MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF BAMBARA GROUNDNUT (Vigna Subterranea L. Verdc) TO SHORT PERIODS OF WATER DEFICIT STRESS DURING DIFFERENT DEVELOPMENTAL STAGES

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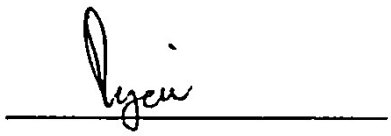
STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at the University of Botswana, Botswana College of Agriculture from August 2009 to June 2010. It is original except where references are made and it will not be submitted for the award of any other degree or diploma of any other University.



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DEDICATION

This work is dedicated to my loving husband Moses for being so selfless and supportive throughout my studies and to my son Bradley for seeking less attention thereby giving me time to do my studies. To all mothers out there, this is to show you that even the sky is not the limit and even if you have children to attend to, you can still fulfil your dreams.

ABSRACT

The response pattern of morphological traits and physiological processes of bambara groundnut (*Vigna Subterranean* L. Verdc) to short periods of water stress imposed at different growth and developmental stages and their recuperative ability after water stress were evaluated. Two greenhouse experiments were conducted in Gaborone, Botswana at in the 2009/2010 cropping season. The treatments consisted of watering plants to 100 % plant available water (PAW), withholding water to 30 % PAW at vegetative, flowering and pod filling growth stages and rewatering the plants after 21 days of each stress treatment. Water stress reduced relative water content, chlorophyll fluorescence, stomatal conductance and the reduction was more pronounced in plants stressed during the pod filling stage and less pronounced in plants stressed during the vegetative stage. Chlorophyll content was not affected by water stress at all stages of growth and development. Proline levels were increased by water stress mostly during the pod filling stage.

Relative leaf expansion rate was reduced by water stress with the pod filling stage having the highest reduction. Leaf numbers, plant height and shoot: root ratio were also reduced by water stress and the reduction was highest during the vegetative stage. When plants were rewatered after each stress treatment, plants stressed during the pod filling stage failed to fully recover the relative water content and chlorophyll fluorescence. All water stressed plants at different growth stages of growth and development fully recovered stomatal conductance and proline concentration. The relative leaf expansion rate of plants stressed at pod filling and flowering stages failed to recover from water stress. Seed yield in all stressed plants was reduced by water stress due to reductions in pods per plant, seeds per pods and seed weight. The highest

yield amongst the stressed plants was obtained in plants stressed during the vegetative stage, followed by the flowering and lastly the pod filling stage. Bambara groundnuts appears to reduce water loss under water stress and has the ability to recover from water stress after rainfall or irrigation and is therefore capable of producing some yield under water limited conditions.

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LIST OF ABBREVIATIONS

ABA: Abscisic acid

ANOVA: Analysis of variance

ATP: Adenosine triphosphate

Ca^{+}: Calcium

cm: Centimetres

CO_2: Carbon dioxide

DAS: Days after sowing

DNA: Deoxyribonucleic acid

EU: European union

F_o: Initial Fluorescence

F_m: Maximum fluorescence

F_v: Variable equal F_m-F_o

F_v/F_m: The ratio of variable to maximum fluorescence

g: Grams

H_2O: Water

K^{+}: Potassium

Kg ha^{-1}: Kilograms per hectare

LSD: Least significant difference

ml: millimetre

mmols/ m^2s^{-1}: millimoles per meter squared per second

MPA: Megapascals

N: Nitrogen

Na^{+}: Sodium

NADP: Nicotinamide adenine dinucleotide phosphate

NADPH: Nicotinamide adenine dinucleotide phosphate reduced

P: Phosphorus

PAW: Plant available water

PS II: Photosystem two

RLER: Relative leaf expansion rate

Rubisco: Ribulose-1, 5-bisphosphate carboxylase/oxygenase

RWC: Relative water content

SAS: Statistical analysis system

Ψp: Pressure potential

Ψw: Water potential

Ψs: Solute potential

µg: Micrograms

µgchl: Micrograms of chlorophyll

µmole: Micromole

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

1.1.1 Origin and Distribution

Bambara groundnut (*Vigna subterranean* L. verdc) is an indigenous African leguminous crop grown primarily for its seeds. Its center of origin is thought to be Bambara, near Timbuktu I central Mali, West Africa (hence its name Bambara groundnut) (De Kock, 2004) but according to Brink *et al.* (2006), the centre of origin of bambara groundnut is probably north-eastern Nigeria and northern Cameroon. It is also found in the wild from central Nigeria eastwards to Southern Sudan. It is now cultivated throughout tropical Africa and is also grown in other parts of the world like the tropical parts of Asia, parts of Northern Australia, and South and Central America (De Kock, 2004).

1.1.2 Morphology of bambara groundnuts

Bambara groundnut is an indeterminate annual herb up to 30 cm in height with creeping stems branching just above ground level (Linnemann and Azam-Ali, 1993). Growth habits vary from bunch type to semi-bunch type and spreading (Cornellisen, 2005). It consists of a tap root with lateral roots lower down with rounded and sometimes lobed nodules. Leaves are pinnately trifoliate, glaborous, with erect, grooved petioles thickened at the base. The terminal leaflet, often larger than the lateral leaflets, may reach a length of 10 cm and a width of 3.5 cm (Linnemann and Azam-Ali, 1993).

The racemes consist of 1-3 (usually 2) whitish to yellow flowers on short axillary peduncle. The flowers open in the early hours of the morning when skies are clear and sometimes flower opening may be delayed due to an overcast sky or low temperature. The flowers produced on the same peduncle do not open on the same day although the interval does not exceed 24 h (Massawe *et al.*, 2003). In many genotypes, flowering is photoperiod-insensitive, while in others it is delayed by long photoperiods (Brink *et al.*, 2006). Flowering starts 30-55 days after sowing and may continue until the plant dies (Linnemann and Azam-Ali, 1993). Recent research suggests that bambara groundnut is self pollinated in most environments (Massawe *et al.*, 2003) and according to Doku (1968) ants may play a vital role in the pollination of the flowers. After pollination and fertilisation the peduncle which has a rounded, glandular apex lengthens, thus bringing the ovaries to or just below the ground level (Linnemann and Azam-Ali, 1993).

The pods usually develop underground and may reach up to 3.7 cm in diameter depending on landrace (Massawe *et al.*, 2003) and are usually almost spherical and contain one round to oblong seed although they may occasionally contain up to 4 seeds (Rassel, 1960). Pods reach their maximum size in about 30 days and seeds expand to reach maturity in the following 10 days. Bambara groundnut seeds reach maturity between 3-6 months from sowing depending on genotype. Podding is retarded by long photoperiods and it may also be delayed by drought (Brink *et al.*, 2006). Mature pods are indehiscent, ranging from yellowish to reddish to dark brown or even black in colour. Seeds are very variable in colour being white, cream, yellow, red, purple, brown or black coloured and the colouration is uniform, mottled, blotched or striped and some have an eye with a dark colour around the white helium (Linnemann and Azam-Ali, 1993).

1.1.3 Physiology of bambara groundnuts

Germination of bambara groundnut is hypogeal and it usually takes 7 to 15 days (Linnemann and Azam-ali, 1993; Kocabas *et al.*, 1999) and germination rate is dependent on temperature, genetic variability, seed size and age when water is not limited (Kobacas *et al.*, 1999; Massawe *et al.*, 2002). The optimum temperature for germination of bambara groundnut is 30–35°C and below 15°C and above 40°C, germination is very poor. Bambara groundnut is cultivated in the tropics at altitudes up to 2000 m. A frost-free period of at least 3 months is necessary. Average day temperatures of 20–28°C and full sun are preferred. The crop is cultivated successfully in areas with an average annual rainfall of 600–750 mm, though optimum yields are obtained when rainfall is higher (900–1200 mm/year). It is also grown in humid conditions, such as Northern Sierra Leone, where the annual rainfall exceeds 2000 mm (Brink *et al.*, 2006).

1.1.4 Yields

The current yields of bambara groundnut are extremely low and variable because of the environments in which it is normally grown which are characterised by various biotic and abiotic stresses (Massawe *et al.*, 2003). Bambara groundnut yields vary considerably between sites, seasons and genotypes. The highest recorded yields under field conditions are close to 4000 kg ha^{-1} and average farm pod yields vary between 300 and 800 kg ha^{-1} for most of the semi-arid tropics (Brink *et al.*, 2006). However, there are large differences between countries. In various studies in Africa, the following yields have been reported : 500 to 800 kg ha^{-1} in Ghana (Doku, 1997), 50-660 kg ha^{-1} in Swaziland (Sesay *et al.*, 1999), 60-110kg ha^{-1} in Zambia (Linnemann and Azam-Ali, 1993) and 71 to 82 kg ha^{-1} in Zimbabwe (Manyepe, 2002). Bambara

groundnut therefore combines the potential to produce substantial yields under favourable growing conditions with the ability to produce a small but stable yield under marginal conditions of limited rainfall (Linnemann and Azam-Ali, 1993).

1.1.5 Who grows the crop?

Bambara groundnut has been grown for centuries as a secondary food crop by subsistence farmers throughout sub-Saharan Africa, but mainly in the semi-arid regions of the continent. The crop is usually cultivated on small farms by the poorer section of the population (Linnemann and Azam-Ali, 1993). Bambara groundnut is considered to be a 'women's crop' (Linnemann, 1990; Drabo *et al.*, 1997; Manthe *et al.*, 2002) thus it is mostly given less value and less priority in allocation of land (De Kock, 2004). According to Brink *et al.* (1996), bambara groundnut fields were recorded as under female ownership in Botswana but in Swaziland, Sesay *et al.* (1999) found that although an average of 74% of the fields were under female ownership there were significant differences in this proportion between regions.

1.1.6 Significance of Bambara groundnuts

Bambara groundnut is the third most important legume in Africa after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) (Howell *et al.*, 1994), but has been neglected and underutilised (Cornellisen, 2005). Experimental results and growers experience have indicated that bambara groundnut is able to produce pod yields where many other crops (including groundnut) may fail altogether (Babiker, 1989; Collinson *et al.*, 1996). This may be because, many of its genotypes are drought tolerant and so give farmers some returns in years when other legume crops like groundnuts fail due to bad rains. It may also be because bambara groundnut is adapted to a wide range of soils, although it thrives most on light sandy and well

drained loams. Bambara groundnut is therefore an ideal 'food security' crop (Linnemann and Azam-Ali, 1993).

Bambara groundnut is grown mostly for human consumption (Brink *et al.*, 2006) and has diverse uses. Primarily it is preferred for its taste and flavour as compared to groundnuts. Freshly harvested pods are cooked, shelled and eaten as snacks and dry seeds are cooked and pounded into flour or eaten as a snack (Linnemann and Azam-Ali, 1993). Reports in literature indicate that the mature seeds are a rich source of protein (16-25% DM) and carbohydrate (42-60% DM); but in comparison with groundnut, the lipid content is low (5-6% DM) (Poulter and Caygill, 1980; Aykroyd and Doughty 1982; Brough and Azam-Ali 1992). The protein in bambara groundnut has high lysine content, and so has a beneficial complementary effect when consumed together with cereals which have low lysine content (Rowland, 1993). Bambara groundnut thus plays an important role in food security particularly meeting the protein requirement of resource-poor farmers in semi-arid Africa.

Vegetable milk and fermented products have been made from bambara groundnut seeds and the seeds have been used to feed pigs and poultry. The leaves and stems have also been used as fodder (Brink *et al.*, 2006). Bambara groundnuts may be used as a source of additional income as the average market price lies well above the world prices of other pulses (Coudert, 1982). Any excess production can therefore be easily sold along roads as a snack to passer-by or in local markets (Linnemann and Azam-Ali, 1993).

Bambara groundnut has been reported to leave nitrogen in the soil as it fixes atmospheric nitrogen with bacteria of the bradyrhizobium group. It is therefore useful in crop rotations because it may improve the nitrogen status of the soil which can then

be used by subsequent crops (Brink *et al.*, 2006). Mukurumbira (1985) reported that in Zimbabwe, bambara groundnut had a higher residual nitrogen effect than groundnut, maize or fallow and that there was no need to apply nitrogen to maize when it was grown after bambara groundnut in rotation. Furthermore because bambara groundnut is relatively free of pests and diseases, its inclusion in the cropping sequence may delay their build-up in succeeding crops in the rotation (Linnemann and Azam-Ali, 1993).

However, despite its importance in the subsistence diet of much of Africa, bambara groundnut is still cultivated from local landraces rather than from varieties bred specifically for particular environments, and farm yields are still low.

1.2 Statement of problem

It is an important and recurring point in the literature that although there is a growing awareness of the potential of bambara groundnut to contribute to increased food production in Africa, a major problem associated with its production is the very low yields often obtained by farmers (NAS, 1979; Linnemann and Azam-Ali, 1993; Sesay *et al.*, 1999; Hampson *et al.*, 2000). According to Linnemann and Azam-Ali (1993) yields typically achieved by subsistence farmers vary between 0.65 and 0.85 t ha^{-1}. and perhaps as low as 0.06-0.11 t ha^{-1}. Yields are also notoriously erratic and total crop failures are not uncommon (Stanton *et al.*, 1966; Johnson, 1968).

Harris and Azam-Ali (1993) attributed the low and unpredictable yields to variation in photoperiod resulting from year to year variations in planting dates among farmers. However, the low and unpredictable yields of bambara groundnut could also be due to intra-seasonal and inter-seasonal variability in rainfall. Semi-arid regions are environments that are susceptible to pronounced variability not only in amount of

rainfall but also in the distribution and intensity within and between seasons (Usman and Reason, 2004). Limitations in water availability during these events could have a significant impact on rain-fed crops. Thus, in crop production, the consistency with which minimally required rainfall is received could be more critical than the total received over the season. Crops are more likely to do well with uniformly spread light rains than with a few heavy rains interrupted by dry periods. Therefore, the timing of the dry spells relative to the cropping calendar or the phenology of crop plants rather than the total seasonal rainfall could be fundamental to crop performance. Thus, in several crop species the impact of moisture stress has been shown to depend at least as much on the stage of development reached by the crop as to the severity of the stress (Nageswara *et al.*, 1985; Nielsen and Nelson, 1998).

Much is known about the adverse impact of drought on plant growth and physiological activities, but much less about the recovery of physiological function following stress relief. The ability to survive and recover rapidly from moisture stress after rainfall or irrigation is critical to the maintenance of growth or production during stress. Thus, selecting traits for improved recovery may be as economically important as selecting for improved growth during drought in semi-arid environments. Whereas there has been extensive research documenting the growth and physiological response of bambara groundnut to terminal moisture deficit stress, there is hardly any report in literature on its response to short periods of drought stress imposed at different growth stages, or on the recuperative ability of the species from drought stress. Information on the response pattern of morphological traits and physiological processes to drought stress imposed at different growth stages might provide a basis for development of strategies to stabilize yields of bambara groundnut in semi-arid environments.

It is hypothesized that the effect of soil moisture stress on morphological traits and physiological processes will not vary with the time of stress application in bambara groundnut. It is also hypothesized that in bambara groundnut re-watering, following moderate drought stress at different growth stages will induce the same levels of recovery of physiological processes.

1.3 Objectives

- To determine the sensitivity of bambara groundnut to soil moisture stress imposed at different developmental stages.
- To evaluate the recuperative capacity of physiological processes in bambara groundnut from drought stress.

CHAPTER 2

LITERATURE REVIEW

2.1. Water stress

Water stress or water deficit stress frequently refers to a condition in which plant cells and tissues have less than full turgor because of transpiration demand in excess of root water uptake adversely affecting the growth and development process, thus limiting productivity (Ahuja *et al.*, 2008). During water deficit stress, water potentials in the rhizosphere are sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development. On a global basis, it is a major factor limiting productivity of agricultural systems and food production (Boyer, 1982).

2.2 Responses of plants to water deficit stress

Water deficit elicits several morphological and physiological responses in crop plants (Jones *et al.*, 1989; Passioura *et al*, 1993; Jones, 2004). Most of these responses are adaptive mechanisms to withstand water deficit or drought, and to ensure both survival and reproduction under conditions of water deficit stress. The responses range from visible expression of plant water deficits, such as wilting, through morphological changes, such as reduction in growth, to physiological responses, such as stomatal closure and osmotic adjustment (Ludlow, 1989; Hsiao and Acevedo 1974; Jones and Turner 1978; Collinson *et al.*, 1997).

A fundamental problem with these adaptive responses is that most are aimed at reducing water use, and consequently affect plant function and productivity through reduction in photosynthesis. However, the adaptive strategies have been classified into three types, either as drought escape, drought tolerance and drought avoidance

(Turner, 1979) or as developmental, morphological and physiological mechanisms (Turner and Begg, 1981). Details of these adaptive strategies have been discussed by Jones (1993) and Kramer and Boyer (1995).

The drought escape or developmental strategy is demonstrated by some short duration dryland crops which have a condensed growth cycle and reach maturity before drought occurs. They complete their life cycle during the period of adequate moisture and form dormant seeds before the onset of the dry season. Crop species demonstrating this type of adaptive strategy tend to be photoperiod-sensitive so that flowering coincides with the average date of the end of the rainy season (Ludlow and Muchow, 1988). Drought avoiding or morphological mechanisms involve maximizing water uptake and minimizing water loss by the plants. Drought-tolerance or physiological mechanisms ensure the survival of drought through dehydration tolerance.

