ASSESSMENT OF WATER DEFICIT EFFECTS ON AGROMORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF SIX BAMBARA GROUNDNUTS (*Vigna subterranea* (L.) *Verdc.)* LANDRACES GROWN IN WESTERN AND COASTAL REGIONS OF KENYA.

BY

OMBIMA JOSEPH

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DEPARTMENT OF BOTANY

MASENO UNIVERSITY

DECLARATION

I certify that this thesis has not been previously submitted for a degree in Maseno University or any other University. The work reported herein has been carried out by myself and all sources of information have been specifically acknowledged by means of references.

Signature……………… Date…………………**OMBIMA JOSEPH**

PG/MSC/031/06
School of physical and biological sciences
Department of Botany,
Maseno University.

This thesis has been submitted for examination with our approval as the university supervisors.

SIGNATURE ………………DATE ………………
PROF. G. W. NETONDO,
School of physical and biological sciences
Department of Botany,
Maseno University.

SIGNATURE ………………DATE………………
PROF. J. C. ONYANGO
School of physical and biological sciences
Department of Botany,
Maseno University.

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In memory of my late father: Joseck Likoko Ombima and Brother Wallace Zadock Ombima whose life and financial sacrifices gave me the best in life.

ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is one of the most neglected and under-utilized African legume with the potential to alleviate food insecurity and poverty in the tropical semi-arid regions of Africa. The crop is reported to be drought tolerant and produce reasonable yields in poor soils. However key morphological and physiological attributes that confer drought tolerance to different landraces is not well established. The main objective of this study was to assess the effects of water deficit on agro-morphological and physiological traits in six Bambara groundnut landraces commonly grown in western and coastal regions of Kenya. The study involved two greenhouse experiments conducted at University Botanic Garden, Maseno. Six commonly cultivated landraces in Kenya were collected from farmers. Six seeds of each landrace were planted in a 20-litre plastic pot and thinned to three plants 20 days after sowing. Greenhouse experiment 1 involved measurements of agro-morphological parameters (plant height, number of leaves per plant, plant leaf area, shoot: root biomass % and total dry matter), while experiment 2 involved measurements of physiological parameters (stomatal conductance, net photosynthesis internal CO_2 concentration and transpiration rate, water use efficiency and leaf chlorophyll content). Soil moisture % was determined after every 10 days in both experiments till 80days after sowing. The experimental design was randomized complete block design with 4 replications and 4 treatments laid in a factorial set up. The main factor was water irrigation imposed after 20days after sowing at four levels (5, 10, 15 days intervals and no irrigation at all) while the sub-factor was six Bambara groundnut landraces. Data collected was subjected to analysis of variance and effects declared significant at 5% level. Least significance difference was used to separate the means. Linear correlation was conducted to determine the relationship between variables. Plant leaf area, shoot to root biomass%, total dry matter significantly ($p<0.05$) decreased as water deficit increased while stomatal conductance, transpiration rate and chlorophyll content significantly ($p<0.05$) decreased as irrigation frequencies decreased. The results obtained from this study demonstrated significant variation between the Bambara groundnut landraces in moisture stress, and also identified some important traits that are useful in selecting for drought tolerance in Bambara groundnut The traits include restriction of leaf area expansion, lowering stomatal conductance, decreased shoot to root biomass% and increasing water use efficiency in response to increasing water stress. Chlorophyll content also proved to be a useful index for evaluating Bambara groundnut responses to reduced water availability. Total dry matter which was the best indicator of yield was also found to reduce as water stress increased indicating that the effects of water stress on growth and physiology of Bambara groundnut ultimately results in reduced yields. Mombasa dark brown and Mumias light brown landraces were identified to have drought tolerant traits which could make them thrive in low rainfall areas hence recommended to farmers in areas prone to moisture stress. The results of this study therefore provided useful data that can be used in optimizing food productivity in drought prone regions.

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LIST OF ABBREVIATIONS AND SYMBOLS

A - Net Photosynthesis

ABA - Abscisic acid

ANOVA - Analysis of Variance

Ci - Internal CO_2 concentration

CO_2 - Carbon (IV) oxide

DAS - Days after Sowing

DF - Degrees of Freedom

E - Transpiration rate

Fig. - Figure

gs - Stomatal conductance

IPGRI - International Plant Genetic Resources Institute

IRGA - Infra-Red Gas Analyzer

KaKR - Kakamega red

KaKB - Kakamega black

KaKDBs - Kakamega dark brown Spotted

LR - Landraces

LSD - Least Significance Difference

MumDB - Mumias dark brown

MUMLB - Mumias Light brown

MOMDB - Mombasa dark brown

NUC - Neglected and underutilized crops

NW - No wetting

RAPD - Rapid Amplified Polymorphic DNA

RCBD - Randomized Complete Block Design

Rep - Replicate

WUE - Water use efficiency

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CHAPTER ONE
INTRODUCTION

Introduction

1.1 Background

Bambara groundnut (*Vigna subterranea* (L.) Verdc.), family *leguminosae* and subfamily *papilionoideae*, is one of the most neglected and underutilized crops (NUC) with a potential to alleviate poverty, malnutrition and contribute to food security in Africa (Linnemann and Azam-Ali, ;1993 Swanevelder, 1998; FAO, 2001; Azam-Ali *et al*., 2001; Mwale *et al*., 2007). However until recently it has received little attention despite its potential as a food security crop. Important attributes of the crop suggested in the literature includes, tolerance to drought, high balanced nutritional value, relative resistance to pests and diseases, a wide agro-ecological potential and great genetic diversity (Linnemann and Azam-Ali, 1993; Anchirina *et al*. 2001). The marginal nature of most of sub-sahara Africa's agricultural land, and the effects of expected climate change (Hassan, 2006), challenge the existence of major crops and their ability to ensure food security in the future. Neglected and under-utilized species (NUS) have been reported to have possibly evolved to tolerate harsh environments, including drought stress, and have been touted as possible future (food security) crops. Climate change is expanding marginal agro-ecological zones hence the need to intensify selection, cultivation and breeding of drought tolerant crops in tropical Africa. It has been reported to contain 17–25% protein, 42–65% carbohydrate and 6% lipid (Linnemann and Azam-Ali, 1993; Mwale *et al.*, 2007). In the past, research has established the possibility of using the crop in various food products, such as vegetable milk, weaning food and processed flour products (Wambete and Mpotokwane, 2003). Bambara groundnut is ranked the third most important legume in Africa after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) (Ntundu *et al.*, 2004). Previous research has shown that Bambara groundnut is capable of producing good yields under conditions where other legumes such as groundnuts failed completely (Collinson *et al.,* 1997; Mwale, *et al*., 2007). This was attributed to drought resistance mechanism such as high Shoot to Root biomass ratio, reduced leaf area index and maintenance of osmotic potential during water stress through osmotic adjustment (Collinson *et al*; 1997). Germplasm improvement and management practices have mainly relied on local experience and indigenous knowledge (Mukurumbira, 1985). Consequently, the crop remains under-utilised and is still mainly cultivated from landraces of which very little is known about their growth, yield and water-use responses under water stress conditions (Mabhaudhi *et al.,* 2013b). The growth responses of Bambara groundnut to water stress have been described in several instances, using growth indices such as plant height, leaf area index and total dry matter (Collinson *et al.,*1999; Mwale *et al.,* 2007; Vurayai *et al.,* 2011, Mabhaudhi and Modi, 2013). However, most of this research has been done under controlled environments (Sesay *et al.*, 2010) and field conditions (Mabhaudhi and Modi, 2013). Existing literature has not adequately addressed agro-morphological, physiological and biochemical responses of Bambara groundnuts to water stress in East Africa, infact there is hardly any report in literature describing the same on Kenyan landraces. There is therefore need to evaluate the effects of water deficit on commonly cultivated landraces in Kenya with the purpose of identifying and selecting suitable landraces for different agro-ecological zones to mitigate the inevitable climate change effects on food crops. This will not only facilitate breeding and improvement programs for Bambara groundnuts, but also assist subsistence farmers to increasing production by offering them suitable drought tolerant landraces for growth in there agro-ecological zones.

1.2 Statement of the problem

The phenomenon of climate change has resulted in rapid expansion of marginal agro-ecological zones in tropical Africa. Bambara groundnut is one of the most neglected and underutilized crops with the potential to alleviate malnutrition and food insecurity prone in the arid and semi-arid regions of Africa, however, physiological and biochemical attributes that confer drought tolerance to different landraces of the crop are not well understood hence unstable, variable and poor yields. Since many of Bambara groundnut's genotypes are drought tolerant, there is need to establish traits which will provide a basis breeding. Research on commonly cultivated landraces to determine traits for drought tolerance could be an important basis for screening to identify landraces suitable for growth in drought prone areas and breeding to develop high yielding cultivars. This will ultimately optimize productivity of Bambara groundnuts for both subsistence and commercial farming hence eradicate poverty.

1.3 Objectives of the study

1.3.1 General objective

To assess the effects of water deficit on agro-morphological and physiological traits in six Bambara groundnut landraces commonly grown in western and coastal regions of Kenya.

1.3.2 Specific objectives

1) To assess water deficit effects on agro-morphological traits of six landraces of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) commonly grown in Kenya.
2) To determine water deficit effects on physiological traits of six Bambara groundnuts landraces (*Vigna subterranea* (L.) Verdc.) Commonly grown in Kenya.

1.4 Hypotheses

a) Water deficit has no effect on agro-morphological traits of the six Bambara groundnut landraces (*Vigna subterranea* (L.) Verdc.) commonly grown in Kenya.

b) Water deficit has no effect on physiological traits of the six common Bambara groundnut landraces (*Vigna subterranea* (L.) Verdc.) grown in Kenya.

1.5 Justification

Global warming and climate change threatens to increase semi-arid agro-ecological regions, crop diseases and pests, which may aggravate the food crisis in Tropical Africa. Research into traditional neglected crops with the purpose of selecting and breeding for drought tolerance (Massawe *et al*., 2003), could be the key to a revolution in African agriculture. Bambara groundnut, an indigenous African legume crop could provide a major food source with highly balanced nutritional value to alleviate poverty, food insecurity and malnutrition in Sub-Saharan countries like Kenya with large tracts of unutilized semi-arid land. The crop is drought tolerant and requires relatively low inputs and yet provides reasonable yields. It also contributes to sustaining of cropping systems by fixing atmospheric nitrogen in symbiosis with *Bradyrhizobium* strains through nodulation process (Gueye *et al.* 1998). Under changing global climatic conditions, neglected and underutilized crops may provide farmers with promising alternatives for enhancing nutrition, food security and income. It is thought that due to their greater genetic diversity, neglected and underutilized crops might provide for better adaptability and resilience to water stress caused by climate change. Research at the interface between climate change and under-utilized crops will provide local farming communities with greater information on the effectiveness of under-utilized crops in certain climate change situations hence improve productivity.

CHAPTER TWO
LITERATURE REVIEW

Literature review

2.1 Origin and taxonomy

Bambara groundnut belongs to the family *Legminosae* and subfamily *Papilinoideae*. (Goli, 1995). In 1963, Linnaeus described it in species *Plantarum* and named it *Glycine subterranean*. (Du Petit Thoaurs, 1806) had proposed the name *Voandzeia subterranean* (L.) Thouars and this was used widely by subsequent researchers over a century. Later, detailed botanical studies were undertaken by (Marechal *et al*., 1978) who found great similarities between Bambara and plant species of the genus *Vigna*. This confirms studies done by Verdcourt, who seized the opportunity in 1980 to propose the current name *Vigna subterranean* (L) Verdc. (Goli, 1995). The common English name Bambara appears to be derived from a tribe of agriculturalists, the Bambara, who nowadays live mainly in Mali (Linnemann, 1993; Goli, 1995). Major producing countries of Bambara are Nigeria, Niger, Ghana, and Côte d'Ivoire, but it is also widely grown in Eastern Africa and Madagascar (Linnemann and Azam-Ali, 1993), and is even found in parts of South and Central America where it was taken by slaves, it's also found in Asia, particularly India, Indonesia, Malaysia, Philippines and Sri-Lanka (Linnemann and Azam-Ali, 1993) and in parts of northern Australia (Linnemann, 1993). Bambara groundnut has not only a wide agro-ecological potential but a great genetic diversity too and this makes it a potential crop for research and breeding programmes.

2.2 Morphology

Bambara groundnut is an annual herb 30 cm in height with creeping, multi-branched, leafy lateral stems just above ground level **(**Plate 1).

Plate 1: Morphological features of Bambara groundnut: (Karunaratne, 2009).

In association with *Rhizobium*, its roots form rounded and sometimes lobed nodules (Linnemann and Azam-Ali, 1993). Bambara groundnut landraces differ in many aspects from each other, with a wide variety of seed and pod colours, with growth habits varying from bunch type, to semi bunch and spreading. The flowers are typically *papilionaceous*, borne on long racemes with hairy peduncles arising from nodes on the stems. The branching ecotypes are usually self-pollinated whilst the spreading ones are cross-pollinated. The nuts which vary from 1 to 5 cm in diameter, are usually round, slightly oval-shaped and wrinkled with one or two seeds borne below ground. It takes 7 to 21 days to germinate and flowering starts from 30 to 35 DAS and may be determinate or indeterminate (Swannevelder, 1998) though it can take longer 40DAS as was in this study. It has small yellow flowers, which are normally carried in pairs, on short peduncles, which arise from the axis formed by the petioles and the stem. Recent research suggests that Bambara groundnut is mainly self-pollinated in most environments (Massawe *et al.*, 2003). The pods of Bambara groundnut develop underground and may be up to 3.7 cm in diameter, depending on the landrace and number of seeds they contain. The pods are spherical or oval in shape and many contain only one seed. Pods with two seeds are also common in some landraces (Massawe *et al*., 2003). Pods with more than two seeds have also been reported (Pasquet and Fotso, 1997). Mature pods are indehiscent, ranging from yellow to reddish to dark brown or even black in colour.

2.3 Agronomy

Germination of Bambara groundnut generally takes 7 to 15 days after sowing (Kocabas *et al.*, 1999) the rate of which, when water is not limited, being dependent on temperature, genetic variability and seed size and age. (Massawe *et al.*, 2002) reports a similar response for germination to temperature, but notes that the response is landrace-dependent. Germination is faster when in continuous darkness, indicating that germination of Bambara groundnut is sensitive to the duration of light (Massawe *et al.*, 2002). Seeds are planted 2.5 to 3.0 cm deep in sandy loam soils. Large seeds are recommended and should be hydrated and treated with a fungicide before sowing. The recommended spacing is 10 – 15 cm in single row 45 to 90 cm a part.

