Seed quality and water use characteristics of a bambara groundnut (*Vigna subterranea* L.) landrace differing in seed coat colour

by

Tendai Polite Chibarabada

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Crop Science

School of Agricultural, Earth & Environmental Sciences

University of KwaZulu-Natal

Pietermaritzburg

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PREFACE

The research contained in this dissertation 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.

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Signed: Supervisor

Date: 30 June 2014

DECLARATION

I, Tendai Polite Chibarabada, 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;

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

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ABSTRACT

Bambara groundnut (*Vigna subterranea* L.) is an underutilised African legume that fits the same ecological niche as *Arachis hypogea* (groundnuts). Because of its reported drought tolerance and high water use efficiency there are now renewed efforts to study bambara groundnut with a view to promoting it as an alternative crop in marginal production areas. It is still cultivated using unimproved landraces, and little is known about their seed quality. There is need for information describing aspects of their seed quality in order for farmers to successfully produce the crop. The study evaluated seed quality and seedling water use characteristics of selected seed coat colours of bambara groundnut. Lastly, the study investigated the effect of water stress imposed on maternal plants on subsequent yield and seed quality of bambara groundnut. A single bambara groundnut landrace was characterised into four distinct selections based on seed coat and speckling colour; plain red, plain cream, cream with brown speckles (brown speckled) and cream with black speckles (black speckled). Seed quality (viability and vigour) was evaluated using the standard germination, electrolyte conductivity and imbibition tests as well as water activity, seed coat thickness and mineralogy. Seedling water use characteristics were evaluated under varying water regimes (25%, 50% and 75% field capacity). Measurements included plant growth and physiological (chlorophyll content index and chlorophyll fluorescence) responses up to 21 days after planting; thereafter seedling water use efficiency was determined. Irrigation was withdrawn thereafter in all water treatments to determine physiological and metabolic responses (total soluble sugars, antioxidants and phenols) to terminal stress. A field trial was grown in 2013/14 summer season under irrigated and rainfed conditions. Yield and yield components as well as subsequent seed quality (viability and vigour) of progeny was determined from harvested material. Darker coloured seeds and seeds with similarly coloured speckles showed better viability while the plain cream landrace selection was more vigorous. Seedling water use efficiency in bambara groundnut improved with decreasing water availability. Drought avoidance strategies and acclimation to water stress were also found to be present at the seedling establishment stage. Yield was negatively affected by water stress. Subsequent seed viability and vigour were respectively higher in seeds produced under irrigated and rainfed conditions. The study concluded that although bambara groundnut is a water use efficient crop, water stress may affect yield and subsequent seed quality.

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CHAPTER 1

INTRODUCTION

1.1 Rational for the Research

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an African grain legume which forms an integral component in many African farming systems, particularly smallholder subsistence farmers who cultivate it using local landraces (Sumberg 2002). Landraces are varieties that have arisen through natural selection with little assistance and/or intervention from humans (Louette *et al.* 1997, Zeven 1998). Typically, landraces exhibit huge variability and are often adapted to local agro–climatic conditions (Zeven 1998), thus making them capable of meeting farmers' social, economic, cultural and ecological needs (Teshome *et al*. 1997). Studies using Random Amplified Polymorphic DNA (RAPD), Flourescence Amplified Fragment Length Polymorphism (FALP) and Single Nucleotide Polymorphisms (SNPs) markers revealed high genetic diversity among different landraces (Amadou *et al.* 2002, Massawe *et al.* 2002, Somta *et al.* 2011). Owing to a lack of bambara groundnut cultivars, several scientific studies on bambara groundnut have used seed coat colour as a selection criterion (Sinefu 2011, Mabhaudhi and Modi 2013, Mabhaudhi *et al.* 2013). Mabhaudhi (2012) reported a significant association between possible drought tolerance and bambara seed coat colour, stomatal regulation and vigorous establishment. Despite evidence suggesting that bambara groundnut is a drought tolerant crop with food security potential, the crop still remains largely underutilised (Mabhaudhi *et al.* 2011, Sinefu 2011).

Other underutilised African grain legumes identified, but not limited to, include cowpea (*Vigna unguiculata*), African yam beans (*Sphenostylis stenocarpa*), lima beans (*Phaseolus lunatus*), sword beans (*Canavalia gladiata)*, pigeon pea (*Cajanu cajan*), jack beans (*Canalia eniformis*) and dolichos lablab (*Lablab purpureus*) (Ajayi *et al.* 2009). Neglected underutilised plant species (NUS) are crops primarily grown in their centres of origin or centres of diversity by traditional farmers. In these areas, NUS have remained important for the subsistence of local communities. Some NUS may be globally distributed but tend to occupy special niches in the local economies and in local production as well as consumption systems. Neglected underutilised species are under researched and conserved by research and conservation but continue to be maintained by socio-cultural preferences and local use

practices (IPGRI 2002). Jughes (2008) characterised NUS as under-exploited species with potential to contribute to food security, nutrition and health, poverty alleviation (income generation) and sustaining the environment.

1.2 Justification

Building a food secure future for developing countries requires focus and action in critical areas such as increasing productivity of small holder farmers, advancing nutrition among children, building sustainable food systems and empowering women and people living in rural areas (UNDP 2012). The conservation and sustainable utilization of plant genetic resources is key to improving agricultural productivity and sustainability thereby contributing to national development, food security and poverty alleviation (FAO 1996). The neglect by researchers has led to a scenario whereby NUS continue to be underutilised and even risk further erosion (Idowu 2008). The neglect is further restricting development options for NUS. There is need to boost current research efforts so as to increase the value and marketability of NUS as well as to make them more widely available. This, in turn, will also broaden its resource base and increase the livelihood options for rural communities (Idowu 2008). This will lead to a need for availability of good seed of known quality with high yielding potential. There is scant information on seed quality of bambara groundnut. Sinefu (2011) evaluated seed quality of one landrace separated into three selections based on seed coat colour. Elsewhere, Zondi (2013) evaluated seed quality of different landrace provenances that were also separated into several seed coat colour selections. Their studies concurred that seed colour was a useful index for determining seed colour although variations existed between landrace provenances. There is need for further research on seed quality of other variations of bambara groundnut seeds and landraces from different locations which have been shown to be highly diverse.

South Africa is located in a predominantly semi-arid part of the world. The country's climate varies from desert and semi–desert in the west to the sub-humid along the eastern coastal area with an average rainfall of 450 mm per year, which is below world average of 860 mm per year (DWAF 2006). Agriculture is a major user of water resources. Climate change is expected to negatively affect water supply and agriculture through changes in seasonal timing of rainfall as well as higher incidence and severity of floods and drought

(OECD 2011). In addition to the agro-ecological niche (semi-arid and arid parts of Africa) of bambara groundnut indicating adaptability to drought, several studies have proven that bambara groundnut can, to a certain extent, adapt to water limited conditions (Mwale *et al.* 2007, Mabhaudhi and Modi 2013, Mabhaudhi *et al.* 2013). Studies have mainly focused on responses and adaptability of bambara groundnut to water stress (Mwale *et al.* 2007, Vurayai *et al.* 2011a). The issue of effect of water stress imposed on maternal plants on subsequent seed quality in bambara groundnut has not yet been fully exploited and there is need to determine whether water stress has an effect on subsequent seed quality of bambara groundnut landraces.

1.3 Aims and Objectives

With water being vital to crop production and most resource–constrained farmers relying on rainfed agriculture, it is important to quantify water use and determine water use efficiency of bambara groundnut under different water production scenarios. Azam-Ali *et al*. (2004) and Mabhaudhi (2012) quantified water use and determined water use efficiency of bambara groundnut under limiting and non-limiting water conditions. Bambara groundnut is cultivated using seeds obtained from farmers' previous harvest and little is known about their seed quality and the effect of production environment on subsequent seed quality. Therefore, the aim of this study was to evaluate seed quality of selected seed coat colours of bambara groundnut, determine water use efficiency during seedling establishment and to determine the effect of water stress on maternal plants on subsequent seed quality of bambara groundnut. It was hypothesised that seed coat colour had no effect on (i) seed performance in terms of germination, vigour and (ii) seedling water use efficiency of bambara groundnut.

The specific objectives of the study were to:

1. determine the effect of seed coat colour of bambara groundnut on seed performance, i.e. germination capacity and vigour,
2. to evaluate water use efficiency and water use characteristics at the seedling establishment of selected seed coat colours of bambara groundnut under varying water regimes in a controlled environment facility,
3. to evaluate yield and yield components of selected bambara groundnut seed coat colours under rainfed and irrigated field conditions, and

4. to evaluate subsequent seed quality of selected bambara groundnut seed coat colours in response to imposed stress on maternal plants under field conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Bambara Groundnut Crop Origins and Ecological Significance

2.1.1 Origin and history

Bambara groundnuts (*Vigna subterranea* (L.) Verdc), also known as jugo beans, izindlubu, round beans in South Africa (Swanevelder 1998) and nyimo in Zimbabwe (Mungate 1995), is a legume crop widely cultivated in Sub-Saharan Africa. Its centre of origin is believed to be Bambara near Timbuktu in central Mali, West Africa hence its name bambara groundnuts (Goli 1995). It was first described by Linnaeus in 1763, and was confirmed to be widely cultivated in most parts of Africa except North Africa, Ethiopian Highlands and temperate regions of South Africa in 1806 (Hepper 1963). It belongs to the family Leguminosae and sub–family Papilionoideae. *Subterranea* is the cultivated species while wild forms belong to the species *spontanea* (Pasquet *et al.* 1999). In much of Africa bambara groundnut is the third most important legume after groundnut (*Arachis hypogea*) and cowpea (Kishinevsky *et al.* 1996, Azam Ali *et al.* 2001). However, bambara groundnut has slowly been replaced by the exotic *Arachis hypogea,* although its cultivation still has a wide distribution from Senegal to the Ethiopian lowlands, as well as in South Africa and Madagascar (Pasquet *et al.* 1999). The top five bambara groundnut producing countries in the world (in descending ranking order) are: Burkina Faso, Cameroon, Mali, Niger and Democratic Republic of Congo, together, producing just over 140 000 MT per annum (FAOSTAT 2011). There are no hybrids/registered cultivars for bambara groundnuts; the Department of Agriculture Forestry and Fisheries (DAFF) (2011) classified varieties according to seed coat colour.

2.1.2 Botany and ecology

Bambara groundnut is a low flat annual plant with compound leaves of three leaflets (Stephens 2012). The petioles are approximately 15 cm long, stiff and grooved and the base is green or purple in colour. Leaves and flower buds arise alternately at each node. After fertilisation the flower stem elongates. The sepal enlarges and the fruit develops above or just below the soil surface (Fig 2.1). The unripe pod is yellowish green with up to six pods while mature pods may be yellowish green or purple (DAFF 2011). The plant can be categorized

into bunch, semi–bunch and open cultivars on the basis of the ratio petiole length/ internode length or canopy diameter 100 days after planting (Pasquet *et al.* 1999). The pods are round, wrinkled and over 1.27 cm long. Each pod contains one or two seeds that are round, smooth and very hard when dried (Stephens 2012). The seeds differ in size, seed coat colour, testa colour and eye pattern around the hilum (Fig 2.2) (Basu *et al.* 2007). Depending on cultivar and season, the plant takes between three to six months to mature (DAFF 2011).

Figure 2.1: Description of bambara groundnut plant; 1= Habit of flowering plant 2= Flower 3=Fruits 4=Seed. (Source: database.prota.org)

Figure 2.2: Seed of bambara groundnut differing in size, seed coat colour and eye pattern around the hilum. (Source: www.agfax.net)

Bambara groundnut grows best in areas receiving 400 - 600 mm of annual rainfall with an average temperature of 25ºC (Kouassi and Zoro Bi 2010). Planting dates for bambara groundnut range from September to February but Sesay *et al.* (2007), Sinefu (2011) recommended planting in November in sub-tropical and tropical regions. The crop does not tolerate water logging (Mabhaudhi 2012, Zondi 2013). It is grown mostly on flat ground but in wetter areas mounds or ridges are preferred (Hillocks *et al.* 2011). Best mounding times were shown to be seven weeks after planting (Oudraogo *et al.* 2012). Subsistence farmers in South Africa prefer growing bambara groundnut on flat ground and mounding times range from 50 to 100 days after planting (Swanevelder 1998). Bambara groundnut is typically a short day plant and studies have shown that long days delay flowering and pod development. Average biomass yield ranges from 1 500 to 8 500 kg ha^{-1} while average pod yield ranges from 300 to 3 000 kg ha^{-1} (Azam-Ali *et al.* 2001). Grain yield ranges from 400 to 4 000 kg ha^{-1} while haulm yield ranges from 2 500 to 8 000 kg ha^{-1} (Masindeni 2006). Sinefu (2011) attained the same yield range as Masindeni (2006), and values were influenced by season, agronomic practices and water availability to the crop.

2.1.3 Uses and importance

Bambara seeds can be eaten fresh when semi-ripe, as a pulse when dry and mature or can be ground into flour (Linnemann and Azam-Ali 1993). The fresh pods are boiled with salt and pepper and eaten as a snack (Hillocks *et al.* 2011). The leaves of bambara groundnut are used as animal feed (Ajayi *et al.* 2009). Bambara groundnut seed contains sufficient quantities of protein (16-25%), carbohydrate (65%) and fat (up to 6.5%) as well as appreciable amounts of micro nutrients as recommended by the FAO/WHO (Fanzo *et al.* 2011). Ijarotimi and Esho (2009) found fermented bambara groundnut flour to have high nutritional quality and recommended its use in weaning food formulation. The seeds of bambara groundnut are also used to produce a paste which is used to prepare akara (a traditional African food) (Alobo 1999). Bambara groundnut seeds can be used to produce vegetable milk for local use (Brough *et al.* 1993). Agunbiade *et al.* (2011) found bambara groundnut milk to be acceptable on the basis of organoleptic scores compared with the standard cow milk; this is further testament to the untapped potential that the crop holds. The Luo tribe in Kenya explore the medicinal properties of bambara groundnut by using water from boiled grain to treat diarrhoea (Dakora 2011). Being a grain legume, bambara groundnut is capable of fixing nitrogen and does not need nitrogen fertiliser application; this implies that even resource-constrained households can participate in production of bambara groundnut (Dakora 2011).

In South Africa, bambara groundnut is popular amongst resource constrained farmers who practice rainfed agriculture because of its tolerance to drought and ability to produce a reasonable crop when grown on poor soils (Swanevelder 1998). Its ability to fix atmospheric nitrogen is an advantage in cases of intercropping as well as for the subsequent crop when excess nitrogen is left in the soil (Graham and Vance 2003). Bambara groundnut is suitable for intercropping with other crops and does not take up large areas of land that could be considered more important or lucrative (Hillocks *et al.* 2011). Karikari (2003) reported that bambara groundnut was an excellent crop for intercropping with maize and other cereal crops. Locally, Mabhaudhi (2012) showed that intercropping taro and bambara groundnut landraces under dry-land conditions was beneficial and productive. In addition, unconfirmed observations by Mungate (1995) reported that bambara groundnut can potentially suppress *Striga species*, a parasitic weed.

The cultivation of indigenous crops has a long history that has been intimately linked to women and their traditional livelihood tasks (Modi *et al.* 2006). Bambara groundnut has also

been reported to be mainly cultivated and domesticated by women (Mukurumbira 1985). They play an increasingly important role as a source of income in rural communities especially those near towns and cities. Varying preservation and preparation methods of bambara groundnut ensure its availability almost throughout the year (Vorster *et al.* 2007). In Nigeria, bambara groundnut is mostly consumed during the period preceding harvest of new crops (Onimawo *et al.* 1998); this makes it an important food security crop.

2.2 Seed Quality

The limited extent of cultivation of NUS can also be related to a chronic lack of high yielding, well-adapted varieties and shortage of quality seed (Hughes and Ebert 2011). Current seed systems for NUS are informal; farmers select and store part of their harvests for future planting, exchange seeds with relatives and other farmer's trade in the local market (Ahmed *et al.* 2008). According to Nkoze and Okoko (2003), often seed produced at farmer's level is of poor quality. They attributed this to poor selection of plants to harvest for seed, improper extraction of seeds, processing and storage. With increasing demand for NUS there is a need for good seed of known quality with high yield potential.

Seed quality is the sum of many differing components including genetic quality, physical purity, germination, vigour, uniformity in size, and freedom from seed-borne diseases (McDonald and Copeland 1997, Basra 2005). High viability, storability and vigour are important characteristics of seeds.

2.2.1 Seed coat colour

Several studies have reported association between seed coat colour and seed performance. In bambara groundnut, Mabhaudhi and Modi (2013) associated seed coat colour with establishment performance. They suggested that this association was probably due to phenolic compounds in darker coloured seeds. Flavonoids mainly flavonols, anthocyanins, phlopaphenes, isoflavones and proanthocyanidins serve as pigments in seeds and grains. Anthocyanins are responsible for the red to purple colour while flavonol glycosides are responsible for the yellow colour and proanthocyanidins, when oxidised, are responsible for the brown colour (Owen 1927, Vandenberg and Slinkard 1990). The effect of flavonoids on

seed quality is not well understood and it is also believed to be associated with the adherence of the testa to the cotyledons (Powell 1989). Tannin concentration was also shown to be correlated with seed coat colour. Tannin concentration is higher in dark coloured seed than light coloured seed and increases as colour intensifies (Nti 2009). Tannin concentration has however been linked with palatability of bambara seeds and not seed quality. Food scientists prefer the cream coloured bambara seeds due to superior taste, aroma (Nti 2009), and milk taste (Brough *et al.* 1993), whereas agronomists recommend cultivation of dark coloured seed for reasons of vigorous agronomic performance (Zengeni and Mupamba 1995, Sinefu 2011; Mabhaudhi and Modi 2013). Therefore, a need to research for varieties that are both vigorous and consumer preferred arises.

In cowpea (Odindo 2007, Ntombela 2013) and maize (*Zea mays*) landraces (Mabhaudhi and Modi 2010), dark coloured seeds generally performed better than light coloured seeds. Zengeni and Mupamba (1995) compared germination capacity and seedling emergence of cream, brown and black seeds of bambara groundnuts and found black seeds to perform better. Recently, Sinefu (2011) compared seed quality of red, white and brown bambara groundnut landraces from the same area and found brown seed to germinate faster than red and white seed. The approach by Zondi (2013) was to evaluate the relationship between seed coat colour and seed quality and how this relationship is influenced by different provenances. The conclusion was that provenances play a significant role in seed performance and seed coat colour is inarguably a selection criterion for seed quality. However, most studies of seed coat colour reported in the literature have focused on plain colours and variations such as speckles and colour of speckles have not received any attention. Given that there is much variation in bambara groundnut, of which speckling is one, there is also a need to investigate the effect of speckles and their colour on seed quality.

2.3 Drought and water scarcity

Drought is a weather related natural phenomena and historically has had the greatest impact on economic and environmental damage in the world. Meteorological drought is a measure of the deviation from the normal precipitation over a period of time. Inadequate soil water to meet the needs of a particular crop at a particular time is termed agricultural drought (Disaster handbook 1998); agronomic drought occurs due to meteorological drought or other management factors that may limit soil water availability. There are various definitions and

measurements of water scarcity but UN-Water (2006) broadly defined it as an imbalance between availability and demand. In either case, whether meteorological or agronomic, the end effect of drought is limitations on crop productions.

South Africa is one of many African countries facing water scarcity. Currently 93% of existing fresh water is consumed by irrigation agriculture while 4.7% is used for domestic use and 2.3% in mines. Commercial agriculture relies on irrigation for optimum production (Walter *et al.* 2011). With increased water shortage irrigation will be affected resulting in decreased capacity to maintain food production. Investigating the water use efficiency and enhancing agricultural water productivity is becoming a priority in water stressed countries (UN-Water 2006). Through natural selection some crops have developed mechanisms for adaptation and survival during periods of water stress (Cattivelli *et al.* 2002). The latter has been proven for African indigenous crops as they are highly adapted to African conditions hence have a higher chance of survival in periods of famine.

2.3.1 Crop responses to water stress

In response to water stress plants optimize the morphology, physiology and metabolism of their organs and cells in order to survive. The responses to water stress differ, depending on the plant species, stage of development and intensity and duration of stress (Lisar *et al.* 2012).

2.3.1.1 Plant growth and development

Plants are made up of cells and plant growth involves an increase in cell numbers through cell division and expansion. Cell expansion is a turgor-driven process hence water deficit places limitations on cell elongation (McGraw-Hill 2005). Under water-limited conditions, growth inhibition is as a result of decrease in cell enlargement caused by reduction in plant cell's water potential and turgor, elevating the solutes' concentrations in the cytosol and extracellular matrices (Lisar *et al.* 2012). The reduction in growth could be a result of photosynthate translocation where phloem translocation depends on gradients of hydrostatic pressure. Water stress reduces source strength by reducing photosynthesis and reducing sink strength by inhibiting growth (Hsiao 1973). Studies on bambara groundnut growth response to water stress indicate that plant height, leaf number and leaf area index (LAI) are significantly reduced by water stress (Mwale *et al.* 2007, Karunaratne *et al.* 2011, Sinefu

2011, Vurayai *et al.* 2011a, Mabhaudhi and Modi 2013, Mabhaudhi *et al.* 2013). However, root: shoot ratio in bambara groundnut has been reported to increase as water availability decreases (Vurayai *et al.* 2011a). Liu *et al.* (2004) also found a higher root: shoot ratio in wheat (*Triticum aestivum*) under water stress attributing this to changes in source and sink relationships with the root being a stronger sink than the shoots. Plants that show an increase in root: shoot ratio with increasing water stress are believed to be more drought tolerant because of their ability to maintain osmotic pressure, and ability to maximise available water and penetrate into deeper soil horizon (Lloret *et al.* 1999).

Karunaratne *et al.* (2010) described the phenological cycle of bambara groundnut into five stages which are emergence, vegetative, flowering, pod filling, and maturity. On average bambara groundnut takes 140-150 days to reach maturity (Karunaratne *et al.* 2010, Mabhaudhi 2012). Water stress imposed at any stage of the phenological cycle results in earliness or delay in phenological events (Wopereis *et al.* 1996). Mabhaudhi and Modi (2013) observed that bambara groundnut landrace selections tended to flower earlier under rain fed than irrigated conditions. This tendency of bambara groundnut flowering and maturing earlier under water limited conditions was also confirmed under rain shelter conditions (Mabhaudhi *et al.* 2013). In addition, the different growth stages respond differently to water stress and also the ability of the plant to survive and recover from water stress. The bambara groundnut plant is more sensitive to water stress at the flowering stage and less sensitive at pod filling stage (Vurayai *et al.* 2011b). Cakir (2004) found contrary results in maize where the plants where more sensitive to water stress at the vegetative stage. Water stress during vegetative, flowering and pod filling has a direct link to yield loss and reduced total dry matter of bambara groundnut (Mwale *et al.* 2007, Karunaratne *et al.* 2011, Vurayai *et al*. 2011b).

2.3.1.2 Physiological responses

Photosynthesis is a process by which plants convert photons into chemical energy for food. Photosynthesis takes places in the leaves and involves a complex set of reactions including reducing carbon dioxide to carbohydrates releasing oxygen, with water being the reducing agent (Campbell 2006). The water used for photosynthesis comes from the roots and is pulled up the plant by a process called transpiration which is loss of water vapour through the stomata of the leaves. Water stress results in metabolic changes along with functional and structural rearrangements of photosynthesizing apparatus (Lisar *et al.* 2012).

