genotype BCA003 had significantly high value for this characteristic, while the other genotypes had similar root:shoot ratios (Table 9). These results indicate that in general the root to shoot ratios similar for the genotypes understudy.

Gaseous exchange (Photosynthesis, stomatal conductance and transpiration) were also determined. There was significant difference ($P<0.05$) in photosynthesis, stomata conductance and transpiration between some of the genotypes under both well watered and drought stress conditions for all the gas exchange parameters (Table 12). In order to find whether gas exchange measurement could be used to confirm BMP cluster analysis that had separated the genotypes into HDT, DT, DM and DS, regression analyses were performed to determine their relationships with BMP. The relationship between BMP and photosynthesis and its percent reduction was very weak or poor under well watered and drought stressed conditions (Figure 13). Similar results were obtained for stomatal conductance (Figure 14) and transpiration (Figure 15). These results indicate that gas exchange measurement may not be used as a screening selection mechanism for drought resistance in cowpea.

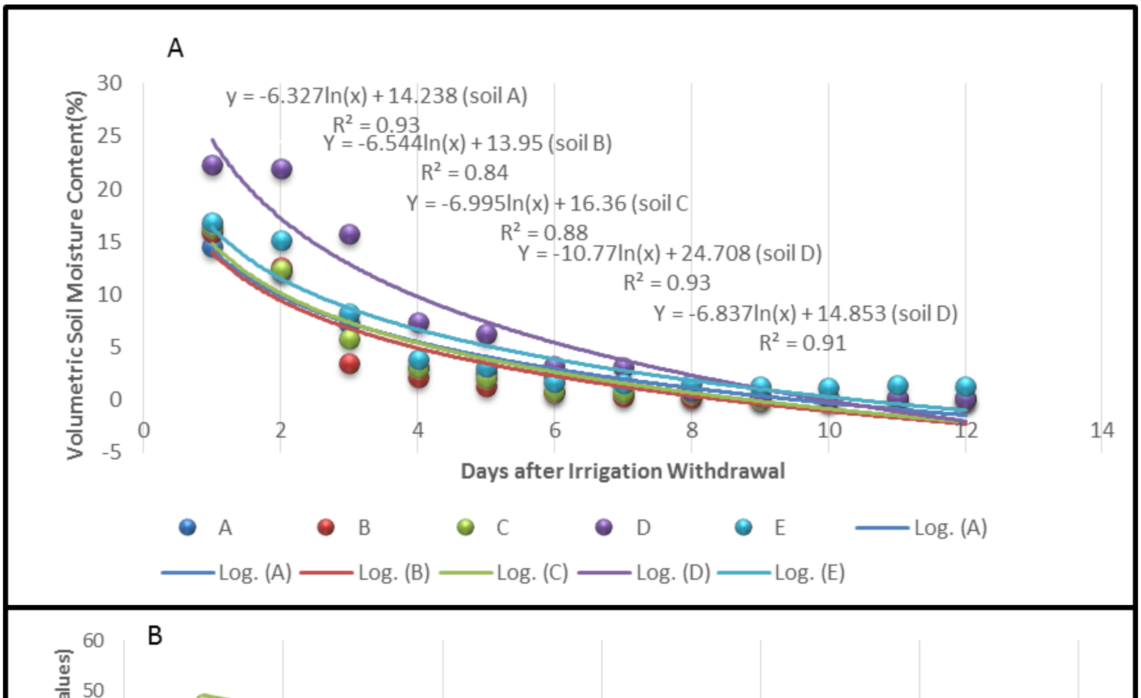
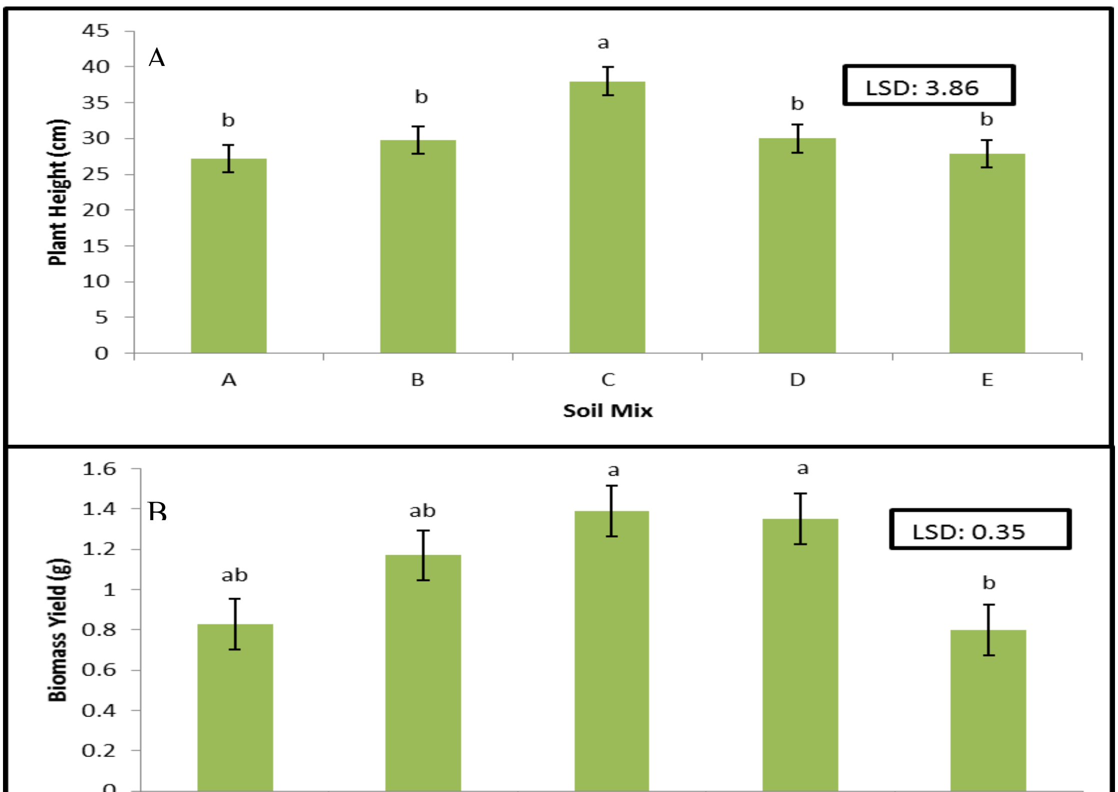


Table 4: Means ranking of the soil moisture % and chlorophyll loss in SPAD meter reading.

	Soil Moisture Content Loss			**Chlorophyll Content**			
	(Y =aLnX + C)			**(Y = aX + C)**			
Soil Mix	Y intercept (C)	Slope (a)	R^2	Y intercept (C)	Slope (a)	R^2	Mean Rank
A	4	1	2	5	5	5	**4**
B	5	2	4	2	2	4	**2**
C	2	4	3	1	1	1	**1**
D	1	5	5	3	3	3	**2**
E	3	3	1	4	2	2	**2**

Soil mixture C is ranked 1 which is the selected soil to be used for subsequent exeperiments**.**



A
LSD: 3.86
Plant Height (cm)
45
40
35
30
25
20
15
10
5
0
b
b
a
b
b
A
B
C
D
E
Soil Mix
B
LSD: 0.35
Biomass Yield (g)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ab
ab
a
a
b

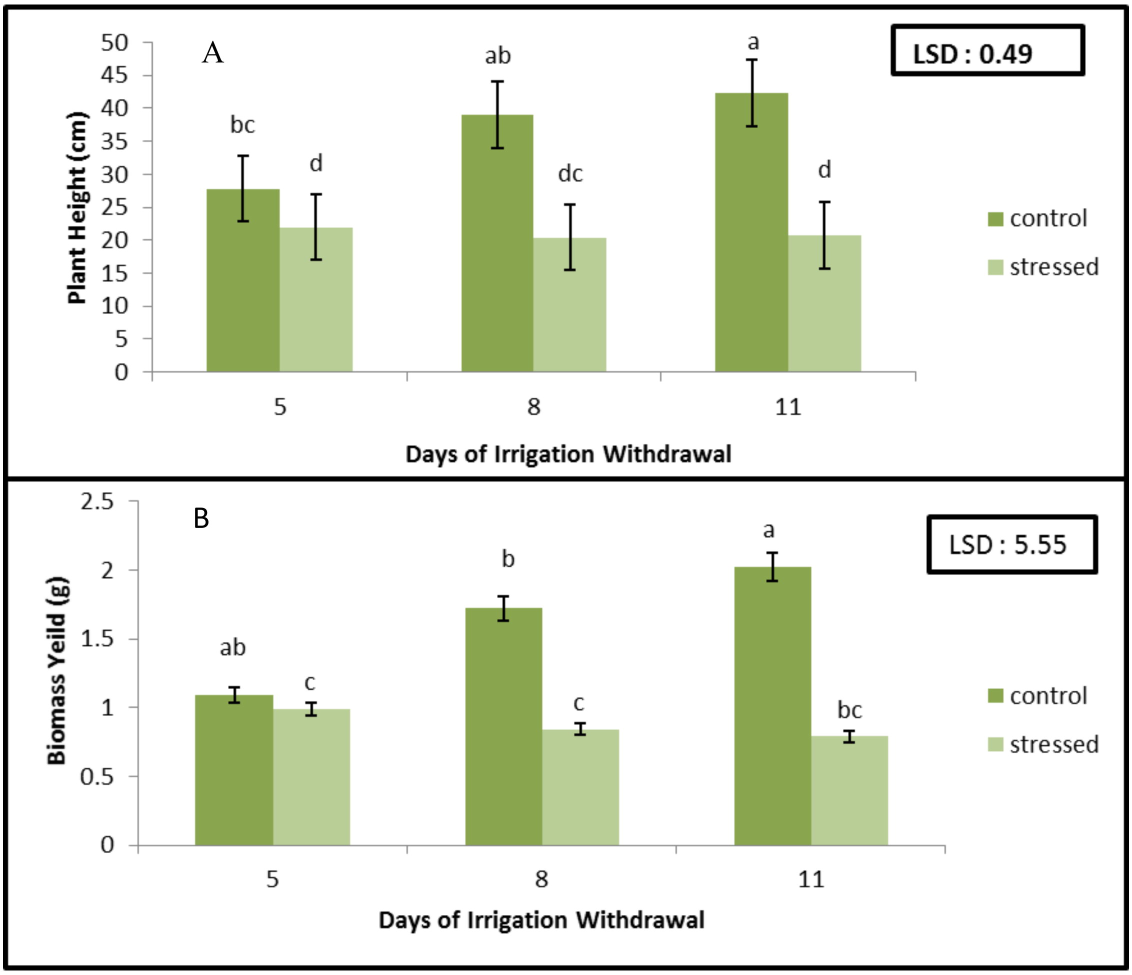


Figure 3**:** Effect of drought stress (water withdrawal days) on cowpea genotype biomass yield and plant height under green house condition. Mean of 4 replications. The error bars represent standard error of the mean. Means with the same letter are not significantly different (P < 0.05).

Table 5 : Lethal drought (LD_{50}) determination on biomass yield. Effect of drought stress treatment on cowpea genotype (blackeye) for days; in establishing the biomass yield reduction percentages for LD_{50} under green house condition.

	Cowpea Genotype	
Treatment	Irrigation withdrawal Days	Biomass Reduction %
A	0	0
B	6	9.8
C	8	51
D	11	61

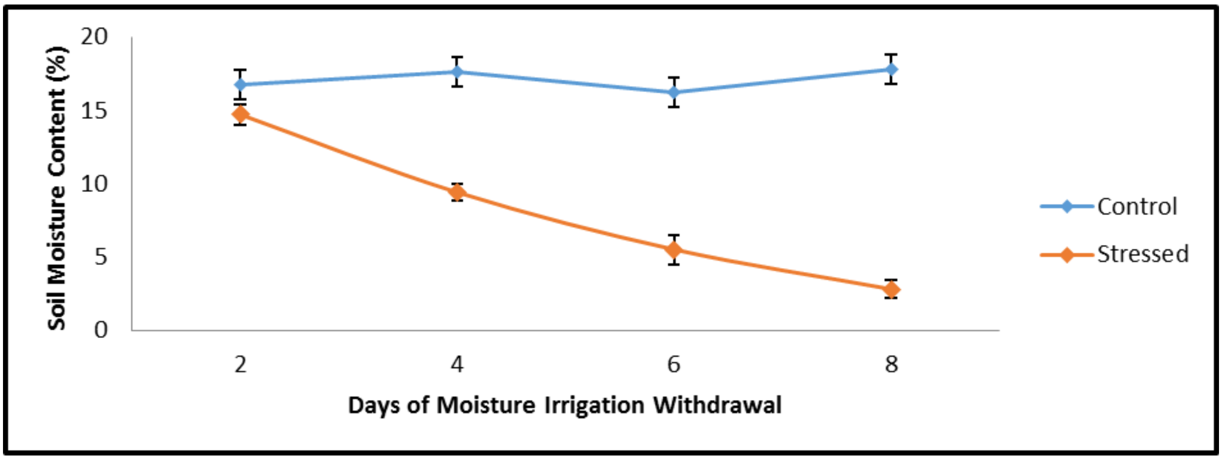


Figure 4 : Effect of irrigation withdrawal on soil moisture loss.Error bar represents standard error of the means.

