CLASSIFICATION OF DROUGHT TOLERANT COWPEA I 'VIGNA UNGUICULATA (L). WALP)' GENOTYPES BASED ON MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES TO WATER DEFICIT

MASTERS OF SCIENCE

IN CROP SCIENCE (AGRONOMY)

BY

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OCTOBER, 2015

UNIVERSITY OF BOTSWANA

BOTSWANA COLLEGE OF AGRICULTURE

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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).

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STATEMENT OF ORIGINALITY

Every bit of work within this dissertation was completed put together by the author 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 diploma in any university around the world.

ACKNOWLEDGEMENTS

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 at 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 me the a scholarship. A humble thanks to Mr. James Dolo of CARI for his serviced during my studied. 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 in 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 investigated during 8-12 days imposition of drought stress at vegetative stage using 20 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 laidout 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 significantly ($P<0.05$) reduced 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 tolerant ; BCA020, BCA014, BCA013, BCA012, BCA007, BCA008, BCA010, BCA005 and BCA013 were sensitive and highly sensitive.

The poor relationship between BMP and gas 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 tolerance rather BMP. The BMP results can be wholly used in crop drought tolerance improvement program and breeding in Botswanna especially under green house condition.

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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 tolerance

DTc: Drought tolerance categories

FAO: Food Agriculture Organization

FW: Fresh weight

HDT: Highly drought tolerant

IITA: International Institute of tropical Agriculture

LD_{50}: Lethal drought 50

LEA: Late embryonic abundant

LSD: Least significant difference

MOA: Ministry of Agriculture

MP: mean productivity

MT: Moderately 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

WUE: Water use efficiency

CHAPTER ONE

INTRODUCTION

1.1. General introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a major economically 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 regions where rainfall resources are characteristically low (300-600 mm) (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, exacerbates the problem. Despite its inherent capacity to survive drought, significant differences exist among cowpea genotypes in drought tolerance (Mai-Kodomi *et al.*, 1999a). 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, where resistance refers to a combination of both avoidance and tolerance. According to Ntombela, 2012 and 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 tolerant plant's response to drought stress, the activation of genes and transmission are involved in the genetic make-up for drought tolerance (Shinozaki and Yamaguchi-Shinozaki, 2007). These genes include those that govern 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 (ROS) (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) for crops like cowpea (Muchero *et al.*, 2008; Mai-Kodomi *et al.*, 1999a; Pungulani *et al.*, 2012).

In cowpea and other plants, drought tolerant morpho-physiological traits, which are genetically controlled, have been determined. These traits include water use efficiency (WUE), relative turgidity, osmotic adjustment, leaf gas exchange, relative water content (RWC), diffusion pressure deficit, chlorophyll stability index and carbon isotope discrimination (Hall et al., 1990; Morgan et al., 1991; Souza *et al.*, 2003; Anyia and Herzog, 2004; Ntombela, 2012). 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 determining 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 (Cisse *et al.*, 1997; Matsui and Singh, 2003). 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 collection of genotypes and some mechanism of tolerance.

1.1. 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 accounts for 66% of total water; this can be as high as 90% in arid region (Shikomanov, 1991; 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.*, 2014). Drought stress also had a 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 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 existence in Africa.

Botswana is still far in cowpea drought tolerance improvement program, which is posing severe threat to cowpea production and utilization. Moreover, the identification of cowpea genotypes among cowpea 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.

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 reseach done at the seedling and reproductive stages of cowpea, the vegetative stage is paramount and it's use in this study; since it has been noted to be most sensitive to drought stress in South Africa (Ntombela, 2012).

Importantly, literature on cowpea agronomy and water stress adaptation in Botswana is limited and this study will help to fill gap . Additionally, researchers have been focused on established legumes such as dry bean (*Phaseolus vulgaris*) and Bamabara groundnut (Vurayai *et al.*, 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 classify cowpea genotypes based on morphological and physiological parameters in response to drought stress during 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 conditions.

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

CHAPTER TWO

LITERATURE REVIEW

2.1. The cowpea crop: Its uses

Cowpea belongs to the family fabaceae. It is one of the members of the three Vigna (genera) in which both the freshly leaves and seeds are consumed (Madamba *et al.*, 2006). The other members are *V. subterranean* (L.) Verdc (Bambara groundnut) and *V. radiata* (L.) Wilczek (Mungbean) and they are considered 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; 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).

2.2. 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 (Ehlers and Hall, 1997; Singh *et al.*, 1997; Ntombela, 2012). In a drought stress screening study, the overall ranking of crops in increasing order of drought tolerant 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 the Savanna and Sahel sub-regions. In fact, drought stress is regarded as a major limitation to crop production in some developing countries and it periodically causes agricultural yield losses in crop like cowpea in 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 are 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 mechanisms to deal with drought stress are 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 activiteics (tissue and cell of the plant) . Previous studies have indicated that proline (Cheulile and Agenbag, 2004; Hamidou *et al.*, 2007), 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 and 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).

2.3. 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 crop production especially in regular limited rainfall of the semi arid regions (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 drier, this would be reduction of about 1.5% yield of crop per decade (Lobell and Gpirdij, 2012). In Southern Africa, among other extremes, there will be limited rainfall, 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 under drought stress. 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).

2.4. 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 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).

2.4.1. Mechanism of drought avoidance

2.4.1.1. 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 (Kumar and Singh, 2003; Farooq *et al.*, 2010; Tuberosa, 2012). 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 (Craz de Carvalho, 2000; Ogbonnaya *et al.*, 2003). 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).

2.4.1.2. 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 cowpea (Hall *et al.*, 2004) and Arabidopsis (Masle *et al.*, 2005; Yoo *et al.*, 2010). 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) and *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) and *Arabidopsis lyrata* (Sletvold and Agren, 2012).

Drought avoidance can also involve rapid phenological development, here referred to as early vigour. Early vigour 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 favour a rapid colonization of space and resources and contribution to improved yield stability in drought prone 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 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 provides evidence that it accumulates in drought stressed soybean than either proline or sucrose; this indicated that it was osmoprotectant (compatible solute that contributes to the stabilization of cell membrane druing drought regime) in crops. 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 (*Medicago 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).

2.5.2.2. 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 against 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).

2.5.2.3. The antioxidant systems and drought tolerance

During drought stress in chloroplasts, limitation of CO_2 fixation, overreduction of the electron transport chain with a high-energy state are transferred to molecular oxygen (O_2) to form 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 damage to cell components, proteins and nucleic acids (Baier *et al.*, 2005). In addition to the chloroplast other sources 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 and programmed cell death.

Plant cells and their components are protected against the detrimental effects of 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 has been associated with phenolic compounds in wheat (Hura *et al.*, 2009), alfalfa (Kang *et al.*, 2011 (e.g. flavonols, (iso) flavones, flavanones, flavan-3-ols proanthocyanidins, and anthocyanin). In cowpea, genotypes water deficit selection study, 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.

2.5.3. Photosynthesis and drought stress

When plant experience drought stress, it exhibit physiological changes in order to response to the drought condition. Stomatal closure is an early indicators of physiological change, it reduces the quality of CO_2 entering the leaves and decreases the transpiration level of the plant. Such physiological change affects respiration and photosynthesis machinery of the plant resulting in low yield, staunted plant, low chlorophyll content, wilted leaves and decrease the dry matter of the plant (Wahid and Rasul, 2005; Ntombela, 2012).

However, stomatal closure due to water stress affects chloroplast development, assimilates allocation, nutrient translocation and accumulation process (Farooq *et al.*, 2008). Drought stress is accompany by high temperature which oxygenase Rubisco, and brings about photoinhition resulting in denaturing the emzymes of the plant and reducing the plant yield.

Evidently, it was clearly indicated by Farroq *et al.*, (2008) that photosynthesis limitations during drought stress is due to stomatal closure. Anjum *et al.*, (2011) highlighted the net photosynthesis (33%), stomatal conductance (25%), transpiration rate (38%) and intercellular Co_2 (6%) in drought stressed studied in maized compared with well-watered.

2.5.4. Conclusion

Generally, drought stress affects plant growth machinery like the cell turgor and meristem depending on the species and the inherent degree of tolerance as well as the magnitude of the water deficit, and how fast the plants exprience this water stress determine the effects on plant height, leaf area, dry matter and yield. However, a crop like cowpea has the inherent ability to performance better under water deficit. Its response to drought stress by displaying morpho-physiological changes (stomatal closure, osmotic adjustment, impaired photosynthesis machinery and chlorophyll content) and biochemical changes (proline, reactive oxygen species, antioxidants and sugar alcohols). These changes in response to drought stress affect the plant height, leaf area, biomass yield, dry matter of the plant, and its triggers the plant to carry on avoidance, escape and resistance mechanisms which makes the plant to be susceptible or tolerant to water stress.

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° 34°'S and latitude 25° 57' at altitude of 994 m above sea level). Two major experiments were conducted in the green house during the vegetative phase of cowpea, 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 2–28 of October, 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. Polythene bags size of 20cm×15cm 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 fully expanded trifoliate leaf after irrigation was withdrawn and followed by drought treatment. For this preliminary growth media were mixed as shown in the Table 1.

Table 1: 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

3.1.2. Experimental Design

The experimental layout was a Completely Randomized Block Design (CRBD), with four replications for each treatment in the green house. The plants were exposed to drought stress by withdrawal irrigation for 12 days. The cowpea genotype (Blackeye) was used as a proxy to determine the suitable soil mixture for the entire experiment due to time and resource limitation. During the 12 days water stress treatment, the following variables were measured:

3.1.2.1. 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 at 5.5 cm depth of the soil in polythene bag at 10–11am 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.

3.1.2.2. Chlorophyll content

During the dry down period, chlorophyll content was monitored with a 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.

3.1.2.2. Plant height

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

3.1.2.3. Plant Biomass Yield

The plant biomass yield was determined by harvesting the plant on the last day of drought stress period, and the plants were oven dried at 80°C for 48 hours, after than it were weights and measured in gram(g).

3.1.3. 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:

$$Y = aX + C$$

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 (LD50)

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 set-up

A Completely Randomized Block Design (CRBD) 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 for 6 days) (DL-1).

Drought Level 2 (irrigation withdrawal for 8 days) (DL-2).

Drought Level 3 (irrigation withdrawal for 11 days) (DL-3).

