Physiological responses of cowpea (*Vigna unguiculata*) to water stress under varying water regimes

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by the Water Research Commission (WRC) of South Africa through WRC Project No. K5/2272//4 'Determining water use of indigenous grain and legume food crops'.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Supervisor

Date: 05 August 2014

DECLARATION

I, Kalanda Ilunga, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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ABSTRACT

Water stress has been reported as one of the most important environmental factors affecting crop productivity in the world, particularly in semi– and arid regions. Climate change, through changes in rainfall amount and patterns, remains a serious threat to crop productivity in these regions that are already food insecure. There is a need to identify and promote more drought tolerant crops with low levels of water use for production in these areas. Cowpea [*Vigna unguiculata* (L) Walp.] has been reported to be more adapted to drought-prone conditions, compared to other crops. Its multi-purpose uses, high protein content and potential to biologically fix nitrogen makes it best suited for production by resource-poor farmers. However, cowpea has not been given the attention it deserves as a crop that has potential to contribute towards food security and improve diets of people living in marginal areas of agricultural production. This study evaluated cowpea physiological responses to water stress under controlled and field conditions. Two cowpea varieties (Brown mix and White birch) were evaluated for seed quality, on a comparative basis of seed coat colour, using standard germination and electrolyte conductivity tests, under laboratory conditions. A pot trial was conducted under controlled environmental conditions (33/27°C day/night; 65% RH) to evaluate cowpea responses to water stress under three water regimes (30% ETc, 60% ETc, and 80% ETc). Thereafter, field trials were conducted to determine the effect of planting date selection on cowpea productivity under irrigated and rainfed conditions. Results of seed quality showed that the Brown mix variety was more viable than White birch. However, results of vigour were contrary to results of viability and indicated that the White birch was more vigorous than the Brown mix. Under controlled environmental conditions, water stress had a negative effect on cowpea stomatal conductance, thereby limiting plant growth and productivity. Water stress had no effect on leaf chlorophyll content index. For all three planting dates, cowpea emergence was affected by temperature; the crop requires warm temperatures for successful stand establishment. Consequently, growth and physiology were also more affected by temperature than water availability. Cowpea performed better under rainfed than irrigated conditions and produced more yield. The Brown mix variety seemed to favour vegetative growth over reproductive growth and thus maybe suitable for production as a leafy vegetable. Overall, the White birch variety was more adapted to limited water availability than Brown mix.

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DEDICATION

This thesis is dedicated to:

My wife FOMA NASIMA. Thank you NASIMA for your sacrifice, understanding and patience during my study. I'm very grateful to have you as a wife.

My son Eden ILUNGA. You were too small and cried every time when I was leaving for Pietermaritzburg. I shall never forget your tears, my son.

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CHAPTER 1

1.1 Introduction

Cowpea [*Vigna unguiculata* (L) Walp.] is still underutilised in many countries including South Africa (Dudje et al. 2009). Cowpea is an important grain legume largely grown in warm and hot regions of Africa, Asia as well as North and South America (Ehlers and Hall 1997). Its agronomic potential relative to current increasing population and climate change that threaten the world make it a crop of choice for agricultural researchers. It is reported to be well-adapted to high temperatures and drought conditions (Surabhi et al. 2009). In addition to being drought tolerant, some varieties have a short production cycle and matures early providing food during the period of hunger when food becomes extremely scarce in semi-arid regions of sub-Saharan Africa (Cisse' and Hall 2002). Its multi-purpose uses make it an attractive alternative for farmers residing in marginal and drought-prone areas. Such areas are usually characterised by low rainfall, high temperatures and less developed or no irrigation systems. Other challenges facing farmers in these areas include, but are not limited to, poor infrastructure, food insecurity and malnutrition (Hallensleben et al. 2009). Its dual purpose production offers versatility through utilisation of both foliage and seed from the same crop (Bubenheim et al. 1990). Both cowpea leaves and grain can play an important role towards meeting the nutritional requirements of humans, especially of resource poor families (Saidi et al. 2010).

Cowpea is an important component in most cereal-legume cropping systems because of its residual nitrogen benefit originating from the decay of its leaf litter, roots and root nodules (Asiwe 2009). Its shade tolerance and compatibility as an intercrop make it the crop of choice for arid zones (Nagalakshmi et al. 2010). It is mostly produced under rainfed conditions by small scale-farmers, and can grow well in poor soils having more than 85% sand, less than 0.2% organic matter and low levels of phosphorus (Singh 2003). In addition, the crop has a high potential to biologically fix nitrogen, and has tri-purpose utilisation, producing vegetable leaves and pods, dry grain and forage. These characteristics distinguish cowpea from many other crops currently grown in Africa (Cisse' and Hall 2002).

Cowpea is slowly gaining popularity in developing countries, especially in arid regions of the world due to its nutritional value (Nagalakshmi et al. 2010). The seeds have high quality protein content that is comparable to other grain legumes and are rich in amino acids such as lysine and tryptophan. Due to the crop's high protein content, it is also used as a nutritional supplement in cereal and animal diets (Davis et al. 1991). Its high protein content is reported to have the capacity to satisfy dietary requirements in food insecure countries, particularly in Africa, where over 200 million people remain malnourished (Atta 2007). This is as a result of food production lagging behind population growth, soaring demand for livestock products, and new stresses brought about by climate change into the already challenging cropping conditions (Fatokun 2010).

Global agricultural production is often limited by environmental factors such as temperature and water stress. The main constraint is water availability and this affects a larger part of the world and limit crop yields (Chebouti and Abdelguerfi 2004). Successive reports from the International Experts Group on Climate Evolution (GIEC) predicted that drought and flooding could disrupt cropping systems in the next decades (Olivier 2008). Under the climate change, drought has been, and is becoming an acute problem mostly constraining plant growth and terrestrial ecosystem productivity, particularly in arid and semi-arid areas (Xu et al. 2010).

South Africa is a country that does not have an abundant supply of water and could well be described as a semi–desert region with a water shortage (World Meteorological Organization 2006). The average annual rainfall of South Africa is 397 mm, compared with a world average of 860 mm. The distribution of it is uneven throughout the country and most of it is received during the summer season between October and March. During this time frame, it fluctuates greatly across time and space usually resulting in sporadic and at times severe episodes of water stress (Laker, 2007). Evaporation (ranging from 1 100 to 3 000 mm annually) is comparatively higher than the worldwide average of 1 130 mm (Babkin 2009). Drought has become a recurrent feature affecting South Africa. In the past, drought has resulted in significant economic, environmental and social impact (World Meteorological Organization 2006).

In response to recurring drought in South Africa, detailed research of the cowpea's physiological responses to water stress is important to help farmers improve its yield and overcome production constraints, since crop physiological changes is results of crop response to environment. This will also help to characterise the crop for water stress since crop physiology is related to drought stress. The crop has the potential to provide food to the ever increasing human population in developing countries. There is currently limited data on the crop's physiology in response to water stress. Where it is available, production data for cowpea is often pooled with that of common bean (*Phaseolus vulgaris*) (FAO 2006). Findings from such a study will contribute to increasing cowpea production.

1.2 Aims and objectives

The aim of this study was therefore to identify the physiological (stomatal conductance and chlorophyll content) changes at different stages of cowpea growth and development in response to water stress under controlled and field conditions. Secondly, the study evaluated the effect of planting date selection as a management tool for managing water stress under field conditions. It was hypothesised that growth, physiology and yield of cowpeas was not sensitive to varying environmental conditions and water regimes. Hence, the specific objectives of this study were:

- to determine seed quality of cowpea varieties and their field planting value,
- to evaluate growth, physiology and yield of cowpea varieties in response to different water regimes under controlled and field conditions, and thereafter, determine the effect of varying water regimes on subsequent seed quality, and
- to determine the effect of planting date selection as a management tool for optimising cowpea yields under water- limited conditions.

CHAPTER 2
LITERATURE REVIEW

2.1 Cowpea

2.1.1 Classification

Vigna unguiculata (L.) Walp, locally known as cowpea is also called caupi, southern pea or black-eyed pea (United states), tua dam, kund*e*, niébé (French speaking Africa), alacín, pericillo, caritas, cabecita negra, macassar bean, rope bean, fríjol (Venezuela), augenbohne or *kuhbohne* (Germany), imbuba (South Africa) and nyemba (Zimbabwe). All these names refer to the same species *Vigna unguiculata* (L.) Walp, which in older references may be identified as *Vigna sinensis* (L.) (Cook et al. 2005). Cowpea is a vascular plant (*Tracheobionta*), belonging to the super-division of seed plants *(Spermatophyte)*, division of flowering plants (*Magnoliophyta*), class of dicotyledon (*Magnoliopsida*), sub–class nitrogen fixing (*Rosidae*), in order cosmopolitan (*Fabales*), family of legumes (*Fabaceae*) (legumes, peas, beans or pulse), *genus Vigna* (United State Department of Agriculture 2009).

2.1.2 Origin and domestication

Cowpea is believed to have probably originated in Africa. Wild plants of *V. unguiculata* are found only in Central and West Africa with the sub–species *missenses* occurring in humid and sub-humid zones and the sub–species *dekindtiana* in seasonally arid regions. A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia and South America as centres of origin (Department of Agriculture, Forestry and Fisheries 2011). Judging from the presence of wild and domesticated cowpea species in Africa, it means that the diversity of the species is greatest there. Within Africa, certain sites have been proposed as centres of diversity and origin of cowpea. These include Ethiopia, West Africa, and Eastern and Southern Africa (Baudoin and Marechal 1985, Valvilov 1926, Weeden 1992, Ng 1995, Pasquet 2000 cited by Ba et al. 2004).

The precise location or region where cowpea was first domesticated is still under speculation. The wide geographical distribution of the cultivar *dekindtiana* throughout sub-Saharan Africa suggests that the species could have been brought under cultivation in any part of the region (Padulosi and Ng 1997). To date, cowpea is cultivated throughout the tropics and subtropics between 35ºN and 30ºS, across Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and South America (Cook et al. 2005). Cowpea is grown in over two-thirds of the developing world as a companion or relay crop with major cereals. However, the centre of maximum diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah regions of Nigeria, Southern Niger, parts of Burkina Faso, northern Benin, Togo, and the north western parts of Cameroon (Ng and Marechal 1995 cited by Padulosi and Ng 1997).

2.1.3 Plant morphology

Cowpea is an annual herb with varying growth forms. It may be climbing, erect as well as prostrate and creeping depending on the cultivar (Eco-crop 2009). It has a strong taproot and many spreading lateral roots. The root system has large nodules and is more extensive than that of soybean. Its specific symbiotic nodular bacteria is Bradyrhizobiuim species (Gomez 2004). The first pair of leaves is simple and opposite while the rest are arranged in an alternate pattern and are trifoliate (has three leaflets). The leaves are usually dark green in colour, smooth, dull to shiny and rarely pubescent. They show considerable variation in size and shape (long, pointed to oval in shape) depending on the variety. The leaf petiole varies from 5 – 25 cm long (Department of Agriculture, Forestry and Fisheries 2009). Stems are striate, smooth or slightly hairy, sometimes tinged with purple (Gomez 2004).

Flowers are arranged in raceme or intermediate inflorescences at the distal ends of 5 – 60 cm long peduncles. They are borne in alternate pairs, with usually only two to a few flowers per inflorescence. They are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple (Department of Agriculture, Forestry and Fisheries 2011). Seeds vary considerably in size, shape and colour. Seed colour varies from red, black, brown, green, white, spotted or blotched. The number of seeds per pod may vary from 8 – 20. Seeds are relatively large (0.2 – 1.6 cm diameter). The testa may be smooth or wrinkled; white, green buff, red brown, black, speckled, blotched, eyed (the hilum is white surrounded by a dark ring) or mottled in colour. Fruits pods vary in size, shapes, colour and texture. They are mostly 6.5 – 25 cm long and 3 – 12 mm wide. They may be erect, crescent-shaped or coiled. Usually yellow when ripe, but may also be brown or purple in colour (Department of Agriculture, Forestry and Fisheries 2009).

2.1.4 Genetic diversity

Vigna unguiculata is different from the other two cultivated forms of cowpea, the sub–species *Vigna catjang* and *Vigna sesquipedalis.* The difference is with respect to shape and length of the pod and seed characteristics (Sheahan 2012). These characteristics are variable and difficult to discern as the plant can readily cross-fertilise and produce fertile hybrids (Sheahan 2012). According to morphological and genetic (DNA) studies done on the crop (Vaillancourt and Weeden 1992, Pasquet 1993 cited by Ba et al. 2004), the species *Vigna unguiculata* includes domesticated forms (*Vigna unguiculata* species *unguiculata* cultivar unguiculata), wild annual forms (species *unguiculata* cultivar spontanea Pasquet), and ten wild perennial sub–species (Ba et al. 2004). The classification of cultivated cowpea is now based on five so-called cultivar-groups (cv.-*gr.*, also *"*cultigroups*"):* Unguiculata, Biflora, Sesquipedalis (the former sub–species *unguiculata*, *catjang* and *sesquipedalis*), Textilis and Melanophtalmus (Cook 2005). The extreme variability of the species has led to a number of commercial cultivars grouped by the variance in bean shape, size and colour (Jefferson Agriculture Institute 1999). For example;

- Brown-eyed peas — pods range in colour from green to lavender and also in length. The immature seeds, when cooked, are a medium to dark brown, very tender and have a delicate flavour.
- Crowder peas — seeds are black, speckled, and brown or brown-eyed. The seeds are "crowded" in the pod and also tend to be globular in shape.

2.1.5 Ecology

Cowpea is primarily a savannah species, highly adapted to marginal environments and depauperate field conditions where other crops do not perform well. It is a widely grown crop in the semi-arid and sub-humid zones of Africa and Asia (D'Andréa et al. 2006). The crop requires temperatures above 10°C for germination to take place, and temperatures varying from 21 to 33°C for the best vegetative growth. Higher temperatures can cause early flowering and flower abscission, resulting in poor pod set (Agriculture Research Council 2008). The crop is adapted to a wide range of soils, from sand to heavy clay soils, well-drained clays, with a preference for lighter soils that favour good root development. It can tolerate a wide range of pH including very acid soil (pH 4), low-fertility, and grows well on heavy textured soils which are strongly alkaline. It is, however, reported to not tolerate salinity (Cook et al. 2005).

Cowpea is moderately drought tolerant but excessive soil water tends to cause harm; it reduces growth and favours infection by fungal diseases (Cook et al. 2005). It is well adapted to a wide precipitation range of (650 - 2 000 mm). When grown for forage use, annual rainfall regimes of 750 - 1 100 mm are preferable. As a food crop for humans, it is often grown in annual rainfall regimes as low as 400 mm (Cook et al. 2005). Compared to other legumes, the crop is sensitive to waterlogging, and does not tolerate extended flooding (Cook et al. 2005). Nitrogen fixation, which is a characteristic of legumes, is inhibited in waterlogged soils (Ajetomodi and Abiodum 2010). It can be grown under both irrigated and non-irrigated regimes (Davis et al. 1991).

2.1.6 World production of cowpea

It has been estimated that the total pulse requirement for consumption by 2010 would be 25 million tons. Among the different pulses grown in the world, cowpea is grown on 14 million ha with production of 4.5 million tons and the productivity of 387 kg per ha (Halemani 2009); Africa accounts for 94% of this. Nigeria is the largest producer and consumer of cowpea, and produced 2.2 million metric tons of dried grain in 2010. Niger, the second largest producer, followed by Burkina Faso, Myanmar, Cameroon, and Mali produced, respectively, 1.800.900, 432.400, 169.900, 135.000, 109.000 metric tons (FAO 2011 cited by Wiley and Sons 2013).

The average yield of cowpea worldwide is estimated at 450 kilograms per hectare, and is the lowest of the major tropical grain legumes. An estimated 38 million households (194 million people) grow cowpea in sub-Saharan Africa, but productivity has not seen sustained growth over the last two decades – total area, yield, and production grew by 4.3%, 1.5%, and 5.8%, respectively (Consultative Group on International Agricultural Research 2011).

2.1.7 Uses and importance

Cowpea is cultivated primarily for the seed, but also as a vegetable (for leafy greens, green pods, fresh shelled green peas, and shelled dried peas), as a cover crop and for fodder (Thomas Jefferson Agricultural Institute 2013). The crop can provide fodder of higher quality than cereals or forage grasses (Akyeampong 2012). Cowpea seed is a nutritious component in the human diet and livestock feed, providing an important source of protein, fat, fibre, carbohydrates and vitamins (Davis et al. 1991) (Table 2.1). The leaves are a source of some vitamins and minerals (Table 2.2). Their protein content (based on total nitrogen) ranges from 29% to 43% on a dry mass basis, with the highest nitrogen content in younger leaves (Nielsen et al. 1996) and have the highest percentage of calories from protein among vegetative foods (Shaw and Monica 2007). It is mainly consumed by rural and peri-urban people in developing countries (Asiwe 2009).

Table 2.1: Nutrients content of mature cowpea seeds (Bressani 1985).

Nutrients	**Percentage**
Protein	24.8
Fats	1.9
Fibre	6.3
Carbohydrates	63.6
Thiamine	0.00074
Riboflavin	0.00042
Niacin	0.00281

Table 2.2: Cowpea raw leaves content (vitamins and minerals) per 100g edible portion (Pandey and Westphal 1989).

Nutrients	**Quantity (mg)**
Calcium	256
Phosphorus	63
Iron	5.7
β-carotene	2.4
Thiamine	0.20
Riboflavin	0.37
Niacin	2.1
Ascorbic acid	56

In addition to being a nutritious crop, cowpea has the capacity to fix atmospheric nitrogen, thereby reducing the demand for nitrogen need by the crop. This makes cowpea popular and enormously valuable to Africa, especially in the rural areas where resource-poor farmers do not have access to fertilizers. According to a study by (Zahran 1999) the amount of N fixed biologically by cowpea ranges between 65 to 335 kg N/ha per year. The crop is a valuable component of farming systems in many areas because of its ability to restore soil fertility for subsequent cereal crops grown in rotation with it (Timko and Singh 2007). It also grows and covers the ground quickly, preventing soil erosion (IITA 2009).

2.2 Agronomy

2.2.1 Cultural practices

2.2.1.1 Propagation and planting

Cowpea is directly grown from seed. Both inter-row and intra-row spacing are determined by the type of variety and growing pattern. Cultivars with upright growth habits have a higher plant population than trailing or semi-trailing types, because the upright cultivars perform much better in narrow rows (Shiringani 2007). The environmental potential of the land to be used can also determine the most favourable plant population for cowpea (Shiringani 2007). Generally for grain production, a plant population of 200 000 to 300 000 plants per ha at 30 to 50 cm inter-row spacing is preferred to wider rows (70 to 100 cm), which could be suitable to the trailing types (Department of Agriculture Forestry and Fisheries 2011). Concerning planting date, manipulation is utilized by farmers for various reasons. The reasons include escape from periods of high pest infestation or to plant cowpea at such a time that harvesting of the crop would coincide with the period of dry weather (Department of Agriculture Forestry and Fisheries 2011).

2.2.1.2 Planting dates

Planting time is an important cultural practice that results in the greatest differences in growth and yield of grain legumes without involving additional costs (Shiringani 2007). However, studies on the effect of cowpea planting dates are not well documented, and are most of the time referred to common bean (*Phaseolus vulgaris*) and other legumes, since legume research focus has been mainly devoted to established legumes such as common bean and soybeans (Ntombela 2012). As a neglected and underexploited crop, cowpea management practices depend on indigenous knowledge. This being so, planting time is usually used by farmers for various reasons including crops ‘protection against biotic (pest, animals) and abiotic stresses (excessive moisture, heat, drought). For examples; - Early season planted groundnut has been shown to have low *Aphid craccivora* infestations, and consequently, little or no groundnut rosette. This practice is probably effective against some of the cowpea pests, and could be used as components of an integrated pest management system (Karungia et al. 2000). Dudge et al. (2009) also reported that when cowpeas are planted early, photosensitive varieties (semi-erect and prostrate varieties) will not flower but grow very leafy and yield may be reduced. The same authors further indicated that, the important criterion for cowpea planting dates is to determine the onset and duration of the rains and, more importantly, the maturity period of used variety. This will help to avoid the crop’s maturity during the rains and the danger of early end of the rains during cowpea growth.

Given the current scourges that threaten the world (food security and water scarcity); many researchers urge to maximize crop yields with minimum possible water consumption. This involves several practices, including the selection of planting time that matches the crop species throughout the entire growing season (Elsnesr et al. 2013).

2.2.2 Fertilization

As a legume cowpea fixes its own nitrogen from the symbiotic relationship between the crop and the bacterium (*Rhizobium species*) within the soil. This is probably the reason why cowpea rarely requires nitrogen supply. In areas where soils are deficient in nitrogen, there is a need to apply a small quantity of nitrogen fertilizer, about 15 kg N per ha as a basal application for a good crop. If too much nitrogen fertilizer is applied, the plant will grow luxuriantly (excessive vegetative growth) and produce poor grain yield (Dugje et al. 2009). Application of phosphate fertilizer is usually beneficial. Cowpea can grow in soils with a pH range of 5.6 to 6.5 (Dugje et al. 2009).

2.2.3 Irrigation

Cowpea is a drought tolerant crop compared to many other crops. It can grow under rainfall ranging from 400 to 700 mm per annum (Department of Agriculture, Forestry and Fisheries 2011). It is usually grown under dryland rather than irrigated conditions. However, a study by Ahmed and Suliman (2010) showed that water deficit experienced during flowering and pod-filling stages (sensitive growth stages) can lead to lower yields. This suggests that the plant may require supplementary irrigation during dry spells, especially those that coincide with critical crop growth stages such as flowering and yield formation.

2.2.4 Pests and diseases

The pest spectrum of cowpea is relatively wide, and practically every part of the cowpea plant has an adapted pest species that can cause substantial damage (Jackai and Daoust 1986). Birds, especially of the parrot family, can be a problem, as they can pull up emerging seedlings and fee*ds* on the developing green pods (Department of Agriculture, Forestry and Fisheries 2011). Common diseases include; stem rot caused by *Phytophtora vignae*, bacterial blight (*Xanthosomonas vignicola*), aphid-borne mosaic virus (most frequent virus), *fusarium wilt*, bacterial canker, *Cercospora* leaf spot, rust and powdery mildew. Cowpea is also susceptible to nematodes and should not be planted consecutively on the same land (Wang and Sorley 2012).

2.2.5 Weed control

Weeds are a permanent constraint to crop productivity in agriculture. They compete for nutrients, space, and light and exert significant harmful effects by reducing the quality as well as quantity of the crop, especially if weed populations are left uncontrolled (Madukwe et al. 2012). Weed control can either be manual or chemical (by application of herbicides). Manual weed control is the most common method used by farmers in cowpea production. It is recommended that cowpea be weeded twice with a hand hoe; first at 2 weeks after planting and at 4–5 weeks after planting, to ensure a clean field. Poor weed control or delay in weeding causes drastic reduction in yield (Dugje et al. 2009). The choice of herbicide in chemical weed control usually depends on the predominant weed species and the availability of the herbicide. Herbicides application is not recommended where leaves are consumed (Dugje et al. 2009). *Striga gesnerioides* and *Alectra species* are the principal parasitic weeds attacking cowpea particularly in the semiarid regions (Department of Agriculture, Forestry and Fisheries 2009).

2.2.6 Harvesting

2.2.6.1 Maturity

Cowpeas vary in growth habits from erect or semi –erect types with short (< 100 days) growth duration, grown mostly for grain, to longer (> 120 days) duration in semi–erect to trailing plants which are normally grown primarily for forage (Department of Agriculture, Forestry and Fisheries 2011). At maturity, leaves will dry down but may not drop off completely. Cowpea is harvested when seed moisture content is about 12 to 14% to minimise cracking and damage to seed (Mullen et al. 2003).

2.2.6.2 Methods of harvesting

Cowpeas can be harvested at all the three stages of its maturity that include small green pods, mature and dried beans (Davis et al. 1991). Most domestic cowpea production is mechanically harvested, however, hand harvested cowpeas suffer less damage and the harvest season may continue over a 1 to 3 week period (Gomez 2004). For the cowpea seed market, quality of seed is important, so care in harvest and post-harvest handling may be important to avoid cracked or split seed (Department of Agriculture, Forestry and Fisheries 2009).