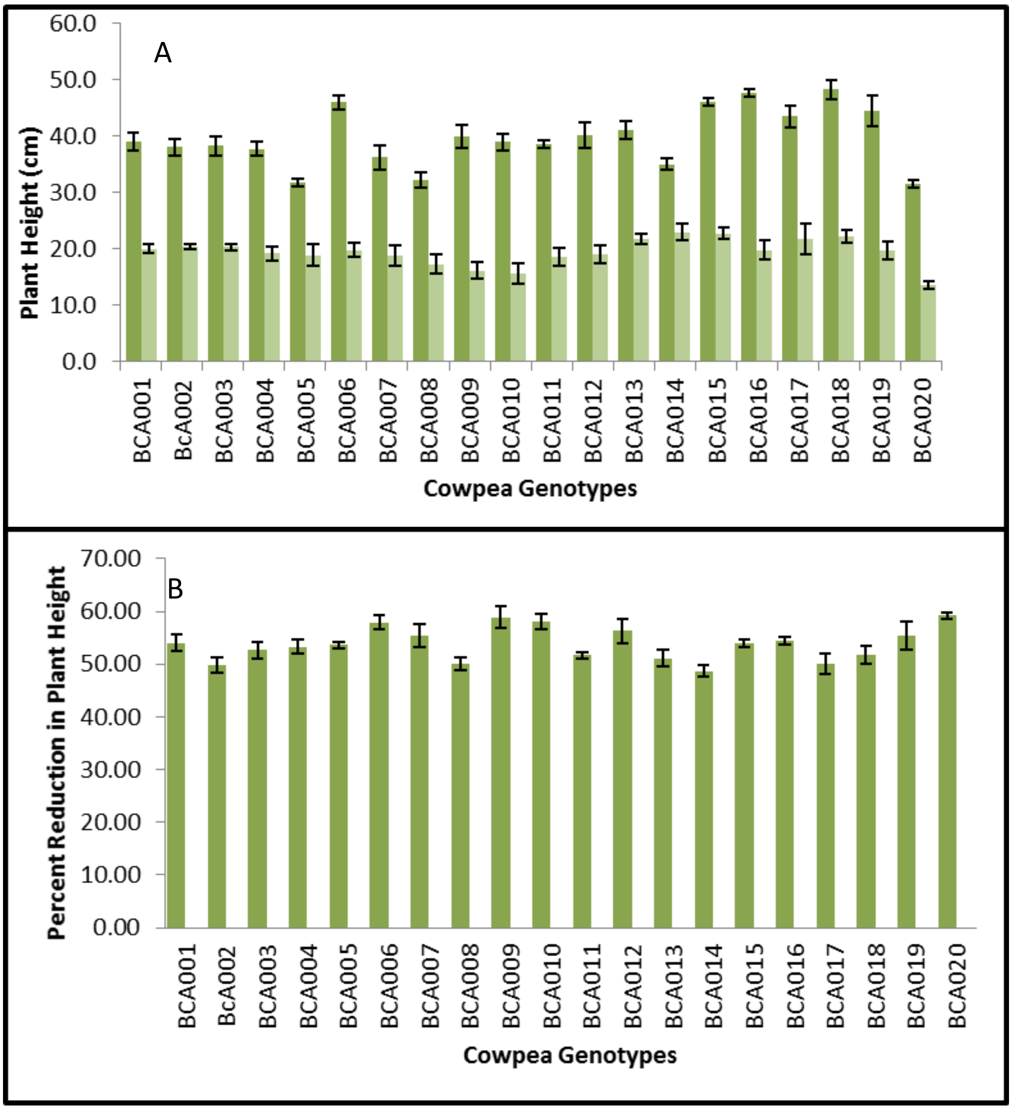


Figure 5: Effect of drought stressed on cowpea genotypes plant height.
A: plant height and B: percentage reduction in plant height due to drought stress. Mean of 4 replicates and plant height (cm) due to well-water and drought stress respectively**.** Bar represents standard error of the means.

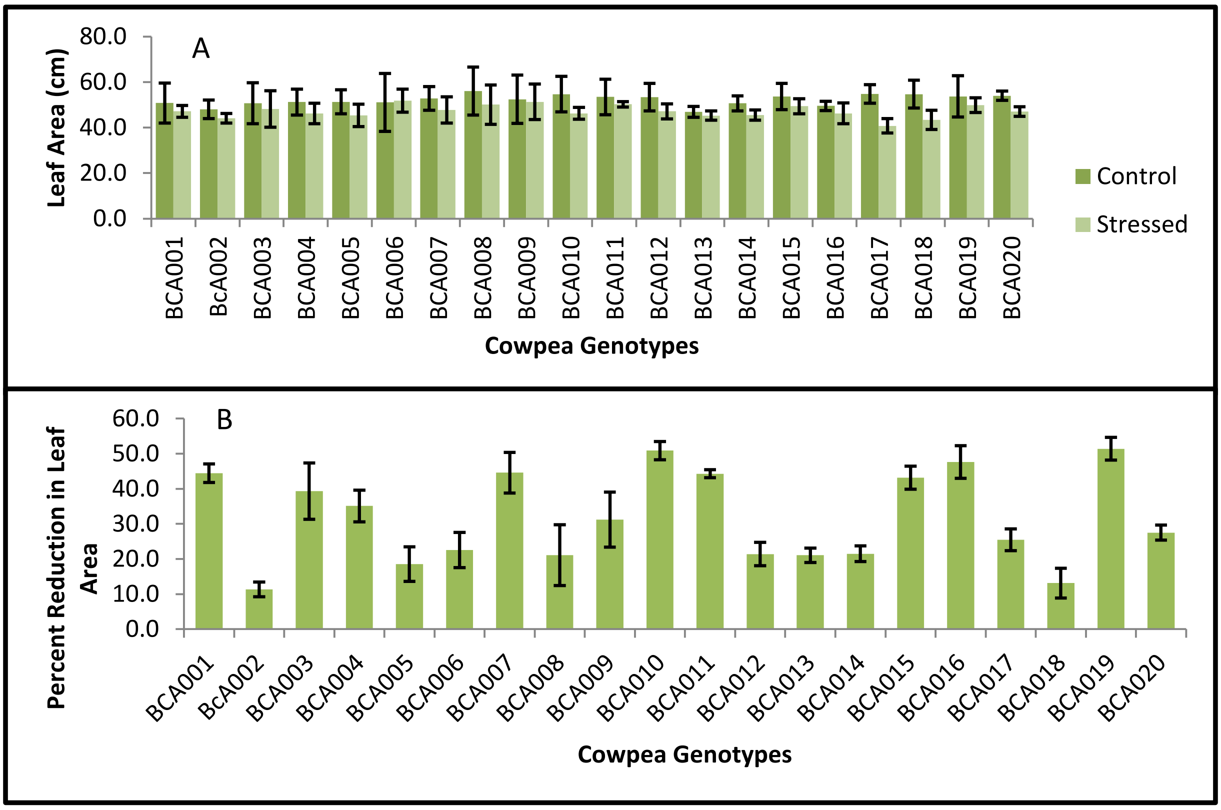


Figure 6 : Effect of drought stress on leaf area. A: Leaf area and B: Percentage reduction in leaf area due to drought stress. Error bar represents standard error of the means

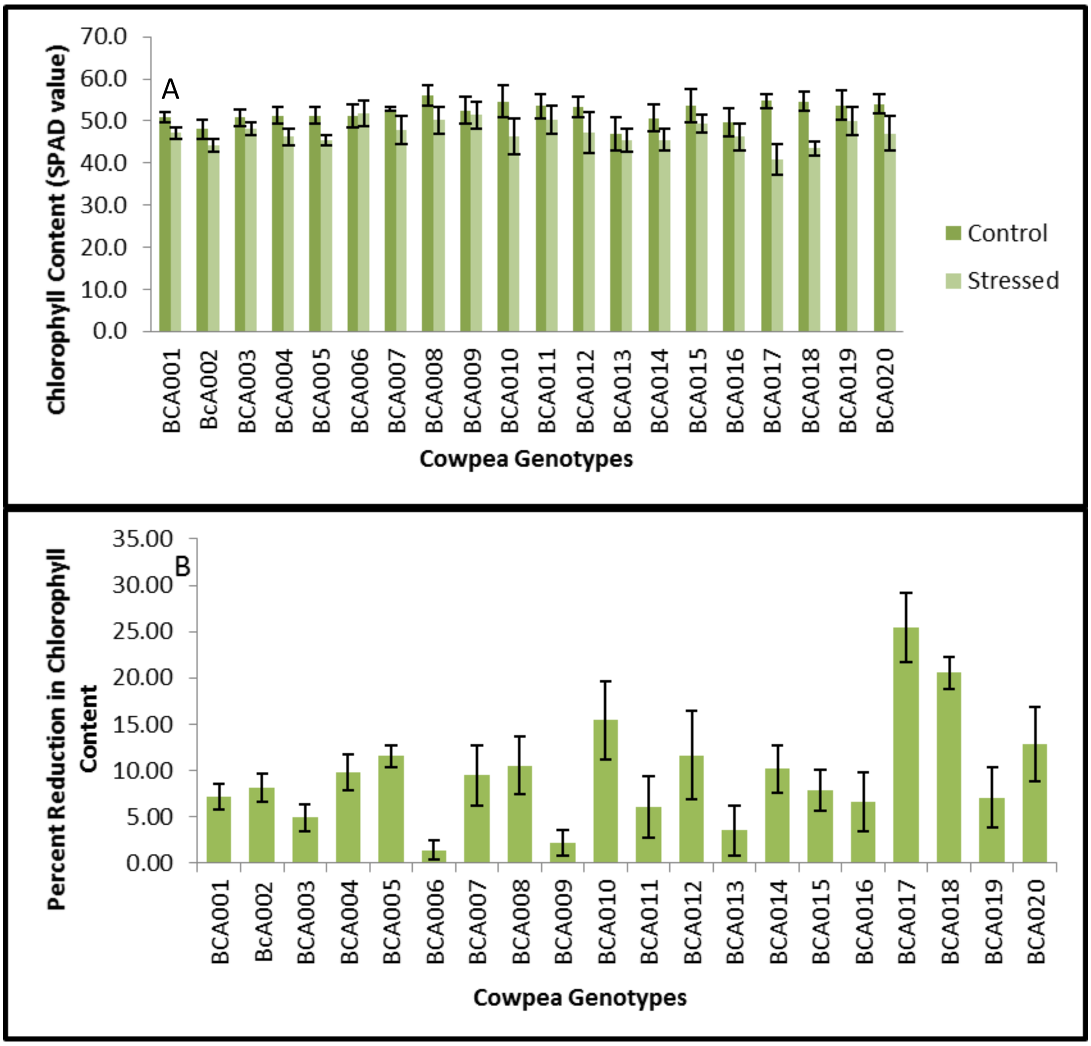


Figure 7: Effect of drought stress on cowpea genotypes chlorophyll content. Error bars respresent standard error of the means. A: chlorophyll content (SPAD value) and B: percentage reduction in chlorophyll content due to drought stressed.

Table 6: Tolerance indices of cowpea genotype under stress and non- stress condition in a green house.

Genotypes	Byp	Bys	TOL	BMP	BSTI	BRDI	BSSI	BDRI	BYR%
BCA001	8.098	2.290	5.808	5.194	16.086	0.750	0.546	3.016	63.72
BCA002	6.664	2.434	4.230	4.549	5.844	0.750	0.288	1.096	51.94
BCA003	7.718	2.725	4.993	5.222	5.649	0.750	0.333	1.059	54.50
BCA004	4.823	1.672	3.151	3.248	5.547	0.750	0.355	1.040	55.28
BCA005	1.891	2.223	-0.332	2.057	18.809	0.750	3.072	3.527	-33.74
BCA006	6.549	1.867	4.682	4.208	4.561	0.750	0.540	0.855	60.21
BCA007	4.446	1.030	3.416	2.738	3.707	0.750	0.665	0.695	62.66
BCA008	2.224	2.236	-0.012	2.230	4.525	0.750	3.952	0.848	-33.32
BCA009	5.530	2.528	3.002	4.029	7.314	0.750	-0.171	1.371	50.52
BCA010	2.814	1.305	1.509	2.060	7.420	0.750	-0.215	1.391	33.69
BCA011	4.185	1.641	2.544	2.913	6.274	0.750	0.178	1.176	49.58
BCA012	3.860	1.529	2.331	2.695	6.338	0.750	0.161	1.188	47.15
BCA013	3.952	1.659	2.293	2.806	6.717	0.750	0.047	1.259	41.62
BCA014	3.163	1.488	1.675	2.326	7.527	0.750	-0.262	1.411	34.11
BCA015	4.590	1.570	3.020	3.080	5.473	0.750	0.371	1.026	57.12
BCA016	6.542	2.288	4.254	4.415	5.596	0.750	0.345	1.049	51.18
BCA017	5.953	1.939	4.014	3.946	5.211	0.750	0.424	0.977	56.64
BCA018	6.173	1.879	4.294	4.026	4.870	0.750	0.488	0.913	59.89
BCA019	6.518	2.165	4.353	4.342	5.315	0.750	0.404	0.996	55.97
BCA020	4.174	1.339	2.835	2.757	5.133	0.750	0.439	0.962	59.42

BYp - biomass yield under well water, BYs - biomass yield under drought stress, TOL – tolerance, BMP - biomass mean productivity, BSTI – biomass stress tolerance index, BRDI –

biomass relative drought index, BSSI – biomass stress susceptability index, DI – drought index and BYR% – Biomass yield reduction percent.