In Swaziland the highest yield were obtained with 50 cm row spacing (Swanevelder, 1998; Masindeni, 2006). Bambara beans will grow on any well-drained soil, but light, sandy loams with a pH of 5.0 to 6.5 are most suitable, the beans grow poorly in calcareous soils and can be cultivated up to 1600m above sea level, an average day temperature of 20 to 28^0C is ideal for the crop. Widespread rain during the growing season (600 – 700mm) is ideal, though too much rain at harvest time may damage the crop (Swanevelder, 1998). It gives best yields on a deeply ploughed field with a fine leveled seedbed (Swanevelder, 1998; Masindeni, 2006).

2.3 Physiology

Bambara groundnut is widely considered to be a drought resistant crop (Collinson *et al.*, 1997). (Begemann, 1988) suggests two traits that help the crop adapt to a dry environment, namely, a short growing season and a deep root system. (Collinson *et al.*, 1997) suggest that drought tolerance of Bambara groundnut is a result of osmotic adjustment, reduction of leaf area index, and low water loss through the stomata. (Nyamudeza, 1989) reported a high root to total dry matter ratio in Bambara groundnut compared to other crops, while (Shamudzarira, 1996) found a high water use efficiency, both of which are characteristics linked to drought resistance. Also, paraheliotropism and higher leaf reflectivity have been observed when the crop is subjected to water stress (Collinson *et al.*, 1999). (Mwale *et al.*, 2003) reported preferential allocation of dry matter to the roots with increase in the intensity of drought. However the above traits have not been determined in the Kenyan landraces hence the purpose of this study.

2.5 Water deficit and plant morphological response

Water deficit influences several plant processes including cell biochemistry, division and expansion (Mwale *et al.*, 2006). The effect of drought on Bambara groundnuts landraces is not yet fully understood (Cornelissen, 2005). (Mwale and Azam-Ali, 2005) compared water deficit effects on an irrigated Bambara plant with a water stressed one on growth and development and linked their findings to physiological measurements of the water status of the crop. Although this was a good start, only irrigated and water stressed treatments were compared hence there is need to quantify the effect of different water regimes on the growth and development of Bambara groundnut.

Generally, water deficit adversely affects vegetative growth as indicated by changes in plant height, leaf area and dry weight and decrease in plant height is proportional to the extent of drought conditions imposed on the plant (Rahman *et al.*, 2000). Extensive investigations of the effect of water deficit on leaf area have been reported by (Brouwer, 1963) in *P. vulgaris* and peanuts (Rao *et al.,* 1988). These studies have established that leaf area decreased under severe water stress and that on the removal of the stress, the rate of growth of the leaf is restored to a value comparable to that of the control. Although there is a noted general reduction in plant growth rates under limited water supply, shoot growth is more inhibited than root growth (Richardson and Macree, 1985). Water deficit delays the rate of leaf production and leaf extension due to the effects occurring in the meristem in plants. Leaf expansion during vegetative stage is very sensitive to water stress. Cell enlargement requires turgor to extend the cell wall and a gradient in water potential to bring water into the enlarging cell. Thus water deficit decreases leaf area, which reduces the intercepted solar radiation (Salisbury and Ross, 1992). Under water stress, plants may modify their water extraction pattern from the soil, minimize water loss by closing their stomata, reduce leaf area expansion and in extreme cases cause leaf loss through abscission and/or senescence such modifications that occur under drought have implications on the overall productivity of a crop (Mwale *et al.,* 2003). The vegetative growth rate of the crop under water stress may be severely restricted resulting in reduced total dry matter (TDM) and smaller leaf area than where water is unlimited (Mwale *et al.*, 2006). Decreases in dry weight of *Phaseolus vulgaris* under drought conditions have been observed by Brouwer (1963). Reduction in both leaf area and dry matter production has been reported in many crops including legumes, such as *Arachis hypogaea* (Collino *et al.,* 2001). Similar results have been reported in Bambara groundnut where TDM and leaf area index (LAI) were reduced by drought (Collinson *et al.*,1996, 1997) but the same has not been reported on Kenyan landraces hence the need to do so to isolate which among them is more tolerant. Reduced biomass due to drought is partly a consequence of restricted leaf area of the plants, which in turn reduces light interception (Singh,1991), and partly a direct effect of low net photosynthesis due to stomatal closure (Anyia and Herzog, 2004). During the reproductive phase, drought can seriously affect dry matter allocation to yield components. In chickpea, for example, drought reduced both seed number and size, which led to a yield loss of up to 80% (Leport *et al.,* 1999). In Bambara groundnut, (Collinson *et al.*, 1996) reported a significant reduction in pod number per plant, harvest index (HI) and final yield due to drought. There is little information on how landraces from different regions of Africa differ in their efficiency to produce yield in different levels of soil moisture content. In this regard the specific vegetative and reproductive adjustments that take place in different Bambara groundnut landraces under water stress have not been elucidated (Mwale *et al.,* 2006). It is not clear whether genotypic differences in Bambara groundnut landraces across Africa are primarily a result of variations in the rates of crop growth and development under water stress (i.e. phonological responses) and or changes in the rates or efficiencies of resource capture and use (i.e. physiological responses). Even where landrace differences in response to drought have been reported (Collinson *et al.,* 1999) their underlying mechanisms have not been adequately established purely under field conditions where the response to a particular abiotic factor, e.g. drought, is difficult to uncouple from other abiotic factors such as heat or biotic factors such as pests and diseases. Knowledge of the extent of variations among landraces of a crop is an important pre-requisite to breeding (Massawe and Azam-Ali, 2005) because it provides a basis to make decisions on where and how the required variation will be sourced and the environmental gradient across which breeding objectives can be tested. Information on genotypic differences within a species and their underlying physiological bases are also useful in the development and validation of crop growth models which, in turn, can assist in the setting of breeding objectives (Mwale *et al.*, 2006).

2.6 Water deficit and plant physiological response

Bambara groundnut is grown in the semi-arid tropics where water is usually in short supply. However, there are only limited reports on Bambara groundnut (Collinson *et al.,*1999) that have attempted to quantify the impact of soil water deficit on the growth and physiology of the crop. At low water potentials stomatal closure and inhibition of chloroplasts activity reduces photosynthesis. Stomatal closure is a plant’s initial response to declining soil water content and has been characterized as a drought avoidance mechanism (Farooq *et al.,* 2009), as well as being a characteristic of increased water use efficiency under drought stress (Blum, 2009). In drought tolerant plants, there is a remarkable resistance of photosynthetic apparatus to dehydration suggesting that plant survival under drought is partly due to maintenance of the photosynthetic capacity by leaves thus allowing rapid recovery after rewetting. Reduction in photosynthetic activity under water stressed condition is as a result of stomatal and non - stomatal effects. Water deficit leads to stomatal closure due to reduced turgidity hence reduced CO_2 absorption markedly diminishing the photosynthetic activity. Water stress more often causes significant reduction of stomatal conductance (Bradbury, 1989) leading to reduced photosynthetic capacity. Photosynthesis may also be limited by the chloroplast capacity to fix CO_2 than by increased diffusive resistance. The influence of water deficit on stomatal conductance can be used as a rapid procedure for screening lines of potential drought tolerance. Photosynthetic pigments of chlorophyll and carotenoids absorb solar energy that is used for photosynthesis. Part of this energy is emitted as chlorophyll fluorescence. In many cases chlorophyll fluorescence increases under stress conditions (Pereira *et al*., 2004). An inverse relationship occurs between chlorophyll content and chlorophyll fluorescence under water stress conditions, in many observed cases chlorophyll content declines with increase in stress conditions (Pereira *et al*., 2004). Chlorophyll fluorescence parameters are direct indicators of the photosynthetic activity and give an indication of status of photosynthetic apparatus. Chlorophyll content of leaves is a useful indicator of both potential photosynthetic productivity and general plant vigour (Alonso *et al.,* 2002). However changes in leaf chlorophyll content often has been regarded as a relatively late mechanism of photosynthetic adaptation (Anderson *et al.*, 1995). Other mechanisms such as regulation of CO_2 supply by stomatal limitation and shifts in photochemistry of photosynthesis are thought to be the primary responses to a changing environment. Stomatal behavior has an obvious influence on transpiration water loss (Hoffman *et al.,* 1984) stomatal closure during water stress is believed to be mediated by abscisic acid (ABA). Increase in the level of ABA in plants has often been associated with water stress; drought resistant plants contain large amounts of ABA (Devlin and Witham, 1983). In dry environments, plants are able to reduce leaf area development during water loss, hence conserve moisture for the reproduction and filing phase (Garrity *et al*., 1984). Reduced transpiration is an important physiological indicator of water stress (Xu *et al.*, 1995). Water deficit induces a significant increase of proline content. For instance proline content in leaves of cowpea increased by 56% in stressed plants compared to well-watered plants (Falalou *et al.,* 2007*).* Proline accumulation is known to provide an efficient mechanism for cellular adaptation to osmotic stress (Martinez *et al.,* 1995). (Marjorie *et al.* 2002) demonstrated that proline accumulated during water deficit had a minor contribution to total osmolytes in drought conditions but plaid a key role after re-watering. (Onyango, 1996) observed that Protein content reduction in Rice (*Oryza sativa*) with a decrease in soil moisture content may have been due to the reduction in polyribosome levels or increase in protein breakdown in plant tissues caused by water deficit. This study seeks to understand the crop's morphology, physiology and biochemistry to generate information useful to plant breeders for the purposes of genetically improving the crop for higher yields in marginal regions.

2.7 Pests and diseases

The crop has a tendency to resist pests and diseases, but very little is known about the kind of pest and disease attacks and the extent of the damage to the plant, pods or seed, only a few authors have reported on the pests and diseases of the crop (Masindeni, 2006) The crop is susceptible to viruses such as cowpea mottle virus (Shoyinka *et al.,* 1978), cowpea mild mottle virus, *Voandzeia* necrotic mosaic virus (Fauquet *et al.*, 1984) and *Meloidogyne incognito* and *M. javanica* are parasitic nematodes on Bambara. Pests attacking Bambara are *Hilda petrel's* (leaf hoppers) and the larvae of *Diacrisia maculosa* and *Lamprosema indicata*. Fusarium wilt disease has been reported in Kenya as one of the major diseases limiting yields of the crop (Cook, 1978), in storage, bruchids (*Callosobruchus maculatus*) are the most important pest attacking the seeds of the crop (Swanevelder, 1998; Lale and Vidal, 2001).

2.8 Uses, nutritional and economic importance

Bambara groundnut (*Vigna subterranea* (L) Verdc) is a major source of vegetable protein in sub-Saharan Africa. The seed is regarded as a complete balanced food because it is rich in iron (4.9-48 mg/100 g), compared to a range of 2.0-10.0 mg/100 g for most food legumes, protein (18.0-24.0%) with high lysine and methionine contents, fibre (5.0-12.0%), potassium (1144- 1935 mg/100 g), sodium (2.9-12.0 mg/100 g), calcium (95.8- 99 mg/100 g), carbohydrate (51-70%), oil (6-12%), and energy (367-414 kal/100 mg) (Rowland, 1993). It has been reported to contain 17–25% protein, 42–65% carbohydrate and 6% lipid (Linnemann and Azam-Ali, 1993; Mwale *et al.,* 2007). The seeds can be eaten fresh or grilled while immature. In many countries in West Africa, fresh pods are boiled with salt and pepper and eaten as a snack. Recent research has established the possibility of using Bambara groundnut in various food products, such as vegetable milk, weaning food and processed flour products (Wambete and Mpotokwane, 2003). Bambara seed and haulm have been used to feed livestock and poultry (Anchirina, *et al.,* 2001). Bambara groundnut fixes atmospheric nitrogen in symbiosis with *Bradyrhizobium* strains through a nodulation process (Gueye *et al.,* 1998) hence useful in crop rotations (Masindeni, 2006; Ntundu, *et al.,* 2006). Despite the importance of the crop as a food grain legume in the traditional farming systems in Africa, no significant efforts have been made scientifically to improve this crop, no commercial production and no industrial use of the crop take place and research is concentrated only on the agronomic aspect, while the processing aspects have been neglected (Masindeni, 2006).

2.9 Yield

Bambara groundnut is harvested when the plant turn yellow (80% of the pods have matured) between 120 -145 DAS depending on landrace type and environmental factors, care is taken to reduce pod loss (Swanevelder, 1998). Harvested pods are sun dried 2-3 days until the seeds become loose in the pods. Seed vigor deteriorates after shelling and shelling should therefore be done prior to planting, otherwise they are susceptible to weevils. The current yields of Bambara groundnut are extremely low and variable, because the environments in which it is normally grown are characterized by various biotic and a biotic stresses (Massawe *et al.,* 2003). However, even under optimum conditions yields are variable and unpredictable due to the variability of growth and development of individual plants within a landrace (Squire *et al.,* 1996). According to (Linnemann and Azam-Ali, 1993) farm pod yields vary between 650 and 850 kg ha^{-1} for most of the semi-arid tropics. However there are large differences between countries, with yields as low as 56 to 112 kg ha^{-1} have been reported in Zambia, while in Zimbabwe 3870 kg ha^{-1} was obtained (Linnemann, 1987). Major factors associated with low production of Bambara groundnuts includes unimproved cultivars, low germination due to poor seed storage, breeding of cultivars through hybridization is very difficult due to the small flowers of the Bambara groundnut, high labor requirements due to the ambiguous character of the plant and therefore costly and Small sized seeds resulting in poor yields (Swanevelder, 1998). Development of high yielding and drought adapted varieties is one of the approaches to resolving the Bambara groundnut shortages.

CHAPTER THREE
MATERIALS AND METHODS

Materials and methods

3.1 Experimental site:

The study was conducted in a greenhouse at University Botanic Garden, Maseno in Western Kenya (0^{o} 1’N – 0^{0}12’S, 34^{o}25’E – 47 ‘E) between April 2008 and September 2009. The area receives a mean annual precipitation of 1750mm with a bimodal distribution. The mean temperature of Maseno is 28.7^{o}C. Daily mean greenhouse temperature was maintained at 29 ^{o}C with diurnal amplitude of ± 5^{o}C. The soils are classified as acrisol (well drained, deep reddish brown clay), with a pH ranging between 4.5 and 5.4 (Mwai *et al.*, 2001). Two greenhouse experiments were conducted here-after referred to as experiments **1** and **2.**

3.2 Experimental plant materials:

Both Greenhouse experiments **1** and **2** were preceded by collection of Bambara groundnut landraces. Based on the information collected from local farmers six landraces commonly grown were collected from the two agro-ecological zones in Kenya where its chiefly grown (Western and Coastal Counties) Figure 3.

