GROWTH AND YIELD RESPONSES OF COWPEAS (*Vigna unguiculata* L.) TO WATER STRESS AND DEFOLIATION

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DECLARATION

I, Zinhle Ntombela, certify that the material reported in this thesis represents my original work, except where acknowledged. I further declare that these results have not otherwise been submitted in any form for any degree or diploma to any university.

Signature ________________

Zinhle Ntombela

I, Professor Albert Thembinkosi Modi supervised the above candidate in the conduct of his dissertation study.

Signature ______________________

Prof. A.T. Modi

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DEDICATION

This dissertation is dedicated to my family, especially my mother, for believing in me and for supporting me throughout the course of my studies.

GENERAL ABSTRACT

Cowpea (*Vigna unguiculata* L.) is an important legume, especially in the hot, dry tropics and subtropics of sub-Saharan Africa. It has been widely reported to be drought tolerant. Cowpea is a highly nutritious, multi-purpose crop, used as a leafy vegetable and grain legume with potential to contribute to food security in marginal areas. However, the crop is still classified as a neglected underutilised species; legume research focus has been mainly devoted to established legumes such as common bean and soybeans. There is a need to collect empirical information on cowpea which could be used to advise farmers on management strategies. This study evaluated cowpea responses to water stress under controlled and field conditions. Initially, two cowpea varieties (Brown and White birch) were evaluated for seed quality using the standard germination that was laid out in a completely randomised design and each variety was replicated for times. Electrolyte conductivity test was also performed under laboratory conditions. Thereafter, a pot trial was conducted to evaluate cowpea response to water stress imposed at different growth stages under varying growth temperatures. The pot trial comprised three factors: temperature [High (33/27℃), Optimum (27/21℃) and Low (21/15℃)], water regimes (no stress, terminal stress, intermittent stress – vegetative and intermittent stress - flowering) and cowpea varieties. Lastly, a field trial was conducted to evaluate cowpea production as well as the effect of sequential leaf harvesting on yield under irrigated and rainfed conditions. The field trial was laid out as a split-plot design, with water regime (irrigation vs. rainfed) as main factors, cowpea varieties as sub-factor and sequential harvesting (no harvest, harvested once and harvested twice), replicated three times. All treatments were arranged in a randomised complete block design. Results of the initial study showed that germination capacity and vigour of cowpea varieties were significantly different ($P < 0.001$). White birch had higher electrolyte leakage than Brown birch. Pot trial results showed that cowpea growth (leaf area, leaf number and plant height) was vigorous in the high temperature regime compared with optimum and low temperature regimes. Chlorophyll content index was higher under high temperature relative to optimum and low temperature regimes, respectively. Under low and optimum temperature regimes, cowpea growth was stunted; cowpea failed to flower and form yield. Whereas, under high temperature regime, cowpea growth was vigorous hence flowered and formed yield. Vegetative growth was more sensitive to water stress than flowering stage. Terminal stress and stress imposed during flowering resulted in increased proline accumulation relative to no stress and stress imposed during vegetative growth. Harvest index was lower when water stress was imposed during vegetative relative to flowering stage. Field trial results showed that cowpea growth was sensitive to water stress. Plant height, leaf number, chlorophyll content index and stomatal conductance were lower under rainfed relative to irrigated conditions. Sequential harvesting of leaves had no significant effect on cowpea yield. It is concluded that tropical temperature conditions are most suitable for cowpea production; the controlled environment study showed best crop performance under 33/27℃. In the context of varieties used for the present study, vegetative growth was the most sensitive stage to water stress. Cowpea performed better under rainfed relative to irrigated conditions with respect to yield formation. Low temperature was found to be more limiting to cowpea growth, development and productivity compared with water stress. Whereas, under high temperature conditions, water stress was more limiting to plant growth and productivity. White birch may be used as a dual purpose crop due to its ability to produce reasonable grain yield regardless of defoliation.

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CHAPTER 1

1.0 GENERAL INTRODUCTION

Cowpea (*Vigna unguiculata*) is one of the most ancient crops known to man and is grown across various climatic zones, most commonly in the dry savanna regions of sub-Saharan Africa (Singh *et al.*, 1997). Its centre of origin is still uncertain, Ng and Marechal (1985) initially thought it to be India, with secondary centres in China and Ethiopia, but later it was believed to have originated and domesticated in Africa, especially Ethiopia. Recent studies, on the other hand, suggest that it might be of central African origin (Ogunkamni *et al.*, 2006).

Cowpea is treated as an important food legume in tropical and sub-tropical regions of the world, especially where drought is prominent due to low and uneven rainfall patterns thus causing major limitation to crop production (Singh *et al.*, 1997). It is widely grown in east Africa and south-east Asia, primarily as a leafy vegetable (Hallensleben *et al.*, 2009) due to its high protein content. Cowpea leaves and seeds are low in fat, high in carbohydrates and proteins and low in anti-nutritive factors (Ohler *et al.,* 1996). As such, consumption in Africa and Asia corresponded to 5 million tonnes of dry cowpea seeds and this represented 30% of total food legume production in lowland tropics (Steele *et al.* (1985).

The total area harvested with cowpea worldwide is about 10 979 841 ha. As a result, annual world cowpea seed production is 364 817 tonnes (FAO, 2010). West and central Africa are the leading cowpea producing regions in the world, accountable for 64% of the annual estimation of 3 million tonnes of cowpea seed (IITA, 2009). Nigeria is the leading cowpea producer in Africa responsible for 68% (Figure 1.1), followed by Ghana, Niger, Senegal and Cameroon (IITA, 2009). Other major producers of cowpea outside Africa are Asia and Central and South America (Figure 1.1).

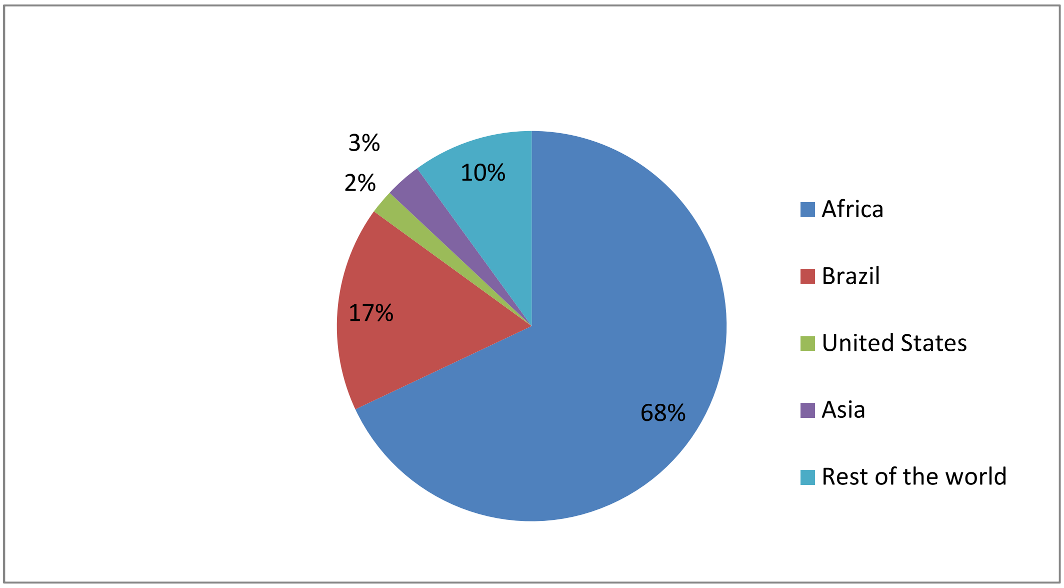

Figure 1.1: World cowpea production.

(FAO, 2004. http://www.fao.org/inpho/content/compend/text/ch32/ch32.htm).

Subsistence farmers in the semi-arid and sub-humid regions of Africa are the major producers and consumers of cowpeas. These farmers not only grow cowpeas for dry seed, but also utilize the leaves and pods as vegetables (IITA, 2009). The other plant parts, besides dry seeds, are commonly consumed as young leaves, immature pods and immature seeds (IITA, 2009). Basically, all components of the plant can be consumed since they are rich in nutrients and fibre.

The world population is increasing and so is the demand for food to meet population growth. Although food production has improved in the past few years, water scarcity still remains a challenge. In the last century, water use has increased worldwide at more than double the rate of population growth (FAO, 2007). On average, agriculture uses about 66% of the total withdrawals; this can be as high as 90% in arid regions (Shiklomanov, 1999). The other 34% is left for domestic households (10%), industry (20%), and/or evaporated from reservoirs (4%) (Shiklomanov, 1999). It is therefore apparent that agriculture is the largest consumer of water and yet it is expected to meet the rising demand for food and industrial goods. However, drought in particular, causes a serious threat to sustainability of agricultural production. Drought is regarded as a major limitation to crop production in most developing countries and it occasionally causes agricultural losses in developed countries (Ceccarelli and Grando, 1996). Since South Africa is a water-stressed country (Bennie and Hensley, 2001), production of crops that are drought-tolerant is a priority in order to meet the growing demand for food and nutrition. Cowpea has a potential to meet both needs – the crop has been reported to be drought tolerant as well as being nutritious (Table 1.1).

Information on cowpea's agronomy and water use is lacking in South Africa, thus, its production is very low. The lack of information may be attributed to the fact that limited research has been conducted on the crop. Most of traditional research has favoured more established legumes such as dry bean (*Phaseolus vulgaris*) and peas (*Pisum sativum*), while cowpea has been left neglected and underutilised. A renewed interest to revisit these neglected and underutilised crops has recently been launched, where these crops need to be evaluated for possible drought tolerance in order to generate information that may be used to promote and encourage their re-introduction.

The aim of the current study was to evaluate two cowpea varieties for their ability to tolerate drought stress. To a limited extent, the two cultivars were also evaluated within the context of their alternative use as a leafy vegetable. Given that South Africa is a water-stressed country where rainfall is poorly distributed (Bennie and Hensley, 2001), it is hypothesized that there are no significant differences between the two varieties in terms of drought tolerance characteristics during plant growth, and drought tolerance is not associated with crop leaf yield and nutritional value at different stages of development.

The specific objectives of the study, over two growing seasons, were to:

(i) compare irrigated production with rainfed production in terms of crop stand establishment,

(ii) compare irrigated production with rainfed production in terms of crop growth as determined by plant height, leaf number and leaf area index,

(iii) compare irrigated production with rainfed production in terms of crop response to water stress as indicated by stomatal conductance and leaf chlorophyll content [these data will be correlated to (i) and (ii) above],

(iv) compare irrigated production with rainfed production in terms of leaf sequential harvesting, crop harvest index and economic yield, and

(v) determine changes in soil water content during each growing season and correlate them with crop growth responses.

In addition to field studies described above, controlled environment studies were undertaken to determine crop growth and yield responses to water stress under different temperature conditions.

1.1 LITERATURE REVIEW

1.1.1 Cowpea origin and diversity

Vigna unguiculata is known by different names throughout the world. In the United States, the crop is called black-eye peas or southern peas (Vorster *et al.,* 2002). In South Africa the crop is known as “swartbekboon” (Afrikaans), “dinawe” (Ndebele), “dinaba” (Shangaan), “imbumba” (Zulu), “intlumayo” (Xhosa), “dinawa” (Tswana and Sotho), “monawa” (Pedi) and “nawa”(Venda) (van Rensburg *et al.*, 2007). The English speaking people in Africa refer to it as cowpea while in other African regions; the name “niebe” is mostly used. In Senegal it is called “sueb” and “niao”, “wake” in Nigeria and “luba hilu” in Sudan. The predominant name used in the literature though is “cowpea” (Van Wyk and Gericke, 2000). Cowpea is a legume crop that belongs to *the Fabacea* earlier known as *Leguminosae* family and is also a member of the genus *Vigna*, which belongs to the family *Phaseolinae* and section Catiang (Verdcourt, 1970). The family consists of other legumes, namely mungbean (*V. radiata*), blackgram (*V. mugo*), adzuki bean (*V. angularis*) and bambara groundnut (*V. subterranea*) (van Rensburg *et al*., 2007).

Cowpea is one of the oldest crops known to man with its centre of origin and domestication being closely related to pearl millet and sorghum in Africa. The exact centre of origin remains unknown. However, botanical and cytological evidence have been used to trace the origins of the crop. Information on its geographical distribution, cultural practices and historical records has also been used to speculate on its origin (Ng and Marechal, 1985). Duke (1981) concluded that cowpea originated and was domesticated in the African savanna. The author based his conclusion on the fact that wild *V. unguiculata* and *V. unguiculata* sub-species were found in southern and south-eastern Africa, whereas the centre and diversity of the cultivated *V. unguiculata* is in West Africa (Ng, 1995).

1.1.2 Crop description

Cowpea is a warm season, annual, herbaceous legume characterized as erect, semi-erect (trailing) and climbing plant. It follows an epigeal emergence pattern which makes it prone to seedling injury especially when the seed bed is not firm (Shiringani, 2007). There is high variability within species, in terms of growth habits. The growth habits range from indeterminate to moderately determinate and non-vining types are more determinate. It has a long taproot, reaching a maximum effective rooting depth of about 2.4 m within eight weeks after planting, especially if drought conditions prevail. The leaves are trifoliate with a smooth surface, dull to shiny and develop alternately (Davis *et al*., 1991). Cowpea has a vigorous growth and can reach a height of about 48 - 61 cm when growing conditions are suitable. Early or late planting may lead to the crop having elongated internodes, more vegetative growth and lower yield than those planted at optimum time (Davis *et al*., 1991)

Cowpea seeds differ in size, and a single pod can contain about 10 - 20 seeds. Seed shape is used as the major characteristic associated with seed development in the pod. Initially, the seed develops into a kidney shape; when the pod is not restrictive, the seed maintains that shape until maturity (Gomez, 2004). But the pod has the tendency of restricting seed shape to a more globular shape. The seed coat can be smooth or wrinkled. Seed colour varies from white, cream, brown, red and black and it is not restricted to uniform colours, they can be speckled, mottled, and blotchy or eyed (black eye, pink eye, purple eye) (Aeling, 1999).

Like most other legumes, cowpea is self-pollinated and is a typical day neutral plant; it may flower within 30 days after sowing when temperatures are around 30°C. Whereas, other photosensitive varieties would flower at approximately 100 days after planting as influenced by time and location. Flowers are borne in alternate pairs at the tip of the branches, and two or more flowers can be found per inflorescence (Gomez, 2004). Flowers are borne on short pedicels with corollas that are either white, dirty yellow, pink, pale blue or purple and are displayed above the foliage such that they can attract insects for pollination. The plant produces smooth, cylindrical and curved pods. As the seeds approach the green-mature stage for use as a vegetable, pod colour may change, most commonly green, yellow or purple. As the seeds dry up, pod colour of the green and yellow types becomes tan or brown (Aeling, 1999).

1.1.3 Agronomic requirements

Cowpea is adapted to different soil types; it has been observed to grow well in sandy soils where root growth is not restricted (DAFF, 2011). It can survive under infertile acid soils but it is reported to be less tolerant to cold soils (DAFF, 2011). The crop requires well-drained soils with a pH of 5.6 - 6.0, but can still produce reasonable yield in waterlogged and heavy soils (Smith, 2006). The optimum rainfall conditions for cowpea range from 400 to 700 mm per annum (Smith, 2006). It is important that the rainfall is well–distributed for normal growth and development. Since South Africa is faced with a problem of uneven rainfall, this may have negative consequences on cowpea growth and yield.

1.2 Importance of cowpea for food security

Food security is defined by Parnell and Smith (2008) as the ability of an individual to access enough food that is nutritious, safe, and personally acceptable in a socially acceptable way. According to Schönfeldt and Pretorius (2011), food insecurity is one of the main causes of malnutrition in South Africa, and statistics shows that one out of two households is experiencing hunger. Since South Africa has the ability to import food, if necessary, to meet basic nutritional requirements of its population (DAFF, 2011), it has been considered as a food secure nation. However, this may not be particularly true since food imports are often expensive and therefore unaffordable to the general populace (Laker, 2007). In 2005, it was reported that one third of South Africans were at risk of hunger and that one out of every five people was food insecure (NFCS-FB-1, 2008; Schonfeldt *et al.*, 2010) and these results give us a clear picture of the situation on the ground.

According to El-Jasser (2011), South Africa is not the only country facing food insecurity, about sixty percent of the world population suffers from food insecurity and this consequently leads to protein malnutrition. Current trends also suggest an increasing gap between human population and protein supply (El-Jasser, 2011). Research studies have been devoted to finding the potential of legumes to curb this problem since they are still not widely used in diets of many populations. Legumes form an essential part of daily diets in many countries. They are a rich source of proteins and carbohydrates that are essential to man. Legumes are becoming popular nowadays because they have been found to be ideal crops that can be consumed and achieve three developmental goals for the targeted population These goals are; reducing poverty, improving human health and nutrition and enhancing ecosystem resilience (Akibode and Maredia, 2011). Of all the domesticated legumes, soybean had a competitive advantage over other legume seeds since it has been given more attention as a protein crop. It is only recent that researchers decided to explore other underutilised legumes such as cowpea (El-Jasser, 2011).

Cowpea is treated as a staple food crop in many regions of Africa (Keller, 2004). It is the most important food legume grown in the tropical savanna zones of Africa. Cowpea is considered as a neglected crop due to limited research and improvement on its potential use as a leafy vegetable. Neglected as it may be, the crop is still one of the highly appreciated crops in several African countries (Keller, 2004; Weinberger and Msuya, 2004). Farmers in drought-prone areas, with less rainfall, and less developed irrigation systems are becoming more interested in cowpea cultivation due to its multipurpose uses. The short life cycle of some varieties are priced for their ability to mature early thus provide food during periods of food scarcity.

Cowpea plays a very important subsistence role in diets of many households in Africa (Kebe and Sembene, 2011). It provides nutrients that are deficient in cereal crops, e.g. iron, calcium and zinc. The seeds contain proteins that are rich in amino acids, lysine and tryptophan, but they lack methionine and cystine when compared with animal protein (Table 1.1). Cowpea seed is therefore, valued as a nutritional supplement to cereals. Combining cowpea with a cereal crop, e.g. rice or maize meal, one can make food with a near-complete or a balanced set of nutrients (Davis *et al.*, 1991).

Table 1.1: Chemical composition of cowpea (%). Sources: Kay (1979); Tindall (1983); Quass (1995); FAO (2004).

	Seeds	Hay	Leaves
Carbohydrates	56-66	-	8
Proteins	22-24	-	4.7
Water	11	18	85
Crude fibre	5.9-7.3	9.6	2
Ash	3.4-3.9	23.3	-
Fat	1.3-1.5	11.3	0.3
Phosphorus	0.146	2.6	0.063
Calcium	0.104-0.076	-	0.256
Iron	0.005	-	0.005

In Senegal, people harvest near-mature green pods of early traditional cowpea varieties at the end of the wet season, and this provides them with food during the time of the year when food becomes extremely scarce (Kebe and Sembene, 2011). With these green pods, subsistence farmers and street vendors even get an opportunity to sell and get cash allowing them to buy other staple food crops such as pearl millet or imported rice. The above mentioned cowpea attributes makes it to be an attractive crop in areas where infrastructure, food security, and diminishing malnutrition are major challenges (Hallensleben *et al.*, 2009).

1.3 Drought

Drought is a meteorological term used to define the period in which there is no considerable rainfall. In the context of crop production, drought refers to a lack of sufficient water in the soil to support normal plant growth (Jaleel *et al.*, 2009). It occurs when available water in the soil is reduced and the atmospheric conditions cause continuous loss of water through transpiration and evaporation. This may be as a result of meteorological drought, uneven rainfall distribution or even inefficient irrigation systems. Plants are able to adapt and survive under drought stress through morphological, biochemical and physiological responses. Mitra (2001) suggested three categories of drought tolerance mechanisms in plants based on earlier descriptions by Levitt (1972), namely: escape, avoidance and tolerance.

1.3.1 Drought escape

Drought escape is when the plant grows rapidly to shorten its life cycle and reproduce before drought stress becomes terminal. This mechanism is closely linked with time to flowering as it allows the plant to escape drought through a short life cycle (Araus *et al.*, 2002). Drought escape makes time to flowering a major trait for crop adaptation to drought stress. It has been proven that early maturing cowpea varieties are very useful in some dry environments due to their ability to escape drought (Singh, 1994). Mortimore (1997) reported that there has been a shift towards growing early maturing cowpea varieties especially in drought prone areas. In a study by Suliman and Ahmed (2010), cowpea varieties grown under water stressed conditions flowered 1-15 days earlier than those grown under well watered conditions. Water stress had a similar effect even on days to maturity, whereby water stressed cowpea matured faster than the control treatment. However, not all cowpea genotypes responded the same way to water stress with respect to maturity. Dadson *et al.* (2005) found that water stress delayed the maturity of certain cowpea genotypes.

1.3.2 Drought avoidance

Drought avoidance is made up of mechanisms that reduce water loss from plants. These mechanisms consist of stomatal control and enhanced water uptake through a broad and prolific root system (Turner *et al.*, 2001; Kavar *et al.*, 2007). The major drought avoidance traits include the root characteristics such as biomass, length, density and depth. In addition to enhanced soil water capture, plants also avoid stress by reducing the size of their canopy. For example, reduced plant size, leaf area and leaf area index are major mechanisms controlling water use and reducing injury under drought stress (Mitchell *et al.*, 1998). These traits allow plants to reduce water use in order to avoid drought stress. According to Lawan (1983), cowpea is a dehydration avoider with strong stomatal sensitivity and reduced growth rate. Separate studies by Lawan (1983) and Boyer (1996) both suggested that possible mechanisms of drought tolerance in cowpea include stomatal closure to minimize water loss through transpiration, cessation of growth and osmotic adjustment and continued slow growth. Mai-Kodomi *et al.* (1999) studied drought tolerance of different cowpea lines which revealed that susceptible lines had a mechanism whereby plants closed stomata to minimize water loss and stopped growing in response to water deficit, whereas tolerant lines had a mechanism that allowed them to have stomatal regulation (partial opening), osmotic regulation and selective mobilization of water to the growing tips and upper leaves.