Table 7: Correlation coefficient between BYp, BYs and tolerance indices

	Byp	***Bys***	***TOL***	***BMP***	***BSTI***	***BRDI***	***BSSI***	***DI***	***BYR%***
Byp	1								
Bys	0.506*	**1**							
TOL	0.97**	0.28	**1**						
BMP	0.98**	0.66**	0.90**	**1**					
BSTI	-0.66**	0.25	-0.81**	-0.52*	**1**				
BRDI	0.51	-0.06	0.59	0.43	-0.60	**1**			
BSSI	-0.43*	0.25	-0.55*	-0.31	0.84**	-0.41	**1**		
DI	-0.66**	0.25	-0.81**	-0.52*	1.00**	-0.60[c]	0.84**	**1**	
BYR%	0.70**	-0.18*	0.83**	0.57**	-0.99**	0.60[c]	-0.85**	-0.99**	1

*and ** significant at 0.05 and 0.01 levels .

BYp - biomass yield under well water, BYs - biomass yield under drought stress, TOL – tolerance, BMP - biomass mean productivity, BSTI – biomass stress tolerance index, BRDI – biomass relative drought index, BSSI – biomass stress susceptability index, DI – drought index and BYR% – Biomass yield reduction percent.

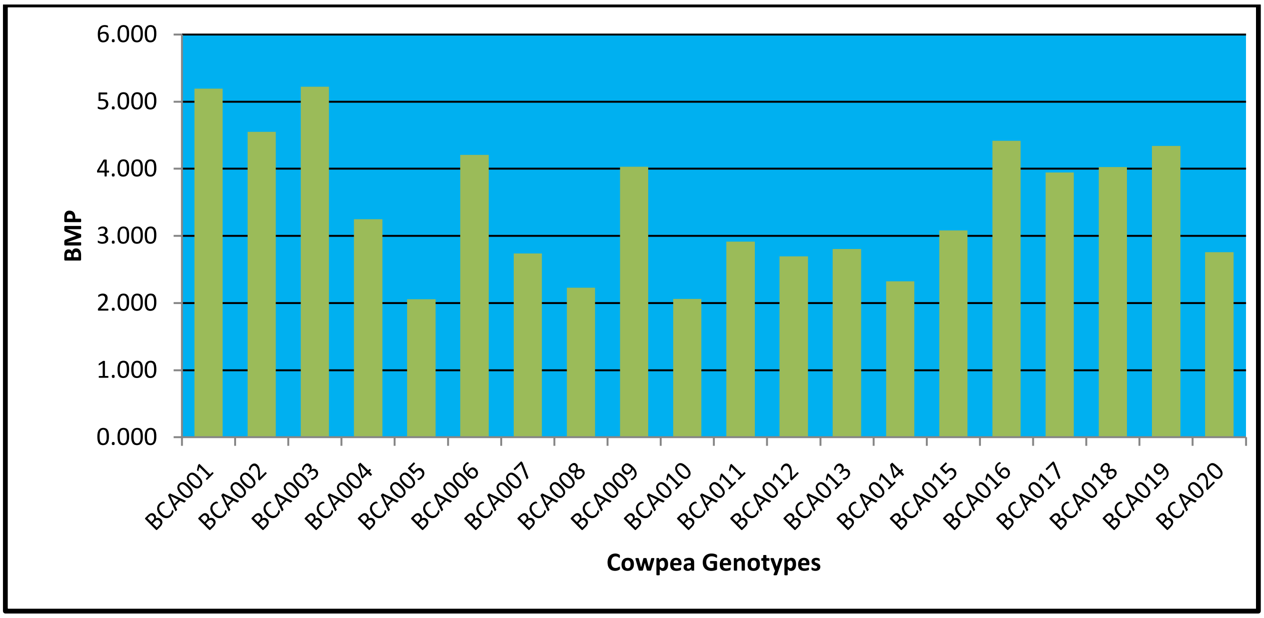


Figure 8: Determination of drought tolerance cowpea genotypes (highly, tolerance, moderate and sensitive) based on significant index (BMP-biomass mean productivity) . BMP value: 2-3 = sensitive, 3-4 = moderate, 4-5 = tolerance and 5-6 highly tolerance.

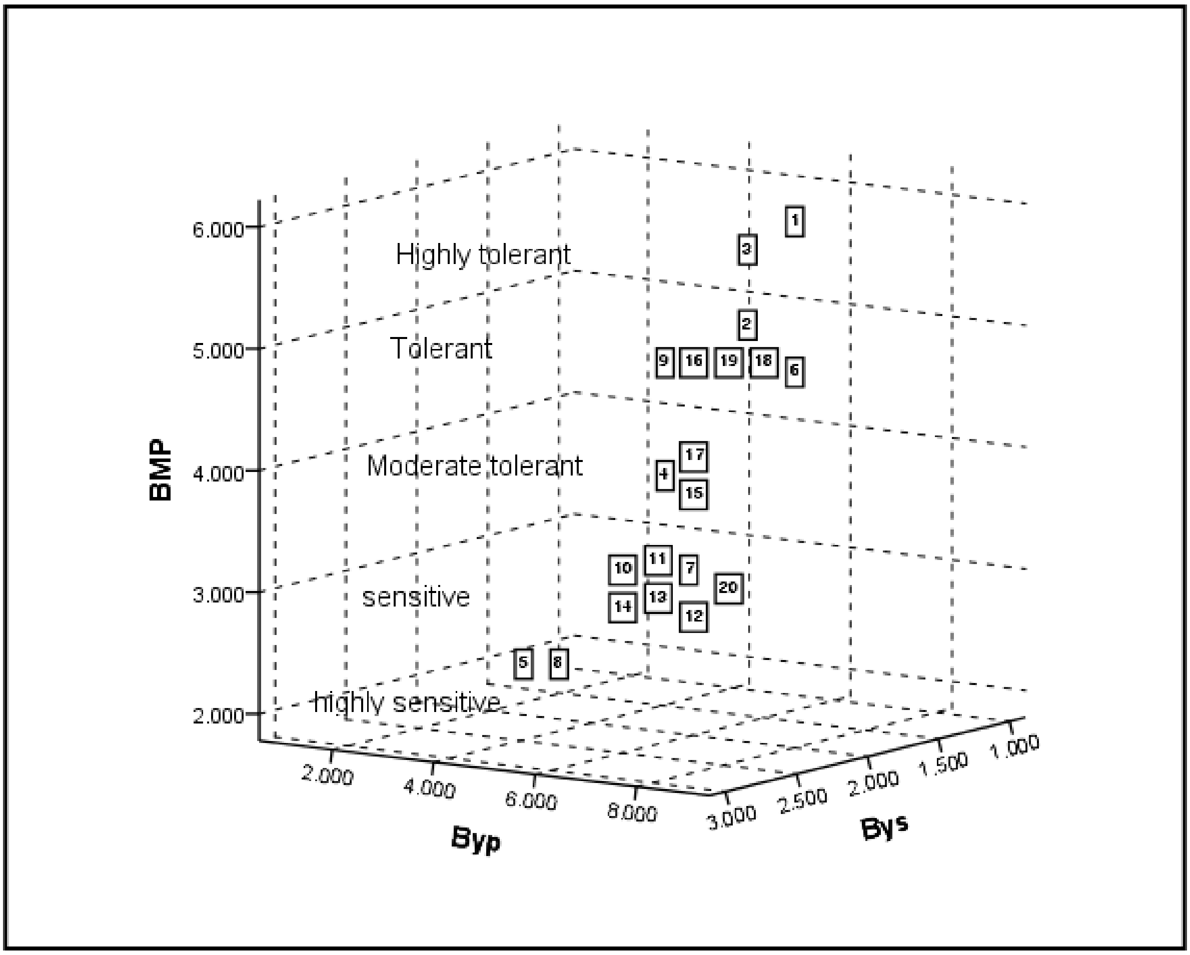


Figure 9: A three dimensional plot among BMP, BYP and BYS. BMP (biomass mean productivity, BYP (biomass yield well water) and BYS (biomass yield under water stress). 1= BCA001, 2= BCA002, 3 = BCA003, 4 = BCA004, 5 = BCA005, 6 = BCA006, 7= BCA007, 8 = BCA008, 9 = BCA009, 10 = BCA010, 11= BCA011, 12 = BCA 012, 13= BCA013, 14 = BCA014, 15 = BCA015, 16 = BCA016, 17 = BCA017, 18 = BCA018, 19 = BCA019 & 20 = BCA020.

Table 8: Drought tolerance biomass mean production index for eight cowpea genotypes.

Genotypes	DTc	Byp	Bys	BMP
BCA001	HDT	4.486	1.920	3.20
BCA003	HDT	3.830	1.270	2.96
BCA002	DT	1.854	1.854	2.92
BCA019	DT	1.629	1.629	2.48
BCA001	DT	1.594	1.594	2.55
BCA017	DM	1.471	1.471	2.55
BCA004	DM	1.624	1.624	2.64
BCA011	DS	1.203	1.203	2.46
CD at 5%		1.03	0.21	
CV%		55	3.75	

HDT = highly drought tolerance; DT = drought tolerant; DM = drought moderate; DS = drought sensitive; DTc = drought tolerance categories.

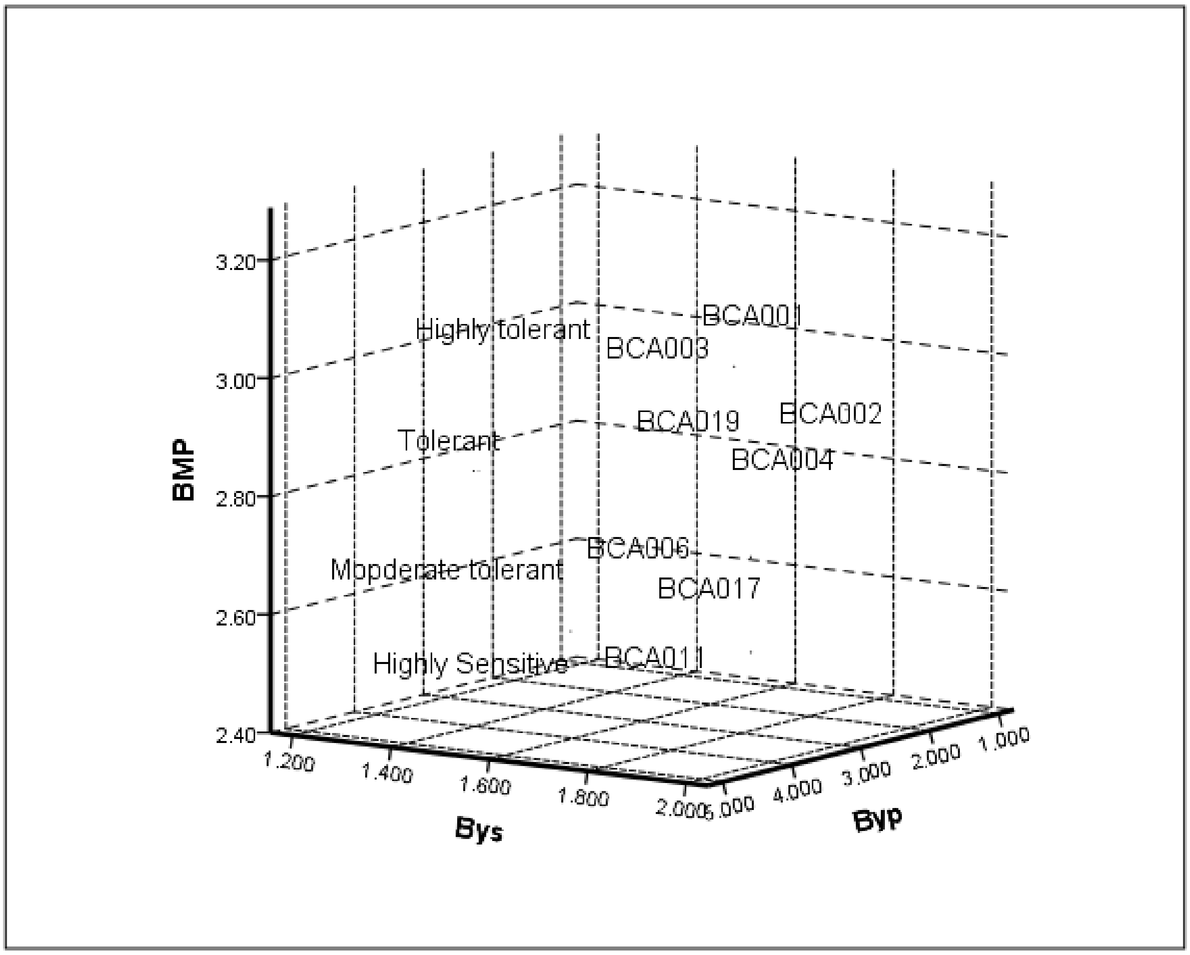


Figure 10: A three dimensional plot among BMP, BYs and BYp. BMP (biomass mean productivity), BYs (biomass yield under drought stress) and BYp (biomass yield under well watered conditions) for eight Cowpea genotypes grown in polythene bags in soil mixed (mixed C).