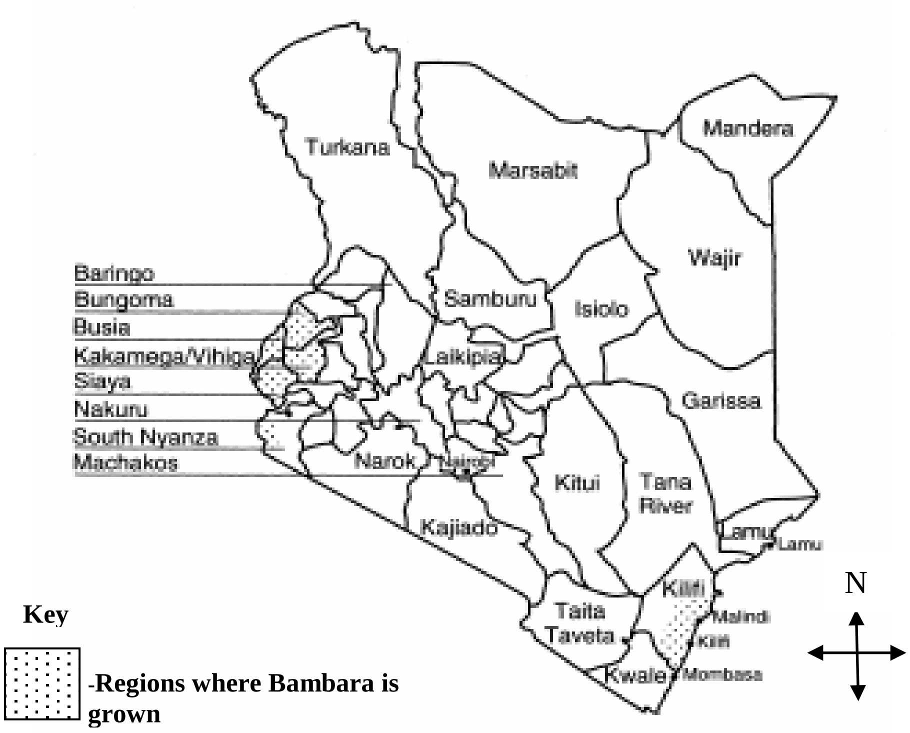


Fig.3 Distribution of Bambara groundnut growing regions in Kenya: (Ngugi, 1995)

Bambara groundnut is still cultivated from local landraces rather than from varieties bred specifically for particular environments and farm yields are still low (Vurayai *et al.,* 2011). Large similar sized seeds harvested in 2007 were sorted out, sterilized for five minutes in 10% sodium hypochlorite (v/v) and then rinsed several times with distilled water before planting. The year of harvest and seed size is important because both seed source and year of harvest can affect growth parameters being measured (Massawe *et al*., 2003). Using colour and eye pattern of the seed testa (Collinson *et al.*, 1996) they were christened as in plate 2.

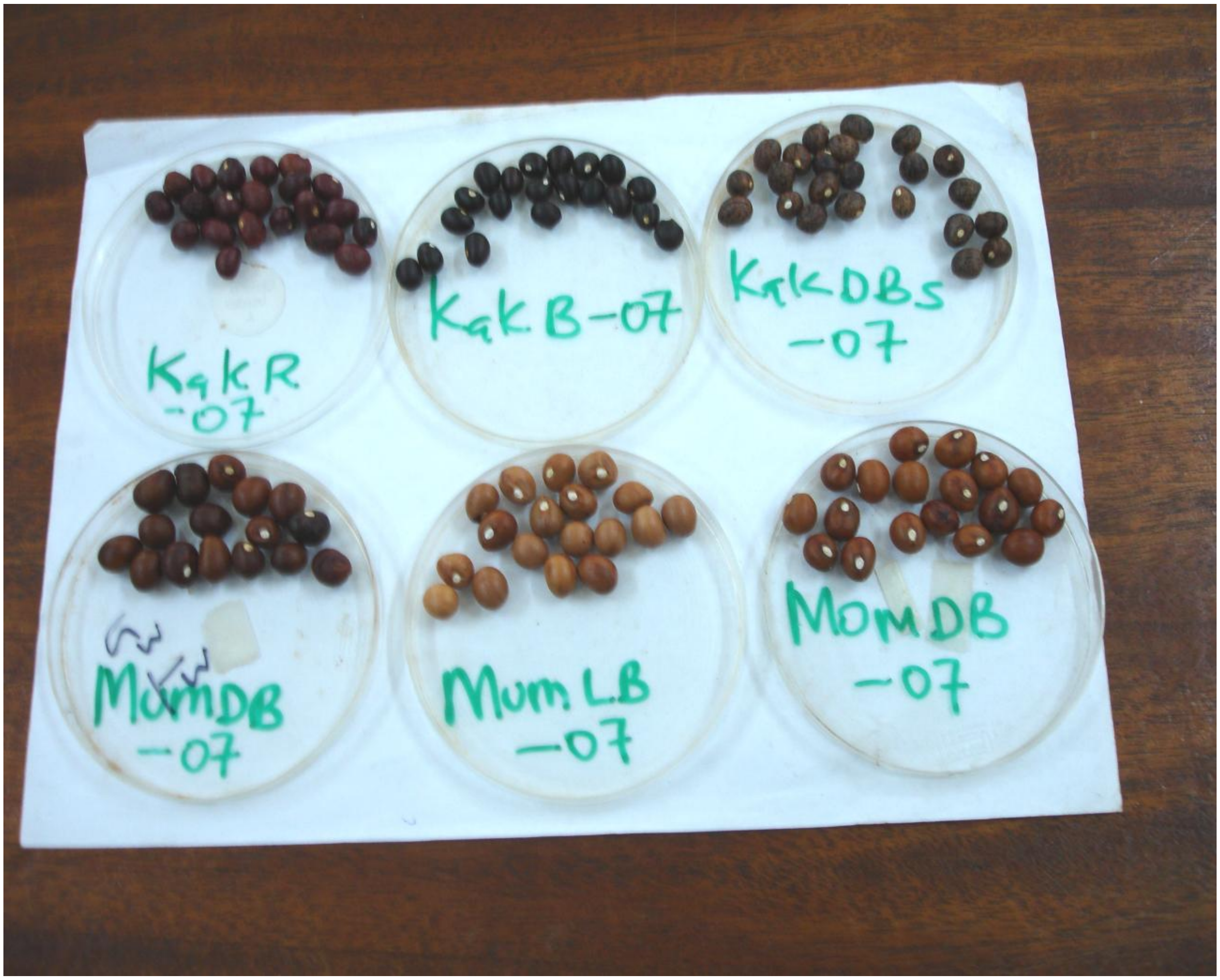


Plate 2: Description of seeds of the six landraces (LR) their origin and year of collection. Where:

LR1 – KaKR 2007 – Kakamega red

LR2 – KaKB 2007 – Kakamega black

LR3 – KaKDBs 2007 – Kakamega Darkbrown Spotted

LR4 – MumDB 2007 – Mumias Darkbrown
LR5 – MUMLB 2007 – Mumias Light brown
LR6 – MOMDB 2007 – Mombasa Dark brown

3.3 Experimental design and treatments:

The experimental design was a Randomized Complete Block Design (RCBD) in a factorial layout with 4 replications. The main factor was water irrigation regimes at four levels - 5control, 10,15 days intervals and droughted 20DAS and sub-factor was the six Bambara groundnut landraces commonly cultivated in Kenya. Before 7-10 days before sowing the soil was fumigated with methyl bromide to kill soil borne diseases, pathogens and weeds. Six seeds of each landrace were planted at a recommended depth of 3cm in a 20-litre PVC pot (Ø 30cm and 40cm height) placed 15cm apart giving a plant population of 12 plants m^{-2}. Each filled with 20kgs mixture of normal fumigated field soil. Each PVC pot was maintained at 100% field capacity daily until 20 DAS to ensure successful germination and establishment of the crop. The seedlings were then thinned to three plants per pot 20 DAS, after which 5 irrigation regimes (Collinson *et al.*, 1997) of 500ml treatments were imposed at: Treatment A - 5 days interval (control), Treatment B - 10 days interval, Treatment C - 15 days interval and Treatment D – droughted from 20 DAS – 80 DAS each replicated four times.

3.4 Greenhouse experiment 1: measurements of growth parameters:

Greenhouse experiment **1** involved measurements of Soil Moisture Content **(SMC%)** and growth and morphological parameters (plant height **(PH)**,leaf number per plant **(LNP)**, plant leaf area**(PLA)**,shoot: root biomass % **(S:RB%),** Total Dry Mass **(TDM)** at 10 days intervals as described in section 3.5 below. Daily mean temperature and relative humidity was also recorded and tabulated table 1 for experiment 1 below, however greenhouse conditions were not maintained at constant.

Table1 Daily mean temperature and relative humidity within experimental period.

Month 2008-2009	**Temperature min-max (^{o} C)**	**Relative humidity min-max (%)**	**Day length(hours/day)**
0ctober	24 - 32.8	65-80	9
November	25.2 - 34.5	70-90	10
December	25.5 - 37.8	69-92	10
January	27.4 - 37.7	63-85	10

3.4.1 Soil moisture content (SMC %)

Soil moisture content in both greenhouse experiments **1** and **2** was monitored from each pot between 8.00 – 10.00 am gravimetrically (Gardner, 1986) at 40DAS and 60DAS at a 10-30 cm depth using a screw auger just before irrigation treatments. Care was taken to minimize root destruction. The samples were placed in polythene bags to minimize moisture loss. Fresh weight 50gms **(M1)** was taken immediately after collection using an electronic weighing balance (Denver instrument, model XL -3100D) and oven dried at 105^{o}C for 48 hours to a constant dry weight to determine dry weight **(M2)**.

Percentage soil moisture content **(M)** was calculated as:

$$\mathbf{M = \frac{M1 - M2}{M1} \times 100}$$

3.4.2 Plant height (PH)

Plant height (cm) was measured per landrace per treatment using a meter rule at 40DAS and 60 DAS on the petiole from the soil surface at the base of the stem to the furthest point vertically from selected/pre-determined plants, which were tagged at 20DAS

3.4.3 Leaf number per plant (LNP)

The trifoliate leaves were counted as one leaf. The number of fully expanded leaflets per landrace per treatment was determined physically from selected/pre-determined plants, which were tagged after emergence (collinson, *et al.*, 1999) at 40DAS and 60DAS.

3.4.4 Plant leaf area (PLA)

Plant leaf area ($\mathbf{A_{plant}}$) was measured after every 40 DAS- 60DAS from leaf width and length, using the model assumptions of (Deswarte, 2001) and (Cornelissen, 2005) as described below:

The Bambara groundnut has leaves with a shape very close to an ellipse (plate 3)

Area for the ellipse: $\mathbf{A = L * W * \pi /4}$**…………………………………………. Equation (1)**

Equation to estimate the leaflet area: $\mathbf{A = \sigma * L * W * \pi /4}$**…………………Equation (2)**

Where: **L** = Length of the leaflet (cm), **W** = Width of the leaflet (cm), $\mathbf{\pi = 3.1416}$ and

$\boldsymbol{\sigma}$ = correction factor (to account for the difference between the actual shape and an ellipse)

- The size of the lateral leaflets is usually closely related to the size of the middle leaflet.

- The plant leaf-area is a function of the leaf number and the single-leaf area. Bambara groundnut has trifoliate leaves, although these three leaflets have the same shape, they do not always have the same dimensions or leaf area. In order to compensate for these differences an extra parameter **τ** is added to Equation 2:

$A = \tau * 3 (\sigma L * W * \pi /4)$…………………………………………………. Equation (3)

The step to leaf area of the whole plant seems to be simply multiplying Equation 3 with the total number of leaves (**N_1**), however an extra parameter (**υ**) needs to be added to compensate for inaccuracy in sample methods. Young not fully unfolded leaves and leaves that look significantly smaller than others are rejected. This can lead to under or overestimation of the leaf area. The equation becomes:

$A_{plant} = \upsilon * N_1 [\tau * 3 (\sigma * Length * Width * \pi /4)]$……………………….. Equation (4)

(Deswarte, 2001) calculated landrace independent values for **σ**, **τ** and **υ**, given in (Table 2) and the relation between actual and estimated leaf area.

Table 2 Landrace independent values for calculating Bambara groundnut leaf area

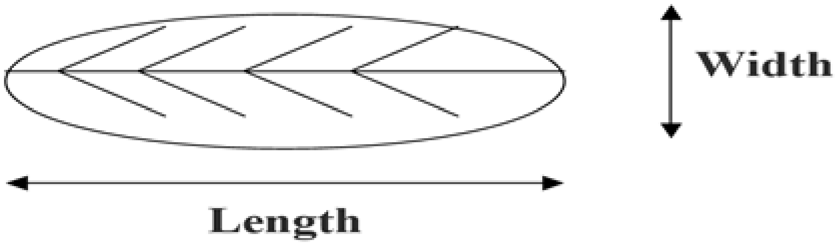
Model Parameters	Landrace independent values
σ	0.95
τ	0.91
υ	0.86
R^2	90.90%

Source: (Deswarte, 2001)

For practical purposes, equation 4 can be simplified by subsuming **σ**, **τ** and **υ** into **δ ($\delta=0.74$):**

$A_{plant} = 0.74 * 3 * N_l (Length * W * \pi /4)$

Plate 3: Length and width measurements of a Bambara groundnut leaflet



3.4.5 Shoot: Root biomass % (S: RB %)

One plant per pot was carefully scoped with all roots intact using a trowel and hand washed over a fine sieve with tap water collecting all roots one at a time in two stages (flowering-40 DAS, podding-60 DAS). The plant was separated into shoot and root, oven dried at 72°C for 48 hours and their dry weights determined using an electronic weighing balance (Denver Instrument. Model XL-3100D). Shoot to Root biomass ratio was computed as percentage.

$$\textbf{Shoot: Root biomass \%} = \frac{\textbf{Shoot dry weight}}{\textbf{Root dry weight}} \times 100$$

3.4.6 Total dry matter (TDM)

One plant per pot was carefully scoped with all roots intact using a trowel and hand washed over a fine sieve with tap water collecting all roots one at a time in two stages (flowering-40 DAS, podding-60 DAS). The plant was then oven dried at 72°C for 48 hours and the dry weights determined using an electronic weighing balance (Denver Instrument. Model XL-3100D). Total dry matter was computed using the formular below (collinson, *et al.*, 1999).

$$\textbf{TDM = shoot dry weight + Root dry weight}$$

3.5 Greenhouse experiment 2: measurements of physiological parameters

Experimental site, plant materials, design and treatments in greenhouse experiment 2 were as in experiment 1 section 3.1, 3.2 and 3.3 respectively. Greenhouse experiment 2 involved measurements of Soil moisture content (as in experiment 1) and physiological parameters (gas exchange measurements (net photosynthesis **(A)**, stomatal conductance **(gs)**, internal CO_2 concentration **(C_i)** and transpiration rate **(E))**, water use efficiency **(WUE)** and leaf chlorophyll content (**CHLc)**. Gas exchange measurements were taken from one marked plant and chlorophyll content from the second marked plant from each pot at 40 and 60 DAS. The monthly greenhouse data was recorded within the study period is presented in table 3 for experiment 2.

Table 3 Daily mean temperature and relative humidity within experimental period.