3.2.2. Calculation of LD_{50}

During the dry-down period, soil moisture 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 at the end of each irrigation withdrawal days for control and treatment using 30 cm rulers.

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 drying for 24 hours at 105.8 °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 genotypes of cowpea seed were obtained from Farmers and Traders, Seed Multiplication Unit Ministry of Agriculture, National Plant Genetic Resources Centre (NPGRC) and The International Institute of Tropical Agriculture (IITA). The genotypes are listed in Table 2.

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

ID No	Genotypes	Source
BCA001	Blackeye	BCA Sec 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

A Completely Randomized Block Design (CRBD) was used for the experiment in the green house from November 2014–January 2015.

The 20 genotypes were planted in wooden boxes (block) with 5 cm in row and 10 cm between rows, each row containing 8 plants per genotype. The screening boxes were 12 cm deep of 85 cm wide and length of 117 cm long. Cowpea was planted at a depth of 4cm. Drought stress was applied after the first trifoliate leaf had fully expanded. The treatment(irrigation withdrawal) for eight days that established LD_{50} in section 3.2.1 was applied according to the experimental procedures and protocols.

3.3.3. Variables measured

Well-watered and non-watered treatments were applied on 20 cowpea genotypes after the first trifoliate leave had actually expanded under greenhouse conditioned. At the end of the experiment, BYp and BYs were measured and used to calculate the following: biomass stress susceptibility index (BSSI), biomass relative drought index (BRDI), biomass Drought Resistance Index (BDRI) biomass stress tolerance index (BSTI), tolerance (TOL), biomass mean production (BMP), and biomass yield reduction percentage (BYR%), 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.

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

No.	Index	Calculation	Reference
1	Biomass Stress Susceptibility Index (BSSI)	$\frac{1 - (BYs/BYp)}{1 - (B\bar{Y}s/ B\bar{Y}p}$	Naghavi *et al.*, 2013
2	Biomass Relative Drought Index (BRDI)	$\frac{(BYs/BYp)}{(B\bar{Y}s/B\bar{Y}p)}$	Naghavi *et al.*, 2013
3	Biomass Stress Tolerance Index (BSTI)	$\frac{(BYs \times BYp)}{(B\bar{Y}s^2)}$	Nazari and Pakniyat, 2010
4	Tolerance (TOL)	BYp – BYs	Nazari and Pakniyat, 2010
5	Biomass Mean Production (BMP)	(BYs+BYp)/2	Darabad, 2014
6	Biomass Drought Resistance Index (BDRI)	(BYs×(Bys/BYp) $B\bar{Y}s$	Farshadfar *et al.*, 2013
7	Biomass Yield Reduction percent (BYR%)	*[BYp – Bys]/ [BYp)] x 100*	Farshadfar *et al.*, 2013

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. Data analysis

The significant index (BMP) was subjected to IBM SPSS statistics 21 analysis in order to construct a dimensional plot, which classified the genotypes according to their tolerance levels.

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 cowpea genotypes of interest were selected from the 20 genotypes in section 3.3. 2 highly drought tolerant (HDT), three drought tolerant (DT), two moderately tolerant and one drought sensitive (DT) were used 3.3. This experiement was meant for morpho-physiological screening in the greenhouse. The same ploythene bag size 20cm×15cm used in 3.2 was used and filled with soil mixure C. The experiement was conducted in the green house from January to March, 2014.

3.4.2. Experimental Design

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

1-(Highly drought tolerant) (HDT)
2-(Drought tolerant) (DT)
3-(Drought moderate) (DM)
4- (Drought sensitive) (DS)

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 eight 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 h after which its turgid weight (TW) was measured. The samples were then oven dried at $82^{O}C$ for 24 h and weighed also measured (DW). Relative water content was calculated according to the formula:

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

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

3.4.3.3. Leaf gas exchange measurement

The full-leaflet of each cowpea genotype 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 gase exchange measurement, whole plants were harvested (leaf separated from stem) and oven dried at 82^{o}C for 48 h, from each root, shoot and root:shoot ratio were calculated: Dry matter of the root / dry matter of the shoot = root:shoot ratio.

3.5. Statistical analyses.

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. Selection of appropriate growth medium

The results presented in Figure 1 and 2 and Table 4 and 5 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 and 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 the 12 days of withholding water, plant perfomance was measured in the five soils. The results showed that plants grown in the medium had the tallest plants and highest yield, which indicated good perfomance compared with other soil media (Figure 3 and 4). Therefore, the soil media C (60% river sand, 30% loam soil, 10% compost) was chosen as appropriate for subsequent experiments in this research project.

4.2. Lethal drought-50 (LD50) of cowpeas

The results presented in Figure 5 and 6 and Table 6 show that the effect of drought stress duration was significant as early as eight (8) days when biomass was reduced by approximately 50% (Figure 5). The reduction in biomass yield was also followed by reduction in plant height, which was also approximately 50% (Figure 6). 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 cowpea genotypes

The results presented in this section, show that drought stress affected soil moisture content (Figure 7) and plant performance in all the 20 genotypes in terms of plant height (Figure 8 and 9), leaf area (Figure 10) and chlorophyll content (Figure 11 and 12). Variations in these parameters were; 48.67–59.20 % (for plant height), 11.3 – 51.4% (for leaf area) and 1. 42 – 25.48% (for chlorophyll content). The result further showed that there were significance between the twenty (20) genotypes for the above parameters. However, the result of plant perfomance could not be used at this level to clearly 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. 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 20 that were screened. Biomass yield under both well watered (Byp) and drought stress (Bys) was measured (Table 7) in order to use it to calculate the various indices, further to determine the most suitable index for drought tolerant classification. Correlations analysis of these was done with each other and TOL, BMP, BSTI, BRDI, BSSI, BDRI, and %BYR determined (8). 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 8). 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) (Naghavi *et al.*, 2013). For the above reason, BMP was selected for further analysis of drought tolerance. 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 13) was further used to generate a three dimesional plot showed interelationships among BMP, Byp and Bys (Figure 14). The interelationship is presented as a cluster into the highest biomass yielding genotypes under both well watered and stress conditions (*highly drought tolerant*: 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 14).

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 9). 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 15). The results confirm the finding in section 4.3.1.

The imposition of drought stress for 12 days caused reduction of soil moisture in drougtht stressed treatments, which ranged from 0.30 to 2.5%. There were significant differences between the genotypes, whereby BCA001 left the highest soil moisture and BCA002 left the lowest, while other genotypes also indcated significant ($P>0.05$) soil moisture content (Figure 16). Reduction in soil moisture content resulted in RWC% reduction. However, there were no significant differences between the eight genotypes (Table 11) Plants were also analysized for their reduction in chlorophyll content due to drought, and it was found that there were significant differences ($P<0.05$) between the eight genotypes for both well-watered drought stressed treatments. 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 ($P>0.05$) differences in chlorophyll content (Table 12). An analysis for 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 18). The reduction in chlorophyll content (%) results showed that there were differences among the genotypyes and those that had less reductions are drought tolerant, and also was not significant ($P<0.05$) with biomass mean production (Table 14).

The biomass characteristics (shoot and root dry weight and shoot:shoot ratio) were also analysized. The result showed that there were significant differences ($P<0.05$) 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 ($P<0.05$) high value for this characteristic, while the other genotypes had similar root:shoot ratios (Table 10). These results indicate that in general the root to shoot ratios were similar for the genotypes under study.

Gaseous exchange (Photosynthesis, stomatal conductance and transpiration) was also determined. There was significant difference ($P<0.05$) in photosynthesis, stomatal conductance and transpiration between some of the genotypes under both well watered and drought stress conditions for all the gas exchange parameters (Table 13). 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 19). Similar results were obtained for stomatal conductance (Figure 20) and transpiration (Figure 21). These results indicate that gas exchange measurement may not be used as a screening selection mechanism for drought resistance in cowpea. But could be used to regulate stomata well and can be used for drought avoidance mechanism.

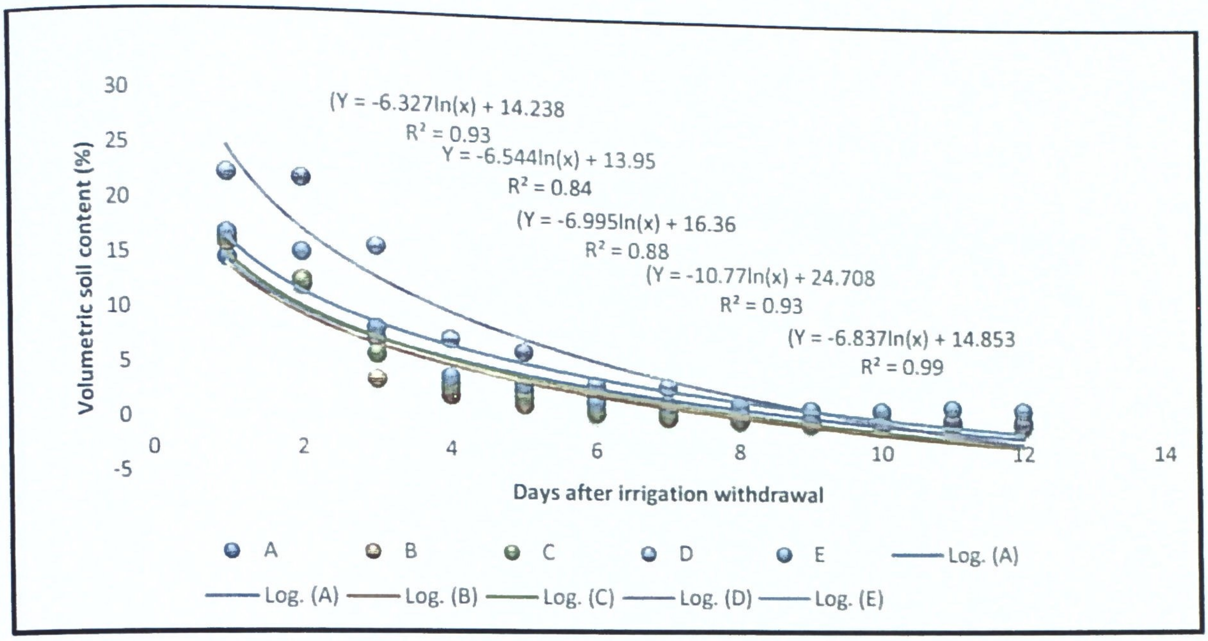

Figure 1: Effect of irrigation withdrawal on soil moisture losses of various medium.