2.3.1.2.1 Stomatal conductance

Stomatal conductance is the rate of passage of either water vapour or carbon dioxide through the stomata. Stomatal conductance allows the leaf to change the partial pressure of carbon dioxide at the sites of carboxylation and the transpiration rate. In cases of water scarcity, abscisic acid (ABA) biosynthesis increases in the roots and is translocated to the shoot via the xylem sending a signal to close the stomata, hence reducing the amount of water lost through the leaves (Farquhar and Sharkey 1982). Cowpea and bambara groundnut have been proven to respond promptly to water stress by lowering stomatal conductance (Diallo *et al.* 2001, Vurayai *et al.* 2011a, Mabhaudhi and Modi 2013, Mabhaudhi *et al.* 2013). Stressing bambara groundnut plants at pod filling stage results in the highest reduction of stomatal conductance compared to plants stressed at the vegetative stage (Vurayai *et al.* 2011a). Mabhaudhi (2012) showed that there was an association between seed coat colour and stomatal regulation; the red landrace selection exhibited greater stomatal plasticity than the brown and light-brown landraces.

2.3.1.2.2 Leaf water potential

Changes in stomatal conductance alter the transpiration rate and consequently affects leaf water potential (Farquhar and Sharkey 1982). Leaf water potential is the driving force for the movement of liquid water through the plants and relates to the volume flux of water through the plant and the characteristics of the pathway of water transfer from soil to leaf. When water lost through transpiration exceeds water absorbed by the roots it causes a reduction in leaf water potential (Jarvis 1976). Leaf water potential decreases with decreasing soil water content but in kidney bean leaf water potential was constant for a few days although soil water content kept decreasing (Miyashita *et al.* 2005). In *Dianthus species*, Alvaret *et al.* (2009) found decreasing leaf water potential to be the first physiological response to decreasing soil water content and hence influencing stomatal conductance. Crops that are able to maintain constant leaf water potential during water stress are believed to be more adaptive to drought (Stoyanov 2005). The latter has been proven in cowpea where leaf water potential was constant in both well watered and water stressed plants (Diallo *et al.* 2001).

2.3.1.2.3 Chlorophyll content and chlorophyll fluorescence

Chlorophyll is the green bio-molecule pigment found in chloroplasts of green plant cells. In plants chlorophyll exists as, chlorophyll a and b; both of them function as photoreceptors during photosynthesis (Khaleghi *et al*. 2012). Chlorophyll content is normally determined quantitatively and is strongly correlated to nitrogen content in leaves. Chlorophyll accumulation was shown to decrease in water-stressed seedlings (Dalal and Tripathy 2012). Reduced chlorophyll synthesis is associated with a decrease in the accumulation of biosynthetic intermediates, such as glutamate-1-semialdehyde (GSA), 5-aminolevulinic acid, Mg-protoporphyrin IX monomethylester and protochlorophyllide (Rahbarian *et al.* 2011). It is also said that, under water stress, chlorophyll biosynthesis is also down-regulated to prevent the accumulation of harmful singlet oxygen generating tetrapyrroles at a very early stage, due to reduced gene expression of early enzymes of chlorophyll biosynthesis pathway (Khaleghi *et al.* 2012). As a result, chlorophyll content may be useful for evaluating plant responses to water stress. Mabhaudhi and Modi (2013) used chlorophyll content to evaluate drought tolerance in bambara groundnut selections. They observed that it was lower, in the early stages of plant growth, in stressed plants relative to unstressed plants. They concluded that chlorophyll was a good indicator of drought tolerance and required more study.

In addition, chlorophyll allows plants to absorb photons (energy from light) especially the blue and red ends of the visible light spectrum. Light energy absorbed by chlorophyll molecules is consumed primarily through three pathways; it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as light commonly known as chlorophyll fluorescence (Dalal and Tripathy 2012). The photochemical efficiency of the system is the ratio of variable fluorescence (Fv) to maximal fluorescence yield (Fm) attained when high intensity flash has been applied. Variable fluorescence (Fv) being the difference between the fluorescence origin and Fm. The Fv/Fm ratio is directly related to the quantum efficiency and is thus a good measure of stress (Butler 1978). The intensity of chlorophyll fluorescence is directly related to the concentration of excited chlorophyll molecules, which suggests that a change in the efficiency of photosynthesis provides a measure of leaf photosynthesis ability of plants (Dalal and Tripathy 2012). It is well known that photosynthetic systems in higher plants are most sensitive to water stress. As stress increases there is a decrease in chlorophyll fluorescence (Khaleghi *et al.* 2012). Insight into the ability of the plant to tolerate environmental stresses and into the

extent to which these stresses have damaged photosynthetic apparatus can also be gained from this measure (Maxwell and Johnson 2000).

A Fv/Fm value greater than 0.79 is a good indicator of photosynthetic efficiency in plants (Bjorkman and Demmig 1987, Ow *et al.* 2011), while a Fv/Fm value less than 0.72 indicates that the plant is failing to cope under stress conditions (Ow *et al.* 2011). Odindo (2007) found Fv/Fm values of non-stressed, intermittent stressed and terminal stressed to be above 0.80. In maize Mabhaudhi (2009) also observed Fv/Fm values greater than 0.80 in water stressed plants and non-stressed plants. Interestingly Mabhaudhi (2009) found the Fv/Fm ratio higher in water stressed compared to non-stressed plants. Both authors found other parameters measured to indicate stress. A combination of water stress and heat stress also showed no effect on Fv/Fm value in wheat (Lu and Zhang 1999). In all cases it can be argued that although plants were stressed the photosynthetic apparatus remained intact and photosynthetically efficient. The effect of water stress on Fv/Fm value of bambara groundnut landraces has not yet been explored.

2.3.1.3 Metabolic responses

Plants also respond to water stress at a molecular level. The ability of plants to tolerate water stress at the molecular level is dependent on several biochemical pathways that facilitate retention of water, protect photosynthetic apparatus and maintain ion homeostasis (Bohnert and Jensen 1996). Accumulation of compatible solutes and specific proteins is a common metabolic adjustment of plants under water stress. The role of compatible solutes and specific proteins in plants under water stress is that of scavenging reactive oxygen species (ROS), restoring metabolism, preservation of cellular turgor by reinstitution of osmotic balance and protection and stabilization of proteins and cellular structures or acting as chaperones (Hare and Cress 1997).

2.3.1.3.1 Antioxidants

Water stress creates an imbalance between light captured and light utilized which inhibits photosynthesis. The excess light energy in the photosynthetic apparatus results in generation of ROS which denature functional and structural molecules especially in the chloroplast and disrupts cellular redox homeostasis. In order to scavenge ROS, plants have developed enzymatic and non–enzymatic antioxidant systems (Gill and Tuteja 2010, Lisar *et al.* 2012). Among enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and

glutathione peroxidise (GPX). Non–enzymatic antioxidants include phenolic defence compounds (Vitamin E, flavonoids, phenolic acids and other phenols), nitrogen compounds (alkaloids, amino acids and amines), caretonoids and chlorophyll derivatives (Panda 2012). Hameed *et al.* (2011) reported that activity of antioxidants differed with species, duration and severity of stress and mode of imposition. Turkan *et al.* (2005) confirmed this report by Hameed *et al.* (2011) when they exposed common bean (*Phaseolus vulgaris*) and tepary bean (*Phaseolus acutifolis*) to different levels and duration of stress, observing variation in SOD and CAT activity. Literature on the activity of antioxidants in bambara groundnut and how it is affected by severity and duration of water stress is limited.

2.3.1.3.2 Total soluble sugars

Soluble sugars are among compatible metabolites and osmolytes which increase when water stress increases (Bray 1997, Rosa *et al.* 2009) and soil water content decreases (Arabzadeh 2012, Naser *et al.* 2010). Accumulation of sugars under water stress lowers osmotic potential of cells and water is attracted into the cell and helps with the maintenance of turgor (Farhad *et al.* 2011). According to the water replacement hypothesis, sugars act as a water substitute by satisfying the hydrogen-bonding requirement of polar groups of the dried protein surface. Sucrose and glucose either act as substrates for cellular respiration and as osmolytes to maintain cell homeostasis, while fructose is not related to osmoprotection and seems related to secondary metabolites synthesis (Rosa *et al.* 2009). The build-up of total soluble sugars with increasing water stress was evident in cowpea (Souza *et al.* 2004). However, increased levels of soluble sugars have unpredicted negative effects on growth and development of plant due to the highly integrated nature of sugar metabolic pathways and its link to decreased carbon dioxide assimilation. Souza *et al.* (2004) observed the latter theory in cowpea.

2.3.1.3.3 Proline

Proline is a DNA encoded α-amino acid. It is synthesised from glutamate in the cytoplasm of chloroplast and catabolized within mitochondrial matrix by action of proline dehydrogenase and pyrroline-5carboxylate dehydrogenase to glutamate (Hare and Cress 1997). During periods of water stress there is transcriptional up regulation of genes for pyrroline-5 carboxylate synthase and pyrroline 5 carboxylate to increase proline synthesis from glutamate and down regulation of genes for pyrroline 5 carboxylate reductase and prohydrogenase to arrest proline catabolism. Overexpression of biosynthetic proline enzymes increase the levels of compatible solutes and improve water stress tolerance in plants (Fraire- Velazquez and

Balderas-Hernandez 2013). In bambara groundnut the levels of proline have been shown to increase up to four times higher in water stressed plants compared to non-stressed plants proving some level of tolerance to water stress (Vurayai *et al.* 2011a). In cowpea the increase in levels of proline was significant in severely stressed plants and not significant in moderately stressed plants indicating the crops' tolerance to water stress (Souza *et al.* 2003).

2.3.1.3.4 Proteins

Generally proteins in the leaves decrease during water stress due to suppressed synthesis. The alteration of gene expression consequently leads to synthesis of new proteins and mRNA. The main types of stress induced proteins are heat shock proteins and late embryogenesis abundant (LEA) type proteins. These proteins have been found to protect macromolecules such as enzymes, lipids and mRNA from dehydration (Lisar *et al.* 2012). LEA type proteins were first discovered in cotton (*Gossypium species*) seeds accumulating during late embryogenesis (Hundertmark and Hincha 2008). Subsequently they were found to accumulate in vegetative tissue of seedlings of barley (*Hordeum vulgare*) and rice (*Oryza sativa*) during periods of environmental stress (Hong *et al.* 1992, Xu *et al.* 1996). As much as researchers have studied proline accumulation in bambara groundnut information on accumulation of LEA type proteins is not readily available.

2.4 Water Use

With the growing world population the pressure on fresh water resources increases. The climate change models predicting decreasing precipitation in future worsens the severity of the situation at hand. Water consumed by the agriculture sector is not sustainable (Pimental *et al.* 2000). This implies that there will be less water available for food production, continuing to put food security at stake. The agriculture sector faces a great challenge; producing more food from less water. The main strategy is to improve the productivity of water use in irrigated and rainfed agriculture (Condon *et al.* 2004). Plant breeders addressing this issue are looking at breeding for high water use efficiency (WUE) (Blum 2005).

2.4.1 Water use efficiency

Water use efficiency is defined in several ways. All definitions have some measure of water being exchanged for some unit of production. Physiologists and biologists define WUE as carbohydrates formed through photosynthesis per unit of transpiration (Davies *et al.* 2002). Agronomist and farmers define WUE as the yield of harvested products achieved from the water made available to the crop through precipitation and/or irrigation (Sinclair *et al.* 1984). Water use efficiency is thus expressed as;

$$\frac{\boldsymbol{Biomass}}{\boldsymbol{Water\ applied}} \qquad \textbf{Equation 2.1}$$

Where biomass is total (above and below ground) biomass (kg) and water applied is total water applied through irrigation and/or rainfall (m^3) (Kriedemann *et al.* 1999).

Scientists working on crop water relations and irrigation management would prefer quantifying WUE as;

$$\frac{Biomass}{ETa} \qquad \textbf{Equation 2.2}$$

where, ETa = crop evapotranspiration/water use/ crop water requirement (Mabhaudhi 2012).

Condon *et al.* (2004), Siahpoosh and Dehghanian (2012), suggested further quantifying WUE on how effectively the biomass is partitioned into the harvested product that is the ratio of grain yield to biomass termed the harvest index (HI). Water use efficiency can therefore be expressed as;

$$\frac{HI * Biomass}{ETa} \qquad \textbf{Equation 2.3}$$

Water use efficiency can be used as a tool for exploring potential increase in crop yields that result from increasing water supply. Farmers can assess whether yield is limited by water supply or by other factors. The unit increment in yield per unit water use reveals the impact and worth of additional water supply. Information on WUE is crucial to farmers in order for them to plan irrigation water management strategies (Alghariani 2007). High water efficiency

in crops is usually achieved by reducing water applied, the crop improving yield under water limited conditions, maximizing yield through enhanced fertility, disease and pest control and optimum planting. Davies *et al.* (2002) and Blum (2005) reported that high WUE is not necessarily drought tolerance, but plants improve water use efficiency by exploiting drought response mechanisms. Some genotypes of cowpea improved WUE under water deficit by approximately 20% indicating some level of drought tolerance (Anyia and Herzog 2004). It was reported that bambara groundnut had a (WUE) of 0.1 kg m^{-3}, a figure that was found to be higher but comparable to that of other established legumes (Azam-Ali *et al.* 2004).

2.5 Drought Response Mechanisms

Pursuing the literature by Davies *et al*. (2002) and Blum (2005); where high WUE is not necessarily drought tolerance, but plants improving WUE by exploiting drought response mechanisms. Drought response mechanisms are divided into three categories namely: escape, avoidance and tolerance (Pouresmaeil *et al.* 2012). Drought escape is the ability of the plant to complete its life cycle before drought stress becomes terminal. The mechanisms involved in drought escape include, rapid phenological development, variation in duration of growth period depending on water availability (developmental plasticity) and remobilization of pre–anthesis assimilates to grain (Mitra 2001, Chaves *et al*. 2002.). Drought avoidance is the ability of plants to maintain high tissue water potential under water stress while drought tolerance is the plant's ability to maintain its normal functions at low tissue water potential. Drought avoidance is usually achieved through plant growth and development changes while drought tolerance is usually achieved by cell and tissue specific physiological, biochemical and molecular mechanisms (Chaves *et al*. 2002, Pouresmaeil *et al.* 2012).

Earlier studies by Collinson *et al*. (1997) found that bambara groundnut was able to withstand drought stress but the response mechanisms were unclear. Later on, bambara groundnut was shown to respond to drought by lowering the rate of leaf area expansion, final canopy size and total dry matter suggesting that the crop explored drought avoidance mechanisms. The latter findings were confirmed by Jorgensen *et al.* (2010) and Mabhaudhi *et al*. (2013). Furthermore, drought avoidance mechanisms were identified on a physiological level where bambara groundnut was able to maintain high tissue water potential through stomatal closure during periods of low soil water availability (Jorgensen *et al*. 2010, Vurayai *et al*. 2011a, Mabhaudhi and Modi 2013, Mabhaudhi *et al*. 2013). Mabhaudhi and Modi

(2013) and Mabhaudhi *et al.* (2013) observed drought escape through rapid phenological development (early flowering, reduced flowering duration, early senescence and early maturity) when bambara groundnut was subjected to varying levels of water stress. Drought tolerance being usually achieved by molecular mechanisms, proline accumulation is the only mechanism that has so far been associated with drought tolerance in bambara groundnut (Vurayai *et al*. 2011a, Sinefu 2011, Zondi 2013).

Response mechanisms are not mutually exclusive as shown by Vurayai *et al*. (2011a) and Mabhaudhi *et al*. (2013) who observed both drought escape and avoidance mechanisms. However, escape and avoidance – high WUE – are often at the expense of biomass production and yield attainment (Blum 2005). If plants are to achieve reasonable yields under water stress, then drought tolerance mechanisms which allow plants to maintain normal metabolic function and yield attainment at low tissue water potential are desirable (Blum 2005). Thus far, these have not been fully explored in bambara groundnut. Mabhaudhi and Modi (2013) put it forward that efforts to study drought tolerance mechanisms in bambara groundnut needed to pay attention to molecular responses such as compatible solutes, proteins and antioxidants. Therefore, there is a need to investigate the drought tolerance in bambara groundnut by studying metabolic responses of bambara groundnut to water stress.

2.6 Conclusion

The review of literature showed that bambara groundnut is an important African indigenous legume. The crop has high nutritional value, drought tolerant characteristics and N-fixation properties; this combination makes it a possible multi-use crop in marginal areas of agricultural production. In addition, the review showed that bambara groundnut, as a crop, has great potential to contribute to food and nutritional security in Africa, more so with predicted climate change. However, despite showing much potential, the review of literature also highlighted the fact that the crop remains underutilized. This is partly due to limited scientific information describing growth, development, yield and water use; there are also no improved varieties and cultivated landraces are often of poor seed quality. This may partly explain the poor uptake of the crop by farmers as well as its diminishing presence within communities that traditionally cultivated the crop. Thus, there is a need to understand the seed quality components of bambara groundnut landraces. In this regard, this study will seek to contribute to efforts on using seed coat colour as a criterion for seed quality by exploring speckling in bambara groundnut.

Crop yield is influenced by water availability of which drought is a major threat to agriculture. This review highlighted that currently, studies on drought tolerance of bambara groundnut have been biased towards ecophysiological and morphological adaptations with little attention being given to metabolic responses. The current study will seek to determine the metabolic responses associated with drought tolerance in bambara groundnut. Identifying metabolites contributing to drought tolerance of bambara groundnut will aid in genetic selection for drought tolerance, as this is intricately linked to high water use efficiency. Determining the effect of water tress on mother plants on subsequent seed quality will also aid in identifying and solving the problem of poor seed quality in bambara groundnut.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Material

Bambara groundnut seeds acquired from a seed company (Capstone Seeds®) were divided into four distinct selections based on seed coat and speckling colour: plain red, plain cream, cream with brown speckles (brown speckled) and cream with black speckles (black speckled) (Figure 3.1). Initial seed characterisation was done to obtain 100 grain mass of all seed lots. Three replicates of 100 seeds of each seed colour were weighed using a sensitive balance (Masskot, FX320, Switzerland) and the mean mass was calculated. One hundred grain mass for the bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) was 76.99, 88.05, 73.29 and 86.42 g, respectively.

Figure 3.1: Seeds of a bambara groundnut landrace. A – seedlot of bambara groundnut before colour selection and B– brown speckled colour selection, C – plain cream colour selection, D – plain red colour selection and E – black speckled colour selection.

3.2 Seed Quality Tests

3.2.1 Standard germination test

Seed germination capacity was determined using the standard germination test under laboratory conditions. A completely randomised design was used for the standard germination experiment. Four replicates consisting of 25 seeds of each landrace selection (plain red, plain cream, brown speckled and black speckled) were placed between two layers of double moistened brown paper towel. Paper towels were rolled, tied with elastic bands on both ends and sealed in zip-lock bags to prevent loss of moisture. Thereafter, zip-lock bags were incubated for the duration of 10 days in a germination chamber set at alternating temperatures [20/30°C day/night (16 hr. day/8 hr. night)]. Germination was assessed daily by counting seeds with radicle protrusion of at least two mm daily. Final germination (total number of germinated seeds) was determined on the tenth day. Following this, germination vigour characteristics of seedling, root and shoot lengths as well as fresh and dry mass were determined. Seedling size was determined by measuring whole, shoot and root (mm). In addition, fresh and dry mass were measured using a sensitive balance (Masskot, FX320, Switzerland). For measurement of dry mass, seedlings were oven-dried for 72 hours at 80ºC (ISTA 2011).

3.2.2 Germination velocity index (GVI)

In order to assess seed vigour, germination velocity index (GVI) (germination speed) was calculated based on Maguire's (1962) formula:

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

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.

3.2.3 Mean germination time (MGT)

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

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

where;

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

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

3.2.4 Electrolyte conductivity

In order to determine the amount of solute leakage from seeds (µs/gram/hour), electrolyte conductivity (EC) of seeds was determined using the CM100-2 EC Meter (Reid and Associates CC, South Africa). Twenty seeds of each landrace selection were individually weighed and put into separate wells. Thereafter, the wells were filled with 2 ml of distilled water and seed electrolyte conductivity was recorded over 24 hours.

3.2.5 Imbibition test

Two imbibition methods (seed testing water bath and the seed soaking) were used to determine the rate of water uptake of each landrace selection.

3.2.5.1 Seed testing water bath method

The imbibition test was done on a seed testing water bath (Grants Instruments, England). Five seeds per landrace selection were placed in a completely randomized design experiment with three replications per seed colour. Seeds were arranged on a Whartman® (Whartman International Ltd Maidstone, England) paper on glass and covered with plastic funnels. Seeds were allowed to imbibe for 0 (control), 30, 60, 120, 240, 480, 960, 1 920, 3 840, 7 680 minutes. Initial and final (after imbibition) seed mass was measured at each time interval. Water activity (a_w) of the same five seeds per landrace selection, per replication used for the imbibition test, was also measured at each time interval using the AquaLab CX2 Water Activity Meter (Decagon Devices, USA).

3.2.5.2 Seed soaking method

Ten seeds per landrace selection, replicated three times, were weighed and immersed in 150 ml of distilled water in a glass flask. This was repeated every hour, for the next 11 hours with a new batch of ten seeds in a new beaker filled with same volume of distilled water. On the 12^{th} hour, water was poured out, each batch of seeds was briefly dried on paper towels to remove surface unabsorbed water. Seeds were re-weighed and final mass recorded (Hershey 2010). Water activity (a_w) of five seeds per landrace selection, per replication per imbibition interval was measured using the AquaLab CX2 Water Activity Meter (Decagon Devices, USA)

For both methods percentage change in seed mass during imbibition was determined using

$$\%\text{change in mass} = [(\text{Final mass} - \text{Initial mass})/\text{Initial mass}]*100 \qquad \textbf{Equation 3.3}$$

3.2.6 Seed coat thickness

To determine the effect of seed coat thickness on imbibition and electrolyte leakage, seed coat thickness was measured using a Zeiss EVO Scanning Electron Microscope (SME) (Zeiss, Germany). Three seeds of each landrace selection (were cryo–fractured in liquid nitrogen and split into two halves. Following this, seeds were mounted onto stubs and secured using a two way insulating tape. Thereafter, seeds were gold-coated using an ion coater (Eiko 1B.3) and viewed under the Zeiss EVO Scanning Electron Microscope (SME) (Zeiss, Germany) in high vacuum mode. Images were captured on the scanning microscope and seed coat thickness was measured using image analysis software -analySIS- soft imaging system (Olympus Germany®).

3.2.7 Seed coat mineral composition

In order to evaluate the effect of seed mineral composition on imbibition and germination, seed mineral proportion of different atomic number elements was evaluated under Zeiss EVO Scanning Electron Microscope using Energy Dispersive X-Ray Spectrotomy (EDX) technique. Three seeds of each landrace selection were cryo–fractured and split into two halves. Seeds were mounted onto stubs and secured using a two way insulating tape, and

viewed under the Zeiss EVO Scanning Electron Microscope (SME) (Zeiss, Germany) in variable pressure mode. Seed coat mineral composition was determined separately for the seed coat and cotyledon structures using INCA software (ETAS Group) which is linked to the scanning microscope.