1.3.3 Drought tolerance

Drought tolerance is the capacity of the plant to maintain or conserve plant function under water deficit conditions. This mechanism is rarely found in crop plants; it usually exists in seed embryo but is eventually lost after germination (Turner *et al.*, 2001; Kavar *et al.*, 2007). Cowpea has not been reported to posses this drought resistant mechanism.

1.4 Effects of water stress on crop growth and development

1.4.1 Crop establishment and growth

Impaired germination and poor stand establishment are the first and foremost effects of drought (Harris *et al.*, 2002). Cell growth is one of the most drought sensitive physiological processes caused by reduction in turgor pressure. When water deficit is high, cell elongation is inhibited due to disturbance of water flow from xylem to surrounding elongating cells (Nonami, 1998). Observations on soybean plants revealed that stem length decreased significantly under water deficit conditions (Specht *et al.,* 2001). In other plants, including potato (Heuer and Nadler, 1995), *Abelmoschus esculentus* (Sankar *et al.*, 2007), soybean (Zhang *et al.*, 2004) and parsley (Petropoulos *et al.,* 2008), it was reported that stem length was significantly reduced by water stress. Other growth parameters affected by water deficit include leaf area expansion which depends mostly on leaf turgor, temperature and assimilate supply. Development of optimum leaf area is important for photosynthesis and dry matter yield. It is common that water stress causes a reduction in fresh and dry biomass production (Zhao *et al.*, 2006). Studies on many plants like populous (Wullschleger *et al.*, 2005), soybean (Zhang *et al.*, 2004), and several others (Farooq *et al.,* 2009), showed that water stress reduced leaf growth and consequently reduce leaf area.

Khan *et al.* (2001) conducted a study with six irrigation levels in maize where it was reported that plant height; stem diameter and leaf area decreased significantly with increasing water deficit.

1.4.2 Photosynthesis

Photosynthesis is highly affected by drought due to a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and reduction in dry matter production (Wahid and Rasul, 2005). Drought stress reduces gaseous exchange due to stomatal closure hence limiting the amount of CO_2 entering the leaves, which reduces photosynthesis. Anjum *et al.* (2011) observed a significant decline in net photosynthesis (33%), transpiration rate (38%), stomatal conductance (25%), water use efficiency (51%) and intracellular CO_2 (6%) in water stressed maize compared with well-watered plants. A combination of stomatal and non-stomatal limitations was shown to decrease photosynthetic activity under drought stress (Ahmadi, 1998; Del Blanco *et al.*, 2000; Samarah *et al.*, 2009). Farooq *et al.* (2008) however, reported small limitations to photosynthesis due to stomatal mechanisms as compared with non-stomatal mechanisms. Stomatal limitations refer to closure of stomata in response to water stress and is one of the first responses to drought stress often resulting in a decreased rate of photosynthesis. Non-stomatal limitations on the other hand include changes in chlorophyll synthesis, functional and structural changes in chloroplast, and interruption in processes of accumulation, transport, and distribution of assimilates (Farooq *et al.*, 2008).

Important photosynthetic pigments such as Chlorophyll a and b and carotenoids are affected by drought stress (Anjum *et al.*, 2003). Fu and Huangi (2001) reported that water stress damaged photosynthetic apparatus, decreased activities of Calvin cycle enzymes, and reduced crop yield (Monakhova and Chernyadev, 2002). The ratio of the photosynthetic pigments was also changed by drought stress (Anjum *et al.*, 2003; Farooq *et al.*, 2009). Sunflower plants recorded a significantly decreased chlorophyll content at high water deficit in (Kiani *et al.*, 2008). Similar results were reported by Manivannan *et al.* (2007) where chlorophylls a and b, and total chlorophyll content of sunflower declined significantly in response to water stress. According to Anjum *et al.* (2011) loss of chlorophyll content under water stress is considered as the main cause of inactivation of photosynthesis Photosynthetic ability is also affected by the loss of balance between the production of reactive oxygen species and antioxidant defence (Reddy *et al*., 2004). This results in accumulation of reactive oxygen species which encourages oxidative stress in proteins, membrane lipids and other cellular components.

1.4.3 Physiological responses

In addition to morphological aspects, water stress also affects the physiology of plants. At the physiological level, plants respond to water stress through osmoregulation and osmotic adjustment, by accumulating metabolites, increasing protein synthesis as well as accumulating both enzymatic and non-enzymatic antioxidants (Blokhina *et al*., 2003). Osmoregulation is whereby the salt concentration in the cell is monitored in such a way that when there is low or high water potential within the cell, the cell allows for addition or removal of salts from the cell sap until the intracellular osmotic potential is nearly equal to the potential of the surrounding cells. Plants adapt to water stress through osmotic adjustment - by reduction of water potential through increased accumulation of solutes in the cell in response to water deficit or salinity (Anjum *et al*., 2011).

Osmotic adjustment involves accumulation of one or more low molecular weight organic solutes known as compatible osmolytes (Naidu *et al*., 1992). Osmolytes play a vital role in counteracting effects of water deficit. Proline is one such compatible osmolyte mainly found in water stressed plants (Yoshiba *et al.,* 1997). Proline accumulation is believed to be a universal plant response to environmental stresses such as water stress, salt stress, extreme temperatures, and high light intensity. It has been speculated that proline accumulation could be a possible mechanism for plant survival during drought stress (Nojaphy *et al.,* 2010). However, questions have been raised on the role of proline as an osmotic regulator (Aspinall and Paleg, 1981). It has been suggested that proline function as an osmotic regulator, a protector of enzyme denaturation, a reservoir of nitrogen and carbon as well as a stabiliser of protein synthesis machinery (Ibarra-Caballero *et al*., 1988).

Studies in higher plants have demonstrated that proline accumulates when plants are exposed to environmental stresses. Proline content increased in maize varieties in response to water deficit (Mohammadkhani and Heidari, 2008). Decreasing soil water potential to -1.76 MPa, resulted to increased root proline content. In maize primary roots, proline levels can increase by a hundred fold when the crop is subjected to low water potential (Voetberg and Sharp 1991). Verslues and Sharp (1999) also reported evidence of proline transport to the root tips while the plant was water stressed. Two pathways were observed to account for accumulation of proline in plants under stress: increased expression of proline synthesis enzymes and suppressed activity of proline degradation (Delauney and Verma, 1993; Peng *et al.*, 1996). Continuous water deficit caused a significant increase in proline content in leaves during post-anthesis stage. Despite the differences ascribed to the role of proline accumulation in plants, proline has emerged as a suitable index in drought selection studies.

1.4.4 Oxidative damage

Plants respond to biotic and abiotic stresses through generation of reactive oxygen species (ROS) namely: superoxide anion radicals (O_2^-) hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), alkoxy radicals (RO) and singlet oxygen (O_2^1) (Munne-Bosch and Penuelas, 2003). Reactive oxygen species are normally produced as by-products of normal oxygen metabolism and have an important role in cell signalling. When plants are water stressed, the level of ROS increase considerably resulting in oxidative damage to proteins, DNA and lipids (Apel and Hirth, 2004).

Reactive oxygen species are highly reactive and cause serious damage to plants by increasing lipid peroxidation, protein degradation, fragmentation and ultimately, cell death. Over-production of ROS is associated with accumulation of malondialdehyde (MDA); as such, MDA content has been considered as an indicator of oxidative damage (Moller *et al.*, 2007). Blokhina *et al.* (2003) reported that reactive oxygen species caused deleterious effects and their production was stimulated under drought stress. Generally, there is a linear relationship between ROS production and severity of drought stress; this leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids and both structural and functional proteins. Literature was num in terms of research done on cowpea with respect to these physiological responses to water stress.

1.4.5 Antioxidants

Reactive oxygen species are always produced by higher plants in organelles such as mitochondria, chloroplast and peroxisomes. The equilibrium between production and scavenging of ROS is highly affected by environmental stresses such as drought, UV radiation, heavy metals, temperature extremes, and nutrient deficiencies. When the environmental conditions are favourable, ROS molecules are scavenged by different antioxidant mechanisms (Foyer and Noctor, 2005) (Figure 1.2).

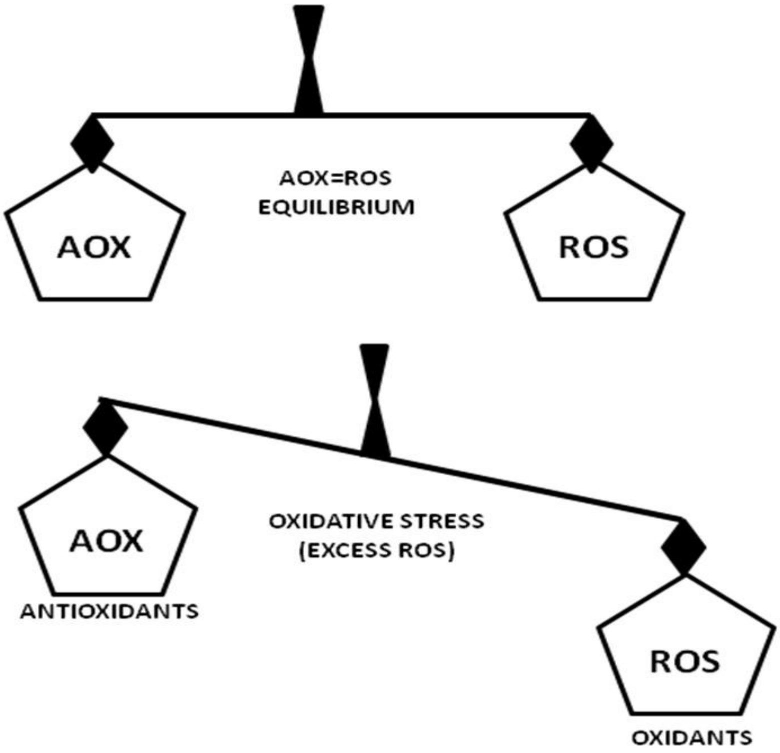

Figure 1.2. The equilibrium between Reactive Oxygen Species and Antioxidants. (Foyer and Noctor, 2005).

Plants prevent the severity of damage caused by environmental stresses through activation of complex antioxidant system which aid to detoxify ROS. These systems include low molecular mass antioxidants and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and enzymes involved in ascorbate glutathione cycle (Foyer and Helliwell, 1976).

In the context of drought stress, the production of reactive oxygen species is directly proportional to the severity of drought stress (Farooq *et al*., 2009). This then enhance the peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins. This also affects the various organelles found in the plant cells such as chloroplasts, mitochondria and peroxomes (Farooq *et al*., 2009).

1.5 Effect of drought on yield

The main purpose of growing crops is to obtain high harvestable yield. Under water stress, crops show great differences in harvestable yield (Jaleel *et al*., 2009). Water stress is one of the most common environmental stresses known to affect plant growth and development (Aslam *et al.,* 2006). A lot of research has been done concerning drought; however, it still remains a challenge to agricultural scientists, in general, and to plant breeders in particular. Drought can be regarded as a permanent constraint to agricultural production especially in developing countries and occasionally causes losses to agricultural production in developed countries (Ceccarelli and Grando, 1996).

The severe effect of drought stress on grain yield occurs when water stress coincides with the reproductive stage (Thomas, 1997). Cell division, expansion and enlargement facilitate growth, at the same time they are very sensitive to drought stress. Drought stress reduces leaf production and expansion this consequently leads to leaf senescence and abscission (Karamonos, 1980). As a result of limited leaf production, leaf area is reduced and thus, reduced biomass accumulation. Since seed production is positively correlated with leaf area, it becomes imperative that reduction in leaf area would result in reduced seed production (Rawson and Turner, 1982).

Yield is obtained through an interaction of many processes that occur during plant growth and development (Anjum *et al.,* 2011). Exposure of plants to water stress compromises the ability of the plant to express yield traits. This occurs primarily due to the disruption of leaf gas exchange which results in loss of harvestable yield. Impaired leaf gas exchange limits the size of the source and sink tissue, phloem loading, assimilate translocation and dry matter partitioning (Farooq *et al.,* 2009). Water stress also inhibits dry matter production through its inhibitory effect on leaf expansion and consequently reduced light interception (Nam *et al.,* 1998).

Great differences occur in different plant species in relation to yield reduction under drought stress (Figure 1.3). If drought stress is imposed during reproductive phase, it becomes very critical because it affects the partitioning of dry matter to the sinks. Anjum *et al.* (2011) observed a substantial reduction in yield and yield components such as kernel rows/cob, kernel number/ row, 100 kernel mass, kernels/cob, grain yield/plant, biological yield/plant and harvest index, when stress was imposed at the tasseling stage of maize growth. Grain yield of maize was found to be greatly reduced by drought stress and this was related to the level of defoliation that occurred in response to water stress at reproductive stage (Kamara *et al.*, 2003; Monneveux *et al.*, 2006).

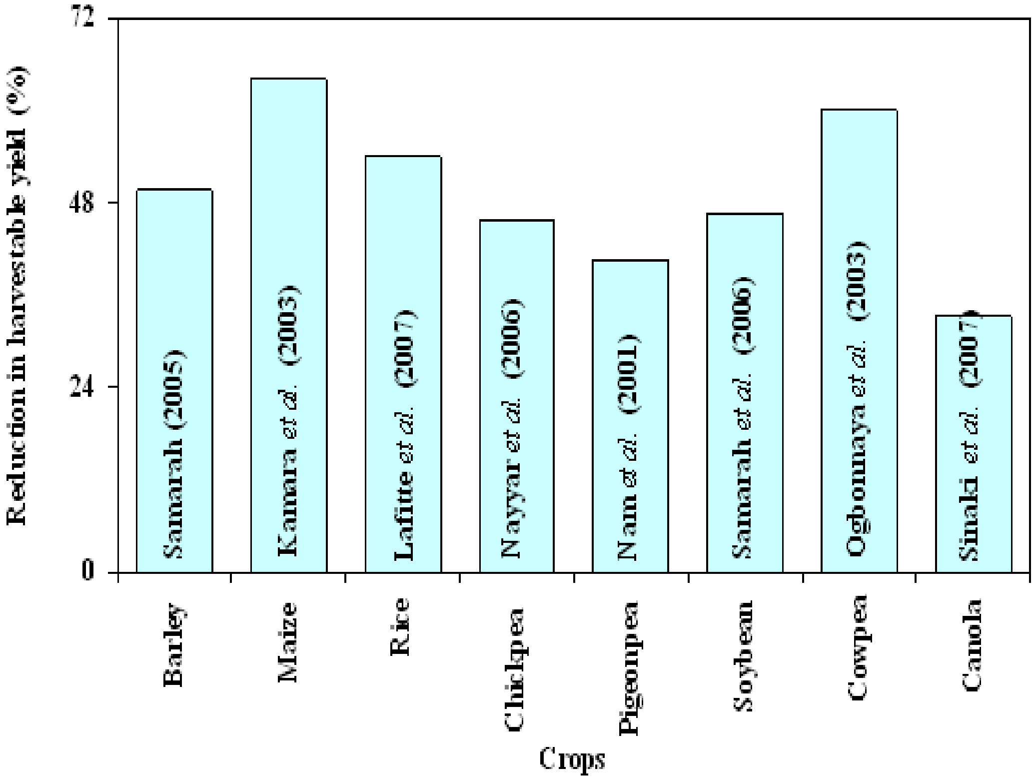

Figure 1.3: Loss of harvestable yield in different crop species when water stress is imposed at reproductive stage. (Jaleel *et al.*, 2009).

Qasem and Biftu, (2010) studied the yield response of cowpea under low water potential and they reported that yield was reduced through decreased pod size and number of seeds per plant .Another study by Suliman and Ahmed (2010) indicated that cowpea seed yield was significantly reduced by water stress. These authors also confirmed the findings of

Qasem and Bift (2010) above that loss in seed yield was due to significant reduction in yield components such as number of pods/plant, number of seed/pod and seed mass.

1.6 Vegetative harvesting

Cowpea can be consumed at various stages of its development, either as green leaves, green pods, green peas or dry grain (Ibrahim *et al*., 2010). In many parts of Africa, cowpea leaves and shoots are consumed as leafy vegetables and as an alternative for spinach (Onwueme and Sinha, 1991). The leaves can be harvested as an alternative to conventional seed harvest and could increase productivity while minimizing waste (Maeda, 1985). Cowpea plants allow for harvesting of leaves and seeds from the same plant but this may reduce harvest index (Ohler *et al*., 1996). Advantages of cowpea vegetative harvesting also extend to improving dietary nutrition since cowpea leaf protein can complement that of cereal grains (Maeda, 1985).

Cowpea leaves have a significant amount of nutritional value, however, this has been ignored due to high water content in leaves and it has been hard to document their production and consumption (Ahenkora *et al.,* 1998). Cowpea leaves provide a good source of amino acids, vitamins, minerals and proteins with a higher nitrogen content in the younger leaves (Ahenkora *et al.,* 1998). The leaves contain much higher protein content than the seeds. It was reported that protein content found in leaves is about 15 times than that found on mature dry seeds and this is because leaves are produced earlier and in much greater quantity than seeds. Protein content in cowpea leaf ranges from 29% to 43%, however these amounts decrease with increasing leaf age (Nielson *et al.,* 1993).

Timing of commencement of leaf harvesting is very important for final yield determination (Matikiti *et al.,* 2009) as it affects the plants' ability to recover from defoliation (Berrett, 1987). Bubenhein *et al*. (1990) initially suggested that cowpea should be strictly grown for either leaves or seeds but not both. However, recent research has shown that by using systematic approaches, both leaves and seeds can be harvested from the same crop. Matikiti *et al*. (2009) concluded that in order to obtain maximum grain yield, leaf harvesting should not be done from two weeks after crop emergence (WACE) up to 8 WACE. They also found that 75% of potential grain yield can be obtained if leaf harvesting is performed from 2 to 3 WACE or from 7 to 8 WACE. Conflicting results regarding the effect of leaf harvesting on seed yield were reported by Bittenbender *et al*., (1984) and they can be attributed to cultivar differences. It was reported that some cultivars are adversely affected by leaf harvesting while others are not. According to Wien and Tays

(1978), determinate types suffer more reduction in seed yield than indeterminate cowpea types following defoliation.

There was a paucity of information in literature describing the effect of water stress on leaf harvesting and seed yield of cowpea. Most literature describes leaf harvesting and seed yield under optimum conditions. However, since cowpea has been touted as a drought tolerant crop with potential for production in marginal areas; it is imperative that information relating to leaf harvesting and seed yield be generated. Therefore, one objective of this study was to determine the effect of water stress, under field conditions, on leaf harvesting and seed yield of two cowpea varieties.

Conclusions

Availability of water is a major limiting factor to crop production and ensuring food security in Africa as a whole. South Africa is a water stressed country characterised by high temperatures and low relative humidity which often result in uneven rainfall distribution as well as very high potential evapo-transpiration (Bennie and Hensley, 2001). This creates a big challenge for crop production and feeding the ever-growing population. Therefore, in order to address this issue it is important that researchers seek to explore ideal crops that can help in achieving the three developmental goals which are; reducing poverty, improving human health and nutrition and enhancing ecosystem resilience. Cowpea is one of the ideal crops that can be exploited in order to achieve these goals since it has been reported to be drought tolerant.

The challenges of an ever-growing human population and climate change have aroused interest in researchers to seek more information about neglected underutilised crops. Cowpea is an underutilised crop with potential drought tolerance. In addition to drought tolerance, cowpea has proven to have a great potential for multiple uses: human food and livestock feed. As a result, cowpea can play a vital role in improving food security, especially in dry arid areas. The crop can be grown to feed people while they are waiting for their staple crops to mature. Cowpeas can also be used as a rotation crop due to beneficial roles of nitrogen fixation associated with legumes.

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CHAPTER 2

EVALUATING SEED QUALITY OF COWPEA

2.1 Introduction

Cowpea (*Vigna unguiculata*) is considered as one of the most important legumes, especially in hot, dry tropics and subtropics in Sub-Saharan Africa (Ogunkamni, 2006). It is commonly cultivated in areas where water stress is the major constraint to crop production (Santos, 2000). It is used mainly as grain (immature or dried), green leaves and for animal feed (Uarrota, 2010). Cowpea is rich in proteins that are readily available and of high biological value due to high levels of lysine and tryptophan (Santos, 2000). As such, the crop has potential to contribute towards food security and improve diets of people living in marginal areas of agricultural production. Unfortunately, there is limited information describing aspects of cowpea growth, seed quality in particular and the crop receives a small fraction of research attention in comparison with other established legumes such as common beans and soybeans. Due to this and despite its potential, the crop is classified as a neglected and underutilised crop.

Seeds are very important in crop production for both commercial and subsistence farmers as they are the first input in crop production. However, seeds encounter different environmental conditions in the field such as temperature, water stress, photoperiod and soil fertility that may affect their ability to germinate successfully (Mbatha, 2010). Therefore, it is important that seeds be of high quality in order to establish successfully under often hostile seedbed conditions. As a result, seed quality can be described as the most important parameter in crop production (Salisbury & Ross, 1991; Bewley & Black, 1994).