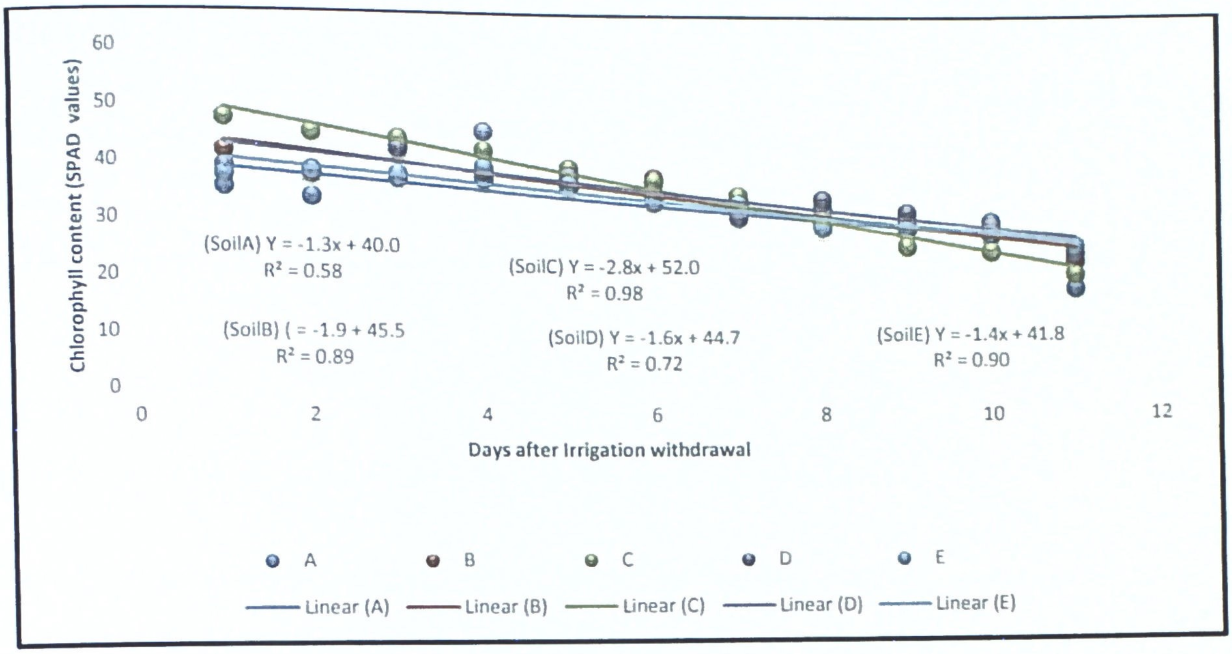

Figure 2: Effect of irrigation withdrawal on chloropyll content of cowpea genotype (backeye).

Table 4 : Means ranking of the soil moisture % .

	Soil Moisture Content Loss			
	($Y = aLnX + C$)			
Type of medium	Y intercept (C)	Slope (a)	R^2	Mean Rank
A	4	1	3	2
B	5	2	4	4
C	3	3	1	1
D	1	5	5	4
E	2	4	4	3

Table 5: Means Ranking of chlorophyll loss in SPAD meter reading.

	Chlorophyll Content Loss			
	($Y = aX + C$)			
Type of medium	Y intercept (C)	Slope (a)	R^2	Mean Rank
A	5	5	5	4
B	2	2	4	2
C	1	1	1	1
D	3	3	3	3
E	4	2	2	2

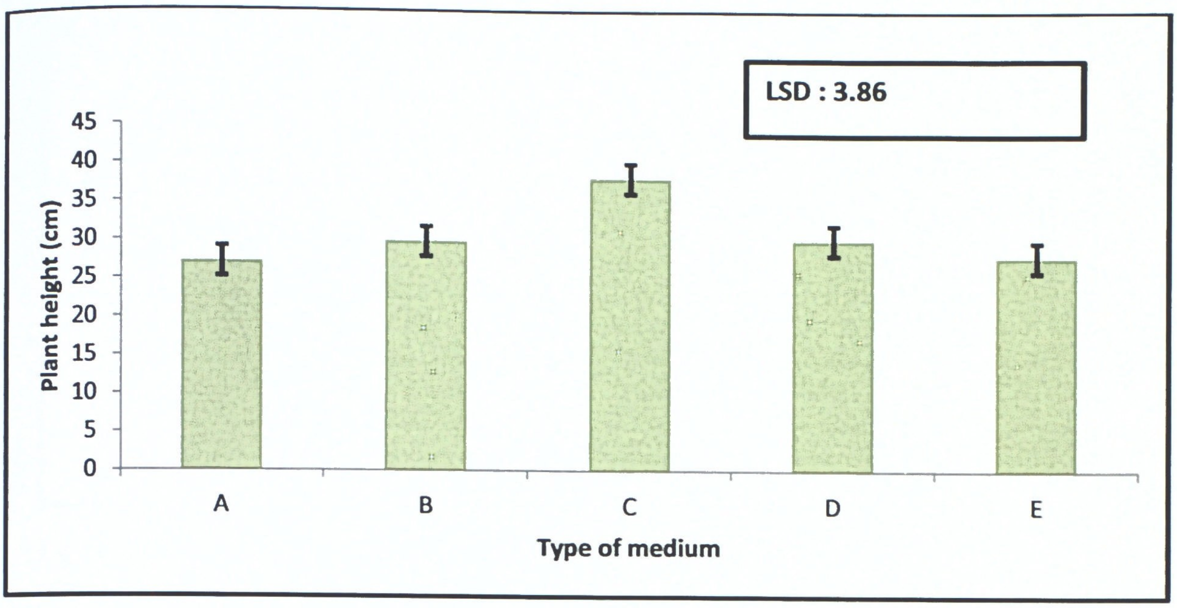

Figure 3: Effect of soil moisture content on plant height. Error bar represents standard error of means of 4 replicates.

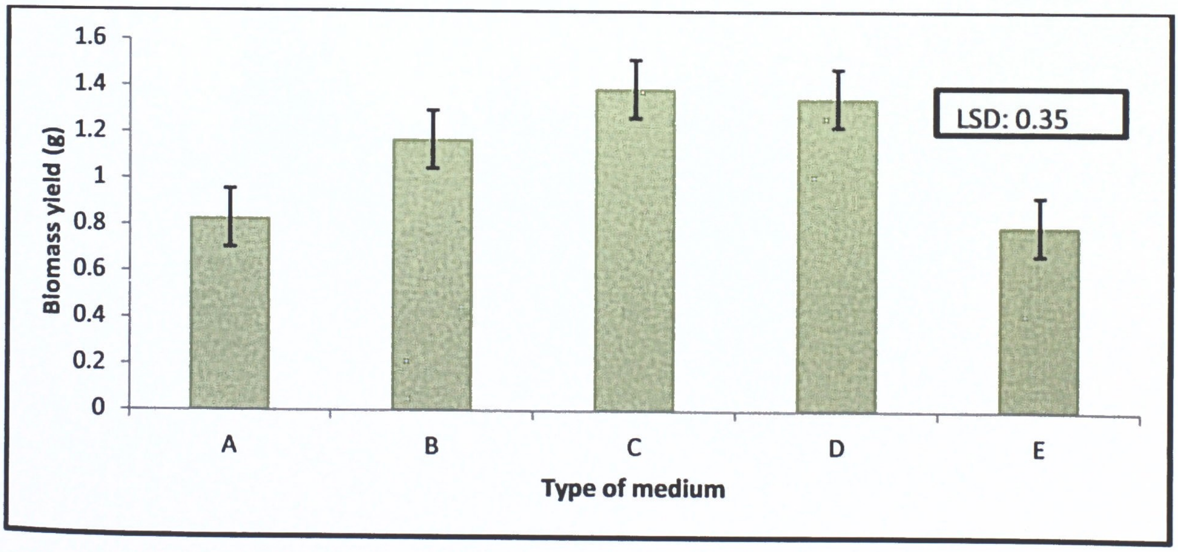

Figure 4: Effect of soil moisture on plant biomass. Error bar respresents standard error of the mean of 4 replicates.

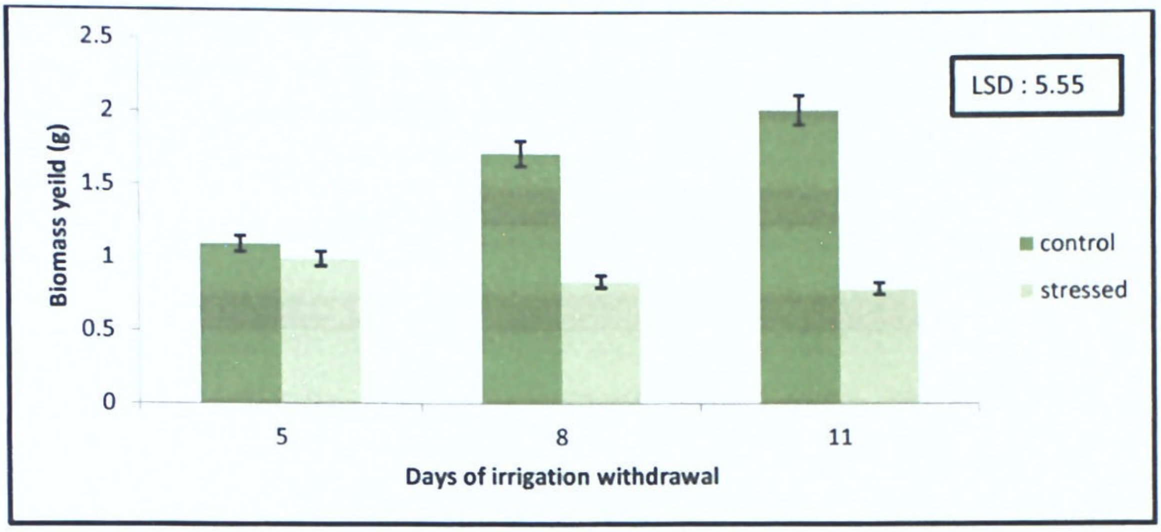

Figure 5: Effect of drought stress (water withdrawal days) on cowpea genotype biomass yield under green house condition. Error bars represent standard error of the mean of 4 replicates.