2.2.1 Morphological responses

There are three main aspects of plant morphological behaviour in relation to drought: the modulation of leaf size, the modulation of root growth and changes in leaf orientation (Turner and Begg, 1981). Passioura (1976) reported that it is the control of leaf area and morphology which is often the most powerful means a mesophytic plant has for influencing its fate when subjected to long term water stress in the field.

The earliest macroscopic response of plants to drought is modulation of the expansion rate of the leaves, and thus reduction in leaf size (Wardlaw, 1969; Turner and Begg, 1978). Reduction in leaf area in response to water deficit has been reported in many crops including legumes such as groundnuts (*Arachis hypogea*) (Collino *et al.*, 2001), faba beans (*Vicia faba*) (Mwanamwenge *et al.*, 1999), cowpea (*Vigna unguiculata*)

(Anyia and Herzog, 2004), chickpea (*Cicer arietinum)* (Singh, 1991) and bambara groundnut (Collinson *et al.*, 1996). The sensitivity of leaf expansion to water deficit generally operates through cell expansion. Ong *et al.*, (1985) showed that cell expansion is extremely sensitive to water stress mainly due to reductions in the hydrostatic pressure or turgor potential necessary for expansion. However, in very young leaves there is a modulation of cell number (Randall and Sinclair, 1988), such that a sustained drought tends to produce leaves that are smaller because they have fewer cells that are of normal size. However, there is evidence in literature indicating little correlation between turgor and expansion rate under certain conditions, suggesting the response of leaves to hormonal signals from roots in the drying soil (Passioura, 1994).

The functions of roots are to anchor the plant, absorb water, synthesize hormones and acting as sensors of water status in the soil (Kramer and Boyer, 1995; Taiz and Zeiger, 2006). The modulation of root growth to maximize water uptake as a response of plants to drought occurs through preferential development of the root over the shoot enabling the crop to explore a greater soil volume for water. Hurd (1974) and Zibaidi *et al.* (1999) reported that drought tolerance in wheat was related to the rooting patterns of the varieties. In both cases the rooting depth and root length density were found to be the important characteristics for high moisture absorption and conferring of drought tolerance. It has long been reported that bambara groundnut has a high root to total dry matter ratio, due to preferential allocation of dry matter to roots in conditions of drought (Nyamudeza, 1989).

The third main aspect of plant morphological behaviour in response to water deficit stress is leaf orientation. Changes in leaf orientation may occur as a result of a passive

wilting response due to general loss in turgor of the leaf tissue (Turner and Begg, 1981). While modulations of leaf size are irreversible, changes in leaf orientation are reversible if there is a return to more favourable conditions.

2.2.2 Physiological responses

Stomatal closure and osmotic adjustment are the two major drought tolerance or physiological responses to water deficit (Ludlow, 1989; Collinson *et al*, 1997).

2.2.2.1 Stomatal closure

Plants generally respond to acute water stress by closing their stomata to conserve water (Raghavendra, 1998). The hormone abscisic acid (ABA) has been implicated in stomatal closure during water stress. The hormone is synthesized or redistributed to the leaves and its accumulation results in ion fluxes in guard cells of the stomata, decreasing turgor pressure thus causing stomatal closure (Taiz and Zeiger, 2006). Stomatal closure increases leaf resistance and closes the pathway for exchange of water, carbon dioxide and oxygen (Raghavendra, 1998). According to Ennahli and Earl (2001), this is detrimental to the plant since it results in a decrease in photosynthesis due to reduced diffusion of and fixation of carbon dioxide. This then result in reduced carbon gain, reduced growth, reduced reproductive success and reduced crop dry matter accumulation.

It should however be noted that (partial) stomatal closure is considered to be a stress adaptive response and can positively impact the potential for survival of plants which must reach reproductive maturity on limited supply of water. This is because stomatal closure typically increases the water use efficiency (increase in plant dry matter per

unit water, on a long term basis) or transpiration ratio (g H_2O transpired per CO_2 fixed, on an instantaneous basis) of crop plants (Raghavendra, 1998).

2.2.2.2 Osmotic adjustment

Osmotic adjustment is a major physiological mechanism underlying plant resistance to water deficit (Turner and Jones, 1980; Zhu *et al.*, 1997). It is a process of active accumulation of solutes or compatible osmolytes in plant cell cytoplasm exposed to water deficit. The compatible osmolytes include the amino acid proline, sugar alcohols like sorbitol and mannitol, amines like glycine and betaine and ions like K^+, Na^+, Ca^{2+}. Cellular water potential is therefore decreased without an accompanying decrease in turgor or decrease in cell volume (Taiz and Zeiger, 2006). As a consequence of solute accumulation, the osmotic potential of the cell is lowered; this in turn attracts water into the cell and thereby, tends to maintain turgor. Accumulation of solutes in roots therefore leads to lowering of the osmotic potential which maintains the driving force for extracting soil water under deficit conditions (Moinuddin and Khanna-Chopra, 2004). Osmotic adjustment has been reported to be an important drought – adaptation mechanism in many crop plants (Ludlow and Muchow, 1990; Subbarao *et al.*, 1995; Hare *et al.*, 1998) as it may enable a continuation of leaf expansion or elongation though at reduced rates (Turner, 1986), maintenance of root development and soil moisture attraction (Morgan and Condon, 1986), stomatal and photosynthetic adjustments (Morgan, 1984), delayed leaf senescence (Hsiao *et al.*, 1984), better dry matter accumulation and yield production for crops in stressful environments (Boyer, 1982).

A positive relationship between osmotic adjustment and grain yield in water-deficit environments has been shown in sorghum (*Sorghum bicolor*) (Tangpremsri *et al.*,

1995), wheat (Blum *et al.*, 1999), and peas (Rodriguez-Maribona *et al.*, 1992). Significantly greater seed yield in groups of genotypes with high osmotic adjustments than in groups with low osmotic adjustments has been reported under water deficit conditions in various crops (Ludlow *et al.*, 1990). This may be because the accumulation of solutes results in sufficient osmotic adjustments to maintain water content and turgor in stressed plants equal to the well watered plants throughout most of the stress period (Allen *et al.*, 1987).

2.2.2.2.1 Proline accumulation

The amino acid proline is one of the major osmolytes in osmotic adjustment and could account for up to 70% of the total amino compounds during periods of stress (Andreas, 1995). Reports in literature indicate a positive correlation between proline accumulation and adaptation to drought stress (Delaune and Verma, 1993). Proline plays a direct and adaptive role in counteracting the effect of water stress, and in transgenic tobacco overproduction of proline resulted in less decrease in the osmotic potential of the leaf sap and also enhanced root biomass and flower production (Kishor *et* al., 1995).

Proline accumulation is caused by both the activation of its biosynthesis and inactivation of its degradation (Mattioni *et al.*, 2008). Proline which accumulates in response to water stress is primarily localised in the cytosol (Ketchum *et al.*, 1991) and serves as a good indicator of water status of the plant as there is an inverse relationship between proline content and water potential (Malinowski and Belesky, 2000). Proline accumulates intensely in all stressed organs of plants especially in leaves as a consequence of increasing breakdown of proteins and conversion of some amino acids ornithine, arginine and glutamate to proline (Abdallah and El- khoshiban,

2007). This may be because at low relative water content redox components are very much reduced and this triggers proline formation from amino acids like glutamate (Pessarakli, 2005). Furthermore, drought stress can cause oxidative stress through the production of reactive oxygen species such as superoxide radicals and hydrogen peroxide (Foyer and Kunert, 1994). Reactive oxygen species can cause lipid peroxidation which leads to membrane injury, protein degradation, enzyme inactivation and disruption of DNA strands (Abdallah and El- khoshiban, 2007). Proline is thought to contribute to detoxification of reactive oxygen species thus protecting the plants from the oxidative stresses caused by water deficit (Molinari *et al.*, 2007). Proline synthesis is also implicated as a mechanism of alleviating cytoplasmic acidosis and may maintain NADP+/NADPH ratios at values compatible with metabolism (Hare and Cress, 1997).

2.3 Responses of bambara groundnuts to water stress

Bambara groundnut is widely regarded as being drought tolerant (Linneman and Azam-Ali, 1993). Collinson *et al.* (1997) suggested that drought tolerance of bambara groundnut is a result of osmotic adjustment, reduction of leaf area index and low water loss through the stomata. Collinson *et al.* (1999) reported that bambara groundnut exhibits higher leaf reflectivity and heliotropism when subjected to water stress. It has also been reported that bambara groundnut has a high root to total dry matter ratio (Nyamudeza, 1989). This would imply the involvement of more than one mechanism, that fall within the adaptation categories of drought tolerance and drought avoidance.

2.4 Measurement of environmental stress effects

Measurements widely used in studies of responses of plants to environmental stresses include relative water content, stomatal conductance, chlorophyll content, chlorophyll fluorescence, and photosynthesis (Sparks, 2007).

2.4.1 Relative water content (RWC)

The accurate measurement of plant and/or soil water status is critical in experiments concerned with understanding the effect of differing water supply. Such measurements, according to Jones (2007), are essential to define the conditions of the experiment both in terms of the treatment applied and in terms of the effects on the plant. A very powerful and widely used method for measuring plant water status is the use of leaf relative water content. RWC expresses the water content in per cent at a given time as related to the water content at full turgor (Gonzalez and Gonzalez-Vilar, 2001). Measuring RWC is applicable more especially where the concern is with the direct effects of leaf water status on physiological processes within the leaf.

Water stress is generally characterised by a decrease in leaf water status or in RWC and pressure potential (Ψp) resulting in wilting, stomatal closure and reduced growth (Kramer and Boyer, 1995). As a general rule, a reduction in RWC from 100 to 90% is associated with closing of stomatal pores in the leaf and reduction in cellular expansion and growth. RWC of 90-80% is correlated with changes in the composition of plant tissues and with alteration in the relative rates of photosynthesis and respiration. Levels of RWC below 80% usually correspond with water potential of the order of -1.5 MPa or less, and this would cause changes in plant metabolism expressed as decline in photosynthesis, increase in respiration and accumulation of proline and abscisic acid (Sparks, 2007).

The maintenance of high RWC values under low soil moisture conditions appears to be a common trait in drought resistant species, as species which exhibit restricted changes in RWC per unit reduction in water potential are often considered to be relatively drought resistant. Bambara groundnut is reported to maintain relatively high RWC despite wide changes in lowered leaf water potential (Collinson *et al.*, 1997).

2.4.2 Stomatal conductance

Stomatal conductance is a numerical measure of the maximum rate of passage of either water vapour or carbon dioxide through the stomata (Chen *et al.*, *1999*). Diffusion of CO_2 into the mesophyll of leaves and water vapour from the leaves to the atmosphere is driven by stomatal aperture which is controlled by a complex system of environmental factors and plant physiological processes. As was mentioned earlier, when plants experience water stress, the hormone ABA is synthesised or redistributed to the leaves. Its accumulation results in ion fluxes in the guard cells of the stomata resulting in decreases turgor pressure thus causing stomatal closure (Taiz and Zeiger, 2006).

Decreasing RWC and water potential of leaves and stomatal closure progressively decrease stomatal conductance, leading to decline in CO_2 molar fraction in chloroplasts, decreased CO_2 assimilation and reduced rate of photosynthesis (Pessarakli, 2005). Thus stomatal conductance generally correlates with photosynthetic capacity and photosynthetic efficiency (Wong *et al.*, 1979).

However, stomatal conductance provides some indication of potential transpiration, the relationship is not straightforward. For instance, stomatal closure may be accompanied by increase in leaf temperature of up to 6°C under conditions of high irradiance which in turn would increase the leaf-to-air vapour pressure gradient during

transpiration, thereby partially offsetting the conservative influence of stomatal closure (Black *et al.*, 1985).

2.4.3 Chlorophyll content

A plant's photosynthetic potential according to Schlemmer *et al.* (2005) is directly proportional to the quantity of chlorophyll present in the leaf tissue. Water stress is said to adversely affect the amount of chlorophyll plants produce. Measuring chlorophyll content is fundamental to understanding a plant's response to the environment in which it resides and so quantifying chlorophyll content can provide information regarding the physiological state of leaves. Instruments have been developed that examine the reaction of light energy with leaf tissues for the purpose of providing rapid estimates of chlorophyll content (Schlemmer *et al.*, 2005). Markwell *et al.* (1995) reported a very strong relationship between the Minolta SPAD-502 chlorophyll meter readings and direct measurements of chlorophyll content in corn and soyabean leaves produced.

2.4.4 Chlorophyll fluorescence

Water stress reduces the photosynthetic capacity of plants by affecting numerous aspects of the photosynthetic processes (Pessarakli, 2005; Taiz and Zeiger, 2006). Identification of metabolic disturbances in leaves provides an indicator of plant photosynthetic performance and this can be accomplished through the measurement of chlorophyll fluorescence emissions kinetics. Measurements of chlorophyll fluorescence using chlorophyll fluorescence technique are therefore a powerful non-destructive and fast method to detect changes in the photosynthetic activity in leaves influenced by changes in the environment (Sparks, 2007).

Water stress causes drastic changes in fluorescence levels and at extreme water deficit; fluorescence changes are more pronounced (Pessarakli, 2005). Chlorophyll fluorescence parameters such as F_o (initial), F_m (maximum) and F_v (variable equal F_m-F_o) are used to determine the ratio F_v / F_m. The dark adapted F_v / F_m measures the efficiency of excitation energy captured by PS II. The decrease in F_v / F_m ratio indicates stress and damage to photosynthetic system (Sparks, 2007).

Among all photosynthetic functions, PS II is believed to be the most stress sensitive (Duraes *et al.*, 2001). The ratio of variable to maximum fluorescence (F_v / F_m) of PS II measures the efficiency of excitation energy captured by PS II. Chlorophyll fluorescence emissions kinetics from plants provides an indicator of plant photosynthetic efficiency or performance (Sparks, 2007). It serves as a more practical means for indirectly assessing leaf photosynthetic rate (Earl and Davis, 2003) and can be used to distinguish tolerant from susceptible hybrids to water deficit (M O'Nell *et al.*, 2006). According to Zrust *et al.* (1988), seven potato genotypes grown underwater stress showed decline in F_v/F_m ratio of leaves with a concomitant decrease in net photosynthesis. Total dry matter production in water stressed potato plants was also correlated with F_v/F_m. Decreasing F_v/F_m under water stress indicated diminishing photosynthetic activity in potato leaves and F_v/F_m values were shown to provide a method for the study of changes in the photosynthetic capacity of potatoes in response to water stress.

2.5 Water stress and crop yield

Soil moisture deficit is an important determinant of crop yield in many environments (Collinson *et al.*, 1996; Mwale *et al.*, 2007). The yields of several crops have been

related to moisture availability in many experiments. Although bambara groundnut has been cultivated successively for centuries in environments where both protracted and/or terminal drought stress is common and has been reported to have favourable drought-tolerance status (Babiker, 1989), the productivity of the crop can be adversely affected by soil moisture stress (Collinson *et al.*, 1996; Mwale *et al.*, 2007). Collinson *et al.* (1996) reported reductions in biomass production of 60 to 79% in bambara groundnut and yield reductions of 94 to 97% in greenhouse experiments due to moisture stress. From a similar series of studies, Mwale *et al.* (2007) also reported a yield loss of 45% in bambara groundnut.

Water stress affects many physiological processes, such as photosynthesis, respiration, translocation, and senescence (Collinson *et al.*, 1996). Water stress also lowers leaf water potential leading to stomatal closure, decreased stomatal conductance, altered chlorophyll fluorescence, photo inhibition of photosystem II, impaired ATP synthesis and ribulose 1,5 – biphosphate (RuBP) regeneration, conformational changes in membrane bound ATPase enzyme complex, as well as a decrease in both activity and concentration of ribulose 1,5 – biphosphate carboxylase/oyxgenase (Rubisco) enzyme (Pessarakli, 2005). Consequently, the vegetative growth, development and yield of the plants may be severely impaired.

Because yield is an integral of several processes, Passioura (1994) defined the components of water-limited yield as follows:

Yield = Water transpired x Water use efficiency x Harvest index

Where:

Water use efficiency = amount of dry matter produced per unit of water transpired

Harvest index = ratio of seed yield to total dry matter

A decrease in any of these components is likely to result in a decrease in yield. According to Passioura (1994), there are four main aspects of the behaviour of plants in relation to drought that can readily be linked to yield via these components. These are: the modulation of leaf area, the modulation of root growth, the efficiency with which leaves exchange water for CO_2, and the processes involved in setting and filling the seeds. However, in several crop species the impact of moisture stress has been shown to depend on the stage of development reached by the crop as on the severity of the stress as shown in groundnuts (Nageswara *et a.*, 1985), maize (Grant *et* al., 1989), chick pea (Singh, 1991), black beans (Nielson and Nelson, 1998), and soyabeans (Brevadan and Egli, 2003).

2.6 Water stress at different growth stages

Developmental stages at which crop plants are more sensitive to water stress as compared to others are known as critical stages. Restricting water supply during these stages affects productivity more severely than during other periods (Stewart and Howell, 2003). In some crops, like dry beans (*Phaseolus vulgaris*), water stress during the vegetative period delays flowering; retards root development as well as vegetative growth (Robins and Domigo, 1956). Retardation of vegetative growth in plants is a result of a reduction in stem and leaf expansion, resulting in reduced plant height and leaf area (Lauer, 2005). Another effect of water stress during the vegetative stage is pollen sterility which is an indirect consequence of water deficit in the vegetative parts (Basra, 1997).