3.3 Controlled Environment Experiments

3.3.1 Description of controlled environment

The experiment was conducted in a growth tunnel at the University of KwaZulu-Natal's Controlled Environment Facility (CEF). The environmental conditions in the tunnel were 33/27°C (day/night) temperatures, 60-82% relative humidity (RH) and natural day length; the environment in the tunnels is representative of a warm tropical climate (Modi 2007).

3.3.2 Experimental design

Seedling establishment was done using 128 unit seedling trays. The seedling trays were filled with a silt–loam soil (Table 3.1). The experimental design was a factorial experiment consisting of two factors: water regimes and bambara groundnut landrace selections laid out in a split-plot design. The experiment was replicated four times. Water regimes were the main factor while bambara groundnut landrace selections were the sub-factors arranged in a randomized complete block design. There were three water regimes: 25%, 50%, and 75% (control) of field capacity (FC). The four bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) were as described in Section 3.1. One seed was planted per planting station. After planting, all trays were watered up to 75% of field capacity for ten days to ensure maximum emergence. Thereafter, the different watering regimes were imposed by allowing soil water depletion until trays had attained their corresponding field capacities. Three extra trays that were treated the same as experimental trays were also established for the purposes of destructive sampling.

Table 3.1: Physical and chemical characteristics of soil used during seedling establishment (Department of Agriculture and Environmental Affairs; Soil Analytical Services, Pietermaritzburg)

FC	pH	P	K	Ca	Mg	Zn	Mn	Cu
(%)	(KCl)	——————————————(mg L^{-1})——————————————						
34	5.75	3	347	859	214	1	31	4.8

3.3.2.1 Watering regimes

Field capacity of the media was determined following the gravimetric field capacity test. Three small drained pots (representing three reps) were used. Each pot was filled with media. Thereafter, water was added to the pots until saturation was achieved. Pots were then left to drain for 12 hours and thereafter mass of soil was measured hourly until a constant mass was reached. At this point it was assumed that the soil was now at field capacity. Following this, the soil was taken out, put it in labelled brown paper bags and the wet mass of the soil determined. Thereafter, brown bags with soil in them were put to dry in an oven set at 80°C for 72 hours after which dry mass of the soil was measured. Gravimetric field capacity (FC) was then calculated as follows:

$$\theta m = \left(\frac{\theta w - \theta d}{\theta d}\right) x\ 100\% \qquad \textbf{Equation 3.4}$$

where: θ_m =gravimetric field water capacity,

θ_w = wet mass of soil, and

θ_d = dry mass of soil.

Water was applied based on gravimetric water content (1 g = 1 mℓ) every two days by weighing trays to determine the amount of water used by the plants and then refilling the trays to their corresponding field capacities. Water added at each irrigation event was recorded in order to determine water use at the end of the experiment. Irrigation was withdrawn in all the

trays, except the control, at 21 days after planting (DAP) until termination of the experiment (at 27 DAP).

3.3.3 Data collection

3.3.3.1 Growth and physiology

Visual counts of emerged seedlings were taken daily from one DAP up to ten DAP. A seedling was considered to have emerged when the cotyledon had fully emerged. Mean emergence time was calculated using the formula by Bewley and Black (1994):

$$MET = \frac{\sum(fx)}{\sum f} \qquad \textbf{Equation 3.5}$$

where: MET = Mean emergence time,

F = number of newly germinating seeds at a given time (day), and

x = number of days from date of sowing.

Thereafter, seedling height was measured weekly (from seven DAP) until the end of seedling establishment (21 DAP). Seedling height was measured using a 30 cm ruler from the base of the main stem to the base of the longest stem. Seedling leaf number was determined weekly (from 14 DAP up to 21 DAP) by visually counting leaves that were fully expanded and with more than 50% green leaf area. Each trifoliate was counted as one leaf. Following that, three seedlings per replication per landrace selection were destructively sampled and total leaf surface area per seedling was measured using an LI-3000C Portable Leaf Area Meter connected to a LI-3050C Transparent Belt Conveyer (LI-COR®, USA). Upon termination of the experiment (at 21 DAP), seedling parameters (seedling length, root and shoot length, root: shoot ratio and seedling dry mass) were determined.

Chlorophyll content index (CCI) was measured weekly (from seven DAP up to 21 DAP) using the SPAD-502Plus Chlorophyll Meter (Konica Minolta, USA) on the adaxial surface of fully expanded, fully exposed and actively photosynthesizing leaves. In order to determine plant photosynthetic efficiency, chlorophyll fluorescence (CF) was measured weekly (from seven DAP up to 21 DAP) using a Pocket PEA-Chlorophyll fluorescence system (Hansatech Instruments, United Kingdom). Chlorophyll fluorescence was measured on the adaxial

surface of young, fully expanded and fully exposed green leaves. Before measuring CF a sample area of the targeted leaf was covered with a lightweight leaf clip (Hansatech Instruments, UK) for 20 minutes to exclude light and allow for dark adaptation. Weekly measurements of CCI and CF were taken before an irrigation event. After 21 DAP, when irrigation was withdrawn (except the control), measurements of CCI, and CF were taken daily until 27 DAP when the experiment was terminated. Measurements of CCI, and CF were routinely taken during midday.

On the 21st day after planting, before irrigation was withdrawn, measurements of pre–dawn leaf water potential (PDLWP) and midday stem water potential (MSWP) were taken using the 3005F01 Portable Plant Water Status Console (Soilmoisture Equipment Corp, USA). Measurements were taken on a healthy, mature, fully exposed terminal leaf. A random order of destructive sampling within each watering regime and among landrace selections was followed for each replication. Pre–dawn leaf water potential readings were taken from 3.30 am to 5 am (before sunrise) based on the assumption that before sunrise the plant is at equilibrium with soil water potential hence PDLWP being a more sensitive indicator of soil water availability (Ameglio *et al*. 1999). At midday stem water potential was preferred because it has been shown to be less susceptible to fluctuations in environmental pressures than leaf water potentials and hence more representative of actual level of stress (McCutchan and Shackel 1992, Chone *et al*. 2001, Williams and Araujo 2002). Midday stem water potential was measured between 12 noon and 1.30 pm.

Before cutting, the selected leaf was wrapped with a moist cloth and secured using cling wrap to minimize further transpiration of the shoot which alters the resultant pressure reading. Thereafter, the leaf petiole was cut using a surgical blade; a uniform length of 5 cm per cut petiole was maintained. The petiole was then quickly placed through the chamber lid and secured tightly with the excised edge of the petiole facing outside and the bagged leaf inside the chamber. The chamber was sealed and then slowly pressurized with nitrogen gas at 10 kPA s^{-1}. During pressurisation, a *x*16 magnifying glass was used to carefully observe the excised surface of the petiole for the appearance of a drop of water (sap). As soon as the drop appeared the corresponding pressure on the chamber gauge was recorded and leaf water potential was expressed as the negative of the corresponding pressure. For MSWP the same procedure as above was followed except that the targeted leaves were covered with a static shield bag (Soilmoisture Equipment Corp, USA) 2 hours prior to measurements being taken.

3.3.3.2 Metabolic responses

In order to determine metabolic responses of bambara groundnut during soil water depletion, starting from 21 DAP, irrigation was withdrawn in all trays except the control. Plant leaf tissue was sampled destructively daily at midday for laboratory analysis of plant metabolites associated with stress acclimation (total antioxidant capacity, total phenolics and total sugars).

3.3.3.2.1 Determination of total antioxidant capacity

Total antioxidant capacity in leaf tissue of bambara groundnut landrace selections was determined using the ferric reducing ability of plasma (FRAP) assay as described by Benzie and Strain (1996). 0.1 g of ground freeze dried leaf tissue was mixed with 5 mℓ 1N perchloric acid was added and homogenised using an Ultra-Turrax (Model T25D, IKA, Germany) for 30 s. The homogenate was centrifuged at 5 000 g for 10 min at 4°C using a SORVALL® RC 5C centrifuge (Sorvall, Newtown, CT, USA). 900 µℓ freshly prepared FRAP reagent was pipetted into cuvettes. 30 µℓ of sample solution was added into the 900 µℓ FRAP and mixed. Cuvettes were incubated for 10 min making sure each cuvette reacts the same period of time. Absorbance was then recorded at 593 nm using a UV–1800 UV–Vis spectrophotometer (Shimadzu, North America). A concentration of $FESO_4$ was in turn plotted against concentrations of the standard antioxidants.

3.3.3.2.2 Determination of total phenolics content

Total phenolics in leaf tissue of bambara groundnut landrace selections were determined using the method of Pérez-Conesa *et al*. (2009). 0.1 g sample of freeze-dried leaf tissue was mixed with 1 mℓ of 1 M HCL, vortexed for 1 min and incubated at 37°C for 30 min. After incubation, 1 mℓ NaOH (2 M in 75% methanol) solution was used for alkaline hydrolysis and the resulting mixture vortexed for 1 min and incubated at 37°C for 30 min. The samples were vortexed before mixing with 1.0 mℓ of 0.75 M metaphosphoric acid and centrifuged at 5 000 rpm (2 510) for 10 min. The supernatants were collected, transferred into a 10 mℓ volumetric flask and the pellets re-suspended in 1.0 mℓ of acetone: water (1:1, v/v), vortexed for 1 min and centrifuged at 5 000 rpm (2 510) for 10 min. Both extracts were combined and made up to 10 mℓ with acetone: water (1:1, v/v). Total phenolics were determined using Folin-Ciocalteu reagent. Briefly, 5 mℓ of nanopure water, 1 mℓ of sample and 1 mℓ of Folin-Ciocalteu reagent were added to a 25 mℓ volumetric flask and allowed to stand for 5-8 min at room temperature. Thereafter, 10 mℓ of a 7% sodium carbonate solution was added, followed by the addition of

8 mℓ of nanopure water placed to volume. The solution was vortexed thoroughly and allowed to stand at room temperature for 2 h before being filtered through a Whatman® 0.45 µm poly filter prior to determination of total phenolics at 750 nm absorbance using a UV–1800 UV–Vis spectrophotometer (Shimadzu, North America). Gallic acid monohydrate was used as the standard to prepare the calibration curve. The results were expressed as milligrams of gallic acid equivalents (GAE) per g DM of leaf tissue.

3.3.3.2.3 Total soluble sugars determination

For total soluble sugars determination 5 g of freeze dried plant samples were sent to Cedara Feed Laboratory, Pietermaritzburg. Samples taken at 21 (the last day of watering), 24 (3 days after withdrawing irrigation) and 27 DAP (6^{th} day after withdrawing irrigation and day of termination) were submitted for analysis.

3.3.4. Seedling water use efficiency

The amount of water used (WU) to establish seedlings across the different watering regimes was converted from mℓ to mm (depth) using conversion factors described by Allen *et al*. (1998) where;

$$1 \text{ mm day}^{-1} = 10 \text{ m}^3 \text{ ha}^{-1} \text{ day}^{-1} \qquad \textbf{Equation 3.6}$$

At the end of seedling establishment (21 DAP), seedling biomass was also determined. Using these measurements, seedling water use efficiency was then determined as follows:

$$\text{Seedling WUE} = \text{Seedling biomass} / \text{Water use} \qquad \textbf{Equation 3.7}$$

where: WUE = water-use efficiency in g mm^{-1},

Biomass = seedling biomass (above and below ground) in g, and

Water use = amount of water (in mm) applied to the respective seedling trays.

3.4 Field Trial

3.4.1 Site description and experimental design

A field trial was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 775 m.a.s.l.) during the summer planting season of 2013/2014. Ukulinga represents a semi-arid environment and is characterised by clay-loam soils (USDA taxonomic system). Average annual rainfall is about 694 mm received mainly during the summer months (mid-October to mid-February). Weather parameters were monitored by an automatic weather station (AWS) (ARC – Institute for Soil, Climate and Water) situated within a 100 m radius of the trials.

The experimental design was a factorial experiment laid out in a split-plot design, replicated three times. Water treatment [full irrigation (IRR) vs rainfed (RF)] was the main factor, with landrace selections (plain red, plain cream, brown speckled and black speckled) as sub-factors arranged in a randomised complete block design. Therefore, the treatment structure was (2*4) replicated three times. Main plots (IRR and RF) measured 81.4 m^2 each, with 10 m spacing between them to prevent water sprays from reaching RF plots. Sprinklers were designed to have a maximum range of 6 m radius. Sub-plot size was 3.6 m^2. Plant spacing was 0.45 m between rows and 0.2 m within rows translating to 55 plants per sub-plot, 27 plants being experimental. During the growing season (18 November, 2013 — 16 March, 2014), 347.5 mm of rainfall was received and supplementary irrigation was scheduled to apply 35 mm per week in the irrigated plot.

Initially, bambara groundnut seeds were planted in seedling trays and established in growth tunnels. Four weeks old seedlings were transplanted in both trials. In order to ensure seedling survival and maximum crop establishment, light irrigation was applied in both trials for one week after transplanting, thereafter irrigation was withdrawn from the rainfed treatment.

3.4.2 Crop management

Land preparation involved disking and rotovating the fields to achieve a fine seedbed. Prior to planting, soil samples were taken and submitted for soil textural and fertility analyses. Gravimetric field capacity of the soil was determined prior to planting. Results of soil fertility analysis revealed that there was no need for fertiliser application to meet bambara groundnuts requirements for macro and micro-nutrients. Therefore, no fertiliser was applied. Plants were

sprayed with Kemprin (Cyphermethrin) at 20 mℓ 10 ℓ^{-1} against cutworm. Weeding was done by hand-hoeing. Ridging commenced eight weeks after transplanting; re-ridging was done thereafter as part of routine weeding.

3.4.3 Yield and yield components

At harvest yield and yield components were measured. These included total biomass per plant (fresh and dry), pod number per plant, number of seeds per pod and the pod mass per plant (fresh and dry). Harvest index was calculated as;

$$\frac{\textbf{Pod dry mass (g)}}{\textbf{Total biomass (g)}} \qquad \textbf{Equation 3.8}$$

3.4.4 Plant material, Standard Germination Test, Germination vigour characteristics, Electrolyte conductivity test, Seed coat thickness, Seed mineral composition

Seeds harvested from the field trial [plain red, plain cream, cream with brown speckles (brown speckled) and cream with black speckles (black speckled)] were subjected to seed quality tests following the same procedures as in section 3.2.1, 3.2.2, 3.2.1, 3.2.4, 3.2.6, 3.2.7. Initial seed characterisation was done to obtain 100 grain mass of all seed lots (Table 3.2).

Table 3.2: One hundred grain mass (g) for the bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) harvested from the rainfed and irrigated trial.

Landrace selection	Rainfed	Irrigated
Plain Red	51 g	47 g
Plain Cream	61 g	57 g
Brown sp	48 g	44 g
Black sp	49 g	46 g

3.5 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using GenStat® Version 16 (VSN International Ltd, UK) at the 5% level of significance (Appendix). Means of significantly different variables were separated using the Duncan's multiple range test in GenStat® at the 5% level of significance.

CHAPTER 4

SEED QUALITY CHARACTERISTICS OF A BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* L.) LANDRACE DIFFERING IN SEED COAT COLOUR

4.1 Introduction

Bambara groundnut (*Vigna subterranea* L.) is an underutilised grain legume, popularly grown by women subsistence farmers in sub-Saharan Africa (Azam –Ali *et al*. 2001). Bambara groundnut is cultivated using landraces which are mainly genetically heterogeneous and retained from farmers' previous harvest (Hillocks *et al*. 2011). Successful production of bambara groundnut is dependent upon availability of good quality seed. To a seed scientist, good quality seed would be the sum of many differing components including genetic quality, physical purity, germination, vigour, uniformity in size, and freedom from seed-borne diseases (McDonald and Copeland 1997, Basra 2005). To a farmer, good quality seeds possess all the physical, physiological, pathological and genetic attributes that contribute to final yield (Basra 1995). Physiological qualities (viability and vigour) refer to aspects of seed performance that are related with emergence and early crop establishment (Tekrony and Egli 1991). It is important to evaluate seed quality before planting, especially in the case of landraces where seed quality is often unknown.

Viable seeds are those that are alive and have the potential to germinate when exposed to favourable germination conditions (Basra 1995, McDonald and Copeland 1997). Desai (2004) defined germination as emergence of embryo from the seed by starting a variety of metabolic activities, including respiration, protein synthesis and mobilisation of food reserves after it has absorbed water. Imbibition is the initial and essential process that sets in motion metabolic events essential for germination to occur (Finch–Savage and Leubner–Metzeger 2006). The kinetics of imbibition are influenced by a number of factors including seed structure, seed coat thickness and hydration matrices (Nonogaki *et al*. 2010, Modi 2013). When water is imbibed into the seed the continuum of its energy states, referred to as the water activity also influences the germination process. The three states of water are; a strongly bound monolayer where the water is tightly bound to other molecules, less strongly bound multilayer where water is deposited on the more tightly bound water and the free water which contributes to early germination events (McGill 2012). Vigour is that quality of the seed responsible for rapid, uniform germination, increased storability, good field emergence and ability to perform

over a wide range of field conditions (AOSA 1980, ISTA 2011). A vigorous seed lot is one that is potentially able to perform well even under sub-optimal environmental conditions (Basra 2005). Speed of germination (Maguire 1962) and dry mass of seedlings (Burris and Black 1976) were initially suggested as indices for evaluating seed vigour. Additional vigour tests have since been developed to better predict seedling emergence under a wide range of field conditions (Copeland and McDonald 2001). Measurement of electrical conductivity of leachates provides an assessment of the extent of electrolyte leakage from seeds and is a method of seed vigour testing widely used in seed laboratories (ISTA 2011). Poor seed quality (viability and vigour) results in uneven or erratic emergence, hence reduced crop stand and ultimately reduced crop yield; this has deleterious effects from which farmers may fail to recover (Mabhaudhi 2009).

Poor crop stand in bambara groundnut has been reported by several authors (Sinefu 2011, Legwaila *et al*. 2013, Zondi 2013, Mabhaudhi and Modi 2013). Poor emergence below 30% was observed by Legwaila *et al*. (2013), although high emergence (>80%) has also been reported (Sesay 2009, Mabhaudhi *et al*. 2013, Ogbuehi *et al*. 2013). Time taken by bambara groundnut to 50% emergence is between 35 days (Mabhaudhi and Modi 2013) and 55 days after sowing (Makanda *et al*. 2009). Bambara groundnut is slow to establish compared to other grain legumes; cowpea (Ntombela 2013) and soybean (Liu 2004) take an average of seven days after sowing to emergence. Sinefu (2011) observed that a combination of low emergence and late establishment led to a poor crop stand. This makes bambara groundnut unattractive for commercial cultivation, and could be partly the reason for low acceptance by farmers (Mabhaudhi and Modi 2013). Differences in bambara groundnut establishment results are attributed to variability in physical, physiological and genetic seed quality characteristics of different bambara groundnut landraces.

Studies on bambara groundnut landraces have suggested that dark coloured seeds maybe more vigorous than light coloured seeds (Sinefu 2011, Mabhaudhi and Modi 2013, Zondi 2013). However, the genetics of seed coat colour in bambara groundnut have not yet been explored and variations in seed coat colours may not necessarily infer genetic differences (Mabhaudhi and Modi 2013). These studies evaluated only plain colours, but this study sought to investigate speckling in bambara groundnut seeds. It was hypothesised that speckling and colour of speckles has no effect on seed quality of bambara groundnut. The aim of the present study was to determine seed quality characteristics (germination and vigour) of a bambara groundnut landrace separated according to four distinct seed coat colours (plain red, plain cream, brown speckled and black speckled).

4.2 Results

4.2.1 Standard Germination test

There were highly significant differences (P<0.001) between landrace selections with respect to daily percentage germination (Figure 4.1). The interaction between landrace selections and time was, however, not significant (P>0.05). The highest final germination (87%) was observed in the black speckled seeds, while the lowest final germination (67%) was observed in the plain cream landrace selection. Final germination of 77% and 80% was observed in the brown speckled and plain red seeds, respectively. For all landrace selections, results showed that, on average, germination commenced at four days with 50% germination being achieved by day six (Figure 4.1).

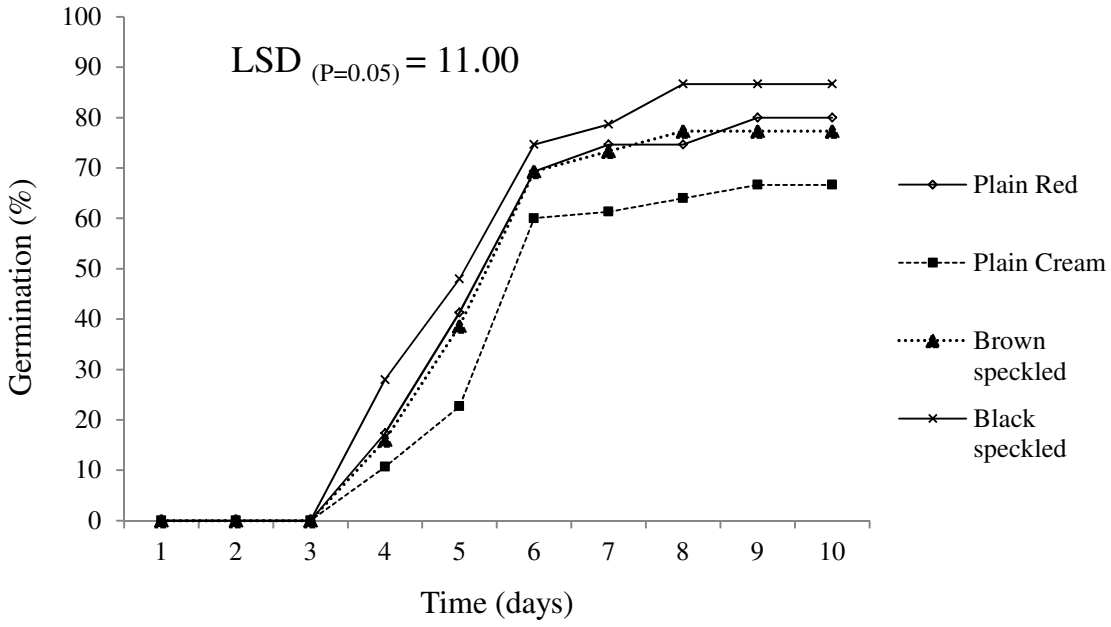

Figure 4.1: Daily percentage germination of the different landrace selections (plain red, plain cream, brown speckled and black speckled) as observed in the standard germination test.

4.2.2 Germination vigour characteristics

There were no significant differences between bambara groundnut landrace selections with respect to (mean germination time) MGT, seedling length, root length and root: shoot ratio (Table 4.1). Results of shoot length and germination velocity index (GVI) showed significant differences ($P<0.05$) among landrace selections (Table 4.1). Black speckled landrace selection was shown to have the longest shoots (33.00 mm), followed by brown speckled and plain red landrace selection (30.60 mm and 31.30 mm, respectively); plain cream landrace selection had the shortest shoots of (20.00 mm) (Table 4.1). Black speckled landrace selection had the highest GVI (17.02) while the plain cream landrace selection had the lowest GVI (11.73).

There were highly significant differences ($P<0.001$) among bambara groundnut landrace selections with respect to dry mass (Table 4.1). Mean separation showed that the plain cream landrace selection had the highest and significant dry mass (2.52 g); dry mass of plain red, brown and black speckled landrace selections was statistically similar. Results of seedling fresh mass showed significant differences ($P<0.05$) across the landrace selections (Table 4.1). Similar to results of dry mass, the highest fresh mass (4.02 g) was recorded for the plain cream landrace selection while the lowest fresh mass (3.22 g) was recorded for the black speckled landrace selection. Fresh mass for the brown speckled and plain red landrace selections was 3.56 g and 3.50 g, respectively.