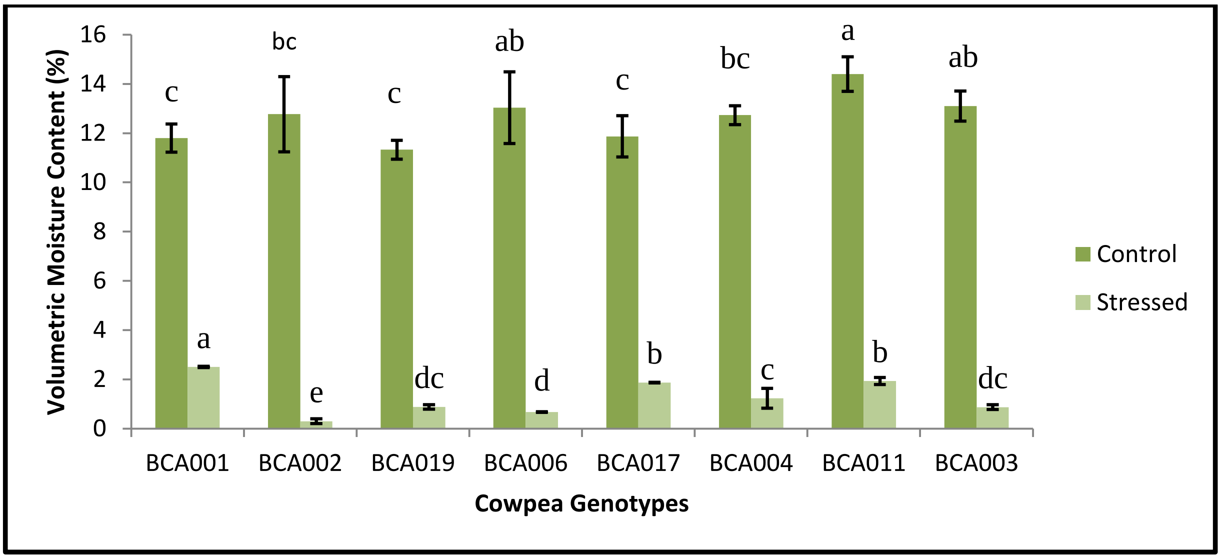


Figure 11: Effect of drought stress on soil moisture content on eight cowpea genotype during eight days of drought stress. Means with the same letter are not significantly different at P<0.05.

Table 9: Effect of water stress on cowpea genotypes morphological traits.

		Well- watered (Control)			Drought Stressed		
Genotype	DTC	Shoot DW (g)	Root DW (g)	Root:Shoot	Shoot DW (g)	Root DW (g)	Root:Shoot
BCA001	HDT	4.87[a]	0.7[bc]	0.16[bc]	2.07[a]	0.78[bc]	0.17[c]
BCA003	HTD	4.12[ab]	0.99[a]	0.24[a]	1.46[b]	0.99[a]	0.34[a]
BC002	DT	4.25[ab]	0.58[c]	0.13[c]	2.01[a]	0.58[c]	0.21[bc]
BCA019	DT	3.71[c]	0.57[c]	0.15[bc]	1.74[ab]	0.57[c]	0.18[bc]
BCA006	DT	4.76[ab]	0.90[ab]	0.19[ab]	1.72[ab]	0.90[ab]	0.25[b]
BCA017	DM	3.85[c]	0.72[bc]	0.19[ab]	1.64[ab]	0.72[bc]	0.23[bc]
BcA004	DM	3.98[bc]	0.57[c]	0.14[bc]	1.36[b]	0.57[c]	0.16[c]
BCA011	DS	4.12[ab]	0.71[bc]	0.17[bc]	1.36[b]	0.71[bc]	0.19[bc]
Significance ($LSD_{0.05}$)		*	**	**	*	***	**

*** , ** and * significant at P<0.001, 0.01 and 0.05 levels.

HDT = highly drought tolerance; DT = drought tolerant; DM = drought moderate; DS = drought sensitive; DTc = drought tolerance categories; DW dry weight. Means with the same letter are not significantly different within columns at P<0.05, $LSD_{0.05}$

Table 10: Effect of drought stress on cowpea genotypes relative water content (RWC%).

		Relative water content(%)	
Genotype	DTc	Well-watered	Drought stressed
BCA001	HDT	89.91^{ab}	58.82^{c}
BCA003	HDT	88.92^{ab}	58.08^{c}
BCA002	DT	86.95^{ab}	50.08^{c}
BCA019	DT	90.91^{ab}	52.36^{c}
BCA006	DT	92.91^{a}	49.68^{c}
BCA017	DM	84.95^{ab}	68.55^{ac}
BCA004	DM	86.69^{ab}	62.57^{bc}
BCA011	DS	88.63^{ab}	59.79^{c}
Significance ($LSD_{0.05}$)		NS	NS

Not Significant (NS) at P>0.05 level.

HDT = highly drought tolerance; DT = drought tolerant; DM = drought moderate; DS = drought sensitive; DTc = drought tolerance categories. Means with the same letter are not significantly different with in columns at P<0.05.

Table 11 : Effect of drought stress on Chlorophyll content of cowpea genotypes

		chlorophyll content (SPAD value)	
Genotypes	DTC	Well-watered	Drought stressed
BCA001	HDT	33dc	33.99^{dc}
BCA003	HDT	38.63^{ac}	40.20^{ab}
BCA002	DT	38.5 ^{ac}	38.69^{ac}
BCA019	DT	39.07^{ac}	35.26^{dc}
BCA006	DT	37.43 ^{ac}	33.67^{dc}
BCA017	DM	39.43^{a}	31.95^{dc}
BCA004	DM	43.25^{a}	31.68^{de}
BCA011	DS	35.43^{c}	30.75^{e}
Significance ($LSD_{0.05}$)		**	**

** significant at P<0.01

HDT = highly drought tolerance; DT = drought tolerant; DM = drought moderate; DS = drought sensitive; DTc = drought tolerance categories. Means with the same letter are not significantly different within columns at P<0.05, $LSD_{0.05}$.

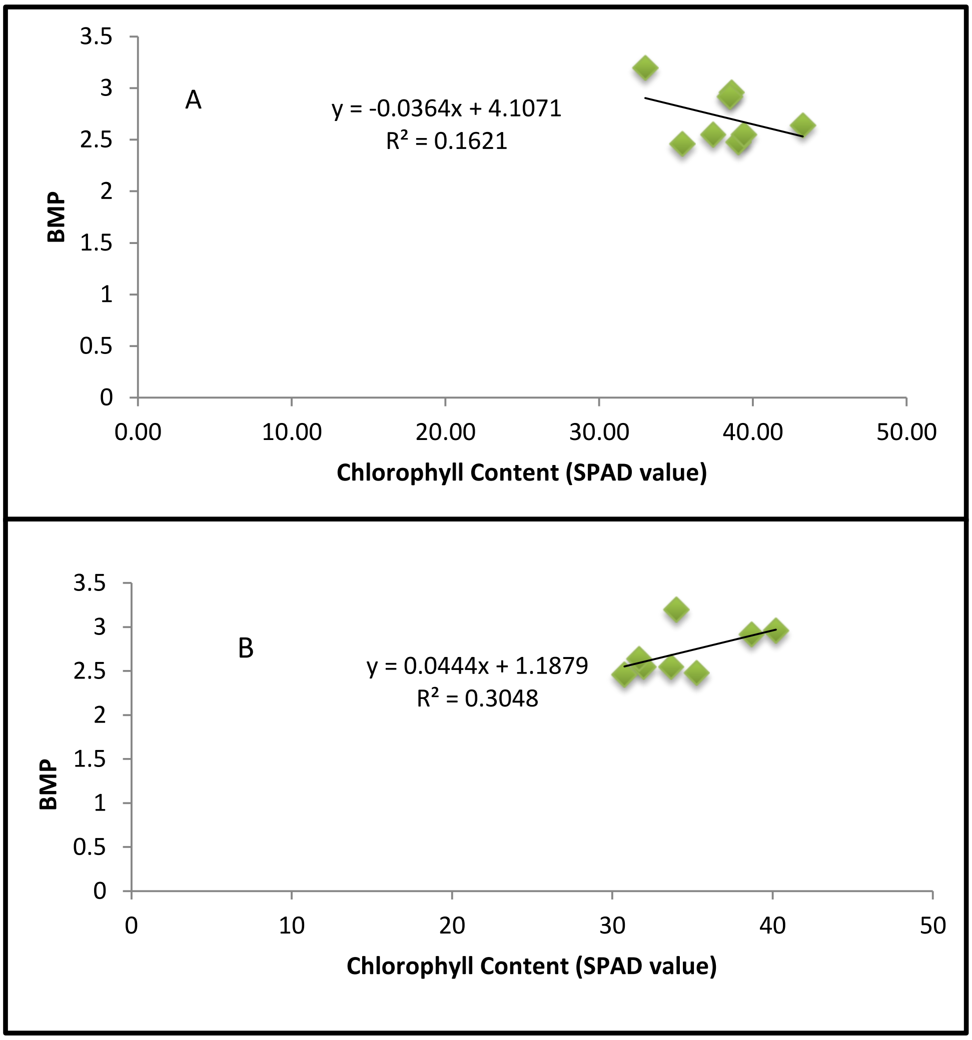


Figure 12 Relationship between chlorophyll content (SPAD value) and biomass mean productivity (BMP). A: Well-watered and B: Drought stress

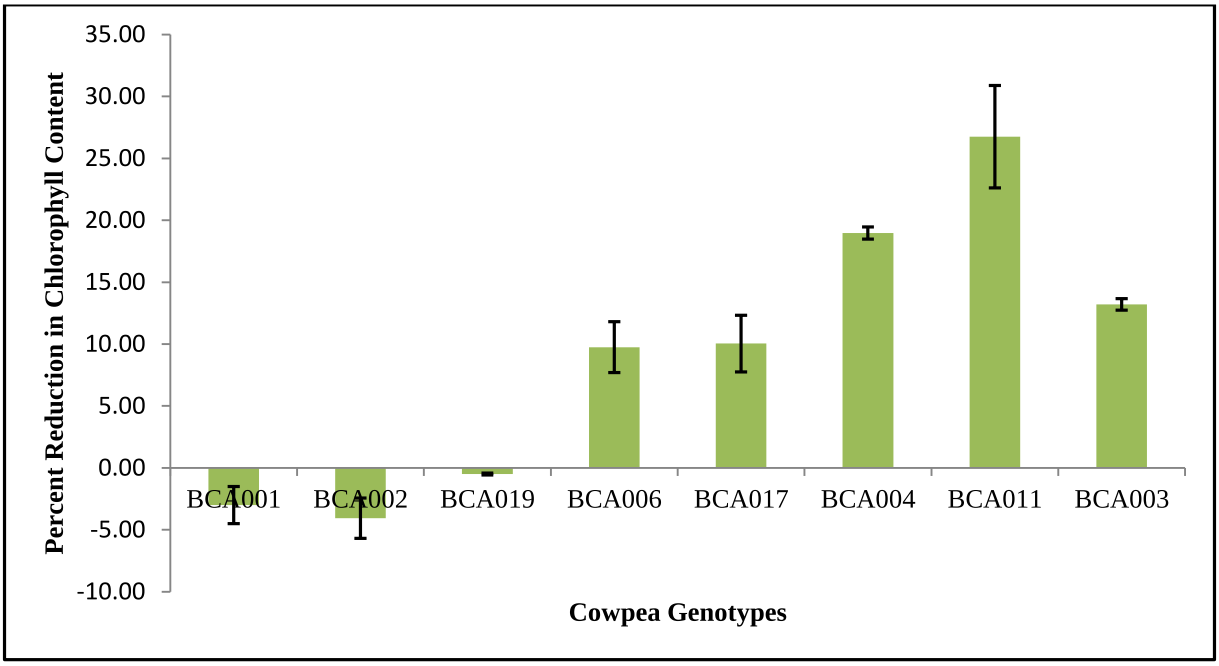


Figure 13: Precentage reduction in chlorophyll content due to drought stress . Error bar respresents standard error of the means.

Table 12: Effect of water deficit on gas exchange (Photosynthesis, stomatal conductance and transpiration).

		Well- watered (Control)			Drought Stressed		
		Net Photosynthesis	Stomata Conductance	Transpiration	Net Photosynthesis	Stomata Conductance	Transpiration
Geontypes	DTC	(mol CO_2. m^{-2}.s^{-1})	(mol H2O.m-2.s-1)	(mol $H_2$0.m-2.s-1)	(mol CO_2. m^{-2}.s^{-1})	(mol H2O.m-2.s-1)	(mol $H_2$0.m-2.s-1)
BCA001	HDT	15.76[ab]	0.12[b]	7.65[a]	9.30[ab]	0.03[bc]	3.54[ac]
BCA003	HDT	14.57[ab]	0.46[ab]	7.84[a]	7.18[ab]	0.06[ac]	5.06[a]
BCA002	DT	9.86[b]	0.26[ab]	6.62[a]	9.41[ab]	0.02[bc]	3.08[ac]
BCA019	DT	14.10[ab]	0.44[ab]	7.15[a]	9.69[ab]	0.08[ab]	4.29[ab]
BCA006	DT	14.03[ab]	0.11[b]	5.58[a]	5.04[ab]	0.006[c]	1.49[c]
BCA017	DM	14.14[ab]	0.69[a]	6.52[a]	7.96[ab]	0.07[ab]	3.14[ac]
BCA004	DM	14.67[ab]	0.21[b]	7.52[a]	7.16[ab]	0.07[ab]	2.27[c]
BCA011	DS	19.77[a]	0.18[b]	7.46[a]	11.31[a]	0.09[a]	5.22[a]
Significance ($LSD_{0.05}$)		NS	NS	NS	NS	*	*

* significant at P<0.05 and NS (not significant) at P >0.05
HDT = highly drought tolerance; DT = drought tolerant; DM = drought moderate; DS = drought sensitive; DTc = drought tolerance categories. Means with the same letter are not significantly different within columns at P<0.05, LSD $_{0.05}$.