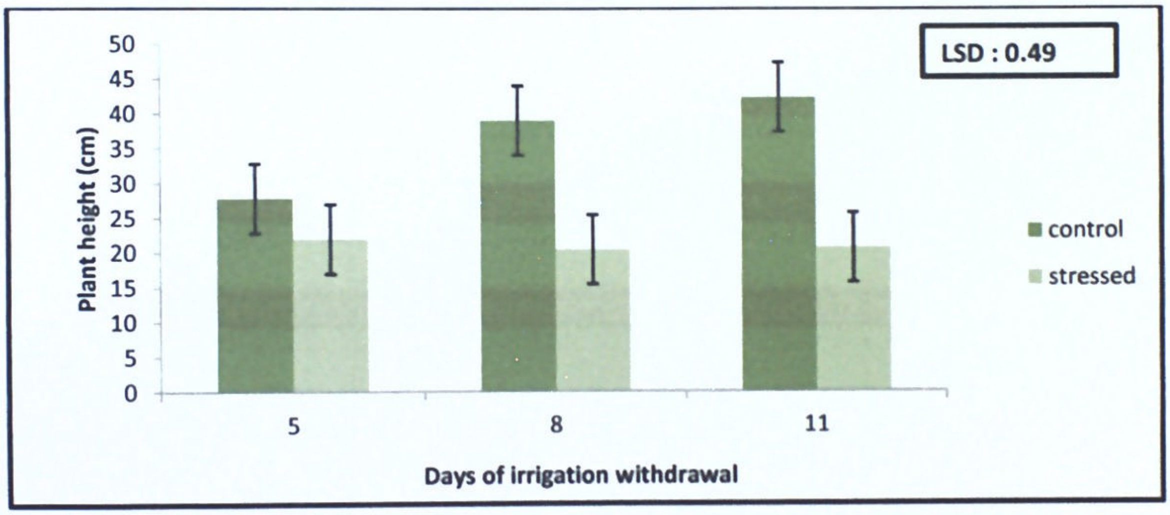

Figure 6: Effect of drought stress on plant height under green house condition. Error bars represent standard error of the mean.

Table 6: 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.

Type of medium	Irrigation withdrawal Days	Biomass Reduction %
A	0	0
B	6	9.8
C	8	51
D	11	61

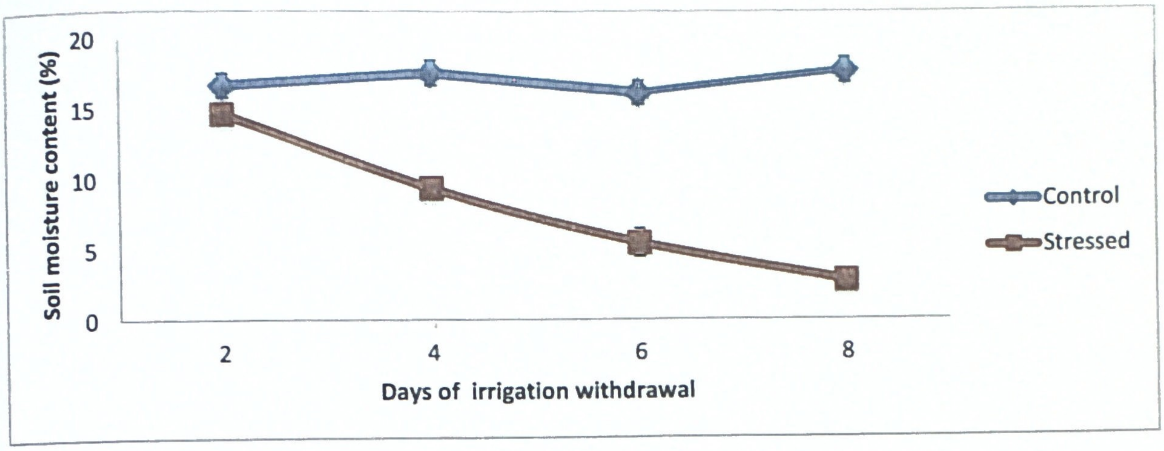

Figure 7: Effect of irrigation withdrawal on soil moisture loss. Error bar represents standard error of the means of 4 replicates.

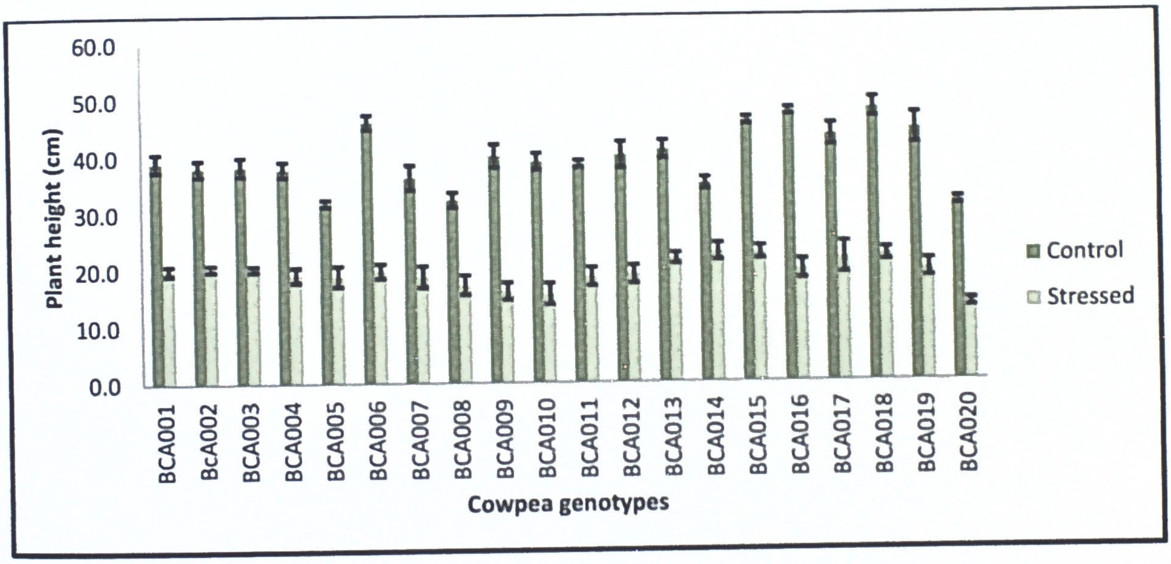

Figure 8: Effect of drought stressed on cowpea genotypes plant height (cm). Error bar represents standard error of the means of 4 replicates.

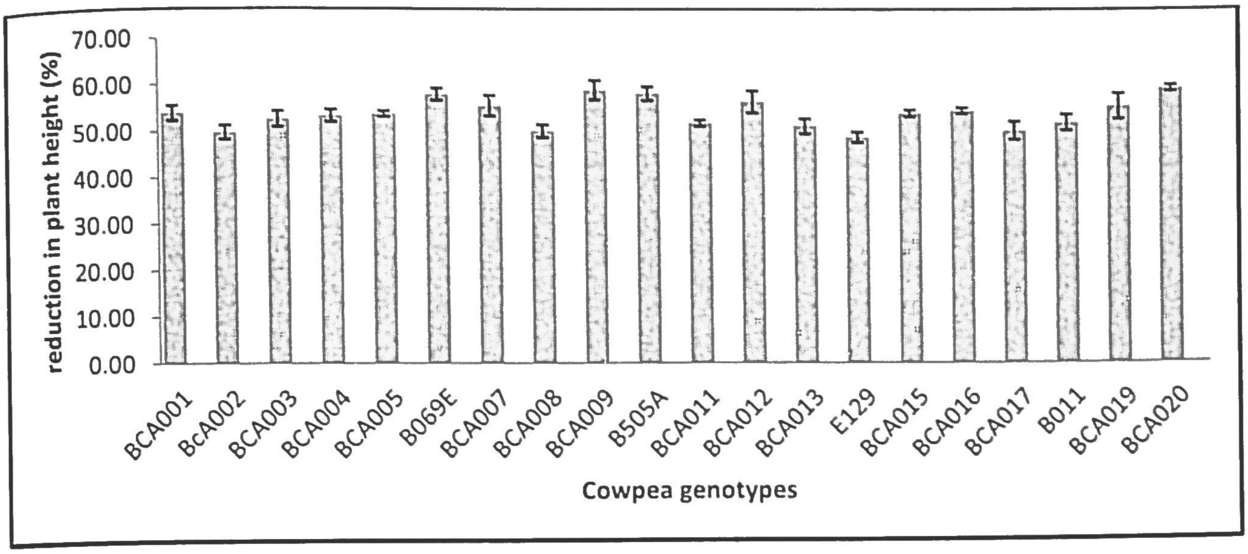

Figure 9: Percentage reduction in plant height due to drought stress. Erroe bar representts standard error of the means of 4 replicates.