Basra (1997) reported that the reproductive phase in the development of a plant starts with the transformation of vegetative meristem into an inflorescence or flower primordium and ends when the fruit or seed reaches maturity. This rather broad period covers several sub-stages, including floral initiation, differentiation of various parts of inflorescence and/or flower, meiosis, development of pollen and embryo sac, pollination, fertilisation and fruit and seed development.

During flowering, depending on species, water stress causes loss of pollen fertility, spikelet death or abortion of newly formed seed. These injuries are associated with a decline in the water status of the reproductive structures (Basra, 1997). In bush beans, water stress which occurs preflowering, flowering or post flowering reduces the total number of pods and pod fresh weight (Dubertz and Mahaille, 1969). Drought that coincides with the flowering period therefore causes serious yield instability at farm level, because it allows no opportunities for farmers to replant or otherwise compensate for loss of yield (Kamara *et al.*, 2003).

During the grain filling period, water stress reduces seed weight and seed yield as reported by Robins and Domigo (1956) and Singh (1995) in dry beans. In maize, water stress during the grain filling period increases leaf senescence, shortens the grain filling period, increases lodging and lowers kernel weight thus reducing yield (Lauer, 2005). Thus there is a strong linear relationship between water applied during flowering and grain filling period and yield (Miller and Burke, 1983).

Plant height and leaf area have been reported to be most sensitive to water stress during vegetative growth as shown with black beans by Nielson and Nelson (1998). According to Sterwart and Howell (2003), developmental stages at which crop plants are most sensitive to water stress depend on crop species. Groundnuts are most

sensitive to water stress during pod development rather than flowering or even pegging. It has been reported that water stress during this stage reduces pod yield by 56% compared to 27% and 45% at flowering and pegging, respectively. Robins and Domigo (1956) showed that yields are reduced most in dry beans when water stress occurs during flowering and that yield reductions during flowering are a result of both fewer pods and seeds per pod. In faba beans, the early podding stage of development was shown to be the most sensitive to water stress causing a reduction in harvest indices and seed yields. Faba bean also showed a better ability to recover from water stress during floral initiation and first flower stages than at early podding (Mwanamwenge *et al.*, 1999).

2.7 Drought recovery

The ability of a plant to recover or grow and yield after a temporary and/or prolonged stress and the rate of recovery are of great importance in crop production (Bielorai and Hopmans, 1975) and the ability to recover during certain stages of plant development is linked to water use efficiency (Jones, 1989). A continuous recovery process in drought active plants may enable plants to quickly use evanescent water resources when they become available. Trade-offs between drought fitness and water use efficiency may be necessary to ensure rapid use of pulses of rainfall after intervening dry periods (Havstad *et al.*, 2006).

The re-establishment of vegetative growth after a period of water stress depends on the recovery of leaf water potential (Ψw) after rewatering (Nulsen and Thurtell, 1978). In Guayule plants, rewatering after a stress period resulted in increase in (Ψw) and solute potential (Ψs) but with values remaining lower than those of non stressed control plants. A resulting lower Ψw and Ψs than non stressed plants is caused by the

active accumulation of solutes due to stress induced loss of water content as passive concentration of cell solutes results in equal Ψs of plants following rehydration of the stressed plants (Allen *et al.*, 1987)

During drought recovery, catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP thus providing energy for recovery from stress-induced damage (Hare and Cress, 1997). Proline is also regarded as a source of carbon and nitrogen for recovering tissues. The younger more actively growing plants are endowed with greater ability to recover after stress or greater potential for proline accumulation than plants at later stages of growth (Andreas, 1995).

Different crop species have been shown to employ different strategies in responding to rewatering. Pigeon pea (*Cajanas cajan*) responded to rewatering by slowly recovering photosynthetic CO_2 exchange rate and leaf diffusive conductance (Lopez *et al.*, 1988). Soyabean plants reversed stress-induced senescence by increasing leaf area, leaf nitrogen and chlorophyll content (Brevedan and Egli, 2003). According to Efeoglu *et al.* (2009), in maize, chlorophyll a, chlorophyll b, total chlorophyll (a+b) and carotenoid contents were reduced under drought but a recovery was observed following rewatering. Growth of maize was also retarded under drought stress conditions but it regained speed during the recovery stage. The RWC of maize was decreased under water stress but equaled the values of non-stressed plants during recovery. In plants the reversible decrease in the photosynthetic activity of the chloroplast occurs at RWC values of 70% to 40% and this is attributed to a reversible inhibitory effect on the enzymatic activity by the increase in cellular solute (Garab, 1998).

In addition, characteristics of rainfall such as size and frequency of rainfall events may elicit different responses from plant species that employ different drought strategies and fitness (Havstad *et al.*, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site and plant material

Two greenhouse trials were conducted between October 2009 to end of May 2010. The first trial was conducted in the greenhouse of the Department of Biological Sciences, University of Botswana and the second trial was conducted in the greenhouse of the Department of Crop Science, Botswana College of Agriculture. The bambara groundnut landrace "Uniswa red" was used in the two trials as this landrace has been widely used in physiological studies published in the literature. The seeds were obtained from the seed stocks of the European Union-funded bambara groundnut research project, (BAMLINK) based at the Botswana College of Agriculture, Gaborone, Botswana.

3.2 Experimental design and treatments

The experiment was arranged in a completely randomised design with four replications. There were five plants (pots) per replication and four treatments giving a total of 80 (plants) pots. The treatments were as follows:

Trial 1:

1. Control-plants were well watered throughout.
2. Stressed during the vegetative stage – plants were stressed for 21 days, starting 25 days after sowing (DAS).
3. Stressed during the flowering stage – plants were stressed for 21 days, starting 46 DAS.

4. Stressed during the pod filling stage – plants were stressed for 21 days, starting 80 DAS.

Trial 2:

1. Control-plants were well watered throughout.

2. Stressed during the vegetative stage – plants were stressed for 21 days, starting 25 days after sowing (DAS).

3. Stressed during the flowering stage – plants were stressed for 21 days, starting 46 DAS.

4. Stressed during the pod filling stage- plants were stressed for 21 days, starting 60 DAS.

In trial 2 plants were stressed for 60 days during the pod filling stage instead of 80 days as in trial 1 because the sowing date was delayed and so there is a reduction in the reproductive period (Harris and Azam-ali, 1993; Collinson *et al.*, 2000; Sesay *et al.*, 2008).

Four seeds were sown per pot at 4cm depth, and seedlings were thinned to one per pot at emergence. The black plastic pots, measuring 225 millimetres in diameter and 450 millimetres in height, were each filled with a 17 kg mixture of sandy loam soil and sand in 5:3 volume ratio. A basal fertiliser (NPK, 2:3:2) was incorporated into the soil at a rate equivalent to 265 kg ha^{-1}. Plants (pots) were spaced 30cm apart on benches to preclude competition effects among treatments. The greenhouse temperature was maintained at 25-28°C in trial 1 but in trial 2 the greenhouse temperature was not controlled.

For each drought treatment, watering was with held until the pots reached a stress level of 30 % of plant available water (PAW)(3.3.1). It took the pots about 10 days to reach 30 % of PAW from beginning of stressing and this stress level was maintained for 15 days. During the experiment each pot was weighed daily at 0900hrs and water was added if necessary to maintain the stress level. Except for the periods of stress, the watering for all treatments was the same as that for the control plants.

3.3 Measurements

3.3.1 Measurement of plant available water (PAW)

Prior to the start of the study, the upper PAW limit was determined by weighing soil from 5 pots two days after they were watered as the amount of water remaining in a soil two days after having been saturated and after free drainage represents the maximum amount of water that soil can store (Cassel and Nielson, 1986). The lower PAW was determined by weighing pots in which plants were allowed to die after transpiring all available water. The PAW for each pot for any other day was calculated according to Rosenthal *et al.* (1987) as follows.

$$PAW\ (\%) = [(WTa - WT1) / (WTu - WT1)] \times 100$$

Where:

WTa – the pot weight on a given date.

WT1 – the pot weight at the lower limit of the PAW.

WTu – the pot weight at the drained upper limit .

All measurements taken on plants were taken before, during and after each stress treatment. During drought recovery, measurements were taken only from leaves existing before rewatering to examine the recuperative ability of stressed tissues and avoid the confounding effects of newly regenerated tissues.

3.3.2 Physiological measurements

All physiological measurements were taken after each water stress treatment and 15 days after recovery from water stress.

3.3.2.1 Relative water content

RWC was determined using 10 leaf discs (about 13mm diameter) from leaves of 3 tagged plants per replication excised using a cork borer from two lateral leaflets of the trifoliate leaves. The leaf discs were placed in pre-weighed vials, sealed and reweighed to derive their fresh weight (FW) before being placed in petri dishes lined with two layers of germinating paper saturated with deionised water. These were sealed with tape to prevent evaporation and left overnight under a light source to allow discs to re-hydrate to their turgid weight (TW). Their dry weight (DW) was obtained after overnight drying at 80°c for 48h. RWC was then calculated according to Turner (1981) as:

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

3.3.2.2 Chlorophyll fluorescence

Chlorophyll fluorescence was measured with a Hansatech Fluorescence Monitoring System. Measurements were taken on terminal leaflets of three fully expanded, fully sunlit leaves from three tagged plants per treatment per replication. All measurements were taken between 1200 and 1400h local time. The plants chosen for evaluation were adapted in the darkness for a minimum of 30 minutes at normal temperature. The dark adapted chlorophyll variable fluorescence/maximal fluorescence (Fv/Fm) ratios and quantum efficiency of photosystem II for each treatment were determined.

3.3.2.3 Stomatal Conductance

Stomatal conductance (mmols/m^2s) was measured with a Decagon Devices Leaf Porometer SC-1. Measurements were taken on fully expanded leaves of 3 tagged plants per treatment per replication. Readings were taken between 1200 and 1400h local time.

3.3.2.4 Leaf Chlorophyll content

The measurements were taken on fully expanded leaves of 3 tagged plants from each treatment per replication.

Two methods were used to determine leaf chlorophyll content.

1. The non-destructive measurement

   This was done using the OPII-SCIENCES CCM-200 chlorophyll content meter to acquire a rapid estimate of leaf chlorophyll content which is expressed as the chlorophyll content index (CCI).

2. The destructive measurement

   This was done by taking a plant sample of about 0.25g and this was macerated using a pestle and mortar. The total pigments were then be extracted with 10 ml of 80% acetone and the crude extract was centrifuged at 3000rpm for 5 minutes. The pellet was discarded whilst the supernatant was kept which is where absorbance was measured by a UV 160 IPC spectrophotometer at 663.6 and 646.6 nanometers which are the major absorption peaks of chlorophyll a and b. The total concentration of chlorophyll was calculated according to Porra et al. (1989) using the following equation:

Chlorophyll a + b (total chl) = (8.29 $A_{663.6}$ + 19.54 $A_{646.6}$)

Where

$A_{663.6}$ = absorbance at a wavelength of 663.6nm

$A_{646.6}$ = absorbance at a wavelength of 646.6nm

Chlorophyll content was expressed in µg chl.g Fwt.

3.3.2.5 **Leaf proline concentration**

Samples of fresh leaf tissue were obtained from fully expanded leaves of 3 tagged plants from each treatment. Proline analysis was carried out according to Bates *et al.* (1973). Prior to analysis, acid-nihydrin was prepared by warming 1.25g of nihydrin in 30 ml of glacial acetic acid and 20 ml of 6 Molar phosphoric acid with agitation until dissolved. The reagent was then kept cool at 4 °C.

Samples of fresh tissue were weighed (0.5 g) and homogenised in 10 ml of 3 % aqueous sulfosalicyclic acid. The homogenate was then filtered through Whatman filter (grade1) paper. 2 ml of the filtrate was reacted with 2ml acid-nihydrin and 2 ml glacial acetic acid in a test tube for an hour at 100 °C in a water bath to develop the colours. Soon after removal from the water bath, the test tubes were cooled in an ice bath and proline was extracted with 4ml toluene, mixed vigorously with a test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance was read in a UV 160 IPC spectrophotometer at 520 nm ,using toluene as a blank. Proline content in fresh tissue was determined by comparing the sample absorbance with the standard proline curve and calculated on a fresh weight basis as follows:

[(µg proline/ml x ml toluene) / 115.5µg/mole] / [(g sample) 5] = µmole proline/g of fresh weight material.

3.3.3 Morphological measurements

All morphological measurements were made on 3 randomly selected plants per replication.

3.3.3.1 Relative leaf expansion rate (RLER)

Relative leaf expansion rate (RLER) was measured during each stress treatment and during recovery from each stress treatment. It was determined non-destructively by measuring the length and width of terminal leaflet of the third most recently unfolded leaf and this was done 3 days apart for 15 days during which PAW was at 30% during each stress treatment and 15 days during recovery for each stress treatment.

The actual leaf area was determined using the following landrace independent formulae (Cornelissen, 2005):

$$A = 0.74 * 3 * N_l (L * W * \Pi/4)$$

Where A = estimated leaf area

N_l = total number of leaves

L = length (cm)

W = width (cm)

$\Pi = 3.1416$

0.74 and 3 = correction factors

RLER was calculated according to the following:

$$RLER = (\ln A_2 - \ln A_1)(t_2 - t_1)$$

Where A is estimated leaf area and t is time, in chronological time (Ober and Luterbacher, 2002).

3.3.3.2 Leaf number.

The total number of leaves (three fully expanded leaflets) was determined by averaging the number for each of the 5 plants per treatment, and was recorded twice weekly (every 3 and 4 days) from thinning until maturity.

3.3.3.3 Plant height

Plant height (cm) was determined by averaging the distance from soil level to the top of each of the five plants.

3.3.3.4 Shoot: Root ratio

At maturity both control and stressed plants were removed from the pots. Plants were separated into two parts, root and shoot and oven dried at 80 C for 72 hours and weighed to determine total root dry weight, shoot dry weight.

3.3.4 Pod yield

At maturity, the pods were harvested and the average number of pods per plant was determined. The pods were said to be mature when the parenchymatous layer surrounding the embryo had disappeared and there were brown patches in the pod (Doku and Karikari, 1970). The pods were then oven dried at 80°C for 48 hours and pods were shelled. The average seed number per plant, 100 seed weight and yield (kg/ha) per treatment was then determined.

3.4 Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS). Treatment means were compared using the Least Significance Difference (LSD) at probability level of 0.05.

CHAPTER 4

RESULTS

4.1 Morphological responses

4.1.1 Relative leaf expansion rate (RLER)

Stressing bambara groundnut plants significantly ($p<0.05$) lowered baseline RLER for the vegetative, flowering and pod filling stages compared to the unstressed control plants in both trial 1 and 2 (Figure 1a, 1b). Depending on the stage of bambara plant development or time when stress occurred, water stress reduced the RLER of the plants by 70.3 - 99.7% (Figure 1a) and 78.7-99.6% (Figure 1 b) with the pod filling stage having the highest reduction and the vegetative stage having the lowest reduction (Figure 1a;b). The RLER for the control fell sharply to 0 after 19 days in trial 1 (Figure 1a) and after 23 days in trial 2 (Figure 1 b). After reaching zero the rate remained stagnant up to the last day of observations (Figure 1a, b).

The RLER for the stressed treatments decreased steadily reaching zero after 15, 11 and 8 days in trial 1 (Figure 1a) after water stressing at the vegetative, flowering and pod filling stages respectively. In trial 2 it took 15 days for RLER to reach zero after water stressing at the vegetative and flowering stages and 4 days after water stressing at the pod filling stage (Figure 1b). After rewatering the RLER for all stressed treatments substantially increased. In both trial 1 and trial 2, the RLER for the vegetative stage was not significantly lower than the baseline RLER for the control plants and so recovered from water stress.

The RLER for the flowering and pod filling stages was however, significantly lower ($p<0.05$) than the baseline RLER for the control plants and so plants which were stressed during the flowering and pod filling stages failed to fully recover from water stress (Figure 1a, 1b). The pod filling stage had the lowest recovery of 33.5 % (Figure

1a) and 13.5% (Figure 1b), while the vegetative stage had the highest recovery of 94.5% (Figure 1a) and 93.6% (Figure 1b). After recovery, the RLER decreased to zero after 15 days (Figure 1a) and 16 days (Figure 1b) for the vegetative stage and 13 days (Figure 1a) and 14 days (Figure 1b) for the flowering and pod filling stages and remained constant up to the last day of observations.