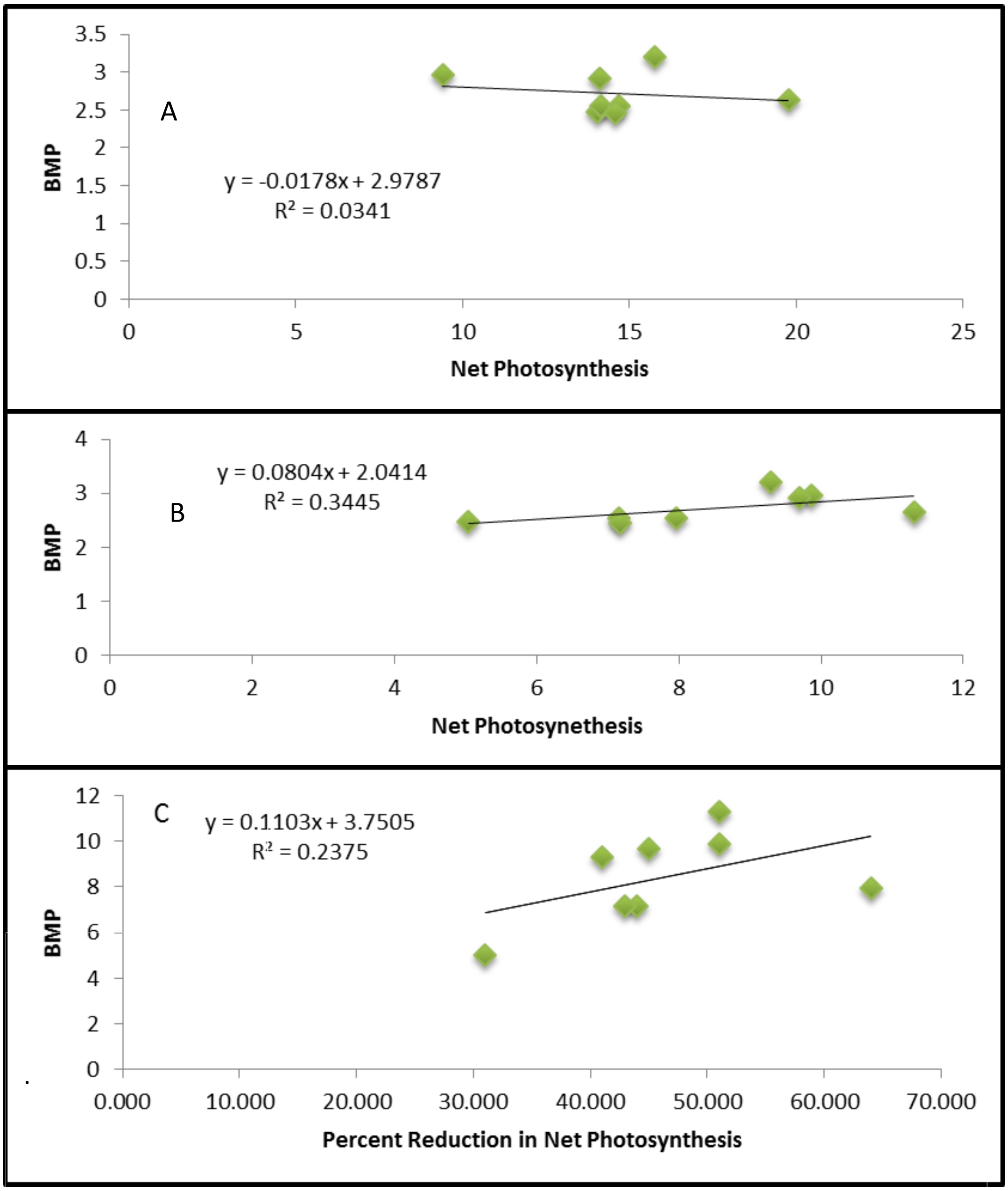


Figure 14: Relationship between photosyntheis and biomass mean production (BMP). A: Well watered, B: Drought stressed, C: Percent reduction due to drought stress.

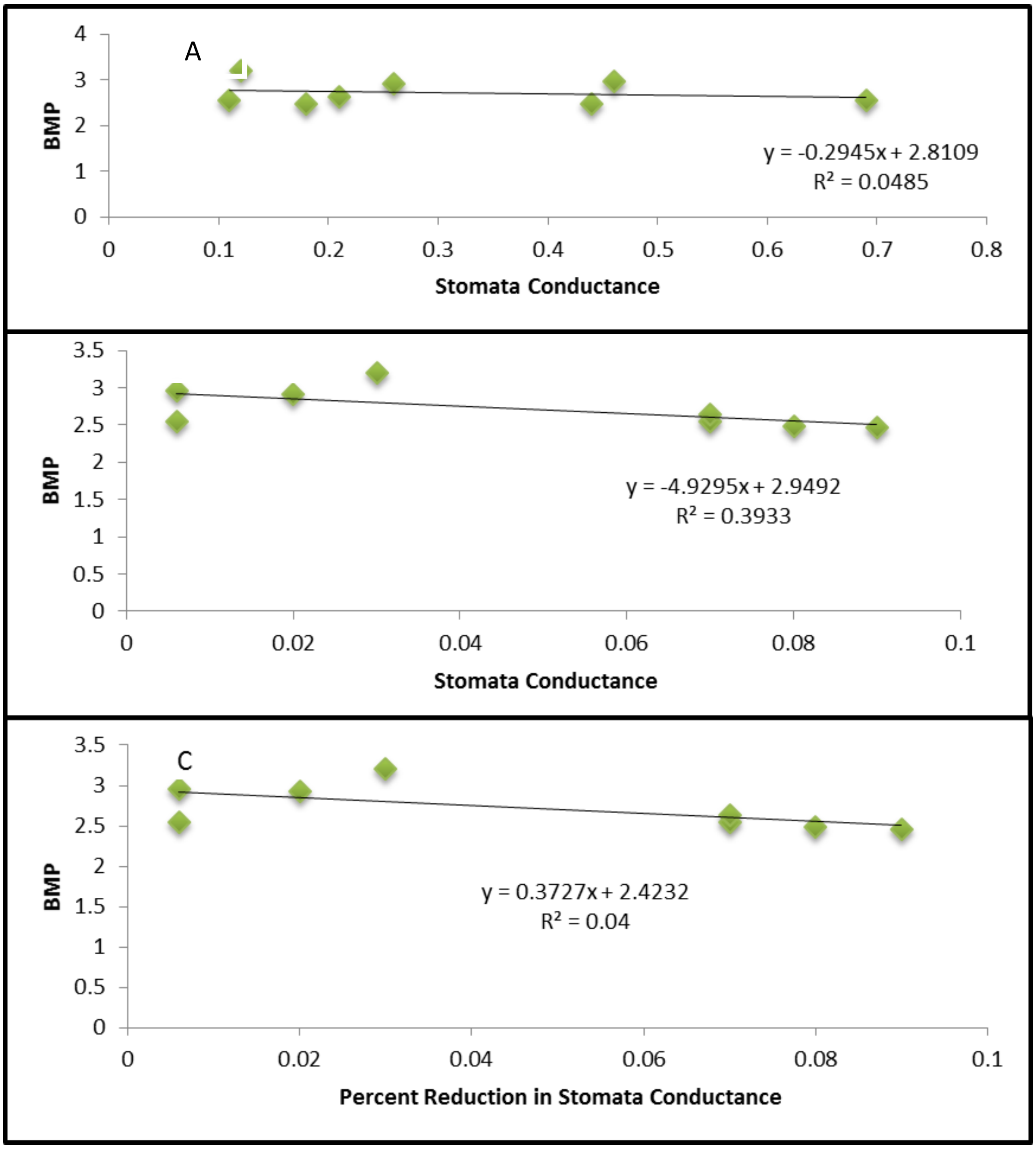


Figure 15: Relationship between stomatal conductance and biomass mean production (BMP). A: Well watered, B: Drought stressed, C: Percent reduction due to drought stress.

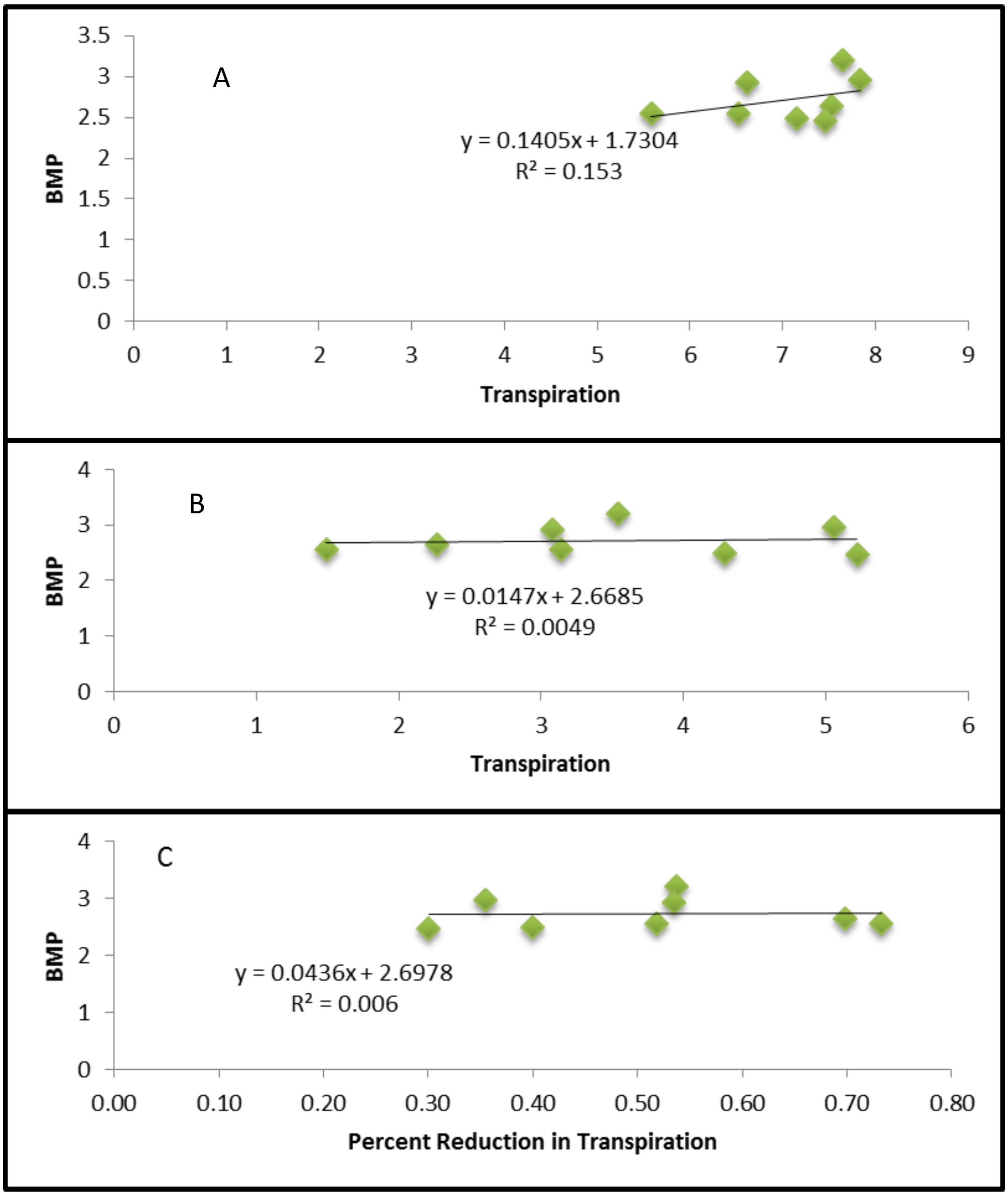


Figure 16: Relationship between Transpiration and biomass mean production (BMP). A: Well watered, B: Drought stressed, C: Percent reduction due to drought stress.

Table 13: Regression analysis for the effect of percent reduction in gaseous exchange (net photosynthesis, stomatal conductance and transpiration) and chlorophyll content on biomass mean productivity.

Statistic	Chlorophyll Content	Net Photosynthesis	Stomatal conductance	Transpiration
Reg. Coef.	-29.96 (30.324)	-0.270 (14.329)[a]	0.179 (0.238)	0.043 (0.719)
Intercept	89.653(11.100)	46.98 (39.147)	0.347(0.649)	2.697 (0.380)
P-value	0.36	0.985	0.499	0.953
F-value	7.154	0.003	0.515	0.004

[a] values in parenthesis are standard errors. Reg. Coef.= Regression Coefficient.

..

CHAPTER FIVE

DISCUSSION

5.1. Establishment of suitable soil mix and its application for lethal drought (LD_{50})

The purpose of this study was to determine the most suitable soil mixture that will support sufficient pre-drought stress plant growth and loose water during drought stress within a reasonable period. Drought stress reduced the soil moisture contents, chlorophyll content, plant height and biomass of all the soil mixtures but in the soil mixed C (60% river sand, 30% sand loamy and 10% compost) (Table 1) showed an optimum plant performance (plant height, biomass yield and chlorophyll content) compared to the others during pre-drought treatment period (Figure 1 and 2). This can be explained by the fact that higher amount of herbage mass and green leaf area would be expected to have higher transpiration because of their greater leaf area. Similar results were obtained in studies comparing soil water loss and plant herbage yield in four leguminous species (alfalfa (*Medicago sativa*), vetch (*Astragalus adsurgens*), sainfoin (*Onobrychis viciaefolia*) and *Lespedeza davurica)* (Xu *et al*., 2006). On the other hand, the differential plant performance as well as rate of soil moisture could also depend on the texture as soil mix C, was speculated to have more sand particle fraction compared to other mixtures. Previous studies with cowpea indicates that its growth performance was significantly increased in soils with high sand content (Eugene *et al*., 2010; Pungulani, 2012)).