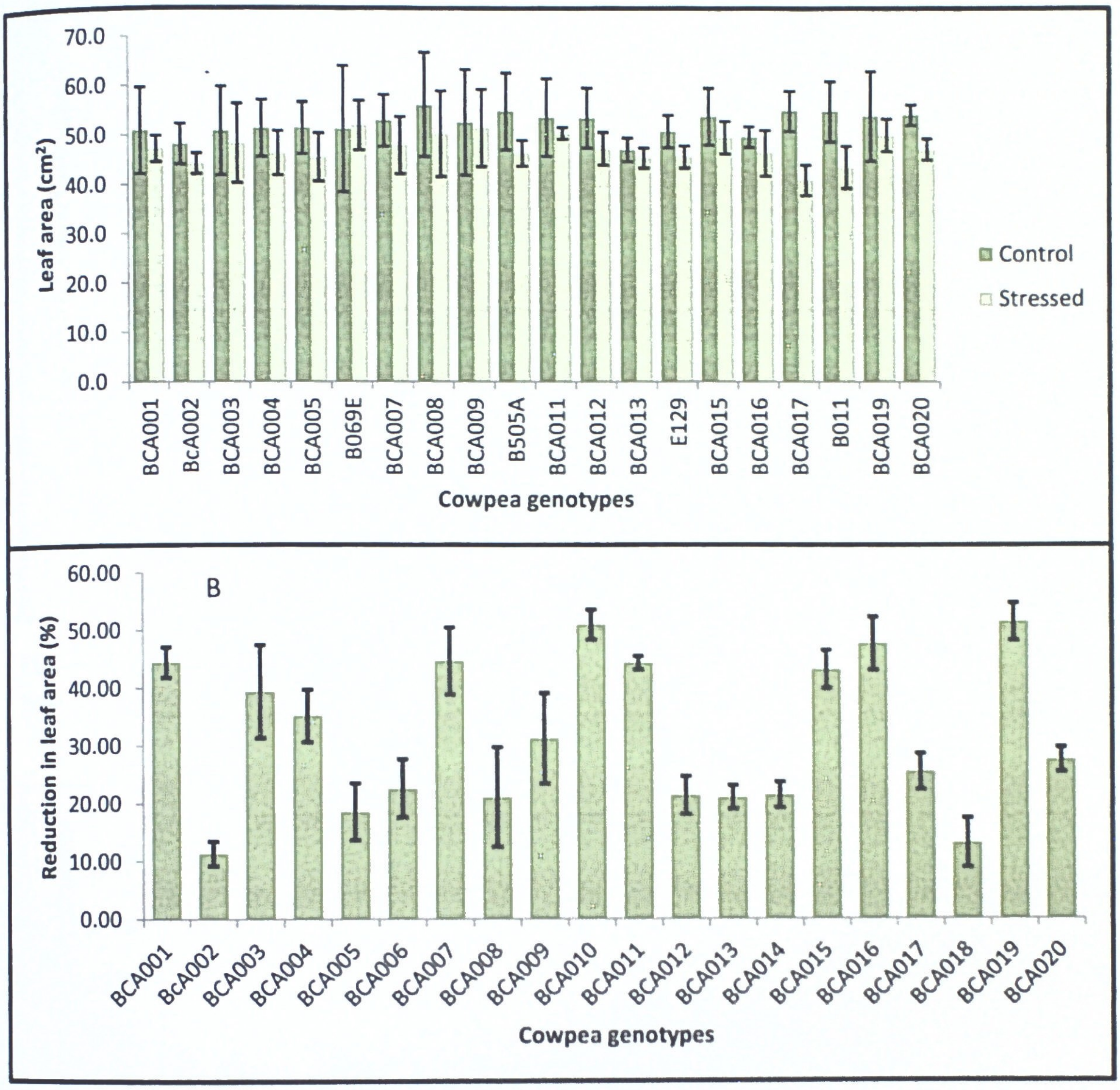

Figure 10: Effect of drought stress on leaf area (A) and their percentage reduction (B). Error bar represents standard error of the means of 4 replicates.

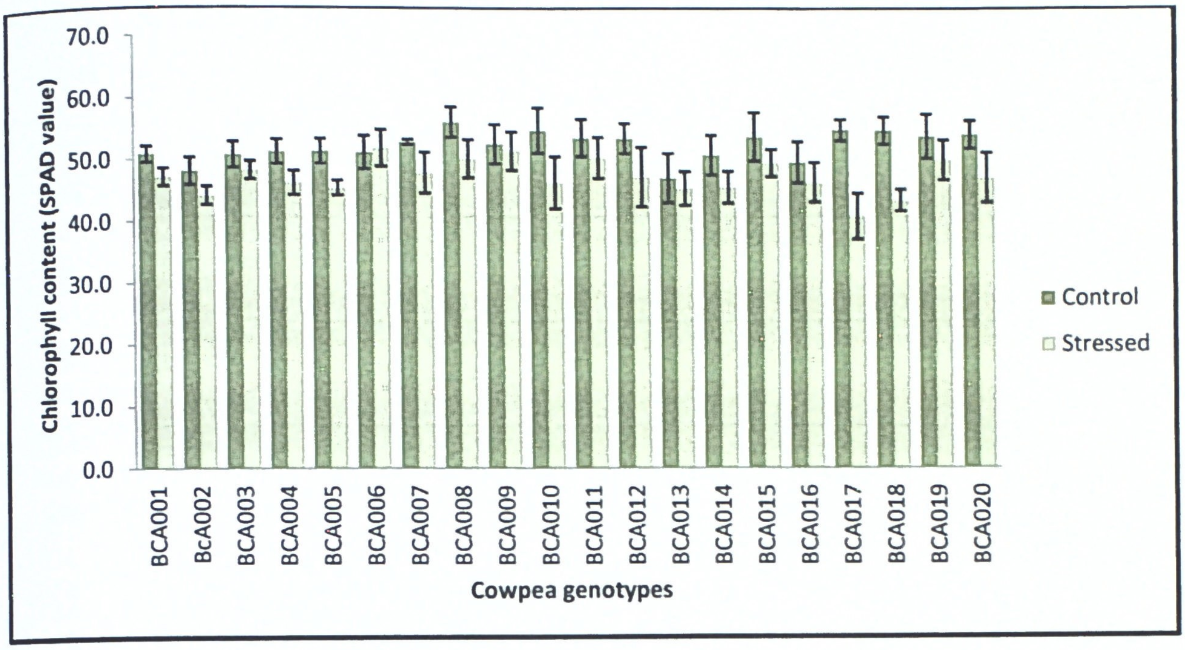

Figure 11. Effect of drought stress on cowpea genotypes chlorophyll content. Error bars respresent standard deviation of the means of 4 replicates.

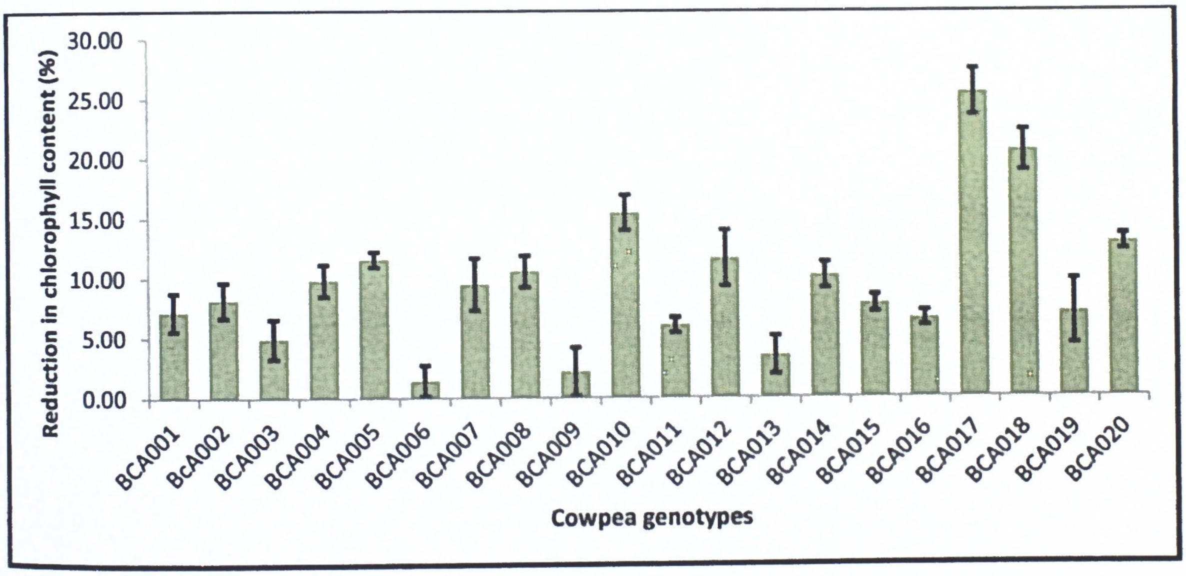

Figure 12: Percentage reduction in chloropyll content due to drought stressed. Error bars represent standard error of the means of 4 replicates.

Table 7: Tolerance indices of cowpea genotype under stress and non- stress condition in a greenhouse.

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, BDRI – biomass drought resistance index and BYR% – Biomass yield reduction percent.

Table 8: 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.

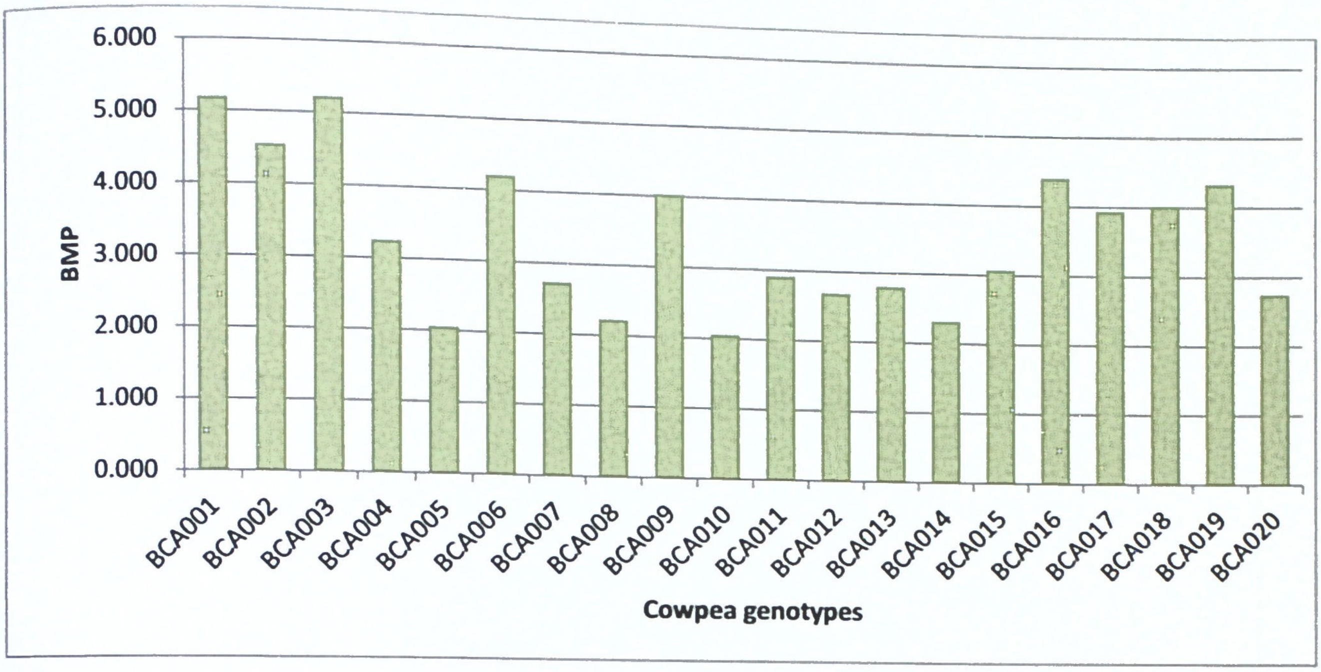

Figure 13: Determination of drought tolerance cowpea genotypes (highly, tolerance, moderate and sensitive) based on significant index (BMP-biomass mean productivity) . BMP value: 2-3 = sensitive, 3-4 = moderate, 4-5 = tolerance and 5-6 highly tolerance.