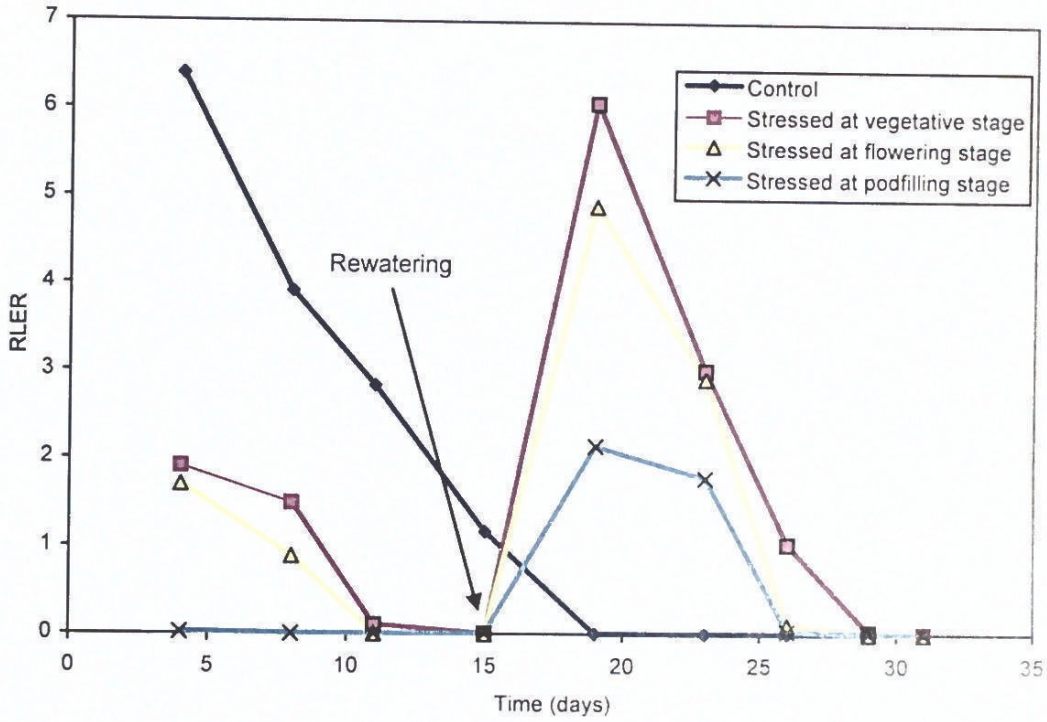

Figure 1a. RLER of bambara groundnut during the vegetative, flowering and pod filling stages during water stress and during recovery from water stress in trial 1.

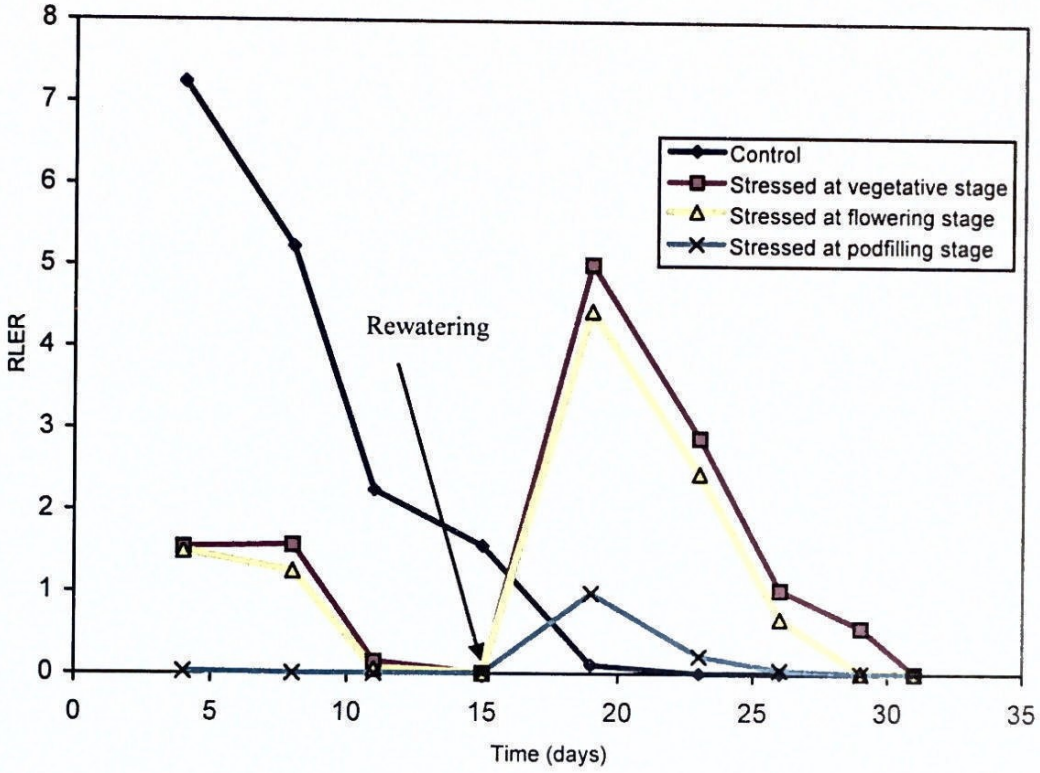

Figure 1 b. RLER of bambara groundnut during the vegetative, flowering and pod filling stages during water stress and during recovery from water stress in trial 2.

4.1.2 Leaf number

Water stress significantly ($p<0.05$) reduced leaf number of bambara groundnut when the plants were stressed for 21 days during the vegetative, flowering and pod filling stages, respectively compared to unstressed control plants in both trial 1 and 2 (Figure 2a, 2b). Varying the time of sowing significantly reduced ($p<0.05$) leaf number per plant of plants in trial 2 at all stages of growth and development as they were sown end of January as compared to plants in trial 1 which were sown end of end of October. The number of maturity days was also lower in trial 2 (95 days) as compared to trial 1 (117 days) and pod filling started earlier at 60 days in trial 2 than 80 days for trial 1 (Figure 2a, 2b).

The maximum leaf number per plant was significantly higher ($p<0.05$) in plants grown in trial 1 (101 days) (Figure 2a) compared to plants grown in trial 2 (72 days) (Figure 2b) both of them being for the control plants. Leaf number reduction was significantly ($p<0.05$) highest when water stress occurred during the vegetative stage of plant development (Figure 2a, 2b).

Once the water stress was removed after rewatering at 46, 67 and 101 days for the vegetative, flowering and pod filling stages, respectively in trial 1 and at 46, 67 and 81 days for the vegetative, flowering and pod filling stages respectively, the number of leaves for the stressed plants at different stages of development was still significantly ($p<0.05$) lower than for the unstressed control plants, showing failure of fully recovery (Figure 2a, 2b). Plants stressed during the pod filling stage had a 0% recovery in leaf number after rewatering as compared to the control plants and all the treatments abscised leaves at the end of the growing season (Figure 2a, 2b). Plants which were stressed during the vegetative stage flowered earlier as compared to plants stressed at the flowering and pod filling stage in both trial 1 and 2. Plants stressed

during the flowering stage resumed flowering after rewatering more so in trial 1 than trial 2 (Figure 2a, 2b).

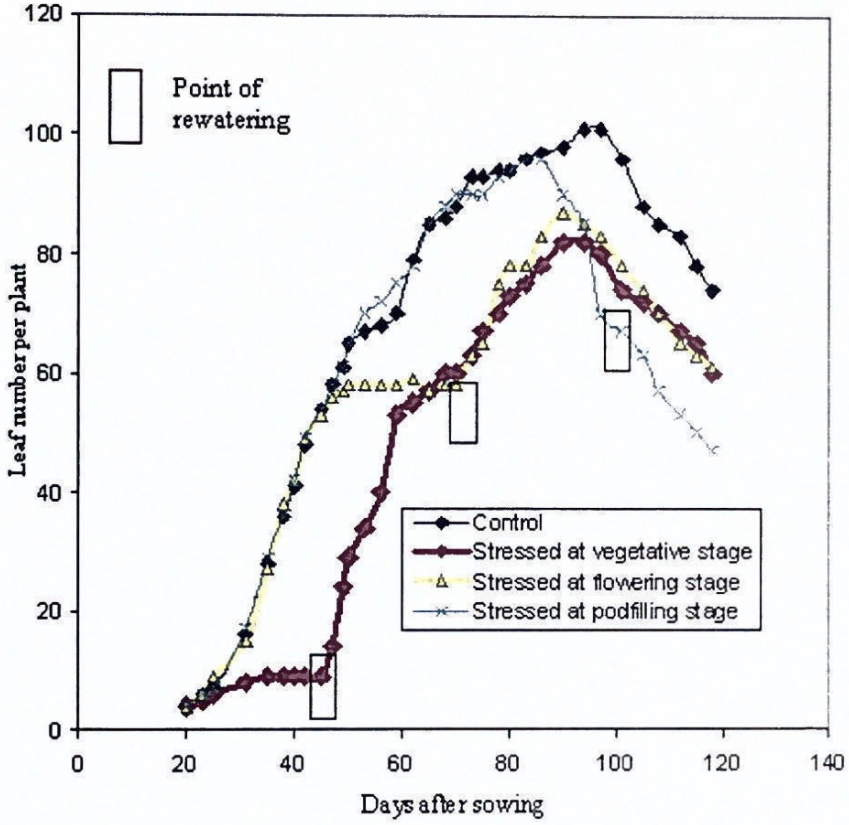

Figure 2a. Effect of water stress at different stages of bambara groundnut plant growth and development on leaf number in trial 1.

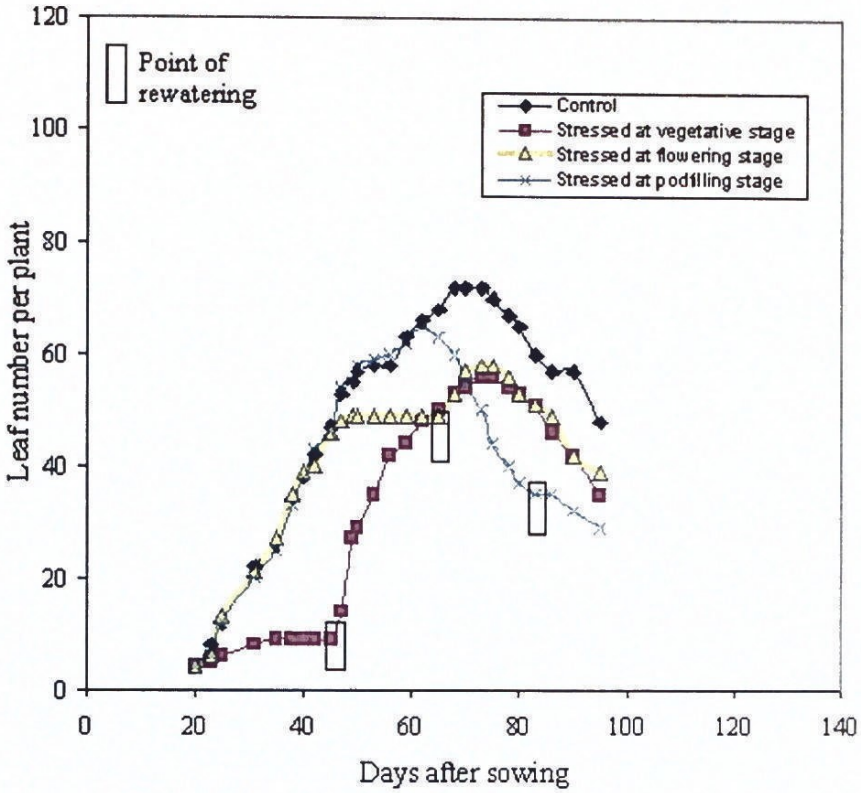

Figure 2b. Effect of water stress at different stages of bambara groundnut plant growth and development on leaf number in trial 2.

4.1.3 Plant height

Water stress at different stages of bambara groundnut plant growth and development significantly reduced ($p<0.05$) plant height compared to non stressed control plants (Figure 3a, 3b). However, the plant height for plants which were grown in trial 2 (Figure 3b) was significantly lower ($p<0.05$) than plants which were grown in trial 1 (Figure 3a).The bambara groundnut plants which were stressed during the pod filling stage were not significantly reduced in plant height as compared to the control plants (Figure 3a, 3b). After rewatering, the plants which were stressed during the vegetative and flowering stages significantly failed ($p<0.05$) to equal the plant height of the control plants and so failed to recover from water stress. Plants which were stressed during the vegetative stage, reached a height which was not significantly different

($p<0.05$) from that of plants which were stressed during the flowering stage after recovering from water stress in trial 1 (Figure 3a) but reached a height significantly different from that of plants stressed at the flowering stage in trial 2 (Figure 3b).

Plants which were stressed during the vegetative stage had a higher rate of increase in plant height (0.44 cm/day) (Figure 3a) and (0.4 cm/day) (Figure 3b) compared to that of the plants which were stressed during the flowering stage (0.16 cm/day) (Figure 3a) and (0.09 cm/day) (Figure 3b).

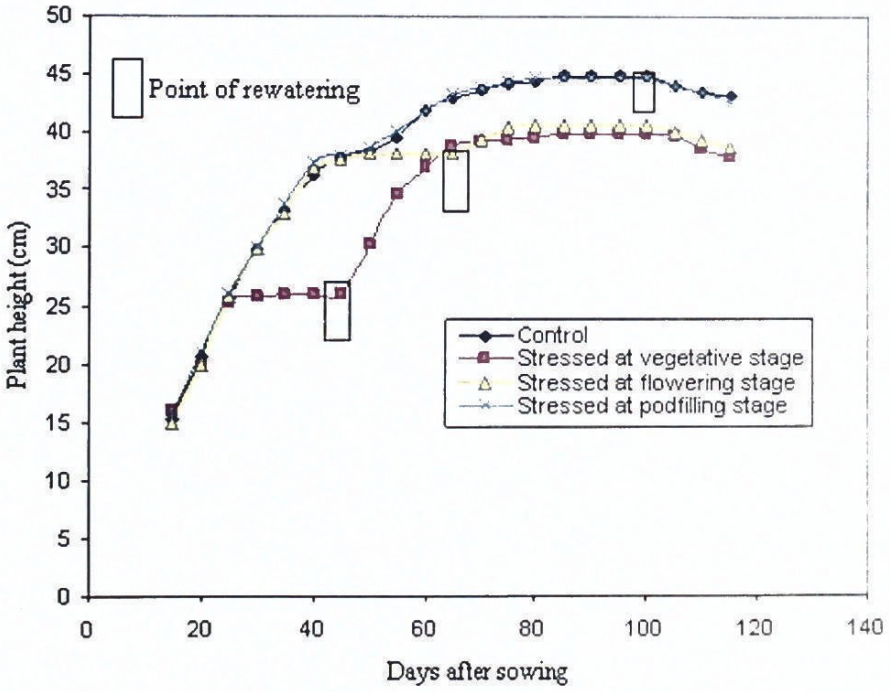

Figure 3a.Effect of water stress at different stages of bambara groundnut plant growth and development on plant height in trial 1.

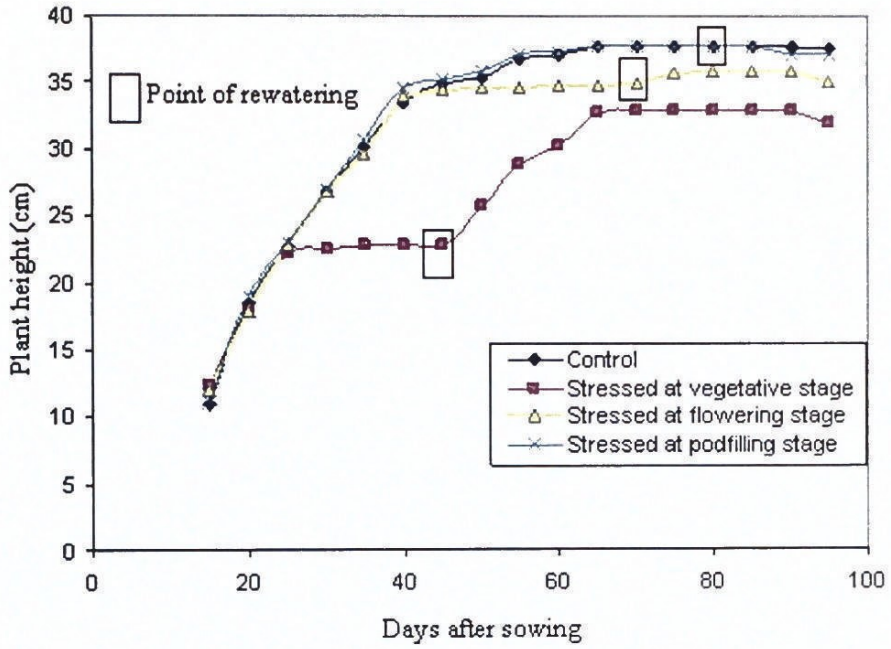

Figure 3b. Effect of water stress at different stages of bambara groundnut plant growth and development on plant height in trial 2.

4.1.4 Shoot: root ratio

The shoot: root ratio was significantly reduced ($p<0.05$) by water stress imposed at vegetative, flowering and pod filling stages compared to the non stressed control plants (Table 1a, 1b). The shoot: root ratio for plants in trial 2 (Table 1b) was significantly lower ($p<0.05$) than plants in trial 1 (Table 1a).The plants which were stressed during the pod filling stage had the lowest decrease in shoot: root ratio (5.36%) (Table 1a) and (4.93%) (Table 1b), while the highest decrease was obtained on plants which were stressed during the vegetative stage (9.15%) (Table 1a) and (21.4%) (Table 1b). There was however, no significant difference ($p<0.05$) on shoot: root ratio of plants which were stressed at the vegetative stage and those stressed during the flowering stage (Table 1a, 1b).

Table 1a. Effect of water stress on shoot: root ratio of bambara groundnuts in trial 1.

Treatment	Shoot: root ratio
Control	3.17
Stressed during the vegetative stage	2.88
Stressed during the flowering stage	2.96
Stressed during the pod filling stage	3
LSD	0.156

Table 1 b. Effect of water stress on shoot: root ratio of bambara groundnuts in trial 2.