Plant grown in soil mix C and drought stress established lethal drought (LD_{50}) within eight days. This was by reducing the cowpea biomass yield to 50% (Table 5). This indicates that soil moisture content have a major role in biomass yield production and its reduction because it is one of the major determinants of plant productivity parameters, such as translocation and assimilation of nutrients (Farouks and Quados, 2013), CO_2 uptake and photosynthetic activity (Lawlor and Cornic, 2002; Abaomi and Abidoye, 2009; Abayomi *et al.*, 2000; Ahmed and

Suliman, 2010) in cowpea and other crop plants (Eric *et al.,* 2010, Farooq *et al.,* 2010). This ultimately leads to retardation of plant growth, reduced biomass yield, and leaf area as observed by (Hayatu *et al.,* 2014) and in maize (Efecoglu *et al.*, 2009), as well as medicinal plants (Koocheki *et al.,* 2008). In view of the foregoing, lethal drought (LD_{50}) (number of days of irrigation withdrawal to reduce plant biomass by approximately 50%) was used in this thesis research as a base for determination of drought resistance and tolerance among twenty cowpea genotypes.

5.2. Identification of the most suitable drought tolerance index and its application in cowpea screening.

This study was carried out in order to evaluate cowpea genotypic reaction to drought stress and to determine the best measures for drought tolerance based on biomass yield in drought stress and non-stress conditions given the established LD_{50}. Twenty genotypes were obtained from the national Plant Genetic Resources Centre and local farmers (Table 2). There was differential responses to drought stress as indicated by reductions in plant height, leaf area and chlorophyll content (Figure 5, 6 and 7). These variations in response parameters can be explained by the fact that drought stress damages plant physiological parameters responsible for growth and maintenance, and genotypic differences observed are also due to different levels of tolerance in materials under study. This could be a result of each genotype ability to affect antioxidant systems *(*Nairs *et al*. 2008), accumulate proline (Costa *et al*., 2011; Farouk *et al*., 2013), pinnitol (Souza *et al*., 2003) aquaporins *(*Simoe-Aranjo et al., 2008). It might have enabled some of the genotypes to have better growth performance as observed in various water stress studies involving cowpea *(*Ogbonnaya *et al., 2*003; Kumar *et al.,* 2008; Hamidou *et al.*, 2007; Muchero *et al.,* 2008) and bambara groundnuts (Vurayai *et al.* 2011).

To identify the most suitable, seven indices were calculated from biomass yield under well watered (Byp) and drought stress conditions (Bys) with modifications. These indices were; tolerance (TOL), biomass mean productivity (BMP), biomass stress tolerance index (BSTI), biomass relative drought index (BRDI), biomass stress susceptibility index (BSSI) drought index (DI) (Blum, 1988) and percentage reduction in biomass due to drought (BYR%) were calculated according to relationships suggested by (Fischer and Maurer, 1978; Rossielle and Hamblin, 1981, Blum, 1988, Harb et al., 2010 and Naghavi et al., 2013). Selection of the most suitable index was based on its positive relationship with Byp and Bys, where it was found that there was strong and positive relationship between the two measurements and BMP (Table 8).

BMP was therefore used as a screening index for cowpea drought tolerant (Figure 8). The results shows that the twenty genotypes can be classified into five categories namely; highly susceptible, sensitive, moderately tolerant, tolerant and highly drought tolerant genotypes. The highly drought tolerant genotypes are those that express uniform superiority in both stress and well watered conditions. A three dimensional plot between BMP, Byp and Bys (Figure 9 and 10), shows that BCA001 and BCA003 are highly drought tolerant compared to the highly sensitive BCA005 and BCA008, while others are classified in between as; tolerant, moderately tolerant and sensitive . These results are consistent with Naghavi *et al.* (2013) in which several indices were correlated with yield under both non-stress and stress conditions. In this case BMP was selected as the index for drought tolerance selection for cowpea genotypes based on its correlation with Byp and Bys. Several studies have shown the use of BMP or MP (BMP) in idenfication of drought tolerance in cowpea (Chiulele et al., 2011) and other crops such as potato (Ghasem, 2014), wheat (*Triticum aestivum L*) (Iiker, 2011; Sio-se Mardeh et al., 2006), barley (Nazari and Pakniyat, 2010), mungbean (Fernandez, 1992). Based on this, the BMP categorized cowpea genotypes in this study as follow: BCA001 and BCA003 are highly tolerant; BCA002, BCA006, BCA009, BCA016, BCA011, BCA019 are drought tolerant;

BCA004, BCA015, BCA017 are moderately drought tolerant and the drought sensitive and highly sensitive are: BCA020, BCA014, BCA013, BCA012, BCA007, B505A, BCA008, BCA010 and BCA005. This categorisation is clearly illustrated by the three dimensional plot (Figure 9 and 10). In conclusion, this study has shown that genetic variability for cowpea drought tolerance existed in the evaluated genotypes. Genotypes were grouped according to their biomass yielding ability and tolerance to drought.

5.3. Differences in chlorophyll content and gas exchange parameters between the cowpea genotypes, but are not associated with BMP index.

This study was investigating whether there is a relationship between drought tolerance (BMP values) and estimated chlorophyll content and gaseous exchange (photosynthesis, stomatal conductance, and transpiration) in plants. Of the environmental factors constraining plant growth, water is usually the most critical and its stress restricts plants growth and yield (BMP). This is partly because transpiration water loss is an inevitable consequence of photosynthesis, through CO_2 diffusion into, and water flux out of stomata. Stomatal conductance thereby acts as a key control on both water loss and carbon gain, while carbon gain is linked to biomass yield or BMP in this particular study.

Drought tolerance is defined as a plant or a group of plants showing better growth and productivity with limited soil moisture than other plants in a given set of similar environments (Kumar, 2005). The results presented in Figure 12, 13, 14 ,15 and 16 showed that the eight genotypes differed in the above parameters. The difference observed in chlorophyll content showed that BCA001, BCA002 and BCA019 had the highest chlorophyll content under drought stressed condition compared to BCA011 and BCA004. This difference can be attributed to several reasons, but the idea among them was that some cowpea genotypes exhibited escape or avoidance mechanism before or after drought stressed was initiated (Ntomebla, 2012 and Vurayai et al., 2011). While this was the case, the parameters; estimated chlorophyll content and gaseous exchange were poorly related to BMP expected (Figure 12, 14, 15 and 16); indicating that potential drought tolerance identification index (biomass mean productivity) does not necessarily result in or supports drought tolerance selection based on physiological traits (chlorophyll content and gaseous exchange). The BMP agro-morphological trait has been used for drought tolerance selection in many crops. According to Fussell *et al.*, 1991, agro-morphological trait response to drought stress is reliable for drought identification compared to physiological traits. However, estimated chlorophyll content and its reduction due to drought stress were treated as one of the key indicators for drought tolerance in this study and others involving cowpea (Ntombela, 2012) , wheat (Talebi, 2009; Farshadfar, *et al.*, 2012a) and peanuts (Songsri, *et al.*, 2008). In addition to this, gaseous exchange parameters in general have also been used as key parameters to be determinants of drought tolerance despite being poorly associated with BMP as a measure of drought tolerance. Other similar studies, also indicates that these parameters were used to screen for drought tolerance in cowpea (Singh et al.,2010; Singh and Reddy, 2011), legumes (Hamidiou *et al.*, 2007; Darwish and Fahmy, 1997; Vurayai *et al*., 2011; Socias *et al.*, 1997); and other crops (Stoll *et al*., 2000; Naianayake, 2007; Kumar *et al*., 2014).

The differences observed in the current research showed that BCA011 had the highest gaseous exchange ability under drought stress condition, while BCA002 had the lowest for stomata conductance and BCA006 had the lowest for transpiration. This could be due to several reasons; key among them was that plants were experiencing the same water deficit and stomatal control of water loss and carbon gain also the same; other plant responses (antioxidant systems, proline, pinnitol, sugar accumulations) which are biochemical by nature played a role in the observed difference in drought tolerance, and species difference or genetics could also account for lack of association of BMP and the physiological parameters.

In view of these observations, BMP is deemed as an appropriate drought tolerance selection index since chlorophyll content (Figure 12) and gaseous exchange (Figure 14, 15 nd 16) showed poor relatioship in this study. It neessary that further studies should be done on cowpea to determine its associated responses mentioned above.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

6.1.1 Drought stress at vegetative stage of cowpea caused a reduction in cowpea genotypes' growth parameters but when it is grown in soil with 60% river sand, 30% loam soil and 10% compost under green house condition exhibits less reduction on growth parameters. It becomes suitable for screening drought toleranance cowpea genotypes during short period water defict regime. In particular, eight days water deficit reduced cowpea genotypes biomass yield by 50% establishing lethal drought. These experimental conditions allowed selection for drought tolerance in twenty cowpea genotypes.

6.1.2 Using biomass yield under well water (Byp) and drought stress conditions (Bys), mean biomass productivity (BMP) was identified as the most suitable index for drought tolerance selection. BMP was further applied to classify the twenty cowpea genotypes as; highly sensitive, moderately tolerant, tolerant and highly tolerant.

6.1.3 The association of BMP and plant physiological parameters (estimated chlorophyll content, photosynthesis, transpiration and stomatal conductance) was analyzed. The results showed that BMP was not associated with any of these parameters.

6.2 Recommendation

Based on the findings of this research, the following recommendations are made;

6.2.1. BMP identified drought tolerance in different genotypes of cowpea at early seedling growth stage. The identified genotypes may be further analyzed for tolerance at reproductive and grain filling stage.

6.2.2. Further research should be done to identify BMP associated physiological and biochemical parameters such as (antioxidant systems, proline, pinnitol, and sugar accumulations).

Reference

Abayomi YA, Afloabi ES and Aderolu (2000). Effects of water stress at different stages on growth, grain yield and seed quality of cowpea genotypes. *NISEB J.* 1: 041-014.

Abebe T, Guenzi AC, Martin B and Cushman JC (2003). Tolerance of Mannitol- accumulating transgenic wheat to water stress and salinity. *Plant Physiol.* 131: 1748-1755.

Aboyomi YA and Abidoye (2009). Evalution of cowpea genotypes for soil moistures stress tolerance under screen house condition. *Afr. J. plant Sci.* 3(13) : 229-237.

Acosta-Diaze E (1998). Early vigor as a selection criteria for daptation to drought stress in common bean. *Annu. Rep. Bean Improv. Coop.* 41: 153-154.

Adeoye PA, Adebayo SE and Musa JJ (2011). Growth and yield response of cowpea (*Vigna unguiculata*) to poultry and cattle manure as amendments on sandy loam soil plot. *Agric. J.* 6: 218-221.

Agbicodo EM, Falokun CA, Muranaka S. Visser R GF and Linden vander CG (2009). Breeding drought tolerance cowpea, constraints, accomplishments and future prospects. *Euphytica.* 167: 353- 370.

Aharoni A, Dixit S, Jetter R, Thoenes E, Arkel G and Pereira A (2004). The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis . *Plant Cell Envron.* 16: 2463-2480.

Ahmed FE and Suliman ASH (2010). Effect of water stress applied at different stages of growth on seed yield and water-use efficiency of Cowpea. *Agric. Biol. JNAM.* 1:534-540.

Akıncı S, and Lösel DM (2012). Plant water-Stress response mechanisms, water Stress. InTech. http://cdn.intechopen.com/pdfs-wm/26970.pdf.

Alscher RK, Euturk N and Health LS (2002). Role of superoxide dismutase (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* 53: 1331-134.

Anyia Ao and Herzog H (2004). Genotypic variation in drought performance and recovery in cowpea uncontrolled environment. *J. Agron. & Crop Sci.* 190:151-15.

Aranjuelo I, Molero G, Erice G, Avices JC and Nogue S (2010). Plant physiology and proteomics reveals the leaf response to drought in alfalfa (Medicago sativa L.). *J. Exp. Bot.* 62: 111-123.

Asch F, Sow A and Dingkuhn M (1999). Reserve mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigor. *Field Crops Res.* 62: 191-202.

Atkinson CJ, Policarpo M, Webster AD and Kingswell G (2000). Drought tolerance of clonal Mulus determined from measurements of stomatal conductance and leaf water potential. *Tree Physiol.* 20:557-563.

Babu CR, Zhang J, Blum A, Ho THD, Wu R and Nguyen HT (2004). HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (Oryza sativa L.) via cell membrane protection. *Plant Sci.* 166:855-862.

Baier M, Kandlbinder A, Golldack D and Dietz KJ (2005). Oxidative stress and ozone: perception, signalling and response. *Plant, Cell & Environ.* 28: 1012-1020.

Bennie ATP and Hensley M (2001). Maximising precipitation utilization in dry agriculture in south Africa- a review. J. *Hydrol.* 241: 124-139.

Belko N, Cisse N, Diop NN, Zombre G, Thiaw S, Muranaka S and Ehlers JD (2014). Selection for postflowering drought resistance in short- and medium-duration cowpeas using stress tolerance indices. Crop Sci. 54: 1-9.

Bezerra FM (2003). Identification of cowpea genotypes for drought tolerance. *Rev. Cienc. Agron.* 34: 1-13.

Bhattarai T and Fettig S (2005). Isolation and characterization of a dehyrin gene from cicer pinnatifidum, a drought_ resistant wild relatives of chickpea. *Physiol. Plant.* 123: 452-458.

Blum A (1988). Plant breeding for stress environments. CRC press, Boca Raton, Florida, USA.

Bohnert HJ and Jensen RG (1996). Strategies for engineering water-stress tolerance in plants. *Trends in Biotechnol.* 14:89–97.

Cheng Z, Targolli J, Huang X and Wu R (2002). Wheat LEA genes, PMA80 and PMA1959, enhance dehydration tolerance of transgenic rice (Oryza sativa L.). *Mol. Breed.* 10:71-82.