4.2.3 Electrolyte conductivity

Results of the electrolyte conductivity showed highly significant differences ($P<0.001$) between landrace selections, time and the interaction between the two factors (Fig 4.2). The highest electrolyte conductivity was measured in the brown speckled landrace selection while the plain cream landrace selection showed the least electrolyte conductivity. Measurements over the 24 hr period showed that the plain cream landrace selection leaked 36 µs/g compared with more than 1400 µs/g measured for the brown speckled landrace selection. Black speckled and plain red landrace selections leaked 701 µs/g and 830 µs/g respectively over the same period.

Table 4.1: Seedling parameters of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) under the standard germination test.

Seed coat colour	[u]GVI	[v]MGT (Days)	Seedling length	Shoot length	Root length	Root: shoot ratio	Fresh mass	Dry mass
			----------(mm)----------				--------(g)--------	
[w]PR	15.26a	7.63a	94.00a	31.30a	63.00a	1.97a	3.50b	1.82c
[x]PC	11.73b	7.77a	70.00a	20.00b	50.00a	2.63a	4.02a	2.52a
[y]Br sp	14.77a	7.59a	93.00a	30.60ab	62.00a	2.05a	3.56ab	1.85b
[z]Bl sp	17.02a	7.47a	134.00a	33.00a	101.00a	2.99a	3.22b	1.72c
LSD	**4.12**	**0.24**	**78.90**	**7.81**	**75.50**	**1.87**	**0.35**	**0.11**

[u]GVI = Germination velocity index; [v]MGT= Mean germination time; [w]PR=Plain Red; PC= [x]Plain Cream; [y]Br sp = Brown speckled; [z]Bl sp = Black speckled. Means in the same column with different letters differ significantly at LSD $_{(P=0.05)}$.

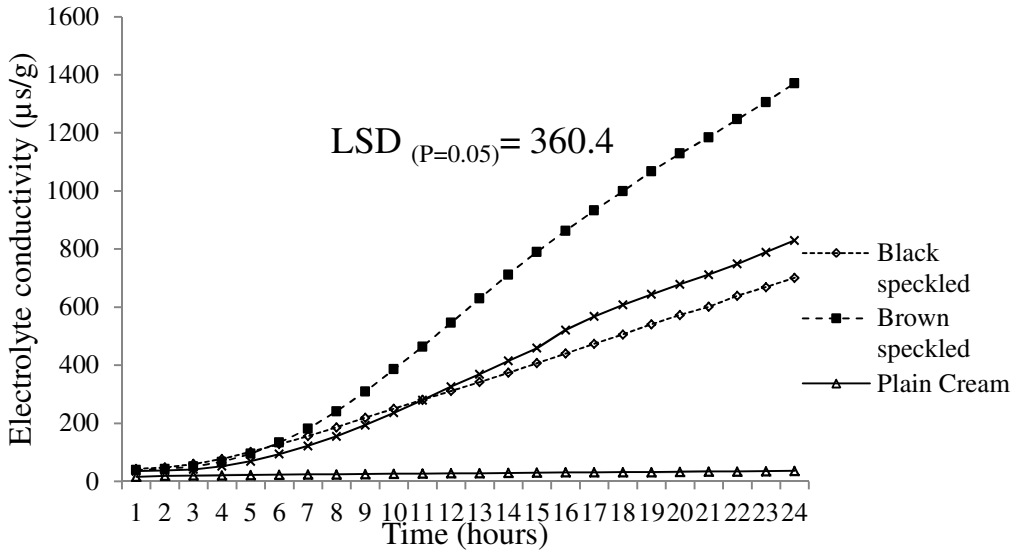

Figure 4.2: Electrolyte conductivity of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) measured hourly for 24 hours.

4.2.4 Seed imbibition

4.2.4.1 Seed imbibition: seed testing water bath method

Results of the imbibition test using the water bath method showed highly significant differences ($P<0.001$) between landrace selections, imbibition time and their interaction (Fig 4.3). A substantial increase (> 3%) in seed mass was observed after 480 minutes for the plain red seeds and 960 minutes for the brown speckled and plain cream landrace selection. The black speckled landrace selection showed an increase in mass between 60 and 120 minutes. Curiously, their seed mass decreased thereafter only to increase again after four hours. A linear increase was observed after 960 minutes (Fig 4.3). The lowest final increase in mass (40.69%) was observed in the plain red landrace selection while the highest change in mass (50.53%) was observed for the plain cream landrace selection.

There were highly significant differences ($P<0.001$) between the landrace selections, time and the interaction between the two, with respect to water activity (Fig 4.4). Water activity was shown to fluctuate. Initial seed water activity for all the landrace selections was ~ 0.4 and then went up to ~ 0.9 during the first 30 minutes then came down again to ~ 0.6. It continued fluctuating between 0.6 and 0.9, but fluctuations were steadier at approximately 0.9 after 960 minutes.

4.2.4.2 Seed imbibition: seed soaking method

Results of the imbibition test using the seed soaking method showed highly significant differences ($P<0.001$) between landrace selections, imbibition time and the interaction between the two (Figure 4.5). The change in mass when seeds were soaked in distilled water was fluctuating between 5% and 70% for duration of the experiment. An initial rapid change in seed mass (12.5%) was observed in the plain red landrace selection within the first hour and then fluctuated between 5% and 50% thereafter. However, at the end of the experiment the lowest change in mass (27%) was observed in the plain red landrace selection and the highest change in seed mass (69.83%) was observed in the black speckled landrace selection. The plain cream and brown speckled landrace selections increased in mass by 56% and 47.57% respectively.

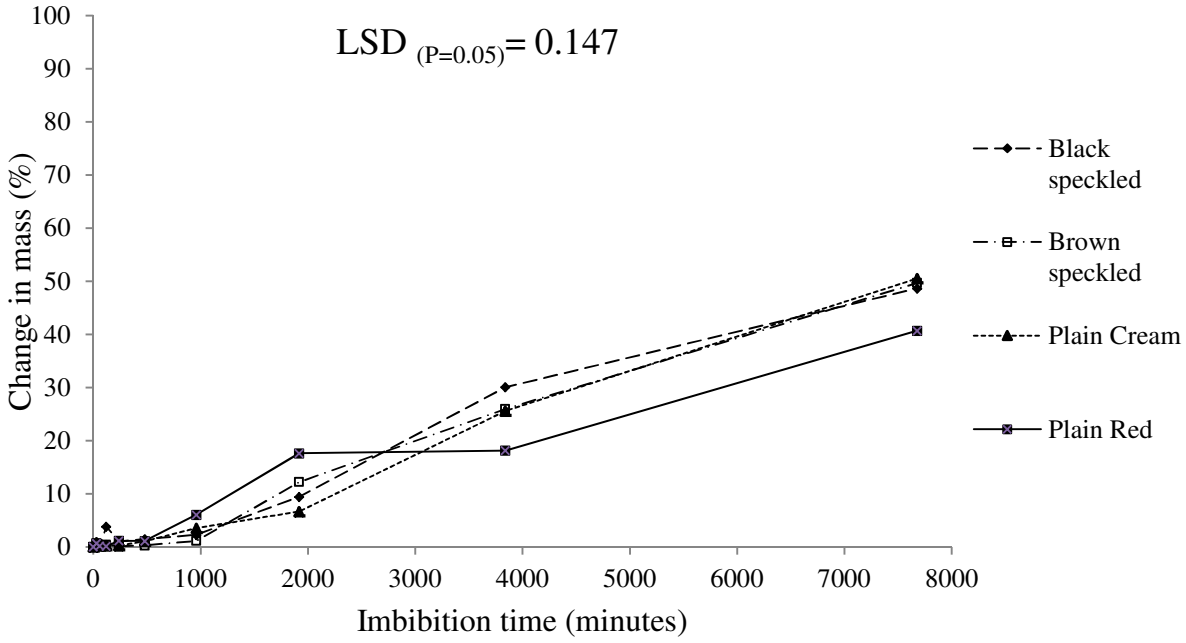

Figure 4.3: Percentage change in mass of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) over a 7680 minutes time period during imbibition on a seed testing water bath.

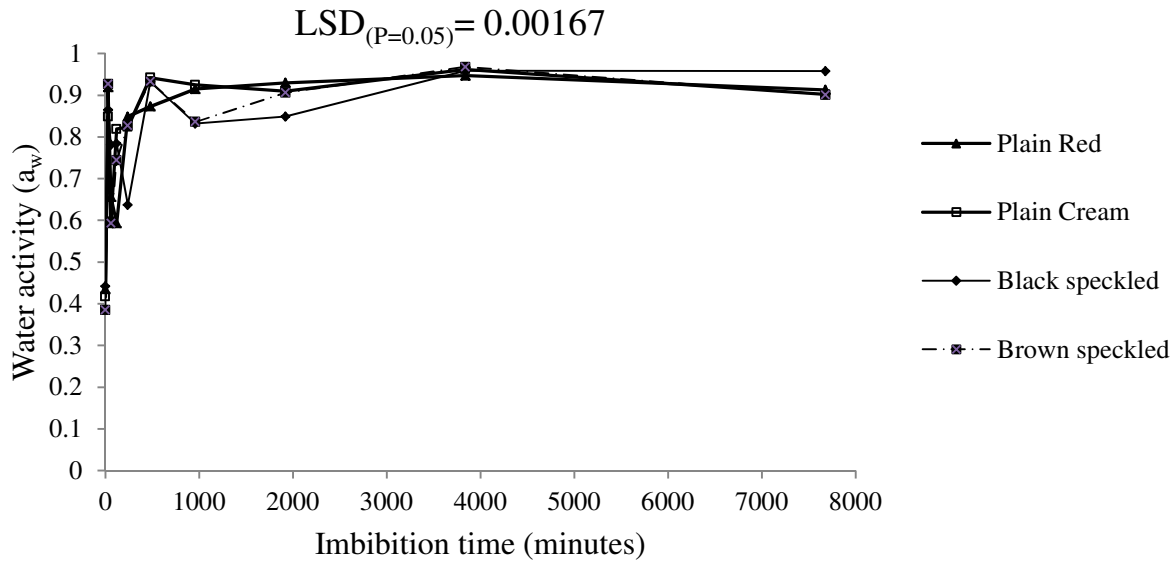

Figure 4.4: Water activity of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) over a 7680 minutes time period during imbibition on a seed testing water bath.

There were highly significant differences (P<0.001) between the landrace selections, imbibition time and the interaction between the two with respect to water activity (Figure 4.6). There was a rapid increase in water activity (from ~ 0.4 to ~ 0.9) within the first hour. Thereafter, the trend was constant, except for the plain cream landrace selection whereby water activity decreased from 0.89 to 0.66 between eight and nine hours before increasing to 0.95 after 10 hours (Figure 4.6).

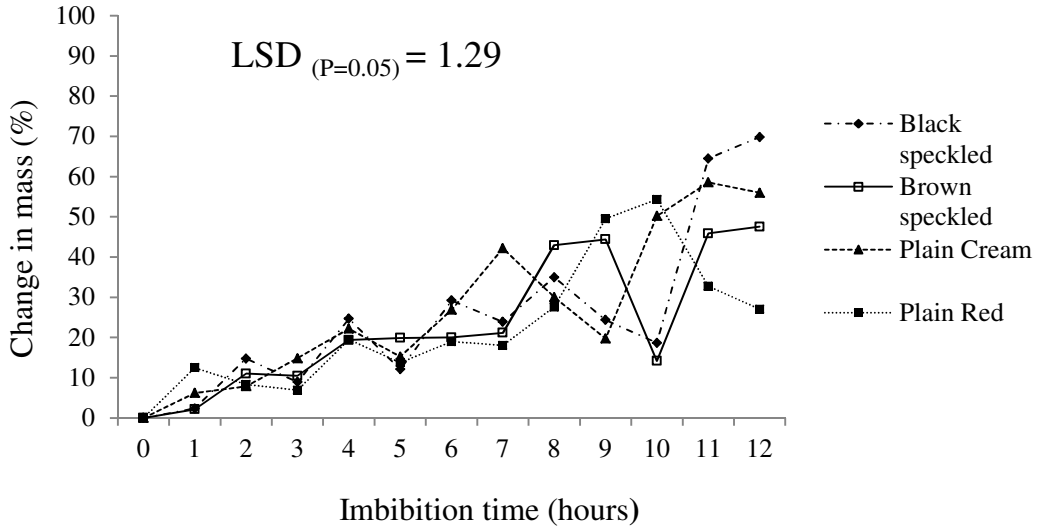

Figure 4.5: Percentage change in mass of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) over a 12 hour time period during imbibition using the seed soaking method.

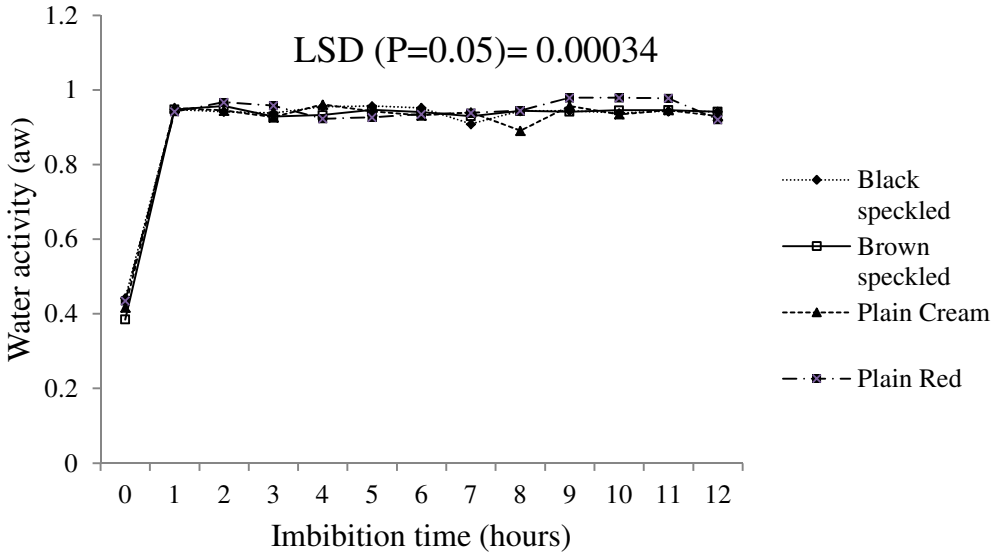

Figure 4.6: Water activity (a_w) of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) in response to imbibition using the seed soaking method over a 12 hour period.

4.2.5 Seed coat thickness

Results of seed coat thickness showed significant differences ($P<0.05$) across landrace selections (Table 4.2 and Figure 4.7). The thickest coat (116.0 μm) was observed in the brown specked landrace selection followed by the plain red landrace selection (111.0 μm) and the thinnest seed coat (107.9 μm) was observed in the plain cream landrace selection (Table 4.2 Figure 4.7).

Table 4.2: Mean seed coat thickness of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) viewed and measured under Scanning Electron Microscope (SME).

Seed coat colour	**Seed coat thickness (µm)**
Black speckled	109.4b
Plain Cream	107.9b
Brown speckled	116.0a
Plain Red	111.0b
LSD $_{(P=0.05)}$	**3.6**

* Means in the same column with different letters differ significantly at LSD (P=0.05).

4.2.6 Seed mineral composition

The seed coat and cotyledon structure had different element weight % across all landrace selections (Table 4.3). Elements such as carbon, oxygen, magnesium, potassium and calcium were present in all landrace selections and in both seed structures. Phosphorous and sulphur were not observed in the plain red seed coat, while iron was observed in the plain red seed coat only. Aluminium was only present in the seed coat of the plain red and brown speckled landrace selections. Chlorine was not present in both structures of the brown speckled landrace selection. Three times more calcium was observed in the seed coat structure of the plain cream landrace selection compared to the other landrace selections (Table 4.3).

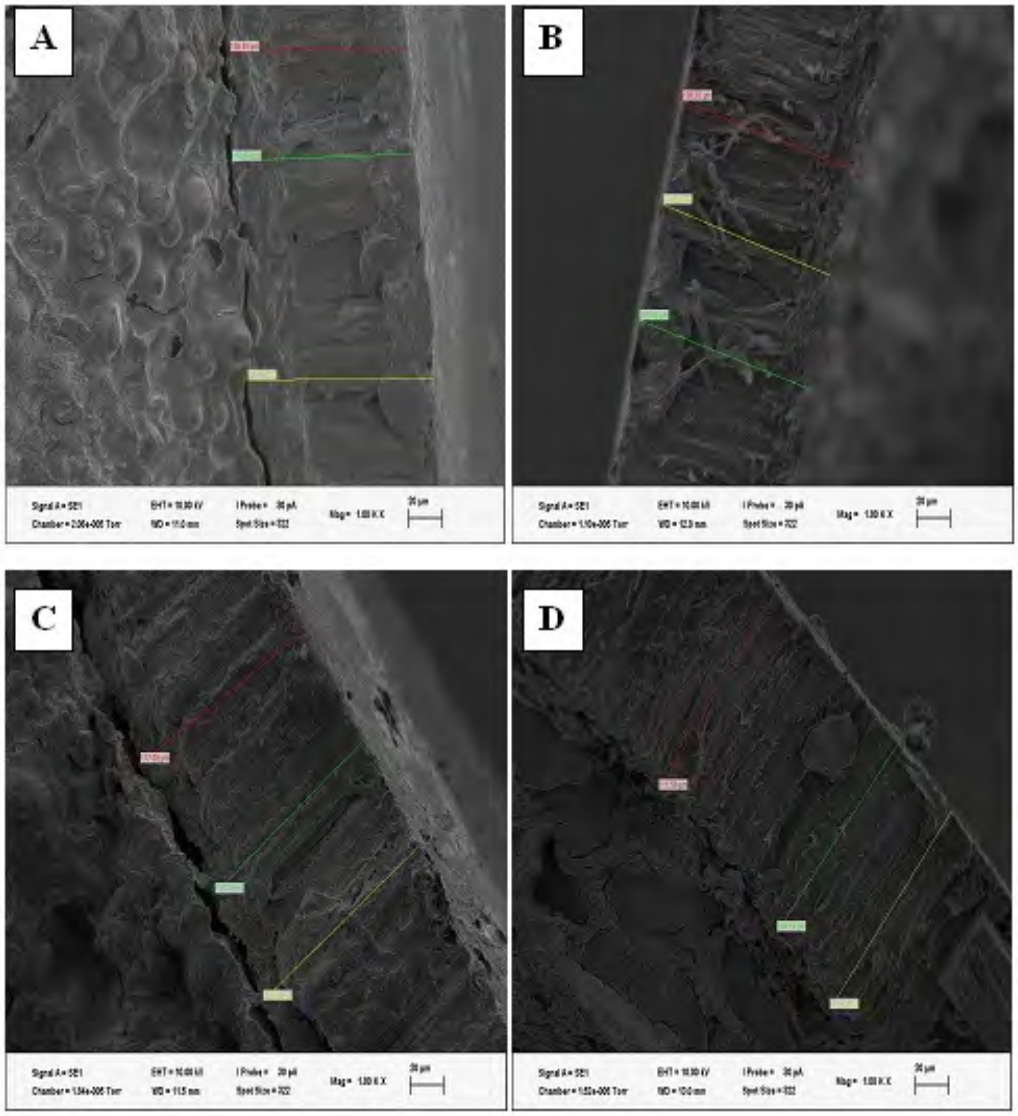

Figure 4.7: Seed coat thickness of (A) black speckled (give mean value), (B) plain cream (mean value), (C) brown speckled (mean value) and (D) plain red (mean value) bambara groundnut landrace selections viewed under scanning electron microscope (SME) at 1000X magnification.

Table 4.3: Seed mineral composition of the seed coat and cotyledon of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) evaluated under Zeiss EVO Scanning Electron Microscope using Energy Dispersive X-Ray Spectrotomy (EDX) technique.

Element (Weight %)	Plain Red		Plain Cream		Brown speckled		Black speckled	
	[x]S C	[y]Cot	S C	Cot	S C	Cot	S C	Cot
Carbon	57.54	59.57	61.55	60.47	61.76	59.67	51.49	59.03
Oxygen	40.60	38.33	35.83	37.15	36.33	38.74	32.06	38.90
Magnesium	0.22	0.42	0.14	0.27	0.25	0.32	0.26	0.44
Phosphorous	-	0.18	0.10	0.26	0.28	0.29	0.18	0.18
Sulphur	-	0.24	0.12	0.22	0.16	0.18	0.18	0.23
Chlorine	0.07	-	0.15	0.23	-	-	0.07	-
Potassium	0.41	0.97	0.63	1.03	0.80	0.52	0.76	0.95
Calcium	0.27	0.29	0.83	0.37	0.13	0.29	0.29	0.27
Silicon	0.18	-	0.16	-	-	-	-	-
Iron	0.10	-	-	-	-	-	-	-
Aluminium	0.60	-	-	-	0.29	-	-	-
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

[x] SC=Seed Coat; [y]Cot =Cotyledon

4.3 Discussion

The objective of the study was to determine the effect of seed coat colour on seed quality of a bambara groundnut landrace. The landrace consisted of plain colours and speckled seeds of different colours. This study aimed at exploring both variations, particularly speckling. The standard germination test is used to determine germination potential of a seed lot, which can then in turn be used to compare seed quality of different seed lots (ISTA 2011). Results of the current study obtained from the standard germination test showed that the plain cream landrace selection had the least germination potential. This suggested that seed viability was lower in the plain cream landrace selection compared to the other landrace selections. Previous studies (Sinefu 2011, Mabhaudhi and Modi 2013, Zondi 2013) that focused on plain bambara groundnut colours reported dark coloured seeds to perform better compared to light coloured seeds. This was related to the tannins present in dark coloured seeds which are polyphenols found in the seeds when exposed to infection, injury, undesirable temperatures and low nutrient activity (Michalak 2006, Mabhaudhi and Modi 2013). In this case, black speckled landrace selection performed better than the other landrace selections, suggesting that speckles could be a useful selection criterion for seed quality in bambara groundnut.

It is interesting to note that the results of seed vigour (dry mass and EC) were contrary to results of viability. Results of viability showed that black speckled landrace selection was more viable than the plain red and plain cream landrace selection. Although the plain cream landrace selection had the lowest germination potential (67%), results of dry mass showed that it had the highest dry mass of all landrace selections. Burris and Black (1976) hypothesized that vigorous seeds were able to synthesize new material and rapidly transfer these new products to emerging embryonic axis, resulting in higher dry mass. Based on this theory alone, the plain cream landrace selection could be more vigorous compared to the other landrace selections. The black speckled landrace selection was more viable and less vigorous based on results of dry mass. Results of electrolyte conductivity (EC) appeared to concur with results of dry mass in that the plain cream landrace selection had the lowest EC compared with other landrace selections. This, again, suggested that the plain cream landrace selection could be more vigorous than the brown and black speckled landrace selections. The results of the current study, with respect to EC, were contrary to the findings by Sinefu *et al*. (2011), who reported that light-coloured seeds had higher EC than dark coloured seeds. Sinefu *et al*. (2011) attributed the high EC to a thin seed coat observed in light-coloured seeds.