Treatment	Shoot: root ratio
Control	3.04
Stressed during the vegetative stage	2.39
Stressed during the flowering stage	2.48
Stressed during the pod filling stage	2.89
LSD	0.137

4.2 Physiological responses

4.2.1 Relative water content (RWC)

Stressing bambara groundnuts for 21 days during the vegetative, flowering and pod filling stages significantly reduced ($p<0.05$) RWC compared to the control or non stressed plants in both trial 1 and 2 (Table 2). Depending on the stage of bambara plant development or time when water stress occurs, water stress reduced the RWC of plants by 9-12.25% in trial 1 and 8.97-12.56% in trial 2. However, stressing bambara groundnut plants at the pod filling stage had the highest reduction of the leaf RWC (Table 2). Water stressing bambara groundnut plants during the vegetative, flowering and pod filling stages in trial 1 and 2 had a significant difference in RWC among the stressed plants when the plants were rewatered after water stress (Table 2). Only the RWC of the plants stressed at the vegetative and flowering stages recovered fully after rewatering attaining values near or close to those observed in control plants (Table 2). However, recovery of RWC for the pod filling stage failed to reach the values for control and had the lowest recovery of 3.65% in trial 1 and 4.08% in trial 2 as compared to control plants (Table 2).

Table 2. RWC (%) of bambara groundnuts after water stress and after recovery from water stress.

Treatment	Trial 1 Stressed (RWC %)	Trial 2 Stressed (RWC%)	Trial 1 Recovered (RWC %)	Trial 2 Recovered (RWC %)
Control	95	95	96	94.87
Stressed at vegetative stage	86	86.48	95	94.49
Stressed at flowering stage	85.75	85.28	95	94
Stressed at pod filling stage	82.75	83.07	92.5	91
LSD	1.9	1.87	1.39	0.96

4.2.2 Chlorophyll fluorescence

There was a 15.2-20.3 % and 10-18.75% significant reduction of chlorophyll fluorescence (dark adapted F_v/F_m ratio) in trial 1 and 2, respectively, in water stressed bambara groundnut plants at different stages of growth and development compared to non-stressed plants (Table 3). However, there was no significant difference in chlorophyll fluorescence among the water stressed plants at different stages of development (Table 3).

Rewatering bambara groundnut plants after water stress during the vegetative and flowering stages significantly ($p<0.05$) increased the dark adapted F_v/F_m ratio to the same level of that of non-stressed plants (Table 3). However, the bambara plants which were stressed during the pod filling stage had the lowest recovery of 3% in trial 1 and 4% in trial 2 and they failed to significantly ($p<0.05$) recover from water stress as compared to non-stressed plants (Table 3).

Table 3. Water stress effects on chlorophyll fluorescence ratio Fv Fm after water stress and after recovery from water stress.

Treatments	Trial 1 Stressed F_v/F_m	Trial 2 Stressed F_v/F_m	Trial 1 Recovered F_v/F_m	Trial 2 Recovered F_v/F_m
Control	0.79	0.8	0.8	0.8
Stressed at vegetative stage	0.67	0.72	0.8	0.8
Stressed at flowering stage	0.66	0.68	0.8	0.79
Stressed at pod filling stage	0.63	0.65	0.77	0.77
LSD	0.11	0.10	0.02	0.02

4.2.3 Stomatal conductance

Stomatal conductance was significantly reduced ($p<0.05$) by water stress and was about 10 times lower in stressed treatments compared to non-stressed plants (Figure 4a, 4b). The plants which were stressed during the pod filling stage had the highest stomatal conductance reduction of 90% in trial 1 and 91% in trial 2 and those stressed during the vegetative stage had the smallest reduction of as compared to the non stressed control plants also in both trial 1 and 2. There was no significant difference in stomatal conductance among the stressed bambara groundnut plants (Figure 4a, 4b). Rewatering bambara groundnuts after water stress resulted in a significant ($p<0.05$) recovery of stomatal conductance in plants which were stressed during the vegetative and flowering stages only. There was however no significant increase or recovery of stomatal conductance in plants which were stressed during the pod filling stage as compared to non stressed plants (Figure 4a, 4b).

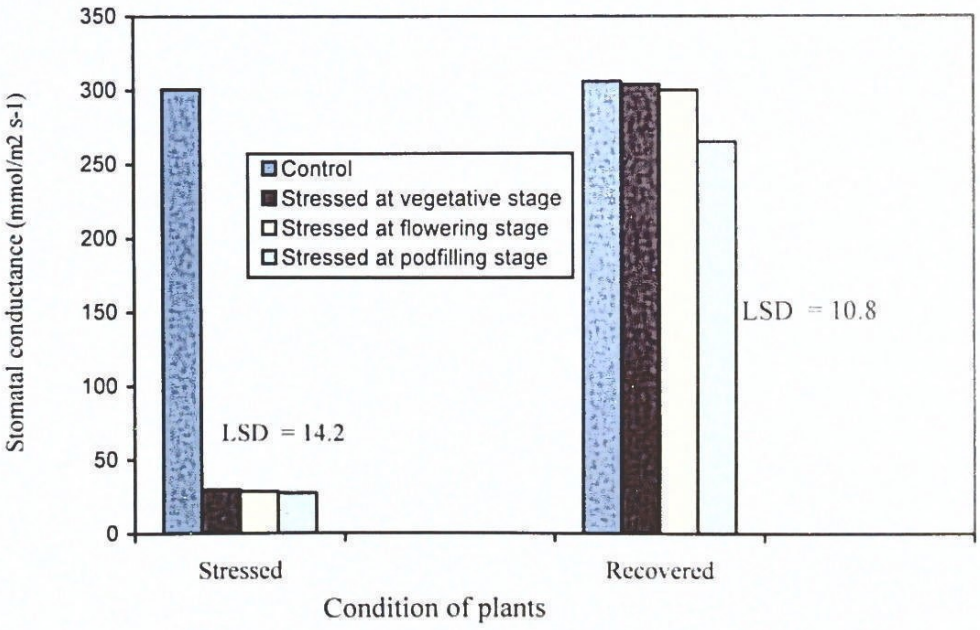

Figure 4a. Stomatal conductance of bambara groundnut leaves at different growth and development stages after water stress and after recovery from water stress in trial 1.

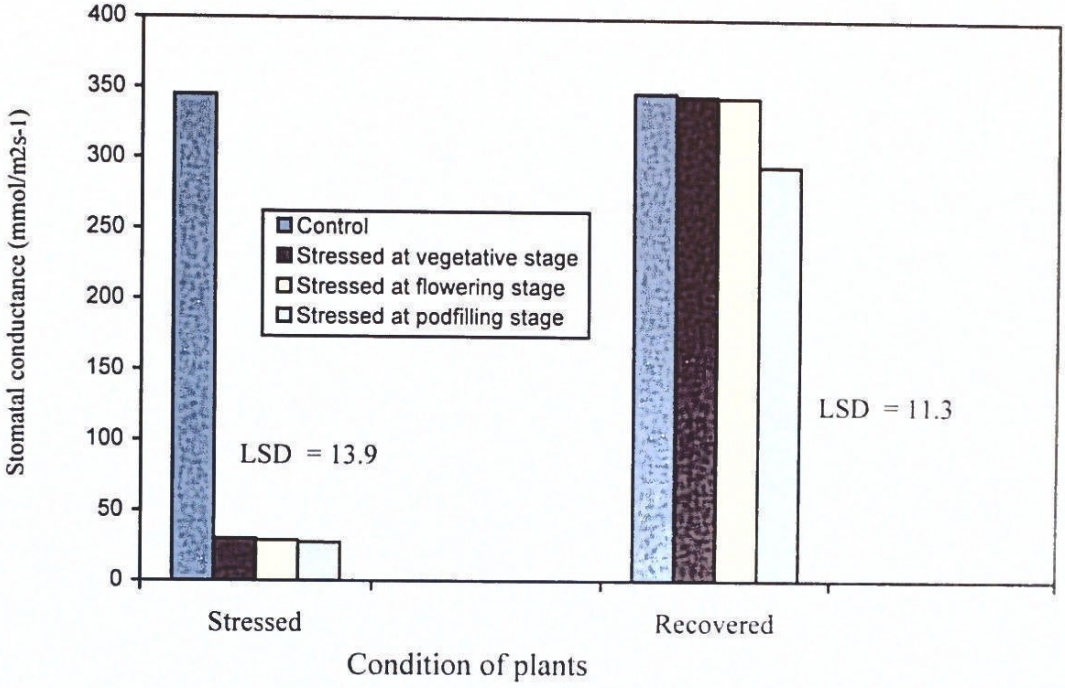

Figure 4b. Stomatal conductance of bambara groundnut leaves at different growth and development stages after water stress and after recovery from water stress in trial 2

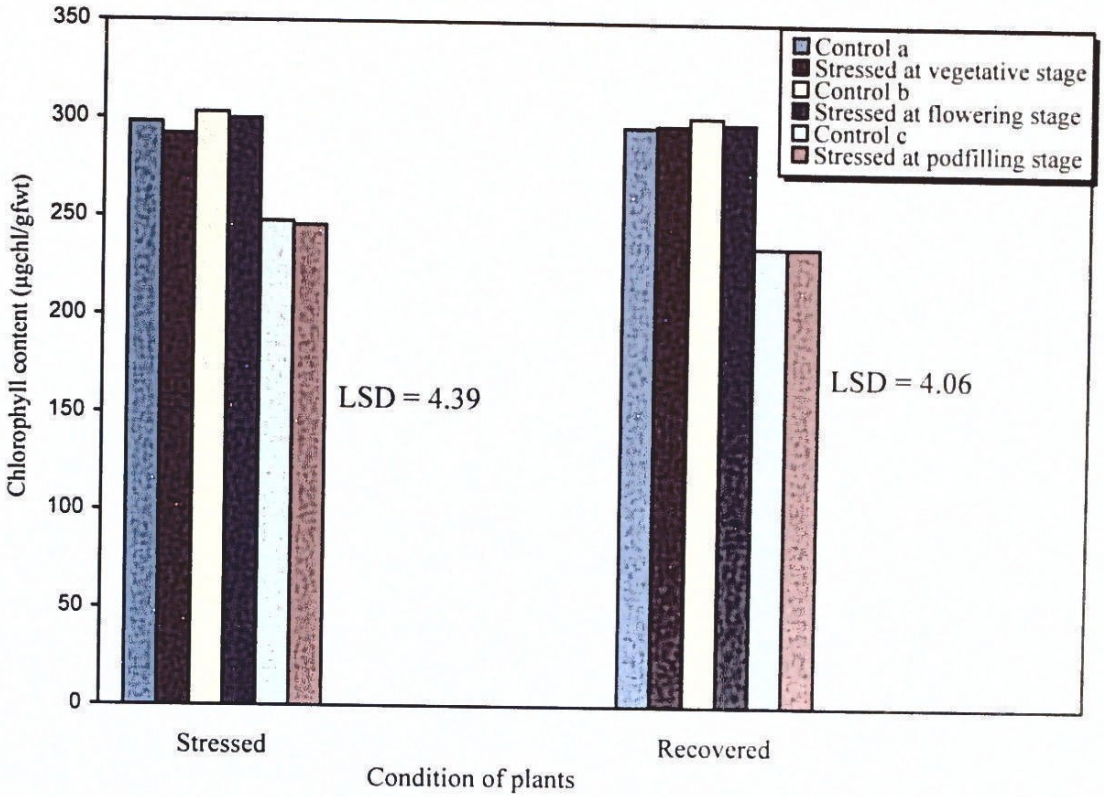

Figure 5 b. Chlorophyll content of bambara groundnut leaves at different stages of growth and development after water stress and after recovery from water stress in trial 2.

Where: control a, control b and control c are chlorophyll contents of the control plants at vegetative, flowering and pod filling stages respectively.

There was however no significant reduction ($p<0.05$) in the chlorophyll content of all stressed treatments as compared to that of the control plants (Figure 6a, 6b). A significant recovery ($p<0.05$) of chlorophyll content (99.7%) in trial 1 and (99.8%) in trial 2 was then observed after the plants were rewatered (Figure 6a, 6b).

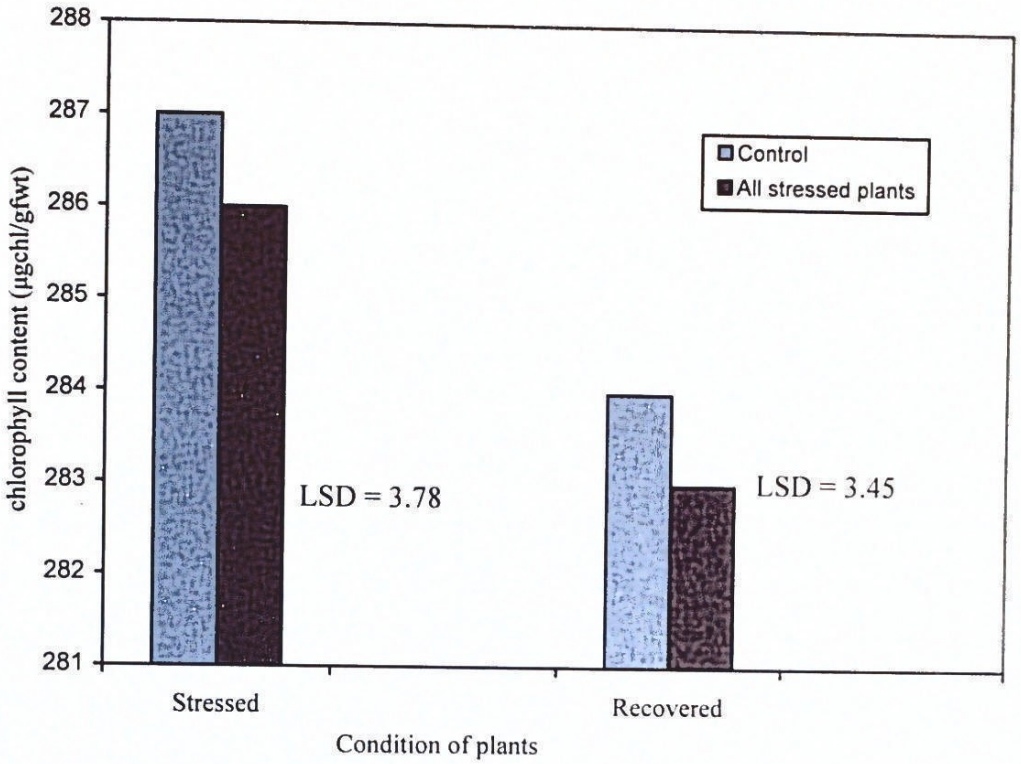

Figure 6 a. The average leaf chlorophyll content of control plants and all stressed treatments of bambara groundnuts after water stress and after recovery from water stress in trial 1.

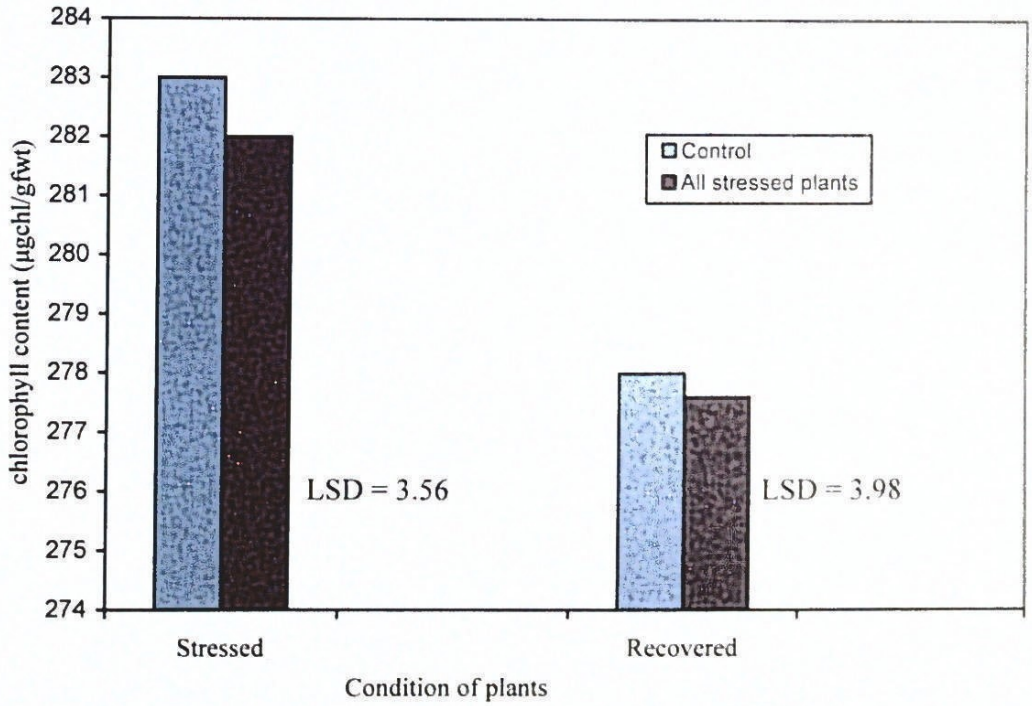

Figure 6 b. The average leaf chlorophyll content of control plants and all stressed treatments of bambara groundnuts after water stress and after recovery from water stress in trial 2.

4.2.5 Chlorophyll content (non-destructive method)

There was no significant effect ($p<0.05$) of water stress on chlorophyll content using the chlorophyll content index (CCI) measurements (Figure 7a, 7b). The CCI values for chlorophyll during the pod filling stage for both control and stressed plants were lower than those for plants water stressed at vegetative and flowering stages of growth and development. These results are consistent with those of destructive method of chlorophyll analysis (Figure 5a, 5b).