2.2.7 Storage

Cowpea storage may be conducted for the purpose of maintaining regular supply throughout the year, sales in times of scarcity at high prices to fetch more money, preservation of seeds for planting in the next cropping seasons and it also encourages price stabilization when Governments buy surplus cowpea at time of harvest at low prices and release them periodically in times of scarcity to force prices down and prevent inflation (Yakubu et al. 2012). The storage life of cowpea depends on its moisture content prior to storage. The lower the moisture content, the better the quality of seeds in storage. In developed countries; one alternative is the use of cold storage. Exposure to -18°C for 6 to 24 hours can reduce pest numbers by more than 99%. The grain can be stored short–term at around 12% moisture or less, with 8 to 9% recommended for long-term storage (Department of Agriculture, Forestry and Fisheries 2011).

2.3 Drought Tolerance and Water Use

2.3.1 Drought effect

Drought is a major environmental stress that affects growth and development of plants (Harb et al. 2010). There are two definitions for drought; meteorological and agronomic drought. Meteorological drought is defined as a period of abnormally dry weather sufficiently prolonged for the lack of water to cause a serious hydrological imbalance in the affected area (Glossary of meteorology 1959). Agronomic drought is when there is a lack of sufficient soil water to meet the demands for crop growth (World Meteorological Organization 2006). Agronomic drought can often occur as a result of meteorological drought, poor rainfall distribution or poor soil water management which may result in insufficient soil water (World Meteorological Organization 2006, Mabhaudhi 2009).

Experts consider a plant to be drought tolerant if it can withstand a moderate period of limited water availability. This does not imply that a drought tolerant plant prefers hot, dry conditions or that the drought will not adversely affect the plant (Fair 2009). Generally, drought has a negative impact on crop growth and development, particularly the reproductive phase. The effect of drought stress on the reproductive stage was also confirmed by a study conducted by de Souza et al. (1997) where water stress was observed to reduce yield by reducing seed size, seed number and shortening the grain filling period. A prolonged drought period will negatively affect plant growth by reducing the plant's capacity to regulate its temperature. Additionally, under limited water availability, the plant can also experience nutrient deficiency, thereby reducing photosynthesis. When photosynthesis is reduced, the plant may become energy starved and be unable to support all its activities (Fair 2009).

Many studies have shown that drying soil can lead to decreased plant water uptake, plant tissue dehydration, photosynthesis and storage reduction (Xu et al. 2010), root system damage, and disruption of cell membrane integrity (Kujawski 2010). In legume crops, in particular, it was found that under severe environmental conditions including drought stress, growth and symbiotic characteristics of most rhizobia bacteria may be suppressed. It was, however, reported that several strains, distributed among various species of rhizobia, were tolerant to stress effects (Zahram 1999). This was further confirmed by Serraj (2003) who reported evidence for nitrogenase activity inhibition in soybean grown under drought conditions.

2.3.2 Water use

Water is required for the germination of seeds and as soon as growth starts it serves as a carrier in the distribution of mineral nutrients and plant food. In addition to that, all processes of metabolism require an aqueous environment in which to function. Water fills a number of important roles in the physiology of the plant roles which only water can play as a result of its unique physical and chemical properties (Sustainable Agriculture Initiative 2010). Under field conditions, there is a positive relationship between water use and dry matter production. Water use efficiency (WUE) is a very important index of the relationship between water consumption and biomass production (Wang et al. 2007 cited by Karatasssiou et al. 2009). Successful agriculture is dependent upon farmers having sufficient access to water. However, water scarcity is already a critical constraint to farming in many parts of the world (Molden 2007), especially in arid and semi-arid countries. Given the role of water in crop production, irrigation remains an effective tool to make agriculture possible. It ensures a constant supply of water, which is essential not only to crops that are still growing, but also to the quality of the crop (Department of Environmental and Primary Industries 2010).

2.3.3 Adaptation mechanism to water stress

It was shown that in response to drought brought about by soil water deficit; plants can exhibit three kinds of adaptation mechanisms, drought escape, drought avoidance and drought tolerance. Drought escape is the ability of plants to complete their life cycle before severe stress sets in. Drought avoidance is achieved through maintenance of high tissue water potential despite soil water deficit (Harb et al. 2010). In contrast; drought tolerance is the ability of the plant to cope with the drought by maintaining its biological functions at low water potential, or by diminishing its metabolic functions which are resumed once water increases (Casares et al. 2011). A study conducted on two annual legumes, *Medicago minima* (L) Barta and *Onobrychis aequidentata* (sibth and Sm) D'urv., showed that these species exhibited an effective use of water to confront drought periods by completing their life cycles, before severe soil and plant leaf water deficit is established (Karatassiou et al. 2009). According to the same author, this drought mechanism was realised using two kinds of adaptation, developmental plasticity and rapid phonological development.

Developmental plasticity is the ability of plants to regulate their development in response to the environment. When plants integrate environmental information into the regulation of these growth and developmental processes, their form can be modulated according to the environment in which they are growing. In this way, the final morphology of these plants depends on the environment, defining it as plastic (de Jong and Leyser 2012). For example, shoots can produce leaves adapted to shade or bright sunlight and, at some point, switch from making leaves to making floral organs. Rapid phenological development is defined as plant reactions in response to environmental effects on their periodic life cycle events (leaf unfolding, flowering, fruit ripening, colour changing and leaf fall in autumn) (Koch et al. 2007). The rapid phenological development can be expressed by reducing flowering time and time to fruits ripening.

Drought avoidance occurs when a plant's roots experience a period of limited soil water availability. The plant hormone abscissic acid (ABA) signals the closure of stomata. Closure of stomata reduces transpiration. Transpiration serves to cool the plant by pulling water and nutrients from the soil throughout the plant (Fair 2009). Thus, tolerance to water deficit is the ability of a plant to grow properly under conditions of water deficit (Weri 1987). Plant growth, photosynthesis and stomatal aperture may be limited under water deficit, and this would be regulated by physical and chemical signals (Xu et al. 2010). The ability to withstand water stress can be associated with an abundant root system, stomatal regulation, and a greater effective use of water or to maintain turgor potential high or finally to a change of biochemical compounds of the plant (Sanou and Dabire 2001).

2.3.4 Chlorophyll content

Chlorophyll, was discovered in 1816 by Joseph Bienaime' Caventou and Joseph Pelletier. The word chlorophyll is of Greek origin, chores – green, and phullon – leaf (Anon 2013). Chlorophyll is a group of green pigments that are found in the chloroplast cells of plants and in other photosynthetic organisms such as cyanobacteria and algae (Oxford dictionary fourth edition 2000). These pigments are an extremely important biomolecule, critical to photosynthesis, which allow plants to absorb energy from light. Leaf chlorophyll content provides valuable information about physiological status of plants (Gitelson et al. 2002). For example, measuring chlorophyll content, also indirectly measures the amount of nitrogen in the plant, since nitrogen is a part of chlorophyll. This measurement helps determine a more efficient fertiliser application program (Analyseur de Teneur en Chlorophylle France 2011). There is literature indicating that water stress can cause reduction in chlorophyll content (Ityrbc et al. 1998). The decrease in chlorophyll under drought stress has been reported to be the result of damage to chloroplasts caused by reactive oxygen species (Mafakheri et al. 2010). However, high chlorophyll content is an indicative characteristic of low degree of photo–inhibition of photosynthetic apparatus, since it reduces carbohydrate losses for grain growth (Farquhar et al. 1989).

2.3.5 Proline accumulation

Proline (proteinogenic amino acid) accumulation is a common physiological response in many plants to a wide range of biotic and abiotic stresses (Verbrugger and Hermans 2008). Under stress conditions many plant species accumulate proline as an adaptive response to adverse conditions (Mattioti et al. 2009). Although a relationship between proline accumulation and stress adaptation has been questioned by some authors. It is generally believed that the increase in proline content following stress injury is beneficial for the plant cell (Handa et al. 1989). However, ever since the early 1980s different research groups have found a significant amount of proline in the reproductive organs of different plant species, raising the possibility that the accumulation of this amino acid may also occur in physiological non-stressed conditions for developmental purposes (Mattioti et al. 2009). Proline accumulation has been observed during conditions of drought, high salinity, high light and ultraviolet irradiation, heavy metals, oxidative stress, and in response to biotic stresses (Szabados and Savoure´2009).

2.3.6 Protein synthesis and accumulation

Different sources of stress, their duration and severity lead to differential expression of genetic information, resulting in changes in gene products, including mRNA and proteins. Such newly synthesised proteins are specific to the particular type of stress and possibly confer enhanced survival value to the plants (Dubey 1999). The same author continues to show that, different environmental stresses can induce the synthesis of new proteins in plants. This can provide evolutionary value to the plants for enhanced survival under adverse environmental situations. The synthesis of such stress-induced proteins has been well documented under salinity stress, osmotic stress, heat shock, low-temperature treatment, anaerobiosis, infection with pathogens, wounding, gaseous pollutants, and ultraviolet (UV) radiation.

2.4 Seed Quality

Seeds are the reproductive unit. They contain all the genetic information to determine yield potential, adaptation to environmental conditions and resistance to pests and diseases (Erker 2008). It is therefore necessary that the seed is of high quality in order to ensure good establishment, and consequently maximum crop production. High quality seeds are characterised by genetic stability, uniform and rapid germination, high seed vigour and freedom from pests and diseases (Balkaya 2004).

Genetic purity of the seed is an important factor in obtaining pure stands of a specific variety Erker 2008). The same author explains that, seeds from varietal mixtures may cause uneven maturity, lower yield potential, susceptibility to disease, insect and pests, and be less adapted to specific environmental conditions. The viability (germination capacity) and physiological vigour are the two most important factors required for high seed quality (Odindo 2007). Viability is the capability of plant structures (seed, cuttings) to show living properties like germination. Seed germination is a process by which a seed embryo develops into a seedling. It involves the reactivation of the metabolic pathways that lead to growth and the emergence of the radicle and plumule (Michael and Peter 2006). Seed germination role in stand establishment, makes it to remains a key to modern agriculture.

Seed vigour provides a very good estimate of the potential field performance and subsequent field planting value (Sun et al. 2007). The Association of Seed Analysts (AOSA) defines seed vigour as comprising of those properties that determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions (Bennett 2011). Seed vigour has been known as a comprehensive characteristic affected by many factors, such as the genetic background, environmental factors during seed development and storage (Wang et al. 2010). It is an important characteristic of seed quality, reflecting potential seed germination, seedling growth, seed longevity, and tolerance to adversity (Wang et al. 2010).

Drought stress has been reported as one of the major factors affecting plant productivity in arid and semi-arid regions of the world (Mohammadizad et al. 2013). Seed germination and early seedling emergence are the most concerned stages for drought stress (Ahmad et al. 2009). Successful establishment is thus related to the capacity of seeds to germinate quick and uniformly, as well as their ability to germinate under low water availability (Fischer and Turner 1978). This might be achieved through the use of water more efficiently by the vigorous seeds in terms of canopy characteristics, and the ability to have a strong tap root in seedlings for effective use of water through enhanced soil water capture (Blum 2005).

Drought stress imposed on the maternal plant during seed development and maturation has been reported to affect seed quality (Alqudah et al. 2011). Several studies confirmed the decrease in seed vigour and germination due to water stress conditions during the reproductive stage (Rassini and Lin 1981 cited by Younesi and Moradi 2009; Drummond et al. 1983). However, these observations were inconsistent since there have been cases whereby findings were contrary to this. Yaklich (1984) showed that drought stress during seed filling had no effect on seedling growth under controlled and field conditions.

It has been suggested that seed coat colour and structure can affect germination (Debeaujon and Koorneef 2000). White seed colour has been reported to have higher water absorption during germination compared with dark coloured seeds (Odindo 2007), leading to the seed secretion and imbibitional injury in the light variety (Mabhaudhi and Modi 2010). As a result, white seed colour has a low emergence rate. In legumes, the good (slow) water uptake observed in dark coloured seeds is attributed to the presence of phenolic compounds and tight adherence of the seed coat to the embryo, resulting in greater rate of imbibition and fast germination (Chachalis and Smith 2000). However, several studies have reported no significant differences between seed colours. Ochuodho and Modi (2013) after conducting germination on three wild mustard species found that all the light coloured seed lots showed high germination percentage and one dark seed lot, which was heavier, showed poor germination.

2.5 Conclusion

Cowpea remains a crop of choice in arid and semi-arid regions of the world where it is grown. This is due to its adaptability to drought-prone conditions, relative to other crops in this environment, and its capability to produce reasonable yields when grown on poor soils. Cowpea grains, as well as the vegetative parts, make major nutritional contributions to diets, and can be harvested as fodder for livestock. Its high potential to biologically fix nitrogen makes it best suited for production by resource poor farmers. Although cowpea is a crop of choice in arid and semi-arid area, it remains underexploited by research in several countries including South Africa (Chen et al. 2007; Dugje et al. 2009). Drought is a major yield-limiting constraint in South Africa and other semi-arid countries (Xu et al. 2010). It continues to affect the production of rainfed crops, and thus threaten food security (Chiulele 2010). Due to climate change and increased frequency of drought, there is a need for farmers to understand the effects of drought on the crop's physiology. Unfortunately, as a neglected crop, the physiological responses of cowpea to water stress conditions are not well–documented. This study will add to the available knowledge and provide more information on crop production under drought stress environment.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Materials

Two varieties of cowpea seed, Brown mix and White birch were obtained from an accredited local seed company and used for this study (Fig 3.1). The 100 grain mass of Brown mix and White birch varieties was determined prior to any of the experiments being done. The mean 100 grain mass was respectively 22.21 g and 18.91 g for Brown mix and White birch, respectively.

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

3.2. Seed Quality Tests

3.2.1. Tetrazolium test (TZ)

Four replicates of three seeds per variety were soaked in 100 mℓ distilled water for 30 minutes. Seeds were then dissected longitudinally into two halves using a scalpel so that the embryo was exposed to the solution. One half of every seed was used for the test and the other half was discarded. The halves were soaked in petri dishes filled with 1% of 2.3.5 triphenil tetrazolium chloride solution. The samples were incubated for 30 minutes at 37°C. Thereafter, seeds were observed for staining using a magnifying glass (5 to 7X). All stained seeds (in normal red colour) were considered alive (viable) (Canadian Seed Institute, 2008).

3.2.2. Standard Germination (SG) test

Four replications of cowpea seeds (25 seeds) of each variety were arranged between double–layered moistened paper towels. The paper towels were rolled and tied on both ends with rubber bands before being put in sealed zip–lock bags (to prevent moisture loss) and incubated in a germination chamber set at 25°C for 8 days. Daily germination was recorded as the time of visible (2 mm) radicle emergence. Final germination recorded on the 8^{th} day was based on normal and abnormal seedlings. Normal seedlings were considered as having all the essential plant structures necessary for the plant to continue to grow normally under favourable conditions (AOSA 1996; ISTA 1996). Upon termination, seedling growth parameters such as shoot and root length, root: shoot ratio as well as fresh and dry mass were measured.

Germination Velocity Index (GVI) was calculated using the following formulae (Maguire 1962).

$$GVI = G_1/N_1 + G_2/N_2 + \ldots + G_n/N_n \qquad \textbf{Equation 3.1}$$

where:

GVI = germination velocity index,

$G_1, G_2 \ldots G_n$ = number of germinated seeds in first, second… last count, and

$N_1, N_2 \ldots N_n$ = number of sowing days at the first, second… last count.

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

$$MGT = \frac{\sum D_n}{\sum n}$$ **Equation 3.2**

where; n = number of seeds which were germinated on day D, and

D = number of days counted from the beginning of germination.

3.2.3. Electrolyte leakage test (EC)

Cell membrane permeability of the two cowpea seed varieties was determined using the R&A CM100 Model Single Cell Analyser. A total of 50 seeds per variety were individually weighed and put into wells filled with 2 ml distilled water. Electrolyte conductivity was measured every 60 minutes for a total duration of 24 hours. Seed electrolyte conductivity was expressed as μS/g of seed.

3.2.4 Seed quality test after harvesting

Following harvest, seeds produced under the varying water regimes were taken and subjected to seed quality tests to evaluate the effect of water stress on maternal plants on subsequent seed quality.

3.3. Controlled Environment Study

3.3.1. Controlled environment conditions

The experiment was conducted in a temperature controlled (33/27°C day/night; 65% RH) glasshouse at the University of KwaZulu-Natal’s Controlled Environment Research Unit (CERU), Pietermaritzburg, South Africa. The temperature of the glasshouse was based on ecological characteristics (environment) of semi-arid tropics where cowpea is commonly grown. In addition, the temperature regime was also based on previous studies that have shown it to be optimum (Ntombela, 2012). Temperature and relative humidity were monitored electronically using HOBO 2K Loggers (Onset Computer Corporation, Bourne, USA).

3.3.2. Experimental design, potting procedure, water stress treatments

The experiment was arranged in a randomized complete block design (RCBD) with two treatment factors: water regime [three levels – 30%, 60% and 80% crop water requirement (ETc)] and variety (two levels – Brown mix and White birch). These were replicated four times giving a total of 24 pots. Each pot was filled with 10 kg of soil whose field capacity had previously been determined using as follows:

$$\theta m = \left(\frac{\theta w - \theta d}{\theta d}\right) x\ 100\% \qquad \textbf{Equation 3.3}$$

where: θ_m =gravimetric field water capacity,

θ_w = wet mass of soil, and

θ_d = dry mass of soil.

Treatments were randomly assigned to pots for every block. Pots were given three weeks to reach full establishment during which all pots were watered at 80% ETc. Thereafter, the three water regimes were imposed. The experiment started on the 7^{th} of April, 2013 and ended on the 30^{th} of August, 2013.

3.3.3. Soil characteristics

The soil used in pots trial was sourced from the University of KwaZulu–Natal's experimental farm – Ukulinga. Soil was classified according to the South African soil taxonomic system. The texture class of the soil was clay loam, with 33% clay, 26% fine silt, and 40% sand.

3.3.4. Crop management

During the experiment, 10 mℓ $5\ell^{-1}$ of Avikayazinon (miticide) was used to spray plants against red spider mites, and 5 mℓ $3\ell^{-1}$ and 20 mℓ $10\ell^{-1}$ of chloriprifos were used to spray plants against Mealy bug and ants. Weeding was done manually as needed (three times during the experiment), to avoid competition and pest harbouring.

3.3.5. Data collection

Leaf number and plant height were measured every week after emergence. Only normal leaves (green leaves or leaves with 50% green area) were counted. Plant height was measured using a metre ruler beginning from soil surface up to the base of the top leaf. Data for stomatal conductance were collected every week using a steady state leaf porometer (Model SC-1, Decagon Devices, USA). Leaf chlorophyll content was measured every week using a chlorophyll content meter (CCM-200 Plus, Opti–Sciences, USA). Soil water content was also determined weekly at the same time when measurements of stomatal conductance and chlorophyll content index were taken. Measurements were taken using a handheld Theta probe (ML 2x). Time to flowering was observed as 50% flowering. Yield components (biomass, Harvest index, pod number, seed number, seed mass and yield per plant) were determined after harvesting.

3.3.5.1 Proline determination

Proline content was determined using the methods of Bates et al. (1973) and Marin et al. (2009) based on proline's reaction with ninhydrin. Freeze-dried seed material (0.5 g) was homogenized in 10 mℓ of 3% sulfosalycic acid (w/v). The homogenate was centrifuged at 11 000 rpm for 10 min at 4°C. For proline colorimetric determinations, a 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100°C for 1 hour. The reaction was arrested in an ice bath and the chromosphere was extracted with 4 mℓ toluene and its absorbance was read at 520 nm using a Shimadzu probe UV-1800 spectrophotometer. Proline concentration was calculated from the standard curve (Appendix 6) on a dry mass basis as follows :[(µg proline/mℓ x mℓ toluene)/ (115 µg/µmole)]/ [(g sample)/5] = µmoles proline/g of dry weight material.

3.3.5.2 Protein determination

Protein concentration was determined according to Kanellis and Kalaitzis (1992). Freeze-dried material was ground to a fine powder in a pre-chilled mortar under liquid nitrogen. Samples of 0.5 g were mixed in 5 mℓ Tris-HCl buffer (pH 7.4) containing 0.2 M NaCl, 20 mM MgSO4, 1mM EDTA,5mM mercaptoethanol, 0.5 mM PMSF, 10 mM leupeptin, and 10% (v/v) glycerol and centrifuged (20 000 rpm for 20 min) at 4°C. The supernatants were collected and considered as seed protein extract. Protein concentration was determined by absorbance at 595 nm (Bradford 1976), with bovine serum albumin as standard.

3.3.5.1 Seed quality tests

Harvested seed from the pot trials was subjected to seed quality evaluation using the tetrazolium test (TZ), standard germination test (SG) and electrolyte leakage (EC). Details of seed quality tests have previously been described in section 3.2.

3.4 Field Experiments

3.4.1. Experimental site description

The experiment was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 845 m a.s.l) during 2013/14. Ukulinga has a warm subtropical climate with an average annual rainfall of about 694 mm received mainly during the summer months. The soil at the field trial site farm was classified according to soil classification, taxonomic system for South Africa 1991. The texture class of the soil was clay loam, with 33% clay, 26% fine silt, and 40% sand.

3.4.2. Weather and soil water content

During the study (April 2013 to February 2014), weather data was obtained from measurements collected by an automatic weather station (AWS) located at the experimental site. Measurements are monthly averages compiled from daily readings. Soil water content was measured using a PR2/6 profile probe connected to an HH-2 moisture meter (Delta-T Devices, UK). Measurements were recorded at depths 10, 20, 30, 40, 60 and 100 cm. Access tubes were inserted in each plot to measure soil water content. Rain gauges were installed in the irrigated plot to quantify the amount of water applied during irrigation.

3.4.3. Experimental design, irrigation and planting

The experimental design was a split-split-plot design with planting date as a main factor, irrigation and rainfed as sub-main factor, and seed variety (Brown mix and White birch) as sub-plots arranged in a randomised complete block design (RCBD), with four replications. Seeds were sown at three different planting dates: April 9, 2013 (first planting), June 19, 2013 (second planting) and August 20, 2013 (third planting). Irrigation was applied after full emergence, for all plantings. Three seeds were planted per station at a spacing of 0.5 m x 0.5 m.

3.4.4. Crop management

During the three plantings, plants were sprayed with Kemprin (Cyphermethrin) at 20 mℓ/10ℓ against cutworm. Weeding was performed as needed using hand hoes, to avoid competition and pest harbouring.

3.4.5. Data collection

Time to 50% emergence was recorded weekly starting from 3 days after planting. The emergence rate was determined when seeds had stopped to emerge. This was considered as full emergence. Leaf number was manually counted every week. Only normal leaves (green leaves or leaves with 50% green area) were considered. Plant height was also measured every week using a metre ruler beginning from soil surface up to the base of a top leaf. Stomatal conductance was measured every week. Measurements were recorded using a steady state leaf porometer (Model SC-1, Decagon Devices, USA). Leaf stomatal conductance (indication of opening and closing of stomata) was reported in mmol m^{-2} s^{-1}. Leaf chlorophyll content index was measured weekly using the CCM-200*Plus* chlorophyll content meter (Opti-Sciences, USA). Soil water content was also determined every week using a PR2/6 profile probe connected to an HH-2 moisture meter and the volumetric water content of the soil was reported in percentage. Time to flowering was recorded when at least 50% of experimental plants were observed to have flowered. At harvest, yield and yield components (biomass, harvest index, pod number per plant, seed number per pod, seed mass per pod, seed mass per plant and dry matter) were measured. After harvest seed moisture content was determined using a grain moisture analyser (KM–21G). Thereafter, seed viability was determined as described in section 3.2.2.

3.4.6. Description of Statistical Analyses

GenStat® 14 edition was used to perform analyses of variance (ANOVA) and the differences between means of significant variables were separated using least significant difference (LSD) at the 5% level of significance (P = 0.05).

CHAPTER 4

SEED QUALITY COMPONENTS OF COWPEA VARIETIES BROWN MIX AND WHITE BIRCH

4.1 Introduction

There is still a need to increase crop productivity in the developing world where production remains largely traditional and is concentrated in the hands of smallholder farmers and pastoralists (Janneh and Ping 2009). Among the constraints faced by this category of farmers is that of poor quality seed (Dobermann 2013). Seed is a basic agricultural input. It is important in crop production systems since a significant contribution to yield can often be attributed to the quality of the seed used (FAO Corporate document repository 1994). It is therefore important to ensure that seed quality remains a priority to modern seed science and a prerequisite for obtaining high yields (Milosevic et al. 2010).