Month 2009	**Temperature min-max (^{o} C)**	**Relative humidity min-max (%)**	**Day length(hours/day)**
January	27.4 - 35.7	63-85	10
February	26.2 - 35.6	62-80	10
March	26.5 - 34.8	69-90	10
April	25.4 - 30.7	70-90	9

Plate 4: Germination of the six landraces 25 DAS in greenhouse experiment 2 layout

3.5.1 Gas exchange measurements

Gas exchange Parameters measured in this study included net photosynthesis **(A),** stomatal conductance **(gs)**, internal CO_2 concentration **(C_i),** and transpiration rate **(E)**.These measurements from one predetermined /tagged plant per replication were taken between 0900 and 1300 hours on the first fully opened and exposed leaf of the main axis at 40 DAS to 60 DAS in brightly lit greenhouse just before irrigation treatments. An open infra-red gas analyzer (IRGA) system (CIRAS 1-PP system, Shortfield, Hitdlin, Herts, UK) was used. This system was connected to a Parkinson leaf chamber whose area is 2.5 cm^2. The intact leaf lamina was sealed in the leaf chamber and all the major veins avoided. The whole cuvette area was covered by the leaf surface. Readings were taken after warming up the equipment for 10 minutes in order to achieve steady-state conditions of gas exchange. Data was stored in the data logger and downloaded into a personal computer for analysis. Three readings were taken at (0900 - 1300HRS), from each leaf for all four replicates.

3.5.2 Water use efficiency

Water use efficiency **(WUE)** represents the ratio of carbon assimilated to water lost by transpiration Turner, (1986). It was calculated at 20 DAS and 60 DAS from data of net photosynthetic rate (**A**) and transpiration rate (**E**) by dividing net photosynthetic rate (**A**) and transpiration rate (**E**) i.e. **WUE = A/E** (Todorov *et al.*,1992).

3.5.3 Leaf chlorophyll content

Leaf chlorophyll content was measured using the methods of Arnon, (1949) as described by Netondo, (1999) and Musyimi, (2007). The third fully expanded trifoliate leaf was collected from each treatment per replication for measurements at 40 and 60 DAS, 0.1g of the fresh leaf tissue was measured and cut into small pieces into specimen bottle. Ten (10) ml of 80% acetone was added and the set up kept in the dark for 7 days for chlorophyll to be extracted by the acetone. One (1) ml of the filtered extract was diluted with 20ml of 80% acetone and absorbance of chlorophyll solution measured using a spectrophotometer (model Novaspec II, Pharmacia biotech, Cambridge England) at 645 and 663 nm to determine the content of chlorophyll *a* and *b* and the total chlorophyll (*tchl*) of the leaf tissue. The respective chlorophyll content in milligram of chlorophyll per gram of leaf collected was calculated using Arnon, (1949) formulae as below:

- **Mg *chl a/g* leaf tissue =12.7 (D663) -2.67(D645) x V/1000XW**
- **Mg *chl b/g* leaf tissue =22.9 (D645) -4.68(D663) x V/1000XW**
- **Mg *tchl/g* leaf tissue =20.2 (D645) +8.02(D663) x V/1000XW**

Where: **D** = absorbance measured at wavelengths 645nm and 663nm.

V = volume (ml) of the acetone extract.

W =fresh weight (g) of leaf tissue from which the extract was made.

3.6 Statistical Analysis of Data

The data collected was subjected to Analysis of Variance **(ANOVA)** at **5%** level of significance using XLSTAT Statistical Package to determine any significant differences between treatments. Least significant difference **(LSD $_{5\%}$)** was used to separate the means and correlation was used to determine the relationship between variables.

CHAPTER FOUR

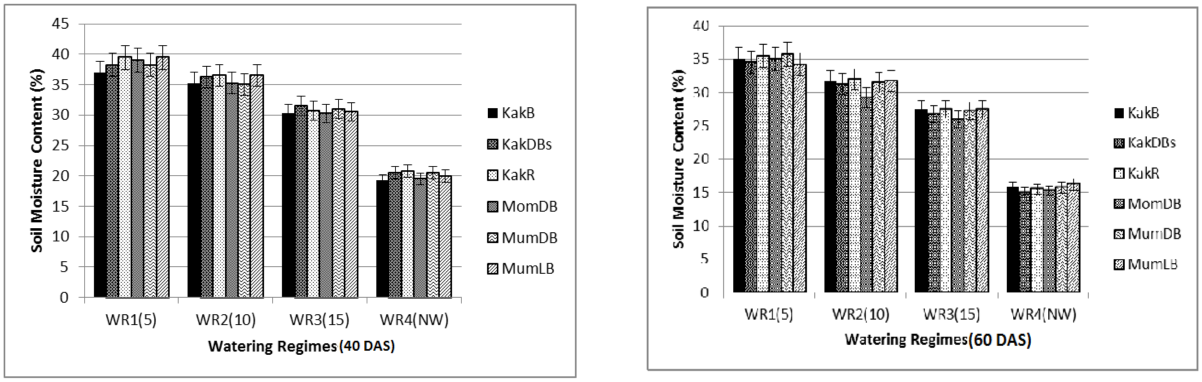
RESULTS

Results

4.1 Greenhouse experiment 1

4.1.1 Soil moisture content (SMC %)

There was no significant difference ($p>0.05$) in the water content within the pots, within the watering regimes both at 40 DAS (Figure 4.1a (1)) and at 60 DAS (Figure 4.1a (2)). However, different watering regimes (WR) recorded significant ($p<0.05$) variations in soil moisture content which decreased as irrigation frequency decreased. There were no significant ($p>0.05$) interactions between the landraces and watering regimes (Appendix 1.1). This indicated that different landraces responded in a similar way to different watering regimes. Significant positive correlations were observed between the soil moisture content and plant height, plant leaf area and plant leaf number (Table 4.1).

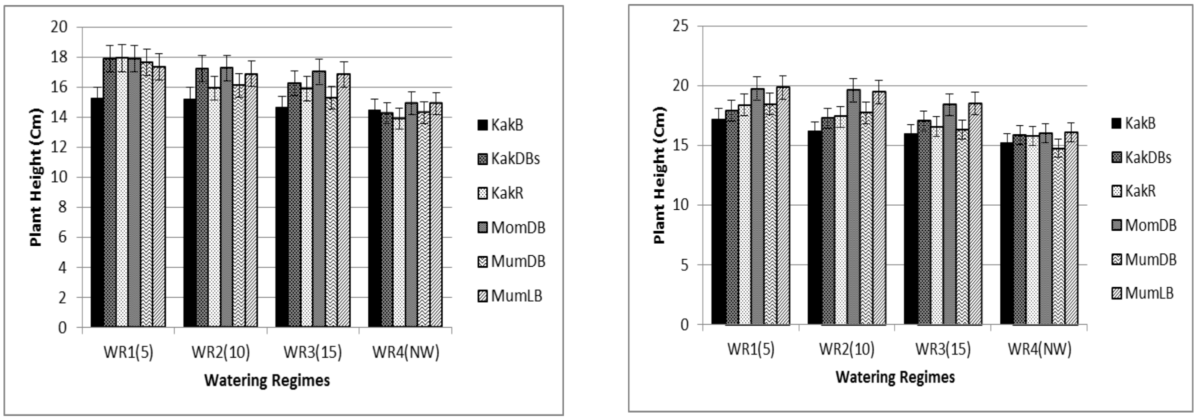

a (1)

a (2)

Fig 4.1 a: Variation in soil moisture content % of different Bambara groundnut landraces as affected by watering regimes. a (1) = 40DASwhile a (2) = 60DAS. Error bars represent standard error of means.

4.1.2 Plant height

The landraces were not significantly (p>0.05) different in plant height both at 40 DAS (Figure 4.1b (1)) and at 60 DAS (Figure 4.1b (2)). Plant height in the four watering regimes were also not significantly different from each other. However, MomDB and MumLB were consistently the tallest under all watering regimes. Analysis of variance conducted with the four watering regimes combined showed that there was no significant (p>0.05) interaction between the plant height of the different landraces and watering regimes (Appendix 1.2). This indicated that the plant height of different landraces was affected in a similar way by different watering regimes. Significant positive correlations were observed between the plant height and both plant leaf area and plant leaf number (Table 4.1).


b(1)

b(2)

Fig 4.1b: Variation in plant height (cm) of different Bambara ground nut landraces as affected by watering regimes. b (1) = 40DAS while b (2) = 60DAS. Error bars represent standard error of means.

4.1.3 Leaf number per plant

The landraces were not significantly (p>0.05) different in number of leaves per plant both at 40 DAS (Figure 4.1c (1)) and at 60 DAS (Figure 4.1c (2)). However, the leaf number was found to decrease as water stress increased both at 40 DAS and 60 DAS. Analysis of variance conducted with the four watering regimes combined showed that there was no significant (p>0.05) interaction between the leaf number of the different landraces and watering regimes (Appendix 1.3). This was an indication that the leaf number of different landraces was affected in a similar way by different watering regimes. Significant positive correlations were observed between plant leaf number and soil moisture content, plant leaf area and plant height (Table 4.1).

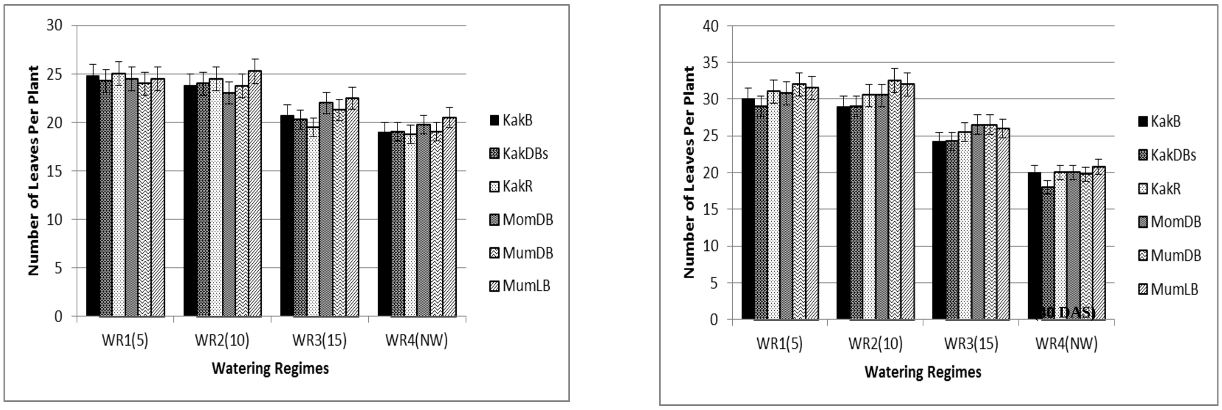
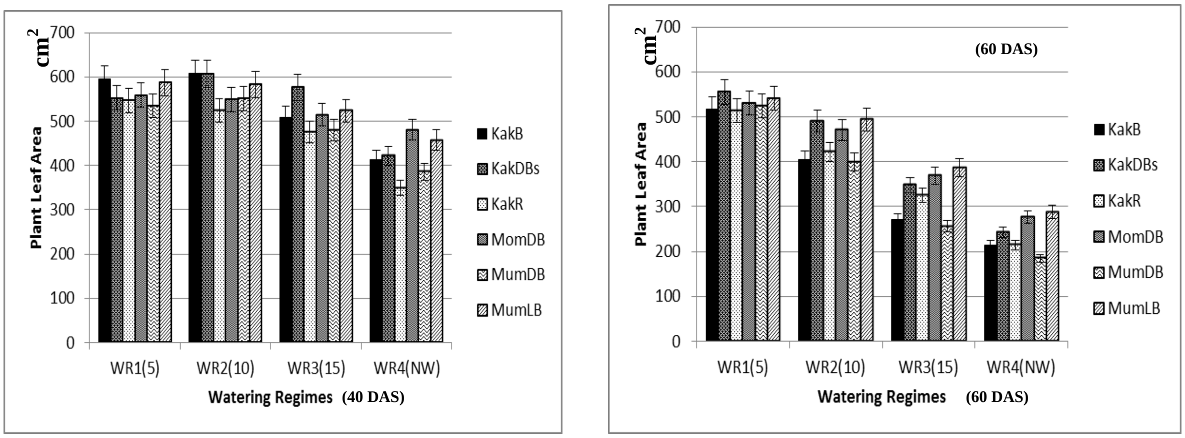

c (1) c (2)

Fig 4.1 c: Variation in leaf number per plant of different Bambara ground nut landraces as affected by watering regimes. c (1) = 40DAS while c (2) = 60DAS. Error bars represent standard error of means.

4.1.4 Plant leaf area

Plant leaf area was found to reduce significantly ($p<0.05$) as water stress increased. The landraces also varied significantly ($p<0.05$) in leaf area under the four watering regimes both at 40 DAS (Figure 4.1d (1)) and at 60 DAS (Figure 4.1d (2)). The landraces MomDB and MumLB recorded the largest leaf area under watering regime 4 (no irrigation) both at 40 DAS (Figure 4.1d (1)) and 60 DAS (Figure 4.1d (2)). There was no significant ($p>0.05$) interaction between the leaf area of the different landraces and watering regimes (Appendix 1.4). This was an indication that the leaf area of different landraces was affected in a similar way by different watering regimes. Significant positive correlations were observed between plant leaf area and soil moisture content, plant leaf number and plant height (Table 4.1).

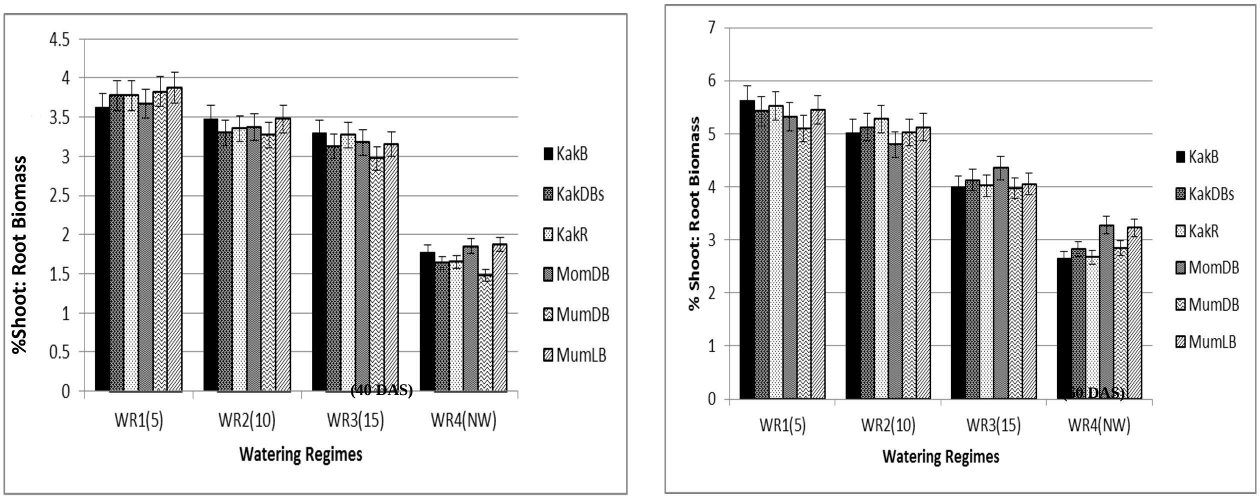

d(1)

d(2)

Fig 4.1d: Variation in plant leaf area of different Bambara groundnut landraces as affected by watering regimes. d (1) = 40DASwhile d (2) = 60DAS. Error bars represent standard error of means.