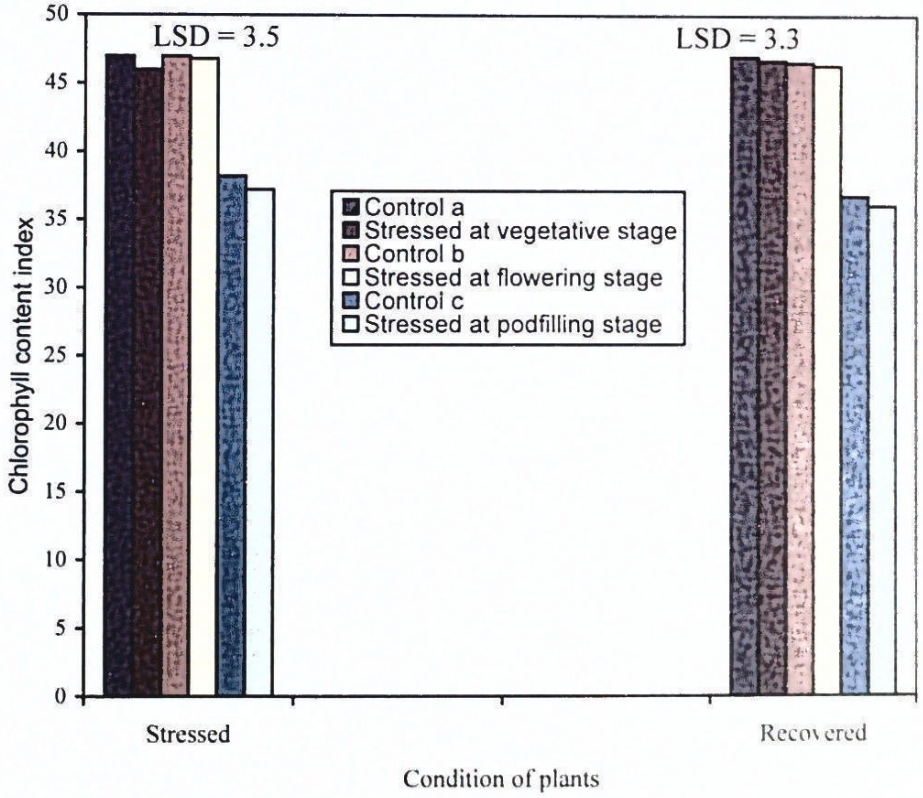

Figure 7 a. Chlorophyll content index values of bambara groundnut leaves after water stress and after recovery from water stress in trial 1.

Where: control a, control b and control c are chlorophyll content index values of the control plants at the vegetative, flowering and pod filling stages respectively.

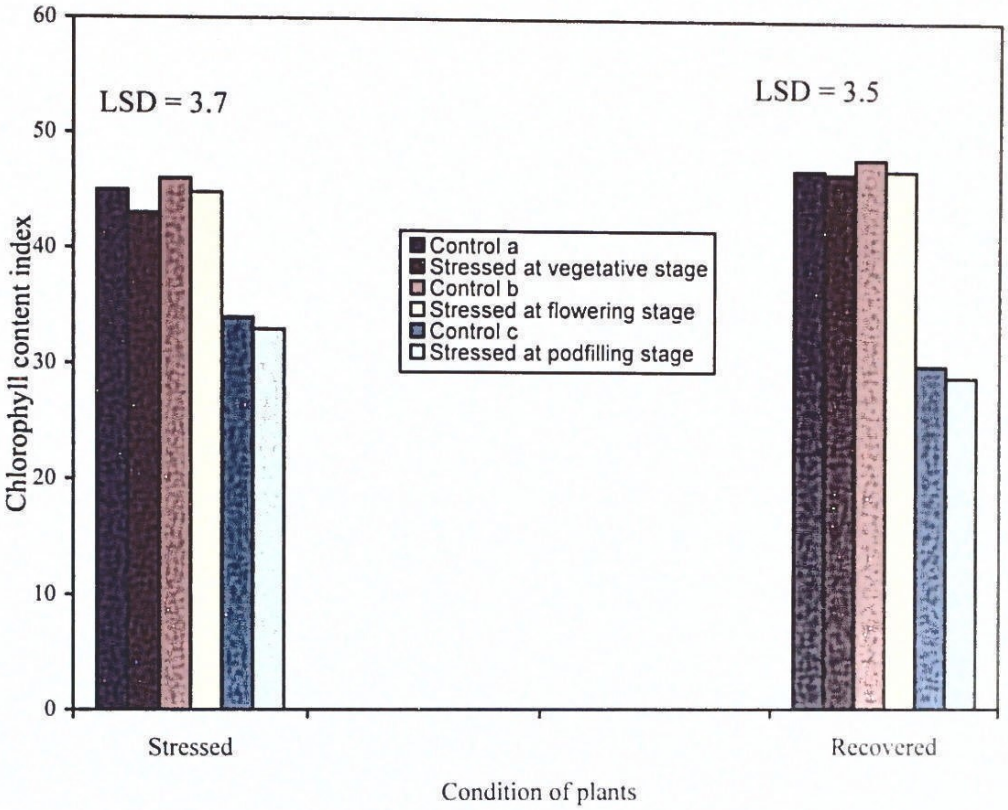

Figure 7 b. Chlorophyll content index values of bambara groundnut leaves after water stress and after recovery from water stress in trial 2.

Where: control a, control b and control c are chlorophyll content index values of the control plants at the vegetative, flowering and pod filling stages respectively.

Water stress did not significantly reduce ($p<0.05$) chlorophyll content of all stressed treatments when compared to the chlorophyll content of non-stressed plants for all stages of growth (Figure 8a, 8b). After rewatering, the mean chlorophyll content of all stressed treatments was not significantly different ($p<0.05$) from the mean chlorophyll content of control plants showing a 97.7% recovery (Figure 8a) and 98.1% (Figure 8b).

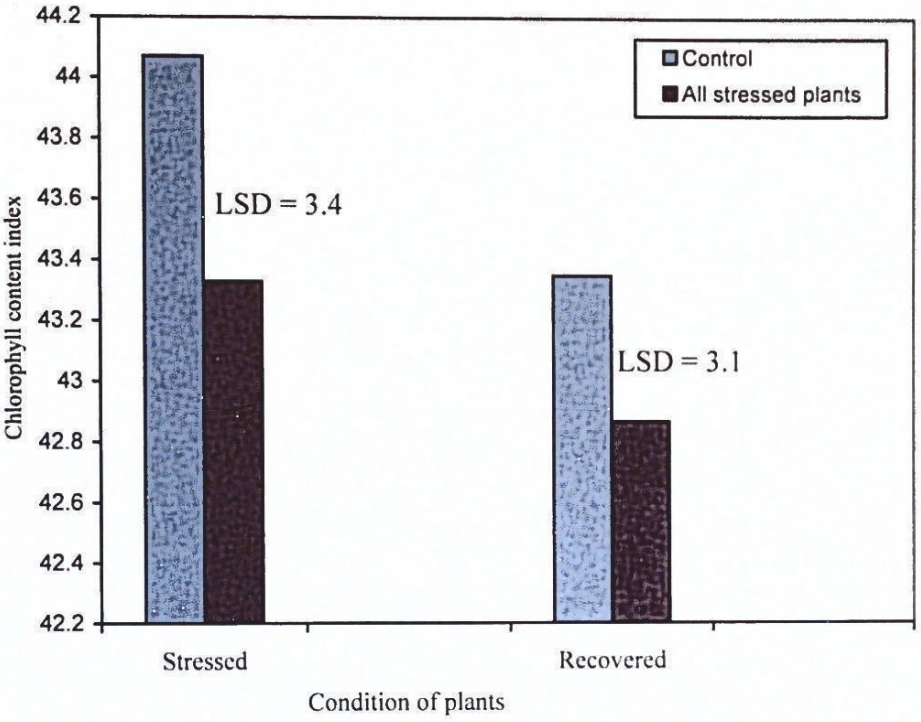

Figure 8 a. The average leaf chlorophyll content index values of control plants and all stressed treatments of bambara groundnuts after water stress and after recovery from water stress in trial 1.

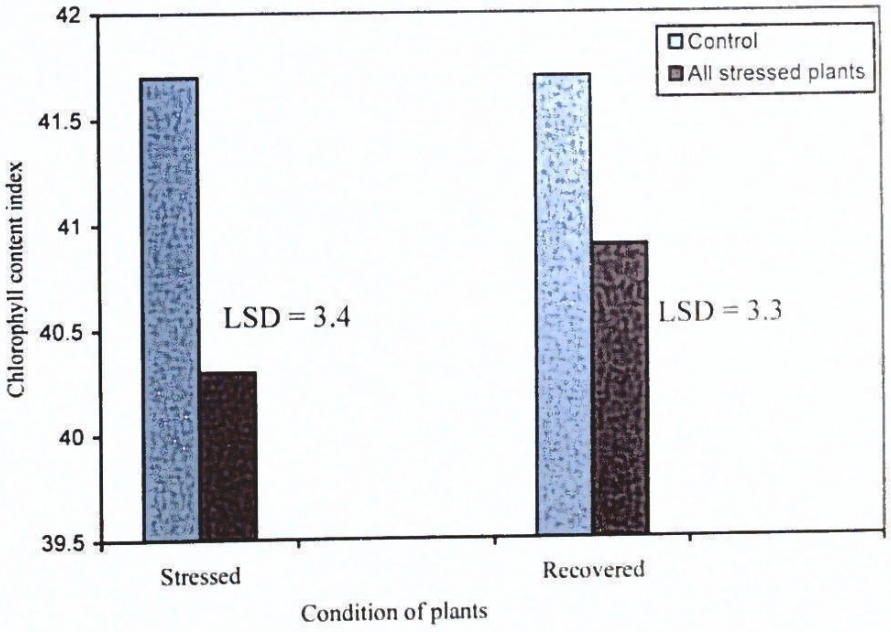

Figure 8 b. The average leaf chlorophyll content index values of control plants and all stressed treatments of bambara groundnuts after water stress and after recovery from water stress in trial 2.

4.2.6 Proline content

Water stress significantly increased ($p<0.05$) proline concentration in bambara groundnut plants water stressed at the vegetative, flowering and pod filling stages compared to the non-stressed plants (Figure 9a, 9b). Depending on the stage of development, water stressed plants produced about four fold increases in the amount of proline compared to non-stressed plants (Figure 9a, 9b). Plants which were water stressed during the pod filling stage had the lowest increase in proline content (368%) (Figure 9a) and (285%) (Figure 9b) while plants which were water stressed during the vegetative stage had the highest increase in proline content (402%) (Figure 9a) and (301%) (Figure 9b). Rewatering significantly ($p<0.05$) reduced proline concentrations in all the previously water stressed plants at different stages of growth and development (Figure 9a, 9b).

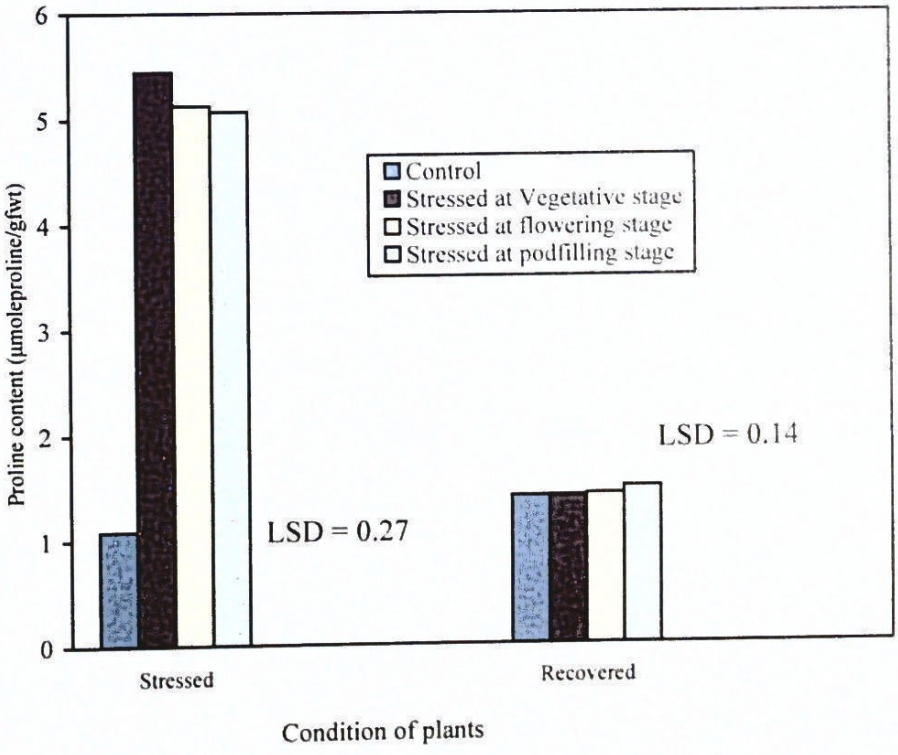

Figure 9 a. Proline concentration in bambara groundnut leaves after water stress and after recovery from water stress in trial 1.

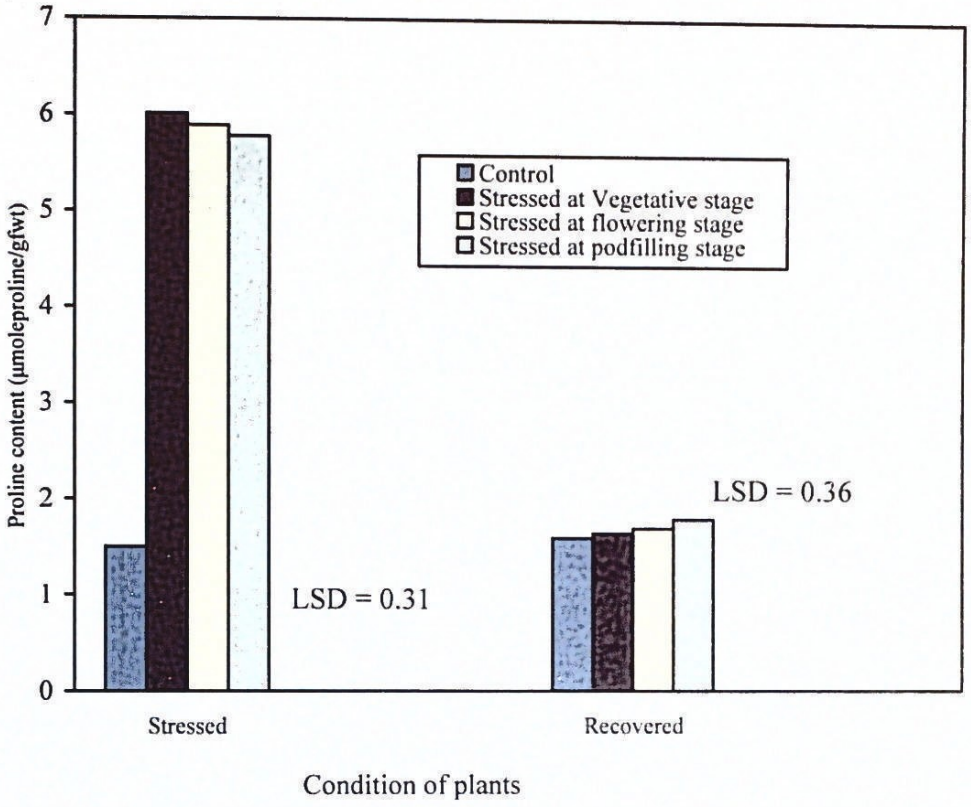

Figure 9 b. Proline concentration in bambara groundnut leaves after water stress and after recovery from water stress in trial 2.

4.3 Yield and yield components

Water stress at the vegetative, flowering and pod filling stages of growth and development of bambara groundnut plants significantly reduced ($p<0.05$) number of pods/ plant, number of seeds/plant and 100 seed weight (Figure 10a, 10b, 11a, 11b). The number of pods and seeds per plant was significantly lower ($p<0.05$) in trial 2 compared to trial 1 at all stages of development (Figure 10a, 10b). The lowest pod and seed number per plant was obtained in plants water stressed during the flowering stage (Figure 10a, 10b). Plants which were stressed during the pod filling stage had the lowest decrease or percentage loss in number of pods (Figure 10a, 10b) but had the highest percentage loss in 100 seed weight compared to plants water stressed at vegetative and flowering stages (Figure11a, 11b). The plants which were stressed at the flowering stage had the lowest pod and seed number per plant in trial 2 (Figure

10b) but had the highest 100 seed weight when compared to other stressed treatments (Figure 11b). There was however, no significant difference ($p<0.05$) between the 100 seed weight of plants stressed at flowering and the control plants in trial 2 (Figure 11 b).

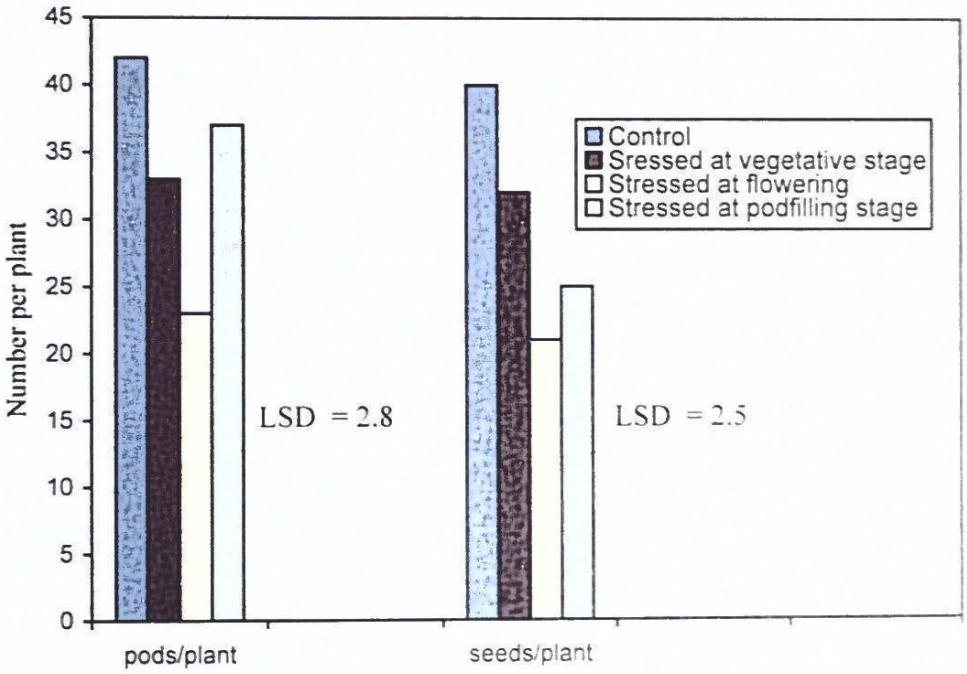

Figure 10 a. Effect of water stress on number of pods per plant and number of seeds per plant of bambara groundnuts in trial 1.