High quality seeds are characterised by genetic stability, uniform and rapid germination, high seed vigour and freedom from pests and diseases (Balkaya *et al*., 2004). Germination capacity and physiological vigour are the two most important factors required for high seed quality (Odindo, 2007). According to Association of Official Seed Scientists (AOSA) (1983), germination capacity is the ability to germinate and produce a normal seedling. On the other hand, seed vigour is an indication of a seed's ability to emerge and form a uniform stand under a wide range of environmental conditions. It provides a clear understanding of the seed's physiological quality, which is defined as genetic purity and constitution of the seed (Odindo, 2007). Seed quality is affected by several external (environmental) and internal (physiological and genetic) factors which occur during seed development on the mother plant, harvest operations and storage (Tekrony, 2003; Powell *et al.*, 2005). Seed quality has been shown to be affected by drought stress (Pervez *et al.*, 2009).

South Africa is water limited and frequent droughts that occur can potentially affect seed quality of cowpea. However, plant response to drought stress, in terms of seed quality, is variable; this can be attributed to different physiological mechanisms that plants posses. Sorghum seeds exposed to mild water stress showed significantly higher germination than those grown under normal conditions (Benech-Arnold *et al.*, 1991). Similarly, water stress imposed during seed development in soybean resulted in reduced seed yield; however, germination and vigour were not affected (Vieira *et al.*, 1992). Drought stress has been shown to affect seedling establishment (Pervez *et al.*, 2009). It was reported that water stress delayed the onset and reduced the rate and uniformity of germination, leading to poor crop performance and yield (Demir *et al.*, 2006). On the other hand, inherent seed traits such as seed colour have also been shown to affect seed quality.

Seed coat colour has been reported as one of several factors affecting seed quality (Pederson and Toy, 2001; Odindo, 2007; Mabhaudhi, 2009; Mbatha, 2010; Zulu, 2010; Sinefu, 2011). Work done by Powell (1986) and Ouveira and Matthews (1986a) showed that light coloured bean seeds succumbed to imbibitional injury as a result of rapid imbibition. This often led to high electrolyte leakage and tissue death and this could explain why they also observed poor emergence in light coloured seeds under field condition. On the other hand, dark coloured seeds were observed to imbibe water relatively slower and had higher emergence under field conditions relative to the light coloured seeds (Powell, 1989). Similar effects of seed colour on seed quality have been reported in several legumes such as cowpea (Asiedu & Powell, 1998), long bean (Abdullah *et al.*, 1993), soybean (Mugnisjah *et al.*, 1987) and radicchio (*Cichorium intybus, L.*) (Pimpini *et al.*, 2002). There have also been cases whereby findings were contrary to this. Pederson and Toy (2001), after conducting warm germination and vigour tests on sorghum, concluded that seed quality could not be attributed to seed coat colour. However, there were few studies concurring with their findings in the literature.

Seed quality has beneficial effects on field performance of many crops including cowpea. It has been hypothesised that seed coat colour is not associated with seed quality of cowpea. Therefore, this research aimed at determining seed quality of two cowpea varieties with different seed coat colour (Brown and White) before they were used in further experiments in the study.

2.2 Materials and Methods

2.2.1 Plant material

Seeds of two cowpea varieties – White birch and Brown birch were sourced from a local seed supplier and used in the studies. The cultivar names were indicative of seed colour. Seeds were of medium size and seed size was uniform across both varieties.

Figure 2.1: Cowpea seed varieties used in the study (A = White birch variety and B = Brown birch variety).

2.2.2 Standard germination (SG) test

Germination capacity of the two cowpea varieties was assessed using the standard germination test (ISTA, 1996). The experiment was laid out in a completely randomised design were forty seeds of each variety were arranged between moistened double-layered paper towels, replicated four times. Thereafter, the paper towels were rolled and tied on other end with rubber bands and placed in zip-lock bags to prevent moisture loss. Following this, seeds were placed in a germination chamber set at 25°C and incubated for 8 days. Germination counts were taken daily until the eighth day to determine germination percentage. Germination was defined as radicle protrusion of 2 mm. After 8 days, seeds were taken out of the chamber, at which point seed vigour parameters of shoot length, root length, root:shoot ratio, fresh and dry mass were determined. Seedling dry mass was determined by oven drying seedlings at 80°C for 72 hours.

Carvalho and Nakagawa (1980) described Gemination Velocity Index (GVI) as the strength of the seed lot. Germination velocity index was calculated according to Maguire's (1962) formula:

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \qquad \textbf{Equation 2.1}$$

where:
GVI = germination velocity index
G1, G2…Gn = number of germinated seeds in first, second… last count.
N1, N2…Nn = number of sowing days at the first, second… last count.

Mean time to germination (MGT) was calculated according to the formula by Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n} \qquad \textbf{Equation 2.2}$$

where:
MGT= mean germination time,
n= the number of seed which were germinated on day D, and
D= number of days counted from the beginning of germination.

2.2.3 Electrolyte conductivity

The electrolyte conductivity (EC) of seeds was measured using the CM100-2 EC Meter (Reid & Associates, South Africa) to determine the amount of solute leakage. Briefly, 50 seeds of each variety (Brown and White birch) were initially weighed and put into wells. Thereafter, the wells were each filled with 2 ml of distilled water. Electrolyte conductivity of the seed was read over at hourly intervals over a period of 24 hours.

2.2.4 Data analysis

Data were analysed using analysis of variance (ANOVA) in GenStat® 14th Edition (VSN International, UK). Treatment means were separated using Duncan's Multiple Range Test in GenStat at the 5% level of significance.

2.3 Results

The ANOVA tables for the seed quality experiments are presented in Appendix 2.1. There were no significant differences ($P > 0.05$) between the two cowpea cultivars, with respect to germination (Figure 2.2). However, there were highly significant differences ($P < 0.001$) with regards to overall daily germination (Figure 2.2). Germination percentage increased rapidly with time, from day 1 to day 2, until maximum germination was reached on day three (3). Results showed that both cultivars were fast to germinate and attained maximum germination within the first three days.

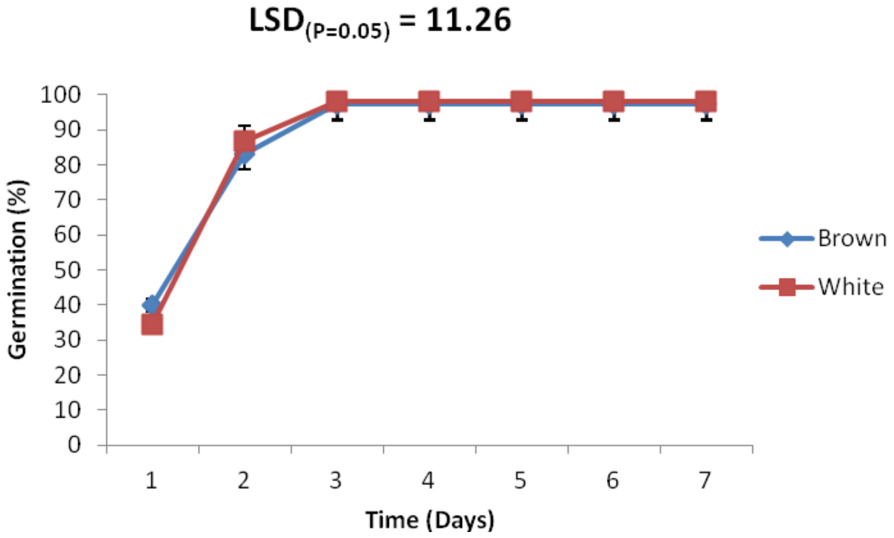

Figure 2.2: Daily germination percentage of two cowpea cultivars (Brown and White birch).

Results of parameters measured on the final day of the standard germination test (Table 2.1) showed no significant differences ($P > 0.05$) between the two cowpea varieties for fresh mass and root:shoot ratio (Table 2.1). However, dry mass, root length and shoot length showed highly significant differences ($P < 0.001$) between the two cowpea varieties (Table 2.1). The White birch variety had longer root and shoot; however, this did not translate to greater dry mass. Although Brown birch variety had shorter root and shoot, than the White birch variety, it had higher fresh and dry mass than White birch (Table 2.1). Although GVI and MGT were not statistically different ($P > 0.05$) across the varieties, White birch variety was shown to germinate faster (low MGT and high GVI) but had low fresh and dry mass.

Table 2.1: Seed performance of two varieties with respect to germination indices.

Variety	**^{x}GVI**	**^{y}MGT (Days)**	**Root length (cm)**	**Shoot length (cm)**	**Root:shoot Ratio**	**Fresh mass (g)**	**Dry mass (g)**
White	72.6a^{z}	4.22a	7.33a	12.62a	1.73a	1.21a	0.56a
Brown	59.2a	4.34a	5.31b	8.87b	1.67a	1.25a	0.76b
LSD$_{(P=0.05)}$	16.950	0.516	1.347	0.546	0.223	0.119	0.094

Note: ^{X}GVI = Germination velocity index; ^{y}MGT = Mean germination time; ^{z}Means followed by the same letter indicate that they were not significantly different ($p < 0.05$) from each other.

There were significant differences ($P < 0.05$) between cultivars with respect to EC (Figure 2.3). White birch had the highest EC relative to Brown birch; indicating that there was more solute leakage in white seeds than in brown seeds.

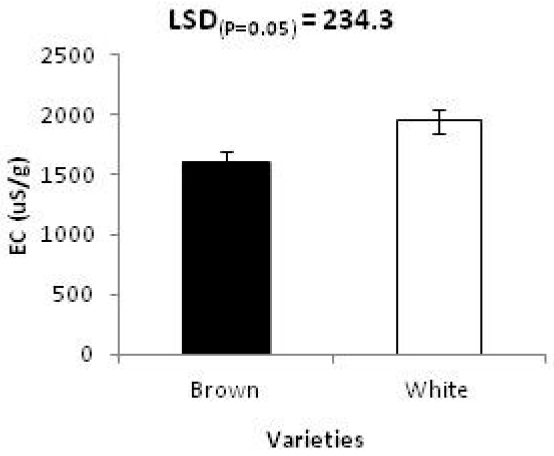

Figure 2.3: Electrical conductivity (uS/g) of the two cowpea varieties – Brown birch and White birch.

2.4 Discussion

The standard germination test is a common test used to measure seed viability. The observed results of this study showed no differences in seed viability between white and Brown birch. This could have been because seeds used were commercial varieties with similar seed characteristics, excluding seed coat colour. However, similar viability between the cultivars cannot be used to assume similar emergence rate (Heydecker, 1972; Perry, 1981). Germination vigour index of White birch was higher than that of Brown birch while the mean germination time was lower. This could have been attributed to differences in genetic make-up between the varieties. Therefore, within the context of this study, White birch had more strength and thus germinated faster.

The observed differences in root and shoot lengths would suggest that White birch seeds were more vigorous than brown seeds. However this did not translate to high fresh and dry mass. The brown seeds germinated slower but accumulated more dry mass which is an advantage in terms of seed vigour because the seed will have more assimilates required for further growth. These results concur with those of Mavi (2010) who reported that the seedling fresh and dry mass of brown water melon seeds was higher than that of light coloured water melon seeds. Dry mass production during germination can also be used as an indicator of high seedling vigour. Accumulation of dry mass indicates that there will be enough reserves for good seedling establishment. As such, the results indicated that the Brown birch was more vigorous than the White birch.

The observed differences in EC showed that seed coat colour is strongly linked with seed quality. The White birch germinated faster meaning that there was rapid water uptake which had a positive impact on germination rate. However, it was not good for seed quality. Rapid water uptake damages membrane cells as a result membranes lose their selective permeability thus permitting leaching of cytoplasmic metabolites into intercellular spaces (Mavi, 2010). Rapid water uptake also result in swelling damage; unpigmented seeds have been found to deteriorate faster and more susceptible to swelling damage than are pigmented seeds (Abdullah *et al.,* 1993; Asiedu and Powell, 1998).

Previous research has shown that differences in seed coat colour can be used as a quality indicator in seeds (Odindo, 2007; Mavi, 2010; Mbatha, 2010; Zulu, 2010; Sinefu, 2011). Mavi (2010) reported a correlation between seed coat colour and properties used to identify seed quality. Seed colour was found to influence germination capacity, emergence rate and seedling elongation in *Trifolium alexandruim* seeds (Daliania, 1980). Sinefu (2011) concluded that seed colour can be used for germplasm selection to grow under various conditions. Findings from the current study are in line with what has been reported by other researchers.

2.5 Conclusion

Results from this study allow us to conclude that seed coat colour in cowpea is associated with seed quality. Based on germination indices (GVI and MGT), it can be concluded that Brown birch had better seed quality than White birch. In addition, results of EC confirmed that light coloured seeds had lower seed quality as compared to dark coloured seed. As such, it may be expected that under field conditions, Brown birch may perform better than White birch, especially at the early establishment stage where seed quality plays a major role. However, more research is required to determine if superior seed quality (viability and vigour) of Brown birch would translate to yield advantages under field conditions. Furthermore, future research should also evaluate the physiological basis for the association between seed coat colour and seed quality.

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CHAPTER 3

COWPEA RESPONSE TO DIFFERENT WATER AND TEMPERATURE REGIMES UNDER CONTROLLED ENVIRONMENTAL CONDITION

3.1 Introduction

Water stress and high temperatures are the major growth limiting factors that plants encounter in semi-arid and arid environments. Although these factors usually occur concurrently, their effects on plant growth and development have often been studied as separate effects (Machado and Paulsen, 2001). Limited information exists in the literature relating to the combined effect of these environmental factors (Rizhsky *et al.,* 2004). Climate change, which will eventually increase global temperatures, has a potential to alter rainfall distribution and intensify drought in arid and semi-arid areas (Wigley and Raper, 2001; Chaves *et al*., 2003). Temperature or heat stress, is regarded as the increase in temperature beyond a threshold level for a period of time, enough to cause severe and irreversible damage to plant growth and development (Wahid *et al*., 2007). Generally, a sudden increase in temperature (e.g. 10 -15°C) above ambient, is considered heat shock or heat stress (Wahid *et al*., 2007). Heat stress has a significant negative effect on plant growth (Xu and Zhou, 2006) and poses a serious threat to crop production worldwide (Hall, 2001). Visible symptoms on plants under heat stress include overall leaf chlorosis, necrotic lesions and tip burning (Wahid *et al.,* 2007). Heat stress greatly affects both qualitative and quantitative characteristics of plants; thereby affecting both primary and secondary metabolic pathways (Wahid, 2007). These morphological changes result in a decline in net photosynthesis and consequently reduced photoassimilates; and out of the limited assimilates, a significant portion is used for stress acclimation mechanisms (Taiz and Zeiger, 2006; Wahid *et al.,* 2007). In order for plants to cope with heat stress, they undergo physiological changes and accumulate osmolytes like proline, soluble sugars, glycinebetaine and proteins (Wahid and Close, 2007).

Water stress affects plant growth starting from the cellular level where it inhibits cell division and enlargement (Jaleel *et al*., 2009). It disturbs the normal functioning of the plant with respect to physiological and biochemical processes which are responsible for plant survival and effective plant growth (Farooq *et al*., 2008; Jaleel *et al*., 2008). Experiments done on several crops have revealed that drought reduces plant growth parameters such as plant height (Heuer and Nadler, 1995; Spetch *et al*., 2001; Wu *et al*., 2008), leaf area, leaf number (Wullschleger *et al*., 2005) and biomass production (Farooq *et al*., 2009). It has been established that the initial growth stage is more sensitive to water stress than the subsequent growth stages (Anjum *et al*., 2003). Due to this reason it is important to recognize critical growth stages. Previous reports suggest that there are genotypic differences in the ability of cowpea [*Vigna unguiculata* (L.) Walp] to withstand drought at the vegetative stage (Watanabe *et al.,* 1997) and at flowering stage (Babalola, 1980). Ahmed and Suliman (2010) reported that the reproductive stage is the most sensitive to water deficit and concluded that cowpea can be subjected to water stress at vegetative growth and this would not significantly affect final seed yield.

Cowpea is an important legume known for its uses as a grain and fodder crop (Singh *et al*., 2003). There is limited information describing the combined effect of water and heat stress on cowpea. Moreover, the crop is grown in areas where water stress is often accompanied by high temperatures. As such, that should justify a study of this nature – an assessment of water stress is incomplete without an assessment of temperature stress. It is hypothesised that there is no interactive effect of water and heat stress on cowpea growth and productivity. As a result the objective of this study was to determine the combined effects of high temperatures and water stress imposed at different growth stages on growth and development of cowpea.

3.2 Materials and Methods

3.2.1 Plant material

Two cowpea varieties differing in terms of seed colour (Brown birch and White birch) were purchased from a local seed supplier, Capstone Seeds, in 2011, and used for the experiment. The varieties were classified as annual determinant legume types.

Figure 3.1: Cowpea seed varieties used in the study (A = White birch and B = Brown birch).

3.2.2 Glasshouse environment

A pot trial experiment was conducted in three controlled environment facilities at the University of KwaZulu-Natal's Phytotron Unit, Pietermaritzburg. Three environmental conditions were created in the glasshouse: (i) High temperature environment (33/27^{o}C day/night; natural daylength; 65% relative humidity), (ii) low temperature environment (21/15°C day/night; natural daylength; 65% relative humidity) and optimum environment (27/21°C day/night; natural daylength; 65% relative humidity). The optimum environment was selected to represent a typical warm sub-tropical climate to grow cowpea (Modi, 2007).

3.2.3 Experimental design, water stress treatments and potting procedure

The experiment was designed as a factorial experiment consisting of three factors: temperature, water regimes and cowpea varieties. There were three temperature environments (High, Optimum and Low) (Section 3.2.2). There were four water regimes: no stress (NS), terminal stress (TS), intermittent stress – vegetative (ISV) and intermittent stress - flowering (ISF). The no stress (NS) treatment involved watering the crop to 100% of crop water requirement. The vegetative intermittent stress (ISV) treatment involved establishing (seedling establishment) the crop at 100% of crop water requirement and then stressing the crop down to 30% of crop water requirement during the vegetative stage before watering it back up to 100% of crop water requirement at the onset of flowering. Flowering intermittent stress (ISF) involved establishing the crop at 100% of crop water requirement and maintaining it throughout the vegetative stage; the crop was only stressed down to 30% of crop water requirement at the onset of flowering. Terminal stress (TS) involved establishing the crop at 100% of crop water requirement; thereafter the crop was stressed down to 30% of crop water requirement for the entire duration of the crop cycle. The two cowpea varieties (Brown and White) were as described in Section 3.2.1. Therefore, the experimental layout was a split-split-plot with a treatment structure of 3*4*2, replicated three times. Whereby temperature was regarded as the main plot; water regimes was the sub-plot and variety sub-sub-plots.

The soil used for this study was collected from the same site where the field trial (Chapter 4) was conducted (Appendix 5). Seventy two (5 L) undrained pots were each filled with 3.5 kg of soil whose field capacity had previously been determined. Two seeds were planted per pot and later thinned to one plant per pot after seedling establishment. At planting, all pots were watered up to field capacity. Soil water content was monitored periodically using an ML-2x Theta probe connected to an HH2 handheld moisture meter (Delta-T Devices, UK). Irrigation scheduling in the pot trial was based on crop water requirement as described by Allen *et al.* (1998). Crop water requirement was calculated as follows:

$$ET_c = ET_o * K_c \qquad \textbf{Equation 3. 1}$$

Where: ET_c = crop water requirement,
ET_o = reference evoptranspiration obtained using the FAO-Penman Monteith method, and
K_c = crop factor obtained from Allen *et al.* (1998).

The irrigation was applied twice daily to meet daily crop water requirement.

3.2.4 Proline determination

Leaf material for determination of free proline content was sampled destructively from the plants in the high temperature (33/27°C) only. This was because at this stage the plants were not comparable, since the plants in other temperature environments stopped growing due to low temperatures. Proline content was then determined according to the method of Bates *et al.* (1973), with minor modifications. Leaf material was ground to a fine powder under nitrogen, mortar and pestle. Thereafter, 0.5 g leaf material was homogenised in 10 ml of 3% aqueous sulphosalicyclic acid. The homogenate was filtered through Whatman® No. 2 filter paper. About 2 ml of the filtrate was then put into a test tube to which 2 ml of glacial acetic acid and acid ninhydrin were added, respectively. The solution was heated in a boiling (100°C) water bath for 1 hour. Following this, the reaction mixture was placed in a cool water bath to terminate the reaction. The reaction mixture was extracted with 4 ml toluene and vortexed for 15 – 20 sec. The chromophere containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank. Proline concentration was calculated from the standard curve (Appendix 6) on a dry weight basis as follows:
[(µg proline/ml x ml toluene)/ (115 µg/µmole)]/ [(g sample)/5] = µmoles proline/g of dry weight material.

3.2.5 Data collection

Seedling emergence counts were performed daily after planting. Length and width of the first true leaf were measured during the establishment phase 21 DAP (days after planting). These measurements were then used to calculate leaf area non-destructively according to Lu *et al.* (2004). Leaf number and plant height were recorded weekly from 21 DAP. Leaf number was counted for leaves with at least 50% green area and height was measured from the base of the plant to the base of the upper most leaf. Plant growth parameters were measured until flowering. Chlorophyll content index and was determined weekly using a CCM-200 *Plus* (Optisciences, USA). It was measured from the abaxial and adaxial leaf surfaces, of the new leaf for the entire duration of the experiment. The plants growing at 33/27°C were the only plants to produce yield. As a result, when they reached maturity, the whole trial was harvested. Yield parameters (total biomass, pod number/plant, pod mass/plant, see number/pod, seed mass/plant and harvest index) at 33/27°C were measured at time of harvest and only total biomass was measured at 27/21°C and 21/15°C.

3.2.6 Data analysis

Data were analysed using analysis of variance (ANOVA) in GenStat® Version 14 (VSN International, UK). Means of significantly different variables were separated using least significant differences (LSD) at a probability level of 0.05.