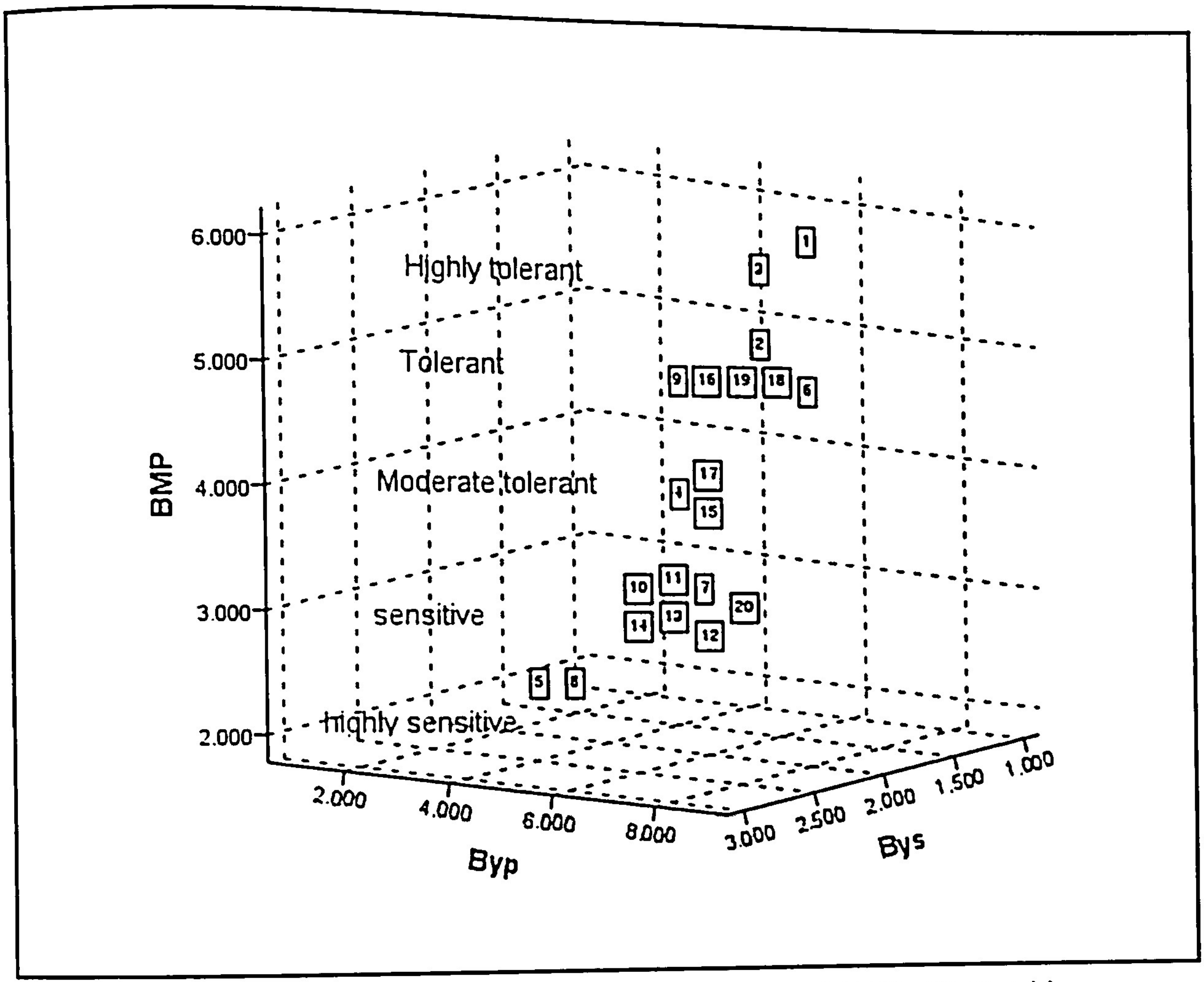

Figure 14: A three dimensional plot among BMP, BYP and BYS. BMP (biomass mean productivity, BYP (biomass yield well water) and BYS (biomass yield under water stress). 1= BCA001, 2= BCA002, 3 = BCA003, 4 = BCA004, 5 = BCA005, 6 = BCA006, 7= BCA007, 8 = BCA008, 9 = BCA009, 10 = BCA010, 11= BCA011, 12 =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

Table 9: 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.

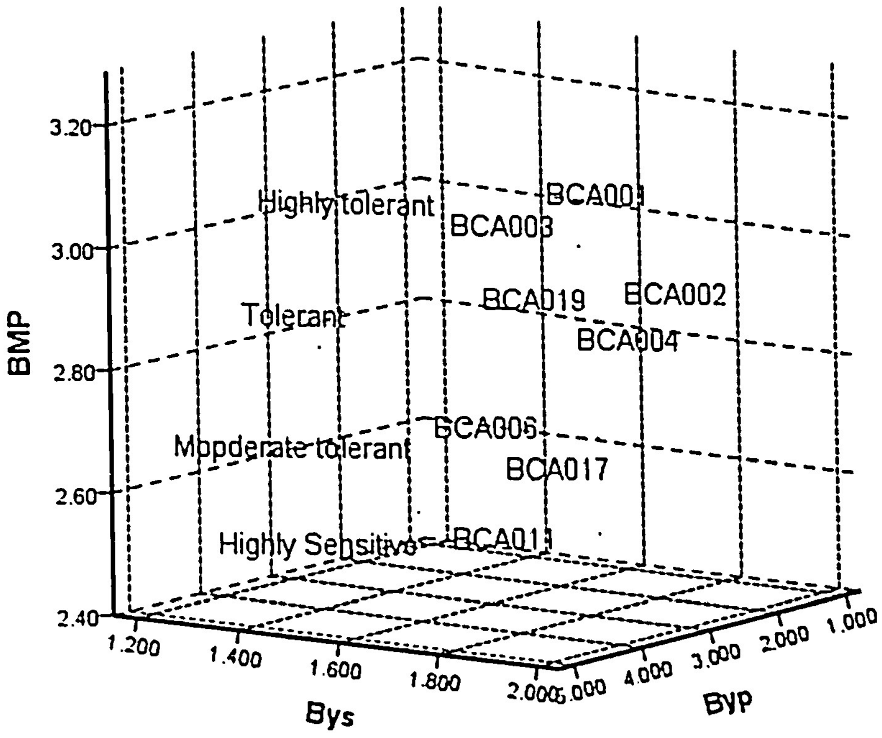

Figure 15: A three dimensional plot among BMP, BYs and BYp. BMP (biomass mean productivity), BYs (biomass yield under drought stress) and BYp (biomass yield under well watered conditions) for eight Cowpea genotypes grown in polythene bags in soil mixed (mixed C).

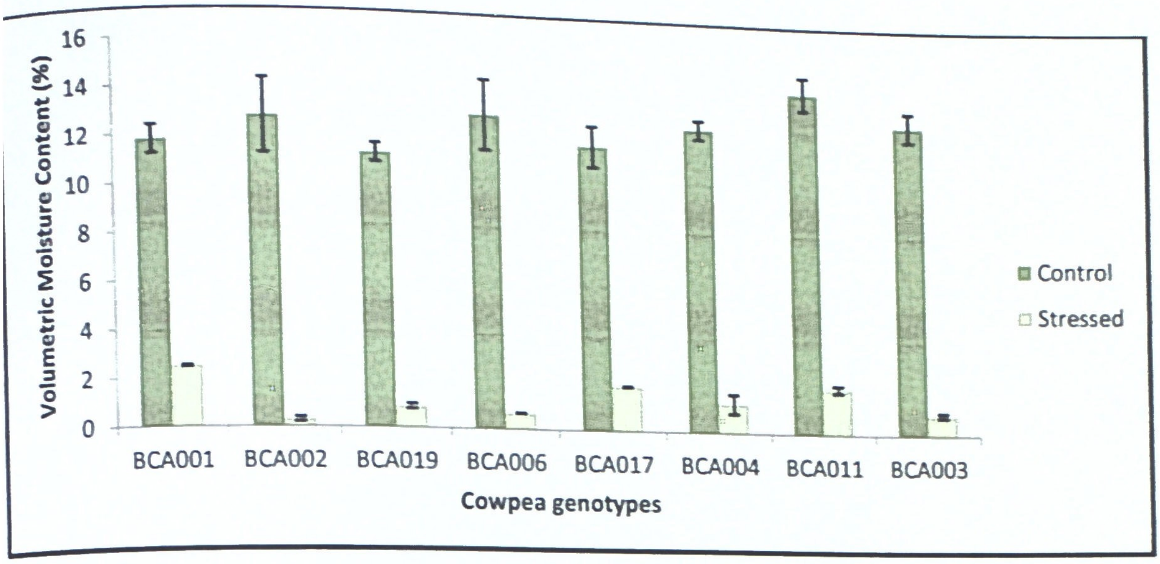

Figure 16: Effect of drought stress on soil moisture content on eight cowpea genotype during eight days of drought stress. Error bars respresent standard error of the means of 4 replicates.

Table 10: 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}

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 11: 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}

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 12: 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^{dc}
BCA011	DS	35.43^{c}	30.75^{c}

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 $P<0.05$.