In the current study, the plain cream landrace selection had the thinnest seed coat. Interestingly high EC was observed in the brown speckled landrace selection, which also had the thickest seed coat. Based on these results, it cannot be said that there was an association between seed coat thickness and EC as previously alluded by Sinefu (2011). The inconclusiveness of these results suggests that there may be a need to evaluate other parameters when determining seed quality. Modi (2013) suggested the use of seed mineral content as a rapid physiological seed quality test in bambara groundnut. Calcium in legumes was shown to enhance cell wall integrity and reduce rate of water movement (Mazibuko and Modi 2005). The high calcium content observed in the plain cream landrace selection could have led to cell wall integrity, hence reduced electrolyte leakage. On the other hand, low calcium content observed in the seed coat of the brown speckled landrace selection suggests that the high EC may have been related to poor cell wall integrity. Poor cell wall integrity may increase permeability of the cell wall.

Absorption of water (imbibition) is the initial and essential process to set in motion the metabolic events essential for germination to occur. The seed coat regulates the uptake of water by the seed (McDonald *et al*. 1988). The rate of water uptake influences metabolic activities, including respiration, protein synthesis and mobilization of food reserves resulting in the emergence of the embryo (Desai 2004, Tan–Wilson and Wilson 2012). The rate of imbibition was different for the different imbibition methods (seed testing water bath and seed soaking method). The seeds absorbed more water when soaked in distilled water as compared to what was observed with the seed testing water bath method. This was possibly due to the hydration matrixes where the degree of contact of the seed with water influences the imbibition rate (Desai 2004). Similar behaviour was observed for chickpea and maize, where the rate of water imbibition was higher when seeds were soaked in distilled water than when they were planted in moist soil (Rahman *et al*. 2011). The large fluctuations in change in mass when seeds were soaked could be due to influx of water into the cells of dry seeds, particularly to membranes, which led to an immediate and rapid leakage of solutes and low molecular weight metabolites into the imbibition solution (Rahman *et al*. 2011). The argument advanced by Rahman *et al*. (2011) could also explain the fluctuations in mass observed in the black speckled landrace selection on the water bath during the early stages of imbibition.

Seed coat colour significantly affected imbibition rate. The slow imbibition rate and highest final change in mass observed in the plain cream landrace selection could be due to

cell wall integrity and minimal electrolyte leakage. The slow imbibition rate observed for the plain cream landrace selection could explain the low GVI; the rate of water uptake was slow, hence the slow speed of germination. This is because (imbibition) is the initial and essential process to set in motion the metabolic events essential for germination to occur (Basra 2005). The inconsistent behaviour of the landrace selections, during imbibition on the seed testing water bath and when seeds were soaked in distilled water, could be attributed to flooding tolerance of different seed coat colours of bambara groundnut (Sesay 2009).

The measurements of water activity during the imbibition intervals were done to determine the amount of water in the seed available for hydration of other substances (Colas *et al*. 2010). The water activity trends for both imbibition methods used in this study were found to be inconsistent. The fluctuations of water activity during imbibition on the seed testing water bath could have been due to the relatively high proteins in bambara groundnut seeds (16 – 24 %). Proteins are zwitterions in that they exhibit both negative and positive charges that attract the highly charged water molecules (Copeland and McDonald 2001). Garnczarska *et al*. 2007 related high water absorbing capacity of lupine seeds (*Lupinus luteus* L.) to the high protein content in the cotyledon. It can therefore be hypothesized that the hydration of water by the proteins in the cotyledons was higher than the rate of water uptake by the seed, hence the fluctuations observed in Figure 4.5. When seeds were soaked, due to matric potential, seeds absorbed water faster compared to molecules within the seed hence a steady water activity for the duration of the experiment (Figure 4.6). The water activity pattern in soaked bambara groundnut seeds reported in this study was consistent with findings by Sinefu (2011).

This study has revealed that (i) seed coat colour has an effect on seed quality, (ii) speckling in bambara groundnut seeds may be associated with seed quality, and (iii) seed viability may not necessarily imply good seed vigour. Based on the results of this study, the black speckled landrace selection of bambara groundnut was more viable and vigorous compared to the brown speckled landrace selection. Contrary to authors who studied seed quality of other bambara groundnut landraces and found dark coloured seeds to be more vigorous than light coloured ones, this study showed that the plain cream landrace selection was more vigorous than the other three landrace selections based on results of dry mass and electrolyte conductivity. This inconsistent behaviour amongst different landrace selections justifies the need to test seed quality in different bambara groundnut landraces. Results of this study are useful for immediate seed selection by farmers and long term crop improvement for

the seed industry. Future studies should evaluate the genetics of seed coat colour and its variations if this characteristic is to be exploited for future crop improvement. There is a need to validate the phenotypic markers using appropriate DNA or genetic markers. The results of seed coat and cotyledon mineralogy and the lack of adequate literature to discuss them suggests that future research should also focus on them.

CHAPTER 5

WATER USE CHARACTERISTICS OF A BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC) LANDRACE DURING SEEDLING ESTABLISHMENT

5.1 Introduction

Uniform and early crop establishment are intricately related to crop yield (Tekrony and Egli 1991). An uneven crop stand limits the crop's ability to fully utilise resources such as light and water. This leads to yield losses from which farmers cannot recover (Mabhaudhi and Modi 2010). Bambara groundnut is slow to establish (Makanda *et al*. 2009, Mabhaudhi and Modi 2013). This leads to significant non–productive water losses through soil evaporation during the establishment stage (Mabhaudhi 2012); this may have negative implications on total water use. As such, strategies to identify bambara groundnut as a water use efficient crop and promote it in dry areas should also consider understanding water use efficiency (WUE) during seedling establishment. Currently, studies that have determined WUE of bambara groundnut have looked at it wholly with none determining WUE during the establishment stage.

Water use efficiency is the yield of harvested products achieved from water made available to the crop (Blum 2005). Water use efficiency in bambara groundnut has been determined by Azam–Ali *et al*. (2004) and Mabhaudhi *et al*. (2013). They found WUE values ranging between 0.09 and 0.1 kg m^{-3}. These were higher than WUE values of 0.0025 kg m^{-3} (Nageswara Rao *et al*. 1993) and 0.0018 kg m^{-3} (Songsri *et al*. 2013) reported for its exotic counterpart *Arachis hypogaea.* This implies that bambara groundnut is a more suitable crop than *Arachis hypogea* during periods of drought. However, these values are for total crop WUE. With the reported slow establishment of bambara groundnut and significant unproductive water lost during seedling establishment, it can be hypothesized that total crop water use could be improved if crop establishment was improved. An initial step is to quantify water use efficiency at the establishment stage. Water use efficiency has been reported to increase with decreasing water supply (Nageswara Rao *et al*. 1993, Mabhaudhi *et al*. 2013, Songsri *et al.* 2013). Genotypic variation (Nageswara Rao *et al*. 1993, Songsri *et al*. 2013) and seed colour (Mabhaudhi *et al*. 2013) in landraces were also shown to influence WUE.

Several drought tolerance mechanisms were shown to contribute to high WUE in bambara groundnut. These were drought escape (Vurayai *et al*. 2011b, Mabhaudhi and Modi 2013) and avoidance mechanisms (Jorgensen *et al*. 2010, Vurayai *et al*. 2011a, Mabhaudhi and Modi 2013). There is scant information on accumulation of plant metabolites in bambara groundnut in response to drought. Plants accumulate a range of osmotically active metabolites under water stress including total soluble sugars and proline in order to maintain water relations under osmotic stress (Nazarli and Faraji 2011). Vurayai *et al.* (2011a) and Zondi (2013) showed that proline was higher in water stressed plants than non–stressed plants. This indicated that proline played a role in drought tolerance of bambara groundnut. There was, however, no information in the literature on accumulation of soluble sugars during water stress. There was also no literature on bambara groundnut's antioxidant defence systems in response to water stress more so at the seedling stage.

Understanding physiological and metabolic responses of bambara groundnut at the establishment stage will aid in understanding water use characteristics of bambara groundnut and how they influence WUE. Previous research, using plain bambara groundnut seed colours, showed there was an association between seed colour and drought tolerance, with darker coloured bambara groundnut seeds being more drought tolerant compared to light coloured seeds (Sinefu 2011, Mabhaudhi and Modi 2013). Mabhaudhi and Modi (2013) further attributed this to presence of phenolic compounds in dark coloured seeds acting as a defensive mechanism against stress.

This study is a sequel to previous studies (Sinefu 2011, Mabhaudhi and Modi 2013, Mabhaudhi *et al*. 2013, Zondi 2013) and investigates whether there is also an association between colour of speckles in bambara groundnut seeds, drought tolerance mechanisms and water use efficiency. A secondary objective was to determine physiological (chlorophyll content index, leaf water potential and photosynthetic efficiency) and metabolic (total antioxidant capacity, total phenolics and total soluble sugars) responses of bambara groundnut to water stress during the seedling establishment stage.

5.2 Results

5.2.1 Seedling establishment

5.2.1.1 Emergence

Figure 5.1 represents total emergence of landrace selections as all trays were watered to 75% FC to ensure maximum emergence hence watering regime was not a factor. Results of seedling emergence showed highly significant differences ($P<0.001$) among landrace selections, time (DAP) and the interaction between the two. The highest (80%) and lowest (63%) final emergence were observed in the plain red and black speckled landrace selections, respectively. The brown speckled and plain cream landrace selections had final emergence of 73% and 67%, respectively. All landrace selections started emerging five DAP. Results of mean time to emergence showed no differences ($P>0.05$) among landrace selections (Fig 5.2).

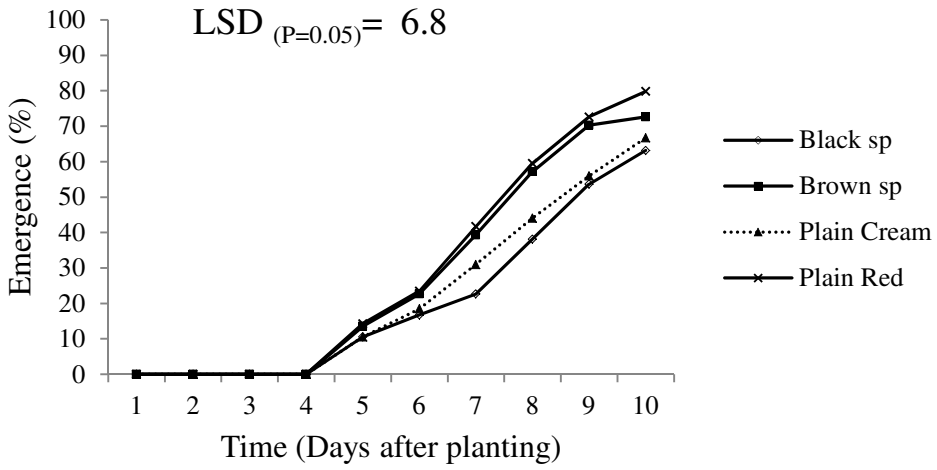

Figure 5.1: Daily emergence of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled). Values are means of landrace selections across water regimes.

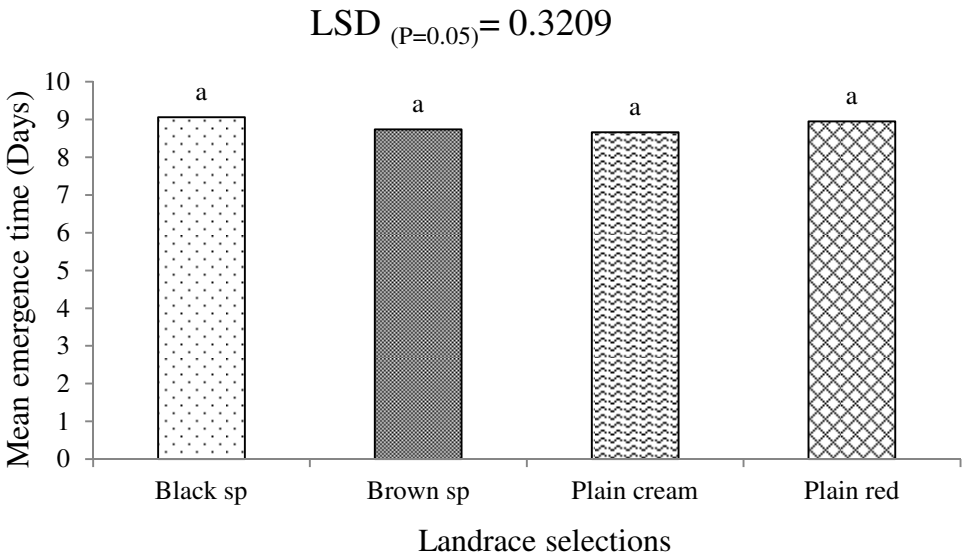

Figure 5.2: Mean emergence time (days) of the bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled). Values are means of landrace selections across water regimes**.**

5.2.2. Growth and physiology

Leaf number showed no significant differences (P>0.05) among landrace selections (Fig 5.3). The differences among watering regimes and time (DAP) were highly significant (P<0.001) (Fig 5.3). The interaction among landrace selections and watering regimes over time was not significant (P>0.05) (Fig 5.3). At the end of the 21 day seedling establishment experiment, the highest leaf number (4) was observed in the 75% FC treatment while the lowest number of leaves (3) was observed in the 25% FC treatment.

Results of seedling leaf surface area were synonymous with results of leaf number (Fig 5.4). Generally, increasing water availability from 25% FC to 50% FC increased leaf surface area by 109%; further increasing water availability to 75% FC increased leaf surface area by 27%. Across all watering regimes and landrace selections, leaf surface area increased with time and the highest leaf surface area (42.31 cm^2) was observed in the non–stressed (75% FC) brown speckled landrace selection, while the lowest (16.43cm^2) was observed in the severely stressed (25% FC) plain cream landrace selection.

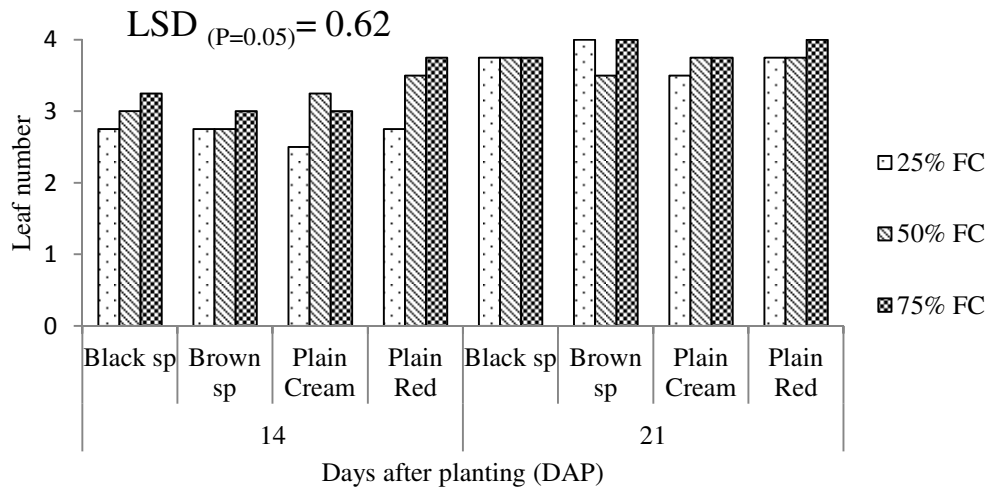

Figure 5.3: Leaf number of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) during seedling establishment under different watering regimes (25%, 50% and 75% FC).

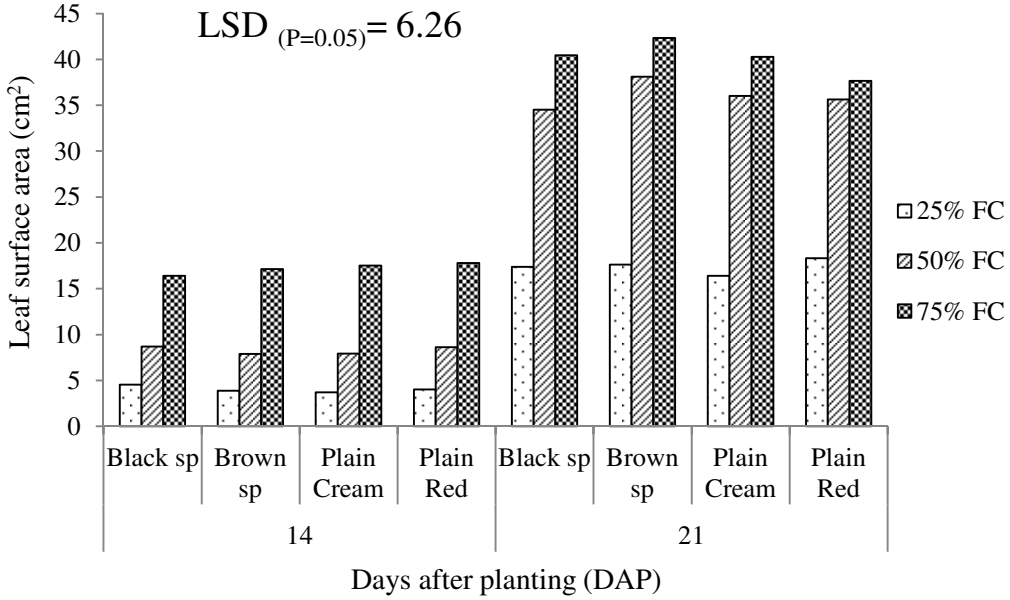

Figure 5.4: Seedling leaf surface area of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) during seedling establishment under different watering regimes (25%, 50% and 75% FC).

There were no significant differences (P>0.05) among landrace selections with respect to plant height (Fig 5.5). The interaction between watering regimes and time (DAP) as well as the interaction among landrace selections and watering regimes over time (DAP) were however highly significant (P<0.001) (Fig 5.5). At the end of the experiment, the tallest seedlings (~17 cm) were observed in the 75% FC treatment while the shortest seedlings (~ 14 cm and ~11 cm) were observed in the moderately (50% FC) and severely stressed (25% FC) watering regimes, respectively. Landrace selections responded differently within the watering regimes but in all watering regimes plant height increased with time (Fig 5.5).

Results of chlorophyll content index showed no significant differences (P<0.05) among landrace selections, watering regimes, time (DAP) and the interaction among the three (Fig 5.6). At 21 DAP, the plain red and brown speckled landrace selections had the highest and lowest CCI, respectively, across all water regimes. Chlorophyll content index did not vary significantly over time.

Chlorophyll fluorescence varied significantly (P<0.05) among landrace selections (Fig 5.7). Watering regimes showed no significant differences (P>0.05) (Fig 5.7). The interaction between landrace selections and watering regimes over time was not significant (P>0.05) (Fig 5.7). Based on means of landrace selections, CF was respectively highest and lowest in the plain red (0.8064) and plain cream landrace selections (0.7925). Results over time (DAP) showed that during the first week CF was 0.8040 and then peaked at 0.8052 14 DAP, only to decrease to 0.7927 at 21 DAP.

With respect to pre–dawn leaf water potential, no significant differences (P>0.001) were observed among landrace selections, watering regimes and the interaction between the two (Fig 5.8). Results of midday stem water potential also showed no significant differences (P>0.05) among landrace selections (Fig 5.9). Watering regimes were, however, highly significantly different (P<0.001) (Fig 5.9). The interaction between landrace selections and watering regimes was not significant (P>0.05) (Fig 5.9). Midday stem water potential was close to zero (–0.276 MPa) in the water stressed watering regime (25% FC) and more negative (–0.443MPa) in the no stress watering regime (75% FC). The moderately stressed watering regime (50%) FC, though slightly lower (–0.419 MPa) was statistically similar to the no stress watering regime (75% FC).

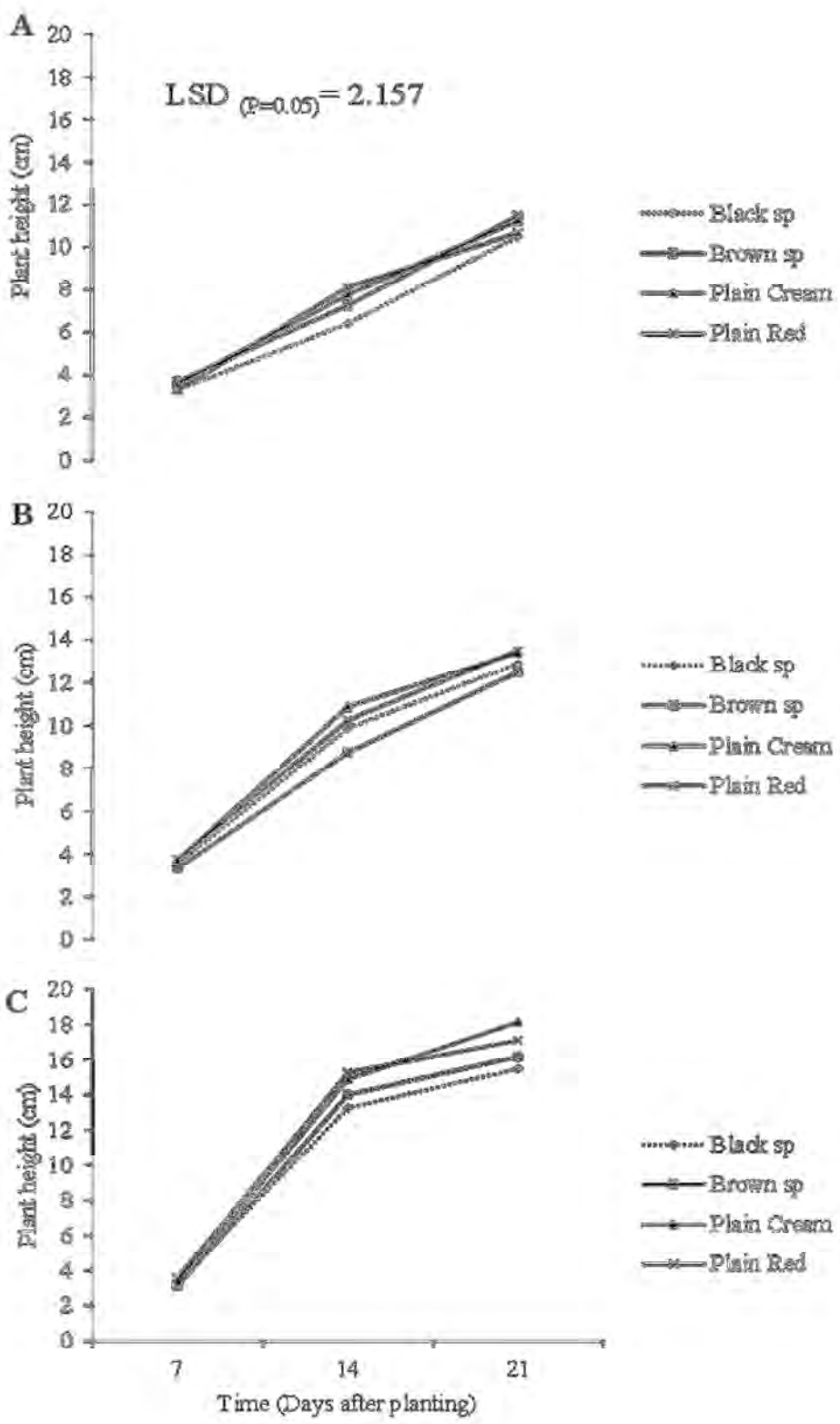

Figure 5.5: Weekly plant height of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) during seedling establishment under different watering regimes: A – 25% FC, B – 50% FC and C – 75% FC.