Cowpea is still classified as an underutilised African legume (Timko et al. 2008). This is because it has not been given the attention it deserves as a crop that has potential to improve livelihoods in Africa. As a result, there is limited information about its seed quality. In most places, cowpea is still cultivated from landraces; these are seeds that have been kept over and handed down generations of traditional subsistence farmers through their informal seed systems. Informal seed systems are defined as systems in which farmers are involved in selection, production and dissemination of seed; whereby sales, exchanges or donations of seed occur within local communities (Louwaars and de Boeuf 2012). This practice, although useful within the context of mutual assistance among farmers, often does not prevent the dissemination of poor quality seed. Seed quality in this system is not controlled by seed experts, and seed selection is based largely on local indigenous knowledge. This situation presents a need for subsistence farmers and cowpea producers to understand seed quality, know when and how seeds are declared quality seed, and the vital role that it plays in agriculture productivity (Ajeigbe et al. 2009).

According to McDonald and Copeland (1997), seed quality is the inclusive worth or appropriateness of a seed lot for its envisioned use. Quality seed ensures good germination, rapid emergence, vigorous growth and good stand establishment (Zecchinelli 2009). The use of good quality seed is essential for maximum crop production and dictates plant productivity (yield, genetic characteristics, market quality and storability) (Mumbi 1994). Zecchinelli (2009) went further to state that seed quality was the only way by which benefits from plant breeding could be transferred to farmers. In practice, seed quality comprises two components – viability and vigour. However, most growers use the terms quality and vigour interchangeably (Santos 2007). Vigour is defined as the totality of those properties that regulate the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions (Association of Official Seed Analysts 2002). It is well known that declines in seed vigour precede those observed in germination as seed deterioration progresses. This phenomenon underscores the importance of using seed vigour tests as a more sensitive measure of seed quality and plant emergence capability of a seed lot (Copeland and McDonald 2001). Odindo (2007) went further to state that seed vigour was the most informative indicator of physiological seed quality. High seed vigour ensures good stand establishment under varying field conditions (Santos 2007).

Studies by Feistritzer (1975) and Cardwell (1984) indicated that seed must have the qualities that allow for rapid germination and seedling establishment (Coolbear et al. 1997). Seed germination is a very important phase in the growth of any plant. It is defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions (Desai et al. 1997). The germination capacity of a seed remains one of the most important indicators of seed quality, since it is an intrinsic property of the seed (Odindo 2007). It is expressed in terms of germination percentage as determined in a standard germination test (Bennett 2001). Many seeds germinate well under ideal laboratory conditions but fail to emerge successfully in the field. It is important therefore, for all seeds used for planting to be tested for quality (viability and vigour) in order to determine the field planting value of a seed lot. The objective of this study was to evaluate seed quality of two cowpea landraces on the basis of seed colour, with respect to viability and vigour.

4.2 Results

4.2.1 Tetrazolium test

All seeds of the Brown mix variety stained normal red, indicating 100% viability. However, only 83.3% of White birch seeds stained positive (Fig 4.1).

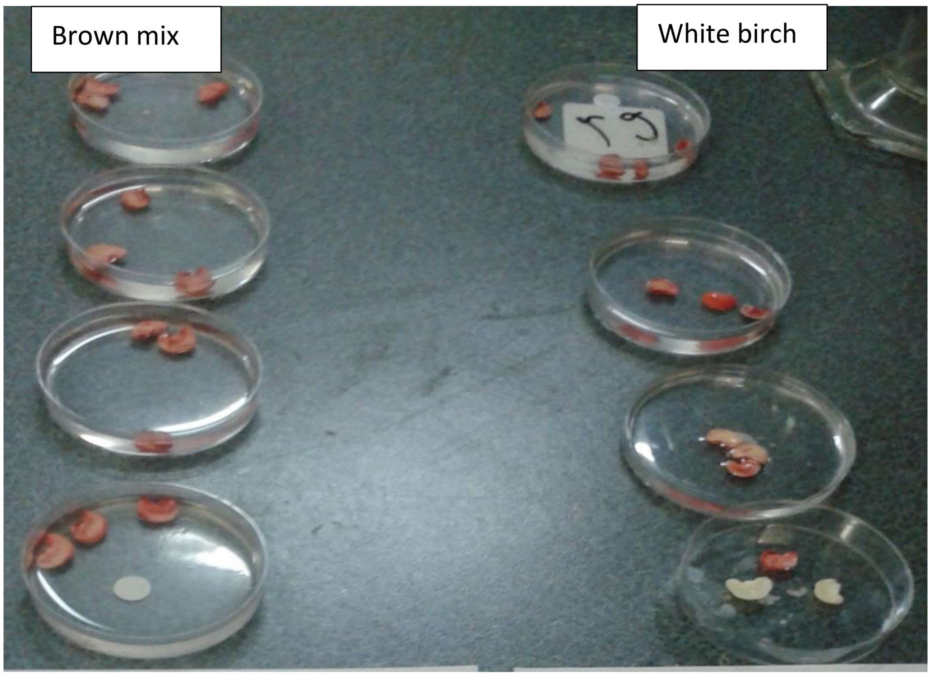

Figure 4.1: Tetrazolium chloride staining results for two cowpea varieties (Brown mix and White birch).

4.2.2 Standard germination test

No significant differences between the two cowpea varieties were found with respect to germination. The Brown mix variety, however, had the higher final percentage germination (100%) compared to the White birch variety (95%) (Figure 4.2; Table 4.1).

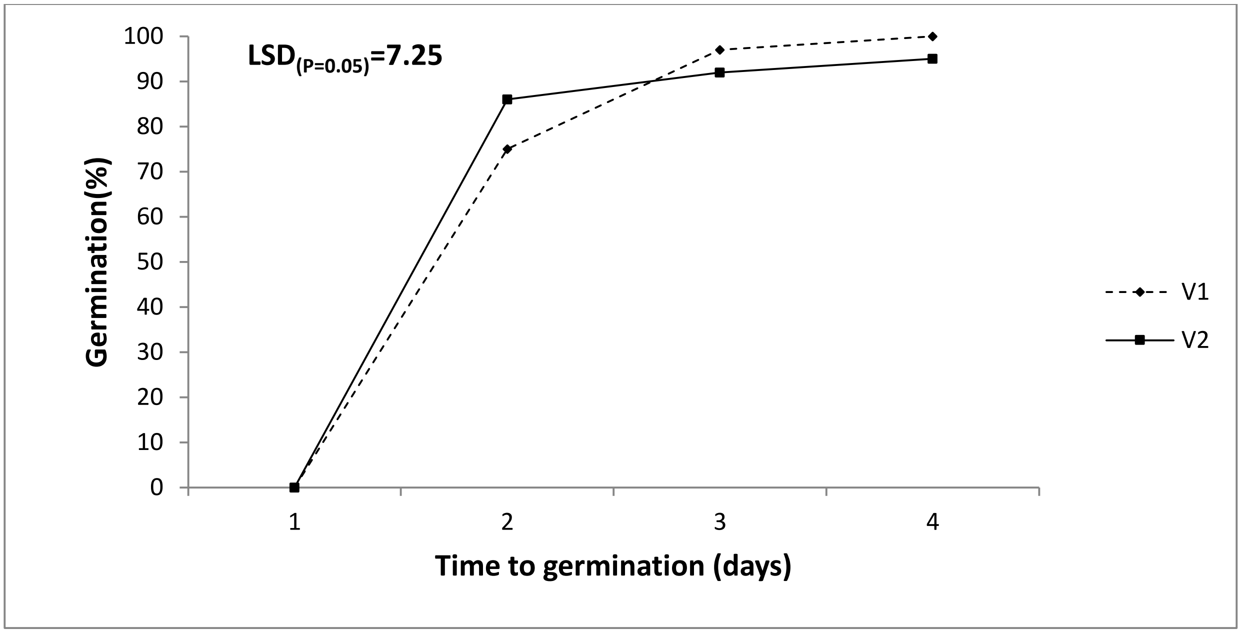

Figure 4.2: Daily germination percentage of two cowpea varieties (Brown mix and White birch) observed during four days of germination. V1 = Brown mix, V2 = White birch, LSD= least significant difference between the means.

Table 4.1: Germination capacity and seed vigour of two cowpea varieties (Brown mix and White birch) obtained from standard germination test.

Variety	**Germination (%)**	**EC (µS/g)**	**MGT (days)**	**GVI**	**Root Length (mm)**	**Shoot Length (mm)**	**Root: Shoot**	**Dry mass (g)**
Brown mix	100 [a]	53.2 [a]	2.10[a]	23.7 a	89.5 [a]	48 [a]	1.93 [a]	2.84 [a]
White birch	95 [a]	18 [a]	2.18 [a]	24.34a	124.9 [a]	62.2 [a]	2.02 [a]	2.97 [a]
$LSD_{(P=0.05)}$	**7.25**	**60.6**	**0.2494**	**5.284**	**19.51**	**24.87**	**0.6195**	**0.2949**

GVI= Germination Velocity Index, MGT= Mean Germination Time, EC=Electrolyte Conductivity. Values sharing the same letter (a) in the same column do not differ significantly at P<0.05.

Seedling growth parameters (root length, shoot length, root: shoot ratio and dry mass) also showed no significant differences between the two varieties. However, dry mass, root and shoot length of the White birch variety were higher than Brown mix variety. Mean time to germination (MGT) and germination velocity index of the two varieties were also not significantly different. The trend was such that the White birch variety germinated faster than the Brown mix variety (Table 4.1).

4.2.3 Electrolyte leakage

Results of electrical conductivity showed significant differences ($P < 0.05$) between the two cowpea varieties. Brown mix had a higher EC relative to White birch (Fig 4.3).

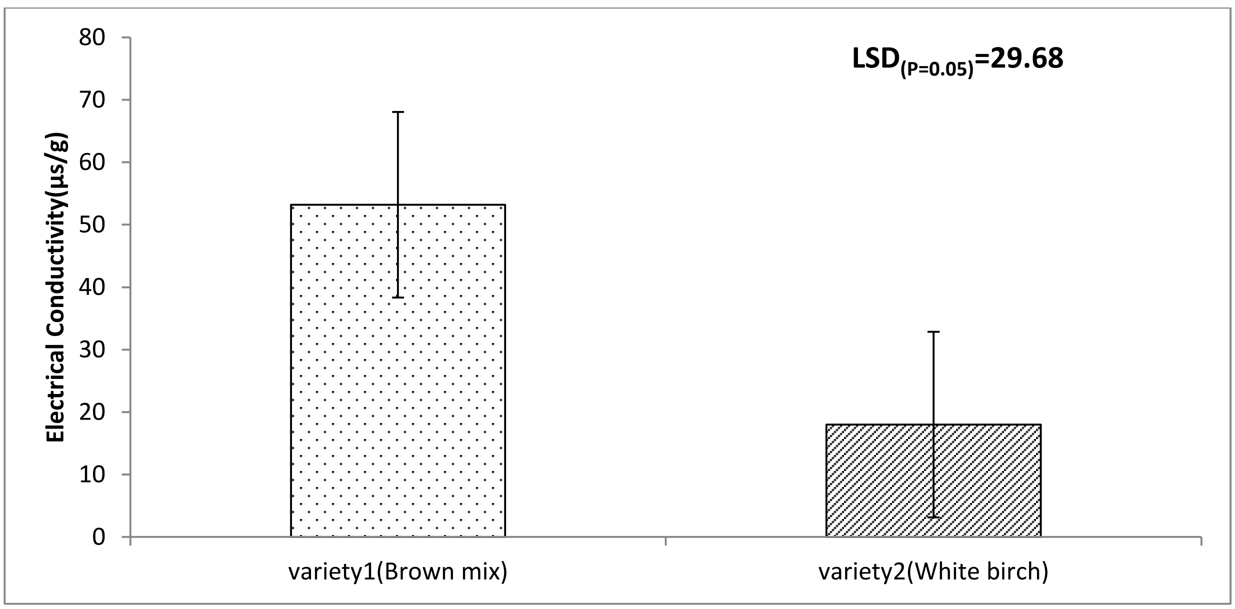

Figure 4.3: Electrolyte conductivity of two cowpea varieties (Brown mix and White birch). EC = Electrolyte conductivity.

4.3 Discussion

The tetrazolium test is a rapid biochemical test that evaluates seed viability based on seed respiration. It determines the percentage of live seeds in a sample that have the potential to produce normal seedlings under favourable germination conditions (Santos 2007). The areas of vital importance in interpreting the staining pattern in monocots are the plumule tips, the portion where the embryo is attached to the scutellum and seminal root region (Patil and Dadlani 2009). Unstained radicle tips in legumes may not allow the radicle to grow. Improper staining in areas such as the radicle and hypocotyl, cotyledons and in the plumule region indicates abnormal seed. In this study, the test showed a difference (16.7%) in viability between the two varieties. Seeds of the Brown mix variety showed 100% viability while White birch seeds had 83.3% viability. The unstained (non-viable) seeds observed in the White birch seed lot may be indicative of physiological deteriorative changes that may be associated with environmental factors (damage during production or storage by machine or pest, age and maturity of seed) that have affected certain seeds (International Rice Research Institute 2008). It should be noted that the test is not a substitute for seed germination test (Peter 1970) and can be ineffective in some cases such as dormancy and recalcitrance (Gosling 2003), since seeds will not germinate even when viable. This is in accordance with Trawatha et al. (1990) who indicated that the viability test should differentiate between poor and good seeds lots.

The germination of seeds is considered as the ultimate measure of viability for any seed lot (Tasmanian 2013). The standard germination test continues to be the most common measure of viability (Penaloza 2005). It gives the information to estimate the field planting value of a seed lot. The test demonstrated 100% of seed germinability for Brown mix and 95% for White birch (Figure 4.2). The trend of result was subsequently confirmed by the result of the tetrazolium test; Brown mix achieved 100% viability and the White bitch 83.3%. All these results agree with reports by Odindo (2007) that dark coloured cowpea seeds in general performed better than light coloured seeds. Several studies have also indicated more viability in dark seed compared with light seed (Sinefu 2011; Zulu and Modi 2010), suggesting a possible effect of seed coat colour on germination. However, a negative relationship between germination and seed vigour was observed between the two seed varieties; White birch variety was more vigorous than Brown mix variety (Table 4.1). Hampton and Tekrony (1995) cited by Takos et al. 2006, explained that the germination test does not thoroughly reflect seed field emergence, since field emergence takes place under variable climatic conditions.

Mean germination time (MGT) has been shown to be highly indicative of emergence performance in seed lots (Demir et al. 2008 cited by Mavi et al. 2010). Germination velocity index (GVI) indicates the relative strength of a seed lot (Carvalho and Nakagawa 1980). Based on results of these two germination indices correlating to seed vigour, White birch performed well compared with Brown mix. The capacity of White birch to germinate faster and uniformly may have been the effect of a more integrated cell membrane (good permeability) observed in solute leakage test, since lower conductivity presupposes less physiological deterioration. This also agrees with results obtained by Sinefu (2011), who reported that white coloured seeds of bambara groundnut performed better than brown and red ones under different water regimes during seedlings establishment.

The electrical conductivity (EC) test is one of the vigour tests included in the International Seed Testing Association Rules for Seed Testing (Matthews and Powell 2006). It is a rapid and well established method of measuring the level of exudates secreted by the seeds, or the leakiness, which is correlated with vigour (Oregon State University 2013). In the present study, Brown mix variety had more leakage (53.2%) than the White birch variety (18%), indicating that seeds of White birch variety had less cell membrane permeability compared with Brown mix variety. The test result was related to performance (MGT, GVI, Shoot length, Root length, Root: Shoot ratio and dry mass) of the White birch variety (Table 4.1). As such, the results indicated that the White birch variety was more vigorous than the Brown mix variety. These results concurred with those of Ntombela (2012) who reported that seedling root length and shoot length of White birch variety were higher than that of Brown mix variety, and that the White birch variety was more vigorous.

However, EC was not a good predictor of germination. This suggests that the association between seed coat colour and electrical conductivity as a measure of performance should be treated with caution (Odindo 2007). In fact, EC measures only the leakage of electrolytes, since the membrane integrity is the basis of the test for seed vigour. However, seed vigour test may reflect several changes at the physiological and biochemical level, which may not involve only membrane integrity, but also food reserve utilization, enzyme activity, metabolic changes in energy or storage compounds. A study by Hamman et al. (2001) was not able to establish a correlation between conductivity of individual soybean seeds and their emergence. According to the authors, seeds with low conductivity performed poorly while those with high values performed well. This could be attributed to seed coat thickness effect, since seed coat plays a role in the control of water imbibition, and consequently on germination (De Souza and Marcos-Filho 2001).

4.4 Conclusion

With respect to the factors determining the quality of seed that were considered in this experiment (viability and vigour), there was statistical difference between Brown mix and White birch. The Brown mix variety was more viable (germinated better and had all seeds stained normal red) compared with White birch. However, results of vigour were not in agreement with results of viability and indicated that the White variety was more vigorous than the Brown mix variety. Results also highlighted the limitations of using seed electrical conductivity as a measure of seed quality. Perhaps EC should be used in conjunction with measurements of seed coat thickness in order to improve the validity of results. This implies that good viability does not always translate to good vigour. Under field conditions it is seed vigour that is more associated with the ability of a seed lot to emerge and form a uniform crop stand. Therefore, farmers would be advised to grow the white variety. However, there is still a need to confirm the findings of this study under field conditions.

CHAPTER 5

EFFECTS OF WATER STRESS ON PHYSIOLOGY, GROWTH AND YIELD OF COWPEA GROWN UNDER CONTROLLED ENVIRONMENT CONDITIONS

5.1 Introduction

Climate change is a global crisis causing extreme environmental conditions such as high temperatures, water deficit, flooding and water logging. This situation exacerbates the water scarcity caused by the fact that one third of the earth's surface is classified as arid and semi-arid (SAHRA 2008). This scenario of increasing water scarcity has resulted in water stress becoming more limiting to agricultural production in semi– and arid areas. There is a need to identify and promote more drought tolerant crops with low levels of water use for production in semi– and arid areas. This will contribute to food security in these marginal agricultural production areas. Drought tolerant grain legumes such as cowpea (*Vigna unguiculata* (L) Walp) have been cultivated in dry and hot regions of the world, mostly in Africa, Asia and the Americas (Dadson et al. 2005). Although they are tolerant to drought, there is evidence that water stress reduces their productivity (Asiwe 2009). Local cultivars of cowpea have not been fully evaluated for their levels of water use and possible drought tolerance in order to see if they can be promoted in dry areas of the country.

Water stress is a major abiotic factor limiting plant growth and crop productivity in South Africa, semi– and arid countries (Kramer 1983). Its effects can be observed at any stage of plant growth and development, including early establishment, vegetative stage, flowering and yield formation. The ultimate effect of water stress occurring at any stage of plant growth is low yields. In rural areas where yields are already low due to lack of fertilisers and other agronomic factors, this could have a devastating effect on household food security. According to Kramer (1983), drought stress can affect plant growth and yield by influencing physiological processes and conditions. However, this influence on a plant's physiology varies depending on the species, degree of tolerance and the magnitude of the water stress (Figueiredo et al. 1999). Water stress was reported by Gomesda et al. (2001) to have a significant effect on cowpea biological nitrogen fixation and consequently caused reduction in leaf chlorophyll content (Sanchez et al. 1983). This was in conformity with the observations of Onuhm and Donald (2009), who reported that root nodulation in legumes correlated with availability of necessary soil components, including soil water. Other reports also indicated that the crop is sensitive to water deficit during the phase approaching flowering, at flowering and pod filling stages (Akyeampong 1986; Figueiredo 1999). Many aspects of plant growth are also affected by drought stress, these include plant height, leaf number and leaf expansion, stem elongation and leaf area index (Loka et al. 2011)). This behaviour was reported to be attributed to the fact that plants growing under water stressed conditions tend to elongate their roots around the growth environment in an attempt to capture water and absorb water from the rhisosphere, thus elongating their stems and roots more than normal (Onuh and Donald 2009).

Water stress remains a constraint to crop production in semi– and arid regions where cowpea is mainly cultivated (Akyempong 1986). Its drought tolerance characteristics could make cowpea a crop of choice for most farmers residing in marginal areas of agricultural production. More arid climates are being predicted due to on–going climate change and variability; cowpea is one of the crops that may contribute to future food security in semi– and arid regions. Although the effects of water stress on cowpea production have been studied, little is known about the effects of water stress on physiological mechanisms, growth and yield components. A better understanding of the effects of water stress on cowpea growth and physiology will provide useful information to farmers on how to manage cowpea production under different environmental conditions. The objective of this study was therefore to evaluate the effect of water stress on physiological changes, growth, yield and yield components of two cowpea varieties (Brown mix and White birch) grown under varying water regimes under controlled environment conditions.

5.2 Results

5.2.1 Soil water content

Soil water content varied quite significantly ($P < 0.001$) over time; it was observed to increase from week 11, reaching the highest level at week 12 where it started to decrease progressively until the end. A similar trend was observed for the interaction between water regime and time. However, there was no significant difference ($P > 0.05$) between varieties under all water regimes (Figure 5.1).

5.2.2 Physiological parameters

Results for stomatal conductance showed no significant differences ($P > 0.05$) between varieties (Figure 5.2). Mean stomatal conductance recorded for Brown mix (47.9 mmol m^{-2} s^{-1}) and White birch (49.1 mmol m^{-2} s^{-1}) were statistically similar. However, there were highly significant differences ($P < 0.001$) between water regimes. Based on mean values of varieties across water regimes, plants grown at 80% ETc had the highest stomatal conductance (60.3 mmol m^{-2} s^{-1}) followed by 60% ETc (48.6 mmol m^{-2} s^{-1}) and then 30% ETc (36.6 mmol m^{-2} s^{-1}). Stomatal conductance was also observed to fluctuate significantly ($P < 0.001$) over time. There were no significant differences ($P > 0.05$) between varieties as well as water regimes, with respect to chlorophyll content index (Figure 5.3). A similar trend was observed for the interaction between the two factors.

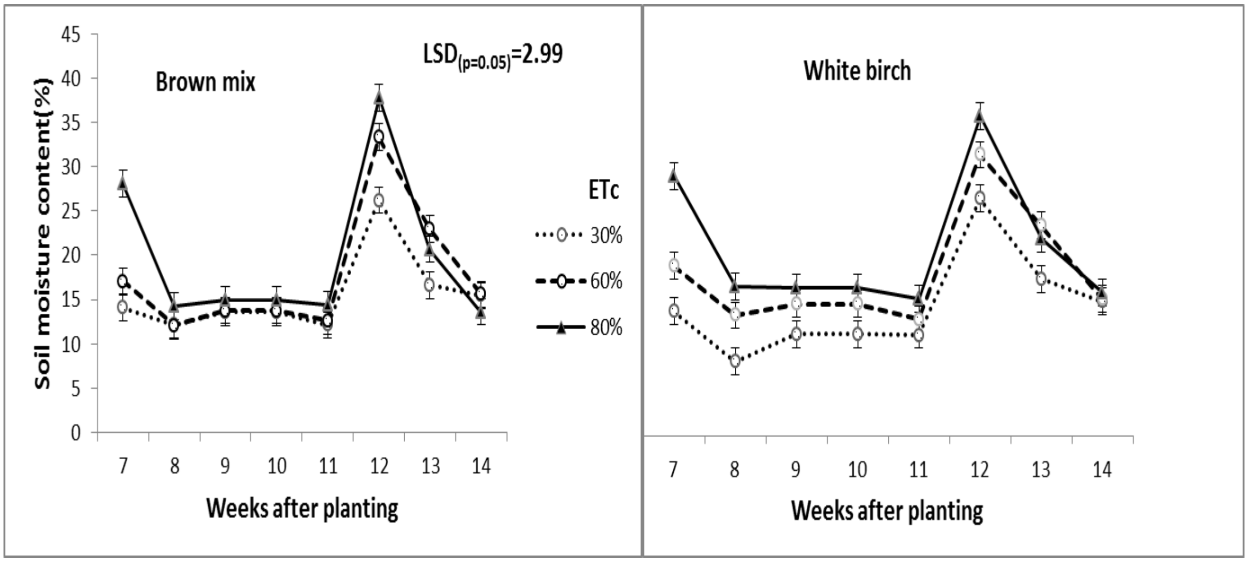

Figure 5.1: Soil water content of two cowpea varieties (Brown mix and White birch) grown under varying water regimes.