4.1.5 Shoot: Root biomass %

Percent shoot to root biomass was found to decrease significantly ($p<0.05$) as water deficit increased (Figure 4.1e). Significant ($p<0.05$) variation in percent shoot to root biomass was only observed under the non-irrigated treatment (WR4) both at 40 DAS (Figure 4.1e (1)) and at 60 DAS (Figure 4.1e (2)). MomDB and MumLB recorded the highest percent shoot to root biomass under moisture stress both at 40 DAS and 60 DAS. The genotypes responded in a similar way in their shoot to root biomass under all the watering regimes at both 40 DAS and 60 DAS and there was therefore no significant ($p>0.05$) interaction between the landraces and watering regimes in shoot to root biomass (Appendix 1.5). However, percent shoot to root biomass of different landraces decreased significantly as water stress increased both at 40 DAS and 60 DAS. Shoot to root biomass was found to be positively correlated to soil moisture content and total dry matter (Table 4.1).

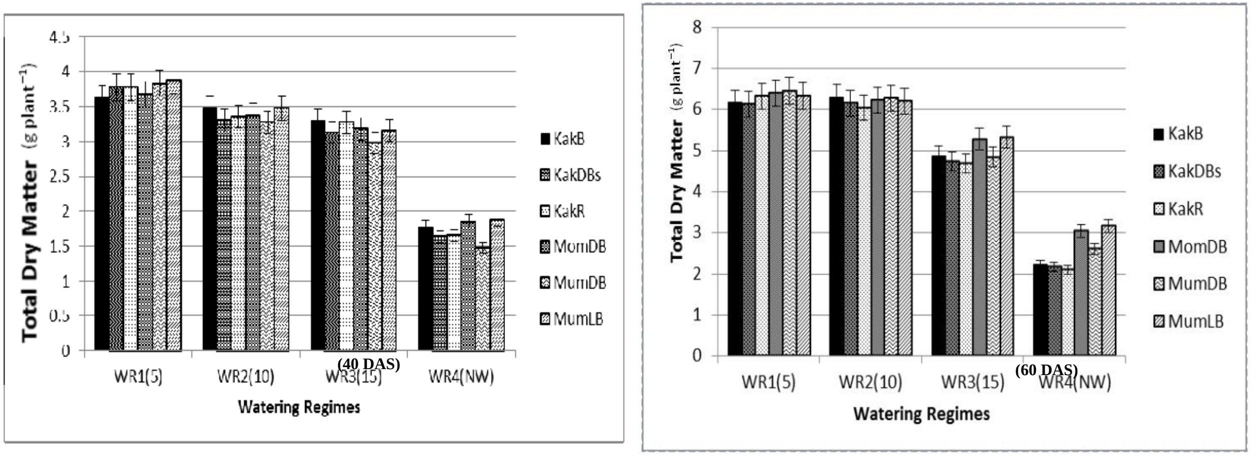

e(1)

e (2)

Fig 4.1e: Variation in Shoot: root biomass % of different Bambara groundnut landraces as affected by watering regimes e (1) = 40DAS while e (2) = 60DAS. Error bars represent standard error of means.

4.1.6 Total Dry Matter

There was significant ($p<0.05$) decrease in total dry matter as water deficit increased (Figure 4.1f).Significant ($p<0.05$) variations in total dry matter were observed among the landraces only under non-irrigated treatment both at 40 DAS (Figure 4.1f (1)) and at 60 DAS (Figure 4.1f (2)). MomDB and MumLB recorded the highest total dry matter under moisture stress both at 40 DAS and 60 DAS while the other landraces did not vary significantly ($p>0.05$) in their total dry matter content. There was no significant ($p>0.05$) interaction between the total dry matter of the different landraces and watering regimes (Appendix 1.6). This was an indication that the total dry matter of different landraces was affected in a similar way by different watering regimes. Total dry matter was found to be positively correlated to soil moisture content and shoot to root biomass (Table 4.1).



f(1) f(2)

Fig 4.1: Variation in total dry matter of different Bambara groundnut landraces as affected by watering regimes. f (1) = 40 DAS while f (2) = 60DAS. Error bars represent standard error of means.

Table 4.1: Correlation coefficient matrix for morphological characteristics

Variables	PH	LNP	PLA	S:R B%	TDM
SMC%	**0.218**	**0.297**	**0.361**	**0.286**	**0.468**
PH		**0.251**	**0.312**	-0.064	-0.100
LNP			**0.525**	-0.037	0.047
PLA				-0.121	-0.015
S:R B%					**-0.321**

NB: Values in bold are different from 0 with a significance level alpha=0.05 where:

PH – Plant height

LNP- Leaf Number per Plant

PLA – Plant Leaf Area

R:SB% - Root to shoot biomass percentage

TDM – Total Dry Mass

4.2 Greenhouse experiment 2

4.2.1 Soil Moisture Content (SMC %)

There was no significant difference ($p>0.05$) in the water content within the pot, within the watering regimes both at 40 DAS (Figure 4.2a (1)) and at 60 DAS (Figure 4.2a (2)). However, different watering regimes (WR) recorded significant ($p<0.05$) variations in soil moisture content which decreased as irrigation frequency decreased. There were no significant ($p>0.05$) interactions between the landraces and watering regimes (Appendix 2.1). This indicated that different landraces responded in a similar way to different watering regimes. Significant negative correlations were observed between the soil moisture content and the water use efficiency (Table 4.2) indicating that the landraces increased their water use efficiency as soil moisture content decreased.

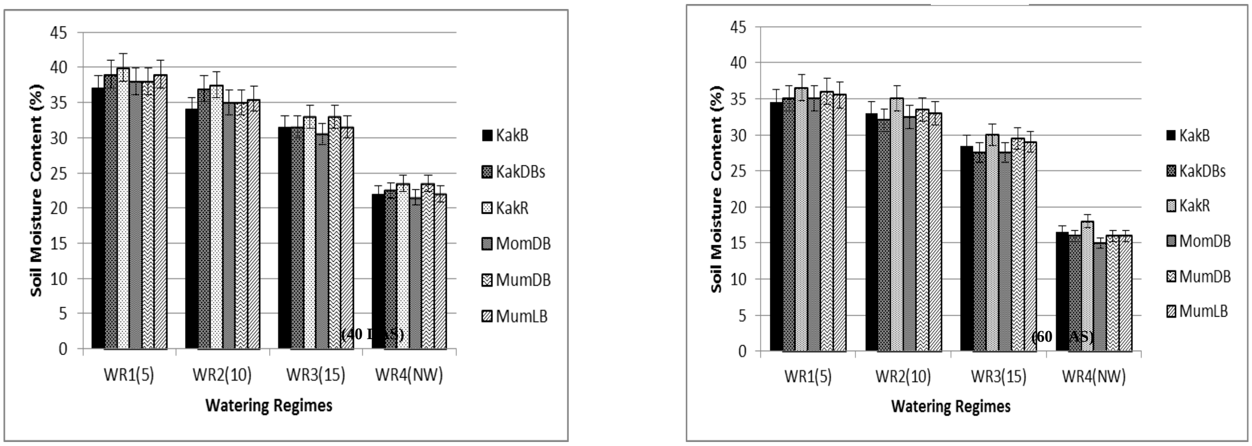
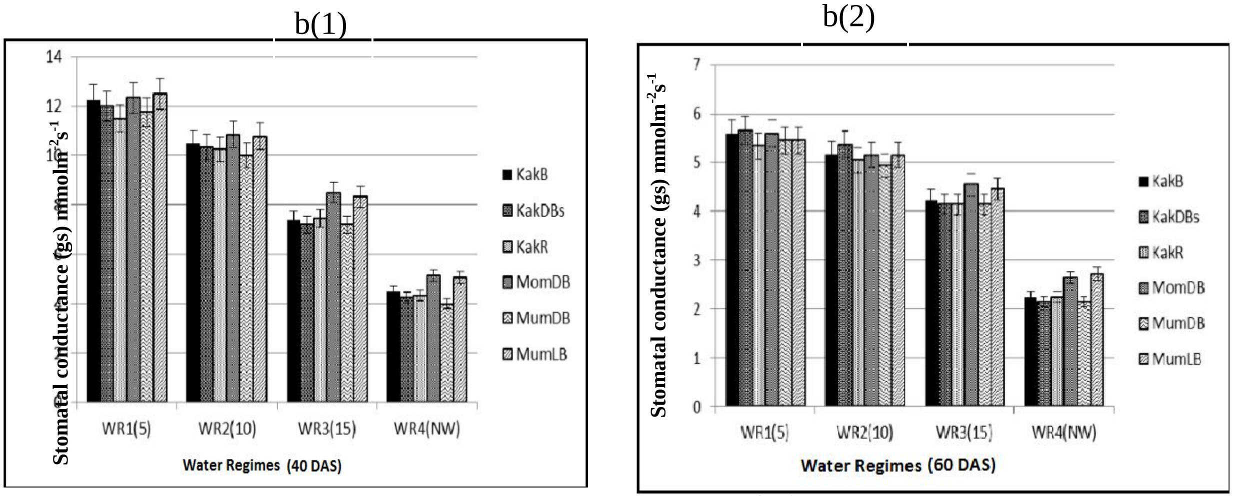

a(1)
a(2)

Fig 4.2a: Variation in soil moisture content (%) of different Bambara groundnut landraces as affected by watering regimes. a (1) = 40DAS while a (2) = 60DAS. Error bars represent standard error of mean.

4.2.2 Gas exchange parameters

4.2.2.1 Stomatal conductance (gs)

Significant ($p<0.05$) variation in stomatal conductance was observed between different Bambara groundnut landraces only under water regime 3 and 4 both at 40 DAS (Figure 4.2b (1)) and at 60 DAS (Figure 4.2b (2)). Watering regimes also differed significantly ($p<0.05$) in their effect on stomatal conductance (Appendix 2.2). Generally, stomatal conductance was found to decrease as irrigation frequency decreased. Significant ($p<0.05$) interaction was also observed between landraces and watering regimes (Landraces x WR) on stomatal conductance (Appendix 2.2) indicating that different landraces responded differently to different treatments. MomDB and MumLB landraces recorded the highest stomatal conductance under moisture stress both at 40 DAS and 60 DAS. Significant positive correlation was observed between stomatal conductance and net photosynthesis and transpiration rate indicating that the latter two parameters increased as the former increased. On the other hand, significant negative correlation was observed between stomatal conductance and water use efficiency indicating that the former decreased as the later increased (Table 4.2).

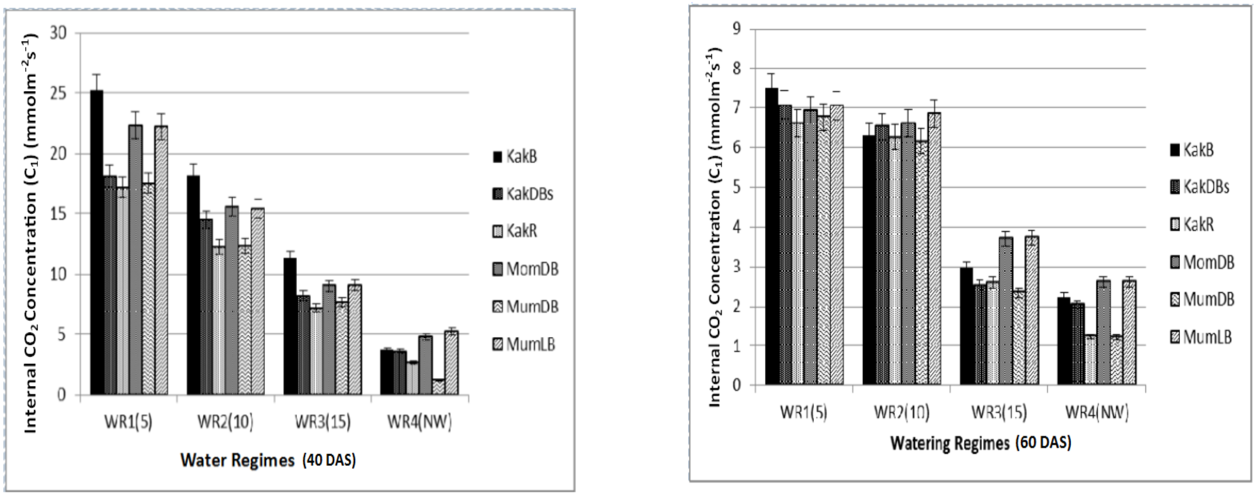


b(1) b(2)

Fig 4.2b: Variation in stomatal conductance (gs) ($mmolm^{-2}s^{-1}$) of different Bambara groundnut landraces as affected by watering regimes. b (1) = 40DAS while b (2) = 60DAS. Error bars represent standard error of means.

4.2.2.2 Internal CO_2 concentration (Ci)

Significant ($p<0.05$) variation in internal CO_2 concentration was observed between different Bambara groundnut landraces both at 40 DAS (Figure 4.2c (1)) and at 60 DAS (Figure 4.2c (2)). KaKB recorded the highest internal CO_2 concentration at WR1, 2 and 3 at 40 DAS followed by MomDB and MumLB. At 60 DAS, there were no significant differences among landraces for internal CO_2 concentration under watering regimes 1 and 2 but MomDB and MumLB had the highest internal CO_2 concentration under water regimes 3 and 4. Watering regimes also differed significantly ($p<0.05$) in their effect on internal CO_2 concentration (Appendix 2.3). Generally, internal CO_2 concentration was found to decrease as irrigation frequency decreased. There was no significant ($p>0.05$) interaction between landraces and watering regimes (Landraces x WR) on internal CO_2 concentration (Appendix 2.3) indicating that different landraces responded in a similar manner at different treatments.