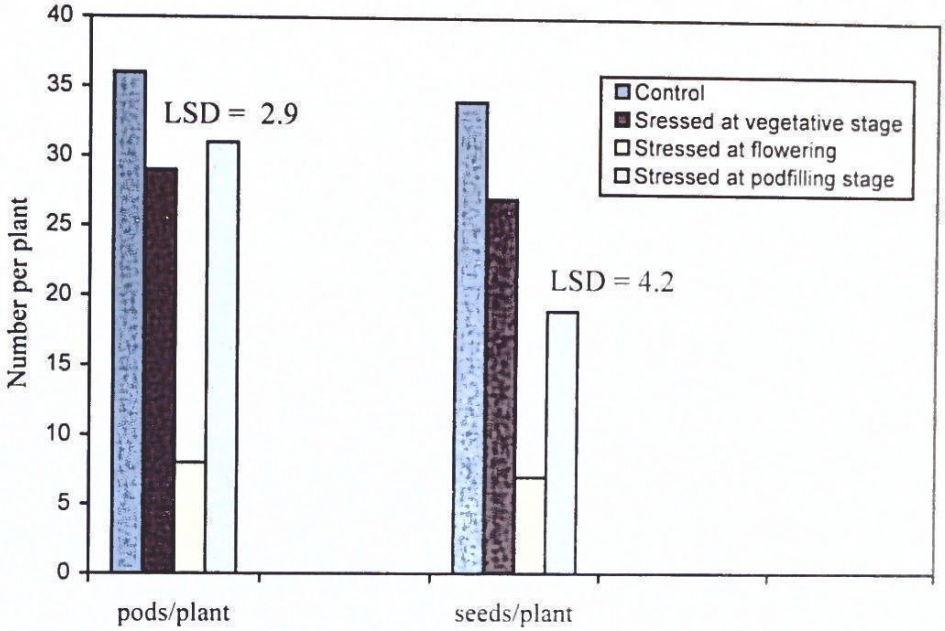

Figure 10 b. Effect of water stress on the number of pods per plant and number of seeds per plant in trial 2.

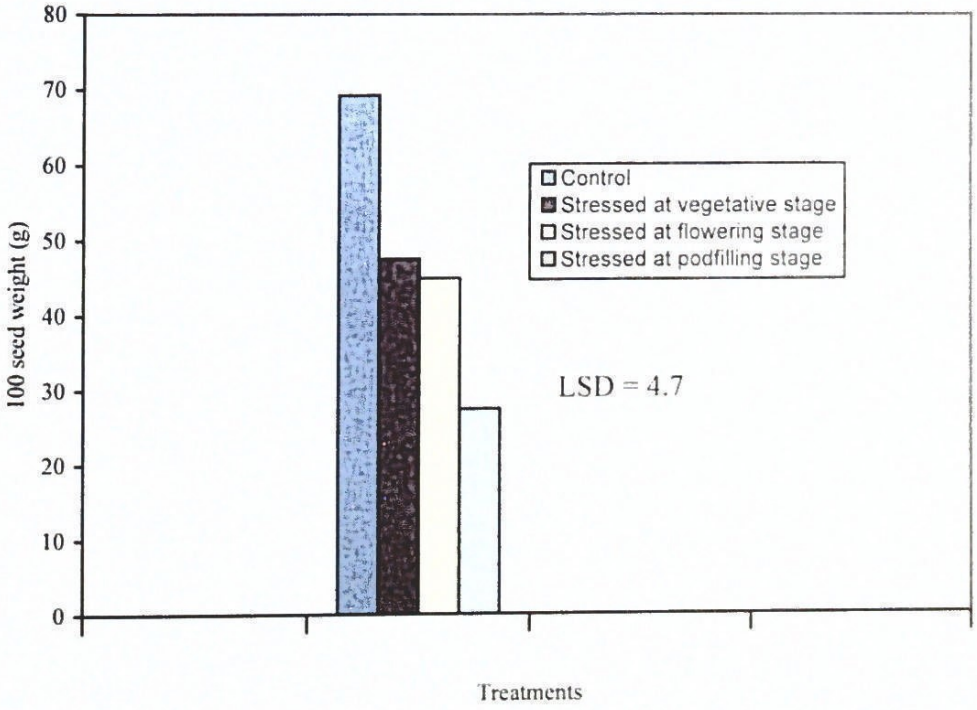

Figure 11 a. Effect of water stress on 100 seed weight of bambara groundnuts in trial 1.

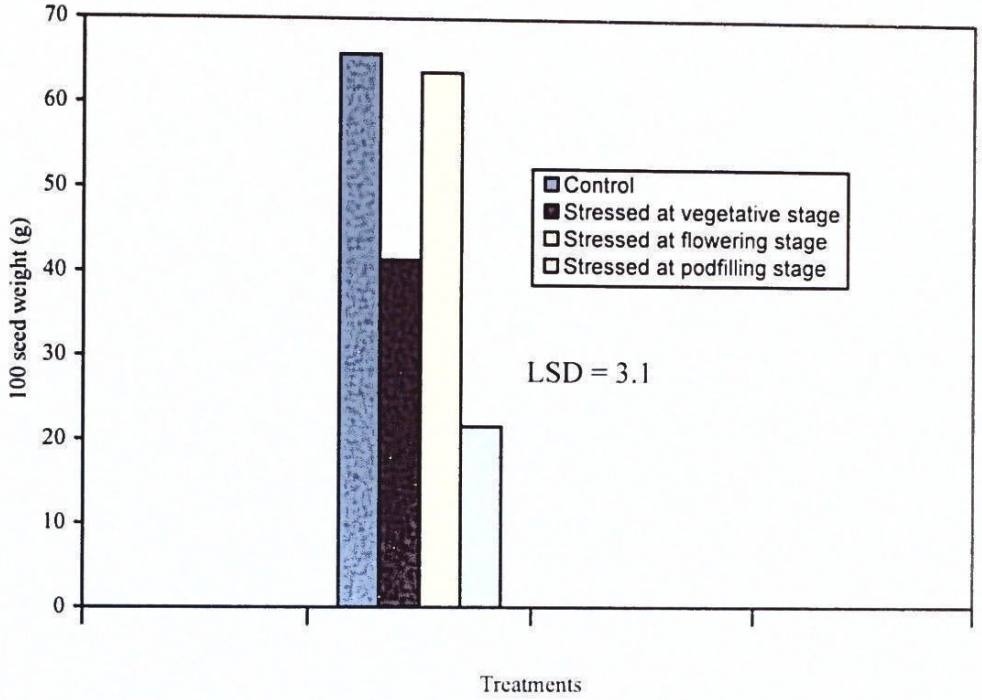

Figure 11 b. Effect of water stress on 100 seed weight of bambara groundnuts in trial 2.

Water stressing bambara groundnuts for 21 days at the vegetative, flowering and pod filling stages of development significantly ($p<0.05$) reduced seed yield (kg/ha) compared to non-stressed plants (Figure 12a, 12b). Yield (kg/ha) for plants in trial 2 was significantly lower ($p<0.05$) than yield for plants in trial 1 at all stages of bambara groundnut growth and development (Figure 12a, 12b). The seed yield loss due to water stress ranged between 45-75 % in trial 1 (Figure 12a) and between 50-82% in trial 2 (Figure 12b) depending with the stage of plant growth and development when water stress occurred. The highest yield reduction occurred on water stressed plants at the pod filling stage was more in trial 2 (82%) (Figure 12b) than trial 1 (75%) (Figure 12a). Plants water stressed at the vegetative stage of growth and development had the lowest seed yield loss compared to non-stressed plants (Figure 12a, 12b).

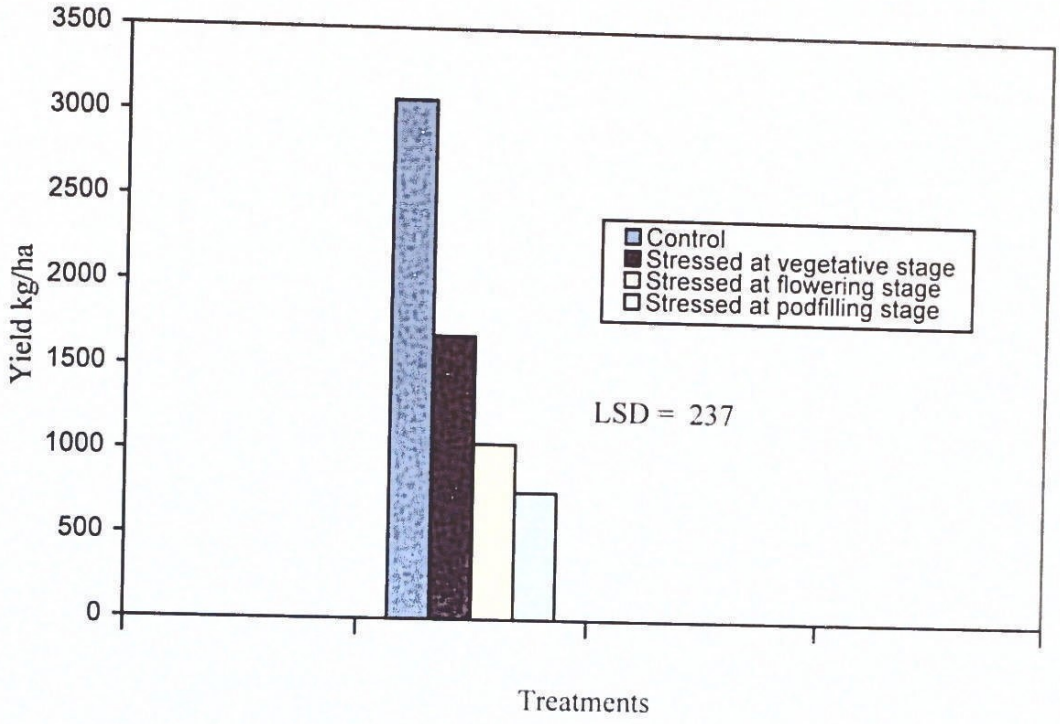

Figure 12 a. Effect of water stress on yield of bambara groundnuts in trial 1.

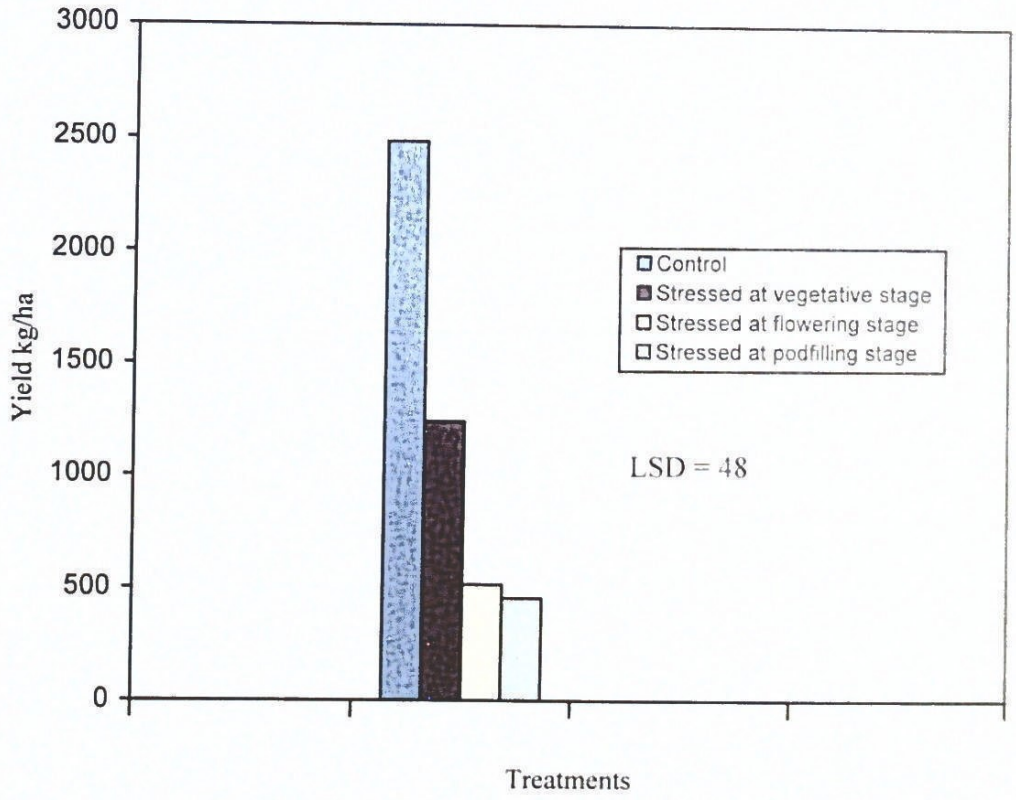

Figure 12 b. Effect of water stress on yield of bambara groundnuts in trial 2.

CHAPTER 5

DISCUSSION

5.1 Relative leaf expansion rate

Water stress which was experienced during the vegetative, flowering and pod filling stages of growth significantly ($p<0.05$) reduced RLER in bambara groundnuts compared to the non stressed plants. Similar results have been reported in bambara groundnuts (Collinson *et al.*, 1997), field beans (*Vicia faba* L.) (Farah, 1981) and soyabeans (*Glycine max*) (Hoogenboom *et al.*, 1987). The rate of expansion of photosynthetic area is an important factor determining productivity or survival of plants (Hesketch and Baker, 1969). Relative leaf expansion rate is important in determining yield since final yield in plants is directly related to the intereception of solar radiation. Yield is determined largely by the rate of development of the crop canopy and the maintenance of functional leaf area. Environmental factors such as saturation deficit that affect leaf growth have a substantial impact on leaf expansion rates. These rates have been found to decline as saturation deficit increases (Taiz and Zeiger, 2006).

The non stressed plants had a significantly ($p<0.05$) very high RLER and this was attributed to the first phase of leaf development. In the first phase of leaf development both cell division rate and the RLER are maximal (Inze, 2007). The reduction in RLER in stressed plants was attributed to turgor reduction which is the earliest biophysical effect of water stress. Turgor dependent activities such as leaf expansion decrease during water stress with consequent decrease in plant growth rate (Taiz and Zeiger, 2006). The resulting smaller leaf area transpires less water thereby effectively

conserving water which is in limited supply during water stress periods. This reduction in leaf area can thus be considered a first line of defence against drought. The RLER for non stressed plants dropped to zero after 19 days of water stress in trial 1 and 23 days in trial 2. In stressed plants RLER dropped to zero after 15, 11 and 8 days for the plants water stressed at vegetative, flowering and pod filling stages, respectively in trial 1 and 4 days for plants stressed at pod filling stage and 15 days for plants stressed at vegetative and flowering stages in trial 2. This may be explained by the fact that the rate of cell division (and hence expansion rate) decline and stops as plants reach final stages of normal development (Inze, 2007). However, this might have taken place earlier in non stressed plants only because, with water available, cell division was more rapid and reached the stationary growth phase earlier. After rewatering the stressed plants, all stressed leaves resumed growth almost immediately and RLER increased probably due to increase in cell size caused by resumption of leaf cell division, culminating in leaf expansion to maximum attainable size. The plants which were water stressed during the vegetative stage had a higher peak RLER after rewatering than the plants water stressed at flowering and pod filling stages, and this can be attributed to young plants having a higher potential to recover after water stress (Jones, 1989).

5.2 Leaf number

Water stress significantly ($p<0.05$) reduced the number of leaves per plant in all stressed bambara groundnut plants more so in plants grown in the second trial. The decrease in leaf production in the second trial may have been caused by declining temperatures which usually occurs later in the season in southern Africa since the temperature was not controlled in the greenhouse used in the second trial (Sesay *et al.*,

2008). Collinson *et al.* (1997) reported that bambara groundnut plants reduced the size of their canopies when subjected to soil moisture stress. Other studies on bambara groundnut have shown that leaf numbers are significantly reduced by soil moisture stress (Collinson *et al.*, 1996 and Mwale *et al.*, 2007). The overall reduction in leaf number may result in yield decrease or decrease in total dry matter production because of the reduction of photosynthetically active leaf area.