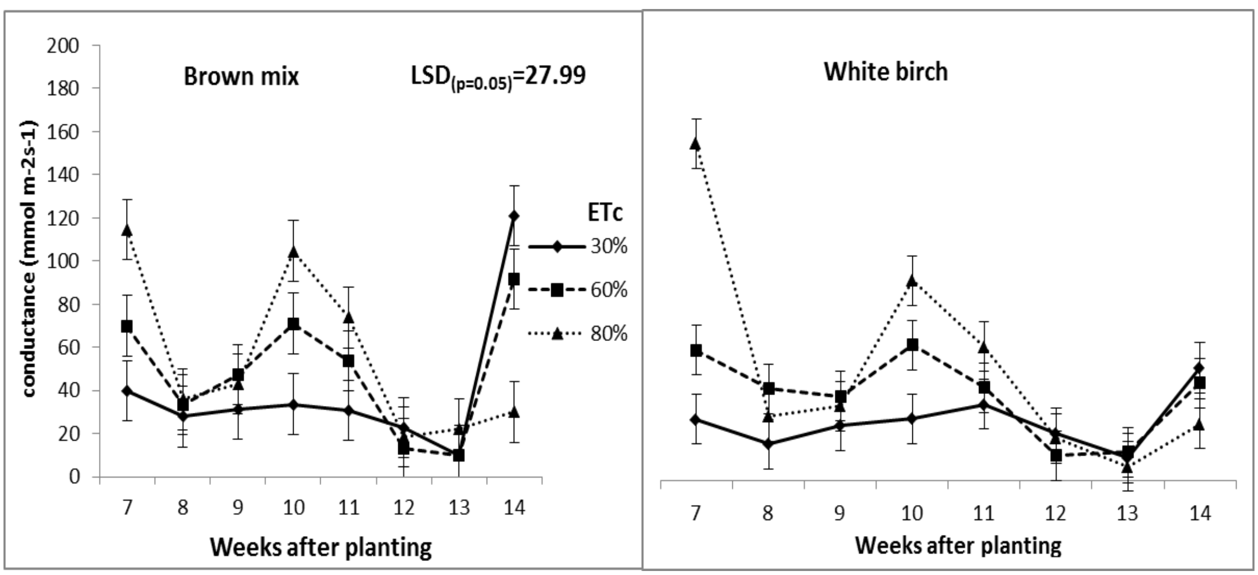

Figure 5.2: Stomatal conductance (mmol m^{-2} s^{-1}) of two cowpea varieties (Brown mix and White birch) in response to varying water regimes

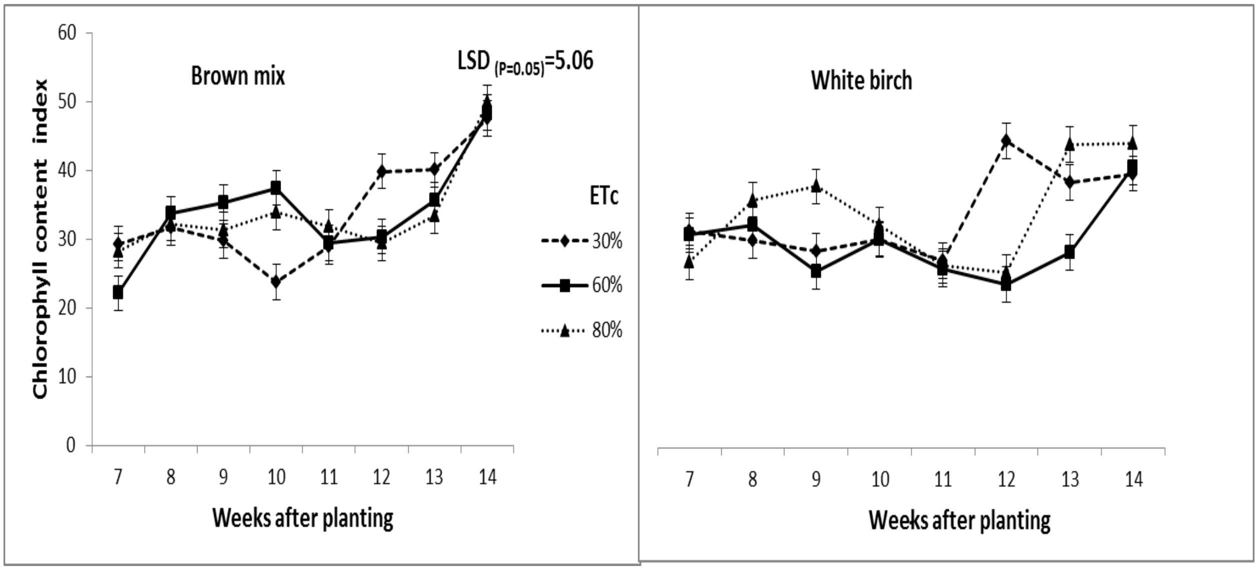

Figure 5.3: Chlorophyll content index of two cowpea varieties (Brown mix and White birch) in response to varying water regimes.

5.2.3 Growth parameters

There was no significant interaction (P > 0.05) between water regimes and varieties over time, with respect to plant height (Figures 5.4, 5.5 and 5.6). However, there were highly significant differences (P < 0.001) between water regimes (Figure 5.5). Plants grown at 80% ETc were taller compared with those grown at 60% and 30% ETc. There were no significant differences (P > 0.05) between the two varieties. For leaf number, there was a significant interaction (P < 0.001) between water regimes and varieties over time (Figure 5.7 and 5.8). The same was observed between water regimes and between varieties (Figure 5.7). The trend was such that 80% ETc > 60% ETc > 30% ETc.

Figure 5.4: Effect of water stress regimes on growth of cowpea. ETc= crop water requirement

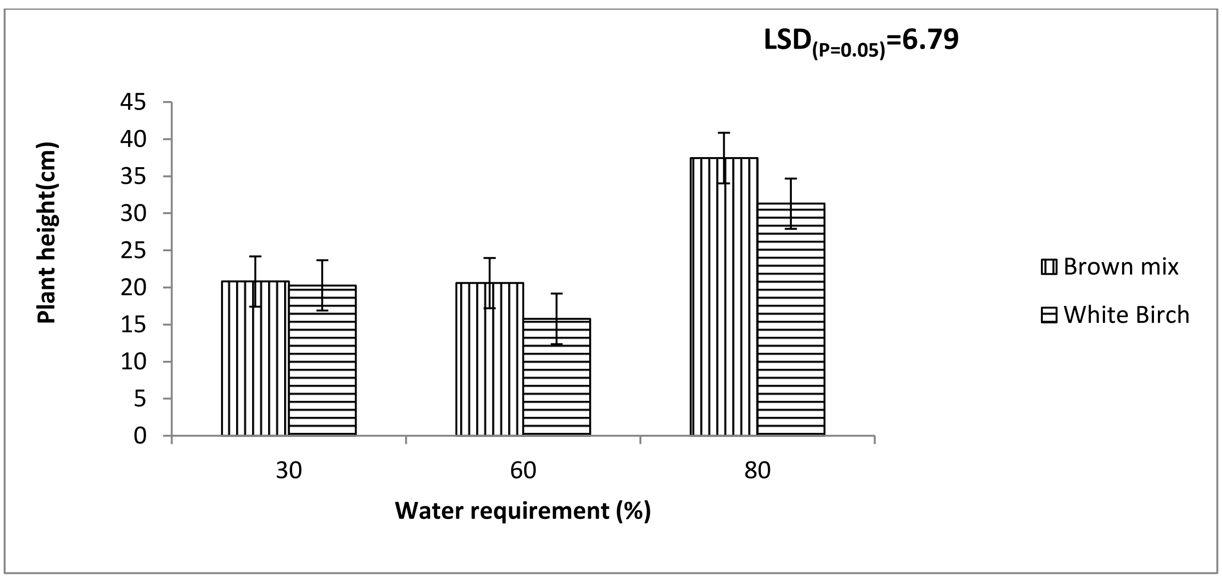

Figure 5.5: Plant height of two cowpea varieties (Brown mix and White birch) grown under water stress regimes.

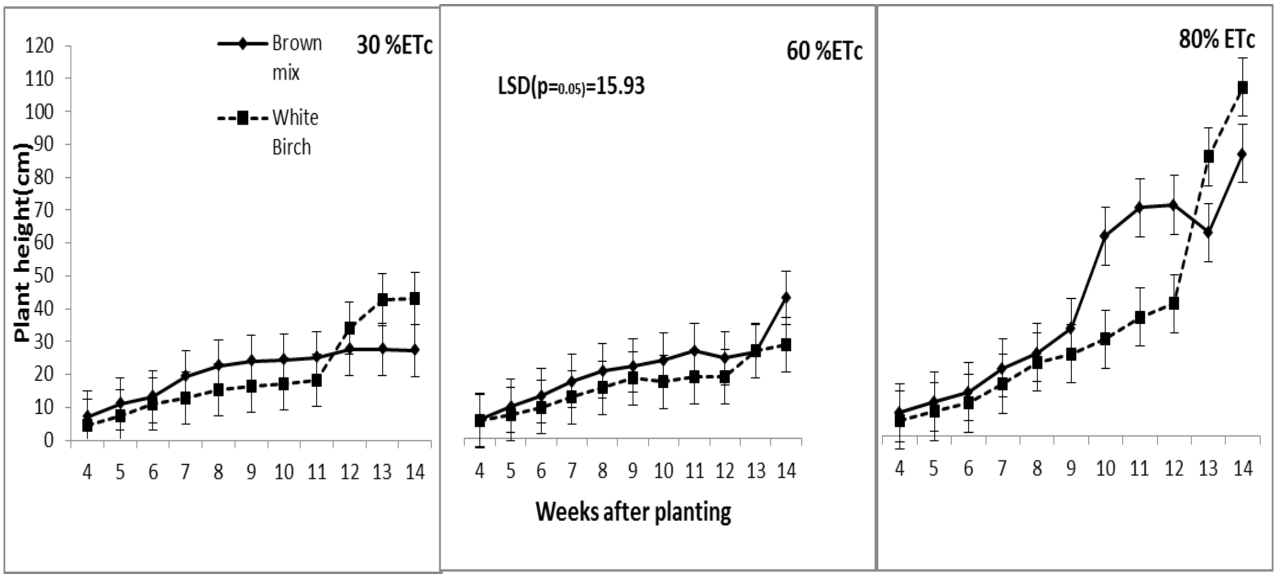

Figure 5.6: Plant height of two cowpea varieties (Brown mix and White birch) in response to varying water regimes.

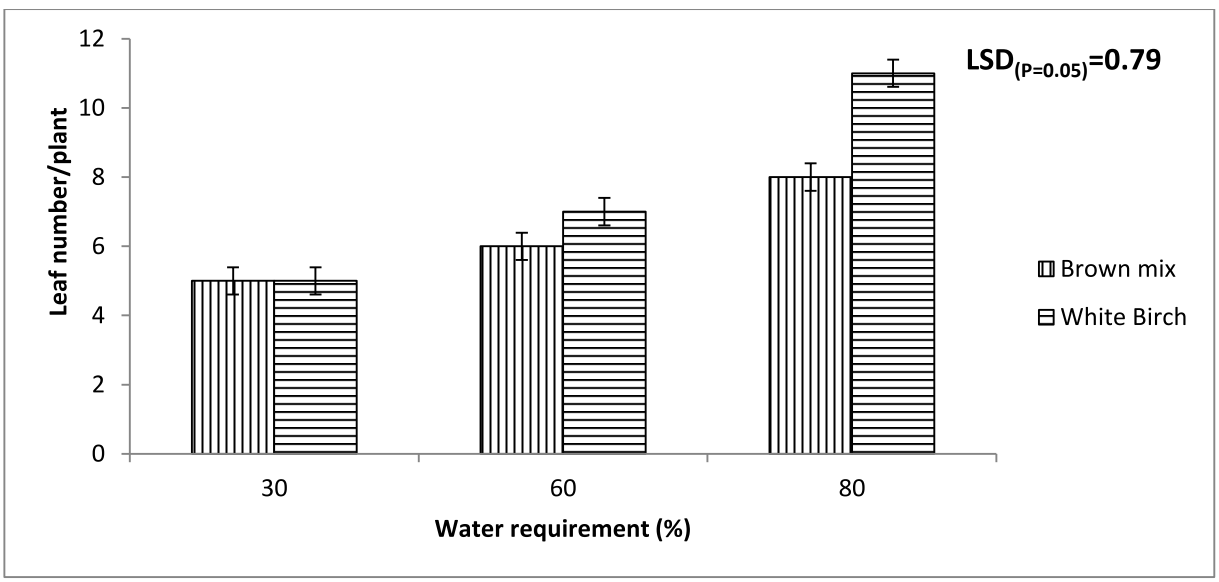

Figure 5.7: Leaf number per plant of two cowpea varieties (Brown mix and White birch) grown under water stress regimes.

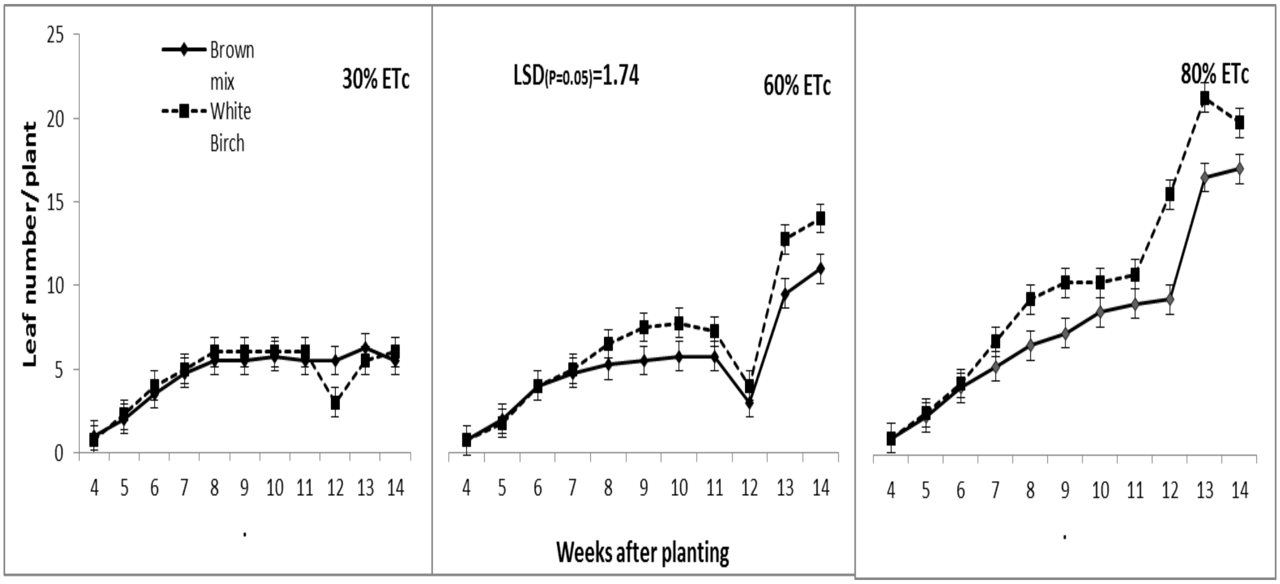

Figure 5.8: Leaf number per plant of two cowpea varieties (Brown mix and White birch) in response to varying water regimes.

5.2.4 Flowering

For both Brown mix and White birch, the average time (days) to flowering was 70 and 77 DAP, respectively (Figure 5.9). Flowering occurred earlier (≈ 70 DAP), for both varieties, at 80% ETc while flowering was slower (≈ 77 DAP) at 30% ETc (Figure 5.9). At 60% ETc, White birch flowered early (≈ 70 DAP) while Brown mix flowered similar to at 30% ETc.

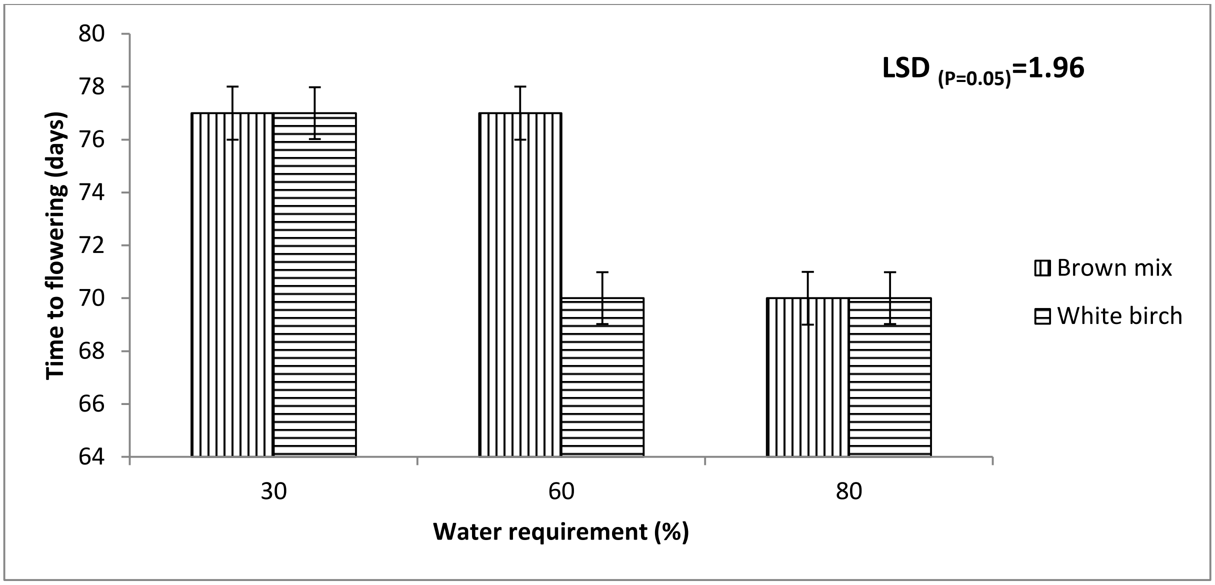

Figure 5.9: Time to flowering (calendar days) recorded for cowpea varieties, Brown mix and White birch, subjected to varying water stress regimes**.**

5.2.5 Yield components

There were highly significant differences ($P < 0.001$) among water regimes for pod number per plant (Table 5.1). Mean values for water regimes across varieties showed that the trend was such that 80% ETc (13 pods) > 60% ETc (6 pods) > 30% ETc (4 pods). Based on mean values of varieties across water regimes, White birch had more ($P < 0.001$) pods (10 pods) per plant than Brown mix (5 pods). The interaction between water regimes and varieties was not significant. With respect to the number of seeds per pod, no significant differences ($P > 0.05$) were observed between varieties and among water regimes. However, Brown mix had more seeds per pod (10 seeds) than White birch (8 seeds) (Table 5.1). Similar to pod number per plant, the interaction between water regimes and varieties was not significant. There were significant differences ($P < 0.05$) between varieties, with respect to seed mass. Based on mean values, Brown mix had the highest seed mass per pod (1.73 g) than White birch (1.02 g). There were no significant differences ($P > 0.05$) among water regimes. Results of HI showed significant differences ($P < 0.05$) between varieties. White birch had higher harvest index than Brown mix. There were highly significant differences ($P < 0.001$) between water regimes. Plants grown under 80% ETc had higher HI, followed by 60% ETc and 30% ETc (Table 5.1).

Table 5.1: Yield components of Brown mix and White birch varieties in response to varying water regimes under controlled environment conditions.

Water regime	Variety	Biomass (g)	*HI	Pod No. Plant^{-1}	Seed No.Pod^{-1}	Seed mass Pod^{-1} (g)	Yield Plant^{-1} (g)
30%ETc	Brown mix	20.57^{a}	25.6^{a}	3^{a}	10^{a}	1.75^{a}	4.14^{a}
	White birch	18.21^{a}	26.9^{a}	5^{a}	6^{b}	0.86^{b}	3.94^{a}
60%ETc	Brown mix	23.8^{a}	31.3^{b}	4^{a}	10^{a}	1.7^{a}	5.01^{b}
	White birch	25.43^{a}	44^{a}	8^{a}	9^{a}	1.06^{a}	8.59^{a}
80%ETc	Brown mix	33.1^{b}	47.4^{a}	9^{b}	9^{a}	1.74^{a}	12.68^{b}
	White birch	38.3 ^{a}	57.9^{a}	17^{a}	9^{a}	1.16^{a}	18.65^{a}
LSD$_{(P=0.05)}$		4.752	0.1117	4.351	2.85	0.7183	2.941

*HI= Harvest Index.

5.2.6 Seed quality tests

For both varieties, for the tetrazolium test, progeny produced under 80% ETc and 60% ETc stained normal red, indicating 100% viability. However, White birch seeds produced under severe water stress conditions (30% ETc) were less viable (75% staining) when compared with Brown mix variety (100%).

Results from the standard germination test showed significant differences ($P < 0.05$) between the two varieties (Figure 5.10). Based on mean values of varieties across water regimes, Brown mix had higher (72.96%) final germination than White birch (67.22%). There were no significant differences ($P > 0.05$) observed among water regimes. The interaction between water regimes and varieties was not significant. However, a closer look at the results showed that germination of Brown mix seeds was not affected by production environment. Germination of White birch was, however, shown to decline in response to increasing water stress imposed on the maternal plants (Figure 5.10)

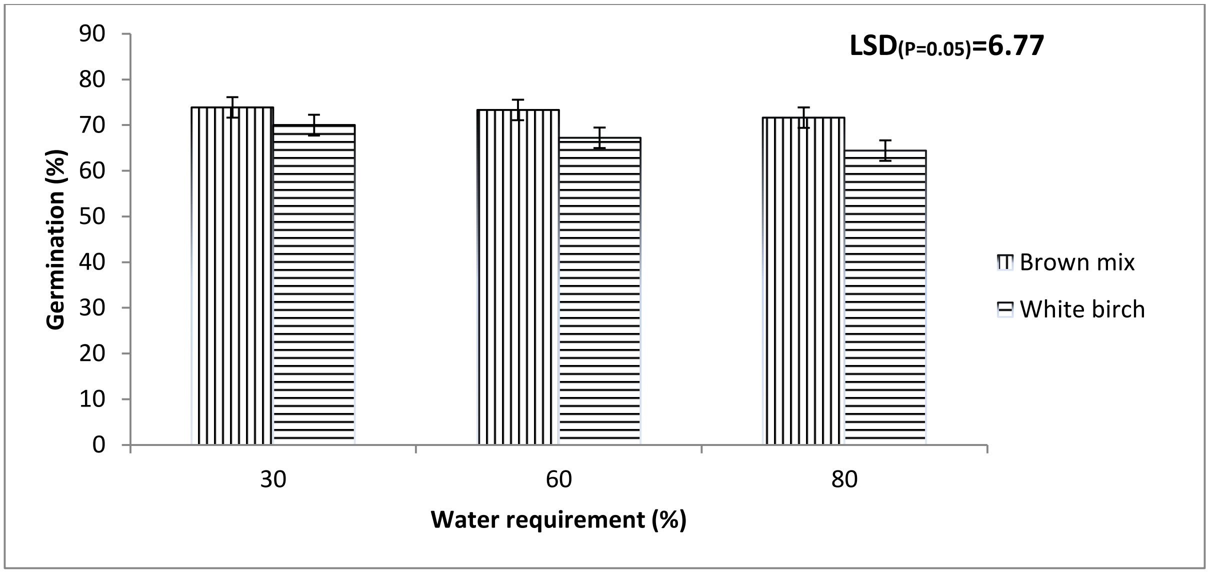

Figure 5.10: Germination percentage of cowpea seeds of Brown mix and White birch varieties grown under water stress regimes, recorded in a germination chamber.

Results of germination vigour indices (root length, shoot length, root: shoot ratio) of the two varieties showed no significant differences ($P > 0.05$) among water regimes (Table 5.2). However, dry mass varied significantly ($P < 0.05$) between varieties and among water regimes. For both varieties, progeny from the 80% ETc water regime had the highest dry mass followed by 60% ETc and 30% ETc (Table 5.2). On average, Brown mix had higher dry mass than White birch. Mean time to germination (MGT) and germination velocity index (GVI) showed no significant differences ($P > 0.05$) between varieties and among water regimes (Table 5.2). The trend was such that the Brown mix variety germinated faster than White birch variety (Table 5.2). The results of electrical conductivity showed no significant differences ($P > 0.05$) between the two cowpea varieties under the different water regimes. Seeds of plants grown under 30% ETc and 60% ETc had lower EC compared with seeds of plants grown under 80% ETc (Figure 5.11).