3.3 Results

The ANOVA tables for the glasshouse experiment are presented in Appendix 3.1

3.3.1 Soil water content

There were highly significant (P<0.001) differences between temperature regimes with respect to soil water content (SWC). Soil water content in the high temperature (33/27°C) environment was 55.5% lower than in the optimum (27/21°C) and low (21/15°C) temperature environments (Figure 3.2). At 33/27°C, SWC was within the range of plant wilting point (PWP) and field capacity or even below the PWP for some water treatments (Figure 3.2); whereas at 27/21°C and 21/15°C, SWC was above field capacity. Differences between water treatments were found to be highly significant (P<0.001). The terminal stress treatment had the lowest (29.44%) followed by 32.35% (ISV), 34.59% (NS) and 35.77% (ISF). There were significant (P<0.05) differences between varieties: pots planted with Brown birch had high SWC than those planted with White birch. The interaction between temperature and water regimes was highly significant (P<0.001). Although water treatments were uniform across all temperature environments, the rate of water loss in the soil differed significantly. At 33/27°C, the rate of water loss was high compared to 27/21°C and 21/15°C where water loss was very minimal (Figure 3.2).

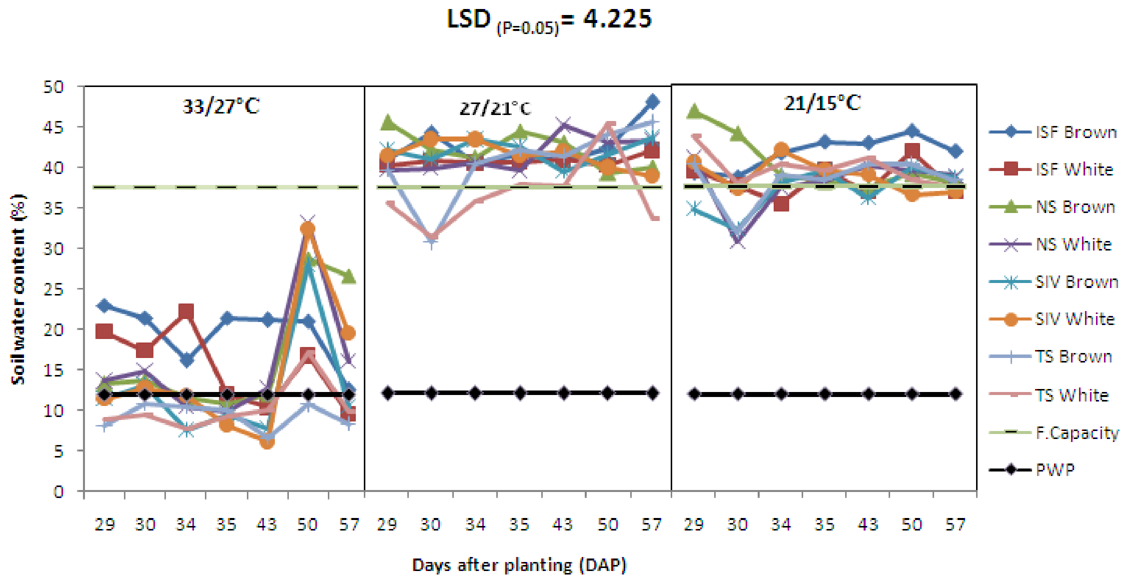

Figure 3.2: Soil water content measured from pots subjected to different water regimes (NS, ISV, ISF & TS) under three temperature conditions[High (33/27°C), Optimum (27/21°C) and Low (21/15°C)].

3.3.2 Establishment and plant growth

Temperature differences between growth environments were found to have a highly significant (P<0.001) effect on percentage emergence (Figure 3.3).

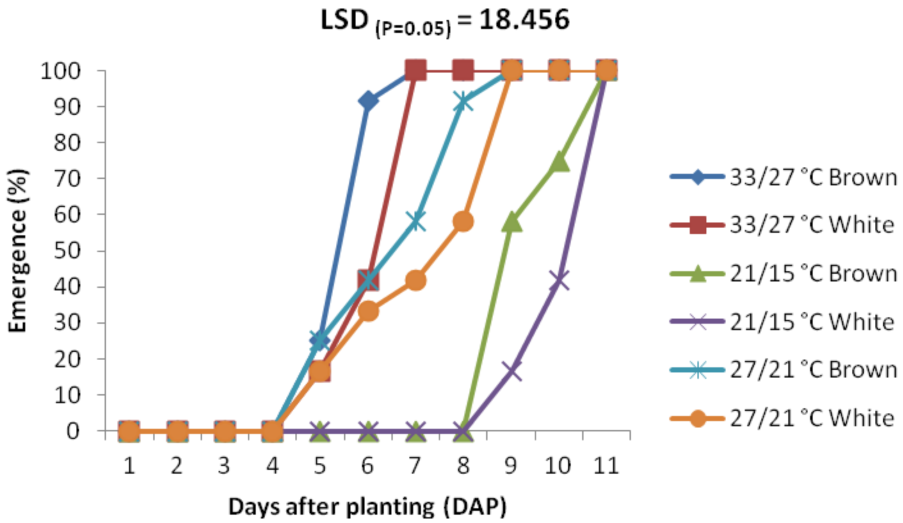

Figure 3.3: Percentage emergence of cowpea varieties (Brown and White birch) grown under different temperature environments [High (33/27°C), Optimum (27/21°C) and Low (21/15°C)].

Differences in percentage emergence and initial leaf area were due to temperature, since initially all the pots were watered up to field capacity. Emergence of plants at 33/27°C was found to be 18% and 67% higher than that of plants at 27/21°C and 21/15°C, respectively. There were highly significant (P<0.001) differences between varieties with respect to emergence (Figure 3.3). Based on mean values of varieties, emergence of Brown birch was 14.6% more than that of White birch. There was a significant (P<0.05) variation in emergence rate over time (Figure 3.3) and there was also a significant interaction (P<0.05) between temperature and variety in emergence over time. At 21/15°C, emergence started 8 DAP while at 33/27°C and 27/21°C emergence started at 4 DAP (Figure 3.3). At 33/27°C and 27/21°C, plants attained full emergence (100%) by 7 and 8 DAP, respectively, whereas at 21/15°C, plants only attained full emergence 11 DAP (Figure 3.3). Temperature regimes had a significant effect (P<0.05) on initial leaf area measured from the first true leaf (Figure 3.4).

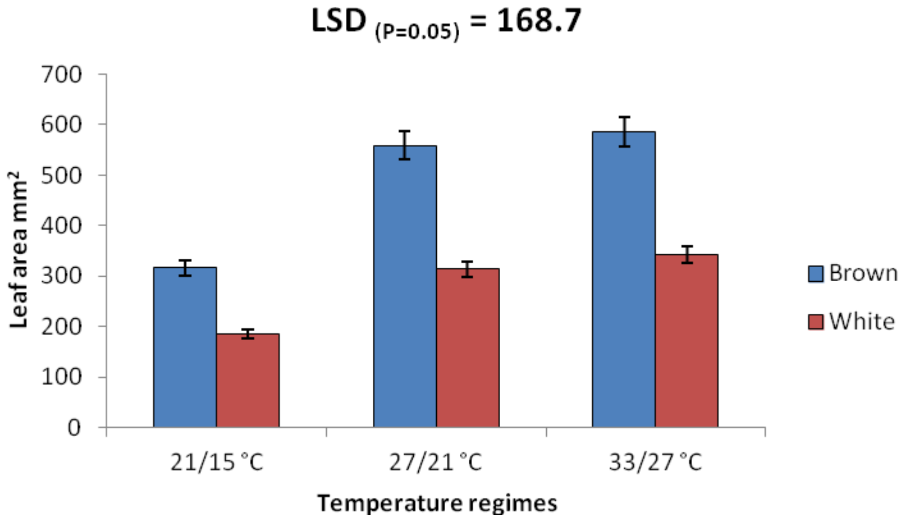

Figure 3.4: Effect of temperature regimes [High (33/27°C), Optimum (27/21°C) and Low (21/15°C)] on initial leaf area measured on the first true leaf of cowpea varieties (Brown and White birch).

Leaf area of plants growing at 21/15°C was respectively 46% and 43% lower than leaf area at 33/27 °C and 27/21 °C (Figure 3.4). The cowpea varieties differed significantly ($P<0.001$) with respect to leaf area. Leaf area of Brown birch was 42% higher than that of White birch (Figure 3.4).

Temperature regimes had a highly significant ($P<0.001$) effect on plant height. Plant height was 61% and 77% lower under 27/21°C and 21/15°C, respectively in relation to 33/27°C (Figure 3.5). The water regimes had a highly significant ($P<0.001$) effect on plant height. The TS water regime had the lowest plant height followed by ISV and ISF (Figure 3.5). Brown birch was 19% taller than White birch. The interaction between temperature and water regimes was shown to be highly significant ($P<0.001$) with respect to plant height. Results showed that, on average, plant height increased with increasing temperature; however, water regimes showed no observable trend for plant height with respect to growth stages (Figure 3.5). Therefore, this shows that cowpea was more affected by temperature than water availability.

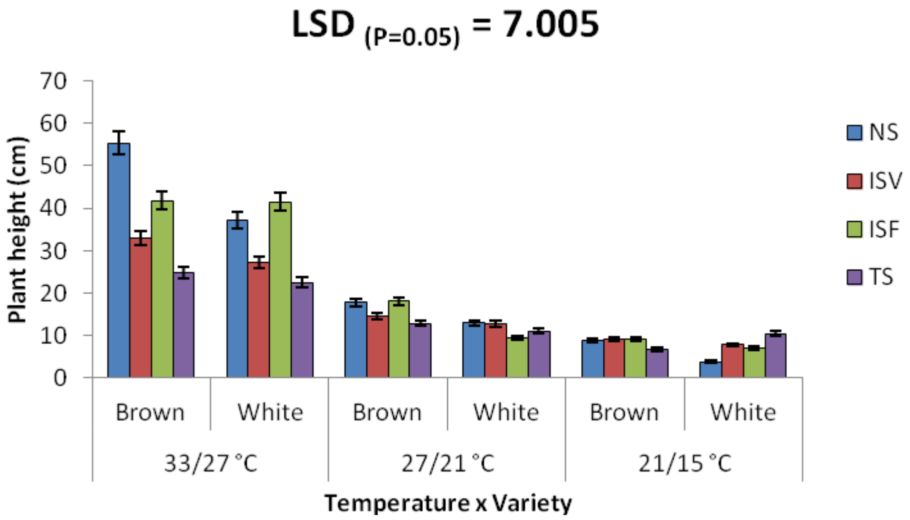

Figure 3.5: Effect of water treatments (NS, ISV, ISF & TS) and temperature regimes [High (33/27°C), Optimum (27/21°C) and Low (21/15°C)] on plant height of cowpea varieties (Brown & White).

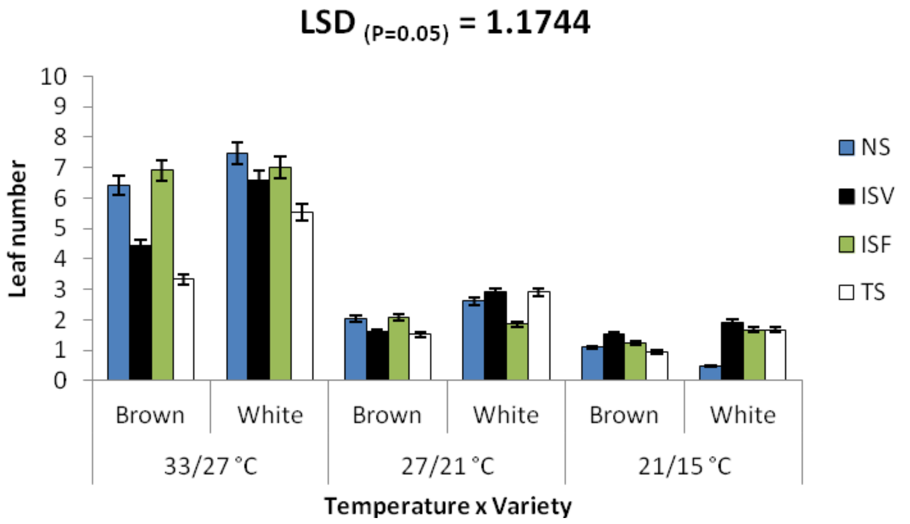

Figure 3.6: Effect of water treatments (NS, ISV, ISF & TS) and temperature regimes [High (33/27°C), Optimum (27/21°C) and Low (21/15°C)] on leaf number of cowpea varieties (Brown & White).

Leaf number was significantly (P<0.001) affected by temperature regimes (Figure 3.6). Plants growing at 21/15°C had the lowest number of leaves (with 1 leaf on average) followed by 27/21°C (2 leaves) and 33/27°C (6 leaves), respectively (Figure 3.6). Leaf number was respectively 63% and 78% lower at 27/21°C and 21/15°C relative to 33/27°C. Water regimes had no significant (P>0.05) effect on leaf number (Figure 3.6). Cowpea varieties showed highly significant differences ($P<0.001$) with respect to leaf number, with White birch having 22% more leaves than Brown birch (Figure 3.6). The interaction between water and temperature regimes was significant ($P<0.05$). The pattern observed for leaf number was similar to that observed for plant height. This further suggests that cowpea growth was more likely affected by temperature than water availability.

Temperature regimes had a highly significant ($P<0.001$) effect on chlorophyll content index (CCI) (Figure 3.7).

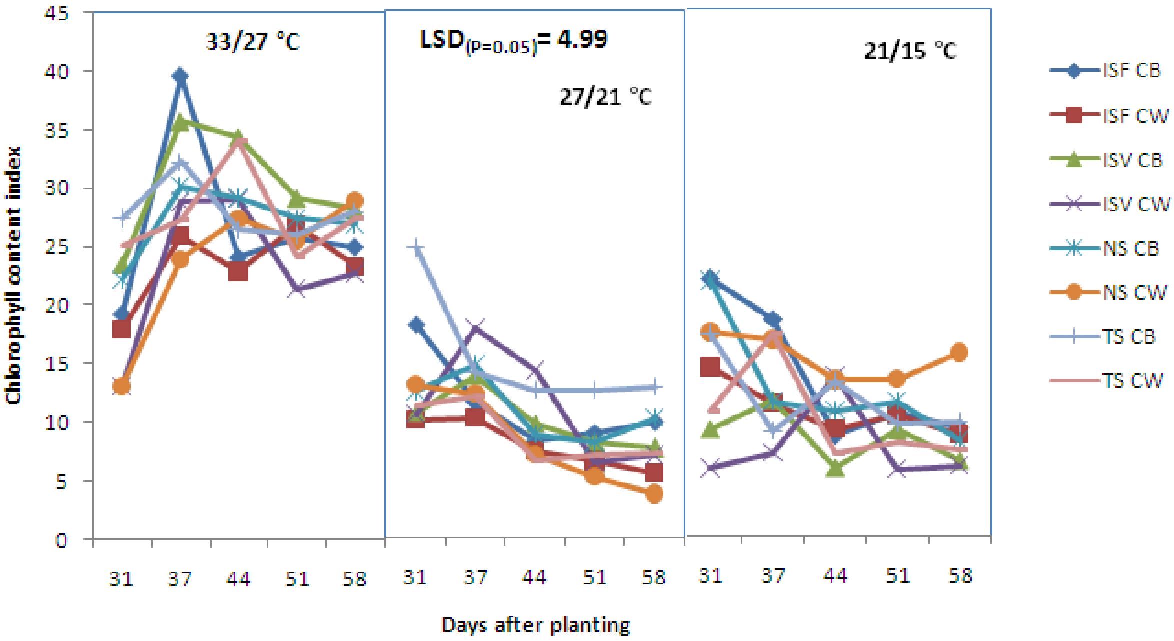

Figure 3.7: Effect of water treatments (NS, ISV, ISF & TS) and temperature regimes [High (33/27°C), Optimum (27/21°C) and Low (21/15°C)] on chlorophyll content index of cowpea varieties (Brown & White) over time (DAP).

The plants at 33/27°C showed ≈ 59.5% higher CCI than plants at 27/21°C and 21/15°C. Mean separation showed that 27/21°C and 21/15°C environments were statistically similar with respect to CCI. There were no significant differences ($P>0.05$) between water regimes with respect to CCI (Figure 3.7). Temperature and water regimes did not have a significant interactive effect ($P>0.05$) on CCI. Cowpea varieties differed significantly ($P<0.001$) with respect to CCI; Brown birch had a 14% higher CCI relative to White birch (Figure 3.7).

There was a significant (P<0.05) interaction between temperature regimes and varieties (Figure 3.7).

3.3.3 Proline

Results of proline showed that there were highly significant (P<0.001) differences of proline concentration (Figure 3.8) across the water treatments.

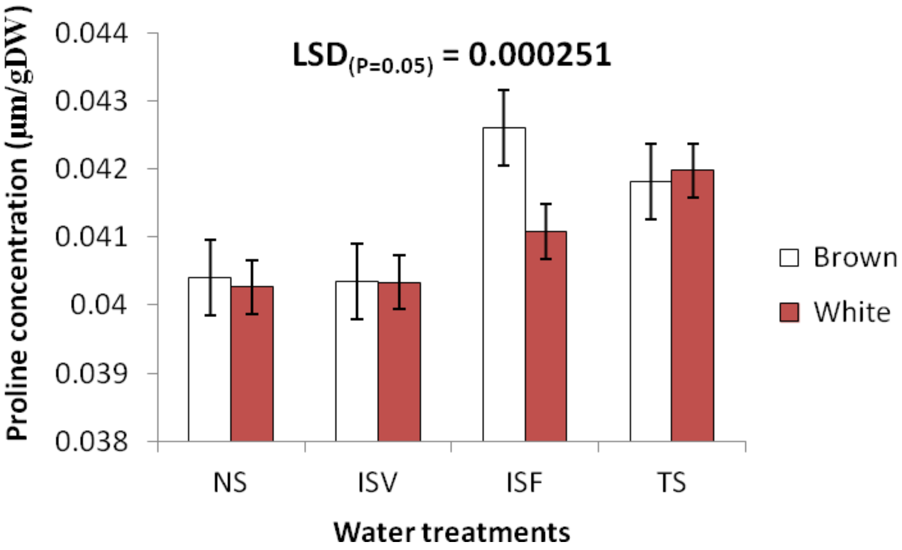

Figure 3.8: Proline concentration of cowpea varieties (Brown & White birch) subjected to different water treatments (NS, ISV, ISF, TS).

Terminal stress (TS) and stress imposed at flowering (ISF) resulted in more proline accumulation relative to NS and ISV (Figure 3.8). Cowpea varieties differed significantly (P<0.001) with respect to proline accumulation under water stress, where Brown birch accumulated more proline in relation to White birch. The interaction between water regimes and varieties was found to be highly significant (P<0.001). Visible differences between varieties were observed under ISF, were Brown birch accumulated about 3.6% more proline than White birch (Figure 3.8).

The plants at the lower temperatures (27/21ºC and 21/15ºC) showed stunted growth and failed to yield. As a result, yield parameters were evaluated for the plants at high temperatures (33/27ºC). With the exception of seed number per pod, water regimes had a significant ($P<0.05$) effect on most yield components (Table 3.1). For all yield components, the interaction between water regimes and varieties was not significant ($P>0.05$). Imposing water stress at different stages of plant growth had a highly significant ($P<0.001$) effect on total biomass (Table 3.1). Total biomass was observed to be 24% (ISV), 38% (ISF) and 69% (TS) lower relative to the NS water regime. Cowpea varieties showed no significant differences ($P>0.05$) for yield components. The interaction between water regimes and varieties was not significant ($P>0.05$); however, the trend of results showed that, with the exception of the NS treatment, Brown birch generally performed better than White birch, with respect to total biomass (Table 3.1).

Imposing water stress during vegetative (ISV) and flowering (ISF) stages resulted in similar pod mass for both varieties compared with the NS water regime; whereas TS resulted in 76% and 63% lower pod mass for brown and White birch, respectively, relative to the NS water regime. Imposing water stress during vegetative stage (ISV) was found to decrease pod mass more than imposing water stress at flowering (Table 3.1); the pattern was similar for both varieties. Brown birch was shown to have 3% higher pod mass than White birch (Table 3.1). However, Brown birch had 39% less pods per plant compared with White birch (Table 3.1). This is because Brown birch produced bigger pods that contained bigger and fewer seeds. Imposing water stress during vegetative (ISV) and flowering (ISF) stages resulted in similar pod number per plant relative to the NS water regime. On the other hand, TS resulted in 77% and 50% less pod number per plant in brown and White birch, respectively, relative to the NS water regime (Table 3.1). Brown birch performed better than White birch with respect to seed number per pod, pod length and seed mass per plant; however, this did not translate to better harvest index for Brown birch.

Cowpea varieties were not significantly ($P>0.05$) different with respect to harvest index (HI); however, it was notable that White birch performed better than Brown birch (Table 3.1). Interesting results were observed with respect to the response of varieties towards ISV and ISF, with respect to total biomass and harvest index when compared with the NS water regime. For Brown birch, imposing water stress during vegetative stage did not affect biomass but it was observed to decrease harvest index by 24% when compared with the NS water regime. ISF decreased biomass significantly ($P<0.05$), by 33%, while harvest index was increased by 26% relative to NS water regime. With the White birch, there was a 48% decrease in biomass in response to ISV however harvest index was kept constant when compared with the NS water regime. There was a 42% decrease in biomass in response to ISF, and the harvest index increased by 43% when compared with the NS water regime. The trend with response to terminal stress for both varieties was constant, whereby biomass was significantly decreased and there was a significant increase in harvest index.

Table 3.1: Yield components of cowpea varieties (Brown and White birch) subjected to different water regimes (NS, ISV, ISF, TS) under high temperature regime (33/27°C).