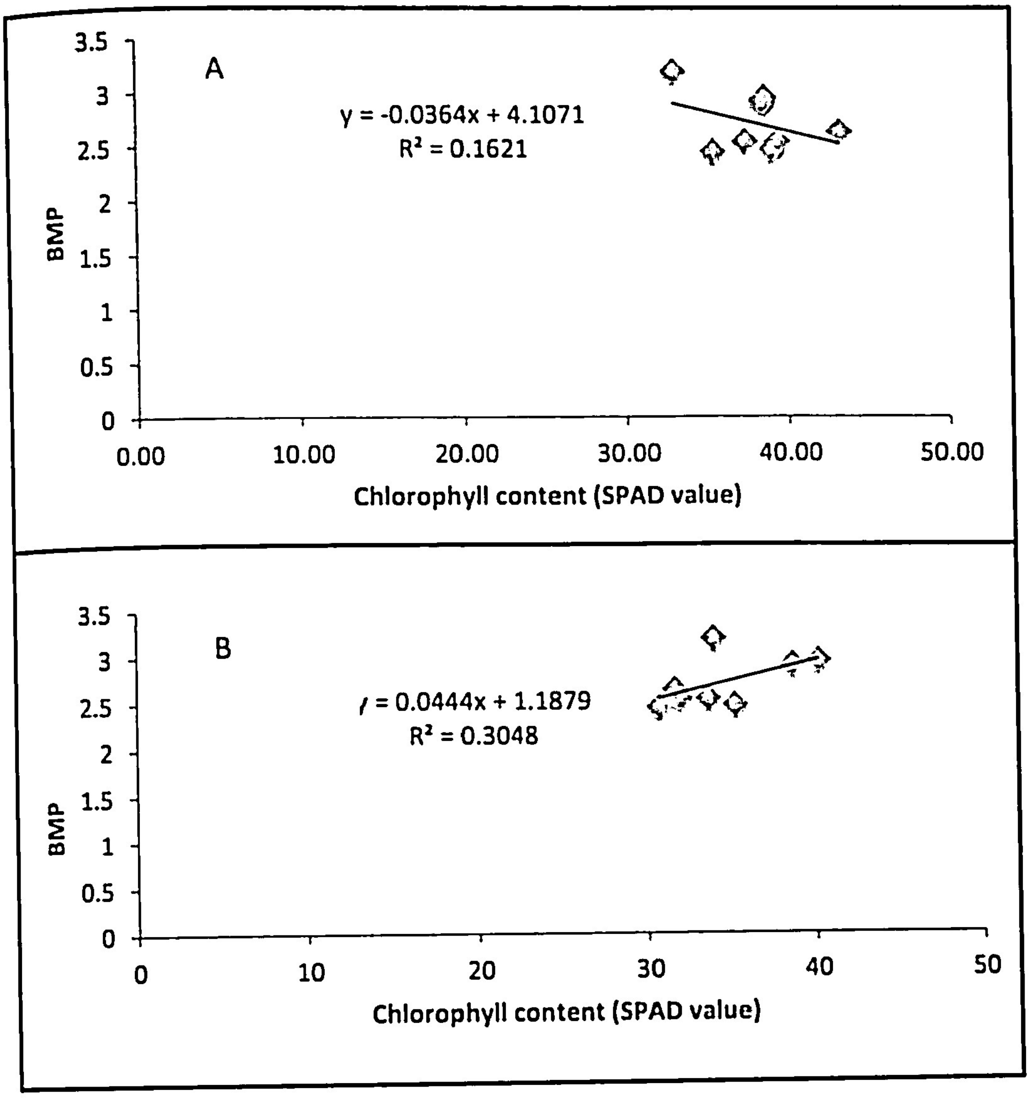

Figure 17: Relationship between chlorophyll content (SPAD value) and biomass mean productivity (BMP). A: Well-watered and B: Drought stress.

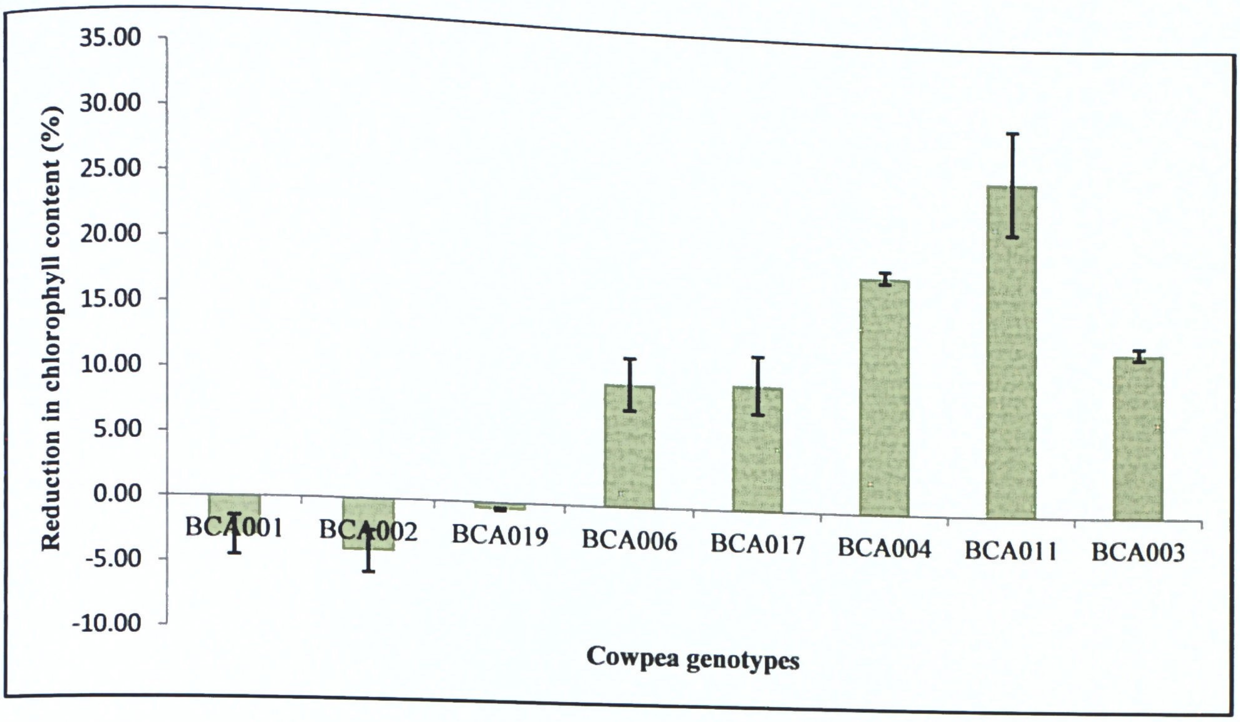

Figure 18: Precentage reduction in chlorophyll content due to drought stress . Error bars respresent standard deviation of the means of 4 replicates.

Table 13: 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 H_2O.m-2.s-1)	(mol H_20.m-2.s-1)	(mol CO_2 m^{-2} s^{-1})	(mol H_2O.m-2.s-1)	(mol H_20.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}

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 $_{0.05}$.

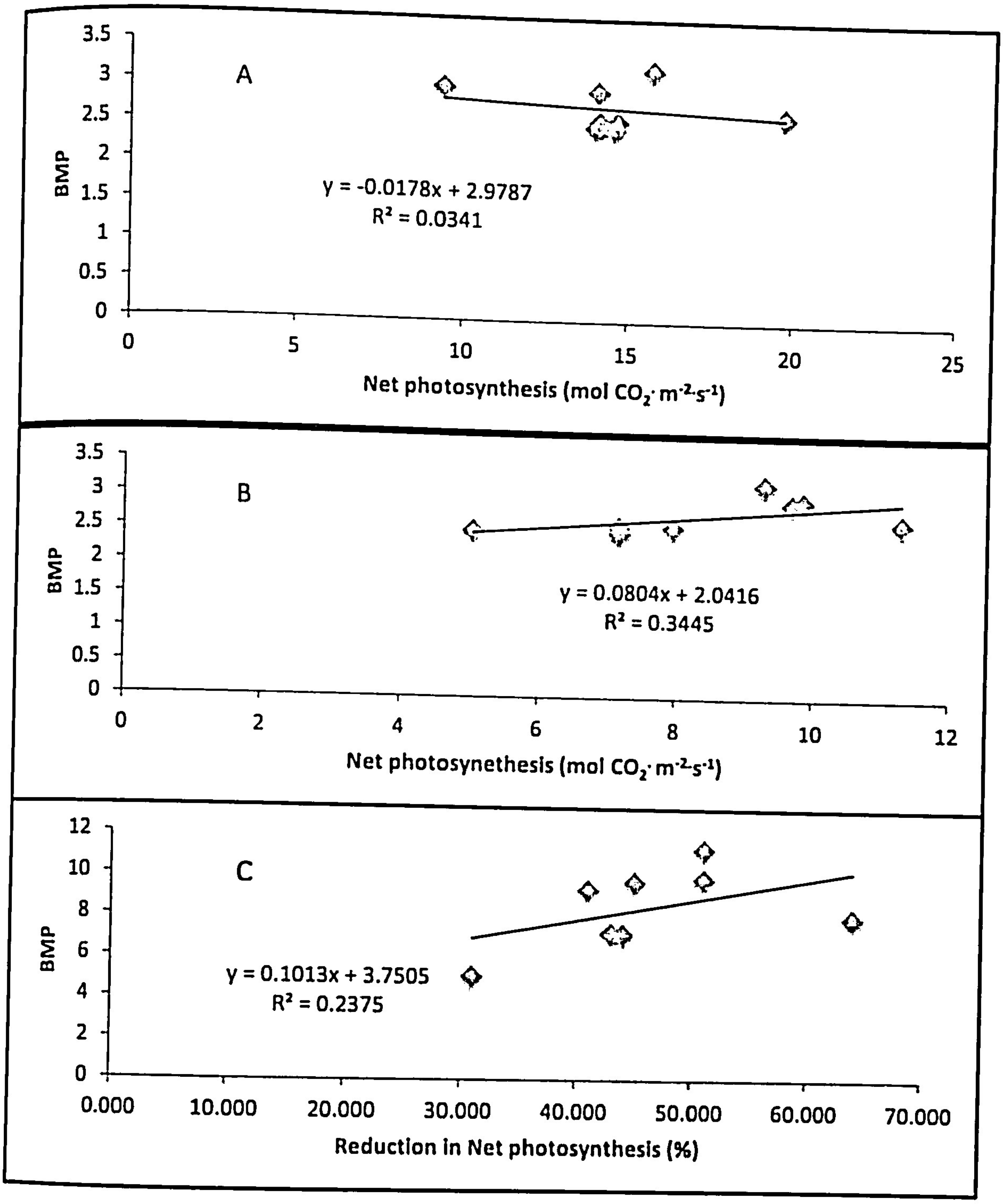

Figure 19: Relationship between net photosyntheis and biomass mean production (BMP). Well-watered (A), drought stressed (B) and reduction in net photosynthesis (C).