Table 5.2: Germination capacity and seed vigour of two cowpea varieties (Brown mix and White birch) recorded during standard germination and seed vigour tests**.**

Water regime	Variety	Germination	EC	MGT	GVI	Root Length	Shoot Length	Root : Shoot ratio	Dry mass
		%	µs/g	days		mm			g
30% ETc	Brown	73.89	307	2.2	3.93	130.8	93	1.41	1.26
	White	70	358	2.2	3.65	116.4	74	1.57	1.09
60% ETc	Brown	73.33	344	2.07	3.93	124.8	97	1.28	1.62
	White	67.22	345	2.07	3.47	131.6	88	1.49	1.22
80% ETc	Brown	71.67	467	2.25	3.77	118.4	89	1.33	2.16
	white	64.44	383	2.05	3.2	136.4	79	1.72	1.33
$LSD_{(P=0.05)}$		4.78	70.8	0.16	0.50	21.4	17.2	0.40	0.21

GVI= Germination Velocity Index, MGT= Mean Germination Time, EC=Electrolyte Conductivity.

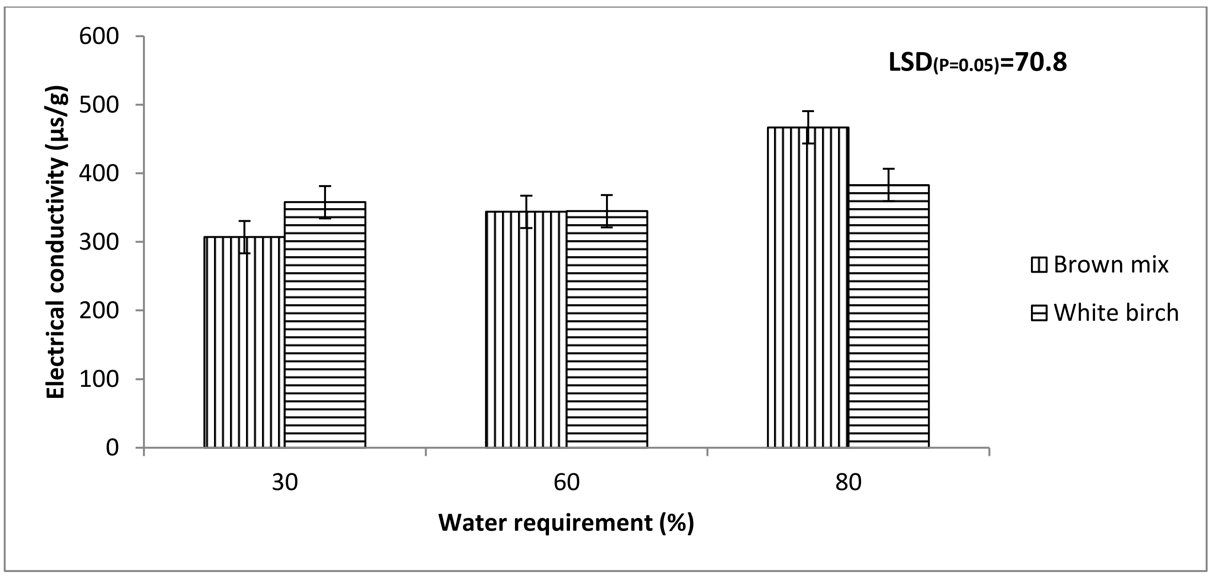

Figure 5.11: Electrical conductivity of cowpea seeds of Brown mix and White birch varieties obtained from plants grown under water stress regimes.

5.2.7 Proline and protein analysis

Proline accumulation showed highly significant differences ($P < 0.001$) between varieties and among water regimes (Figure 5.12). The interaction between varieties and water regimes was also highly significant ($P < 0.001$). For all varieties, proline concentration was lowest at 80% ETc and highest at 30% ETc; the only exception was Brown mix which had higher levels of proline accumulation at 60% ETc than 30% ETc (Figure 5.12). Based on means of varieties across water regimes, Brown mix had the higher proline concentration than White birch variety.

Highly significant differences ($P < 0.001$) were observed between varieties and water regimes, with respect to protein content. The interaction between varieties and water regimes was also highly significant ($P < 0.001$) (Figure 5.13). For both Brown mix and White birch, the trend in protein content was such that 80% ETc > 60% ETc > 30% ETc (Figure 5.13). At 80% and 60% ETc, Brown mix had less protein content than White birch; at 30% ETc the two varieties had similar protein content (Figure 5.13).

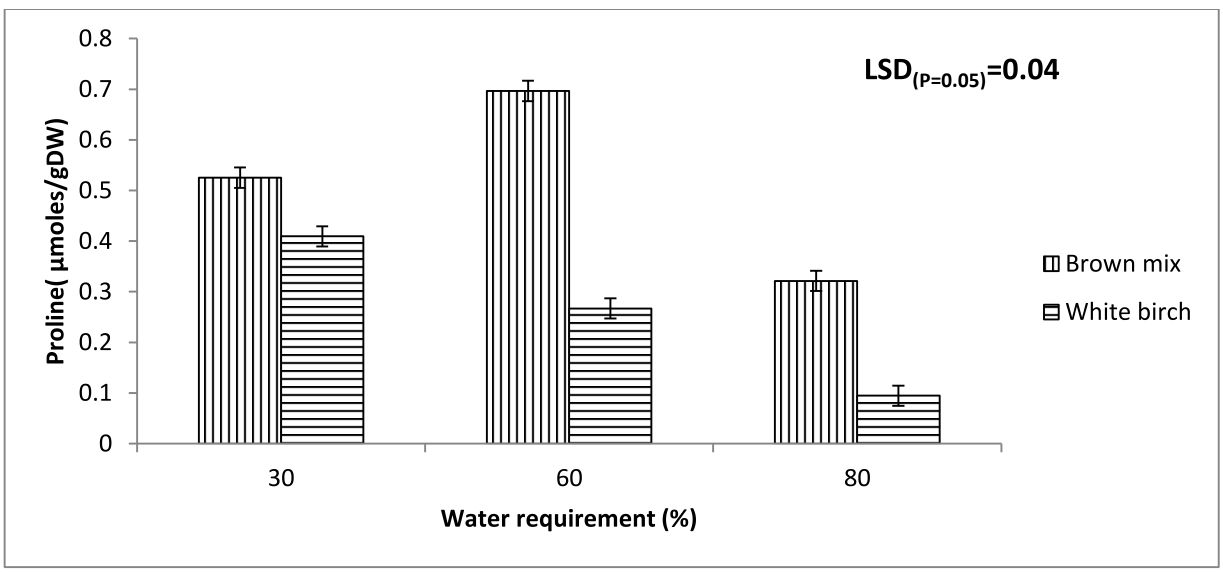

Figure 5.12: Proline concentration in two cowpea varieties (Brown mix and White birch) grown under water stress regimes.

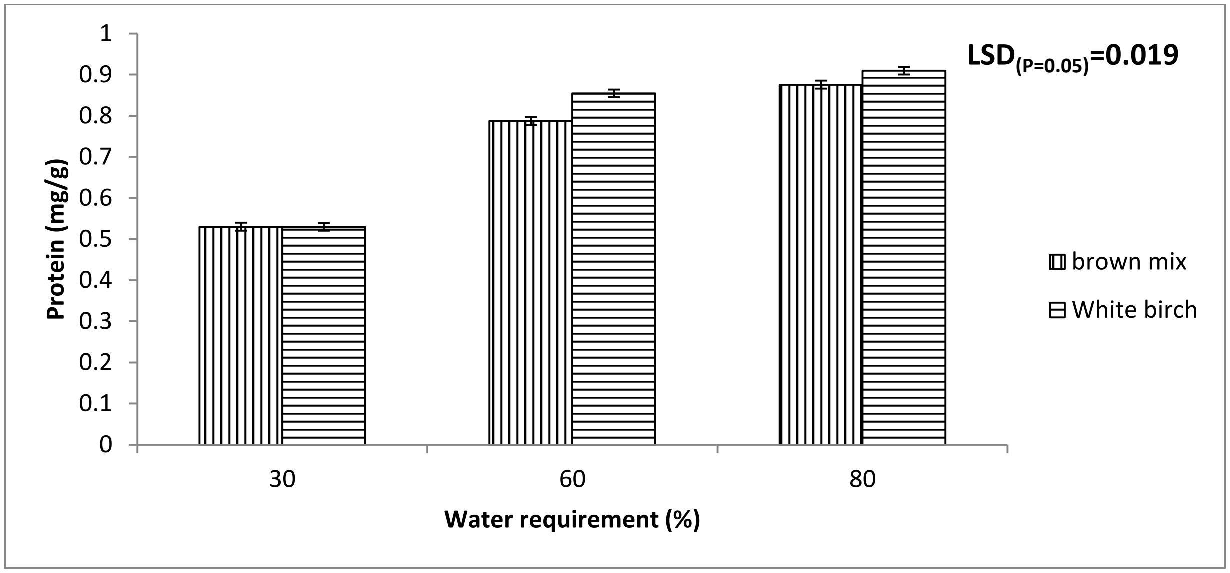

Figure 5.13: Protein content in two cowpea varieties (Brown mix and White birch) grown under water stress regimes.

5.3 Discussion

It is well known that in response to depleting soil water content, plants close their stomata to limit water losses through transpiration. On the negative side, stomatal closure also deprives leaves of carbon dioxide as intracellular carbon dioxide is reduced (Anjum et al. 2011). Several studies have associated stomatal regulation with improved plant water use and drought tolerance under water limited conditions (Medrano et al. 2002; Kumar et al. 2012; Cordona–Ayala et al. 2013). In the current study, stomatal conductance was negatively affected by water stress; stomatal conductance was lowest at 30% ETc. Lower stomatal conductance suggests that cowpea varieties were avoiding water stress by lowering their water use. In addition, stomatal conductance was shown to vary over time suggesting that the effects of water stress on cowpea varied according to plant growth stage. Ntombela (2013) showed that cowpea sensitivity to water stress varied with growth stage, with the reproductive stage being most sensitive to water stress.

In addition to stomatal regulation, leaf chlorophyll content also provides valuable information on physiological status of plants (Gilson et al. 2003). Results of this study showed that leaf chlorophyll content index was stable across the varying water regimes. This observation was inconsistent with previous studies on cowpea (Ntombela 2013) and bambara groundnut (Mabhaudhi and Modi 2013), whereby chlorophyll content index was lower in response to limited soil water availability. Results observed in this study may be attributed to cowpea drought avoidance mechanisms which allow cowpea to endure long periods without significant rainfall and continue with their normal metabolic activities (Kumar et al. 2012). The lack of sensitivity in chlorophyll content index in response to water stress suggests that, in cowpea, leaf chlorophyll content index may not be a useful index for water stress tolerance. However, the variation in chlorophyll content index over time, especially the decrease observed 11 weeks after planting, suggests that chlorophyll content index might be useful for predicting crop maturity.

There is a wide consensus that water stress leads to substantial physiological and morphological changes in plants, which are reflected on the yield (Sinhababu and Banerjee 2013). Morphological changes include growth parameters such as leaf number and plant height (Anjum et al. 2011). In the current study, plant height was adversely affected by water stress. Previous studies on other legumes (Anjum et al. 2010; Mabhaudhi and Modi 2013) also reported a reduction in plant height due to water stress. The reduction in plant height under water stress has been attributed to inhibition of cell division and expansion under water stress (Manivannan et al. 2007). Reduction in plant height also contributes to reduced canopy size, a drought avoidance mechanism (Blum 2005). In the current study, plant height of Brown mix and White birch did not differ significantly, although Brown mix generally had taller plants implying higher levels of water use relative to White birch. Leaves are the most important photosynthetic plant structure for fixing carbon dioxide for plant growth. Results of this study showed that leaf number was significantly affected by water stress at 60% and 30% ETc. Previous studies shown reduction in leaf number due to water stress (Mbatha and Modi 2010; Ntombela 2013). Interestingly, despite White birch having shorter plants, results showed that it generally had more leaves than Brown mix.

Time to flowering is considered as an important descriptor of plant adaptation to a particular agro-ecological zone (Ishiyaku et al. 2005). The current study showed that flowering occurred earlier under optimum than water stressed conditions. This observation was contrary to reports in the literature that flowering is usually hastened under water limited conditions (Mabhaudhi and Modi 2013). Such phenological plasticity has been associated with drought escape mechanisms (Mabhaudhi and Modi 2013). Our observations of delayed flowering in cowpea under water stress may be associated with reports of delayed leaf senescence in cowpea under water limited conditions (Odindo 2007). Under such conditions, the crop may delay leaf senescence and flowering until conditions (soil water availability) are favourable.

Results of plant growth and physiology correlated well with results of yield; cowpea plants grown at 60% ETc and 30% ETc produced fewer pods per plant compared to plants grown at 80% ETc; this trend was consistent for harvest index. The White birch variety had greater pod number per plant and harvest index than Brown mix; this was consistent with higher leaf number observed for White birch. In general, high leaf number implies greater solar radiation interception and more photo-assimilate production hence greater yields (Babaji et al. 2011). In several legumes, the number of pods per plant remains the most important component in determining yield (Mathew et al. 2000). Previous studies (Ahmed and Suliman 2010; Wofia et al. 2013) reported that in legumes pod number per plant was correlated with yield. White birch had more pods compared with Brown mix; this may suggest better adaptation to water stress in White birch compared with Brown mix. On the other hand, the difference between the two varieties may be related to their different genetic potential (Mac William et al. 1999).

Seed yield is determined by the number of seed per unit area and seed mass. Previous studies have been indicated the decrease in seed number per pod when water stress was imposed at flowering and post flowering (Bartel and Caesar 1987; Nciizah 2007). In the present experiment, seed number per pod was not significantly affected by water regimes, indicating that seed number per pod was genetically controlled. This was in conformity with reports by Ferry (1985) that, in cowpea, seed number per pod was heritable under several environmental conditions. Brown mix had more seeds per pod compared with White birch. This also suggests that seed number per pod was genetically controlled. Results of seed mass, across all water regimes, showed that seeds of Brown mix variety had more mass than White birch. This implies that seed mass was not influenced by environmental factor in this case, but was genetically controlled (Pedersen and Lauer 2004). Harvest index (HI) is considered as a measure of reproductive efficiency in grain crops and is generally lower in pulses than cereals (Ghafoor et al. 1993). In the present study, HI decreased with decreasing water availability.

Harvested seeds were evaluated to determine statistically whether water stress imposed during growth and development of the maternal plant can influence the quality (viability and vigour) of the subsequent generation. Results obtained showed seed quality (viability and vigour) did not decrease in relation to water stress. This is confirmed by the fact that progeny from plants grown under 30% ETc and 60% ETc had less electrolyte leakage and higher germination percentage compared to progeny of plants grown under 80% ETc. This observation is in line with Dombos et al. (1989) who indicated that in most of the cases, seeds of plants under water stress had high germination quality. It was observed that although water stress did not negatively affect seed quality of progeny, overall, there was a decline in viability and vigour relative to initial seed quality reported in Chapter 4 (Table 4.1). The higher electrical conductivity values observed in harvested seeds may be attributed to the effect of water stress during seed development and maturation. According to Tang (1982), reduction in seed vigour due to water stress was attributed to both low nutrient accumulation and small embryo size at seed maturity. It could be the cause of low germination percentage observed in harvested seed compared to the initial seed results.

Cowpea seeds produced under 30% ETc showed greater proline accumulation relative to other water regimes. Previous studies have also reported increase in proline concentration under stress conditions (Druge 1998; Chuilele and Agenbag 2004). This observation suggests possible adaptive response of cowpea to water stress. Results of proline are also supported by stomatal conductance results where plants grown under 30% ETc had very low stomatal conductance. Brown mix accumulated more proline compared with White birch. This observation explains the sensitivity to drought stress observed in Brown mix compared with White birch. These observations correlated with results of leaf number and yield, suggesting better adaptation in White birch than Brown mix.

Environmental stresses have been reported to lead to changes in protein synthesis in plants (Dubey 1999). Several studies have indicated the quantitative reduction in the rate of protein synthesis due to water stress (Dhindsa and Cleland 1974; Ezzine and Ghorbel 2006). In the present study, progeny from the 80% ETc water regime had more protein than progeny from plants grown under 60% ETc and 30% ETc. This suggests that protein synthesis was inhibited by water stress. The two varieties differed significantly with respect to protein concentration; White birch had higher protein concentration than Brown mix. Results obtained were related to stomatal conductance, confirming more drought tolerance in White birch compared with Brown mix.

5.4 Conclusion

Crop physiology helps to understand the genetic potential of plants and their interaction with environmental factors. Results of this study showed that water stress had a negative effect on stomatal conductance, thereby reducing plant growth and productivity. Although cowpea is a drought tolerant crop, water stress can negatively affect yield. Chlorophyll content index is not a sensitive parameter to water stress but may be useful for predicting crop maturity. Time to flowering was influenced by water stress; plants grown under severe water stress flowered later compared to plants under moderate stress. Seed quality of cowpea was not significantly affected by production environment although optimum conditions generally led to better seed quality characteristics. The White birch variety proved to be better adapted to water stress and produced good harvest index compared to Brown mix. It is recommended that farmers growing cowpea in water scarce areas could be advised to include White birch in their selection.

CHAPTER 6

EFFECTS OF PLANTING DATES ON PHYSIOLOGY, GROWTH AND YIELD COMPONENTS OF COWPEA

6.1. Introduction

Climate change, through the decrease in water availability (Bates et al. 2008), remains a serious threat to crop productivity in semi– and arid regions that are already food insecure (Knox et al. 2012). Water stress has been reported as one of the most important environmental factors affecting crop productivity in many countries of the world, particularly in these regions (Yang et al. 2006). Many crops, even drought-tolerant ones such as cowpea, still suffer considerable damage due to frequent drought or climate variability which has resulted in shorter and less frequent rainy seasons (Agbicodo 2009; Dadson et al. 2005). Under these conditions, cowpea still remains a crop of choice since it is inherently more drought tolerant than other crops (Singh and Matsui 2002). In addition, it has the potential to provide food to the ever increasing human population in developing countries (Burness communication 2010). Crop adaptation and yield in response to this scourge is among significant challenges facing agricultural researchers. The use of drought-tolerant crops and planting date management are some adaptive strategies (practices) that are envisaged to assist farmers to cope with limited water resources (reduced rainfall and soil water availability) in semi– and arid regions.

Planting date is one of the crucial aspects that need to be considered in management decisions for crop production. It is more important especially in regions with environmental constraints such heat, limited water resources and late or early cold at the beginning and end of the season (Tayebi et al. 2012). It is among the most important factors influencing yield and yield components of crops (Zhang et al. 2008). A study by Tsimba et al. (2013) reported a decrease in total biomass and harvest index of maize due to manipulation of planting date. Ntare and Williams (1992) had earlier pointed out that cowpea yield was reduced by more than 50% if there was a two week delay in sowing. Another study done in Australia reported a 35% reduction of canola seed yield if sowing was done in May and 67% reductions if done in July.

These comparisons were done against canola sowing conducted in April (Hocking and Stapper 2001). Some reports showed that time of planting had significant effects on growth parameters such as plant height, stem diameter, leaf number, and leaf area index (Ada 2012; Moradpour et al. 2013; Tayebi et al. 2012). There are other reports, however, which indicate no relativity between growth parameters and planting date, for example, a study on maize showed no effect on plant height at harvest due to planting date (Pedersen and Lauer 2004).

Generally, planting time is a strategic cultural practice used by farmers in developed and developing countries for protecting crops against biotic (pest, animals) and abiotic stresses (excessive moisture, heat, drought) (Linker et al. 2009; Pedersen 2007). In Western Colorado for example, the selection of corn planting date ensures physiological maturity before fall frost (Anapalli et al. 2005). Inappropriate planting date for cowpea was found to be the cause of crop infection which consequently affected yield and yield component in Northern Nigeria. This was revealed by Mbong et al. (2010), who reported that early sown cowpea had higher scab infection incidences compared with late sown cowpea. From this point of view, the choice of appropriate planting date for a crop is of great importance in crop production. It has a considerable influence on crop survival and hence on yield components (Klebesadel 1992).

An appropriate planting date of a crop is defined as a date when the plants can establish well and their susceptible growth stages do not coincide with adverse environmental conditions (Seghatoleslami et al. 2013). Seghatoleslami et al. (2013) reported an increase in stomatal conductance of roselle due to delayed sowing. Work done by Wilson et al. (2012) on pigeon pea also showed higher stomatal conductance in late planted plots compared to earlier planted ones. Reports by El- Khoby (2004), however, indicated decreases in chlorophyll content of rice when planting date was delayed. Changes in plant functioning also affected the quality of seeds produced. According to Sidibe et al. (1996), seed quality of soybean was improved by delaying planting date. However, in Pakistan (Peshawar) contrasting observations were made by Muhammad (2008), who showed that maximum vigour test values of soybean were recorded in early planting.

Time of planting varies depending on the climatic conditions of the region (Sadeghi and Niyaki 2013). Water is one of the most important climate factors for ecosystem. Both its shortage and excess affect the growth and development of plants directly, and consequently its yield and quality. In cowpea, good yield has been reported to be obtained by determining the onset and duration of the rains (Dugje et al. 2009). Other criteria also considered for planting dates includes; temperature, rainfall distribution, and the maturity period of the varieties used (Tayebi et al. 2012; Sadeghi and Niyaki 2013). Therefore, adjusting the ideal time for planting either to early or late season remains the key for optimising and stabilising cowpea yield especially in severe adverse climatic conditions or management challenges (Tsimba et al. 2013).

South Africa's climate is predominantly semi-arid characterized by large fluctuations in annual rainfall (Palmer and Ainslie 2002). This leads to variability in average date of onset of the rainy season than its cessation (Shiringane 2007). The same author further indicated that time of onset and cessation of rains remains the most important factor for predicting growth and subsequent crop yield of rainfed crops. To maximise yields and profitability, it is therefore necessary to take into consideration the planting date. Significant progress has been made up to now in the determination of planting dates effect on cowpea (Mbong et al. 2010; Peksen et al. 2002). Information about these effects on the interaction of physiological response, growth, yield and water use is still limited. The primary objective of the study was to evaluate the effect of planting date selection as a management tool for optimising cowpea yields under water limited conditions. Secondary to this, the study evaluated the influence of sowing date on cowpea physiology under two different water regimes (irrigation and rainfed). Results from this study will provide more knowledge to cowpea producers on how planting date and water use affect cowpea physiology and grain yield in South Africa and other semi-arid countries.

6.2. Result

6.2.1 Weather data

Rainfall decreased significantly from April to July. This coincided with the first and the second planting date (Figure 6.1). The first planting date took place in early April while the second planting date happened in mid-June. In August the rainfall started to increase and this coincided with the third planting date which took place in late August. Average temperatures from April decreased also significantly and started to increase in august (Figure 6.1).

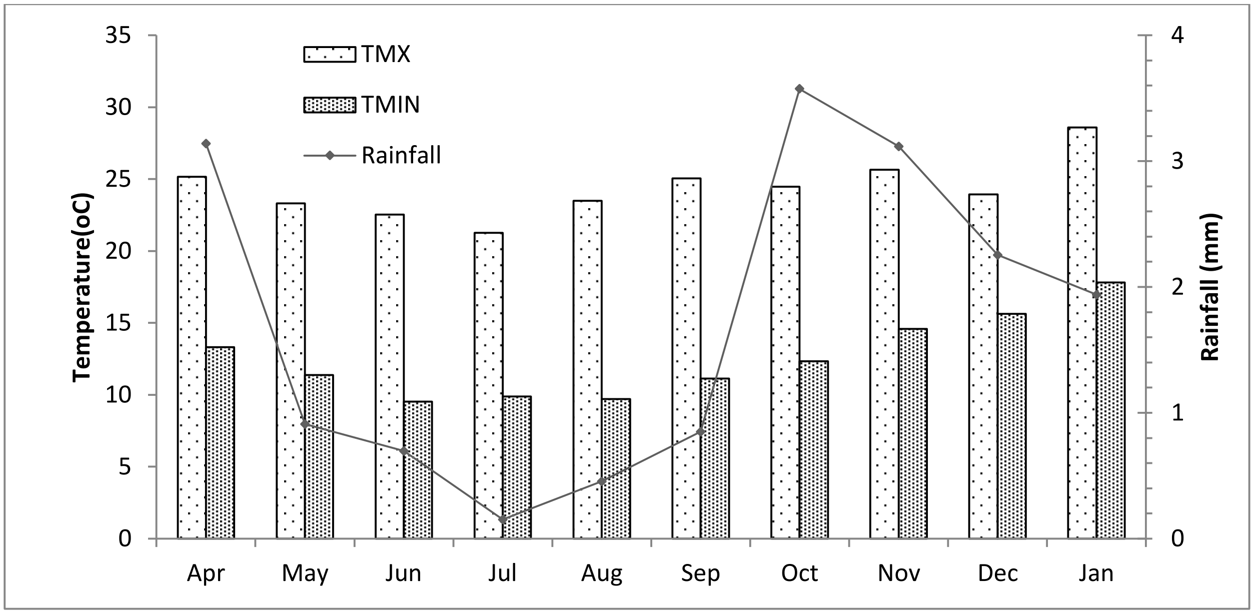

Figure 6.1: Monthly weather data (From April 2013 to January 2014).