Variety	Water treatment	Total Biomass (g)	Pod mass (g)	Pod No. Plant^{-1}	Seed No. Pod^{-1}	Pod length (cm)	Seed mass plant^{-1}	Harvest Index (%)
BROWN	**NS**	18.19a	7.63a	4.33a	9.50a	14.52a	6.05a	32.60b
	ISV	18.47a	5.43a	3.67ab	8.33a	12.50b	4.29ab	24.70b
	ISF	12.02b	6.12a	3.33a	10.44a	19.36a	5.12a	41.40a
	TS	7.44c	1.50b	1.00b	7.33a	12.08b	1.29b	18.60a
	Mean	**14.03^{a}**	**5.17^{a}**	**3.08^{a}**	**8.90^{a}**	**13.87^{a}**	**4.19^{a}**	**29.30^{a}**
WHITE	**NS**	19.54a	7.09a	6.67a	8.03a	12.00a	5.53a	27.70b
	ISV	10.14b	3.78a	4.67ab	5.70a	9.96b	2.90ab	27.40b
	ISF	11.32b	6.97a	5.67a	9.00a	13.54a	5.61a	49.00a
	TS	4.25c	2.20b	3.33b	6.08a	9.60b	1.76b	43.30 b
	Mean	**11.31^{a}**	**5.01^{a}**	**5.08^{a}**	**7.20^{a}**	**11.27^{b}**	**3.95^{a}**	**36.80^{a}**
LSD$_{(P=0.05)}$ (Water*Var)		**6.388**	**3.811**	**1.407**	**4.154**	**2.668**	**1.493**	**18.74**

3.4 Discussion

The objective of this study was to evaluate crop growth and yield responses of cowpea varieties to water stress under different temperature environments. Furthermore, a secondary objective was also to evaluate the interactive effect of water and temperature on cowpea growth and yield. Water stress and high temperature present an excellent example of two environmental stresses that often occur simultaneously under field conditions (Xu and Zhou, 2006). The amount of water applied for all treatments (NS, ISV, ISF and TS) and frequency of irrigation was the same across all temperature environments (33/27°C, 27/21°C and 21/15°C); however, the rate of water loss was different. Water loss in pots increased with increasing temperatures, this is usually the case under field conditions. Plants tend to compete for water with the evaporative demand caused by high temperatures and this competition becomes worse when there is limited water supply.

The trend in emergence showed that it was affected by temperature differences between the growing environments. At lower temperatures (21/15°C), seeds took longer to emerge, while emergence proceeded relatively faster in the warmer environments (33/27°C and 27/21°C). Since water was not a factor during establishment, this suggests that temperature was the main factor affecting emergence rate of cowpea varieties. Low temperatures resulted in slow and uneven emergence, which means that cowpeas emergence is sensitive to low temperatures. This may impose a challenge to the farmers since uniform emergence and hence good stand establishment is of economic importance to the farmers. Therefore, cowpea varieties used for the current study requires high temperatures for optimum, fast, uniform emergence and stand establishment. Ismail *et al.* (1997) also reported that the rate of emergence was slower and erratic under 15ºC when compared to more favourable (28ºC) temperatures.

The effect of temperature on cowpea growth was observed initially from the development of the first true leaf. Consistent with observations of emergence, low temperatures resulted in slow establishment as evidenced by the low initial leaf area relative to the warmer environments (33/27°C and 27/21°C); leaf area of the first true leaf increased with increasing temperature. Leaf area of the first true leaf was measured with the assumption that it would indicate possible growth rate of the plants. As such, based on these observations, it could be suggested that plants growing at 33/27ºC and 27/21ºC were expected to grow faster than plants at 21/15ºC. Similar observations were reported in the study by Xiong *et al.* (2000) where higher temperatures increased leaf area 3.4-5.5 times compared with plants at lower temperatures.

Cowpea has been reported to be adapted to drought, high temperatures and other biotic stresses compared with other crops (Ehlers and Hall, 1997). Cowpea growth, in terms of plant height and leaf number, was observed to improve with increasing temperatures. In this study, it was expected that cowpeas would perform better at 27/21ºC as these were depicted as optimum temperatures; however, it was found that 33/27ºC favoured cowpea growth. The trend of results observed from this study showed that plant height and leaf number were more affected by water stress imposed during the vegetative than during flowering stage. These results are in tandem with reports by Vurayai *et al.* (2011) that plant height and leaf number were reduced following imposition of water stress at vegetative, flowering and pod filling stages, respectively, in bambara groundnut. In the current study, a possible explanation for growth not being interrupted in response to water stress imposed at flowering (ISF) could be the fact that vegetative growth had already ceased at this stage.

Cowpea varieties used for this study showed that they grow optimally at high temperatures (33/27ºC). These findings were also confirmed by chlorophyll content index (CCI) results which showed low CCI at lower growth temperatures (27/21ºC; 21/15ºC) compared with high temperatures (33/27ºC). This was contrary to reports in the literature that high temperatures reduced chlorophyll content in pea (*Pisum sativum*) and faba bean (*Vicia faba*) (MacDonald and Paulsen, 1997). Within the context of the current study, high chlorophyll content index could be related to rapid growth (MacDonald and Paulsen, 1997) which was observed at high temperatures (33/27ºC). Plants from 33/27ºC had high CCI and demonstrated vigorous growth whereas plants at 27/21ºC and 21/15ºC had low CCI and showed stunted growth.

Since growth was more vigorous at 33/27ºC relative to the other temperature regimes, proline accumulation was only evaluated in the 33/27ºC environment. As such, differences in proline accumulation reported in the current study can only be related to water stress imposed at different growth stages rather than temperature. Terminal stress and stress imposed at flowering stage (ISF) resulted in higher levels of proline for both cowpea varieties. Notable differences between cowpea varieties were observed when water stress was imposed at flowering stage (ISF); Brown birch accumulated more proline than White birch (Figure 3.8). This observation suggests a possible stress tolerance mechanism for Brown birch. Chickpea varieties were also reported to accumulate proline in response to water stress imposed during vegetative stage and anthesis (Mafakheri *et al.*, 2010). Mafakheri *et al.* (2010) also reported that proline accumulation was higher when stress was imposed at flowering than at vegetative growth in chickpea.

In this study, there was no yield in the 27/21ºC and 21/15ºC environments. Plants in these environments showed stunted growth and therefore failed to form yield. Hence, the yield components presented in this study were obtained from the 33/27ºC environment. Total biomass for both varieties was significantly lower under water stress relative to no stress. It was also reported with other cowpea varieties that water stress also reduced dry mass production (Ahmed *et al.*, 2010). Imposing water stress at different stages of growth resulted in reduction of most yield components. Imposing water stress at different growth stages (SIV and ISF) and terminal stress reduced pod number, pod mass and consequently seed mass per plant in relation to the NS water regime. In cowpea, harvest index is determined by the number of pods per plant, seed number per pod and the extent to which grains are filled (Ahmed *et al.*, 2010). Supporting evidences were also reported by many other researchers (Turk and Hall, 1980; Ziska and Hall, 1983).

Response of cowpea varieties to water stress imposed during vegetative (ISV) and flowering stage (ISF) showed that, the plants favoured assimilate translocation towards yield formation to maintain or increase yield while compromising biomass accumulation. Reduction in harvest index in response to ISV when compared with ISF imply that farmers using deficit irrigation can briefly impose water stress at flowering stage without affecting the target yield. Water stress during vegetative growth also reduced yield components in bambara and this was attributed to reduced plant growth in response to water stress (Vurayai *et al.*, 2011). It has been reported that the reproductive stage is the most sensitive stage in cowpea (Ahmed *et al.*, 2010). However, this cannot be generalised, there are genotypic differences with respect to responses of cowpea varieties towards water stress imposed at different growth stages. This is because varieties used for the present study showed more sensitivity towards ISV than ISF. Therefore it cannot be assumed that cowpea in general is mostly sensitive to water stress imposed during reproductive stage. Cowpea varieties used for this study showed an interesting response towards terminal stress (TS); whereby they strived in order to reproduce. White birch managed to obtain a harvest index of 43.3% under TS and this suggests that White birch grows best under water limiting environments.

3.5 Conclusions

High temperatures improved cowpea growth and productivity. It can be concluded that cowpea grows best under high temperatures. In the context of this study, 33/27°C was found to be the optimum temperature environment for cowpea growth and productivity. Imposing water stress at vegetative growth stage reduced growth and productivity of cowpea. Water stress imposed during flowering stage had a positive effect on growth parameters and yield of cowpea. Terminal stress reduced growth parameters, however, the plants managed to produce reasonable yield. Based on these results it can be concluded that the vegetative growth stage is the most sensitive stage to water stress. Farmers growing cowpea in water scarce areas could save irrigation water by deficit irrigation, since water stress can be imposed briefly during the flowering stage and still obtain reasonable economic yield. However, it must be stressed that this hypothesis requires further data to confirm. White birch is well adapted to water stress and it is able to produce satisfactory harvest index compared with Brown birch. Based on the results obtained from this study it can be concluded that temperature is more influential to growth, development and productivity compared to water stress when temperature conditions are low. Whereas under high temperatures; water stress is more influential to plant growth and productivity.

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CHAPTER 4

EFFECT OF WATER STRESS AND DEFOLIATION ON GROWTH, DEVELOPMENT AND YIELD OF COWPEA VARIETIES

4.1 Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important legume known for its uses as a grain and fodder crop (Singh *et al*., 2003). It is cultivated worldwide in tropical and subtropical regions (Ogunkamni *et al.,* 2006). Cowpea has a potential to contribute significantly towards food security. It is also a potential cash crop and animal feed in rural areas of Africa (Inaizumi *et al*., 1999). Since the crop is grown mainly by small scale farmers, it could provide dietary support as a relatively cheap protein source for rural households (Sebetha *et al*., 2010); it is also a rich source of vitamins and minerals (Bressani, 1985). The crop can be consumed in several ways - for its grain (22 - 23% protein content) or as a leafy vegetable during vegetative growth. Cowpea is reported to be a drought tolerant, and hot weather crop due to its adaptation to semi-arid regions where other food legume crops do not perform well (Singh *et al*., 2003). Although both cowpea leaves and grain contain significant amounts of nutrients, the crop still remains neglected in terms of research and crop improvement (Barrett, 1990; Schippers, 2002). As such, many cowpea varieties are still damaged by drought, especially during reproductive development.

Water scarcity is the single most critical threat to crop production in the arid regions of sub-Saharan Africa (Chaves and Oliveira, 2002). Sufficient water supply is required in the root zone to facilitate processes of germination, transpiration, nutrient absorption, root growth, organic matter decomposition and nutrient mineralisation (Rashidi and Seyfi, 2007). All these processes are required to sustain crop growth (Fitter, 1981). Water stress affects all aspects of plant growth (Rahman *et al*., 2004) from emergence (Harris *et al*., 2002; Mabhaudhi, 2009), plant growth (Manivannan *et al*., 2007), phenology (Blum, 2005) leading up to yield (Anjum *et al*., 2011). Plants exposed to water stress undergo physiological and morphological changes in response to limited water availability; physiological changes include decreased photosynthesis and respiration (Hall *et al.,* 1990).

Limitations to photosynthesis have primarily been attributed to stomatal closure (Chaves, 1991). Stomatal closure is the plant's primary response to water stress and results in decreased CO_2 assimilation and availability (Anjum *et al*., 2011). Consequently, there is a decline in the rate of photosynthesis due to low intracellular CO_2 while favouring photorespiration (Anjum *et al*., 2011). A series of field experiments conducted on field crops such as maize (Tardieu *et al*., 1991), grapevine (Correira *et al*., 1995; Stoll *et al*., 2000) and clover (Socias *et al*., 1997) all confirmed that, under water stress, stomatal closure was the major limitation to photosynthesis. In addition, metabolic impairment of photosynthesis also occurs when photosynthetic pigments such as chlorophylls a and b and carotenoids are altered under conditions of limited water availability. These pigments are very important for light harvesting and production of reducing powers; however, they are sensitive to soil dehydration (Farooq *et al*., 2009). There was an increase in chlorophyll b content while chlorophyll a content remained unaffected hence the Chl a: b ratio was significantly reduced under water stress (Estill *et al*., 1991; Ashraf *et al*., 1994). Total chlorophyll content declined in a number of sunflower varieties under water stress. Loss of chlorophyll is a drought avoidance mechanism also associated with energy dissipation (Manivannan *et al.,* 2007).

Drought stress negatively affects growth parameters such as leaf number and size, stem extension, plant height and root proliferation (Anjum *et al.,* 2011). Mabhaudhi and Modi, (2010) reported that water stress reduced plant height and leaf number of maize landraces and hybrid varieties, while Mbatha and Modi (2010) reported similar response in wild mustard landraces. Kirnak *et al.* (2001) had earlier found that water stress reduced plant height, stem diameter, leaf expansion rate and dry matter production in egg plant (*Solanum melongena* L.). A similar pattern with leaf number and area were reported in several crops, including soybean (Zhang *et al.,* 2004), cowpea (Manivanna *et al*., 2007), wheat and maize (Sacks *et al*., 2007). Furthermore, water stress imposed during vegetative growth hinders the accumulation of biomass required for reproductive growth and yield (Kamara *et al*., 2003).

Reduction of yield components and yield under water stress conditions is attributed to stomatal closure (Chaves, 1991; Conic, 2000; Flexas *et al*., 2004). Long periods of drought reduce plant growth and affect phenological development, leading to reduced flower production and grain filling, translating to smaller and fewer grains. Drought stress disturbs the normal assimilate partitioning and activities of sucrose and starch synthesis enzymes hence reduced grain filling (Anjum *et al*., 2011). Yield components of cantaloupe (*Cucumis melo*) such as number of fruit per plant, fruit mass and fruit thickness were significantly reduced by drought stress (Rashidi and Seyfi, 2007). Grain number and size were reduced under pre-anthesis drought stress treatments in wheat (Edward and Write, 2008). Specht *et al*. (2001) also reported a reduction in pod formation and consequently reduced seeds per unit area in soybean. Drought stress occurring at flowering caused bareness in pear millet [*Pennisetum glausam* (L.)] and the major cause of this was the reduction in assimilate flux to the developing grain (Yadav *et al.,* 2004).

In many parts of Africa, it is a common practice to remove young cowpea leaves for use as a vegetable (Barret *et al.,* 1997). Previous research on cowpea has revealed that cowpea leaves contain carbohydrates and protein content comparable to that in cowpea grain (Bubenheim *et al.,* 1990). The use of cowpeas as a leafy vegetable may provide nutritional and harvest versatility that is not available with other vegetable crops like cabbage and lettuce (Bubenheim *et al.,* 1990). However, sequential leaf harvesting may have a negative impact on grain yield if the crop is grown for both purposes. It has been reported that grain yield is reduced by leaf harvesting (Bittenbender, 1992). Several studies have been conducted on cowpea to improve the methods of sequential leaf harvesting without imposing a significant damage on grain yield. These include suitable plant growth stage for leaf harvesting (Matikiti *et al*., 2009; Ibrahim *et al*., 2010) and intensity of harvesting (Nielsen *et al*., 1997; Ibrahim *et al*., 2010).

While the effect of leaf harvesting on grain yield of cowpea has been studied, few studies have evaluated the combined effect of water stress and leaf harvesting on grain yield of cowpea. Since cowpea is known to be drought tolerant, these two factors: sequential harvesting and drought stress need to be well understood so as to maximise their combined effect on total grain yield. Such information would be useful in advising farmers who grow cowpea; such that they understand the potential of the crop to produce both green leafy vegetables and grain yield because most of them cultivate the crop in marginal areas. Therefore, the objective of this study was to evaluate the effect of water stress and sequential leaf harvesting on plant growth and grain yield of two cowpea varieties.

4.2 Materials and Methods

4.2.1 Plant material

Two cowpea varieties differing in seed colour (Brown birch variety and White birch variety) were purchased from a local seed supplier, Capstone Seeds in 2011 and used for the experiment. The varieties were classified as annual determinant legume types.

Figure 4.1: Cowpea seed varieties used in the study (A = White birch variety and B = Brown birch variety).

4.2.2 Field description and experimental design

A field trial was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 775 masl). Ukulinga soils are characterised as clay loam. Ukulinga has a warm subtropical climate with an average annual rainfall of about 694 mm received mainly during the summer months (mid-October to mid-February). The long term weather data showing growing seasons, potential evapotranspiration (PET) as well as rainfall distribution for Ukulinga is presented in Figure 4.2.

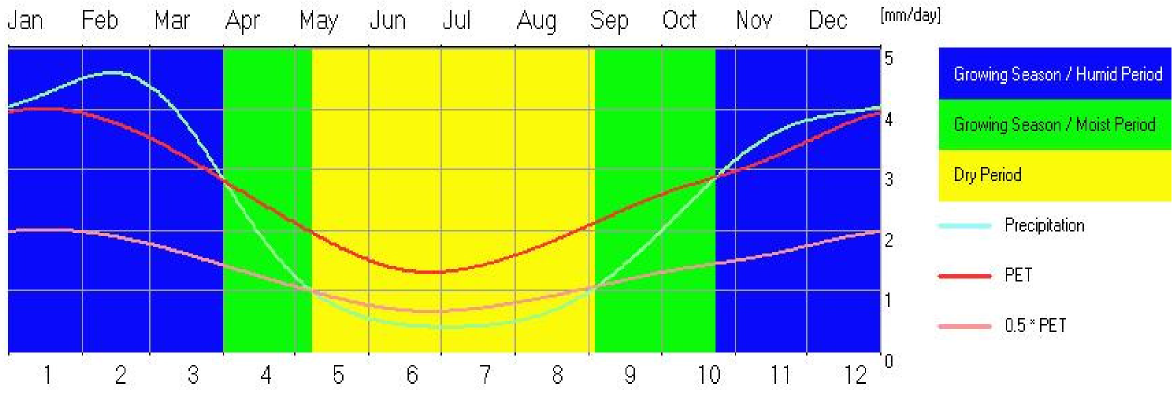

Figure 4.2: Ukulinga growing season, potential evapotranspiration (PET) and precipitation (mm/day).

The experimental design was a factorial experiment (three factors) laid out in a split-plot design, replicated three times. Water treatment [full irrigation (IRR) vs rainfed (RF)] was the main factor, with cultivar (white and Brown birch variety) as sub-factors. The third factor, sequential harvesting, had three levels: no harvest (HO), harvested once (H1) and harvested twice (H2), during plant growth. All treatments were arranged in a randomised complete block design. Therefore, the treatment structure was (2*2*3). The total size of the field trial was 868 m^2. Main plots (IRR and RF) measured 356.5 m^2 each, with 10 m spacing between them to prevent water sprays from reaching RF plots. Sprinklers were designed to have a maximum range of 6 m radius. Sub-plot size was 13.5 m^2 with an inter-plot spacing of 1 m, and plant spacing of 0.45 m x 0.35 m, translating to 122 plants per plot. Irrigation scheduling for the IRR treatment was scheduled to meet 100% of crop water requirement (ET_c) based on reference evapotranspiration (ET_o) and a crop factor (K_c) (Allen *et al*., 1998). During the growing season (December to March) 373.3 mm of rainfall were received and supplementary irrigation in the IRR treatment amounted to 260 mm**.** Both trials were established under full irrigation until the seedlings were fully established, and then irrigation was withdrawn in the rainfed treatment.

4.2.3 Data collection

Emergence counts were taken weekly starting from seven (7) days after planting (DAP) until full emergence. Full emergence was defined as when crops had achieved at least 90% emergence. Thereafter, measurements of plant height and leaf number were taken weekly until 50% of the plants had flowered. Leaf area index (LAI), stomatal conductance (SC) and chlorophyll content index (CCI) were measured weekly. Leaf area index was measured using the LAI2200 canopy analyser (Li-Cor, USA & Canada). Stomatal conductance and chlorophyll content index were measured using a steady state leaf porometer (Model SC-1, Decagon Devices, USA) and the CCM-200 *Plus* (Optisciences, USA), respectively. Sequential harvesting of leaves for the H1 treatment was performed at 55 DAP and the second harvest (H2) was done at 69 DAP. Sequential harvesting was done by carefully removing all the leaves from the plants whilst leaving the nodes intact to allow for new leaves to form. Yield components (total biomass, pod number/plant, pod mass/plant, seed number/pod, seed mass/plant and harvest index) were measured at harvest. Harvest index was calculated using the following formula: HI = (Pod mass/Total biomass)*100

4.2.4 Crop management

Prior to planting, soil samples were taken and submitted for soil textural and fertility analyses. Results of soil fertility analysis revealed that there was no need for fertiliser application to meet cowpea requirements for macro and micro-nutrients. Therefore no fertiliser was applied. Plants were sprayed with Kemprin (Cyphermethrin) at 20 ml/10L against cutworm and weeding was performed manually.

4.3.5 Weather and soil water content

Weather data for the duration of the experiment were obtained from an automatic weather station (AWS) located within a 50 m from the experimental site. Soil water content (SWC) was measured using a PR2/6 profile probe connected to an HH-2 moisture meter (Delta-T Devices, UK) at depths of 10, 20, 30, 40, 60 and 100 cm. Access tubes were inserted in each plot for the purpose of measuring soil water content. Rain gauges were installed in the irrigated trial for the purpose of quantifying amount of water applied at each irrigation event.

4.2.6 Data analysis

Data were subjected to analysis of variance (ANOVA) using GenStat® (Version 14, VSN International, UK). Means were separated using least significant differences (LSD) at a probability level of 0.05.

4.3 Results

4.3.1 Weather data and soil water content

Daily minimum and maximum air temperatures and rainfall were measured during the course of the study from an automatic weather station (Figure 4.3). The minimum temperature for cowpea germination is 9°C and the optimum temperature for vegetative growth is 21-33°C. When the crop was planted minimum temperature was above the base temperature (10°C), therefore providing favourable conditions for successful germination and emergence. Maximum temperatures were within the range of optimum temperature for cowpea growth. The overall rainfall received during the growing season was 373.3 mm.