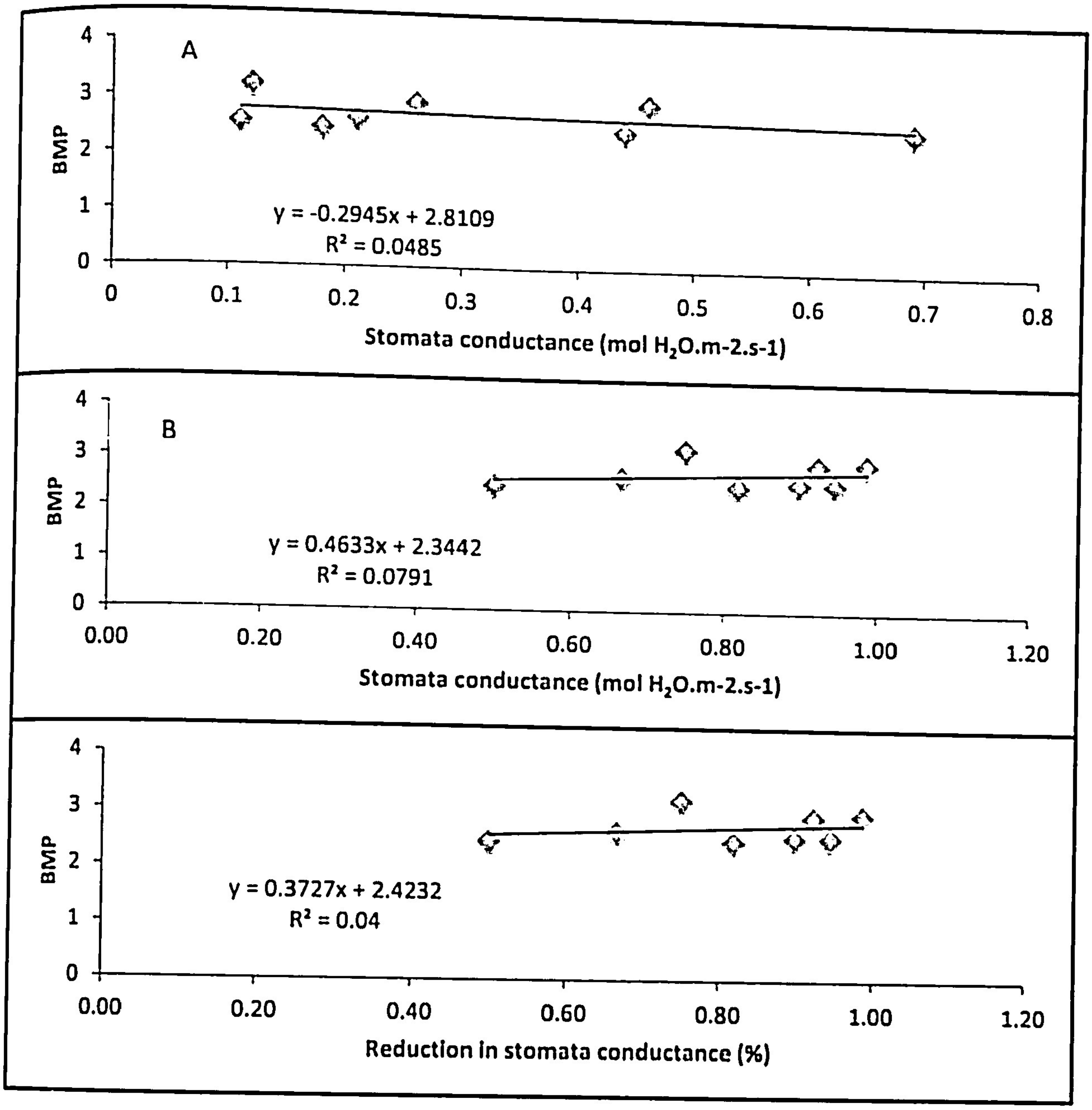

Figure 20: Relationship between stomatal conductance and biomass mean production (BMP). Well-watered (A), drought stress (B) and precentage reduction due to drought stress (C).

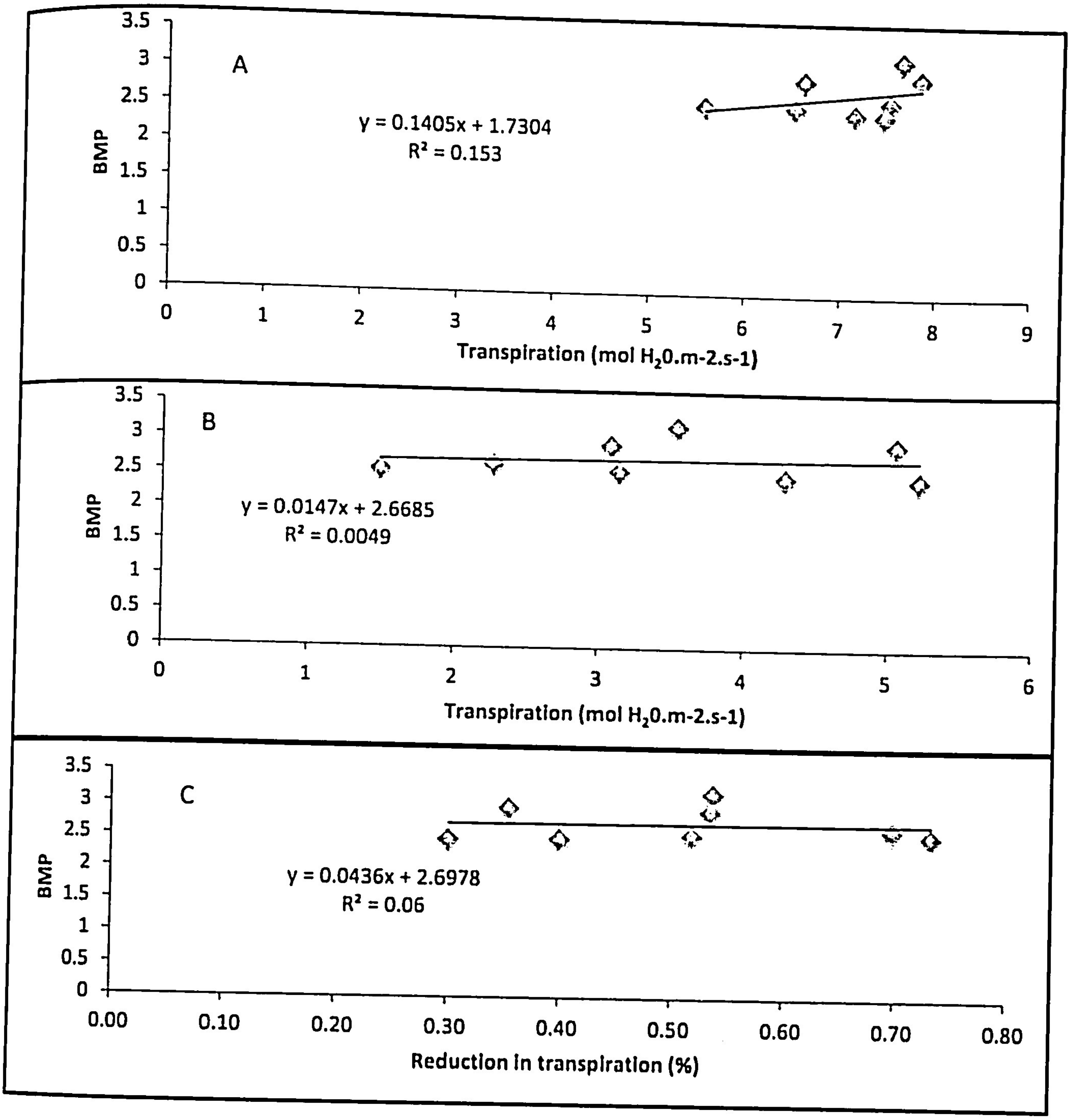

Figure 21: Relationship between transpiration and biomass mean production (BMP). Well-watered (A), drought stress (B) and percentage reduction in transpiration due to drought stress (C).

Table 14: Regression analysis for percent reduction in biomass yield and gaseous exchanges (net photosynthesis, stomatal conductance and transpiration) and chlorophyll content in well-watered and drought stressed conditions.

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	0.036	0.985	0.499	0.953
F-value	7.154	0.003	0.515	0.004

[a] values in parenthesis are standard errors and significant at ($P<0.05$).

Reg. Coef.= Regression Coefficient.

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 20 cowpea genotypes.

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

There were differential responses to drought stress as indicated by reductions in plant height, leaf area and chlorophyll content (Figure 8, 9, 10, 11 and 12). 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 the materials under study. This could be a result of each genotype's ability to affect antioxidant systems (Nairs *et al.*, 2008), accumulate proline (Costa *et al.*, 2011; Farouk *et al.*, 2013), pinnitol (Souza *et al.*, 2003) and 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.*, 2003; Kumar *et al.*, 2008; Hamidou *et al.*, 2007; Muchero *et al.*, 2008) and bambara groundnuts (Vurayai *et al.*, 2011).

The results shows that the 20 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 14 and 15), 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 14 and 15). 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

The results presented in Figure 18 showed that the eight genotypes differed in reduction in chlorophyll content (parameter). 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 was attributed to several reasons, but the idea among them was that some cowpea genotypes exhibited avoidance mechanism before or after drought stressed was initiated (Vurayai *et al.*, 2011; Ntomebla, 2012). The chlorophyll content and gas exchange were poorly related to BMP expected (Figure 19, 20 and 21), 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 (Socias *et al.*, 1997; Hamidiou *et al.*, 2007; Darwish and Fahmy, 1997; Vurayai *et al.*, 2011); 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 was 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 seemed as an appropriate drought tolerance selection index since chlorophyll content (Figure 17) and gaseous exchanges showed poor relatioship in this study (Figure 19, 20 and 21).

CHAPTER SIX

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 greenhouse 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 20 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 20 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. The genotypes BCA001, BCA003 and BCA002 be used as drought tolerant genotypes for breeding cowpea, and distributed to farmers in Botswana.

6.2.2. 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.

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

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