6.2.2 Soil water content

Soil water content varied significantly ($P < 0.05$) between rain fed and irrigated trial for the first 100 mm depth in all planting dates (Figure 6.2). This was particularly apparent to the first and second planting date. Soil water content varied significantly also ($P > 0.05$) between the three plantings. Third planting date was observed to have high water content than all plantings followed by first planting while second planting had least water content (Figure 6.2). First and second planting dates coincided with winter season where rainfall and temperature were very low (Figure 6.1).

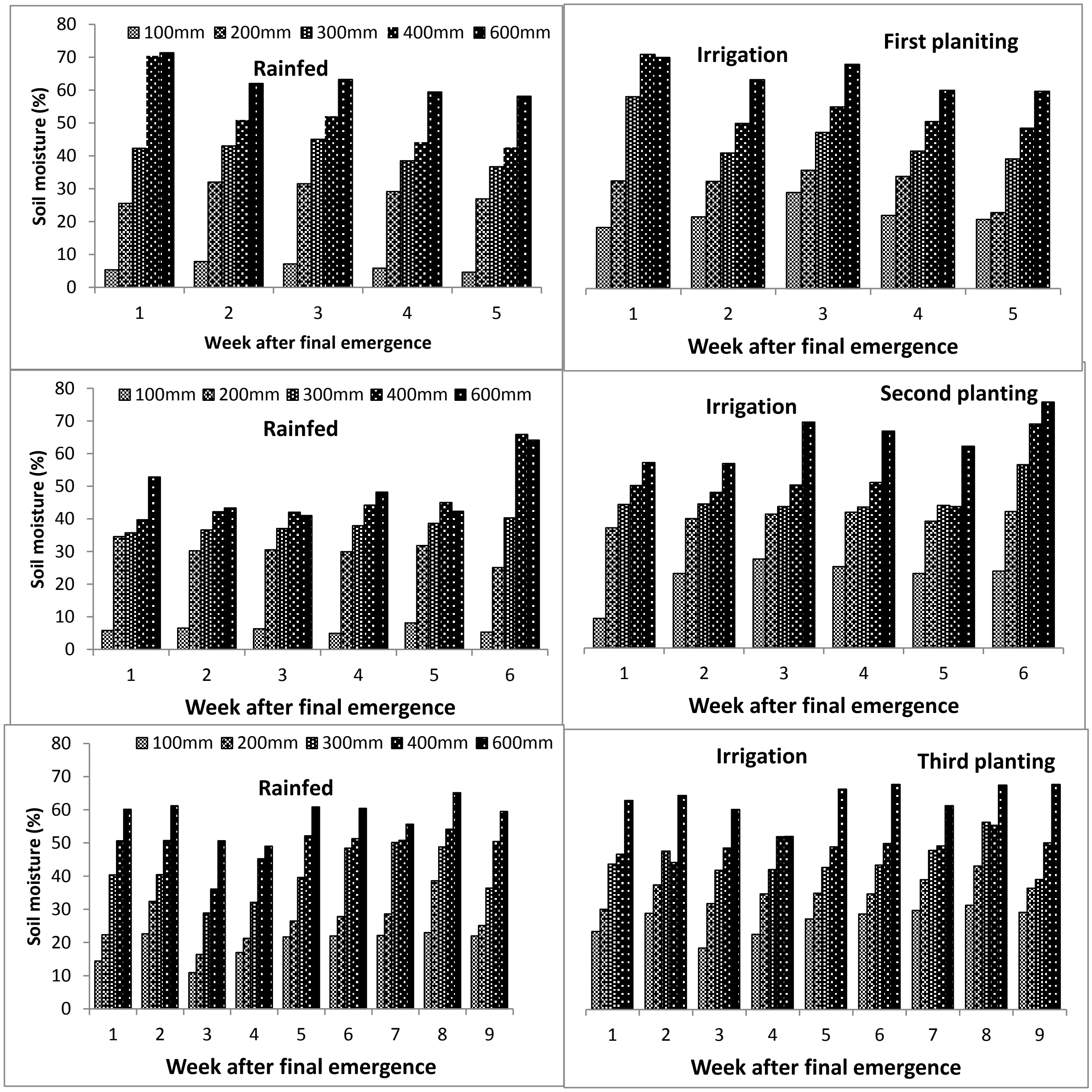

Figure 6.2: Soil moisture at the experimental site at three planting dates.

6.2.3 Emergence

Time to emergence was significantly (P>0.001) influenced by planting dates (Figure 6.3). Seedlings emerged earlier for the first planting date (7 DAP), followed by the second (21 DAP) and third planting dates (29 DAP). There were no significant differences (P>0.05) between the two varieties with respect to time to emergence. However, during the second and the third planting, White birch emerged faster than Brown mix. The interaction between variety and planting dates was not significant (P>0.05).

Planting dates significantly (P<0.001) affected the emergence. The first planting had higher final emergence (85%) followed by the third (58%) and second planting dates (25%) (Figure 6.4). The two varieties did not differ significantly (P>0.05) with respect to percentage emergence. However, during the first planting Brown mix performed better than White birch, while White birch had the highest emergence in the second planting. The interaction between variety and planting date was not significant (P>0.05) with respect to emergence.

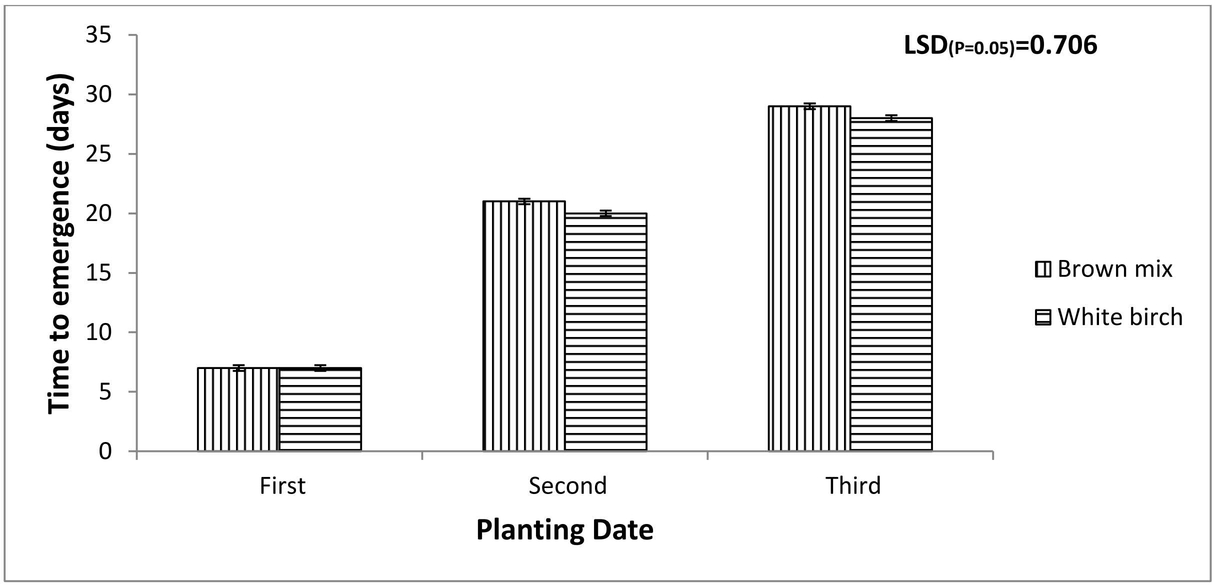

Figure 6.3: Days to emergence of cowpea seeds observed at three planting dates.

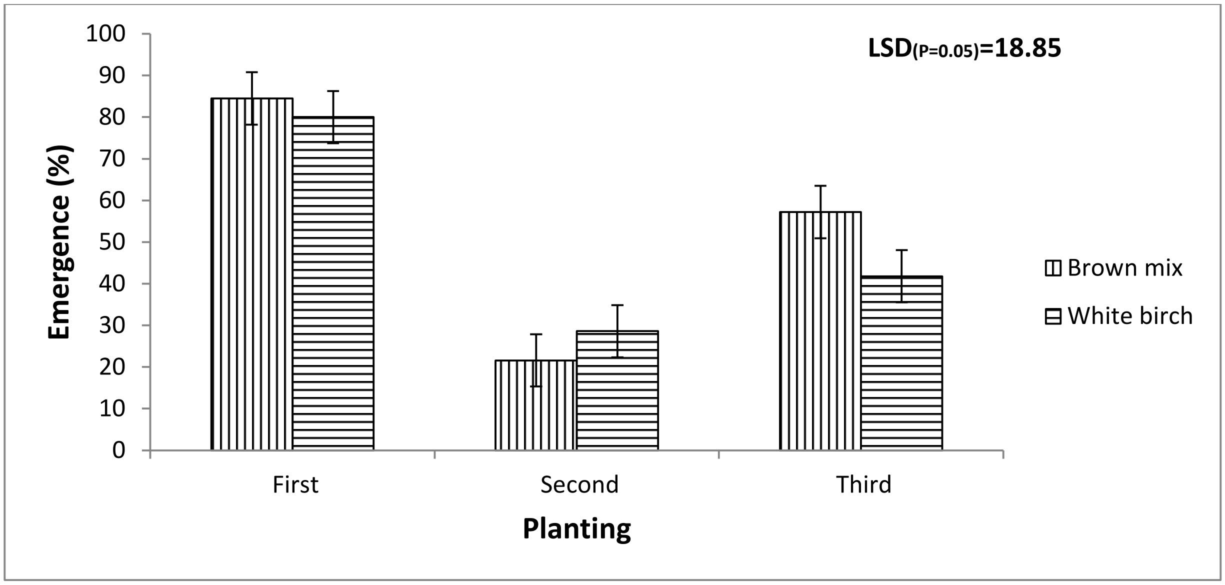

Figure 6.4: Emergence rate of cowpea seeds observed at three planting dates.

6.2.4. Physiological Parameters

6.2.4.1 Stomatal conductance

Stomatal conductance was observed to be affected by different planting dates. It was more reduced during the first planting date, followed by the second and third planting dates respectively (Figure 6.5). Under rainfed and irrigation water regimes, stomatal conductance showed no significant ($P > 0.05$) difference between the two varieties for all three plantings. However, White birch had higher stomatal conductance especially under rainfed compared with Brown mix. For all plantings, the interaction between variety and water regime was not significant $P > 0.05$).

6.2.4.2 Chlorophyll Content Index (CCI)

The CCI differed significantly ($P < 0.05$) on different planting dates. It decreased significantly during the first planting dates, and was observed to increase in the second and third planting dates respectively (Figure 6.6). For all planting dates, there were no significant differences ($P > 0.05$) between water regimes, except for the third planting. Significant differences ($P < 0.05$) in CCI were observed between the two varieties for all three planting dates. Brown mix had the highest CCI relative to White birch.

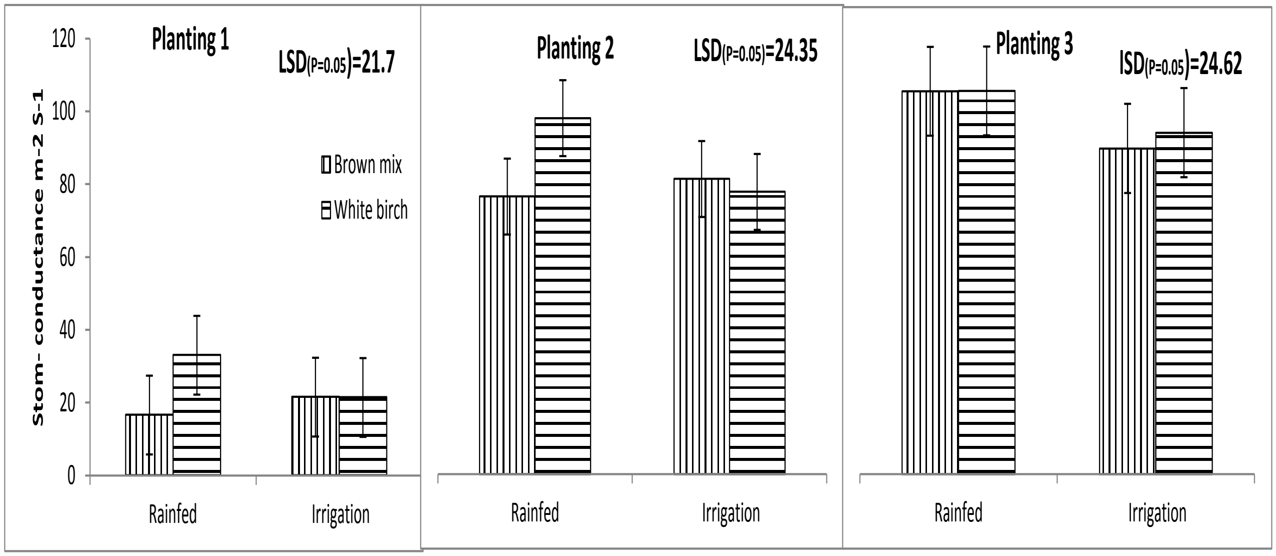

Figure 6.5: Stomatal conductance of cowpea plants grown at the three planting dates.

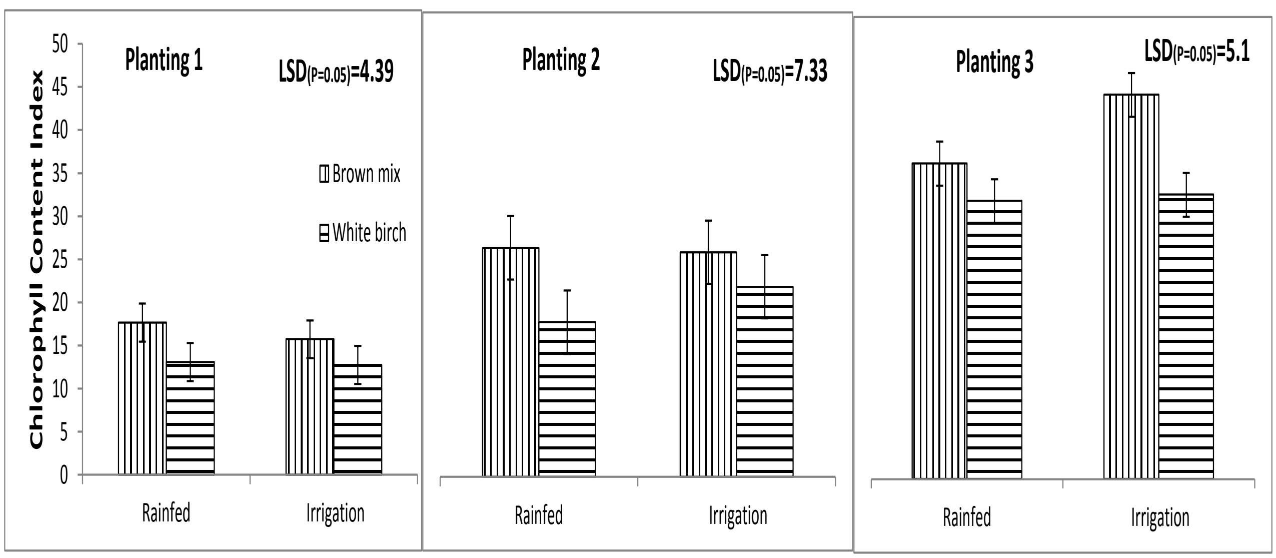

Figure 6.6: Chlorophyll Content Index of cowpea plants observed at three planting dates.

6.2.5 Growth Parameters

6.2.5.1 Leaf number

Leaf number was found to be affected significantly by planting dates. Plants in the third planting date had more leaves, followed by the second and first planting dates (Figure 6.7). No significant differences (P>0.05) were found between the two water regimes. There were no significant differences (P>0.05) between the two varieties. The interaction between planting dates, water regimes and varieties was not significant (P>0.05).

6.2.5.2 Plant height

The pattern observed for Plant height was similar to that observed for leaf number. The third planting date had the tallest plants compared with the first and second planting (Figure 6.8). No significant differences (P>0.05) were observed between the two varieties for rainfed and irrigated plots, during all three plantings. Same trend was found for the interaction between variety and water regime.

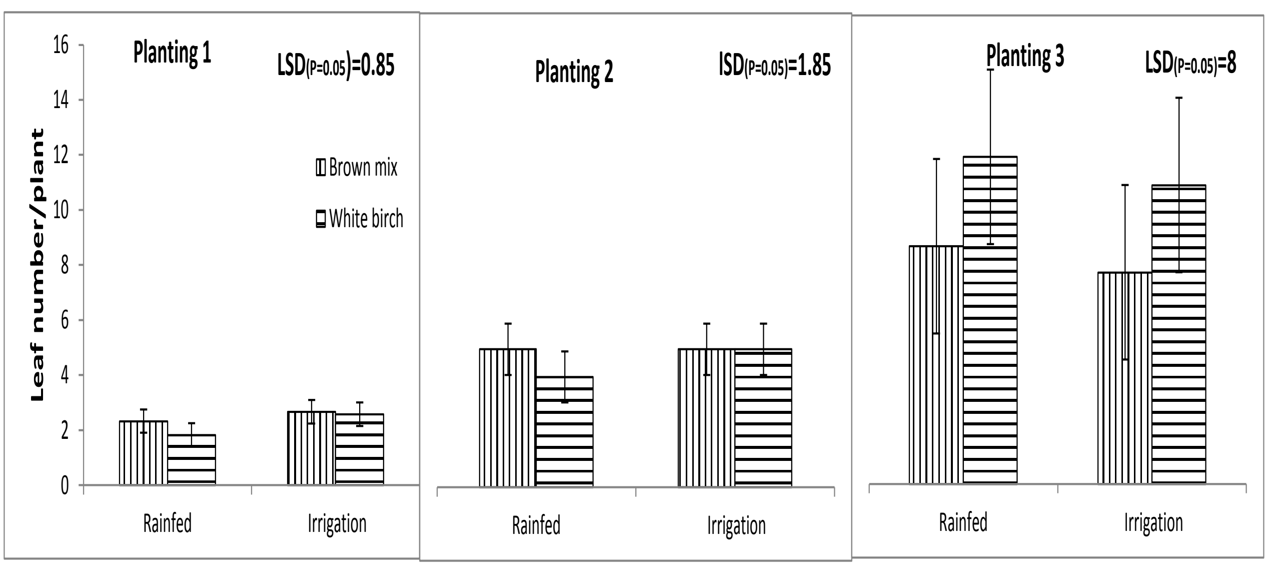

Figure 6.7: Leaf number of cowpea plants observed at three planting dates.

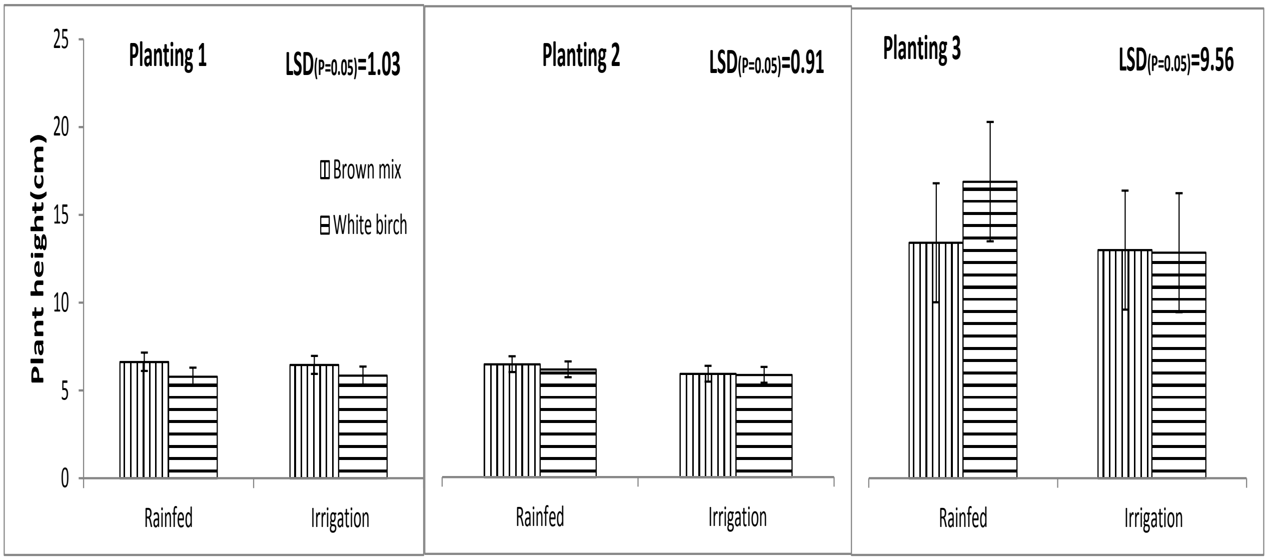

Figure 6.8: Plant heights of cowpea plants at the experimental site at three planting dates.

6.2.6. Flowering

During the first planting date, although plants managed to emerge, they were killed off by frost at 56 DAP under irrigated and 77 DAP under rainfed conditions. For the second planting date, plants emerged, grew, but failed to flower. Therefore, results of flowering and yield are only for the third planting date.

Time to flowering was significantly affected ($P<0.05$) by water regimes. Plants under rainfed flowered early (7 days) compared with plants under irrigation (Figure 6.9). No significant difference was observed between varieties. However, White birch variety flowered early (122 DAP) relative to Brown mix (127 days), especially under rainfed regime.

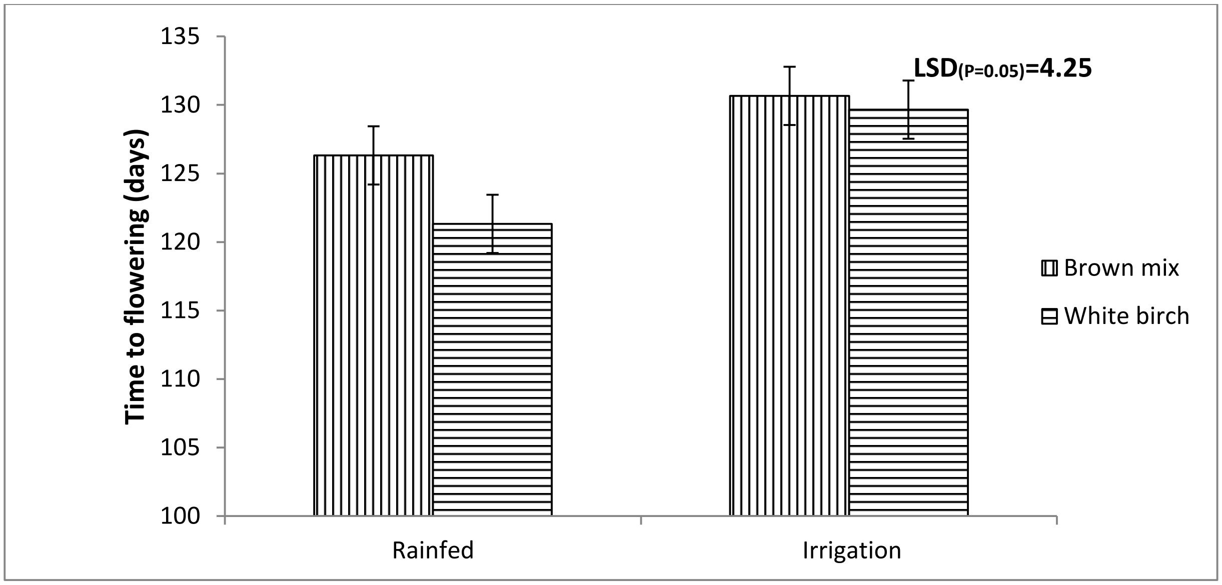

Figure 6.9: Time to flowering observed in cowpea under two water regimes (rainfed and irrigation).

6.2.7 Yield components

6.2.7.1 Biomass

Plants in the third planting date had more biomass compared with the plants of second planting date (Figure 6.10). No significant differences ($P>0.05$) were found between water regimes for both planting dates. However, in the second planting date, plants of irrigated plots had more biomass than plants under rainfed, contrary to the third planting where plants under rainfed had more biomass compared with plants under irrigation. Results of second planting date showed highly significant difference ($P<0.001$) between the two varieties, with Brown mix having the highest biomass compared with White birch, while no significant difference ($P>0.05$) was found between the two varieties for the plants of third planting. The interaction between variety and water regime had no significant effect on plants biomass, for both planting dates.