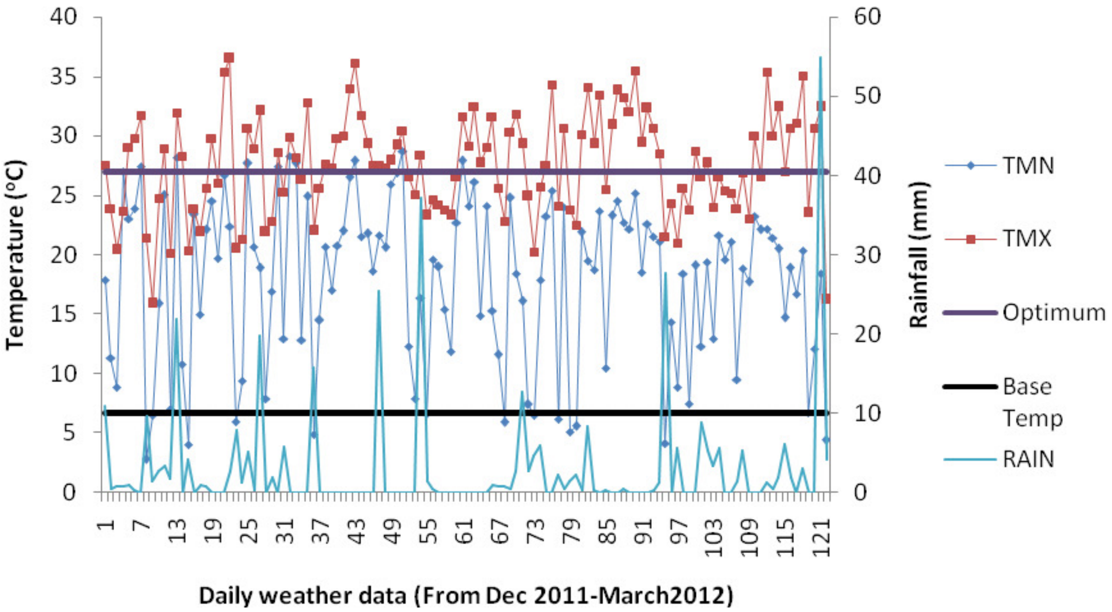

Figure 4.3: Changes in daily water patterns measured during the cowpea growing period.

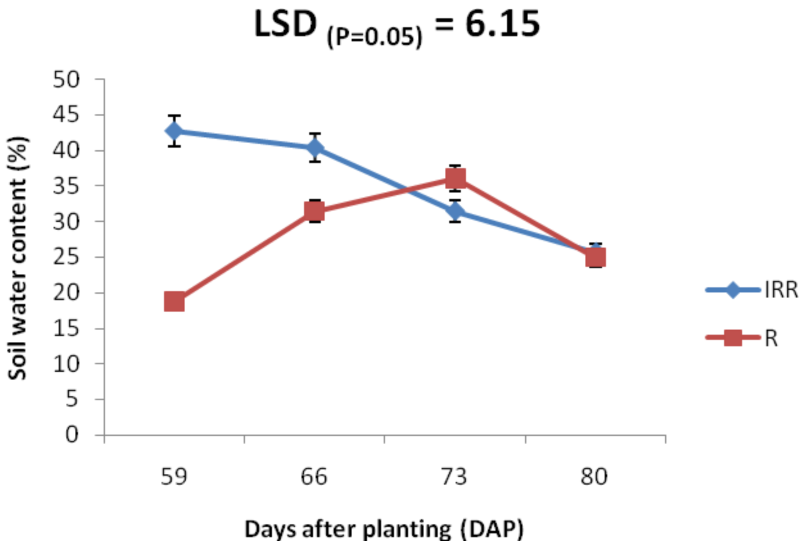

Figure 4.4: Shows the changes in soil water content over time.

Water regimes showed a highly significant ($P<0.001$) effect on soil water content (SWC). First measurement of soil water content took place on the 59th day after the crop was planted. Differences between irrigated and rainfed experiment were very high, with irrigated experiment had the highest SWC than rainfed experiment (Figure 4.4). An increase in SWC with time was observed under rainfed conditions (Figure 4.4) from 59 to 73 DAP; thereafter, a decrease was observed as time progressed. While under irrigated conditions, SWC was initially very high, but it decreased gradually with time (Figure 4.4).

4.3.2 Emergence

As stated in Section 4.3.1, plants in both water regimes (Irrigated and Rainfed) were established with full irrigation until 90% emergence was attained. Therefore, results of emergence reported here only show differences between varieties and not between water treatments (Figure 4.5). Results showed that there were no significant differences ($P>0.05$) in the emergence of two cowpea varieties (Figure 4.5). The crop established very fast, at 7 DAP about 80% of plants had emerged (Figure 4.5); by 21 DAP 100% emergence was reached.

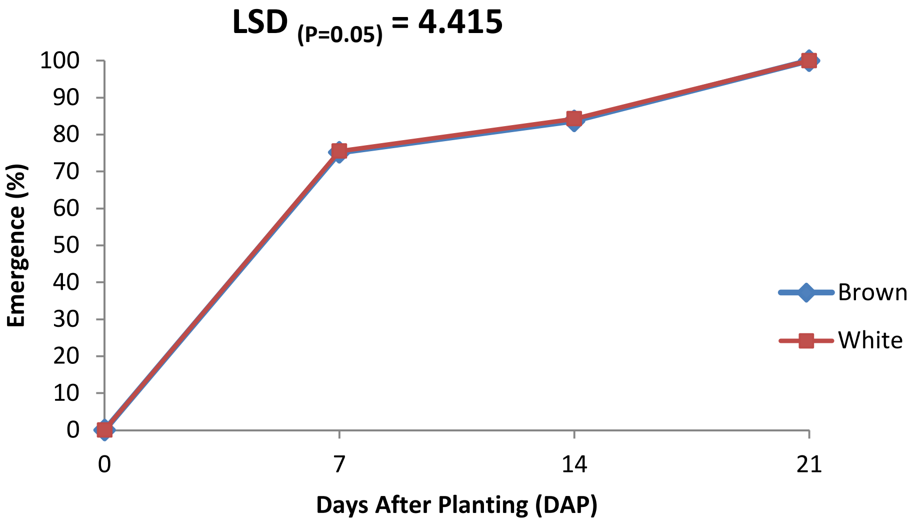

Figure 4.5: Percentage emergence of cowpea varieties (Brown & White) over time.

4.3.3 Plant growth

Response of plant height to water regimes showed highly significant ($P<0.001$) differences (Figure 4.6). Plants grown under irrigated conditions performed better than those under rainfed conditions (Figure 4.6). Cowpea varieties also differed significantly ($P<0.001$) in response to plant growth. While results of emergence showed no differences between varieties, withdrawal of supplementary irrigation in the rainfed treatment resulted in decreased plant growth in terms of plant height compared with the fully irrigated treatment. It was observed that, on average, Brown birch variety performed better than White birch variety. This shows an interesting trend since the emergence results showed no differences between varieties (Figure 4.6). The interaction between water regimes and varieties was also significant ($P<0.05$) (Figure 4.6). Plant height of brown and White birch variety decreased by 23% and 20%, respectively, under rainfed conditions relative to irrigated conditions.

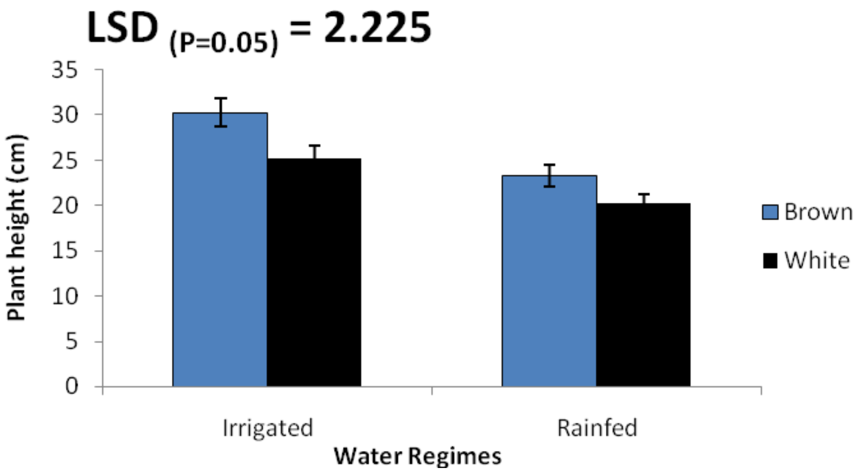

Figure 4.6: Effect of water regimes (Irrigation & Rainfed) on plant height of two cowpea varieties (Brown & White).

There were no significant differences (P>0.05) between water regimes with respect to leaf number (Figure 4.7). However, highly significant (P<0.001) differences in terms of leaf number were observed between cowpea varieties (Figure 4.7). Although the interaction between water regimes and variety was not significant (P>0.05), Brown birch variety had fewer leaves than White birch variety under both irrigated and rainfed conditions (Figure 4.7). Over-all, leaf number of Brown birch variety decreased by 22% whereas leaves of white variety increased by 21% under rainfed conditions relative to irrigated conditions. The performance pattern of both varieties in terms of leaf number was uniform under the rainfed and irrigated conditions.

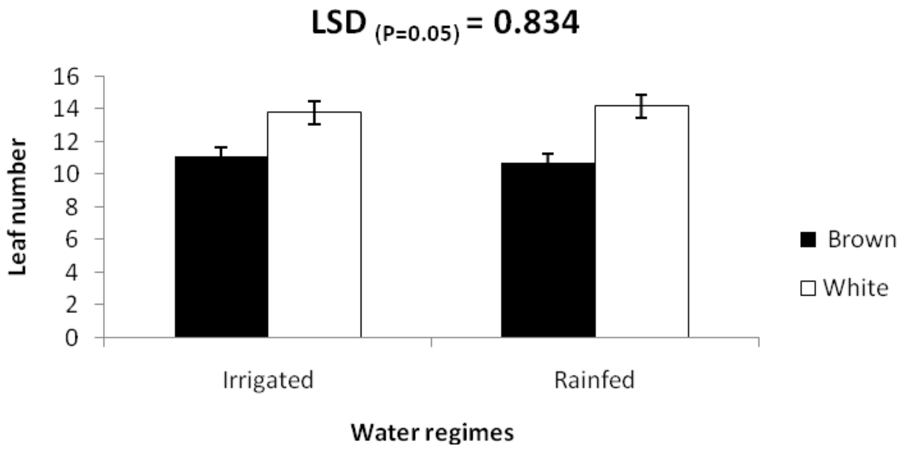

Figure 4.7: Effect of water regimes (Irrigated & Rainfed) on leaf number of two cowpea varieties (Brown & White).

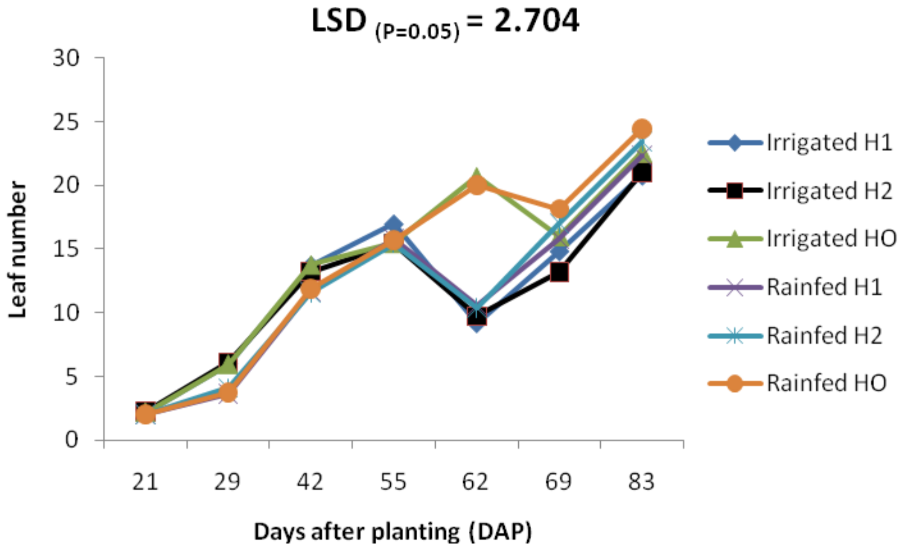

Figure 4.8: Effect of sequential harvesting (HO, H1 & H2) and water regimes on the overall leaf number over time (DAP).

Before performing sequential harvesting it was observed that leaf number increased as growth progressed. However, it was apparent that sequential harvesting caused highly significant ($P<0.001$) differences on the overall leaf number under both water regimes (Figure 4.8). There was a sharp decrease in leaf number 7 days after harvesting the leaves (62 DAP); however an exponential growth was observed 14 days after (83 DAP). Leaf growth in the HO treatment continued to increase until 62 DAP when a decrease was observed. Second harvest (H2) was performed at 69 DAP and the plants were allowed to regenerate for 14 days and leaf number increased exponentially (Figure 4.8).

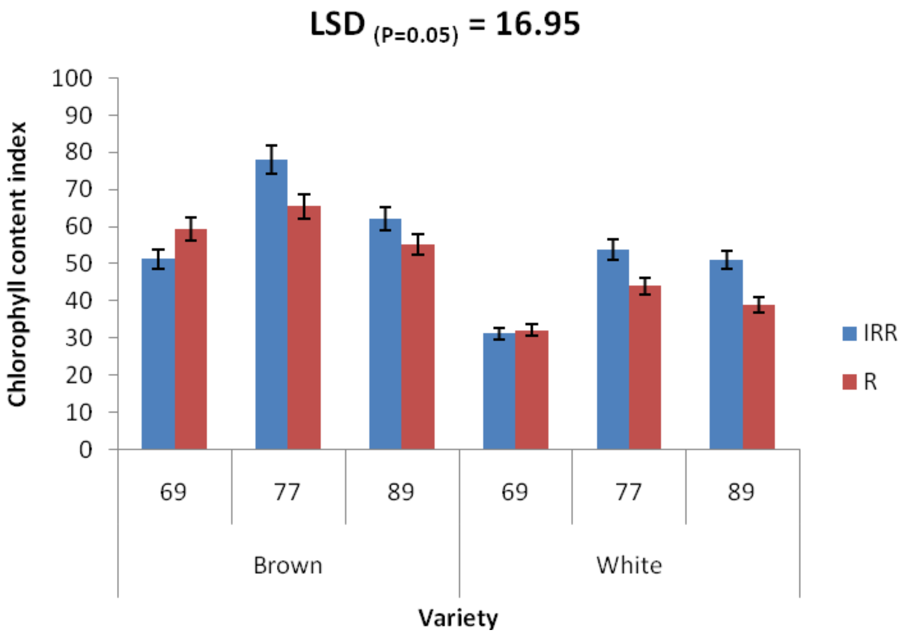

Figure 4.9: Effect of water regimes on chlorophyll content index (CCI) of cowpea varieties over time (DAP).

Differences between water regimes were not significant (P>0.05) (Figure 4.9) with respect to chlorophyll content index (CCI). However, the overall pattern showed that irrigated conditions had higher CCI than rainfed conditions (Figure 4.9). Highly significant (P<0.001) differences in CCI of white and Brown birch varieties was observed. Where the Brown birch variety was superior to White birch variety (Figure 4.9). It was also observed that time had a significant (P<0.05) effect on CCI. Chlorophyll content index increased with time up to a certain point (77 DAP) and then decreased (Figure 4.9). The decrease in CCI was observed during the reproductive phase; as such this decrease can be associated with plant maturity. The interaction between water regimes, variety and DAP was shown e not to be statistically significant (P>0.05).

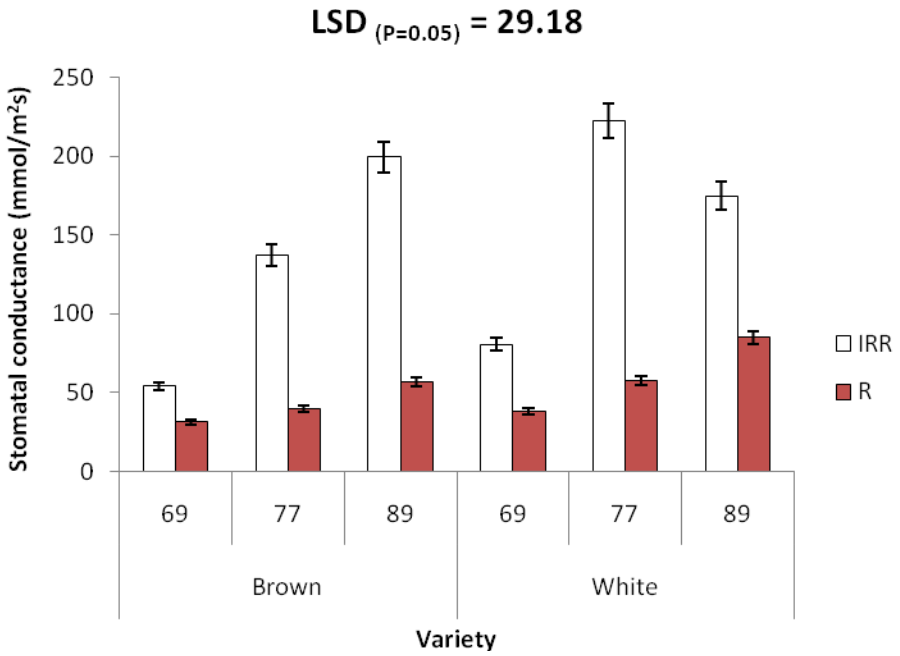

Figure 4.10: Effect of water regimes on stomatal conductance different days after planting (DAP: 69, 77 and 89).

Water regimes had a highly significant (P<0.001) effect on stomatal conductance (SC). Stomatal conductance was higher under irrigated conditions than in rainfed conditions (Figure 4.10). Both varieties showed highly significant differences (P<0.001) in SC; however, there was no clear trend with respect to their SC response to water regimes. Highly significant differences (P<0.001) were also observed for SC over time. These observations can be related to weather conditions at which SC measurements were made. The first record was done at 69 DAP and it coincided with a period where there was no rainfall received for few days. Irrigated trial showed higher SC (Figure 4.10) because of the supplementary water received from irrigation, whereas rainfed trial showed lower SC. At 77 DAP, SC slightly increased under both water regimes and this increase can be justified by the amount of rainfall (1.18 mm) received at 69 DAP. At 77 DAP, an average of 2.38 mm of rainfall was recorded; this then explains the increase in SC observed under rainfed conditions. The interaction between the factors (water regimes x variety x DAP) presented in Figure 4.10 was highly significant (P<0.001) with respect to SC.

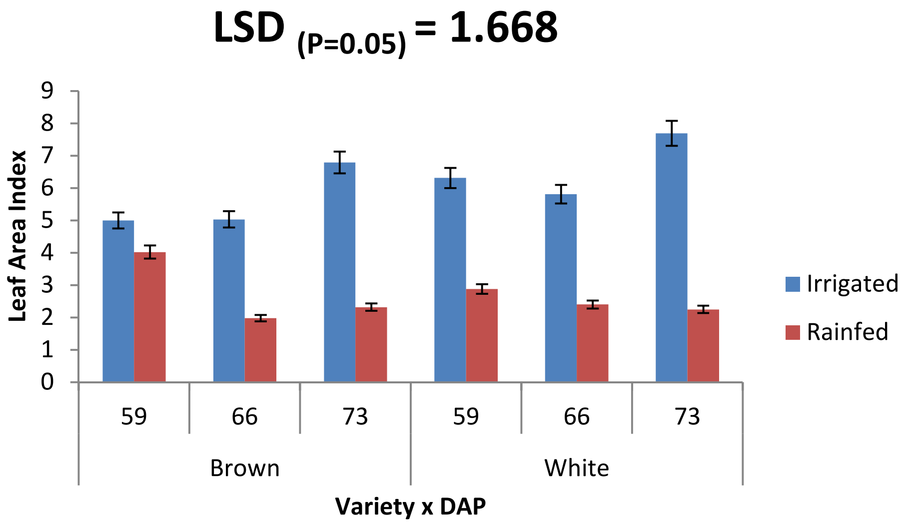

Figure 4.11: Effect of water regimes on leaf area index (LAI) of cowpea varieties over time (DAP: 59, 66 and 73).

Results of leaf area index (LAI) showed that water regimes had a significant ($P<0.05$) effect on LAI. Plants grown under irrigated conditions had higher LAI compared with those grown under rainfed conditions (Figure. 4.11). There were no significant differences ($P>0.05$) between varieties with respect to LAI. Under irrigated conditions, White birch variety had the highest LAI (6.6) followed by the Brown birch variety (5.61); whereas under rainfed conditions Brown birch variety had slightly higher (2.77) LAI than White birch variety birch variety (2.51). The leaf area index of brown and White birch varieties decreased by 50% and 62% respectively, under rainfed conditions relative to irrigated conditions. Leaf area index also varied significantly ($P<0.05$) over time (Figure 4.11). The lower LAI at 66 DAP observed under both water regimes corresponded with the time when leaf number decreased (Figure 4.8) due to sequential leaf harvesting (Figure 4.11).

4.3.4 Yield components

The interaction between water regimes, variety and sequential harvesting showed no significant ($P>0.05$) differences. With the exception of total biomass and pod mass, sequential harvesting had no significant effect on yield components of cowpea (Table 4.1). Sequential harvesting only had a significant ($P<0.05$) effect on total biomass. The no harvest treatment (HO) showed the highest total biomass followed by harvested once (H1) and harvested twice (H2) treatments, respectively (Table 4.1). There were no significant differences ($P>0.05$) between water regimes with respect to total biomass (Table 4.1). The differences between cowpea varieties were highly significant ($P<0.001$) with respect to total biomass. Brown birch variety accumulated more biomass than White birch variety (Table 4.1).