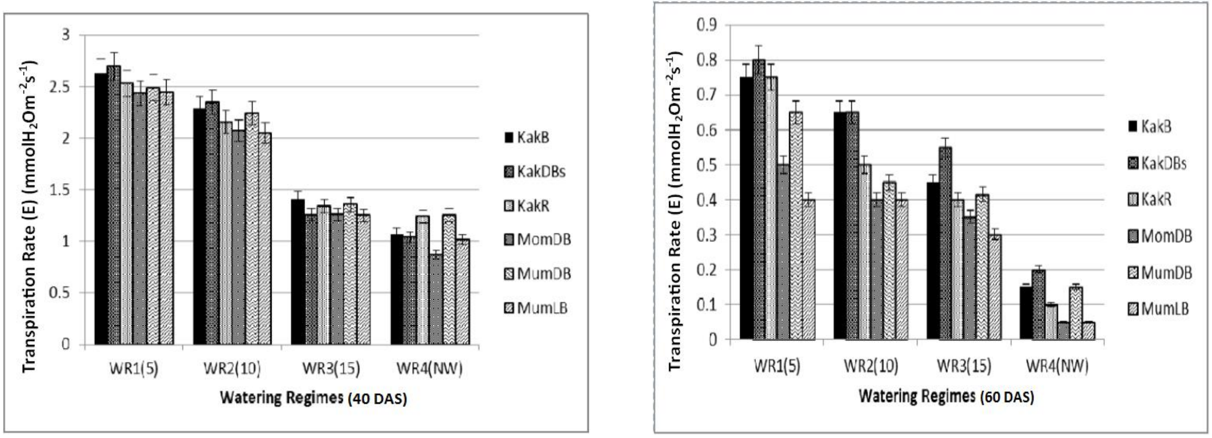

c(1)

c(2)

Fig 4.2c: Variation in Internal CO_2 concentration (Ci) of different Bambara groundnut landraces as affected by watering regimes. c (1) = 40DAS while c (2) = 60DAS. Error bars represent standard error of means.

4.2.2.3 Transpiration rate (E)

There was significant ($p<0.05$) variation among Bambara groundnut landraces for the rate of transpiration at 40 DAS (Figure 4.2d (1)) and at 60 DAS (Figure 4.2d (2)). Watering regimes also differed significantly ($p<0.05$) in their effect on the rate of transpiration (Appendix 2.4) which was found to decrease as irrigation frequency decreased. There was no significant ($p>0.05$) interaction between landraces and watering regimes (Landraces x WR) on transpiration rate (Appendix 2.4) indicating that different landraces responded in a similar manner at different treatments. MomDB and MumLB recorded the lowest rate of transpiration under moisture stress at 40 DAS and in all the treatments at 60 DAS. Significant positive correlation was observed between transpiration rate and both stomatal conductance and net photosynthesis indicating that each of these parameters increased as the other increased. On the other hand, significant negative correlation was observed between transpiration rate and water use efficiency indicating that the former decreased as the later increased (Table 4.2).



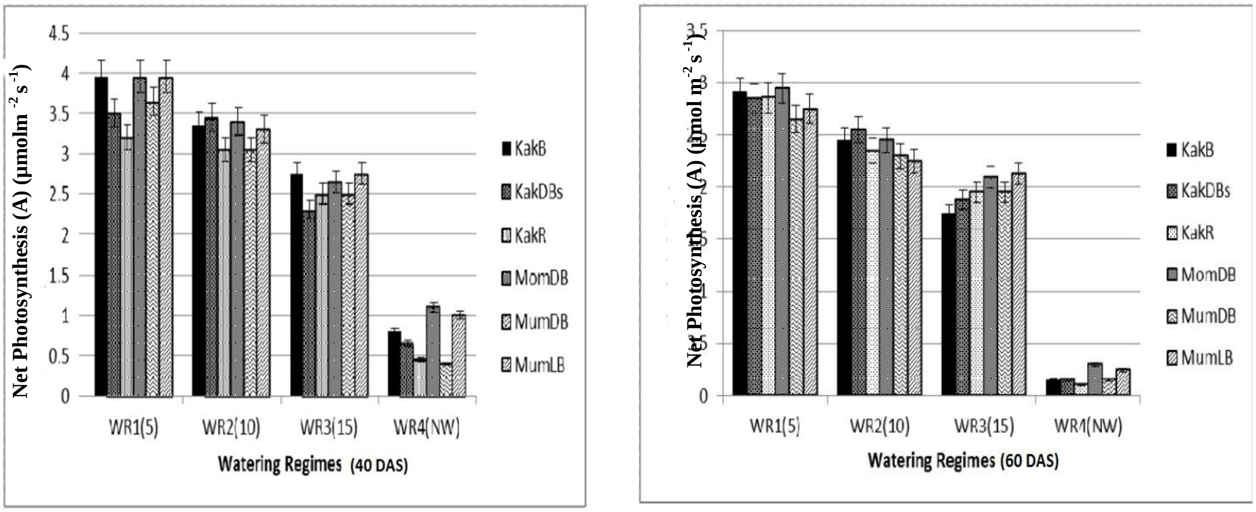
d (1)

d (2)

Fig 4.2d: Variation in Transpiration rate (E) of different Bambara groundnut landraces as affected by watering regimes. d (1) = 40DAS while d (2) = 60DAS. Error bars represent standard error of means.

4.2.2.4 Net Photosynthesis (A)

There was significant ($p<0.05$) variation among Bambara groundnut landraces on net photosynthesis both at 40 DAS (Figure 4.2e (1)) and at 60 DAS (Figure 4.2e (2)). MomDB and MumLB consistently recorded higher net photosynthesis that the rest of the genotypes. Watering regimes also differed significantly ($p<0.05$) in their effect on net photosynthesis (Appendix 2.5) which was found to decrease as irrigation frequency decreased. There was also significant ($p<0.05$) interaction between landraces and watering regimes (Landraces x WR) on net photosynthesis (Appendix 2.5) at 60 DAS indicating that different landraces responded differently to different treatments at this stage. Significant positive correlations were observed between net photosynthesis and water use efficiency, stomatal conductance and transpiration rate (Table 4.2).



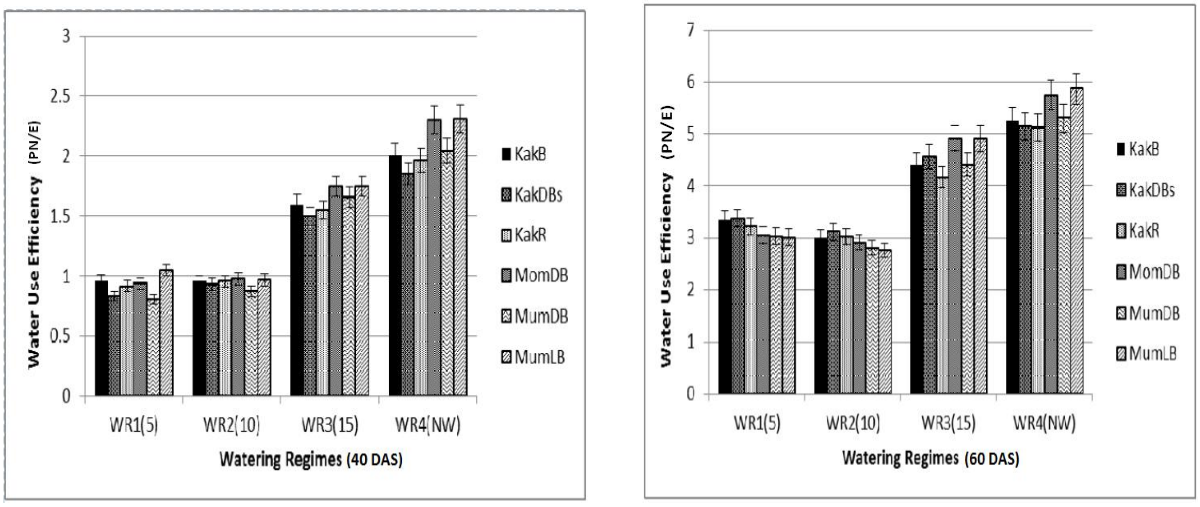
e(1)

e(2)

Fig 4.2e: Variation in Net Photosynthesis (A) of different Bambara groundnut landraces as affected by watering regimes. e(1) = 40DASwhile e(2) = 60DAS. Error bars represent standard error of means.

4.2.3 Water use efficiency (WUE)

There was significant ($p<0.05$) variation among Bambara groundnut landraces on water use efficiency under water stressed treatments but not under well watered treatments at both 40 DAS (Figure 4.2f (1)) and 60 DAS (Figure 4.2f (2)). Watering regimes also differed significantly ($p<0.05$) in their effect on water use efficiency (Appendix 2.6) which was found to increase with moisture stress. There was also significant ($p<0.05$) interaction between landraces and watering regimes (Landraces x WR) on water use efficiency (Appendix 2.6) indicating that different landraces responded differently to different treatments. MomDB and MumLB consistently recorded higher water use efficiency in most of the treatments both at 40 DAS and 60 DAS. Correlation coefficients demonstrated significant increase in water use efficiency as soil moisture content, stomatal conductance and transpiration rate decreased and vice versa. However, net photosynthesis and chlorophyll contents increased significantly as water use efficiency increased (Table 4.2).



f(1)

f(2)

Fig 4.2f: Variation in Water use efficiency (PN/E) of different Bambara ground nut landraces as affected by watering regimes. f (1) = 40DAS while f (2) = 60DAS. Error bars represent standard error of means.

4.2.4 Chlorophyll Content

4.2.4.1 Chlorophyll a

The content of chlorophyll a was found to vary significantly ($p<0.05$) among different landraces (Figure 4.2g). MomDB and MumLB consistently recorded higher chlorophyll a content under moisture stress both at 40 DAS (Figure 4.2g (1)) and 60 DAS(Figure 4.2g (2)).Watering regimes also differed significantly ($p<0.05$) in their effect on chlorophyll a content (Appendix 2.7). There was also significant ($p<0.05$) interaction between landraces and watering regimes (Landraces x WR) on chlorophyll a content (Appendix 2.7) indicating that different landraces responded differently to different treatments. Generally, chlorophyll a content decreased with decrease in irrigation frequency and as DAS increased. Correlation coefficients demonstrated significant increase in chlorophyll a as chlorophyll b and hence total chlorophyll increased and vice versa. Chlorophyll a was also found to increase as water use efficiency increased and vice versa (Table 4.2).

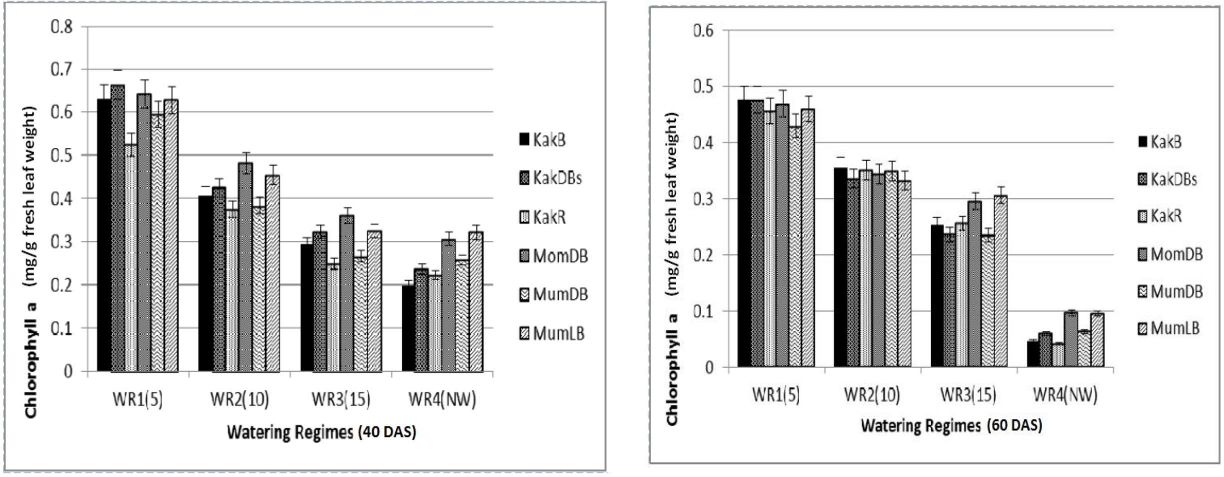
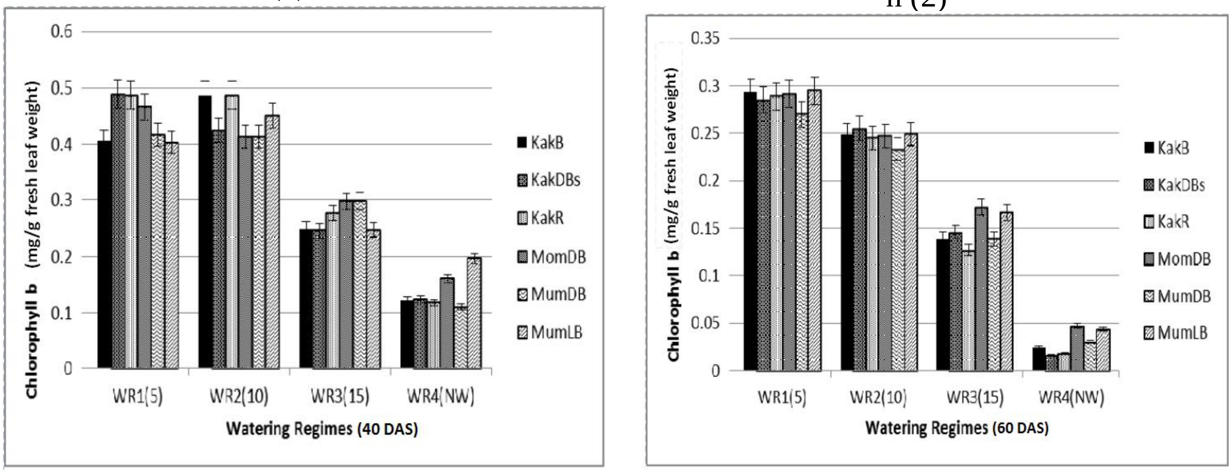

g (1) g (2)

Fig 4.2g: Variation in chlorophyll a of different Bambara groundnut landraces as affected by watering regimes. g (1) = 40DAS while g (2) = 60DAS. Error bars represent standard error of means.

4.2.4.2 Chlorophyll b

The content of chlorophyll b was found to vary significantly ($p<0.05$) among different landraces (Figure 4.2h). There was no consistent trend in change of chlorophyll b content among landraces under watered treatments but MomDB and MumLB consistently recorded higher chlorophyll b content under moisture stress both at 40 DAS (Figure 4.2h (1)) and 60 DAS(Figure 4.2h (2)). As it was the case with chlorophyll a, the content of chlorophyll b was found to decrease as irrigation frequency decreased and as DAS increased. Watering regimes also differed significantly ($p<0.05$) in their effect on chlorophyll b content (Appendix 2.8). In addition, there was significant ($p<0.05$) interaction between landraces and watering regimes (Landraces x WR) on chlorophyll b content (Appendix 2.8) indicating that different landraces responded differently to different treatments. Correlation coefficient demonstrated significant increase in chlorophyll b as chlorophyll a and hence total chlorophyll increased and vice versa. Chlorophyll b was also found to increase as water use efficiency increased and vice versa (Table 4.2).