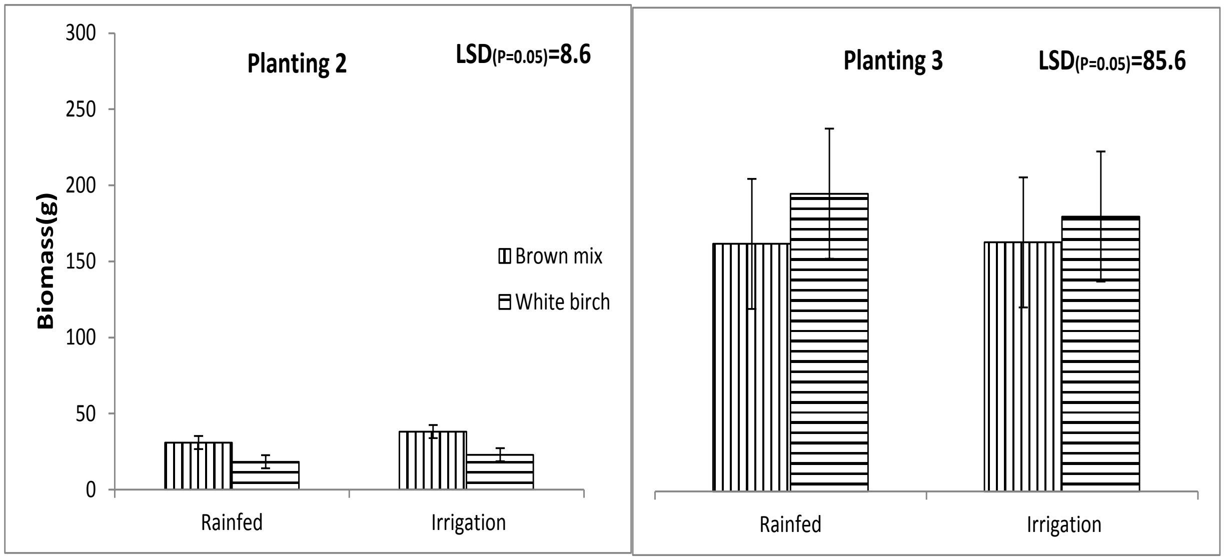

Figure 6.10: Biomass of cowpea plants accumulated at the two planting dates (second and third plantings).

6.2.7.2 Pod number per plant

No significant difference (P>0.05) was found between water regimes, with respect to the number of pods per plant. Highly significant difference (P<0.001) was found between the two varieties. White birch had more pod number compared with Brown mix (Table 6.1). The interaction between variety and water regimes had no effect on pods number

6.2.7.3 Seed number per pod

Water regime had no significant (P>0.05) effect on the number of seed per pod. Seed number per pod was not significantly affected by the varieties. Based on mean values, White birch had more seed per pod relative to Brown mix (Table 6.1). No significant (P>0.05) interaction was observed between variety and water regime.

6.2.7.4 Seed mass per pod

Water regimes had no significant (P>0.05) effect on the mass of seed per pod. However, both varieties had more seed mass under rainfed relative to irrigated plots. No significant difference was found between varieties with respect to the mass of seed per pod. Based on mean values, Brown mix had the highest seed mass compared with White birch (Table 6.1). No significant interaction (P>0.05) was found between variety and water regime.

6.2.7.5 Seed weight per plant (Yield)

Water regimes had no significant effect (P>0.05) on seed weight per plant. There was no significant difference (P>0.05) between varieties, for both rainfed and irrigated plots. White birch variety had the highest values relative to Brown mix (Table 6.1). The interaction between variety and water regimes showed no significant P>0.05) differences with respect to seed weight per plant.

Table 6. 1: Yield components of two cowpea varieties under two water regimes (rainfed and irrigation), observed during the third planting date.

Water regime	**Variety**	**Pod number.plant^{-1}**	**Seed number.pod^{-1}**	**Seed mass.pod^{-1} (g)**	**Yield.plant^{-1} (g)**
Rainfed	Brown mix	36.2	12.25	2.01	68
	White birch	90.2*	13.25	1.86	135
Irrigation	Brown mix	34.2	11.5	1.75	69
	White birch	109.5*	13.25	1.69	109
$LSD_{(P=0.05)}$		44.27	3.396	0.4172	120.8

*: Significant difference

6.2.8. Seed quality test (viability and moisture content)

6.2.8.1 Seed viability

Germination test

Significant difference (P<0.05) was observed between the two water regimes, with respect to germination (Figure 6.16). Based on mean values, seeds of plants under rainfed water regime had the highest percentage (68.3%) relative to seeds of plants from irrigated plots (65%). No significant differences (P >0.05) were found between the two cowpea varieties under both rainfed and irrigated plots. White birch variety (67.29%) germinated better than Brown mix (66.04%). There were no significant differences (P>0.05) for the interactions between days to germination and water regime, variety and water regime.

6.2.8.2 Seed moisture content

Seed moisture content was not significantly (P>0.05) influenced by water regimes. However, based on mean value, seeds from irrigated plants had more moisture, compared to the seeds from non-irrigated plants (Figure 6.17). No significant difference (P>0.05) was found between the two varieties (Brown mix and White birch), with respect to seed moisture content. The interaction between variety and water regime had not effect.

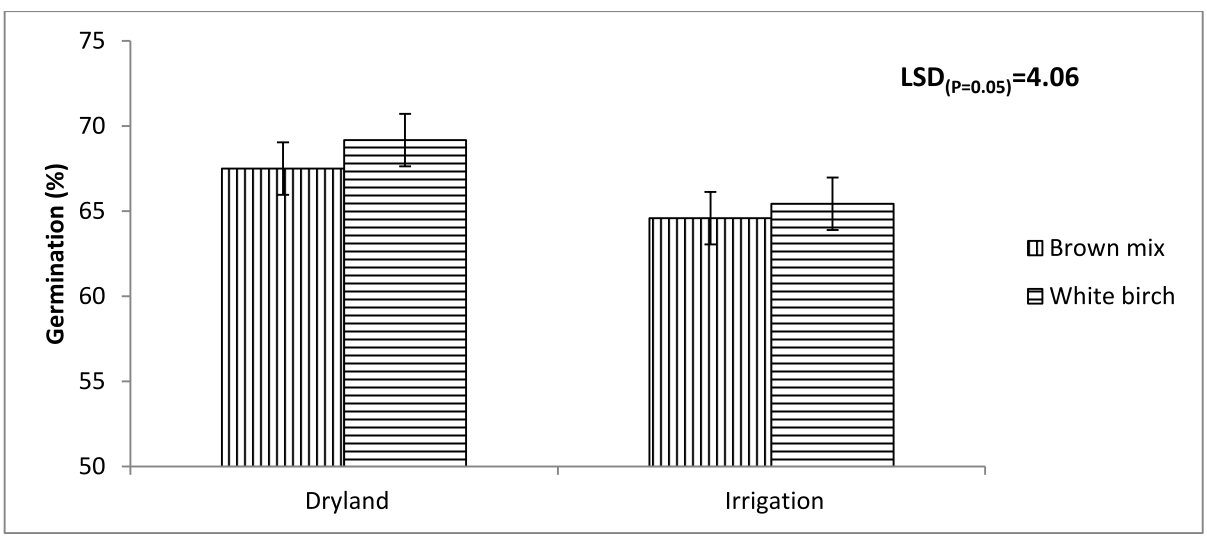

Figure 6.11: Seed germination of cowpea plants grown under two water regimes (rainfed and irrigation).

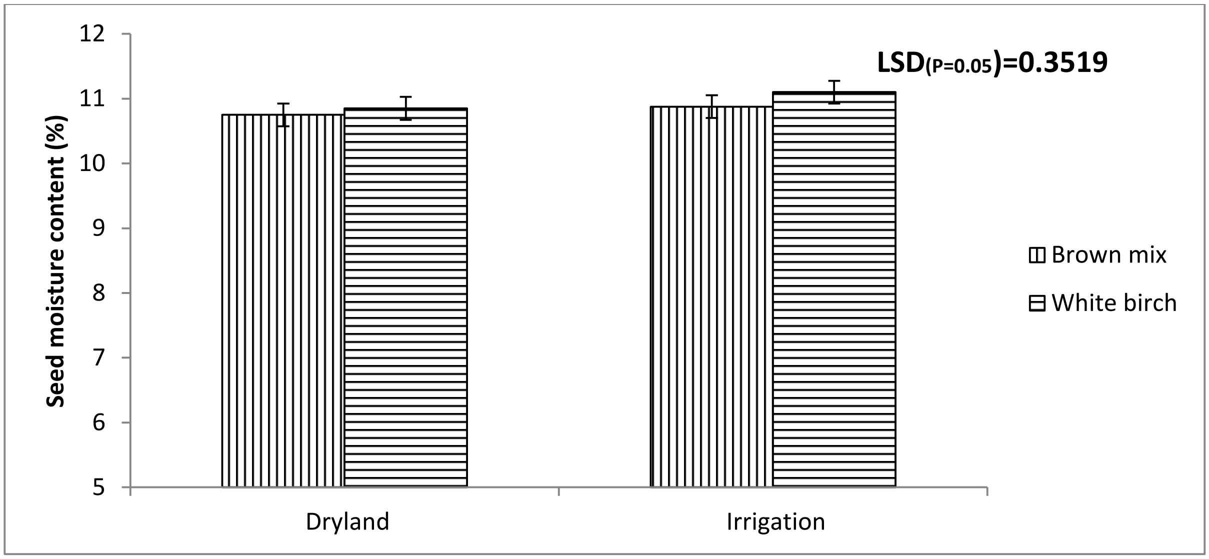

Figure 6.12: Seed moisture content of cowpea plants grown under two water regimes (rainfed and irrigation).

6.3. Discussion

To a larger extent, there is information that seeds contain genetic information that determines adaptability to environmental conditions (Erker 2008). Seedling emergence in relation to environmental conditions and timing remain therefore important agriculture traits. During this study, time to emergence was found to be affected by different planting dates. It was found to vary significantly with different times of planting. The differences in emergence between planting dates may be attributed to different temperatures and water availability observed during the three planting dates. This is accordance with the findings by Finch savage et al. (2001), who defined that emergence time of individual seedling is the result of a complex inter-action of ambient weather conditions (temperature and moisture). Both factors can separately or jointly affect the emergence (Shaban 2013). For example, seed of Lima bean (*Phaseolus lunatus* L.) was observed to emerge better at low soil temperatures when the moisture was at 12% (Bennett and Waters 1984). It can be extrapolated that cowpea seeds have the ability to emerge over a wide range of environmental conditions but the emergence is inhibited by inadequate soil moisture and low temperature.

White birch variety emerged early compared with Brown mix, especially at the second and the third planting date. The two planting dates were characterised by the lack of rains (less than 1mm) with temperatures below 10^0C (the limit for cowpea seed germination). Results obtained were in agreement with seed quality test results observed in this experiment (chapter 4), which showed more vigour in White birch variety compared to Brown mix. Pervious study by Aliabadi et al. (2011) established that vigorous seed can produce a better seedling under stress conditions than the non-vigorous seedlings. This may imply that seed vigour may be associated with physiological responses during stress conditions.

The emergence was greater at the first planting compared to other plantings (Figure 6.3). Poor emergence observed during the second and the third planting may be due to the advent of low temperatures (-10^{o}C) from June (second planting) through August (third planting). According to Guan et al. (2009), temperature plays a major role in determining the periodicity of seed emergence and the distribution of species. This observation may confirm the sensitivity of cowpea emergence to low temperatures, as indicated in several reports (Ehlers and Hall 1998; Ntombela 2012).

Seed emergence has been reported to improve due to increases in soil moisture (Bennet and Water 1984; Wilson and Trawatha 1991; Khan et al. 1992). However, in this experiment; water was not a factor for cowpea emergence. The planting date with more soil water (Second planting) had low emergence relative to the planting date with less water (third planting). This confirms the influence of temperature compared to other factors in cowpea seed emergence. White birch had higher emergence rate during the second planting, compared with Brown mix. It is important to note that, the second planting happened in middle of winter period, which is a stressful environmental period for plant growth. Emergence results were similar to the results of day to emergence observed in the present experiment, which indicated also more vigour in White birch variety compared to Brown mix variety. This leads to a conclusion that White birch variety can be recommended for selection under severe environmental conditions.

Stomatal control has been reported to be the early response of plant to drought stress (Chaves et al. 2002). In this study, stomatal conductance was more reduced during the first planting in both rainfed and irrigation plots, compared with the second and the third planting. This may be attributed to lower temperatures (<10°C) observed during the first planting. Low temperatures may have affected functional stability of cell membrane (Liu et al. 2013), which may have inhibited the conductance of the stomata. It was observed that soil water deficit was not a factor contributing to the low stomatal conductance. This is confirmed by the same level of stomatal conductance when comparing the rainfed and irrigation plots, although their soil water content were different (Figure 6. 5). These results are in line with what has been reported in literature that cowpea seedlings subjected to low temperatures (< 10°C) are inhibited (Oliveira et al. 2010). The study by Ntombela (2012) concluded that temperature was more influential to growth, development and productivity compared to water stress when temperature conditions were low. The fact that stomatal conductance increased from the second planting up to the third planting (Figure 6.5) may be related to the increase in temperatures observed during these plantings (Figure 6.1),which became favourable for a normal internal functioning of the crop such as cell membrane permeability, photosynthesis, and chlorophyll synthesis (Hurry and Huner 1991). It is in accordance with the observations in the present study that all crops of the first planting date were inhibited, while the crops for the second planting date grew well but failed to form yield, and the crops of third planting date grew normally and were able to form yield. From this study, it may be concluded that low temperature (cold) is an environmental stress that limit growth for cowpea.

The results for chlorophyll content index (CCI) showed similar trend to that of SC. Leaf CCI was reduced substantially during the first planting date compared to other planting dates, for both rainfed and irrigated plots (Figure 6.6). This may suggest the influence of temperature compared to water deficit in the environment where temperatures are very low. Previous studies have reported the inhibition of chlorophyll induced by low temperatures (Hurry and Huner 1991). According to Liu et al. (2013), low temperature leads to many changes of physiological indices including chlorophyll content. The final effect of environmental stresses on yield has been found to occur by reducing the canopy photosynthetic rate (Board and Khalon 2011). This is confirmed by yield results observed in the present study, where only the crops of third planting formed yield. Brown mix variety had higher chlorophyll content when compared to White birch variety, during all three planting dates. Results obtained may be related to the leave’s character which may have influenced the amount of chlorophyll in the leaves. Since, chlorophyll content per unit leaf area have been observed to correlate significantly with the number of cells under unit leaf area, and chlorophyll per unit volume with mesophyll cell size (Wilson and Couper 1969). This may suggest more cell number per unit leaf area, or larger mesophyll cell size in Brown mix relative to White birch.

Plant growth is controlled by internal regulators that are modified according to environmental conditions (Manske 1997). Cowpea growth in height and leaf number was found to be affected by different times of planting. Third planting date crops performed better compared to those of the second and the first planting. Differences may be attributed to temperature differences observed during the growing seasons. Previous studies on cowpea reported a decrease in plant heights with the decrease in temperature (20/10°C) (Marsh et al. 2006). According to Hasanuzzaman et al. (2013), plant growth and development involves biochemical reactions that are sensitive to temperature. Temperature and water have been observed to act together to affect physiological and ecological status of plants (Tavili 2007). A possible explanation for growth being increased during the third planting could explain that the full crop requirement (Soil water and temperature) was met, resulting in normal biochemical process in the plant. This may confirm that growth remains an important process to be considered in predicting plant responses to environment.

Time to flowering remains an important component of the adaptation of a variety to a particular agro-ecological zone (Ishiyaku et al. 2005). Under rainfed conditions, White birch variety flowered early compared to Brown mix variety. In dry environments, early flowering or maturing cultivars of cowpea have been found to be useful due to their ability to escape drought (Hall and Patel 1985). Results obtained may be indicative of more adaptation traits to water stress. These results are in line with the findings on controlled environmental conditions (chapter 5) where the same variety showed better performance compared to Brown mix, when subjected to water stress regimes.

In this study biomass was obtained for second and third planting because plants in the first planting date were destroyed by frost. Large biomass was obtained during the third planting and this may be related to plant growth results (plant height and leaf number) as observed during the two planting dates (Figures 6.7 and 6.8). Huge biomass during the third planting may also be due to the fact that these plants produced pods while the ones of second planting date did not produce pods. Biomass production is dependent upon the availability or amount of resources accumulated (Mubeen et al 2013). This was related to favourable environmental conditions observed during the third planting date relative to the second planting date. There is evidence that biomass production and water use by crop stands are closely related (Ehlers and Goss 2003). A study on faba bean showed a decrease in biomass due to water deficit (Gozelani et al. 2009). In this study, biomass of plants under irrigation was similar to plants under rainfed for both plantings. These results concurred with findings of seed mass, per plant observed in the present experiment (Table 6.1). It may be attributed to more stomatal conductance observed in plants under rainfed compared with plants under irrigation, since photosynthesis is a main factor in biomass production in plants (Zarei et al. 2012). More biomass accumulated in Brown mix compared to White birch during the second planting, could be due to the ability of Brown mix to favour vegetative growth more than pod formation. This is in line with study by Ntombela (2012), which indicated that Brown variety favoured vegetative growth more than pod formation. From this observation, farmers may be advised to choose the Brown mix variety when growing cowpea as a vegetable crop. On the other hand, more biomass found in White birch in the third planting date, compared to Brown mix may be a result of more pods (not seed included) per plant observed in White birch relative to Brown mix.

In this study, plants were observed to form seed yield only in the third planting. This correlated positively with the increase in temperature and soil water content in the soil. Pod number per plant is the most important component in determining yield in the legume crop (Mathew et al. 2000). Numerous studies have been established the relationship between soil water and pod number (Abayomi and Abidoye 2009; Bastos 2011). The lack of significant difference between water regimes, may suggest that soil water was not influential in this particular growing environment. This is supported by the observations of stomatal conductance in this experiment, which showed more stomatal conductance for plants under rainfed compared with the ones under irrigation. These assumptions may confirm the widely accepted belief among crop scientists that cowpea is a widely adapted crop legume in the semi-arid tropics (Department of Agricultural, Rural Development and Land Administration 2012).

White birch had the highest number of pods relative to Brown mix. This could be attributed to more leaf number and plant height observed in White birch relative to Brown mix (Figures 6.7 and 6.8). Results obtained were in line with the study by Babaji et al. (2011) who reported that cowpea yield decreased in relation to leaves and branches number. More leaves and plant height may have resulted in higher light interception and more photo-assimilate production that may have increased yield. In addition, White birch flowered late compared with Brown mix. Variety which takes more time on vegetative stage would have more pods per plant (Oyiga and Uguru 2011). Another reason of more pods per plant in White birch relative to Brown mix could be its ability to allocate a greater proportion of its total dry matter to grain yield less to vegetative plant parts, as evidenced by the finding of controlled environmental conditions (chapter 5). In a plant, there is a limited amount of resources to spend on growth, maintenance, and reproduction. Allocation to one function is mostly at the expense of another function.

The effects of water regimes on seed number per pod were not observed (Table 6. 1). This is consistent with previous report that in legumes, the number of seed per pod was heritable under several environmental conditions (Ferry 1985). Results obtained are in tandem with observations in controlled environmental conditions (Chapter 5), where water stress imposed on cowpea had no effect on the number of seed per pod. Similar results were observed by Marsh (1993), who showed that cowpea number of seed per pod was not influenced by soil moisture. White birch producing more seed number per pod compared with Brown mix, may be indicative of more adaptation to environmental conditions. This is in line with primary results on seed quality where White birch was expected to perform well in field conditions (Chapter 4).

The observation that both cowpea varieties had more seed mass under rainfed compared with irrigation is consisted with several reports (Cisse and Hall 2002; Behdoudian et al. 2001). Cowpea is primarily grown in drier regions and possesses high yield plasticity under diverse environmental conditions (Ahmed and Suliman 2010). On the other hand, these results may suggest that plants under irrigation did not use efficiently soil water, as indicated by stomatal conductance results (Figure 6. 5). The highest seed mass recorded in Brown mix variety may confirm that seed mass was a reproductive character, as indicated in chapter 5.

Seed weight per plant was not significantly influenced by water regimes. This is similar to findings on pods number per plant in this study (Table 6.1), confirming that irrigation was not influential to crops yield. These results may be also indicative of cowpea natural competitiveness under rainfed conditions, as was earlier reported. More seed weight per plant observed in White birch compared with Brown mix may be attributed to more pod number. Results in this study concurred with findings of Manjeru et al. (2007) and Oyiga and Uguru (2011), who associated seed weight per plant with number of pod per plant in legumes. This may confirm that in grain legumes, the number of pod per plant is the main contributor toward seed yield.

At harvest, seeds were assessed to evaluate the effect of water stress on the quality of seeds produced. It has been reported that the conditions experienced by the mother plant during seed development and maturation can be carried over and influence physiological potentials of subsequent generations (Wulf 1995). Seed germination test showed more viability for seeds from rainfed plots compared with seeds from irrigated plots (Figure 6.11). This observation may be supported by the findings in the present study, where seed masses of plants under rainfed were higher than seeds of plants under irrigation. It suggests that under low temperature, irrigation may have contributed to further decrease in soil temperature, affecting thus the quality of seed. This is in line with previous study Baskin and Baskin (1998), who reported that many plant species that grow under higher temperatures produced seed with higher germinability, compared to plants grown under low temperatures. On the other hand, Dombos et al. (1989) argued that, although water stress has negative effects on seed number and size in soybean; however, the seeds produced in most of the cases have high germination quality. This may suggest that, cowpea seed produced under rainfed are more viable compared to seed of plants under irrigation.

Seed moisture content is generally used as a measure of seed maturity. It has been found to decline after the completion of maturation and depending on species and weather conditions (Egli 1998 cited by Odindo 2007). Although no significant difference was found between the two water regimes; however, irrigated plants had the highest seed moisture compared with non-irrigated plants (Figure 6.12). These results concurred with previous report, which indicated that the moisture content of snap beans grown under the conditions of optimum irrigation was higher than those that were deprived of irrigation (Eskin 1989). Results obtained could be attributed to cowpea natural competitiveness under rainfed conditions.

6.4. Conclusion

This study has demonstrated that cowpea emergence was more influenced by temperature than water stress, during the three plantings dates. It may be concluded that for a successful cowpea production, the choice of planting date should consider the minimal temperature (above 13°C) for a successful cowpea emergence. During the three plantings, cowpea growth and physiology were more dictated by temperature relative to water stress. Farmers may be advised to plant cowpea at the end of winter season (when temperature starts to increase) instead of planting at the beginning (when temperature starts to decrease).This strategy may allow seedlings to meet the crop full requirements for their continued growth when temperatures are already favourable. Only third planting date (20 August), because of appropriate growth conditions, grew well and produced satisfactory yield. The crops were more affected by irrigation compared to rainfed regime. However, it was expected that plants would perform better under irrigation regime. Contrary to this expectation, plants performed better under rainfed conditions and produced more yield compared to plants under irrigation. This may lead to the conclusion that cowpea is well adapted to rainfed conditions. When comparing varieties, the White birch variety was more adapted to environmental stresses compared to Brown mix. It had higher emergence and produced satisfactory yield, while Brown mix favoured more vegetative growth. Under several environmental conditions, growers may be advised to promote white variety in their selection. Seed produced by plants under rainfed were more viable and had less moisture content relative to seed of plants under irrigation, leading to the conclusion that cowpea good quality seed is obtained under rainfed.

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

Cowpea remains an important crop in arid and semi-arid regions as evidenced by the literature review. This is due to its adaptability to drought-prone environmental conditions, compared to other crops. Its multi-purpose uses, high protein content and potential to biologically fix nitrogen makes it best suited for production by resource-poor farmers. Although cowpea is a crop of choice in arid and semi-arid areas, it still receives little research attention in comparison to other grain legumes such as common beans and soybeans. It has not been given the attention it deserves as a crop that has potential to contribute towards food security and improve diets of people living in marginal areas of agricultural production and therefore remains underutilised (Dugje et al. 2009).

In several developing countries, cowpea production remains largely traditional and is concentrated in the hands of smallholder farmers and pastoralists (Janneh and Ping 2009). One of the constraints faced by smallholder farmers is that of poor quality seed (Dobermann 2013). In most places, cowpea is still cultivated from local landraces rather than from improved varieties suitable for a given environments; these are seeds that have been kept over many years and handed down to many generations of traditional subsistence farmers through their informal seed systems. Seed quality in this system is not controlled by seed experts, and seed selection is based largely on local indigenous knowledge.