Water regimes had a highly significant ($P<0.001$) effect on harvest index. Harvest index was observed to be higher under rainfed conditions than irrigated conditions (Table 4.1). The differences between varieties with respect to harvest index were also highly significant ($P<0.001$). There was also a significant interaction ($P<0.05$) between water regimes and variety with respect to harvest index (Table 4.1). Under irrigated conditions, Brown birch variety had zero harvest index while White birch variety did not produce satisfactory yield but it had harvest index of 19.1% (Table 4.1). Interesting results were observed under rainfed conditions whereby both varieties performed better than in irrigated conditions. Although there was no supplementary irrigation, Brown birch variety had a harvest index of 7% (compared with 0% under irrigated conditions) whilst White birch variety had a harvest index of about 30% under rainfed conditions compared with 19.1% under irrigated conditions (Table 4.1).

Results of pod mass showed significant ($P<0.05$) differences in response to water regimes (Table 4.1). Lower pod mass was recorded in irrigated plants than rainfed plants (Table 4.1). Varieties also showed significant differences ($P<0.05$) in terms of pod mass, with White birch variety showing higher pod mass than Brown birch variety. Although the interaction between water regimes and variety was not significantly ($P>0.05$) different, the varieties showed better pod mass under rainfed than under irrigated conditions. Pod number per plant was significantly ($P<0.05$) affected by water regimes; the rainfed plants continued to perform better than irrigated plants (Table 4.1). The trend of the effect of water regimes and varieties was similar for all yield components (Table 4.1), whereby rainfed plants gave better yield than irrigated plants and White birch variety performed better than Brown birch variety. Other yield components such as grain number per pod and total grain mass per plant also followed the above mentioned trend, in terms of their response to water regimes and varietal differences (Table 4.1).

Table 4. 1: **Yield components of cowpea varieties (Brown & White birch variety) grown under Irrigated and Rainfed conditions at Ukulinga Research Farm and subjected to different levels of sequential harvesting (HO, H1 & H2).**

Water regime	Variety	Harvest	Total biomass (g)	HI (%)	Pod mass (g)	Pod no./Plant	Grain no./Pod	Total grain mass/Plant
Irrigated	**Brown**	**HO**	61.5a	0.04a	2.17a	0.33a	3.67a	0.56a
		H1	38.2a	0.0a	0.0a	0.00a	0.00a	0.00a
		H2	33.8a	0.0a	0.0a	0.00a	0.00a	0.00a
		Mean	**44.5^{a}**	**0.013^{a}**	**0.72^{a}**	**0.11^{a}**	**1.22^{a}**	**0.19^{a}**
	White	**HO**	28.0a	23.6a	6.88a	5.42a	3.67a	3.35a
		H1	25.4a	27.8a	6.39a	5.35a	5.00a	2.07a
		H2	19.2a	5.9a	1.09a	1.33a	6.50a	0.43a
		Mean	**24.2^{b}**	**19.1^{b}**	**4.79^{b}**	**4.03^{b}**	**5.06^{b}**	**1.95^{b}**
Rainfed	**Brown**	**HO**	50.9a	5.7a	6.65a	3.17a	3.00a	2.94a
		H1	36.1a	12.4a	2.27a	3.67a	6.33a	4.30a
		H2	37.5a	5.0a	2.70a	1.89a	1.67a	1.88a
		Mean	**41.5^{a}**	**7.70^{a}**	**3.87^{a}**	**2.91^{a}**	**3.67a**	**3.04^{a}**
	White	**HO**	30.6a	53.3a	17.04a	8.75a	9.54a	12.41a
		H1	28.4a	23.8a	8.25a	4.30a	8.02a	6.15a
		H2	23.5a	43.8a	10.07a	5.90a	8.48a	7.33a
		Mean	**27.50^{b}**	**30.30^{b}**	**11.79^{b}**	**6.32^{b}**	**8.68^{b}**	**8.63^{b}**
LSD (Water*Var)$_{(P=0.05)}$			8.26	10.35	4.494	2.252	0.853	2.909
LSD (Water*Var*Harvest)$_{(P=0.05)}$			14.93	17.92	2.654	3.900	4.331	5.039

4.5 Discussion

The objective of this study was to compare irrigated and rainfed production of cowpeas in relation to crop stand establishment, crop growth, crop response to water stress as indicated by stomatal conductance and chlorophyll content while monitoring soil water content in order to correlate it with growth responses. Furthermore, a secondary objective was to determine the interaction between water regimes and sequential leaf harvesting on harvest index and economic yield of cowpea varieties. Rainfed production is usually faced with drought stress which plays an important role in determining emergence and seedling development (Aboutalebian *et al*., 2012). Emergence is an important stage in plant growth and is a pre-requisite to obtaining optimal crop stand. Optimal crop stands are critical to yield attainment (Aboutalebian *et al*., 2012). Therefore, an optimum seedbed with optimal water availability is crucial to successful crop establishment (Mabhaudhi, 2009). In this study, seedbed conditions and water were made optimum. Therefore, emergence results were only affected by varietal differences. It was observed that the varieties performed similarly with respect to emergence, given that growing conditions were homogenous.

Previous research (Odindo, 2007; Mabhaudhi & Modi, 2010; Mbatha & Modi, 2010; Zulu & Modi, 2010; Sinefu, 2011) suggested that seed colour may be associated with seed quality. Preliminary lab experiments (Chapter 2) to assess seed quality of the two varieties showed that Brown birch variety had higher vigour than White birch variety. As a result, Brown birch variety was expected to perform better than White birch variety under field conditions. The results showed no differences in performance of the two varieties with respect to emergence rate. Based on these observations, it can be extrapolated that; given optimum growing conditions cowpea varieties performed the same irrespective of differences in seed colour.

Rainfed conditions are usually associated with water stress, mainly due to unevenness of rainfall distribution; as such, results obtained from this study can be related to other water stress studies done previously. Cowpea requires 550-775 mm and 550-850 mm of rainfall for seed and hay production, respectively (Smith, 2006). Therefore, rainfall received during this study (373.3 mm) was 32 % lower than the minimum requirement; as such the rainfed treatment was representative of drought. Results from the study agree with previous reports (Mabhaudhi and Modi, 2010) that water stress reduced plant height. Rainfed conditions were found to have a negative effect on plant height of cowpea varieties. These results concurred with the findings of Specht *et al.* (2001) who reported similar results where stem length of soybean decreased significantly due to water deficit. In other plants such as potato [*Solanum tuberosum* L.] (Heuer and Nadler, 1995), *Abelmoschus esculentus* (Sonkar *et al*., 2007), soybean [*Glycine max*] (Zhang *et al*., 2004) and parsley [*Petroselinum hortense*] (Petropoulos *et al.,* 2008), stem length was also significantly reduced by water stress. Reduction in plant height is a stress avoidance mechanism associated with reduced water use (Blum, 2005).

Leaf number has been reported to also decrease in response to water stress (Mabhaudhi and Modi, 2010; Mbatha and Modi, 2010). Contrary to this expectation, results of the study showed that leaf number was not negatively affected by reduced water availability under rainfed conditions. However, despite leaf number being unaffected, a trend was observed for leaf area index (LAI) showing lower LAI under rainfed conditions compared to irrigated conditions. This trend could mean that while cowpea varieties were able to produce a similar number of leaves under both water regimes, the ability of these leaves to expand was affected by limited water availability under rainfed conditions. Water stress has been reported to affect cell division and expansion (Nonami, 1998). Similar observations were reported by Hossain *et al.* (2010) on sunflower with respect to LAI, plants which received full irrigation produced significantly higher LAI than plants subjected to drought stress.

Cowpea varieties differed significantly ($P<0.05$) with respect to leaf number and plant height with White birch variety being superior to Brown birch variety. These differences can be related to their growth habits, the two varieties possessed different growth habits; White birch variety being a runner type and Brown birch variety being a bushy and upright type. Consequently, White birch variety was shorter but with more leaves since it grew sideways. Whereas Brown birch variety, on the other hand, was taller but with fewer leaves relative to White birch variety. However, despite differences in growth habits, both varieties were able to attain full canopy cover, as a result there were no differences in leaf area index between them.

Although the effect of water regimes on chlorophyll content index was not statistically significant, the over-all pattern showed that chlorophyll content index was lower for rainfed plants compared with irrigated plants. This trend was in line with reports in the literature of decreasing chlorophyll content in response to water stress in crops such as cotton (Massacci *et al.*, 2008), sunflower (Kiani *et al.*, 2008) and *Vaccinium myrtillus* (Tahkokorpi *et al.,* 2007). Cowpea varieties showed highly significant differences in chlorophyll content index; Brown birch variety had higher chlorophyll content index than White birch variety. Chlorophyll content index increased with time, reaching a maximum of 78 and 65 at 77 DAP for Brown birch variety under irrigated and rainfed conditions, respectively; a similar trend was observed for White birch variety. However, at 89 DAP chlorophyll content index decreased for both varieties and this decrease can be associated with plant growth stage. From such results it can be hypothesised that chlorophyll content increased during vegetative growth, reaching a peak before decreasing as the crop started to mature. Therefore, chlorophyll content index may be a useful indicator for crop maturity in cowpea.

Results of stomatal conductance (SC) were consistent with reports in the literature. Irrigated conditions had higher SC than rainfed conditions; these observations suggest stomatal regulation as a drought tolerance mechanism in cowpeas. Reduction of SC under rainfed conditions implies that plants were able to close their stomata in order to minimise water losses. Hamidou *et al.* (2007) reported that five cowpea varieties possessed a drought avoidance mechanism which involved decreasing stomatal conductance in response to water deficit conditions. Genotypic differences with respect to SC were observed in this study and since the varieties differ in seed colour, these differences can be associated with seed colour. However, despite varietal differences, the overall pattern showed that stomata closed in response to water stress which is consistent with previous studies. Cowpea is known to have good stomatal regulation (Hall *et al.*, 1997; Scotti *et al.*, 1999; Cruz de Carvalho, 2000; Sarr *et al.*; 2001; Ogbonnaya *et al.*, 2003).

Stomatal conductance was found to vary significantly over time; these variations can be explained with the aid of the weather data. The first measurement of SC was done at 69 DAP which coincided with a period where there had been no rainfall received during the past few days. As such, SC was low under rainfed conditions meaning that stomata were closed in order to avoid water loss. Stomatal conductance was high under irrigated conditions owing to supplementary irrigation and high soil water content. At 77 DAP, SC slightly increased in both water regimes; this increase can be justified by rainfall (1.18 mm) received at 69 DAP. At 77 DAP, an average of 2.38 mm of rainfall was recorded; this then explains the increase in SC observed in rainfed cowpeas.

One of the objectives of this experiment was to determine the interactive effect of water regimes and sequential leaf harvesting on growth and yield of cowpea varieties. Results of the study showed that there was no interaction between these factors with respect to leaf number and yield. However, leaf harvesting was found to have a highly significant (P<0.001) effect on leaf number. As expected, sequential harvesting of leaves resulted in lower leaf number relative to crops where there was no leaf harvesting. The capacity of the crop to recover from leaf harvesting suggested that the two varieties used in this study may be suitable for cultivation as leafy vegetables although sequential leaf harvesting was found to decrease pod yield. These observations were expected since leaf harvesting is a form of plant manipulation which alters the source-sink relationship (Shibles *et al.*, 1981). Within the context of this study, sequential harvesting of leaves slowed down and reduced vegetative growth which accounts for biomass accumulation and assimilate reserves. As a result, photosynthates were used to replenish the lost vegetation as opposed to pod formation and filling; thus, the canopy was a stronger sink than the pods. It was also reported that leaf removal alters hormone balance, starch, sugar, protein and chlorophyll content of the source leaves as well as stomatal resistance and senescence rate (Mondel *et al.,* 1978; Selter *et al.,* 1980).

4.6 Conclusions

The varieties used in this study are mainly used for pastures and fodder; however, we were seeking to explore the possibility of using the varieties as dual purpose crops. The results obtained from the study showed that Brown birch variety cannot be used as a dual purpose crop, especially under irrigated conditions. This variety favoured vegetative growth more than pod formation. White birch variety, on the other hand, can be used as a dual purpose crop since the crop was able to form pods despite having its leaves harvested. White birch variety also performed well under rainfed conditions. These were interesting observations since it was expected that plants would perform and yield better under irrigated than rainfed conditions. Contrary to these expectations, Brown birch variety produced satisfactory yield under rainfed with White birch variety yielding better under rainfed compared with irrigated conditions. It can be concluded therefore, that rainfed conditions are favourable for cowpea growth and yield formation.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The findings of the present study revealed that cowpea is a potential crop that can be used to deal with and possibly overcome the challenges faced by farmers in marginal areas of agricultural production. These challenges include water scarcity, food insecurity and malnutrition. Cowpea shows great potential since the crop is drought tolerant, nutritious and has multi-purpose uses. Despite such potential, the crop still remains a neglected and underutilised crop. The general aim of the current study was to evaluate two cowpea (Brown birch and White birch) varieties for their ability to withstand drought stress. The two cultivars were also evaluated within the context of their alternative use as leafy vegetables. Water stress and high temperature stress have always been studied as separate entities, although they often occur simultaneously under field conditions, especially in areas where cowpea is mostly cultivated (Machado and Paulsen, 2001). As a result, this study also undertook to evaluate crop growth and yield responses of cowpea to water stress under different temperature regimes.

The overall objectives of the study were:

- to compare the quality of cowpea varieties that differed in terms of their seed coat colour,
- to evaluate the response of the crop to water stresses imposed at different stages of growth and high temperatures; and therefore find the interactive effect of temperature and water stress on growth productivity of the crop, and
- to compare the dry land and irrigated production of cowpea and further determine the effect of sequential harvesting on plant growth and grain yield of cowpeas.

Aspects of seed quality consist of basic information required when studying aspects of any crop. This is because growth parameters such as emergence and good stand establishment depend on the quality of a seed lot. Therefore, it was imperative for the current study to determine the quality of cowpea seeds; this was done on a comparative basis with respect to seed coat colour (Chapter 2). The two cowpea varieties (Brown and White birch) were not significantly different with respect to germination capacity. Although not statistically significant, observations of mean germination time (MGT) and germination velocity index (GVI) showed that Brown birch had higher seed vigour compared with White birch. White birch had a high electrolyte conductivity compared with Brown birch. These results suggested that light coloured seeds of cowpea had lower seed quality compared with dark coloured seeds.

A water stress study is incomplete without temperature evaluation. This is because water stress is often associated with high temperatures. Therefore, the effect of water stress imposed at different growth stages and varying growth temperatures was evaluated in Chapter 3. The objective of this study was to determine the combined effects of high temperatures and water stress on growth and development of cowpea. It has been reported that the vegetative stage is the most sensitive to water stress (Turk *et al*, 1980; Ahmed and Suliman, 2010). However, Watanabe *et al*. (1997) reported that some cowpea varieties were not able to survive drought imposed at the vegetative stage. In the current study, imposing water stress during vegetative growth stage reduced growth and productivity of cowpea. Interestingly, water stress imposed during the flowering stage had a positive effect on growth and yield of cowpea. Terminal stress was found to reduce growth and yield of cowpeas; however, the plants were still able to produce reasonable yields. Results of this study revealed that cowpeas perform well in terms of growth and productivity under high temperatures (33/27ºC). Ehlers and Hall (1997) also reported that cowpea is adapted to high temperatures. Results obtained from this study suggested that temperature was more limiting to growth, development and productivity compared with water stress when temperatures are low. Whereas under high temperatures; water stress was more limiting to plant growth and productivity. The latter scenario is more typical of the conditions that prevail in most marginal areas of agricultural production. As such, the fact that cowpea performed well under high temperatures and still produced reasonable yield under water stress suggests that it may be a suitable crop for production in these areas.

Cowpea possesses two major attributes that make it a potentially suitable crop to fight against poverty and food insecurity. The crop is drought tolerant and has nutritious leaf material and grain. There have been several studies investigating drought tolerance in cowpea (Dadson *et al.,* 2005; Lisokwe and Lawn, 2008). There have also been studies investigating uses of cowpea as a leafy vegetable (Inaizumi *et al.,* 1999; Ibrahim *et al.,* 2010; Matikiti *et al.,* 2009). However, research has seldom investigated the effect of water stress and sequential leaf harvesting on plant growth and grain yield, as such a study of this nature was undertaken (Chapter 4). Results obtained from Chapter 2 suggested that Brown birch had higher vigour than White birch. As a result, it was expected that Brown birch would perform better than White birch under field conditions. However, results of the field study were contrary to expectation; White birch was observed to perform better with respect to yield under both water regimes (Table 4.1). Therefore, based on these observations, seed quality results alone cannot be used to extrapolate yield potential of the crop.

Results for growth parameters (plant height and leaf number) obtained from the pot trial (Chapter 3) verified results obtained from the field trial (Chapter 4) with respect to differences in growth pattern of cowpea varieties. Brown birch was observed to be taller and had few leaves and White was shorter with many leaves. Therefore, it can be concluded that differences in varieties were due to differences in growth pattern.

One of the aims of this study was to explore the possibility of using cowpea varieties (Brown and White birch) as dual purpose crops. The results showed that Brown birch is not suitable for use as a leafy vegetable and for grain, especially under irrigated conditions. Brown birch favours vegetative growth more than pod formation, and this becomes prominent when water is not limiting. On the other hand, White birch showed a potential for use as a dual purpose crop. Cowpea grows well under water limited conditions because even Brown birch produced satisfactory yield under rainfed conditions, with White birch yielding better under rainfed compared with irrigated conditions.

High temperatures improved cowpea growth and productivity. For the varieties used in the current study, the vegetative growth stage was found to be the most sensitive to water stress. The interaction between temperature and water stress showed that temperature is more influential to growth, development and productivity compared to water stress when temperature conditions are low. However, under high temperatures water stress is more influential on plant growth and productivity. Brown birch variety cannot be used as a dual purpose crop, especially under irrigated conditions. White birch has a potential for use as both leafy vegetable and grain crop. Rainfed conditions are favourable for cowpea growth and yield formation.

Recommendations

- Brown birch variety was found to be better than white birch with respect to vigour. However, more research is required to determine if superior seed quality (viability and vigour) of brown birch would translate to yield advantages under field conditions.
- Furthermore, future research should also evaluate the physiological basis for the association between seed coat colour and seed quality.
- More research is required to evaluate the physiological basis of cowpea response to water and temperature stress interaction.
- 33/27ºC tends to become an optimum temperature for cowpea growth and productivity. Further research to evaluate response of cowpea to temperatures higher than 33/27ºC is recommended.
- Data obtained from this study may be very useful to people who are interested in cultivating cowpea.

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APPENDICES

Appendix 2.1: List of ANOVAs for seed quality experiments (Chapter 2)

Variate: Germ%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	258.04	86.01	1.39	
Rep.*Units* stratum					
Variety	1	0.45	0.45	0.01	0.933
Day	6	24552.90	4092.15	66.03	<.001
Variety.Day	6	94.87	15.81	0.26	0.954
Residual	39	2416.96	61.97		
Total	55	27323.21			

Variate: Dry_mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.04554	0.01518	0.72	
Rep._units_ stratum					
Variety	1	0.40240	0.40240	18.97	<.001
Residual	35	0.74241	0.02121		
Total	39	1.19036			

Variate: Fresh_mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.23779	0.07926	2.30	
Rep._units_ stratum					
Variety	1	0.01222	0.01222	0.35	0.556
Residual	35	1.20837	0.03452		
Total	39	1.45837			

Variate: Root:Shoot ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.7543	0.2514	2.08	
Rep._units_ stratum					
Variety	1	0.0363	0.0363	0.30	0.588
Residual	35	4.2389	0.1211		
Total	39	5.0295			

Variate: Root_length (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	43.086	14.362	3.26	
Rep._units_ stratum					
Variety	1	140.625	140.625	31.93	<.001
Residual	35	154.149	4.404		
Total	39	337.860			

Variate: Shoot_length (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.9827	0.6609	0.91	
Rep._units_ stratum					
Variety	1	41.0063	41.0063	56.73	<.001
Residual	35	25.3007	0.7229		
Total	39	68.2898			

Variate: EC_(uS_g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	2872514.	2872514.	8.25	0.005
Residual	98	34142204.	348390.		
Total	99	37014719.			