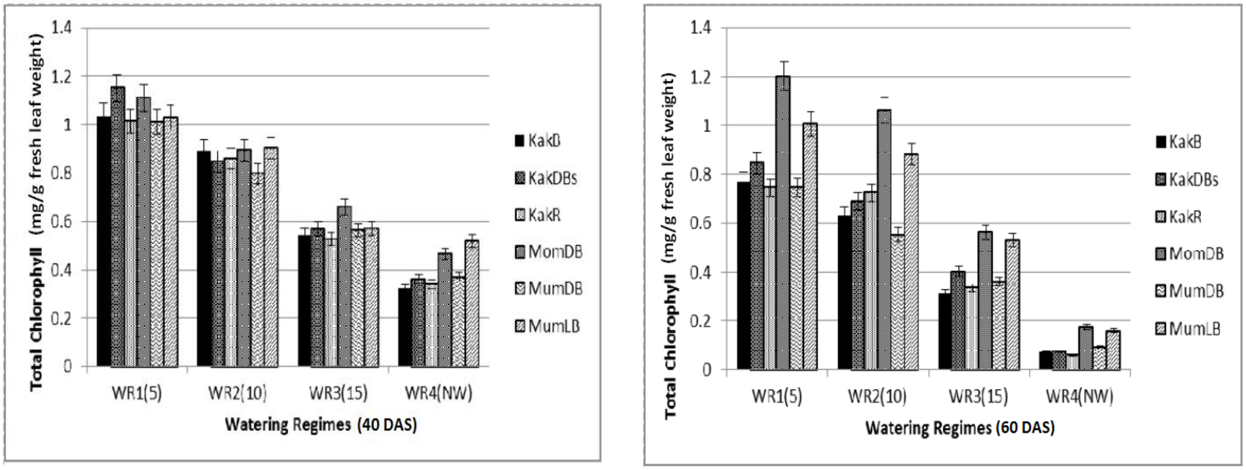

h (1)

h (2)

Fig 4.2h: Variation in chlorophyll b of different Bambara groundnut landraces as affected by watering regimes. h (1) = 40DAS while h (2) = 60DAS. Error bars represent standard error of means.

4.2.4.3 Total Chlorophyll

Different Bambara groundnut landraces varied significantly ($p<0.05$) in total chlorophyll (Figure 4.2i). There was no discernable trend in change of total chlorophyll content among landraces under watered treatments but MomDB and MumLB recorded higher total chlorophyll content under moisture stress both at 40 DAS(Figure 4.2i (1)) and 60 DAS(Figure 4.2i (2)). Like chlorophyll a and b, different watering regimes differed significantly ($p<0.05$) in their effect on total chlorophyll (Appendix 2.9). There was no significant Landraces x WR interaction on total chlorophyll content both at 40 and 60DAS. Significant positive correlations were observed between the total chlorophyll and both chlorophyll a and chlorophyll b contents as well as with water use efficiency (Table 4.2).


i (1)

i (2)

Fig 4.2i: Variation in total chlorophyll of different Bambara groundnut landraces as affected by watering regimes. i (1) = 40DAS while i (2) = 60DAS. Error bars represent standard error of means.

Table 4.2: Correlation coefficient matrix for physiological characters studied

Variables	A	gs	Ci	E	WUE	CHLa	CHLb	TCHL
SMC%	-0.233	0.041	-0.128	0.067	**-0.428**	0.065	-0.141	-0.014
A		**0.308**	-0.061	**0.543**	**0.418**	-0.158	-0.109	-0.144
gs			0.034	**0.644**	**-0.304**	-0.143	-0.198	-0.170
Ci				-0.045	-0.052	0.182	0.187	0.191
E					**-0.522**	-0.102	-0.193	-0.142
WUE						**0.291**	**0.313**	**0.304**
CHLa							**0.854**	**0.978**
CHLb								**0.944**

NB: Values in bold are different from 0 with a significance level alpha=0.05 where:

SMC% - Soil moisture content percentage

A - Net photosynthesis

gs - Stomatal Conductance

Ci - Internal CO_2 Concentration

E - Transpiration rate

WUE - Water use efficiency

CHLa - Chlorophyll a

CHLb - Chlorophyll b

TCHL - Total Chlorophyll

CHAPTER FIVE

DISCUSSION

Discussion

5.1 Greenhouse experiment 1

5.1.1 Soil moisture content percentage (SMC %)

The water consumption for the six landraces was similar in all the four treatments including the most water stressed treatment. This was an indication that all the six landraces were tolerant to soil moisture deficit to some extent. Similar results were obtained by Jorgensen *et al*. (2010) though on only two landraces. Different watering regimes (WR) recorded significant variations in soil moisture content which decreased as irrigation frequency decreased. This shows that the different irrigation treatments imposed to the landraces had a potential of causing significant morphological and physiological differences among Bambara groundnut landraces to enable determination of drought tolerance traits in the landraces being tested.

5.1.2 Plant height (PH)

Although the landraces were not significantly different in plant height in all the treatments, soil moisture deficit was found to have a significant effect on the plant height of Bambara groundnut as irrigated treatments recorded higher plant height than the treatment which was not irrigated at all. This was an indication that Bambara groundnut is generally tolerant to water stress and this finding was confirmed by lack of significant interaction between the plant height of the different landraces and watering regimes. In a similar study but conducted under field conditions, (Mabhaudhi and Modi, 2013) also observed no significant interaction between landraces and water regimes. Beyond 60 DAS, the plants that were severely stressed (WR4) dried off. This showed that the plants at this stage required more water for metabolism but this water was not available thus imposing a condition of severe water stress which led to permanent wilting and eventual desiccation and death. Similar observation was made by Madukwe *et al.* (2011). In this study, it was, however, difficult to use plant height to select the potentially drought tolerant landraces.

5.1.3 Leaf number per plant (LNP)

The highest number of leaves was recorded in water regimes 1, 2 and 3 where plants were irrigated after every 5, 10 and 15 days respectively, while the lowest number was recorded in water regime 4 where no irrigation was applied after 20 DAS. This could be perceived as an adaptive feature by plants to water stress condition. This observation is in line with the findings of Madukwe *et al.* (2011). According to Holbrook *et al.* (2012) drought tolerant plants produce fewer leaves so as to conserve water. However, in this study, the six landraces did not show significant differences in leaf number even in the treatment with the highest water stress. It was therefore difficult to select the potentially drought tolerant landraces using their leaf number. Jorgensen *et al.* (2010) argued that an adaptive mechanism to withstand drought periods plants, can reduce the leaf area index through either reduction of leaf number or reduction of the individual leaf size. It can therefore be argued that the landraces evaluated in this study adopted reduction of individual leaf size rather than reduction of leaf number as evident in section 4.1.4 of this thesis.

5.1.4 Plant leaf area (PLA)

Plant leaf area was found to decrease as irrigation frequency decreased. Similar results were obtained by Jorgensen *et al.* (2010). Jongrungklang *et al*. (2008) also observed that drought caused a reduction in the leaf area in peanut. Although there was no significant difference in leaf area among the six landraces in the frequently irrigated treatments, MomDB and MumLB landraces recorded the lowest leaf area in the severest water stressed treatment (water regime 4). Reduced leaf area expansion to progressive soil drying either by reduced leaf number or individual leaf size has been shown to be an adaptive mechanism to withstand drought periods as the transpiration rate will drop together with the reduced leaf area (Davies and Zhang, 1991; Jorgensen *et al.*, 2010). Earlier studies have also shown that Bambara groundnut can maintain leaf turgor pressure through a combination of osmotic adjustment, reduction in leaf area and effective stomatal regulation (Jorgensen *et al*., 2010). Significant reduction of leaf area by MomDB and MumLB landraces was therefore a positive trait of water stress tolerance in the two landraces.

5.1.5 Shoot: Root biomass % (S: RB %)

Shoot to root ratio is a measure of the distribution of dry matter between the root and the shoot systems and it is a good indicator of effects of certain treatments on root and shoot dry weights (Boutraa *et al*., 2010). In this study, percent shoot to root biomass was found to decrease as the water stress increased. However, the landraces did not differ significantly in shoot to root biomass except in the water stressed treatment (WR4) where MomDB and MumLB recorded significantly higher shoot to root biomass compared to the rest of the landraces. This was an indication that water stress prompted more root growth as a strategy y the plants to acquire more water. Similar results were obtained by Vurayai *et al.* (2011) and Mabhaudhi and Modi, (2013). However, Collinson *et al.* (1997) reported that Bambara groundnut allocated greater fraction of its total dry weight to roots than other groundnuts irrespective of available soil moisture. Reduction of shoot to root biomass followed a similar trend as the one observed for stomatal conductance, chlorophyll content and plant growth parameters. According to Mabhaudhi and Modi, (2013), the combination of reduced CO2 assimilation, low chlorophyll content and a smaller canopy size ultimately meant that Bambara groundnut landrace selections produced less biomass under water stressed conditions relative to irrigated conditions. They concluded that this is the reason why researchers have previously ascribed stomatal limitations to photosynthesis as the chief yield limiting factor under conditions of limited water availability. Blum, (2005) stated that drought avoidance mechanisms had the downside of reduced biomass production. This is because, in order for the plant to avoid drought, it would need to minimise water losses through stomatal closure and reduced canopy size, both of which ultimately reduce the amount of biomass produced by the plant.

5.1.6 Total dry matter

In this study, total dry matter was found to decrease as the water stress increased. However, the landraces did not differ significantly in total dry matter except in the water stressed treatments where MomDB and MumLB recorded significantly higher total dry matter compared to the rest of the landraces. Jongrungklang *et al.* (2008) also observed that drought reduced total dry matter of peanut. Boutraa *et al.* (2010) observed that water stress led to decline in the shoot and root dry weights as well as total dry weight of wheat.

5.2 Greenhouse experiment 2

5.2.1 Gas exchange measurements:

5.2.2.1 Stomatal conductance (gs) and Transpiration rate (E)

The mild water deficit (WR1 and WR2) did not significantly affect stomatal conductance which was found to reduce as water stress increased. This tendency of reduction of stomatal conductance under water stress is consistent with observations made by Collinson *et al*. (1997) and Cornellisen, (2005) in Bambara groundnuts. Decreased stomatal conductance results in lower net carbon dioxide assimilation rate, lower intercellular carbon dioxide and lower chloroplastic carbon dioxide tension. The carbondioxide insufficiency will reduce photosynthetic efficiency and dry matter production and may have negative impact on plant growth and yield (Vurayai *et al*., 2011). According to Collinson *et al*. (1997), Bambara groundnuts adopt a drought avoidance mechanism to survive drought through dehydration tolerance. Mabhaudhi and Modi, (2013) reported that stomatal closure is designed to reduce water losses through transpiration. This means that the Bambara groundnut landraces used in this study were able to adapt to limited water availability under water stressed conditions by closing their stomata. Similar observations of stomatal regulation in Bambara groundnut were reported by Collinson *et al.* (1997), Jørgensen *et al.* (2010) and Mabhaudhi and Modi, (2013). They also concluded that stomatal closure in Bambara groundnut was an important strategy for survival during intermittent stress. The fact that stomatal conductance was lower in severe water stressed treatments relative to more frequently irrigated conditions implies that Bambara groundnut landraces demonstrated a degree of stomatal control and hence regulation of transpiration losses. Stomatal closure is regarded as a plant's first line of defense in response to developing water stress (Vurayai *et al*., 2011; Mabhaudhi and Modi, 2013). Surprisingly, MomDB and MumLB landraces maintained higher stomatal conductance under water stressed conditions than other landraces but recorded the lowest transpiration rate. This implies that there could be another mechanism applied by landraces to regulate stomatal conductance and reduce transpiration rate apart from ordinary closing of the stomata. This could be through osmotic adjustment which assists in turgor maintenance, hence allowing stomatal opening and photosynthesis to be maintained over a wider range of soil moisture stress than in more susceptible species.

In this study, transpiration rate (**E**) of all the landraces was found to decrease as water stress increased. According to Davies and Zhang, (1991), Jørgensen *et al.* (2010) and Mabhaudhi and Modi, (2013), reduction in leaf area is a drought avoidance mechanism applied by groundnuts to reduce the rate of transpiration under water stress conditions. Water stress in plants is commonly attributed to situations where the water loss exceeds sufficient absorption intensity, causing a decrease in plant water content, turgor reduction and, consequently, a decrease in cellular expansion and alterations of various essential physiological and biochemical processes that can affect growth or productivity (Madukwe *et al*., 2011). Vurayai *et al.* (2011) observed that under water stress, Bambara groundnut transpired less water, which they considered as a first line of defense against drought. Davies and Zhang, (1991) and Jorgensen *et al.* (2010) argued that reduced transpiration rate either through reduced leaf area or reduced leaf number or is an adaptive mechanism to withstand drought periods. MomDB and MumLB landraces maintained similar leaf number as that of other landraces but recorded the lowest transpiration rate. These two landraces were therefore considered potentially drought tolerant than all other landraces evaluated in this study.

5.2.1.2 Internal CO2 concentration (Ci)

Internal CO_2 concentration decreased as water stress increased. Mabhaudhi and Modi, (2013) reported that apart from reducing transpiration, stomatal closure also decreases flow of CO_2 into leaves resulting in decline in net photosynthesis and ultimate reduction in plant growth. Reduction in intracellular CO_2, due to stomatal closure, results in reduced substrate availability for photosynthesis. Therefore, there is a need to down-regulate photosynthesis in line with reduced substrate availability. In this regard, chlorophyll content has been reported to decrease in water-stressed plants (Farooq *et al.*, 2009). The six landraces evaluated in this study were not significantly different in internal CO_2 concentration in mild water deficit treatments but significant differences were recorded in severe water stressed treatments. MomDB and MumLB landraces recorded significantly higher internal CO_2 concentration than that of other landraces under water stressed conditions both at 40 DAS and 60 DAS. This was probably due to the ability of these landraces to maintain high stomatal conductance in water stressed conditions as earlier discussed.

5.2.1.3 Net photosynthesis (A)

Net photosynthesis was also found to reduce as moisture stress increased. Similar observations were made by Chaves and Oliveira, (2004) and Mabhaudhi and Modi, (2013). It can therefore be concluded that the landraces demonstrated an ability to down-regulate photosynthesis in line with reduced chlorophyll content and CO_2 availability caused by stomatal closure. Although there was no significant correlation between chlorophyll content and photosynthesis, it was expected that reduction in chlorophyll would result in reduced photosynthesis. Mabhaudhi and Modi, (2013) reported that decreased CO_2 availability necessitates a down-regulation of photosynthesis by lowering the levels of photosynthetic pigments, chiefly chlorophyll. The lower plant growth observed under water stressed conditions may have been due to reduced photosynthesis.