Water stress is one of the most important environmental factors affecting crop productivity in arid and semi-arid regions of the world (Yang et al. 2006). It is generally defined as the condition where a plant’s water potential and turgor are decreased enough to inhibit normal plant functions (Loka et al. 2011). Although cowpea is tolerant to drought, water deficit was observed to reduce its productivity (Neto and Bartels 1992).

Planting date management is one of the management practices used by farmers to cope with limited water resources (low rainfall, soil water content and water availability) in arid and semi-arid regions. In these areas, large fluctuations are observed in average annual rainfall (Palmer and Ainslie 2002), and these fluctuations were found to cause the variability in the average date of rain onset than its cessation (Shiringane 2007). It is therefore necessary to take into consideration the planting date. Since the best planting date allows growth stage to coincide with favourable environmental conditions

7.2 Aim and objectives

The aim of this study was therefore to identify the physiological changes at different stages of cowpea growth and development in response to water stress under controlled and field conditions. Secondary to this, the study also to evaluate the effect of planting date selection as a management tool for managing water stress under field conditions. The specific objectives of this study were:

- to determine seed quality of cowpea varieties and their field planting value,
- to evaluate growth, physiology and yield of cowpea varieties in response to different water regimes under controlled and field conditions, and thereafter, determine the effect of varying water regimes on subsequent seed quality, and
- to determine the effect of planting date selection as a management tool for optimising cowpea yields under water limited conditions.

7.3 Challenges

- Wild animals that interfered with some of the trials.

7.4 Future teaching, learning and research possibilities

The following recommendations may be made, based on observations made during the study;

- Only two cowpea varieties were used in the present study; future research should include more varieties to enable effective assessment of water stress on cowpea physiology.
- Cowpea formal seed systems need to be developed to support smallholder farmers and pastoralists in rural areas, who still rely on landraces or recycled seeds from previous harvests.
- Future research should include more physiological (leaf water potential, chlorophyll fluorescence) and growth parameters (branch number, Leaf Area Index), in order to have more data on crop physiological responses to water stress.
- Leaves of Brown mix variety were found to have more chlorophyll content than White birch. However, future study should evaluate the biological basis for the association between leaf chlorophyll content and variety.
- The interval between plantings dates used in the present experiment was two months; it is also recommended that future studies should reduce these intervals, in order to have a broad knowledge on planting date effects on cowpea physiology.
- In addition, future research should also associate planting dates with different sites.

7.5 Final comments and summary conclusions

Seed quality components were evaluated in chapter 4. This was done on a comparative basis with respect to seed coat colour. Previous research (Odindo 2007) indicated that seed colour was associated with seed quality. With respect to the viability and vigour, there was statistical difference between Brown mix and White birch. The Brown mix variety was more viable (germinated better and had all seeds stained normal red) compared with White birch. However, results of vigour were not in agreement with results of viability and indicated that the White variety was more vigorous than the Brown mix variety. This was confirmed by more performance (MGT, GVI, and seedling growth parameters) observed in White birch variety during seed quality test (Table 4.1). Electrolyte Conductivity should be used in conjunction with measurements of seed coat thickness in order to improve the validity of results. It implies that good viability does not always translate to good vigour.

The effects of water stress on cowpea physiological mechanisms, growth and yield components were evaluated in chapter 5.Water stress had a negative effect on cowpea stomatal conductance, thereby reducing plants growth and productivity. This study showed that, although cowpea can be a drought tolerant crop, water deficit however, does reduce its agronomic performances (yield and yield components) significantly. Water stress had no effect on leaves chlorophyll content; this could explain drought avoidance mechanism of cowpea, which allows the crop to endure long periods without significant rainfall and continue with their normal metabolic activities (Kumar et al. 2012). It was also reflected on maintaining the quality of seed produced (viability, germination and vigour) as observed during seed quality test. Proline is known to accumulate in plants subjected to unfavourable environmental conditions such as water shortage, salinity and extreme temperature (Druge 1998). Plants grown under severe water stress (30% ETc) accumulated more proline followed by plants grown under 60% ETc and then plants under 80% ETc. This observation suggests possible adaptive strategy of cowpea plants in response to water deficit. Highly significant differences was observed between water stress regimes, with respect to protein content; seeds of plants grown under 80% ETc synthesised more protein followed by the seeds of plants under 60% ETc and then 30% ETc. This raises the possibility that seed protein accumulation gradually decreases in relation to water deficit. It can be an indication that water plays a major role in the metabolism of amino acids and their incorporation into protein. White birch variety proved to be well adapted to water stress and produced good harvest index compared to Brown mix.

The effects of planting date on crop physiology, growth and yield components were studied in chapter 6. The aim was to determine the effect of planting date selection as a management tool for optimising cowpea yields under water limited conditions. During all three planting dates, cowpea emergence was more influenced by temperature than water stress. It may be concluded that the choice of planting date should consider the minimum temperature for cowpea emergence. Growth and physiology were more dictated by temperature relative to water stress. Farmers may be advised to plant cowpea at the end of winter season (when temperature starts to increase) rather than at the beginning (when temperature starts to decrease). This strategy may allow for successful seedling establishment. Plants from the first planting date (09 April) did not grow, those from the second planting date (12 June) grew but failed to form yield, while plants from the third planting date (20 August) grew well and produced satisfactory yield. Cowpea was adversely affected by irrigation compared to dryland conditions. However, it was expected that plants would perform better under irrigation regime. Contrary to this expectation, plants under dryland performed better and produced more yield compared to plants under irrigation. This may lead to the conclusion that cowpea is well adapted to dryland conditions. White birch variety was more adapted to environmental stresses compare to Brown mix. It had higher emergence rate and produced satisfactory yield, while Brown mix favoured more vegetative growth and this can be useful to rural communities for vegetative consumption. Under several environmental conditions, growers may be advised to promote white variety in their selection, as indicated in chapter 5.

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APPENDICES

Appendix 1: Analysis of variance tables for chapter 4

Variate: Germination(%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	117.5	39.17	1.61	
Rep.*Units* stratum					
Variety	1	0.5	0.5	0.02	0.887
Day	3	50821.5	16940.5	696.87	<.001
Variety.Day	3	341.5	113.83	4.68	0.012
Residual	21	510.5	24.31		
Total	31	51791.5			

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	99	5131642	51835	1.11	
REP.*Units* stratum					
VARIERTY	1	2803904	2803904	60.21	<.001
Residual	99	4610362	46569		
Total	199	12545908			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	17.7193	5.90643	480.85	
REP.*Units* stratum					
VARIETY	1	0.01125	0.01125	0.92	0.409
Residual	3	0.03685	0.01228		
Total	7	17.7674			

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	1791.408	597.136	108.32	
REP.*Units* stratum					
VARIETY	1	0.813	0.813	0.15	0.727
Residual	3	16.538	5.513		
Total	7	1808.76			

Variate: Root Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	101.61	33.87	0.45	
REP.*Units* stratum					
VARIETY	1	2513.41	2513.41	33.45	0.01
Residual	3	225.41	75.14		
Total	7	2840.43			

Variate: ShootLength

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	127.9	42.6	0.35	
rep.*Units* stratum					
variety	1	411.8	411.8	3.37	0.164
Residual	3	366.3	122.1		
Total	7	906.1			

Variate: Root: Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	0.1754	0.05847	0.77	
REP.*Units* stratum					
VARIETY	1	0.01805	0.01805	0.24	0.659
Residual	3	0.22735	0.07578		
Total	7	0.4208			

Variate:Dry mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	0.05504	0.01835	1.07	
REP.*Units* stratum					
VARIETY	1	0.03014	0.03014	1.75	0.277
Residual	3	0.05152	0.01717		
Total	7	0.13669			

Appendix 2: Analysis of variance tables for chapter 5

Variate: Soil Moisture Content (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	154.556	51.519	5.63	
Rep.*Units* stratum					
Variety	1	1.821	1.821	0.20	0.656
ETc	2	961.175	480.588	52.51	<.001
WAP	7	6976.937	996.705	108.91	<.001
Variety.ETc	2	42.109	21.055	2.30	0.104
Variety.WAP	7	18.793	2.685	0.29	0.956
ETc.WAP	14	781.792	55.842	6.10	<.001
Variety.ETc.WAP	14	63.150	4.511	0.49	0.934
Residual	141	1290.397	9.152		
Total	191	10290.731			

Variate: Stomatal Conductance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2510.7	836.9	1.04	
Rep.*Units* stratum					
Variety	1	80.3	80.3	0.10	0.752
ETc	2	17959.1	8979.6	11.20	<.001
WAP	7	113459.5	16208.5	20.22	<.001
Variety.ETc	2	2048.7	1024.4	1.28	0.282
Variety.WAP	7	9607.5	1372.5	1.71	0.111
ETc.WAP	14	84630.0	6045.0	7.54	<.001
Variety.ETc.WAP	14	10821.1	772.9	0.96	0.493
Residual	141	113019.8	801.6		
Total	191	354136.7			

Variate: Chlorophyll Content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	299.18	99.73	1.27	
Rep.*Units* stratum					
Variety	1	106.51	106.51	1.35	0.247
ETc	2	172.52	86.26	1.10	0.337
WAP	7	5051.91	721.70	9.17	<.001
Variety.ETc	2	214.63	107.32	1.36	0.259
Variety.WAP	7	387.63	55.38	0.70	0.669
ETc.WAP	14	1795.34	128.24	1.63	0.078
Variety.ETc.WAP	14	873.59	62.40	0.79	0.675
Residual	141	11094.18	78.68		
Total	191	19995.49			

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2838.7	946.2	3.62	
Rep.*Units* stratum					
ETc	2	13459.6	6729.8	25.76	<.001
Variety	1	974.8	974.8	3.73	0.055
WAP	10	50858.1	5085.8	19.47	<.001
ETc.Variety	2	379.0	189.5	0.73	0.485
ETc.WAP	20	17118.6	855.9	3.28	<.001
Variety.WAP	10	3759.4	375.9	1.44	0.165
ETc.Variety.WAP	20	3890.4	194.5	0.74	0.776
Residual	195	50934.2	261.2		

Total 263 144212.9

Variate: Leaf number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	36.405	12.135	3.87	
Rep.*Units* stratum					
ETc	2	905.053	452.527	144.22	<.001
Variety	1	91.004	91.004	29.00	<.001
WAP	10	2950.364	295.036	94.03	<.001
ETc.Variety	2	58.280	29.140	9.29	<.001
ETc.WAP	20	1006.114	50.306	16.03	<.001
Variety.WAP	10	41.955	4.195	1.34	0.213
ETc.Variety.WAP	20	78.886	3.944	1.26	0.212
Residual	195	611.845	3.138		
Total	263	5779.905			

Variate: Days to flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.333	0.167	0.14	
Rep.*Units* stratum					
Variety	1	24.5	24.5	21	0.001
ETc	2	147	73.5	63	<.001
Variety.ETc	2	49	24.5	21	<.001
Residual	10	11.667	1.167		
Total	17	232.5			

Variate: Biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	16.437	5.479	0.55	
Rep.*Units* stratum					
Variety	1	17.63	17.63	1.77	0.203
Water_regime	2	1124.526	562.263	56.56	<.001
Variety.Water_regime	2	57.67	28.835	2.9	0.086
Residual	15	149.107	9.94		
Total	23	1365.371			

Variate: H I

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.031033	0.010344	1.88	
Rep.*Units* stratum					
Variety	1	0.041667	0.041667	7.58	0.015
Water_regime	2	0.275275	0.137637	25.05	<.001
Variety.Water_regime	2	0.011408	0.005704	1.04	0.378
Residual	15	0.082417	0.005494		
Total	23	0.4418			

Variate: Pod number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	7	2.333	0.28	
Rep.*Units* stratum					
Variety	1	140.167	140.167	16.82	<.001
Water_regime	2	326.083	163.042	19.57	<.001
Variety.Water_regime	2	39.083	19.542	2.35	0.13
Residual	15	125	8.333		
Total	23	637.333			

Variate: Seed number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.125	1.375	0.38	
Rep.*Units* stratum					
Variety	1	18.375	18.375	5.14	0.039
Water_regime	2	4.083	2.042	0.57	0.577
Variety.Water_regime	2	15.75	7.875	2.2	0.145
Residual	15	53.625	3.575		
Total	23	95.958			

Variate: seed mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0581	0.0194	0.09	
Rep.*Units* stratum					
Variety	1	2.9681	2.9681	13.07	0.003
Water_regime	2	0.0902	0.0451	0.2	0.822
Variety.Water_regime	2	0.1051	0.0525	0.23	0.796
Residual	15	3.4067	0.2271		
Total	23	6.6282			

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	14.439	4.813	1.26	
Rep.*Units* stratum					
Variety	1	58.375	58.375	15.33	0.001
Water_regime	2	590.257	295.129	77.53	<.001
Variety.Water_regime	2	38.912	19.456	5.11	0.02
Residual	15	57.103	3.807		
Total	23	759.086			

Variate:
Gerrmination (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	134.57	67.28	0.99	
Rep.*Units* stratum					
Variety	1	593.21	593.21	8.73	0.005
ETc	2	182.72	91.36	1.34	0.271
day	3	119601.85	39867.28	586.9	<.001
Variety.ETc	2	34.57	17.28	0.25	0.776
Variety.day	3	999.38	333.13	4.9	0.005
ETc.day	6	429.63	71.6	1.05	0.404
Variety.ETc.day	6	320.99	53.5	0.79	0.584
Residual	46	3124.69	67.93		
Total	71	125421.6			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	53.92125	17.97375	726.21	
Rep.*Units* stratum					
Variety	1	0.03375	0.03375	1.36	0.261
ETc	2	0.0625	0.03125	1.26	0.311
Variety.ETc	2	0.0675	0.03375	1.36	0.286
Residual	15	0.37125	0.02475		
Total	23	54.45625			

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	128.5388	42.8463	189.31	
Rep.*Units* stratum					
Variety	1	1.1704	1.1704	5.17	0.038
ETc	2	0.3981	0.1991	0.88	0.435
Variety.ETc	2	0.084	0.042	0.19	0.833
Residual	15	3.395	0.2263		
Total	23	133.5862			

Variate: Root Length (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.027	0.513	0.18	
Rep.*Units* stratum					
Variety	1	0.5	0.5	0.18	0.68
ETc	2	0.744	0.372	0.13	0.876
Variety.ETc	2	7.929	3.964	1.43	0.285
Residual	10	27.781	2.778		
Total	17	37.98			

Variate: Shoot Length (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.385	1.193	0.66	
Rep.*Units* stratum					
Variety	1	9.317	9.317	5.17	0.046
ETc	2	1.819	0.909	0.5	0.619
Variety.ETc	2	0.609	0.304	0.17	0.847
Residual	10	18.036	1.804		
Total	17	32.166			

Variate: Root: Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0931	0.0466	0.46	
Rep.*Units* stratum					
Variety	1	0.313	0.313	3.08	0.11
ETc	2	0.0635	0.0318	0.31	0.738
Variety.ETc	2	0.0143	0.0071	0.07	0.933
Residual	10	1.0152	0.1015		
Total	17	1.4992			

Variate: Dry mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0417	0.02085	0.74	
Rep.*Units* stratum					
Variety	1	0.97069	0.97069	34.66	<.001
ETc	2	0.9811	0.49055	17.51	<.001
Variety.ETc	2	0.35141	0.17571	6.27	0.017
Residual	10	0.2801	0.02801		
Total	17	2.625			

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	4	140926	35232	2.2	
Rep.*Units* stratum					
Variety	1	3989	3989	0.25	0.619
Water_traitment	2	255005	127503	7.95	<.001
Variety.Water_traitment	2	118161	59081	3.69	0.028
Residual	140	2244507	16032		
Total	149	2762588			

Variate: Proline

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2.00	0.0027918	0.001396	204%	
Rep.*Units* stratum					
Variety	1.00	0.2980023	0.298002	43619%	<.001
Water_regime	2.00	0.285076	0.142538	20863%	<.001
Variety.Water_regime	2.00	0.0760106	0.038005	5563%	<.001
Residual	10	0.0068319	0.000683		
Total	17	0.6687127			

Variate: Protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0002956	0.0001478	1.32	
Rep.*Units* stratum					
Variety	1	0.005078	0.005078	45.49	<.001
Water_regime	2	0.4427971	0.2213986	1983.15	<.001
Variety.Water_regime	2	0.0034003	0.0017001	15.23	<.001
Residual	10	0.0011164	0.0001116		
Total	17	0.4526873			

Appendix 3: Analysis of variance tables for chapter 6

Variate: Time to emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.4583	0.1528	0.7	
Rep.*Units* stratum					
Variety	1	1.0417	1.0417	4.75	0.046
Planting	2	1877.5833	938.7917	4278.04	<.001
Variety. Planting	2	0.5833	0.2917	1.33	0.294
Residual	15	3.2917	0.2194		
Total	23	1882.9583			

Variate: Emergence (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1373.4	457.8	2.93	
Rep.*Units* stratum					
Variety	1	112.9	112.9	0.72	0.409
Planting	2	13156.8	6578.4	42.06	<.001
Variety. Planting	2	500.5	250.3	1.6	0.234
Residual	15	2346	156.4		
Total	23	17489.7			

Variate: Stomatal_Conductance (1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	959.9	320	1.74	
Rep.*Units* stratum					
Variety	1	265.4	265.4	1.44	0.26
Water_traitment	1	44.1	44.1	0.24	0.636
Variety.Water_traitment	1	269.2	269.2	1.46	0.257
Residual	9	1656.6	184.1		
Total	15	3195.3			

Variate: Stomatal_Conductance(2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1817	606	0.41	
Rep.*Units* stratum					
Variety	1	2230	2230	1.49	0.226
Water_traitment	1	1619	1619	1.08	0.301
Variety.Water_traitment	1	4278	4278	2.87	0.095
Residual	73	108946	1492		
Total	79	118890			

Variate:
Stomatal_Conducatnce(3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1905	635	0.29	
Rep.*Units* stratum					
Variety	1	135	135	0.06	0.803
Water_traitment	1	5218	5218	2.42	0.123
Variety.Water_traitment	1	128	128	0.06	0.808
Residual	105	226684	2159		
Total	111	234070			

Variate: Chlorophyll
Content (1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	97.42	32.47	1.14	
Rep.*Units* stratum					
Variety	1	170.29	170.29	5.98	0.019
Water_traitment	1	15.56	15.56	0.55	0.464
Variety.Water_traitment	1	7.82	7.82	0.27	0.603
Residual	41	1167.11	28.47		
Total	47	1458.2			

Variate: Chlorophyll Content (2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	224.3	74.8	0.55	
Rep.*Units* stratum					
Variety	1	795.1	795.1	5.88	0.018
Water_traitment	1	65.7	65.7	0.49	0.488
Variety.Water_traitment	1	104	104	0.77	0.383
Residual	73	9872.4	135.2		
Total	79	11061.4			

Variate: Chlorophyll Content (3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	342.25	114.08	1.23	
Rep.*Units* stratum					
Variety	1	1789.84	1789.84	19.29	<.001
Water_traitment	1	532.57	532.57	5.74	0.018
Variety.Water_traitment	1	367.97	367.97	3.97	0.049
Residual	105	9742.64	92.79		
Total	111	12775.28			

Variate: Leaf number (1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.562	0.521	0.48	
Rep.*Units* stratum					
Variety	1	1.021	1.021	0.94	0.337
Water_traitment	1	3.521	3.521	3.25	0.079
Variety.Water_traitment	1	0.521	0.521	0.48	0.492
Residual	41	44.354	1.082		
Total	47	50.979			

Variate: Leaf number (2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3.5	1.167	0.13	
Rep.*Units* stratum					
Variety	1	3.2	3.2	0.37	0.545
Water_traitment	1	3.2	3.2	0.37	0.545
Variety.Water_traitment	1	0.2	0.2	0.02	0.88
Residual	73	632.1	8.659		
Total	79	642.2			

Variate: Leaf number (3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	47.8	15.9	0.1	
Rep.*Units* stratum					
Variety	1	324	324	2.01	0.161
Water_traitment	1	30	30	0.19	0.667
Variety.Water_traitment	1	0	0	0	0.993
Residual	73	11766.6	161.2		
Total	79	12168.5			

Variate: Plant height (1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	5.491	1.83	1.16	
Rep.*Units* stratum					
Variety	1	6.402	6.402	4.06	0.05
Water_traitment	1	0.037	0.037	0.02	0.879
Variety.Water_traitment	1	0.155	0.155	0.1	0.755
Residual	41	64.644	1.577		
Total	47	76.729			

Variate: Plant Height (2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	9.957	3.319	1.12	
Rep.*Units* stratum					
Variety	1	0.956	0.956	0.32	0.57
Water_traitment	1	5.121	5.121	1.73	0.191
Variety.Water_traitment	1	0.371	0.371	0.13	0.724
Residual	105	309.951	2.952		
Total	111	326.357			

Variate: Plant height (3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	106.6	35.5	0.13	
Rep.*Units* stratum					
Variety	1	139.4	139.4	0.5	0.481
Water_traitment	1	238.5	238.5	0.86	0.357
Variety.Water_traitment	1	155.3	155.3	0.56	0.457
Residual	89	24735	277.9		
Total	95	25374.8			

Variate: Days to flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.5	0.75	0.17	
Rep.*Units* stratum					
Variety	1	27	27	5.96	0.05
Water_traitment	1	120.333	120.333	26.58	0.002
Variety.Water_traitment	1	12	12	2.65	0.155
Residual	6	27.167	4.528		
Total	11	188			

Variate: Biomass (2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	443.98	147.99	5.12	
Rep.*Units* stratum					
Variety	1	772.84	772.84	26.73	<.001
Water_traitment	1	134.56	134.56	4.65	0.059
Variety.Water_traitment	1	6.76	6.76	0.23	0.64
Residual	9	260.23	28.91		
Total	15	1618.38			

Variate: Biomass (3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	34590	11530	4.03	
Rep.*Units* stratum					
Variety	1	2459	2459	0.86	0.378
Water_traitment	1	212	212	0.07	0.792
Variety.Water_traitment	1	247	247	0.09	0.776
Residual	9	25746	2861		
Total	15	63253			

Variate: Pod number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1231.5	410.5	0.67	
Rep.*Units* stratum					
Variety	1	31862.2	31862.2	52.07	<.001
Water_traitment	1	272.2	272.2	0.44	0.521
Variety.Water_traitment	1	324	324	0.53	0.485
Residual	9	5507	611.9		
Total	15	39197			

Variate: Seed number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	6.688	2.229	0.49	
Rep.*Units* stratum					
Variety	1	7.562	7.562	1.68	0.227
Water_traitment	1	0.562	0.562	0.12	0.732
Variety.Water_traitment	1	0.562	0.562	0.12	0.732
Residual	9	40.562	4.507		
Total	15	55.938			

Variate: Seed mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.25207	0.08402	1.24	
Rep.*Units* stratum					
Variety	1	0.00141	0.00141	0.02	0.889
Water_traitment	1	0.06126	0.06126	0.9	0.367
Variety.Water_traitment	1	0.00766	0.00766	0.11	0.745
Residual	9	0.61221	0.06802		
Total	15	0.93459			

Variate: Yield (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	13285	4428	0.78	
Rep.*Units* stratum					
Variety	1	11605	11605	2.03	0.188
Water_traitment	1	611	611	0.11	0.751
Variety.Water_traitment	1	725	725	0.13	0.73
Residual	9	51338	5704		
Total	15	77564			

Variate: Germination (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2.0	7.29	3.65	0.27	
REP.*Units* stratum					
Day	3	71504.17	23834.72	1746.67	<.001
VARIETY	1	18.75	18.75	1.37	0.25
Water_regime	1	133.33	133.33	9.77	0.004
Day.VARIETY	3	10.42	3.47	0.25	0.858
Day.Water_regime	3	79.17	26.39	1.93	0.145
VARIETY.Water_regime	1	2.08	2.08	0.15	0.699
Day.VARIETY.Water_regime	3	2.08	0.69	0.05	0.985
Residual	30	409.37	13.65		
Total	47.0	72166.67			

Variate: Seed
Moisture Content (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.13187	0.04396	0.91	
Rep.*Units* stratum					
Variety	1	0.10562	0.10562	2.18	0.174
Water_regime	1	0.14062	0.14062	2.91	0.122
Variety.Water_regime	1	0.01562	0.01562	0.32	0.584
Residual	9	0.43562	0.0484		
Total	15	0.82937			