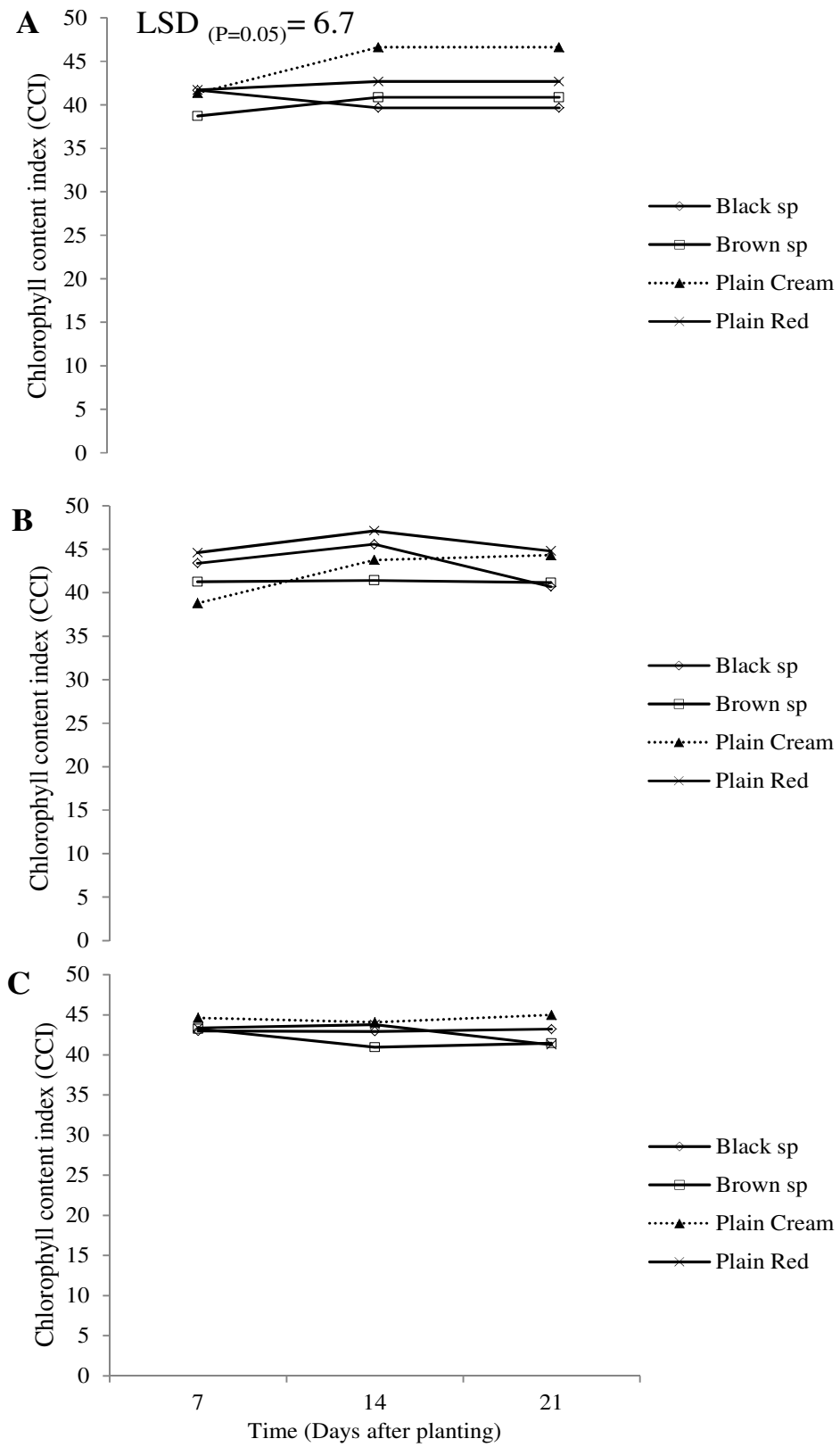

Figure 5.6: Weekly CCI of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) during seedling establishment under different watering regimes: A – 25% FC, B – 50% FC and C – 75% FC.

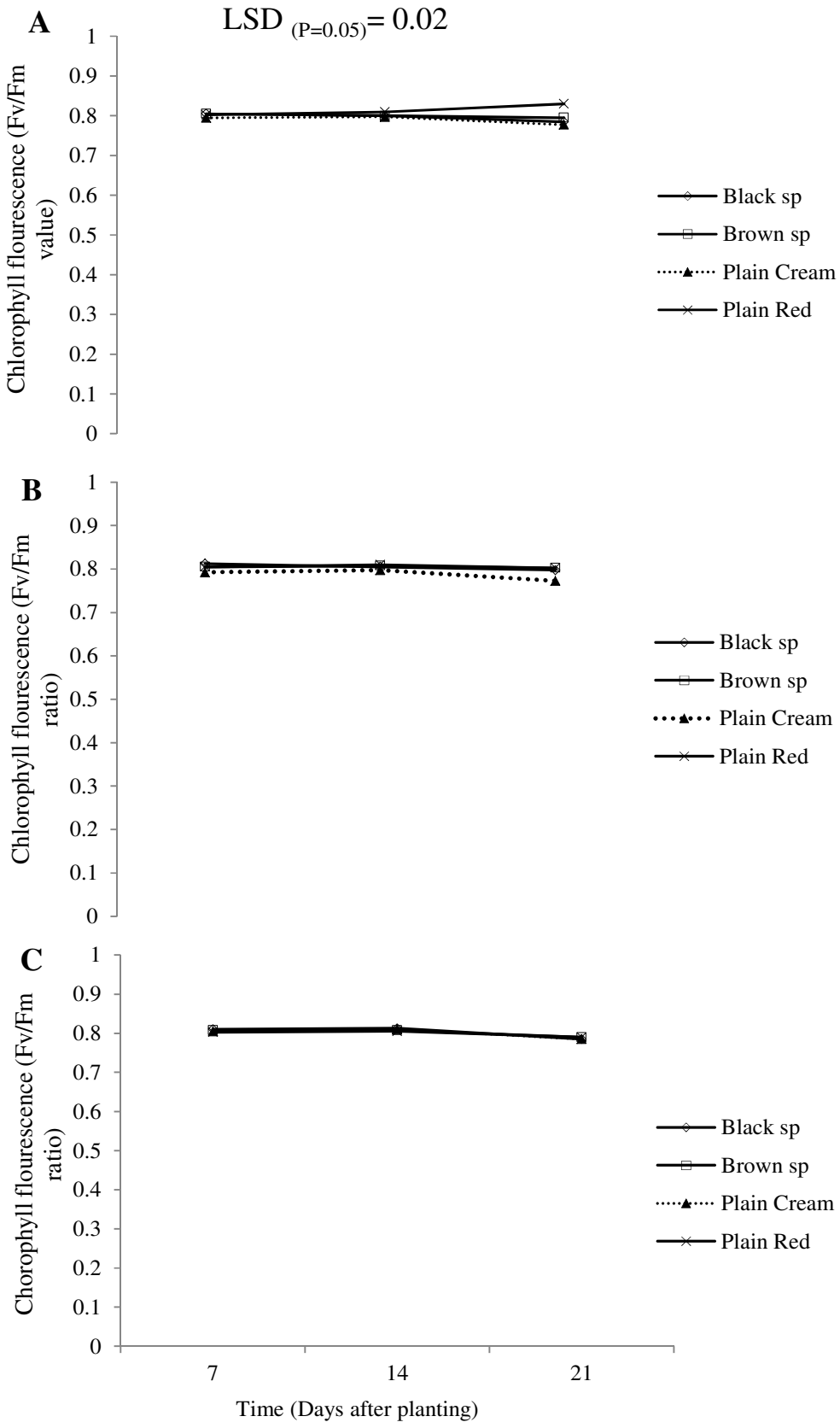

Figure 5.7: Weekly chlorophyll fluorescence of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) during seedling establishment under different watering regimes: A – 25% FC, B – 50% FC and C – 75% FC.

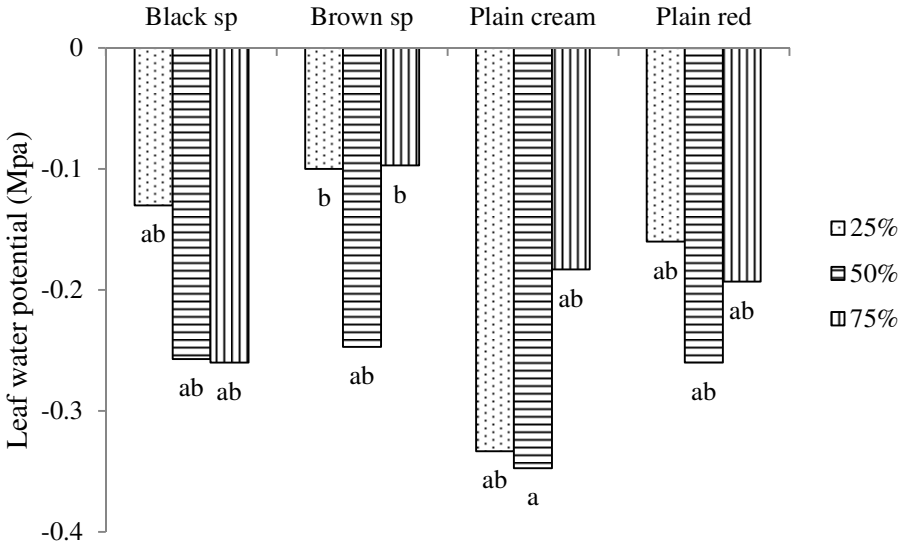

Figure 5.8: Predawn leaf water potential of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) measured on day 21 of seedling establishment under different watering regimes (25%, 50% and 75% FC).

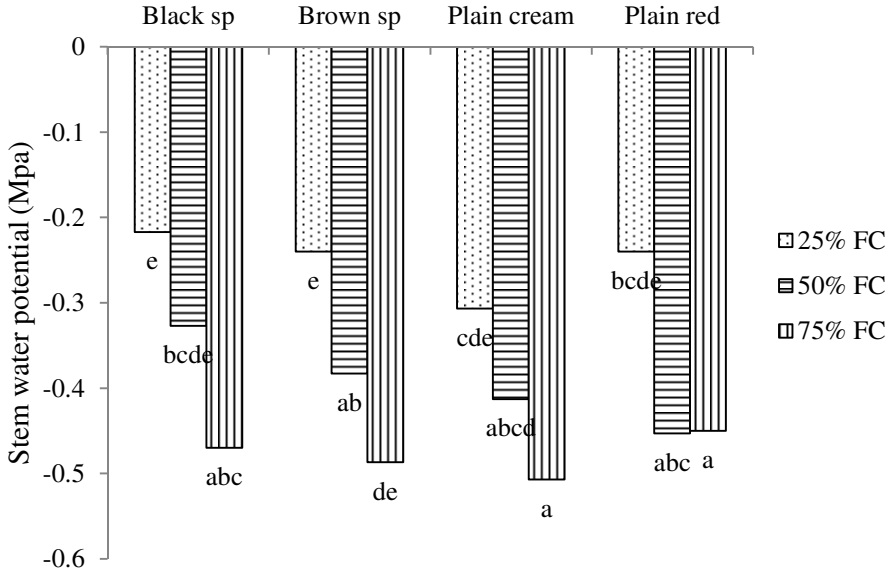

Figure 5.9: Midday stem water potential of bambara groundnut landrace selections (plain red, plain cream, black speckled and brown speckled) measured on day 21 of seedling establishment under different watering regimes (25%, 50% and 75% FC).

5.2.3 Seedling parameters

A consistent pattern was observed for results of seedling growth parameters (seedling length, root and shoot length and root: shoot ratio). Landrace selections were not significantly different (P>0.05), watering regimes were highly significantly different (P<0.001) and the interaction between landrace selections and watering regimes was not significant (P>0.05) (Table 5.1). Seedling length was longest (21.36 cm) in the no stress watering regime (75% FC) and shortest (17.98 cm) in the stressed water regime (25% FC). Shoot lengths also followed a similar pattern where longest shoots (16.90 cm) were observed in the no stress water treatment (75% FC) and the shortest shoots observed in the stressed water regime (25 % FC). Results of root length were the inverse of seedling and shoot lengths whereby the longest roots (6 cm) were observed in the stressed water regime (25% FC) and the shortest roots (4.5 cm) observed in the no stress water regime (75% FC). Root length in the moderately stressed watering regime (50%) was 5.5 cm. Consequently, the trend in root: shoot ratio was such that 25% FC (0.5) > 50% FC (0.35) > 75% FC (0.27). Results of seedling dry mass showed no significant differences (P>0.001) among landrace selections, watering regimes and the interaction between the two (Table 5.1).

Table 5.1: Seedling growth and yield parameters of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) after seedling establishment under three watering regimes (25%, 50% and 75% FC).

[v]Field Capacity (%)	Seed coat colour	Seedling length	Shoot length	Root length	Root: Shoot Ratio	Dry mass
		----------(cm)----------				(g)
25	[w]PR	17.08eb	11.68e	5.41abc	0.47ab	0.59a
	[x]PC	18.47de	12.25de	6.22a	0.52a	0.42a
	[y]Br sp	17.91de	11.60e	6.31a	0.55a	0.52a
	[z]Bl sp	18.48de	12.38de	6.09a	0.50a	0.50a
50	PR	19.79bcd	14.20bcd	5.56ab	0.39bc	0.51a
	PC	18.55de	13.17cde	5.37abcd	0.41b	0.38a
	Br sp	20.10abcd	14.73bc	5.37abc	0.37c	0.53a
	Bl sp	19.46cd	13.38bcd	5.64ab	0.41b	0.53a
75	PR	20.01bcd	15.65ab	4.36e	0.28d	0.56a
	PC	21.39abc	16.95a	4.45ce	0.27d	0.44a
	Br sp	21.89ab	17.50a	4.39e	0.25d	0.49a
	Bl sp	22.16ab	17.80a	4.66bcde	0.26d	0.52a
	$LSD_{(P=0.05)}$	**1.94**	**1.78**	**0.88**	**0.08**	**0.16**

[v]FC = Field capacity; [w]PR = Plain Red; [x]PC = Plain Cream; [y]Br sp = Brown speckled; [z]Bl sp = Black speckled. Means in the same column with different letters differ significantly at LSD (P = 0.05).

5.2.4 Irrigation withdrawal

5.2.4.1 Physiological responses

Following withdrawal of irrigation, results of CCI showed significant differences ($P<0.05$) among landrace selections, while highly significant differences ($P<0.001$) were observed among watering regimes and time (Fig 5.10). However, the interaction among landrace selections and watering regimes over time (DAP) was not significant ($P>0.05$) (Fig 5.10). Results of CCI showed huge variability among landrace selections across all watering regimes. The plain cream and brown speckled landrace selections respectively had the highest (39.98) while and lowest (38.40) CCI. A significant decline in CCI (~42 – ~28) was first observed in the previously no stress water regime (75% FC) three days after withdrawing irrigation. In the previously moderately stressed water regime (50% FC), a significant reduction in CCI (~42 – ~30) was observed four days after withdrawing irrigation. In the previously severely stressed (25% FC) water regime a significant decrease in CCI (~40 – ~32) was observed six days after withdrawing irrigation. The control treatment maintained the highest (> 40) CCI, compared to the treatments were irrigation was withdrawn.

After withdrawing irrigation, results of chlorophyll fluorescence, showed highly significant differences ($P<0.001$) among landrace selections, watering regimes, time (DAP) and the interaction of the three (Fig 5.11). Based on means of landrace selections, the highest (0.7140) and lowest (0.6954) CF was observed in the plain red and black speckled landrace selections, respectively. Chlorophyll fluorescence was relatively constant in the control treatment while it decreased significantly (from ~0.8 – ~0.57) in the previously no stress (75% FC) water regime three days after withdrawing irrigation. At the end of the experiment (7 days after withdrawing irrigation) chlorophyll fluorescence had decreased to ~0.48 in the previously severely stressed water regime (25% FC), ~0.51 in the previously moderately stressed water regime and ~0.35 in the previously no stress watering regime (75% FC).

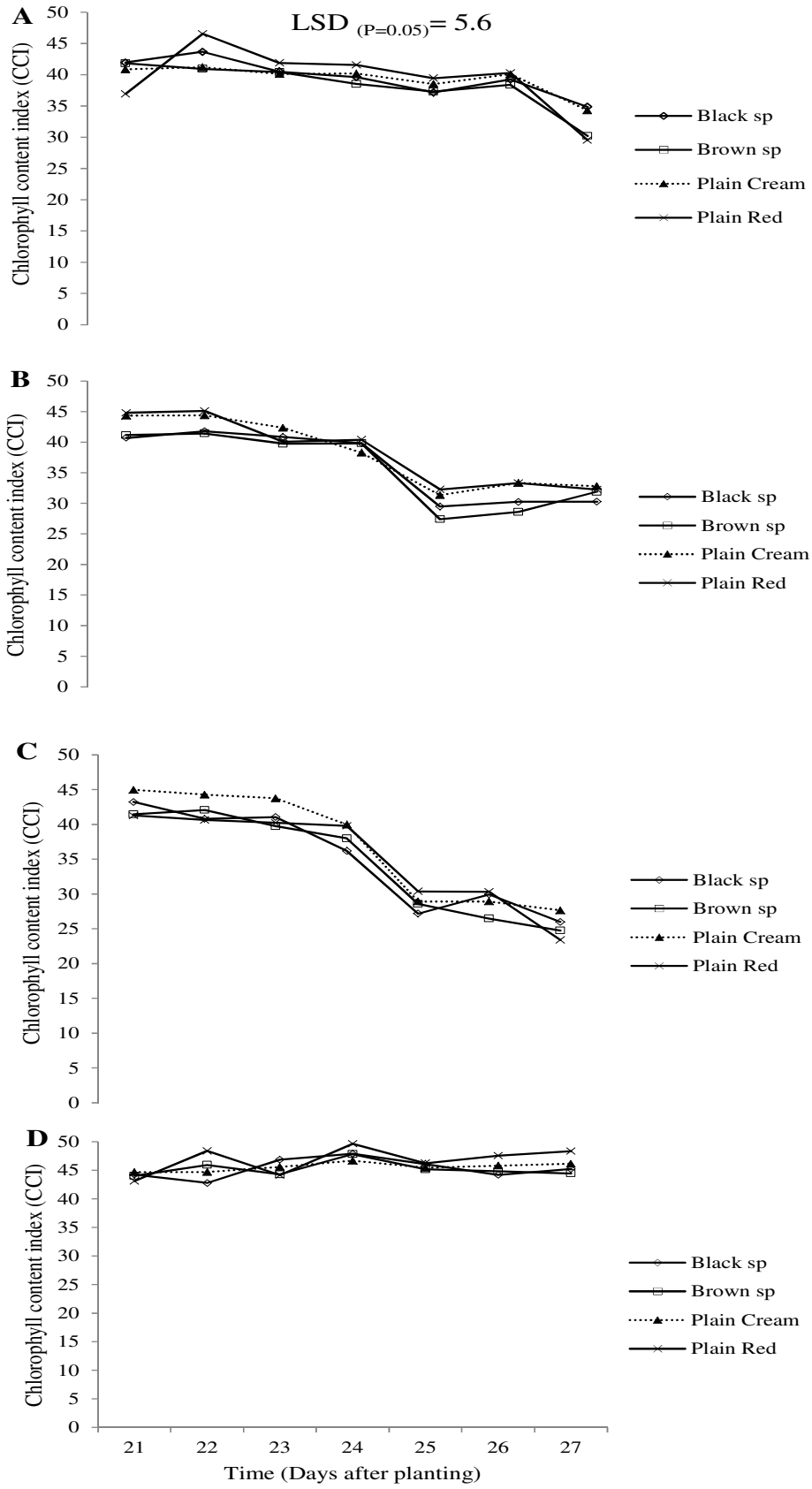

Figure 5.10: Daily changes in content index of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) after withdrawing in all watering regimes (except control): A – 25% FC, B – 50% FC, C – 75% FC and D – control.

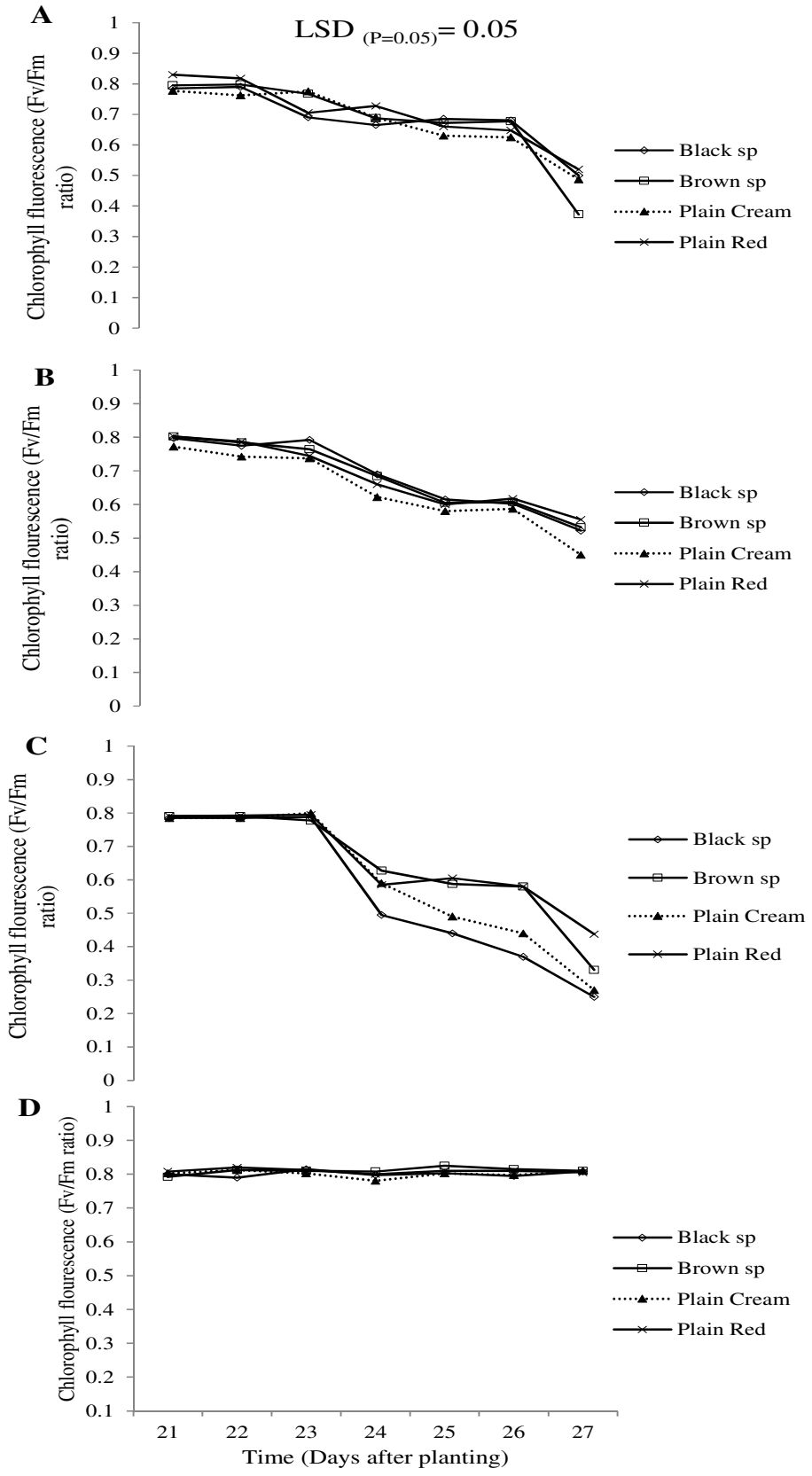

Figure 5.11: Daily changes in chlorophyll fluorescence of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) after withdrawing in all watering regimes (except control): A – 25% FC, B – 50% FC, C – 75% FC and D – control.