5.2.2 Water use efficiency (WUE)

The results showed that the WUE was not affected by the mild water stress treatments in any of the landraces investigated in this study. Similar findings were reported by Boutraa *et al.* (2010). However, WUE was found to increase as water stress increased. High water use efficiency under limited water conditions is linked to reduced canopy size (plant height, leaf number, LAI), reduced transpiration losses (low stomatal conductance) as well as a shortened growth duration (Blum,2009). Increased WUE often occurs at the expense of yield potential (Blum, 2009) .These results contradict the findings of Boutraa *et al.* (2010) who reported that WUE decreased as a result of water stress in wheat cultivars. MomDB and MumLB recorded higher WUE under water stress both at 40 DAS and 60 DAS. Some drought-tolerant plants reportedly increase their WUE as water stress increases, while some drought-sensitive ones decrease the WUE during drought (Boutraa *et al.*, 2010).Correlation coefficients demonstrated significant increase in water use efficiency as soil moisture content, stomatal conductance and transpiration rate decreased and vice versa. However, net photosynthesis and chlorophyll contents increased significantly as water use efficiency increased. These results partly agree and partly disagree with Akram *et al.* (2013) who found a strong and positive correlation between WUE and photosynthetic rate and stomatal conductance but a negative correlation between WUE and transpiration rate.

5.2.3 Chlorophyll content (CHLc)

Assessing alterations in chlorophyll pigment composition and content is an effective means of evaluating plant responses to stresses (Chen *et al.*, 2007). Results of this study showed that chlorophyll (both a and b) content was significantly high in mild water deficit treatments (WR1 and WR2) and lower in severe water stressed treatments especially in WR4 which was not irrigated at all after 20 DAS. Several other researchers also reported that water stress decreased chlorophyll content (Anjum *et al*., 2003; Kiani *et al*., 2008; Farooq *et al*., 2009 and Mabhaudhi and Modi, 2013). In separate experiments conducted on barley by Anjum *et al*. (2003) and Farooq *et al*. (2009), water stress was shown to induce changes in the ratios and quantities of chlorophyll a and b as well as carotenoids. Mensha *et al.* (2006) reported decreased chlorophyll content in sesame subjected to water stress. Chlorophyll content was also shown to decrease in sunflower plants subjected to water stress (Kiani *et al.,* 2008). Unlike these researchers, Vurayai *et al*. (2011), working on pot trials of the same crop in glasshouse, reported that water stress did not have a significant effect on chlorophyll content index (CCI) of Bambara groundnut landraces. They concluded that CCI was not reduced by water stress at all stages of growth. However, they recommended that their observations be evaluated further under field conditions. This study shows that Chlorophyll content of MomDB and MumLB landraces was significantly higher than that of other landraces both at 40 DAS and 60 DAS. The two landraces were therefore able to adapt to water stress better than the other four landraces evaluated. According to Vurayai *et al*. (2011), Bambara groundnut maintain high amounts of chlorophyll content despite the development of moisture deficit stress and this trait can be considered to be a line of defense against drought which probably enable it to resist drought. Mabhaudhi and Modi, (2013) reported that decreased CO_2 availability necessitates a down-regulation of photosynthesis which involves lowering the levels of photosynthetic pigments, chiefly chlorophyll. However, reduction in chlorophyll content and subsequent reduction of photosynthesis results in reduced growth. Significantly higher chlorophyll content recorded in MomDB and MumLB landraces was attributed to the fact that the two landraces had relatively higher stomatal conductance which resulted in higher internal CO_2 concentration as earlier reported.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

Conclusions and recommendations

6.1 Conclusions

This study provided evidence of Bambara groundnut landraces adaptability to conditions of limited water. The result of this study indicated that Bambara groundnut is generally sensitive to severe water stress but can thrive under moderate stress condition. The death of most plants in severe water stressed treatments (WR3 and WR4) beyond 60 DAS which forced the termination of the two experiments was a clear evidence of this. All the landraces demonstrated drought avoidance and escape mechanisms under severe water stress compared to mild stressed. Drought avoidance was demonstrated by minimising water losses by restricting leaf area expansion, lowering stomatal conductance, decreasing shoot: root biomass % and increasing water use efficiency in response to reduced water availability. Chlorophyll concentration also proved to be a useful index for evaluating crop responses to water stress. Total dry matter which was the best indicator of yield was also found to reduce as water stress increased indicating that the effects of water stress on growth and physiology of Bambara groundnut ultimately results in reduced yields.

It was also evident that different landraces are affected differently by moisture stress thus there is a potential of selecting landraces with high moisture stress tolerance. MomDB and MumLB landraces were identified as the most tolerant to water stress, however there was a lot of variability in the other landraces in terms of water tolerant traits. The two are therefore recommended to researchers for further evaluation including under field conditions. The two are also recommended to farmers for adoption especially in drought prone areas. However, this study only reports reactions during the vegetative stage of crop development, studies on water deficit effects should be conducted at germination, vegetative, flowering and podding stages both in controlled and field environments as this will be necessary to evaluate drought tolerant traits in the six landraces effectively.

6.2 Recommendations

MomDB and MumLB landraces morphologically and physiologically exhibited great tolerance to water deficit as compared to the rest of landraces in this study hence the two may be recommended to researchers for further evaluation on their productivity and farmers for cultivation in dry prone areas.

6.3 Suggestions for future research

In future the following should be considered for further research on Bambara groundnut landraces in Kenya:-

1. Field experiments are recommended in different agro-ecological regions in order to select the most suitable landrace for a particular region.
2. Water deficit effects on growth and development of Bambara groundnut should be done based on their various developmental stages (germination, vegetative, flowering and pod filling stage) because this might provide a basis for development of strategies in order to increase yields.
3. Though this study didn’t present data on seed yield due termination at 80DAS due to death of plants in WR 3 and 4 ,there is also need to investigation on seed yield of Bambara groundnut in response to soil water deficit, as the landrace differences in pod setting may be larger than what can be explained by plant physiology alone.

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APPENDICES

Appendix 1: Analysis of Variance for Parameters Studied in greenhouse Experiment 1

1.1 Soil Moisture Content (%)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	12.208	2.442	1.566	0.071*
	WR	3	277.250	92.417	59.273	< 0.0001***
	Rep	3	6.917	2.306	1.479	0.228^{ns}
	Landraces*WR	15	17.875	1.192	0.764	0.711^{ns}
60	Landraces	5	9.833	1.967	1.273	0.084^{ns}
	WR	3	370.792	123.597	79.983	< 0.0001***
	Rep	3	3.375	1.125	0.728	0.539^{ns}
	Landraces*WR	15	18.333	1.222	0.791	0.683^{ns}

1.2 Plant Height (cm)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	43.606	8.721	2.015	0.062^{ns}
	WR	3	2.882	0.961	0.222	0.881^{ns}
	Rep	3	48.809	16.270	3.756	0.015*
	Landraces*WR	15	63.707	4.247	0.980	0.485^{ns}
60	Landraces	5	35.566	7.113	0.869	0.506^{ns}
	WR	3	23.537	7.846	0.959	0.417^{ns}
	Rep	3	25.937	8.646	1.057	0.373^{ns}
	Landraces*WR	15	139.593	9.306	1.137	0.342^{ns}

1.3 Leaf Number per Plant

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	243.458	48.692	2.310	0.053^{ns}
	WR	3	229.083	76.361	3.620	0.024*
	Rep	3	21.833	7.278	0.345	0.793^{ns}
	Landraces*WR	15	398.792	26.586	1.261	0.251^{ns}
60	Landraces	5	1075.927	615.185	1.749	0.169^{ns}
	WR	3	265.948	88.649	2.868	0.044*
	Rep	3	54.531	18.177	0.588	0.625^{ns}
	Landraces*WR	15	606.365	40.424	1.308	0.222^{ns}

1.4 Plant Leaf Area (cm^2)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	742103.900	148420.800	3.614	0.023*
	WR	3	379169.500	126389.800	3.061	0.031*
	Rep	3	1686684.948	62228.320	1.507	0.154^{ns}
	Landraces*WR	15	597462.531	39830.835	0.970	0.495^{ns}
60	Landraces	5	533155.200	106631.000	3.595	0.032*
	WR	3	404953.400	134984.500	4.548	0.011*
	Rep	3	73015.125	24338.375	0.820	0.487^{ns}
	Landraces*WR	15	476479.375	31765.292	1.071	0.399^{ns}

1.5 Shoot: Root Biomass (%)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	4.705	0.941	1.213	0.312^{ns}
	WR	3	6.253	2.084	2.688	0.001**
	Rep	3	2.176	0.725	0.935	0.429^{ns}
	Landraces*WR	15	26.753	1.784	2.300	0.010*
60	Landraces	5	23.578	4.716	0.599	0.701^{ns}
	WR	3	56.818	18.939	2.406	0.010*
	Rep	3	14.381	4.794	0.609	0.612^{ns}
	Landraces*WR	15	147.723	9.848	1.250	0.258^{ns}

1.6 Total Dry Matter (%)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	8.296	1.659	1.776	0.129^{ns}
	WR	3	6.184	2.061	2.208	0.034*
	Rep	3	2.404	0.801	0.858	0.467^{ns}
	Landraces*WR	15	9.910	0.661	0.707	0.769^{ns}
60	Landraces	5	25.869	5.174	1.798	0.125^{ns}
	WR	3	33.259	11.086	3.853	0.028*
	Rep	3	6.889	2.296	0.798	0.499^{ns}
	Landraces*WR	15	34.541	2.303	0.800	0.673^{ns}

Appendix2: Analysis of Variance for Parameters Studied in greenhouse Experiment 2

2.1 Soil Moisture Content (%) (SMC %)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	31.000	6.200	3.056	0.059^{ns}
	WR	3	116.917	38.972	19.208	< 0.0001***
	Rep	3	8.333	8.333	4.107	0.054^{ns}
	Landraces*WR	15	14.333	0.956	0.471	0.932^{ns}
60	Landraces	5	30.250	6.050	3.576	0.015*
	WR	3	175.750	58.583	34.623	< 0.0001***
	Rep	3	10.083	10.083	5.959	0.023*
	Landraces*WR	15	6.250	0.417	0.246	0.996^{ns}

I. 2.2 Stomatal conductance (gs) (mmolm^{-2}s^{-1})

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	1.585	0.317	3.388	0.046*
	WR	3	1.134	0.378	4.021	0.015*
	Rep	3	0.315	0.315	3.367	0.079^{ns}
	Landraces*WR	15	7.108	0.474	5.061	0.061^{ns}
60	Landraces	5	0.493	0.099	3.339	0.039*
	WR	3	1.738	0.579	19.618	< 0.0001***
	Rep	3	0.219	0.219	7.406	0.012*
	Landraces*WR	15	3.454	0.230	7.797	0.059^{ns}

2.3 Internal CO2 concentration (Ci) (mmolm^{-2}s^{-1})

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	45874.100	9174.821	4.339	0.013*
	WR	3	30361.230	10120.410	4.786	0.009**
	Rep	3	1598.521	1598.521	0.756	0.394^{ns}
	Landraces*WR	15	35689.646	2379.310	1.126	0.389^{ns}
60	Landraces	5	168883.167	33776.630	4.464	0.019*
	WR	3	84088.000	28029.330	3.705	0.015*
	Rep	3	28812.000	28812.000	3.808	0.063^{ns}
	Landraces*WR	15	107691.500	7179.433	0.949	0.439^{ns}

2.4 Transpiration rate (E) (mmolH$_2$Om^{-2}s^{-1})

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	9705.604	1941.121	23.856	< 0.0001***
	WR	3	1829.896	609.965	7.496	0.001**
	Rep	3	88.021	88.021	1.082	0.309^{ns}
	Landraces*WR	15	871.479	58.099	0.714	0.377^{ns}
60	Landraces	5	236.188	47.238	19.761	< 0.0001***
	WR	3	21.229	7.076	2.960	0.033*
	Rep	3	3.521	3.521	1.473	0.237^{ns}
	Landraces*WR	15	69.396	4.626	1.935	0.067^{ns}

2.5 Net Photosynthesis (A) (µmolm^{-2}s^{-1})

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	1.760	0.352	11.040	0.003**
	WR	3	1.380	0.460	29.016	< 0.0001***
	Rep	3	0.052	0.052	1.643	0.189^{ns}
	Landraces*WR	15	3.685	0.246	7.707	< 0.0001***
60	Landraces	5	0.899	0.180	11.336	0.003**
	WR	3	0.916	0.305	19.251	< 0.0001***
	Rep	3	0.061	0.061	1.924	0.154^{ns}
	Landraces*WR	15	327.183	21.812	1375.253	< 0.0001***

2.6 Water use efficiency (WUE)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	41.202	8.240	3.780	0.012*
	WR	3	30.085	10.028	4.600	0.012*
	Rep	3	0.394	0.394	0.181	0.675^{ns}
	Landraces*WR	15	94.435	6.296	2.888	0.011*
60	Landraces	5	1290.785	258.157	6.295	0.001**
	WR	3	1276.475	425.492	10.375	0.000***
	Rep	3	77.840	77.840	1.898	0.182^{ns}
	Landraces*WR	15	3961.840	264.123	6.440	< 0.0001***

2.7 Chlorophyll a (mg/g fresh leaf weight)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	0.595	0.119	5.243	0.002**
	WR	3	0.376	0.125	5.549	0.001**
	Rep	3	0.044	0.044	1.948	0.176^{ns}
	Landraces*WR	15	0.455	0.030	1.336	0.258^{ns}
60	Landraces	5	0.539	0.108	5.203	0.002**
	WR	3	0.443	0.148	7.128	0.001**
	Rep	3	0.028	0.028	1.342	0.259^{ns}
	Landraces*WR	15	0.416	0.028	1.339	0.257^{ns}

2.8 Chlorophyll b (mg/g fresh leaf weight)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	0.193	0.039	3.886	0.011*
	WR	3	0.121	0.040	4.183	0.001**
	Rep	3	0.006	0.006	0.598	0.447^{ns}
	Landraces*WR	15	0.216	0.014	1.452	0.205^{ns}
60	Landraces	5	9.349	1.870	4.456	0.001**
	WR	3	4.660	1.553	3.702	0.023*
	Rep	3	0.464	0.464	1.104	0.304^{ns}
	Landraces*WR	15	8.152	0.543	1.294	0.281^{ns}

2.9 Total Chlorophyll (mg/g fresh leaf weight)

DAS	Source	DF	Sum of squares	Mean squares	F	Pr > F
40	Landraces	5	1.420	0.284	5.182	0.002**
	WR	3	1.157	0.386	7.067	<0.0001***
	Rep	3	0.083	0.083	1.507	0.232^{ns}
	Landraces*WR	15	1.250	0.083	1.521	0.178^{ns}
60	Landraces	5	6.132	1.226	2.794	0.041*
	WR	3	3.514	1.171	2.668	0.047*
	Rep	3	0.719	0.719	1.637	0.213^{ns}
	Landraces*WR	15	11.128	0.742	1.690	0.125^{ns}