Cheulele RM and Agenbag GA (2004). Plant water relations and proline accumulation on two cowpea (Vigna uniguiculata (L). Walp.) Cultivars as a response to water stress. *S. Afr. J. Plant & Soil* 21: 107-113.

Chiulele RM, Mwangi G, Tongoona P, Ehlers JD and Ndeve AD (2011). Assessment of farmer's perceptions and preferences of cowpea in Mozambique. *Afr. crop sci. Conf. Proc.* 10:311-318.

Cisse Ndiaye M, Thiaw S and Hall AE (1997). Registration of melakh-cowpea. *J. Crop Sci.* 2: 197-28.

Clifford SC, Arndt SK, Corlett JE, Joshi S, Sankhla N, Popp M and Jones HG (1998). The role of solute accumulation, osmotic adjustment and change in cell wall elasticity in drought tolerance in Zizphus mauritiana (LamK). *J. Exp. Bot.* 49: 967-977.

Condon AG, Richards RA, Rebetzke GJ and farquhar GD (2004). Breeding for high water-use efficiency. *J. Exp. Bot.* 55: 2447-2460.

Costa RCL, Lobato AKS, Silveria JAG and Laughinghouse WHD (2011). ABA- mediated proline synthesis in cowpea leaves exposed to water deficiency and rehydration. *Turk J. Agric*. 35: 309-317.

Cruz De Carvalho MH (2000). Etude physiologique, biochimique et moléculaire de la réponse à la sécheressechez *Phaseolus vulgaris* L. et *Vigna unguiculata* L. Walp. Implication de l_aspartique protéinase. Mise au point del_étape préalable à la transgenèse : régénération in vitro des plantes. Thèse doct. physiol. cellulaire et moléculaire des plantes. Paris, univ. VI, France: Paris.

D'arcy-Lameta A, Ferrari – Iliour R, Contour-Ansel D, Pham-Thi AT and Zuily-Fodily (2006). Isolation and characterization of four ascorbate peroxidise CDNAs responsive to water deficit in cowpea leaves. *Ann. Bot.* 29: 133-140.

Da M, Bao Y-X and Han Lie-Bao (2011). Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. *Hortsci.* 46:1027–1032.

Darwish DS and Fahmy GM (1997). Transpiration decline curves and stomatal characteristics of faba bean genotyeps. *Biol. Plant.* 39(2): 243-249.

Des Marais DL, McKay JK, Richards JH, Sen S, Wayne T and Juenger TE (2012). Physiological genomics of response to soil drying in diverse Arabidopsis accessions. *Plant Cell Environ*. 24: 893–914.

Dingkuhn, M, Johnson, DE, Sow A and Audebert AY (1999). "Relationships between upland rice canopy characteristics and weed competitiveness. *Field Crops Res.*. 61: 79-95

Ehleringer J and Bjôrkman O (1978). A comparison of photosynthetic characteristics of *Encelia* species possessing glabrous and pubescent leaves. *J. Plant Physiol.* 62: 185-190.

Efeoglu B, Ekmekçi Y, Çiçek N (2009). Physiological responses of three maize cultivars to drought stress and recovery. *S. Afr. J. Bot.* 75:34–42.

Ehlers JD and Hall AE (1997). Cowpea (Vigna unguiculata L. Walp). *Field Crops Res.* 53: 187-204.

El-Tajeb N (2006). Differential response of two Vicia faba cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. *Acta Agron. Hung. 54: 25–37.*

Eric, Louahlia, Irigoyen JJ, Sanchez-Daiz M and Avice JC (2010). Biomass partitiong, morphology and water status of four alfalfa genotypes sunmitted to progressive drought and subsequent recovery. *J. plant Physiol.* 167 (2) : 114-20.

Eugene NN, Jacques E, Desire TV and Paul B (2010). Effects od some physical and chemica characteristcs of soil on productivity and yield of cowpea (Vigna unguiculata L. Walp.) in coastal region (Cameroon). *Afr. J. Environ. Sci. & Technol.* 4(3): 108-114.

FAO (2007). Agricultural and water scarcity: A programmatic approach to water use efficiency and agricultural productivity. Paper No. 7. FAO/Rome.

FAO (2012). FAO STAT agriculture database food and Agriulture Organization of the United Nation FAO. http://www.fao..org/2012.

Farooq M, Kobagashi N. Ito O, Wahid A and Serrai R (2010). Broader leaves result in Better performance of indica rice under drought stress. *J. Plant physiol*. 1: 13: 167 (13).

Farouk S, Amira MS and Qados A (2013). Osmotic adjustment and yield of cowpea inresponse to drought stress and chitosan. *Indian J. Appl. Res.* 3: 1-5.

Farshadfar E, Farshadfar M and Dabiri S (2012a). Comparison between effective selection criteria of drought tolerance in bread wheat landrancs of Iran. *Ann. Biol. Res.* 3(7):3381-3389.

Fernandez GCJ (1992). Effective selection criteria for assessing plant stress tolerance. In: Kuo CG , editor, Proceedings of an International Symposium on Adaptation of Vegetables and Other Food Crops to Temperature Water Stress, Taiwan. 13–16 Aug. 1992. Asian Vegetable Research and Development Center, Tainan Taiwan. pp. 257–270.

Forester BP, Thomas WTB and Chlaipek O (2005). Genetic controls of barley root systems and their associations with plant performance. *Asp. Appl. Biol*. 73:199-204.

Fussell LK, Bidinger FR and Bieler P (1991). Crop physiology and breeding for drought tolerance: research and development. *Field Crops Res.* 27(3): 183-199.

Garg, AK, Kim Kj, Owens TG, Ranwala AP, Choi YD and Kochian LV (2002). Trehalos accoumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci. USA.* 99: 15898-15903.

Gharibi S, Tabatabaei BDS , Saeidi G, Goli SAH and Talebi M (2012). Effect of drought stress on some physiological properties and antioxidant activity of Achillea tenuifolia Lam. *J. Herbal Drugs* 7: 181-190.

Ghasem RD (2014). Evaluting the best indicators and indntiying the most tolerant vareties to drought in potato varieties. *IJB.* 7(4): 282-288.

Ghen H and Jiang JG (2010). Osmotic adjustment and plant adaptive to environment changes related to drought and salinity. *Environ. Rev.* 18:309-319.

Gillespie KM, Chae JM and Ainsworth EA (2007). Rapaid measurement of tatol antioxidant capacity in plants. *Nat. Protoc.* 2: 870 – 874.

Guar PM, Krishnamurthy L and Kashiwagi J (2008). Improving drought-avoidance root traits on chickpea (Cicer arietinum L)- current states of research of ICRISAT. *Plant Prod. Sci.* 11 : 3-11.

Guo C, and Oosterhuis DM (1997). Pinitol occurrence in soybean plants as affected by temperature and plant growth regulators. *J. Exp. Bot.* 46: 249-253.

Hall AE (1993). Is dehdration tolerance to genotypic differences in leaf sensecence and crop adaptation to dry environments? pp.1-10. In: Close TJ and Bray EA (ed). Plant responses to cellular dehydration during environmental stress. The America Soc. Plant pathologists. Rockville, mary land.

Hall AE, Ismail AM, Ehler JD, Marto KO, Cisse N, Thiaw S and Close TJ (2002). Breeding cowpea for tolerance to temperature extremes and adaptation to drought. Old.iita.org/details/cowpea_pdf/cowpea_1.2 .pdf.

Hall AE, Thiaw AM, Ismail and Ehlers JD (1997). Water use efficiency and drought adaptation of cowpea pp 87:98. In Singh BB (ed) *advance in cowpea research*. IITA Ibadan, Nigeria.

Hall AE, Zhu H, Zhu XW, Royce T, Gerstein M, and Synder M (2004). Regulation of gene expression by mrtabolic enzymes. *Science* 306: 482-484.

Hall AJ, Conner DJ and Whitfield DM (1990). Root respiration during grain filling in sunflower: the effect of water stress. *J. Plant Soil* 121-57:66.

Hamidou G, Zombre and Braconnier (2007). Physiological and Biological responses of cowpea genotypes to water stress under glasshouse and field conditions. *J. Agron. Crop Sci.* 193: 227-239.

Hanin M, BriniF, Ebel C, Toda Y, Takeda S and Masmoudi K (2011). Plant dehydrins and stress tolerance: Versatile proteins for complex mechanisms. *Plant Signal. & Behav.* 6: 1503-1509.

Harb A, Krishnan A, Ambavaram MMR, Pereira A (2010). Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Plant Physiol.* 154: 1254–1271.

Hayatu M, Muhammad SY and Habibu UA (2014). Effect of water stress on the leaf relatives water content and yield of some cowpea (Vigna ungiculta L) walp) genotype. *Intl. J. sci. & Technol. Res*. 3 (7) : 2277-8616.

Hura T, Hura K and Grzesiak S (2009). Leaf dehydration induces different content of phenolics and ferulic acid in drought-resistant and -sensitive genotypes of spring triticale Z. *Naturforsch.* 64: 85 – 95.

Ilker E, Tatar O, Aykut Tonk F, Tosun M and Turk J (2001). Determination of tolerance level of some wheat genotypes to post-anthesis drought. *Turkish J. Field Crops* 16(1):59-63.

Impa SM, Nadaradjan and Jagadish SVK (2012). Drought stress induced reactive oxygen species and anti-oxidant in plants. *Abiotic Stress Response in Plant:* pp 131-147*; D*oi 10: 1007/1978-1-4614-0634-1-7.

IPCC (2007). Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds Solomon SD Qin M, Manning M Chen Z, Marquis M, KB Averyt KB, Tignor B and . Miller HL). Cambridge, UK and New York, USA: Cambridge University Press.

Jaradat MR, Feurtado JA, Huang D, Lu Y and Cutler AJ (2013). Multiple roles of the transcription factor AtMYBR1/AtMYB44 in ABA signaling, stress responses, and leaf senescence. *BMC Plant Biol.* 13:192-110.

Johansson I, karlsson M, Johanson U, Larsson C and kiellbom (2000). The role of acquaproins in cellular and whole plant water balance. *Bochim. Biophys. Acta* 1465: 324-342.

Kameli A and Losel DM (1993). Carbohydrates and water status in wheat plants under water stress. *New Phytol.* 125: 609-614.

Kang Y, Han Y, Torres-Jerez, Wang M, Tang Y, Monteros M, and Udvard M (2011). System responses to long-term drought and re-watering of two contrasting alfalfa varieties. *Plant J.* 68: 871-889.

Keller F and Ludlow MM (1993). Carbohydrate metablism in drought-stressed leaves of pigeon (Cajanus canjan). *J. Exp. Bot.* 44: 1351-1359.

Koocheki A, Nassiri-Mahallati M and Azizi G (2008). Effect of drought, salinty and defoliation on growth characteristic of some medicinal plants of Iran. *IJHSMP* 14: 37-53.

Kumar A and Singh BB (2003). Root characteristics in cowpea related drought tolerance at the seedling stage. *Exp. Agric.* 39: 29-38.

Kumar D (2005). Breeding for drought resistance. In: Ashraf M and Harris PJC (eds), Abiotic stresses: plant Resistance through Breeding and Molecular Approaches, pp. 145-175. The Haworth Press, New York.

Kumar s, Dwived SK, Singh SS, JHA SK, Leskshamy S, Elachenzhian R, Singh ON, and Bhatt BP (2014). Identification of drought tolerant rice genotypes by analyzing drought tolerance indices and morphol-physiological traits. *SABARAO J. Breeding & Genet.* 42(2) 217-230

Lawlor DW and Cornic G (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environ.* 25: 275–294.

Lee SB, Kim H, Kim RJ and Suh SH (2014). Overexpression of Arabidopsis MYB96 confers drought resistance in *Camelina sativa* via cuticular wax accumulation. Plant Cell Reports [epub ahead of print].

Lenka, SK, Katiyar A, Chinnusamy V and Bansal KC (2011). Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. *Plant Biotechnol*. 9:315–327.

Levitt J (1972). Responses of plants to environmental stresses. NewYork, NY :Academic Press. PP 34-46.

Liu C, LiU Y, Gu K, Fan D, Li G, Zheng Y, Yu L and Yang R (2011). Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. & Exp. Bot.* 71: 174-183.

Lobell DB and Gpirdij SM (2012). The influence of climate change on Global crop productivity. *Plant Physiol.* 60: 1686-1697.

Maroufi K, Farahani HA and Moradi O (2011). Increasing of seedling vigor by hydro priming method in cowpea (Vigna sinesis L). *Adv. Environ. Biol.* 5 : 3668-3671.

Madamba R, Grubben GHJ, Asante IK and Akormah R (2006). *Vigna unguiculata* (L) Walp. [internet] record from prota 4U. Brink M & Belay G (editors). PROTA (Plant Resources of Tropical Africa).

Mahdieh M, Mostajeran A, Horie T and Katsuhara M (2008). Drought stress alters water relations and expression of pip-type aquaproin genes in Nicotiana tabacum plants. *Plant Cell Physiol.* 49 : 801 -13.

Mai-kodomi Y, Singh BB, Myers O, Yopp JH, Gibson PJ, and Terao (1999a). Two mechanism of drought tolerance in cowpea. *Indian J. Genet.* 59: 309-316.

Masle JJ, Gilmore SR and Farquhar GD (2005). The Erecta gene regulates plant transpiration efficiency in Arabidopsis. *Nat. Plant* 436*: 866-870.*

Matsui T, Singh BB (2003). Root characteristics in cowpea related to drought tolerance at the seedling stage. *Exp. Agric.* 39: 29-38.

Maurel C, Veroucq L, Luu DT and Saytoni V (2008). Plant aquaproins: membarane channels with multiple integrated functions. *Ann. Rev. Plant Biotechnol.* 59:595-624.