5.2.4.2 Metabolic responses

Results of TAOC showed highly significant differences ($P<0.001$) among watering regimes over time (DAP) (Fig 5.12). Landrace selections showed no significant ($P>0.05$) variation nor was the interaction among watering regimes and landrace selections over time (DAP) (Fig 5.12). For all treatments, TAOC increased following withdrawal of irrigation. Total antioxidant capacity reached its peak on different days across the watering regimes. The previously not stressed (75% FC) and moderately stressed water regimes (50% FC) attained peak TAOC (~0.9 mg g^{-1} DW) at 24 DAP, corresponding to 3 days after withdrawing irrigation. The previously severely stressed water regime (25% FC) reached peak TAOC (~0.8 mg g^{-1} DW) 25 DAP (4 days after withdrawing irrigation). The control treatment did not show much fluctuation for the duration of the experiment; it was constant between 0.2 mg g^{-1} DW and 0.3 mg g^{-1} DW.

It is worth noting that at 21 DAP TAOC was different across watering regimes; the trend of TAOC was such that 25% FC > 50% FC > 75% FC. With respect to landrace selections generally the plain red had the highest TAOC under moderate and severe stress while the plain cream landrace selection had the least. Differences among landrace selections were more pronounced in the previously severely stressed watering regime (25%), with plain red landrace selection being superior and black speckled landrace selection being inferior.

Total phenolics varied significantly ($P<0.001$) among water regimes, landrace selections, time (DAP) and the interaction of the three (Fig 5.13). Across all water regimes, a noticeable decline in phenolics was observed at 23 and 24 DAP (2 and 3 days after withdrawing irrigation), although the patterns of decline were different. In the previously severely stressed water regime (25% FC), the decline was gradual. A sharp decline was observed at 23 DAP (2 days after withdrawing irrigation) in the previously no stress water regime (75% FC). In the control treatment, total phenolics were constant between 80 and 130 µg GAE g^{-1} DW. At 21 DAP total phenolics were higher (~120 µg GAE g^{-1} DW) in the severely stressed watering regime (25% FC), compared to ~80 µg GAE g^{-1} DW in the moderately stressed watering regime (50% FC) and 100 µg GAE g^{-1} DW in the no stress watering regime (75% FC). Similar to TAOC, differences among landrace selections were more pronounced in the previously severely stressed water regime (25% FC). Again, landrace selections exhibited huge variability across water regimes except for the plain red landrace selection which showed consistently high total phenolics across all watering regimes.

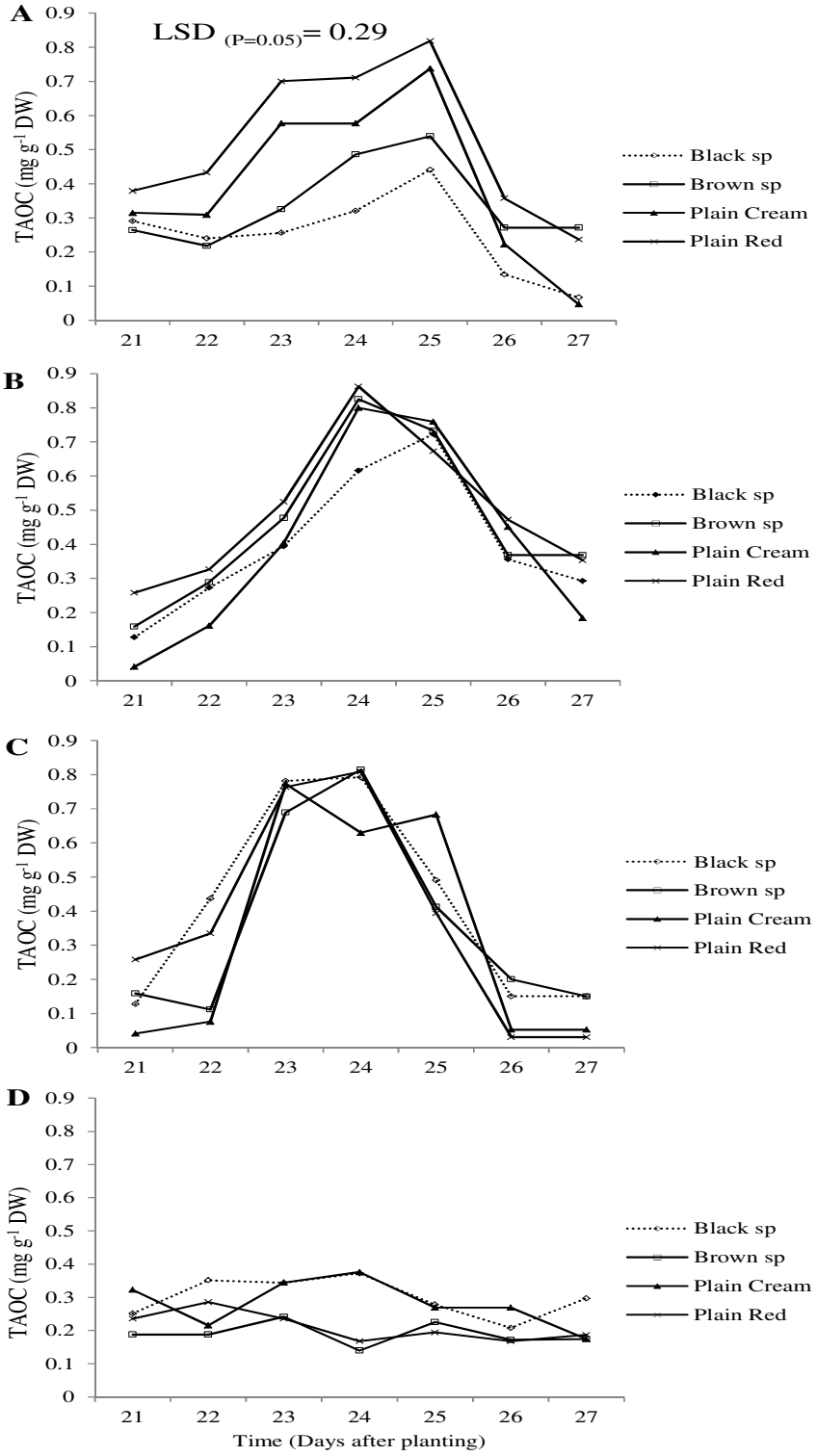

Figure 5.12: Daily changes in total antioxidant capacity of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) after withdrawing in all watering regimes (except control): A – 25% FC, B – 50% FC, C – 75% FC and D – control.

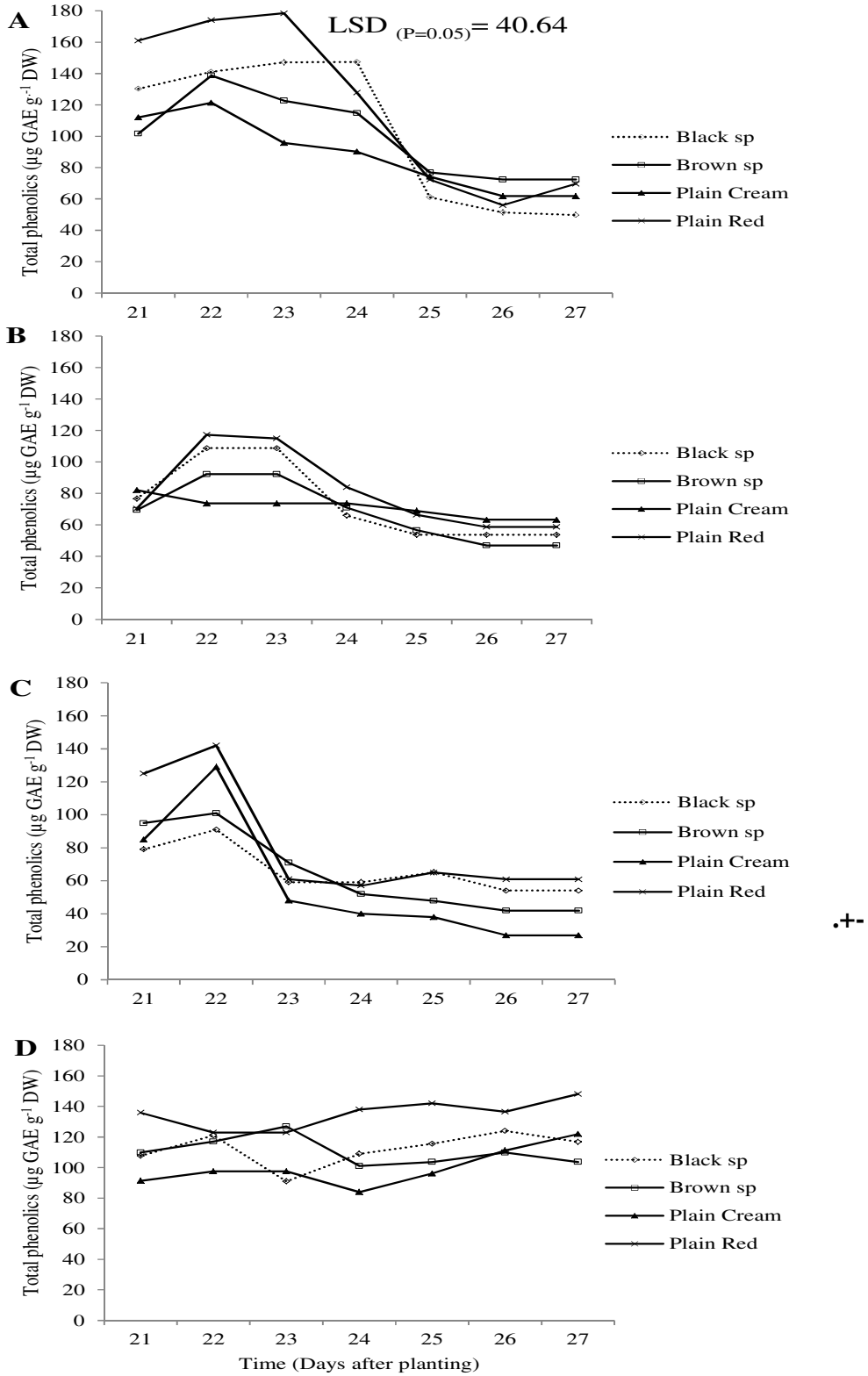

Figure 5.13: Daily changes in total phenolics of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) after withdrawing in all watering regimes (except control): A – 25% FC, B – 50% FC, C – 75% FC and D – control.

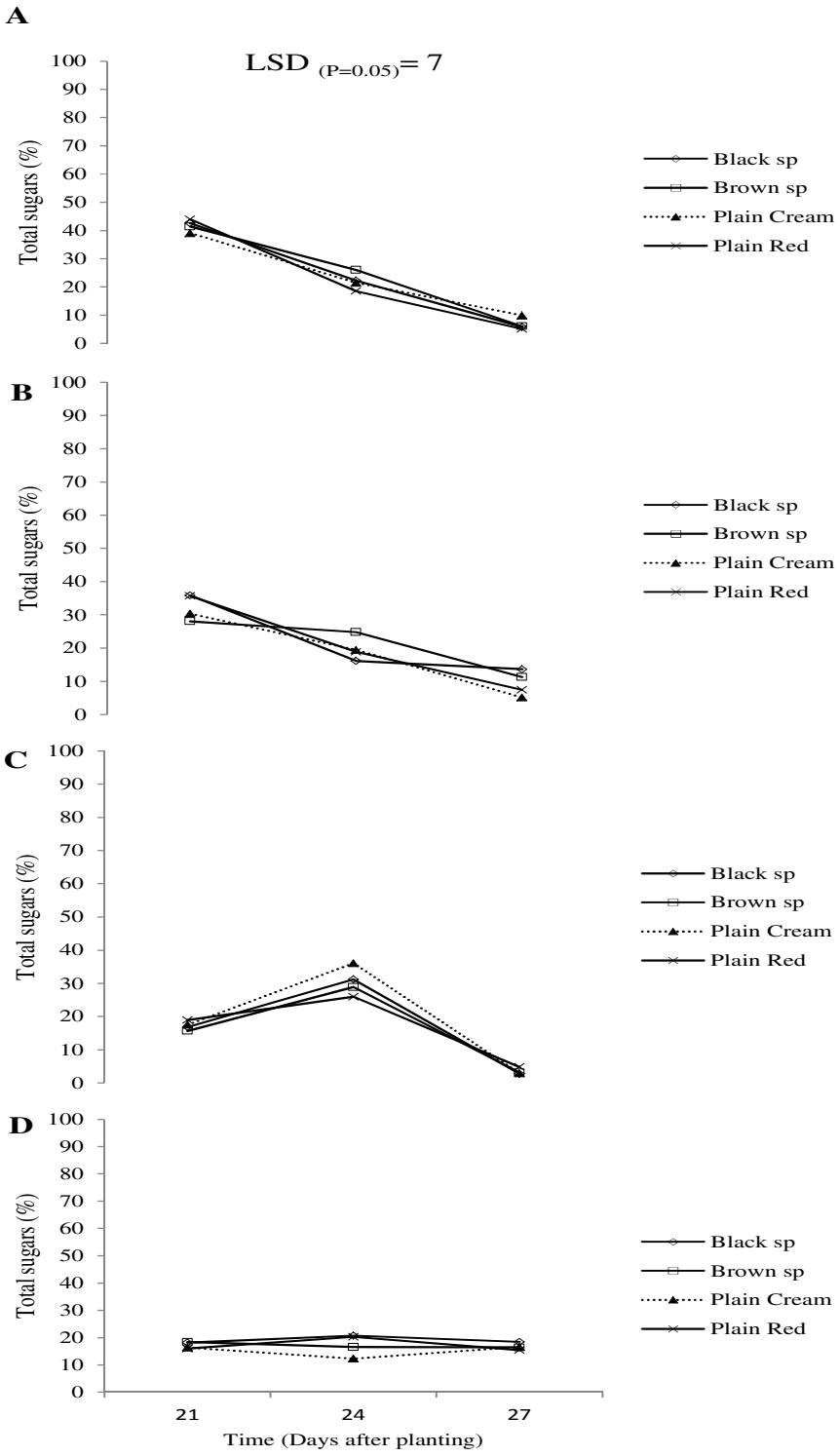

Figure 5.14: Changes in total sugars of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) after withdrawing in all watering regimes (except control): A – 25% FC, B – 50% FC, C – 75% FC and D – control.

There were highly significant differences (P<0.001) among watering regimes and time (DAP), with respect to total sugars (Fig 5.14). Landrace selections were not significantly different (P>0.05) nor was the interaction among watering regimes and landrace selections over time (DAP) (Fig 5.14). When irrigation was withdrawn, total sugars declined (~ 40% – ~ 5%) in the previously severely stressed (25% FC) and moderately stressed water regimes (50% FC). In the previously no stress water regime (75% FC), total sugars went up from ~15 % – ~ 30% during the period 21 to 24 DAP (3 days after withdrawing irrigation), and then declined from ~ 30% – ~3% by 28 DAP. Total sugars in the control treatment did not show much variation for the duration of the experiment (Fig 5.14).

5.2.5 Seedling water use efficiency

Seedling water use efficiency showed significant (P<0.05) variation among water regimes and landrace regimes. The interaction between water regimes and landrace selections was, however, not significant (P>0.05) (Fig 5.15). Seedling water use efficiency was highest in the severely stressed (25% FC) water regime (0.00616 g mm^{-1}) and lowest in the non–stressed (75% FC) watering regime (0.00342 g mm^{-1}) while it was 0.00436 g mm^{-1} in the moderately stressed (50% FC) watering regime. All landrace selections showed improved WUE in response to decreasing water availability. At 25% FC, seedling WUE was statistically different to that at 50% FC and 75% FC; seedling WUE at 50% was statistically similar to that at 75% FC (Fig 5.15). Based on means of landrace selections across water regimes, the trend in seedling WUE was such that plain red (0.00551 g mm^{-1}) > brown speckled (0.00491 g mm^{-1}) > black speckled (0.00427 g mm^{-1}) > plain cream landrace selection (0.00390 g mm^{-1}).

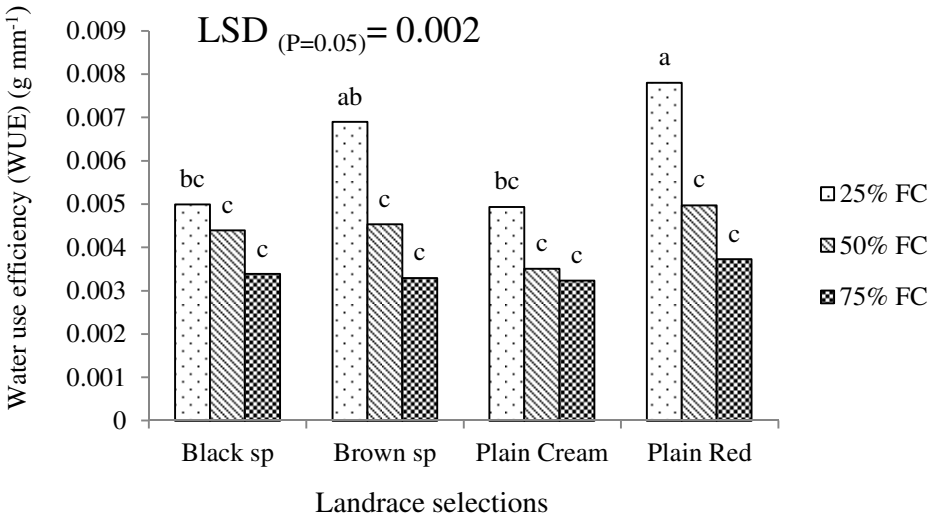

Figure 5.15: Seedling water use efficiency (WUE) of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) during establishment under three different watering regimes (25% FC, 50% FC and 75% FC).

5.3 Discussion

The objective of this study was to determine growth, physiology and water use characteristics of a bambara groundnut landrace differing in seed coat colour, during establishment under different water regimes. A secondary objective was to determine physiological and metabolic responses of bambara groundnut to terminal water stress.

With respect to seedling final emergence, results of this study concurred with the findings by Pillay (2003), Sinefu (2011) and Zondi (2013) where dark coloured seeds had better emergence (80%) compared to the light coloured seeds (63%). This was related to the tannins present in dark coloured seeds which are polyphenols and act as antioxidants under stress conditions (Mabhaudhi and Modi 2013). This study went further to determine the amount of phenolics present in leaf tissue of the different bambara groundnut landraces. True to expectation, high phenolic content and high total antioxidant capacity were observed in the plain red landrace selection. Our observations of high phenolic content in the plain red landrace selection concur with Mabhaudhi and Modi's (2013) hypothesis. This implies that the use of seed colour as a seed selection criterion has merit. Final emergence (> 63%) observed in this study was relatively higher compared to that reported by Legwaila *et al*. (2013) of < 42%. These differences may be attributed to different landraces used in the two studies, and landraces originating from different locations typically show huge variability (Zondi 2013). However, what is evident from both studies is the fact that bambara groundnut establishes poorly. This reduces crop water use efficiency as more water is lost through evapotranspiration during this period with minimal crop biomass gain. Mabhaudhi and Modi (2013) suggested this as a possible reason for low uptake by farmers and recommended that strategies to improve emergence should be explored. Improving seedling emergence will improve crop water use efficiency. In their study, Legwaila *et al.* (2013) managed to improve emergence in bambara groundnut landraces by hydro-priming them.

Water is vital to plant growth and development. It drives the turgor process responsible for cell division and cell expansion. Under water limited conditions plant growth and development are compromised. Studies on the effect of water stress on bambara groundnut seedling growth have shown that seedling growth was affected by water availability; growth rate increased with increasing water availability (Mwale *et al*. 2007, Sinefu 2011, Zondi 2013). The results of this study confirmed these previous studies. However, our study also showed that physiological responses such as CCI and CF were not very sensitive to water

stress. This behaviour suggests that reported drought avoidance in bambara groundnut landraces is also present at the seedling stage. Bambara groundnut seedlings were able to reduce plant growth (leaf number, leaf surface area and plant height) in order to maintain high tissue water potential under stress (Blum 1996, 2005). Results of leaf water potential confirmed this as there were no significant differences observed across the water regimes with respect PLWP and MSWP. The results of total sugars was such that 25% FC > 50% FC > 75% FC at the end of the seedling establishment. It could be assumed that accumulation of TSS under water stress facilitated retention of water, protected photosynthetic apparatus and maintained ion homeostasis in stressed seedlings, contributing to high tissue water potential (Bohnert and Jensen 1996). Results of MSWP could be related to the longer roots observed in the stressed treatments compared to the no stress treatments, implying that there was enhanced soil water capture in the stressed treatment. The higher root: shoot ratios observed in the stressed water treatments suggests that the plant favoured root growth to shoot growth in order to manipulate the source and sink strength and hence utilise resources efficiently (Yordanov *et al.* 2000, Chaves *et al.* 2002).

Seedling WUE is defined as a ratio of biomass accumulation expressed in total seedling biomass (g) to water added in mm. High WUE is largely a function of reduced water use and net improvement in plant production (Blum 2005). This was the case in this study as results of the numerator (total dry mass) were not statistically different among landrace selections and watering regimes. The differences in WUE were influenced by the denominator (water use), with WUE increasing with decreasing water supply. The high WUE could be attributed to the drought avoidance strategies (reduced leaf number, leaf surface area and plant height and sugars accumulation) observed on the stressed seedlings. Mabhaudhi *et al*. (2013) observed a similar trend when they determined whole WUE of the same species. With respect to landrace selections, WUE was higher in the plain red landrace selection. This lends further credence to the hypothesis by Mabhaudhi *et al*. (2013) that dark coloured seeds performed better and exhibited enhanced drought tolerance compared to light coloured seeds.

Lastly, we determined the physiological and metabolic responses of bambara groundnut landrace selections to terminal stress following withdrawal of watering. Generally, the previously severely stressed plants responded better to terminal stress, both physiologically and metabolically, compared to the previously non-stressed plants. This was as a result of ‘acclimation’ where plant adaption to any stress situation is mediated by defences. The

effectiveness of these defences can be enhanced by pre-exposing plants to specific mild stimuli before imposing the full strength of the stress. This was the case for plants that had previously been subjected to 25% FC. Acclimation to water stress has been shown in Arabidopsis, where plants alleviated the effect of water stress due to prior exposure to water stress at an early stage (Harb *et al.* 2010).

Maxwell and Johnson (2000) advanced that the ability of the plant to tolerate environmental stresses and the extent to which these stresses have damaged photosynthetic apparatus can be gained from measuring CF. Based on the latter, our results indicate that the previously moderately and severely stressed plants were better able to tolerate terminal stress compared to the previously not stressed plants. This supports the theory of acclimation to water stress. Our findings also support the assertion by Khaleghi *et al.* (2012) that CCI and CF could be used to evaluate the photosynthetic process under water stress and thus be used as a rapid technique for detecting plant tolerance to drought stress.