Reduction in leaf number may have been a result of reduction and termination of new leaf production. The probable reason for reduction in leaf number is leaf abscission which was evident in bambara groundnut plants which were water stressed during the pod filling stage. Water deficit stimulates leaf abscission which results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene. Drought stress has been reported to induce stress ethylene in a variety of species and tissues (Apelbaum and Yang, 1980; Hoffman *et al.*, 1983; Kacperska *et al.*, 1989). Water stress stimulates the biosynthesis and activity of the enzyme ACC synthase which catalyses the conversion of S- adenosyl methionine to 1-aminocyclopropane-1-carboxyclic acid (ACC-immediate precursor of ethylene biosynthesis), hence increased ethylene biosynthesis (Hoffman and Yang, 1980; Emongor, 1995). Drought stress induced ethylene stimulates the abscission of various plant parts such as leaves, flowers or fruits. Ethylene promotes abscission of leaves by inducing the formation of the abscission layer via induction of hydrolytic enzymes that cause cell wall hydrolysis in specific cells found at the base of leaves and petioles (Jankiewicz, 1985; Reid, 1985).

If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. The same may have happened in bambara groundnut plants which were stressed during the pod filling stage. The resulting decrease in leaf area is one of the mechanisms of moderating water loss from the crop canopy and averting excessive drought induced injury to the plant. Such leaf area adjustment is an important long-term change that improves the plant's fitness in a water-limited environment.

After rewatering both bambara groundnut plants which were stressed during the vegetative and flowering stages increased leaf numbers. This is an important trait for bambara groundnuts as plants are capable of developing a large leaf area very quickly, therefore are better suited to take advantage of occasional wet summers. With bambara groundnut plants which were stressed during the pod filling stage, leaf senescence could not be stopped by rewatering and so the plants failed to recover from water stress. Stoddart and Thomas (1982), suggested that stress induced senescence could be reversed if the stress is relieved before senescence progresses too far. Senescence had apparently progressed too far enough after 15 days of stress in the current study, therefore leaf senescence became non reversible, and eventually the leaves abscised.

5.3 Plant height

Water stress significantly ($p<0.05$) reduced plant height in plants which were stressed during the vegetative and flowering stages of growth. This was attributed to reduction of stem and leaf expansion (Lauer, 2005). Water stress did not affect plant height during the pod filling stage because the plants had ceased growing vegetatively by

this time. Recovery of plant height did not occur in bambara groundnut plants which were stressed during the pod filling stage as the plants had reached maximum height. After rewatering, the plants which were stressed during the vegetative and flowering stage increased in plant height. This may be attributed to resumption of stem cell division and elongation plus leaf expansion

5.4 Shoot: root ratio

Bambara groundnut plants which were stressed during the vegetative, flowering and pod filling stages had a significantly ($p<0.05$) lower shoot: root ratio as compared to the non stressed control plants. Water deficit modulates root length and density and so in times of water stress, plants allocate carbon to the roots for new growth. The potential growth rate of the above ground portion of the plant is therefore reduced resulting in a decrease in shoot: root ratio (Ahuja *et al.*, 2008). This shows that bambara groundnut responds to drought by partitioning more assimilates into roots relative to shoots. A greater soil volume can therefore be exploited which is an important adaptation in drought spells. The results of the current study are in agreement with the results of Nyamudeza (1989) and Collinson *et al.* (1996) in bambara groundnuts which showed that bambara groundnuts have low shoot: root ratio under water stress.

There was no significant ($p<0.05$) difference in shoot: root ratio between plants which were stressed during the vegetative and flowering stages and this may be explained by the short term reductions in shoot growth rate of plants which were stressed during the vegetative stage followed by compensatory growth after rewatering. Plants which were stressed during the pod filling stage had lower shoot: root ratio as compared to

the non stressed control plants even though water was withheld well after the plants had stopped growing vegetatively. The irreversible leaf senescence caused by water stress may have reduced shoot dry matter.

There was also a significant ($p<0.05$) difference in shoot: root ratio between plants in trial 1 and trial 2 and declining temperatures which occurred later in the season might have reduced leaf production and leaf size, thus dry matter production resulting in lower shoot: root ratio for plants in trial 2.

5.5 Relative water content

Water stress during the vegetative, flowering and pod filling stages of growth and development of bambara groundnut plants significantly ($p<0.05$) decreased the RWC. Kramer and Boyer (1995) reported that water stress is characterised by a decrease in RWC and that the ability of plants to survive water deficit depends on their ability to restrict water loss. Reduction of RWC is a common phenomenon in bambara groundnuts as was also reported by Muriuki (1997) and Collinson *et al.* (1997). Similar results were also reported in other crops like rice (*oryza sativa*) (Lafitte, 2002), groundnuts (*Arachis hypogea*) (Pimratch *et al.* 2008) and maize (*Zea mays*) (Efeoglu *et al.* 2009).

Plants which were stressed during the pod filling period had a significantly ($p<0.05$) lowest RWC amongst the stressed treatments and did not fully recover after rewatering. This may be because the plants were on their last stage of growth hence they were aged compared to those at the vegetative and flowering growth stages and the plant's ability to recover is a function of plant age (Jones, 1989).

As there were RWC reductions of between 9-12.3 % in trial 1 and 9-12.6 % in trial 2, this showed that bambara groundnut plants can maintain relatively high RWC values despite the development of moisture stress as was also reported by Collinson *et al.*, (1997). This is a very important trait which indicates drought resistance as species which exhibit restricted changes in RWC per unit reduction of water potential are often considered to be relatively drought resistant.

5.6 Chlorophyll fluorescence

In all water stressed bambara groundnut plants, there was a significant ($p<0.05$) decline in chlorophyll fluorescence (F_v/F_m). The ratio F_v/F_m has been demonstrated to be proportional to the quantum yield of photochemistry (Genty *et* al., 1989) and shows a high degree of correlation with the quantum yield of net photosynthesis in intact leaves (Bjorkman and Demming, 1987). This decrease in F_v/F_m is evidence of the down regulation of photosystem II activity and impairment of photochemical activity which indicates damage in the functionality of the photosynthetic apparatus. This is because water stress reduces photosynthesis directly because dehydrated protoplasm has a lowered photosynthetic capacity (Bjorkman and Demming, 1987). The reduction in chlorophyll fluorescence in bambara groundnuts is consistent with observations from other crops under water stress like potatoes (Zrust *et al.*, 1988), cowpeas (Andrade *et al.*, 1999) and maize (Duares *et al.*, 2001,).

The bambara groundnuts' F_v/F_m significantly ($p<0.05$) recovered after rewatering in plants which were stressed during the vegetative and flowering stages while the plants which were stressed during the pod filling stage failed to fully recover. This

recovery pattern is similar to that observed in cowpeas (Andrade *et al.*, 1999) after rewatering of stressed plants. The increase in F_v/F_m usually results in increase in dry matter production because of return to normal photosynthetic rate. Failure of plants which were stressed during the pod filling stage to recover may indicate damage to the photosynthetic apparatus, and full photosynthetic recovery may have been limited by irreversible down regulation of photosystem II activity during stress (Andrade *et al.*, 1999).

5.7 Stomatal conductance

Water stress experienced during the vegetative, flowering and pod filling stages of growth of bambara groundnuts significantly ($p<0,05$) reduced stomatal conductance. This tendency is consistent with observations made by Collinson *et al.*, (1997) and Cornellisen *et al.*, (2005) on bambara groundnuts, and also on other legumes like groundnuts (Black *et al.*, 1985) and pigeon peas (Lopez *et al.*, 1988). During water stress, stomatal conductance is reduced due to stomatal closure which reduces the rate of passage of water vapour and carbon dioxide through the stomata. Stomatal closure allows plants to maintain high water status during water stress thus avoiding drought. However, stomatal closure can reduce photosynthesis to below compensation point (Pessarakli, 2005).

Decreased stomatal conductance results in lower net carbon dioxide assimilation rate, lower intercellular carbon dioxide and lower chloroplastic carbon dioxide tension (Pessarakli, 2005). The carbon dioxide insufficiency will reduce photosynthetic efficiency and dry matter production and may have negative impact on plant growth and yield. Plants which were stressed during the vegetative stage and flowering stage

significantly ($p<0.05$) recovered their stomatal conductance after rewatering. Recovery of stomatal conductance may result in increased carbon dioxide diffusion into the leaves to attain higher photosynthetic rates which favours higher biomass and higher crop yield. However the plants which were stressed during the pod filling period did not fully regain stomatal conductance after rewatering. This may be because the plants were in their final stage of development: the older the plant the lower the regenerative capacity (Jones, 1989).

5.8 Chlorophyll content

Results from both the destructive and non destructive methods of measuring chlorophyll showed that water stress did not significantly ($p<0.05$) reduce bambara groundnut leaf chlorophyll content. Cornellisen *et al.*, (2005) reported similar results on bambara groundnuts. This shows that bambara groundnut plants maintain high amounts of leaf chlorophyll content despite the development of moisture deficit stress and this trait can be considered to be a line of defence against drought which can result in drought resistance. However since leaf number and hence total leaf area and mass (shoot) were affected, it implies that total plant chlorophyll content was reduced by water stress.

Lower amounts of chlorophyll content were observed in the control plants during the grain filling stage as compared to other control plants for other stages of growth. This may be because at this stage some leaves were senescing. Degradation of photosynthetic apparatus which takes place during yellowing of leaves may have caused a reduction in chlorophyll due to ageing process.

5.9 Proline content

In the current study the concentration of free proline accumulation in stressed bambara groundnut leaves were significantly ($p<0.05$) higher by up to 4 times than in non stressed plant leaves. The higher levels may be a feature of decreased RWC which triggers the increased breakdown of proteins and conversion of some amino acids like arginine and glutamic to proline (Abdallah and Khoshiban, 2007). Proline is reported to accumulate in plant cells exposed to water stress in crops such as soyabean (Waldern *et al.*, 1974); sorghum (Blum and Ebercon, 1976) cotton (Elmore and McMichael, 1981); and maize (Efeoglu *et al.*, 2009). The accumulation of proline is reported to result in drought tolerance and changes in proline content in several crops have been correlated with their capacity to tolerate and adapt to arid environments (Kishor *et al.*, 1995). Although proline's role in plant osmotolerance remains controversial it is however, thought to contribute to osmotic adjustment, detoxification of reactive oxygen species and protection of membrane integrity during water stress (Hare and Cress, 1997).

Plants which were stressed during the vegetative stage significantly ($p<0.05$) accumulated more proline than those stressed during the flowering and pod filling stages. This was attributed to plant age, as plants were still young during the vegetative stage and therefore, more actively growing resulting in more proline accumulation than bambara plants at later stages of growth and development (Andreas, 1995).

Rewatering the plants completely eliminated the effects of water stress on proline accumulation and proline levels dropped and equalled those of non-stressed plants.

The decrease in proline levels due to rewatering may be a result of its rapid catabolism during recovery to provide nitrogen for recovering tissues, reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress-induced damage (Hare and Cress, 1997; Hare *et al.*, 1998).

5.10 Yield and yield components

Water stressing bambara groundnuts significantly ($p<0.05$) reduced the number of pods, seeds per plant, 100 seed weight and also seed yield (kg/ha) in all stressed treatments as compared to the non stressed control plants. Plants which were stressed during the flowering stage had the lowest pod and seed number per plant and water stress during this period may have resulted in death of pegs before they initiated pods culminating in reduction of pods and seed number per plant. After rewatering the plants in trial 1 resumed flowering but the plants reached physiological maturity whilst having small pods without mature seeds inside. Plants which were stressed during the pod filling stage had a significantly ($p<0.05$) higher number of seeds per plant as compared to plants water stressed at vegetative or flowering stage. This may be because water was withheld when most of the pegs had formed pods and so they managed to form seeds. The number of seeds per plant was however, less than the control and might have been caused by abortion of newly formed seeds due to water stress. The plants which were stressed during the vegetative stage also had a reduced pod and seed number per plant. This is because water stress during the vegetative period reduced plant height and canopy growth resulting in reduced plant growth therefore delaying and reducing the appearance of nodes. The reduction in pod number and seed number per plant, 100 seed weight and seed yield in water stressed

plants was also attributed to the reduction in the quantum yield of net photosynthesis in water stressed plants as evidenced by the reduction in chlorophyll fluorescence.

The 100 seed weight of all stressed plants was significantly ($p<0.05$) reduced by water stress as compared to the non stressed plants. The 100 seed weight of plants which were stressed during the pod filling stage was the lowest amongst the stressed treatments (60 % in trial 1 and 67 % in trial 2). This reduction was a result of smaller seeds and earlier maturity relative to non stressed control plants. The smaller seeds may have been a consequence of reduced availability of assimilates for translocation to pods for proper seed development. This reduction of assimilates may be attributed to reduction in photosynthesis possibly caused by reduction in stomatal conductance, chlorophyll fluorescence and RWC during water stress. Another explanation for smaller seeds observed may be stress induced leaf senescence which reduced the photosynthetically active leaf area. Reduction of assimilates for translocation to the seeds may also have been caused by competition for assimilates between roots and seeds. Rewatering the plants which were stressed during the pod filling stage after water stress did not completely eliminate the effects of water stress on senescence, stomatal conductance and RWC, therefore this further reduced production of photoassimilates for pod filling.

The reduction in 100 seed weight of plants which were stressed during the vegetative stage may have been a result of fewer leaf numbers and therefore less photosynthetically active leaf area and reduction in assimilates for pod filling. The 100 seed weight of plants which were stressed during the vegetative stage was not significantly ($p<0.05$) different from plants which were stressed during the flowering

stage in trial 1. This may be because during pod filling, their plants had re-established their top growth after rewatering to almost the same level. However, the 100 seed weight for plants stressed during the flowering stage in trial 2 was not significantly affected by water stress. This may be because the plants had very few pods and seeds and so the sink (seeds) was reduced so the developing seeds received more assimilate from the leaves (source) during pod filling as the sink: source ratio was increased.

Water stress significantly ($p<0.05$) reduced seed yield (kg/ha) in all stressed treatments with an average reduction of 62% in trial 1 and 70 % in trial 2. The reduction in seed yield in stressed treatments in the current study agrees with some reports on legumes under water stress such as black beans (Nielson and Nelson. 1998); faba beans (Mwanamwenge *et al.*, 1999); bambara groundnuts (Mwale *et al.*, 2007) and cereals like oats (Sandhu *et al.*, 1977) and maize (Kamara *et al.*, 2003). The decrease in RLER and leaf number resulted in a decrease in total bambara plant leaf area which decreases the photosynthetically active leaf area and therefore resulted in decreased photosynthesis and photosynthates production which resulted in low seed yield (kg/ha). Reduced RWC , stomatal conductance and chlorophyll fluorescence may also have caused reduced photosynthesis which then resulted in very low seed yields. This is because RWC of 90-80 % as was observed in all the stressed treatments is correlated with alteration in relative rates of photosynthesis (Sparks, 2007) and stomatal conductance is correlated with photosynthetic capacity and photosynthetic efficiency (Wong *et al.*, 1979). On the other hand a decrease in chlorophyll fluorescence indicates stress and damage to the photosynthetic system (Sparks, 2007).

Generally, plants which were grown in trial 2 produced significantly ($p<0.05$) lower pod and seed number per plant, 100 seed weight and seed yield (kg/ha). This might have been caused by reduction in the dry matter production which might have been a consequence of the effect of sowing date on leaf production, canopy development and the substantial reduction in the reproductive period as sowing was delayed (Harris and Azam-ali, 1993; Collinson *et al.*, 2000; Sesay *et al.*, 2008). The reduction of the reproductive period has a major impact on the productivity of bambara groundnut since pod filling is dependent more on partitioning of assimilates from current photosynthesis than from remobilization of stored assimilates from vegetative organs (Brink, 1999; Sesay *et al.*, 2004).

The various amounts of bambara groundnut yield (kg/ha) obtained on different treatments showed that bambara groundnut is capable of producing worthwhile yield even if it has been affected by stress at any stage of growth. The current study has shown that the production of yield by bambara groundnuts under water stress may be linked to maintenance of relatively high RWC, chlorophyll content and root: shoot biomass under water stress and also small leaf area which restricts transpirational water loss. It can also be linked to bambara groundnut's ability to recover RWC, chlorophyll fluorescence and leaf area after receiving water after stress.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSIONS

There are appreciable differences among the vegetative, flowering and pod filling stages of growth in respect to their response to short periods of water stress. Water stress during the vegetative, flowering and pod filling stages of bambara groundnut growth caused a reduction in leaf numbers, RLER, plant height and shoot: root ratio. The physiological responses to water stress at the vegetative, flowering and pod filling stages were reduction in RWC, stomatal conductance and chlorophyll fluorescence and an increase in proline content. Leaf chlorophyll content was however not reduced by water stress at all stages of growth and development. Bambara groundnuts have the ability to recover from water stress after rainfall or irrigation in plants which are stressed during the vegetative and flowering stages only and not in plants stressed during the pod filling stage. The nature and extend of damage and the ability of bambara groundnut to recover from water stress depends on the developmental stage at which the plant encounters water deficit. Water stress experienced by bambara groundnut plants has cumulative effects which are ultimately manifested by a reduction in yield and yield components. Bambara groundnuts are most sensitive to water stress during the pod filling stage, followed by the flowering stage and less sensitive in the vegetative stage. The variations in response to drought found in the current study can be practically exploited in crop improvement especially in the semi-arid and arid environments where rainfall is erratic and unreliable.

6.2 RECOMMENDATIONS

The author recommends that adequate water must be available to bambara groundnuts at all developmental stages in order to obtain an optimum yield.

If water is in short supply as is common in semi arid regions, irrigation (if available) should be done just prior to the flowering and pod filling stages because they are the critical stages of bambara groundnut plant growth and development that affects the yield and yield components. The author further recommends that research should be done on water stress under field conditions and also using various bambara groundnut landraces for comparison.

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