Mcmanus MT, Bieleski RL, Caradus JR and Barker DJ (2000). Pinitol accumulation in mature leaves of white clover in response to a water deficit. *Environ. & Exp. Bot.* 43: 11-18.

Miller G, Suzuki N, Cifti-Yilmazi S and Mittler R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* 33: 453-467.

MOA (2014). 2012 Annual agriculture survey report. pp. 110-111. Agriculture statsistics section, Ministry of Agriculture, Department of Research, statistics and policy development. Statistics Botswana, Gaborone.

Mohamed HI and Akladious SA (2014). Influence of garlic extract on enzymatic and non enzymatic antioxidants in soybean plants (*Glycine Max*) grown under drought stress. *Life Sci. J.* 11: 46-56.

Mohammed MF, Kentgen N, Tawfik AA and Noga G (2002). Dehydration- avoidance responses of tepary bean lines differing in drought resistance. *J. plant physiol.* 159: 31-38.

Morgan JM, Rodriquez-Maribaona B and knights EJ (1991). Adaptation to water deficit in chickpea breeding lines by osmoregulation: relationship to grain yields in the field. *J. Field Crops Res.* 27:61-70.

Morrison1 JIL, Baker NR, Mullineaux1 PM and Davies WJ (2008). Improving water use in crop production. *Philos. Trans. R. Soc.* 363: 639 - 658.

Muchero W, Ehlers JD and Roberts PA (2008). Seedling stage drought induced phenotype and drought-responsives genes in diverse cowpea genotypes. *J. Crop Sci.* 48:541 -552.

Munne-Bosch S and Alegre L (2004). Die and let live; leaf senescence contributes to plant survival under drought stress. *Funct. Plant Biol.* 31: 203-216.

Munne-Bosch S, Falara V, Pateraki I, Lopez-Carbonell M, Cela J and Kanellis AK (2009). Physiological and molecular responses of the isoprenoid biosynthetic pathway in a drought-resistant Mediterranean shrub, *Cistus creticus* exposed to water deficit. *J. Plant Physiol.* 166:136 -145.

Naghavi M R, Aboughadareh A P and Khalili M (2013). Evaluation of drought tolerance indices for screening some of corn (Zea Mays L.) cultivars under environment conditions. *Not . Sci. Bio*l. 5: 388-393.

Nair SA, Abraham TK and Jaya DS (2008). Studies on the changes in lipid peroxidation and antioxidants in drought stress induced cowpea (Vigna unguiculata L.) varieties. *J. Environ. Biol.* 29: 689-691.

Nazari L and Pakniyat H (2010). Assessment of Drought Tolerance in Barley Genotypes. *J. Appl. Sci. 10: 151-15.*

Neumann PM (2008). Coping mechanisms for crop plants in drought-prone environments. *Ann. Bot.* 101: 901-907.

Ng and Marechal R (1985). Cowpea taxonomy, origin and germplasm. In: Singh SR, Rachie KO (eds) Cowpea Research, Production and Utilization. John Wiley and Sons, Ltd., Chichester, NY, PP. 11-21.

Ngugen TTT, Klueva N, chamareck V, Aarti A, Magpantay G, Millena ACM, Pathan MS and Ngugen HT (2004). Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Mol. Genet. & Genomics* 272: 35-46.

Nguyen A. and Lamant A (1988). Pinitol and *myo*-inositol accumulation in water-stressed seedlings of maritime pine. *Phytochem.* 27: 3423-3427.

Ntomebla Z (2012). Growth and yield responses of cowpeas (*Vigna unquiculata L*) to water stress and defoliation, MSc. Thesis, Unuversity of Kwazulu-Natal SA.

Nyugen HT, Babu RC and Blum A (1997). Breeding for drought resistance in rice; Physiology and molecular genetics considerations. *J. Crop Sci.* 37: 1426-1434.

Ogbonnaya CI, Sarr B, Brou C, Diouf O, Diop NN and Roy-Macauley H (2003). Selection of cowpea genotypes in hydroponics, pots and field for drought tolerance. *Crop Sci.* 43: 1114-1120.

Pandy S, Chen J, Jones AM and Assmann SM (2006). G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and post germination development. *J. Plant Physiol.* 141: 243–256.

Passioura JB (1982). The role of root system characteristics in the drought resistance of crop plants. In Drought in crops with emphasis on rice. International Rice Research Institute, Manila. pp. 71-82.

Picotte JJ, Rosenthal DM, Rhode JM and Cruzan MB (2007). Plastic responses to temporal variation in moisture availability: consequences for water use efficiency and plant performance. *Oecologia* 153: 821-832.

Pungulani LLM, Milliner JP and William WM (2012). Screening cowpea (Vigna unguiculata) germ plasm for canopy maintenance under water stress. *Agron. N. Z.* 42: 110-122.

Reyazul RM, Mainassara ZA, Nese S, Trethowan R and Varshney RK (2012). Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *J. Crop Sci.* 47: 1210-1952.

Rosielle AA and Hamblin J (1981). Theoretical aspects of selection for yield in stress and non-stress environment. *Crop Sci.* 21: 943-946.

Sabaghpour SH, Kumar J and Rao TN (2003). Inheritance of growth vigor and its association with other characters in chickpea. *J. Plant Breed.* 26: 542- 544.

Sadok W and Sinclair TR (2012). Crops yield increase under water-limited conditions: review of recent physiological advances for soybean genetic improvement. *Adv. Agron.* 113:313-337.

Shao H, Chu L, Jaleel CA and Zhao C (2008). Water deficit stress-induced anatomical changes in higher plant. *C. R. Biol.* 331: 215-225.

Sheveleva E, Chmara W, Bohnert HJ and Jensen RG (1997). Increased salt and drought tolerance by D-ononitol production in transgenic Nitotiana tabacum L. *J. Plant Physiol.* 115:1211-1219.

Shiklomanov IA (1999). World Water Resources: Modern Assessment and Outlook for the 21st Century, (Summary of World Water Resources at the Beginning of the 21st Century, prepared in the framework of the IHP UNESCO). Federal Service of Russia for Hydrometeorology & Environment Monitoring, State Hydrological Institute, St. Petersburg.

Shinozaki K and Yamaguchi-shinozaki K (2007). Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* 58: 221-227.

Silvente S, Sobolev AP and Lara M (2012). Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. *PloS ONE* 7: e38554. doi:10.1371/journal.pone.0038554.

Simoe-Aranjo Jk, Alves –Ferreira M, Rumjanek NG and Margis-pinheirr M (2008). Vunlp1 (NOD 26-like) and VuHSP17.7 gene expression are regulated in response to heat stress in cowpea nodule. *Environ. & exp. Bot.* 63: 256-265.

Singh BB (2005). Cowpea [*Vigna unguiculata* (L.) Walp. In: Singh RJ, Jauhar PP (eds) Genetic Resources, Chromosome Engineering and Crop Improvement. Volume 1, CRC Press, Boca Raton, FL, USA, pp. 117–162.

Singh SK, and Reddy KR (2011). Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. *J. Photochem. & Photobiol. B:Biol.* 105:40-50.

Singh SK, Kakani VG, Surabhi GK, and Reddy KR (2010). Cowpea (*Vigna unguiculata* [L.] Walp.) genotypes response to multiple abiotic stresses. *J.Photochem. &Photobiol. B:Biol.* 100:135-146.

Singh, BB, Chamblis OL and Sharma B (1997). Recent advances in cowpea breeding. Pp 30–49 *in* Advances in Cowpea Research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, and Japan International Research Centre for Agricultural Sciences (JIRCAS) copublication. Available at IITA, Ibadan, Nigeria.

Singh, BB, Mai-Kodomi Y and Terao T (1999). Relative drought tolerance of major rainfed crops of the semi-arid tropics. *Indian J. Genet.* 59: 437-444.

Sio-Se Mardeh A, Ahmadi A, Poustini K, Mohammadi V (2006). Evaluation of drought resistance indices under various environmental conditioning. *Field Crops Res.* 98: 222-229.

Sletvold N and Jon Agren J (2012). Variation in tolerance to drought among Scandinavian populations of Arabidopsis lyrata. *Evol. Ecol.* 26: 599-577.

SociasX, Correia MJ, Chaves MM, Medrano H (1997). The role of abscisic acid and water relations in drought responses of subterranean clover. *J. Exp. Bot.* 48: 1281–1288.

Songsri P, Jogloy, Holbrook CC, Keshmala T. Vorasoot N, Akasaeng C and A.patanothai (2008*).* Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. *Agric. Water Manag*. 96: 790-798.

Souza RP, Maechado EC, Silva JAB, Lagoa AMMA, and Silveria JAG (2003). Photosynthesis gas exchange, chlorophyll indluence and cowpea (Vigna unguiculata) during water stress and recovery. *J. Exp. Bot.* 51: 45-56.

Stoll M, Loveys B and Dry P (2000). Hormonal changes induced by partial rootzone drying of irrigated grapevine. *J. Exp. Bot*. 51: 1627-1634.

Streeter JG, Lohnes DG and Fioritto RJ (2001). Pattern of pinitol accumulation in soybean plants and relationships to drought tolerance. *Plant Cell Environ.* 24: 429-438.

Swindale LD and Bidinger FR (1981). Introduction: the human consequence of drought and crop research priorties for their alleviation in the physiology and biochemistry of drought resistance in plant. Paleg LG and Aspinall D (eds). Academic press. Sydney, Australia pp. 1-13.

Talebi R , Fayaz F and Naji AM (2009). Effective selection criteria for assessing drought stress tolerance in durum wheat (Triticum durum Desf.). *Gen. Appl. Plant Physiol*. 35: 64-74.

Tuberosa R (2012). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers Physiol.* 10: 3389-00347.

Tuberosa R and Selvi S. (2006). Genomics approaches to improve drought tolerance in crops. *Trends in Plant Sci.* 11: 415-412.

Van Jaarsveld AS, Briggs R, Scoles RJ, Bohensky E, Reyers B, Lynam, T, Musvoto C and Fabricius C (2005). Measuring conditions and trends in ecosystem services at multiple scales: the Southern African Millennium Ecosystem Assessment (SAfMA) experience . Transactions of the Royal Society. *Biol. Sci.* 360: 425-441.

Varshney R K, Thudi M , Nayak S N, Gaur P M, Kashiwagi J , Krishnamurthy L, Jaganathan D, Koppolu J, Bohra A,Tripathi S, Rathore A, Jukanti A K, Jayalakshmi V, Vemula A, Singh S J, Yasin Md, Sheshshayee M S and Viswanatha K P (2014). Genetic dissection of drought tolerance in chickpea (Cicer arietinum L.). *Theor. Appl. Genet.* 127: 445-462.

Veeranagamallaiah G, Prasanthi J, Reddy KE, Pandurangaiah M, Babu OS, Sudhakar C. (2011). Group 1 and 2 LEA protein expression correlates with a decrease in water stress induced protein aggregation in horsegram during germination and seedling growth. *J. Plant Physiol.* 168: 671–677.

Volaire F and Thomas H (1995). Effects of drought on water relations, mineral uptake, water soluble carbohydrate and survival of two contrasting populations of cockfoot (*Cactylis glomerata* L. *Ann. Bot.* 75: 513-534.

Vurayai R, Emongor V and Moseki B (2011). Effect of water stress imposed at different growth and development stages on morphological traits and yield of bambara groundnuts(*Vigna subterranean* L. Verdc). *Am. J. Plant Physiol*. 6:17-27.

Yang S. Vandderbeld B, Wan J and Huang Y (2010). Narrowing down the targets: towards sucessful gentic engineering of drought-tolerant crops. *Mol. Plant* 3: 469- 490.

Watanable I, Hakoyama T, Terao and Singh BB (2012). Evalution methods for drought tolerance in cowpea. pp 141-146 in advances in cowpea research, edited by B.B Singh, D.R. Mohan Rajan, K.E. Dashiell and L.E.N. Jackai. Copublication of International Institute of Research Centre for Agricultural Science (JIRCAS). IITA, Ibandan, Nigeria.

Weidner S, Karolak M, Karamac M, Kosinska A, Amarovik R (2009). Phenolic compounds and properties in grape vine (Vitis Vinifera L.) under drought stress followed by recovery. *Acta Societatis Soc. Bot. Pol.* 78: 97-103.

Xu BC, Gichuki L, Shan L and Li FM (2006). Above ground biomass production and soil water dynamics of four leguminous forages in semiarid region, northwest china. *S. Afr. J. Bot.* 72: 507-516.

Xu C, Zhang R, Qu Y, Miao Z, Zhang Y, Shen X, Wang T, Dong J (2014). Overexpression of MtCAS31 enhances drought tolerance in transgenic Arabidopsis thaliana mm embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt in transgenic rice. *J. Plant Physiol.* 110: 249-257.

Yoo CY, Pence HE, Jin JB, Miura K, Gosney MJ, Hasgawa PM Mickelbert MV (2010). The Arabidopsis GTLT transcription factor regulates water use efficiency and drought tolerance by modulating stomatal density via transepression of SDD1. *Plant Cell environ.* 22: 4125-4133.

Zhang X, Ervin E H, Evanylo GK, and Haering KC (2009). Impact of biosolids on hormone metabolism in drought-stressed tall fescue. *J. Crop Sci.* 49:1893-1901.

Zhou S, Hu W, Deng X, Ma Z, Chen L, Huang C, Wang C, Wang J, He Y, Yang G and He G. (2012). Overexpression of the Wheat Aquaporin Gene, TaAQP7, Enhances drought tolerance in transgenic tobacco. PLoS ONE 7: e52439.

Doi: 10.1371/journal.pone.0052439.