Appendix 3.1: List of ANOVAs for the controlled environment trial (Chapter 3)

Variate: SWC

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		92.55	46.27	1.67	
Rep.*Units* stratum						
Temp	2		90062.48	45031.24	1623.07	<.001
Treatment	3		4608.62	1536.21	55.37	<.001
Variety	1		130.09	130.09	4.69	0.031
DAS	10		6868.73	686.87	24.76	<.001
Temp.Treatment	6		3849.25	641.54	23.12	<.001
Temp.Variety	2		148.72	74.36	2.68	0.070
Treatment.Variety	3		387.67	129.22	4.66	0.003
Temp.DAS	20		8170.05	408.50	14.72	<.001
Treatment.DAS	30		2236.93	74.56	2.69	<.001
Variety.DAS	10		152.11	15.21	0.55	0.856
Temp.Treatment.Variety	6		231.72	38.62	1.39	0.216
Temp.Treatment.DAS	60		4668.25	77.80	2.80	<.001
Temp.Variety.DAS	20		420.30	21.01	0.76	0.765
Treatment.Variety.DAS	30		517.50	17.25	0.62	0.944
Temp.Treatment.Variety.DAS	60		1555.41	25.92	0.93	0.617
Residual	483	(43)	13400.55	27.74		
Total	748	(43)	134088.08			

Variate: Emegernce

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3134.9	1567.5	9.29	
Rep.Temp stratum					
Temp	2	282182.5	141091.3	836.59	<.001
Residual	4	674.6	168.7	0.07	
Rep.Temp.Vareity stratum					
Vareity	1	11428.6	11428.6	4.72	0.073
Temp.Vareity	2	119.0	59.5	0.02	0.976
Residual	6	14523.8	2420.6	3.13	
Rep.Temp.Vareity.*Units* stratum					
DAP	6	397182.5	66197.1	85.52	<.001
Temp.DAP	12	118650.8	9887.6	12.77	<.001
Vareity.DAP	6	4404.8	734.1	0.95	0.460
Temp.Vareity.DAP	12	25714.3	2142.9	2.77	0.001
Residual	450	348333.3	774.1		
Total	503	1206349.2			

Variate: Leaf Area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	143399.	71700.	5.83	
Rep.Temp stratum					
Temp	2	643474.	321737.	26.15	0.005
Residual	4	49215.	12304.	0.65	
Rep.Temp.Variety stratum					
Variety	1	763760.	763760.	40.17	<.001
Temp.Variety	2	52507.	26253.	1.38	0.321
Residual	6	114080.	19013.	0.40	
Rep.Temp.Variety.*Units* stratum	54 .	2573933.	47665		
Total	71	4340368.			

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	188.26	94.13	0.36	
Rep.Temp stratum					
Temp	2	70701.44	35350.72	135.14	<.001
Residual	4	1046.37	261.59	1.80	
_Rep.Temp.Treatment stratum					
Treatment	3	4882.60	1627.53	11.22	<.001
Temp.Treatment	6	9038.92	1506.49	10.38	<.001
Residual	18	2611.51	145.08	1.90	
_Rep.Temp.Treatment.*Units* stratum					
Variety	1	2029.62	2029.62	26.60	<.001
DAS	6	19712.45	3285.41	43.05	<.001
Temp.Variety	2	616.79	308.39	4.04	0.019
Treatment.Variety	3	1386.50	462.17	6.06	<.001
Temp.DAS	12	6037.30	503.11	6.59	<.001
Treatment.DAS	18	2901.07	161.17	2.11	0.006
Variety.DAS	6	295.17	49.20	0.64	0.694
Temp.Treatment.Variety	6	1349.98	225.00	2.95	0.008
Temp.Treatment.DAS	36	5793.42	160.93	2.11	<.001
Temp.Variety.DAS	12	211.30	17.61	0.23	0.997
Treatment.Variety.DAS	18	1008.78	56.04	0.73	0.775
Temp.Treatment.Variety.DAS	36	1429.04	39.70	0.52	0.990
Residual	312	23809.19	76.31		
Total	503	155049.72			

VARIATE: LEAF NUMBER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1706	0.0853	0.02	
Rep.Temp stratum					
Temp	2	2043.1111	1021.5556	219.23	<.001
Residual	4	18.6389	4.6597	0.71	
Rep.Temp.Treatment stratum					
Treatment	3	48.9266	16.3089	2.47	0.095
Temp.Treatment	6	163.4603	27.2434	4.13	0.009
Residual	18	118.8095	6.6005	8.38	
Rep.Temp.Treatment.*Units* stratum					
Variety	1	77.0020	77.0020	97.77	<.001
DAS	6	684.5476	114.0913	144.87	<.001
Temp.Variety	2	27.4921	13.7460	17.45	<.001
Treatment.Variety	3	41.8631	13.9544	17.72	<.001
Temp.DAS	12	84.0000	7.0000	8.89	<.001
Treatment.DAS	18	32.3095	1.7950	2.28	0.002
Variety.DAS	6	15.1508	2.5251	3.21	0.005
Temp.Treatment.Variety	6	18.0952	3.0159	3.83	0.001
Temp.Treatment.DAS	36	66.7619	1.8545	2.35	<.001
Temp.Variety.DAS	12	4.0635	0.3386	0.43	0.951
Treatment.Variety.DAS	18	17.2619	0.9590	1.22	0.245
Temp.Treatment.Variety.DAS	36	17.2381	0.4788	0.61	0.964
Residual	312	245.7143	0.7875		
Total	503	3724.6171			

VARIATE: CHLOROPHYLL CONTENT INDEX

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		57.87	28.93	0.64	
Rep.Temp stratum						
Temp	2		19920.75	9960.38	219.75	<.001
Residual	4		181.30	45.33	0.86	
Rep.Temp.Treatment stratum						
Treatment	3		257.18	85.73	1.64	0.216
Temp.Treatment	6		710.95	118.49	2.26	0.084
Residual	18		943.41	52.41	1.21	
Rep.Temp.Treatment.*Units* stratum						
Variety	1		508.87	508.87	11.74	<.001
Temp.Variety	2		263.40	131.70	3.04	0.049
Treatment.Variety	3		118.05	39.35	0.91	0.437
Temp.Treatment.Variety	6		386.84	64.47	1.49	0.182
Residual	303	(9)	13128.04	43.33		
Total	350	(9)	35880.32			

Variate: %4m_gDW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.185E-08	5.927E-09	2.39	
Rep.*Units* stratum					
Water_treatment	3	1.414E-05	4.715E-06	1897.84	<.001
Variety	1	8.624E-07	8.624E-07	347.16	<.001
Water_treatment.Variety	3	2.705E-06	9.018E-07	363.00	<.001
Residual	14	3.478E-08	2.484E-09		
Total	23	1.776E-05			

VARIATE: TOTAL_BIOMASS_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	46.12	23.06	1.73	
Rep.*Units* stratum					
Water_treatments	3	531.50	177.17	13.31	<.001
Variety	1	44.34	44.34	3.33	0.089
Water_treatments.Variety	3	78.46	26.15	1.97	0.166
Residual	14	186.29	13.31		
Total	23	886.71			

VARIATE: SEED # per POD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.050	3.025	0.54	
Rep.*Units* stratum					
Water_treatments	3	37.099	12.366	2.20	0.134
Variety	1	17.300	17.300	3.07	0.101
Water_treatments.Variety	3	1.792	0.597	0.11	0.955
Residual	14	78.766	5.626		
Total	23	141.007			

VARIATE: SEED_MASS/PLANT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.570	0.785	0.23	
Rep.*Units* stratum					
Water_treatments	3	67.917	22.639	6.77	0.005
Variety	1	0.341	0.341	0.10	0.754
Water_treatments.Variety	3	3.659	1.220	0.37	0.779
Residual	14	46.783	3.342		
Total	23	120.270			

VARIATE: POD # /PLANT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.083	0.542	0.18	
Rep.*Units* stratum					
Water_treatments	3	35.167	11.722	3.95	0.031
Variety	1	24.000	24.000	8.08	0.013
Water_treatments.Variety	3	2.000	0.667	0.22	0.878
Residual	14	41.583	2.970		
Total	23	103.833			

VARIATE: POD_MASS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.351	0.675	0.14	
Rep.*Units* stratum					
Water_treatments	3	107.961	35.987	7.60	0.003
Variety	1	0.150	0.150	0.03	0.861
Water_treatments.Variety	3	6.208	2.069	0.44	0.730
Residual	14	66.308	4.736		
Total	23	181.978			

VARIATE: HI_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	116.6	58.3	0.51	
Rep.*Units* stratum					
Water_treatments	3	1263.7	421.2	3.68	0.038
Variety	1	340.9	340.9	2.98	0.106
Water_treatments.Variety	3	709.0	236.3	2.06	0.151
Residual	14	1603.2	114.5		
Total	23	4033.4			

VARIATE: POD_LENGTH_CM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.392	3.696	1.59	
Rep.*Units* stratum					
Water_treatments	3	65.542	21.847	9.42	0.001
Variety	1	40.289	40.289	17.36	<.001
Water_treatments.Variety	3	0.109	0.036	0.02	0.997
Residual	14	32.483	2.320		
Total	23	145.815			

Appendix 4.1: List of ANOVAs for the field trial (Chapter 4)

VARIATE: PLANT HEIGHT (CM)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	268.839	134.420	14.19	
Rep.*Units* stratum					
Treatment	1	1616.822	1616.822	170.71	<.001
Variety	5	754.801	150.960	15.94	<.001
DAP	4	16645.278	4161.319	439.36	<.001
Treatment.Variety	5	113.256	22.651	2.39	0.042
Treatment.DAP	4	599.903	149.976	15.83	<.001
Variety.DAP	20	229.088	11.454	1.21	0.259
Treatment.Variety.DAP	20	80.093	4.005	0.42	0.985
Residual	118	1117.627	9.471		
Total	179	21425.707			

VARIATE: LEAF NUMBER

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		74.835	37.418	6.67	
Rep.*Units* stratum						
Treatment	1		0.005	0.005	0.00	0.977
Variety	1		600.292	600.292	106.95	<.001
Harvest	2		213.841	106.920	19.05	<.001
DAP	6		10349.984	1724.997	307.33	<.001
Treatment.Variety	1		8.881	8.881	1.58	0.211
Treatment.Harvest	2		5.017	2.508	0.45	0.640
Variety.Harvest	2		12.779	6.389	1.14	0.323
Treatment.DAP	6		158.919	26.486	4.72	<.001
Variety.DAP	6		352.539	58.757	10.47	<.001
Harvest.DAP	9	(3)	712.163	79.129	14.10	<.001
Treatment.Variety.Harvest	2		2.948	1.474	0.26	0.769
Treatment.Variety.DAP	6		49.095	8.182	1.46	0.197
Treatment.Harvest.DAP	9	(3)	18.014	2.002	0.36	0.953
Variety.Harvest.DAP	9	(3)	11.378	1.264	0.23	0.990
Treatment.Variety.Harvest.DAP	9	(3)	35.578	3.953	0.70	0.704
Residual	142	(24)	797.024	5.613		
Total	215	(36)	9840.762			

VARIATE: CCI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	707.7	353.9	3.53	
Rep.*Units* stratum					
Treatment	1	257.8	257.8	2.57	0.123
Variety	1	3599.9	3599.9	35.87	<.001
DAP	2	1711.4	855.7	8.53	0.002
Treatment.Variety	1	25.0	25.0	0.25	0.622
Treatment.DAP	2	444.6	222.3	2.22	0.133
Variety.DAP	2	186.2	93.1	0.93	0.410
Treatment.Variety.DAP	2	42.5	21.2	0.21	0.811
Residual	22	2207.8	100.4		
Total	35	9183.0			

Variate: SC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	801.2	400.6	1.35	
Rep.*Units* stratum					
Treatment	1	78227.1	78227.1	263.50	<.001
Variety	1	4862.6	4862.6	16.38	<.001
DAP	2	41144.7	20572.4	69.30	<.001
Treatment.Variety	1	298.2	298.2	1.00	0.327
Treatment.DAP	2	16951.4	8475.7	28.55	<.001
Variety.DAP	2	3917.7	1958.8	6.60	0.006
Treatment.Variety.DAP	2	5449.8	2724.9	9.18	0.001
Residual	22	6531.3	296.9		
Total	35	158183.9			

Variate: LAI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.132	1.066	0.16	
Rep.Treatment stratum					
Treatment	1	323.995	323.995	49.46	0.020
Residual	2	13.100	6.550	4.29	
Rep.Treatment.Variety stratum					
Variety	1	3.659	3.659	2.40	0.197
Treatment.Variety	1	10.666	10.666	6.98	0.057
Residual	4	6.110	1.528	0.86	
Rep.Treatment.Variety.*Units* stratum					
DAP	2	18.246	9.123	5.11	0.008
Treatment.DAP	2	34.729	17.365	9.72	<.001
Variety.DAP	2	1.229	0.615	0.34	0.710
Treatment.Variety.DAP	2	5.128	2.564	1.44	0.244
Residual	88	157.181	1.786		
Total	107	576.177			

Variate: TOTAL BIOMASS (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	547.82	273.91	3.52	
Rep.*Units* stratum					
Treatment	1	0.20	0.20	0.00	0.960
Harvest	2	1320.49	660.24	8.49	0.002
Variety	1	2642.65	2642.65	33.98	<.001
Treatment.Harvest	2	93.90	46.95	0.60	0.556
Treatment.Variety	1	91.20	91.20	1.17	0.291
Harvest.Variety	2	451.77	225.88	2.90	0.076
Treatment.Harvest.Variety	2	61.40	30.70	0.39	0.679
Residual	22	1711.15	77.78		
Total	35	6920.58			

VARIATE: HARVEST INDEX (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	97.8	48.9	0.44	
Rep.*Units* stratum					
Treatment	1	2080.2	2080.2	18.57	<.001
Harvest	2	292.5	146.3	1.31	0.291
Variety	1	6364.2	6364.2	56.80	<.001
Treatment.Harvest	2	366.7	183.4	1.64	0.217
Treatment.Variety	1	508.4	508.4	4.54	0.045
Harvest.Variety	2	363.5	181.7	1.62	0.220
Treatment.Harvest.Variety	2	840.4	420.2	3.75	0.040
Residual	22	2464.8	112.0		
Total	35	13378.5			

VARIATE: SEED MASS PER PLANT (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	18.398	9.199	1.04	
Rep.*Units* stratum					
Treatment	1	204.347	204.347	23.08	<.001
Harvest	2	36.642	18.321	2.07	0.150
Variety	1	121.698	121.698	13.74	0.001
Treatment.Harvest	2	4.165	2.083	0.24	0.792
Treatment.Variety	1	32.967	32.967	3.72	0.067
Harvest.Variety	2	28.435	14.217	1.61	0.223
Treatment.Harvest.Variety	2	19.462	9.731	1.10	0.351
Residual	22	194.821	8.855		
Total	35	660.934			

VARIATE: SEED NUMBER PER POD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	16.359	8.180	1.25	
Rep.*Units* stratum					
Treatment	1	194.277	194.277	29.70	<.001
Harvest	2	4.522	2.261	0.35	0.712
Variety	1	71.205	71.205	10.88	0.003
Treatment.Harvest	2	24.608	12.304	1.88	0.176
Treatment.Variety	1	3.139	3.139	0.48	0.496
Harvest.Variety	2	4.428	2.214	0.34	0.717
Treatment.Harvest.Variety	2	37.262	18.631	2.85	0.079
Residual	22	143.918	6.542		
Total	35	499.719			

VARIATE: POD NUMBER PER PLANT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	13.113	6.556	1.24	
Rep.*Units* stratum					
Treatment	1	58.039	58.039	10.94	0.003
Harvest	2	27.439	13.719	2.59	0.098
Variety	1	120.890	120.890	22.79	<.001
Treatment.Harvest	2	6.851	3.426	0.65	0.534
Treatment.Variety	1	0.595	0.595	0.11	0.741
Harvest.Variety	2	12.727	6.364	1.20	0.320
Treatment.Harvest.Variety	2	21.637	10.819	2.04	0.154
Residual	22	116.704	5.305		
Total	35	377.995			

VARIATE: POD MASS (g)

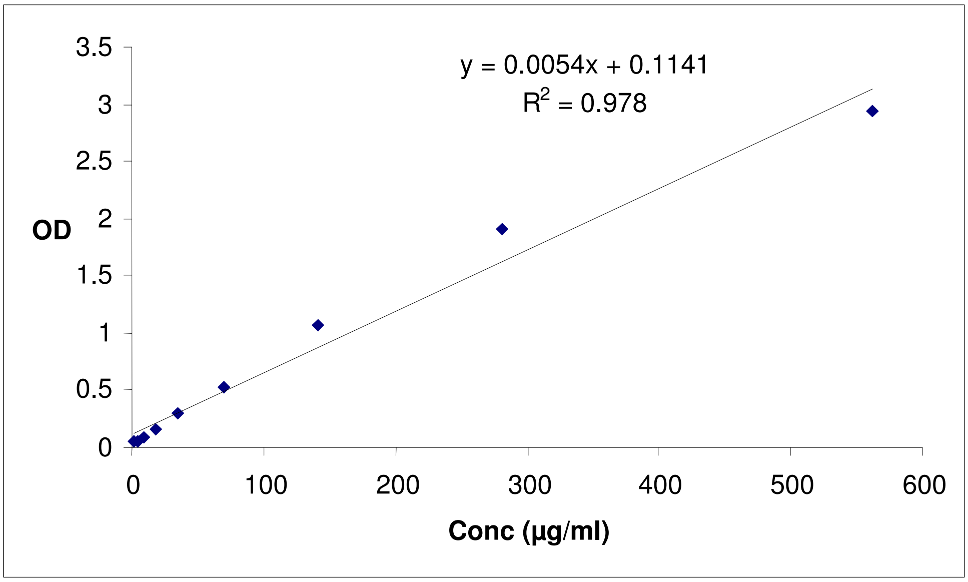
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	58.80	29.40	1.39	
Rep.*Units* stratum					
Treatment	1	296.76	296.76	14.05	0.001
Variety	1	254.93	254.93	12.07	0.002
Harvest	2	136.53	68.27	3.23	0.059
Treatment.Variety	1	14.29	14.29	0.68	0.420
Treatment.Harvest	2	15.95	7.97	0.38	0.690
Variety.Harvest	2	22.34	11.17	0.53	0.597
Treatment.Variety.Harvest	2	54.11	27.05	1.28	0.298
Residual	22	464.79	21.13		
Total	35	1318.50			

Appendix 5: Physical characteristics of the soil used for the pot trial experiment

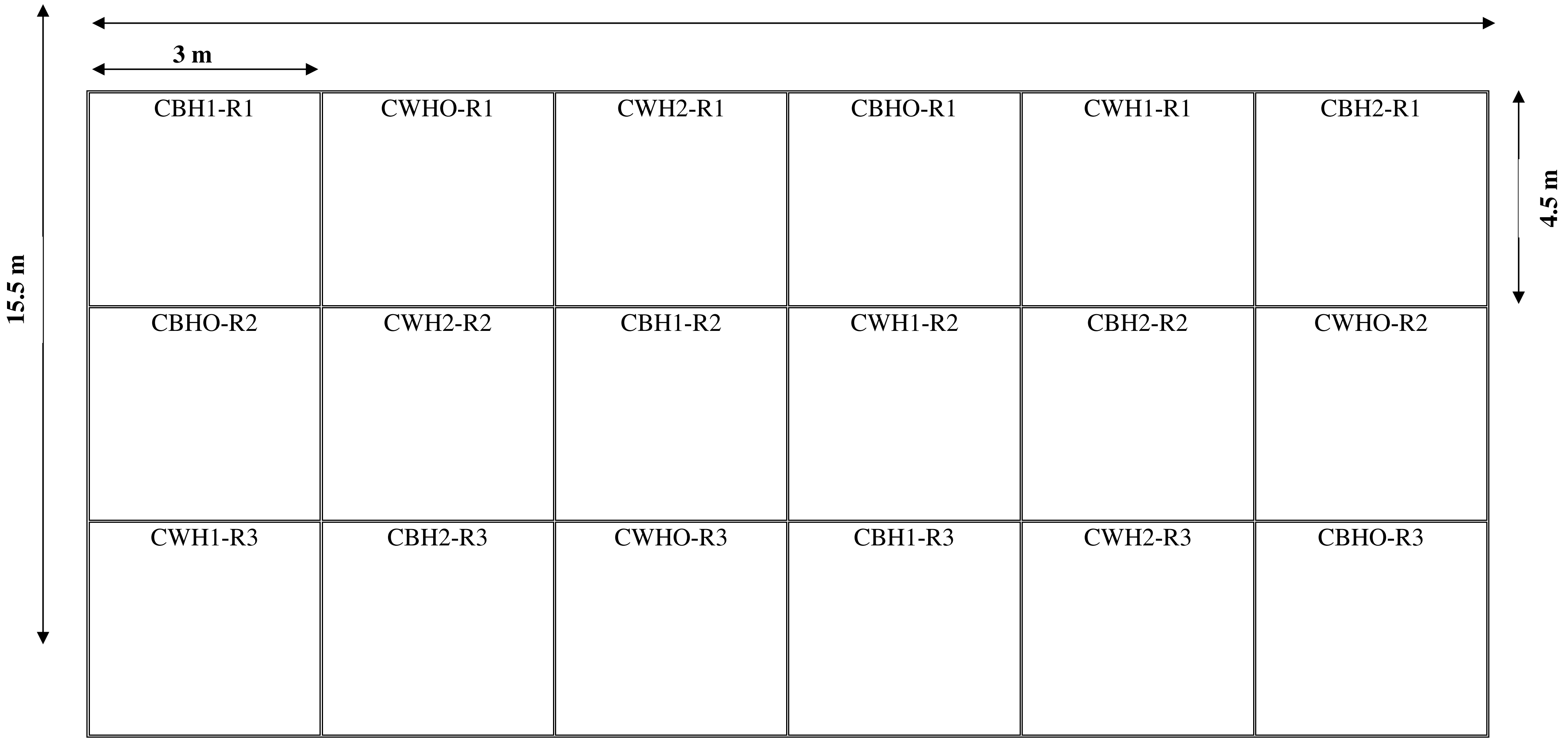
	^{v}PWP	^{w}FC	Clay	Sand	Silt
Textural class	——— vol % ———			———%———	
Clay Loam	28.3	40.6	43.5	24	32.5

*SA Taxonomic system; ^{v}PWP – permanent wilting point; ^{w}FC – field capacity.

Appendix 6: Proline standard curve



Appendix 7: Field trial layout of cowpea



CBH1-R1	CWHO-R1	CWH2-R1	CBHO-R1	CWH1-R1	CBH2-R1
CBHO-R2	CWH2-R2	CBH1-R2	CWH1-R2	CBH2-R2	CWHO-R2
CWH1-R3	CBH2-R3	CWHO-R3	CBH1-R3	CWH2-R3	CBHO-R3