Major findings of this study are: (i) dark coloured seeds performed better than light coloured seeds, especially under stress conditions, (ii) drought avoidance strategies and, (iii) acclimation to water stress were present in bambara groundnut seedlings. The superior performance of dark coloured seeds was associated with high phenolic content. Withdrawal of irrigation led to changes in physiological and metabolic responses, with previously stressed seedlings showing better acclimation to terminal stress compared to previously non–stressed seedlings. Water use efficiency of bambara groundnut seedlings increased with decreasing water supply; this was mainly influenced by changes in water use (denominator) and not biomass (numerator). Improved WUE under water limited conditions was associated with smaller seedling canopy size, longer roots, and higher root: shoot ratio as well as accumulation of solutes which allowed for maintenance of high tissue water potential. Although landrace selections showed variability in their responses, the red landrace selection may be recommended due to its high emergence and ability to tolerate drought. Future studies will determine WUE of bambara groundnuts propagated using seedlings under field conditions so as to determine whether transplanting improves WUE.

CHAPTER 6

EFFECT OF WATER STRESS ON YIELD AND SUBSEQUENT SEED QUALITY OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC)

6.1 Introduction

Bambara groundnut (*Vigna subterranea*) is cultivated using landraces due to a lack of improved varieties. Its cultivation is characterised by informal seed systems where the most common source of seed is farmers' own recycled seed and recycled seed from friends and relatives. According to Nkoze and Okoko (2003), often seed produced at farmers' level is of poor quality. They attributed this to poor growth environment of the parent plant, improper extraction of seeds, processing and storage. Bambara groundnut is grown in rural areas in semi and arid regions where water is the most limiting resource during its production. It is possible that under these conditions, farmers may be recycling seed of inferior quality due to stress occurring on the mother plant during production.

The effect of water stress on the mother plant on subsequent seed quality of progeny is not well understood and is largely species dependant. In cowpea (*Vigna unguiculata*), water stress on maternal plants did not affect viability of progeny but seed vigour decreased (Tang 1982). In a separate study on groundnut (*Arachis hypogaea*), both viability and vigour of progeny were lowered by water stress on the maternal plant (Pallas *et al*. 1977). Several studies on bambara groundnut have looked at the effect of water stress on yield and yield components (Madukwe *et al*. 2011, Vurayai *et al*. 2011b, Mabhaudhi *et al*. 2013, Ngwako *et al*. 2013). However, none of these evaluated the effect of water stress on subsequent seed quality. Zondi (2013) evaluated germinability and vigour of seeds obtained from mother plants subjected to 30%, 60% and 100% crop water requirement (ETc) (Mabhaudhi *et al*. 2013), and found that seeds obtained from 30% ETc had better germinability and vigour than seeds produced at 60% and 100% ETc. This implied that stress may have a positive effect on subsequent seed quality. However, Zondi (2013) did not go further to give reasons as to this positive effect.

The fact that bambara groundnut is cultivated in marginal areas and is currently being promoted as a drought tolerant crop necessitates a study such as this. In this study we hypothesized that even though bambara groundnut is drought tolerant, water stress affects germination rate and vigour of progeny and this could be a possible explanation for the

reported poor seed quality of bambara groundnut (Sinefu 2011, Legwaila *et al.* 2013, Zondi 2013). The objective of this study was therefore to determine yield and subsequent seed quality (viability and vigour) and seed quality characteristics of progeny of different seed coat colours produced under rainfed and irrigated conditions.

6.2 Results

6.2.1 Weather data

Weather data showed that temperatures were cooler during the first month after transplanting. Average maximum temperature (Tmax) during this period was 22°C and minimum temperature (Tmin) was 14°C. Light rains (< 3 mm per day) were received during this period. After 31 days after transplanting (DAT), average maximum and minimum temperatures increased to 27°C and 17°C, respectively. There was a dry period between 43 and 67 DAT. A heavy storm, 72 mm of rain, was measured at 98 DAT. Thereafter, there was a period of sustained rainfall events (> 5 mm per day) (Fig 6.1).

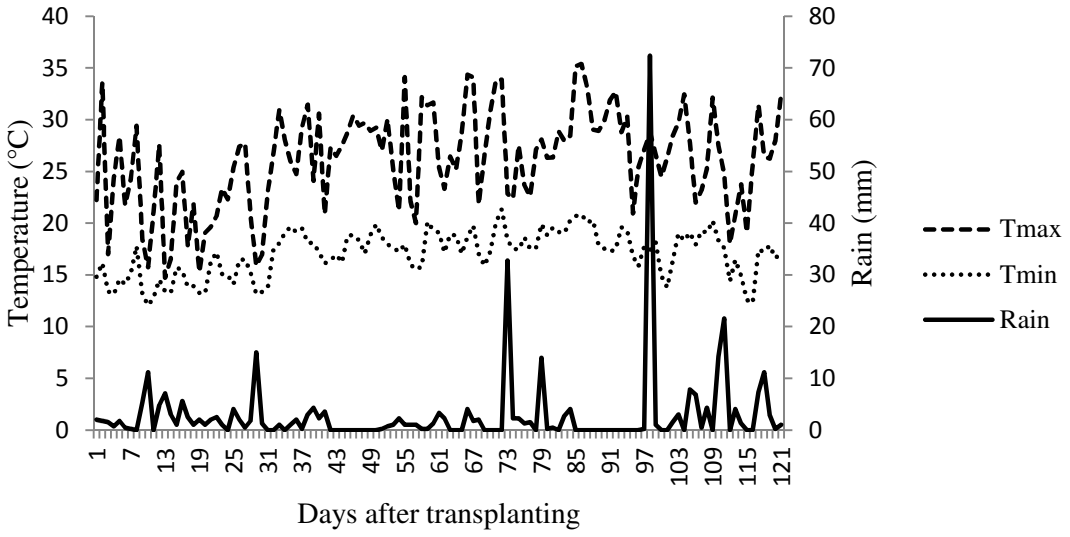

Figure 6.1: Changes in daily weather parameters (Tmax, Tmin and rain) observed during the growing season (18 November 2013 to 16 March 2014) at Ukulinga, Pietermaritzburg.

6.2.2 Yield and yield components

Analysis of variance showed no significant differences (P>0.05) among landrace selections, the water regimes and the interaction between the two with respect to pod mass and number, seed mass and number and harvest index (Table 6.2). However, significant differences (P<0.05) were observed between water regimes with respect to total biomass per plant. Landrace selections as well as the interaction between landrace selections and water regimes was not significant (P>0.05) (Table 6.2). Based on means of water regimes across varieties, total biomass per plant was 106% higher under irrigated compared to rainfed conditions. The plain cream landrace selection had the highest total biomass under both irrigated (33 g) and rainfed (20.4 g) conditions. The black speckled landrace selection had the lowest total biomass per plant (10.3 g) under rainfed conditions. The decline in pod mass was not as pronounced as that for biomass; pod mass was 36% lower under rainfed than irrigated conditions.

The relative stability of pod mass under rainfed conditions resulted in a positive effect on harvest index. Under rainfed conditions, HI increased by, on average, 31% compared to under irrigated conditions. Pod number was 38% lower under rainfed than irrigated conditions. This trend was consistent for seed number per plant; seed number was 39% lower under rainfed than irrigated conditions. However, the ratio between pod and seed number, showed that grain filling was not affected by stress. Interestingly, although seed number was lower under rainfed conditions, the seeds were larger and weighed more, on average, than under irrigated conditions. Results of 100 GM showed that it was higher under rainfed than irrigated conditions (Table 3.2).

Table 6.1: Yield parameters of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) under rainfed and irrigated field conditions

Treatment	Landrace selection	Total biomass plant^{-1} (g)	Pod mass plant^{-1} (g)	Pod number plant^{-1}	Seed mass plant^{-1} (g)	Seed number/ plant	[v]HI (%)
Rainfed	[w]PR	13.1abcd	6.7	11	4.60	11.52	46.1a
	[x]PC	20.4abcd	7.0	14.2	4.47	13.68	37.8a
	[y]Brown sp	11.2bcd	7.0	10.2	5.23	11.26	50.0a
	[z]Black sp	10.3bd	3.9	10	2.85	9.08	40.5a
Mean		**13.8**	**6.1**	**11.3**	**4.29**	**11.4**	**43.6**
Irrigated	[w]PR	31.3ab	9.4	17.6	4.97	18.23	31.0a
	[x]PC	33.2a	9.2	21.6	5.89	21.34	27.3b
	[y]Brown sp	18.1abcd	9.1	14.2	6.79	15.90	41.0a
	[z]Black sp	31.2abc	10.3	19.5	6.75	19.30	33.9a
Mean		**28.5**	**9.5**	**18.2**	**6.10**	**18.7**	**33.3**
LSD (P=0.05)		**18.73**	**NS**	**NS**	**NS**	**NS**	**21.7**

[v]HI = Harvest Index; [w]PR = Plain Red; [x]PC = Plain Cream; [y]Br sp = Brown speckled; [z]Bl sp = Black speckled. Means in the same column with different letters differ significantly at LSD (P = 0.05).

6.2.3 Standard Germination Test

Highly significant differences (P<0.001) were observed between previous water treatments, landrace selections and the interaction between the two over time with respect to the standard germination test (Fig 6.2). Based on mean values of water treatments across landrace selections, seeds produced under irrigated conditions had 6% higher germination compared with seeds produced under rainfed conditions. The interaction between water treatments and landrace selections showed that, with the exception of brown speckled seeds, germination was higher for seeds produced under irrigated relative to rainfed conditions. The brown speckled landrace selection daily germination was lower in the progeny of the irrigated trial than the rainfed trial. After day 7 germination % did not increase and the plain cream and plain red irrigated progeny had attained 100% emergence on day 7. Final germination from the rainfed progeny was above 90% across all landrace selections with the highest (96.67%) being observed in the plain red landrace selection and the lowest (91.67%) in the black speckled landrace selection.

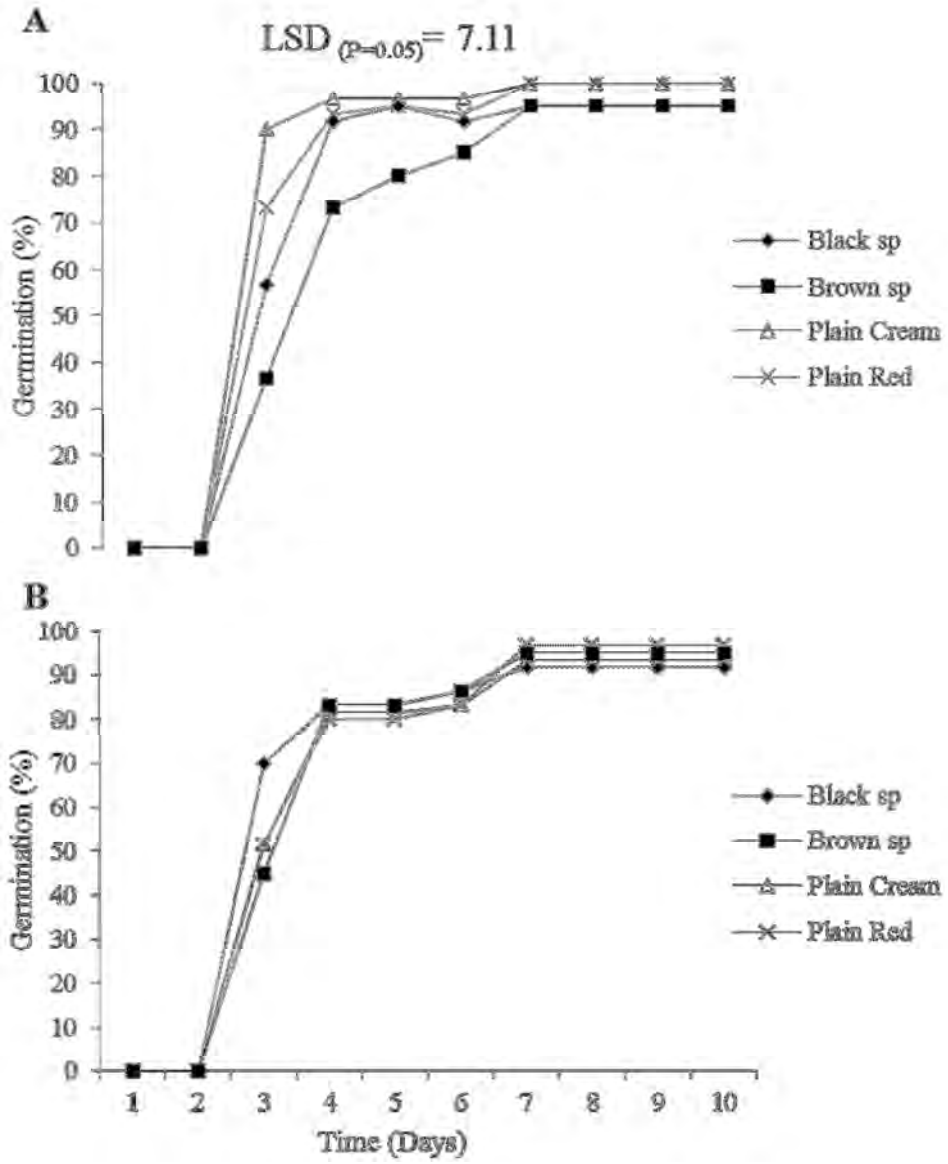

Figure 6.2: Daily percentage germination of the different landrace selections (plain red, plain cream, brown speckled and black speckled) harvested from A- Irrigated and B- Rainfed plot as observed in the standard germination test.

6.2.4 Germination vigour characteristics

There were no significant differences (P>0.05) among bambara groundnut landrace selections with respect to MGT, seedling length, root length, shoot length and root: shoot ratio (Table 6.2). Highly significant differences (P<0.001) among landrace selections were observed only for GVI and fresh and dry mass. Results of vigour indices such as seedling fresh and dry mass as well as seedling, shoot and root lengths all showed significant differences (P<0.05) between the two production environments (irrigated and rainfed). The trend in these indices was such that they were all higher for seeds produced under irrigated relative to rainfed conditions. The only exception was root: shoot ratio which, although not statistically significant (P>0.05), was higher for seeds produced under rainfed relative to irrigated conditions (Table 6.2). Seeds produced under irrigated conditions had 11% higher GVI than seeds produced under rainfed conditions. For all seed colours, GVI was lower for seeds produced under rainfed relative to irrigated conditions. The plain red landrace selection had the highest GVI (18.77) while the lowest GVI (12.60) was observed in the brown speckled landrace selection. However with respect to fresh and dry mass the brown speckled landrace selection was more superior (12.70 g and 4.020 g, respectively) while the plain cream landrace selection was inferior with fresh and dry mass of 9.76 g and 3.321 g respectively. Mean seedling fresh mass from the progeny of the irrigated plot was 11.99 g while it was 10.22 g in the progeny of the rainfed trial. Dry matter accumulation during germination was also greater (3.74 g) in progeny of the irrigated plot compared to 3.35 g in progeny of the rainfed plot. Seedling length and root length was respectively 19% and 25% higher for irrigated than rainfed progeny (Table 6.3).

Table 6.2: Performance of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) harvested from an irrigated and rainfed plot, under the standard germination test.

Treatment	Landrace selection	[u]GVI	[v]MGT (Days)	Seedling length	Shoot length	Root length	Root: shoot ratio	Fresh mass	Dry mass
				——————mm——————				——(g)——	
Irrigated	[w]PR	19.53a	6.55ab	198.80a	96.00a	102.80a	1.07a	11.87b	3.63bc
	[x]PC	19.29a	6.34a	222.80a	105.25a	117.50a	1.10a	9.90c	3.26d
	[y]Br sp	17.19bc	6.81cb	240.50a	100.25a	140.20a	1.49a	12.75ab	3.90ab
	[z]Bl sp	17.62b	6.78bc	241.20a	104.50a	136.80a	1.36a	13.43a	3.93ab
Rainfed	[w]PR	18.01b	6.95c	185.00a	96.00a	98.80a	1.59a	10.04c	3.27d
	[x]PC	16.29bc	6.95c	183.00a	88.75a	94.20a	1.22a	9.61c	3.38cd
	[y]Br sp	17.19bc	6.96c	197.00a	85.75a	111.20a	1.49a	12.75ab	3.90ab
	[z]Bl sp	16.32cd	7.00c	191.00a	95.50a	94.50a	1.02a	8.60d	2.83e
$LSD_{(P=0.05)}$		**1.15**	**0.30**	**NS**	**NS**	**NS**	**NS**	**1.00**	**0.31**

[u]GVI = Germination velocity index; [v]MGT= Mean germination time; [w]PR=Plain Red; PC= [x]Plain Cream; [y]Br sp = Brown speckled; [z]Bl sp = Black speckled. Means in the same column with different letters differ significantly at $LSD_{(P=0.05)}$.

6.2.5 Electrolyte conductivity

Electrolyte conductivity was shown to vary significantly ($P<0.001$) between seeds produced under irrigated and rainfed conditions as well as among landrace selections (Fig 6.3). The interaction between the two factors was also significant ($P<0.001$). Mean values of water treatments across landrace selections showed that EC was higher (648 $\mu s.g^{-1}$) for seeds produced under irrigated than rainfed conditions (521 $\mu s.g^{-1}$) (Fig 6.3). A closer analysis of the interaction showed that plain seeds (cream and red) had higher EC under irrigated than rainfed conditions; the opposite was true for speckled seeds. The highest final EC (2253 $\mu s.g^{-1}$) was observed in the plain cream landrace selection harvested from the irrigated plot while the lowest final EC (744 $\mu s.g^{-1}$) was observed in the plain red landrace selection harvested from the rainfed plot. An interesting observation with respect to time and previous water treatments was that from the first hour to the 7^{th} hour EC was higher in seeds from the rainfed plot and then after the 7^{th} hour the inverse was true.

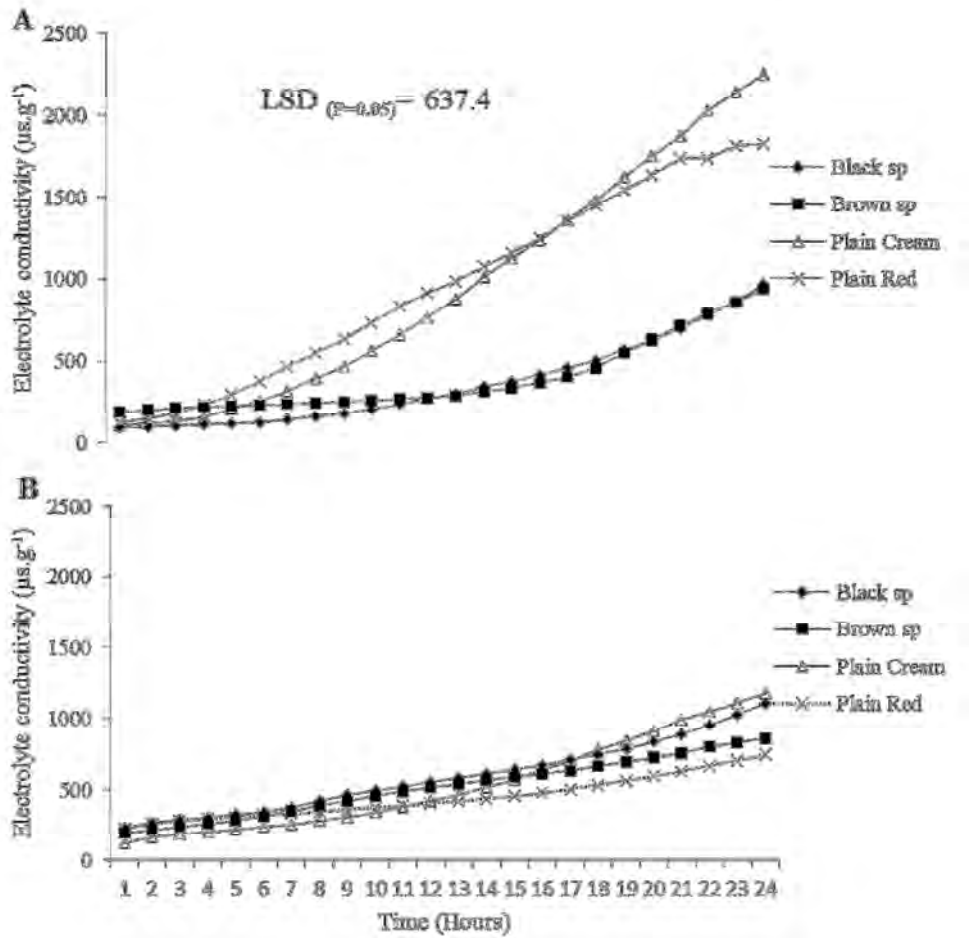

Figure 6.3: Electrolyte conductivity of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) harvested from A- Irrigated plot and B- Rainfed plot measured hourly for 24 hours.

6.2.6 Seed coat thickness

Significant differences (P<0.05) were observed among landrace selections with respect to seed coat thickness. The previous water treatments and the interaction between landrace selections and production environments was not significantly different (P>0.05) (Table 6.3). Although not statistically different progeny from the irrigated plot had slightly thicker seed coats (3% more) compared to progeny from the rainfed plot. Based on means of landrace selections, the thickest seed coat (92.8 µm) was observed in the brown speckled landrace selection while the thinnest seed coat (69.1 µm) was observed in the plain cream landrace selection. The seed coats of the black speckled and plain red landrace selections were 87.3 µm and 71 µm, respectively.

Table 6.3: Seed coat thickness of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) harvested from irrigated and rainfed plot, viewed and measured under Scanning Electron Microscope (SME).

Treatment		Landrace selection	Seed coat thickness(µm)
Irrigated		Plain Red	88.94ab
		Plain Cream	68.99b
		Brown sp	92.33ab
		Black sp	74.90ab
	Mean		**81.3**
Rainfed		Plain Red	85.67ab
		Plain Cream	69.30b
		Brown sp	93.19a
		Black sp	67.19b
	Mean		**78.8**
		$LSD_{(P=0.05)}$	**22.81**

* Means in the same column with different letters differ significantly at LSD (P=0.05).

6.2.7 Seed mineral composition

Seed produced under rainfed and irrigated conditions had different seed mineral composition. Furthermore, seed coat and cotyledon structure had different element weights across all landrace selections (Table 6.5). Elements such as carbon, oxygen, magnesium, potassium and calcium were present in all landrace selections and in both seed structures. Chlorine and sodium were not observed in any structure and landrace selection produced under irrigated conditions while it was only present in the seed coat of the plain cream landrace selection grown under rainfed conditions. The same was observed in iron in seed coat of the plain red landrace selection produced under rainfed conditions.

Table 6.4: Seed mineral composition of the seed coat and cotyledon of progeny of bambara groundnut landrace selections (plain red, plain cream, brown speckled and black speckled) under irrigated and rainfed conditions evaluated under Zeiss EVO Scanning Electron Microscope using Energy Dispersive X-Ray Spectrotomy (EDX) technique.

Treatment	Element (Weight %)	Plain Red		Plain Cream		Black sp		Brown sp	
		[y]SC	[z]Cot	SC	Cot	SC	Cot	SC	Cot
Irrigated	Carbon	59.47	57.71	53.85	59.30	59.84	57.54	58.67	57.01
	Oxygen	39.27	40.75	45.45	38.69	39.21	40.57	40.44	40.99
	Magnesium	0.15	0.30	0.11	0.26	0.15	0.31	0.30	0.33
	Phosphorous	-	0.19	-	0.31	-	0.24	-	0.24
	Sulphur	0.06	0.21	-	0.14	-	0.21	-	0.29
	Chlorine	-	-	-	-	-	-	-	-
	Potassium	0.67	0.66	0.28	1.18	0.27	0.78	0.18	0.97
	Calcium	0.24	0.19	0.20	0.11	0.37	0.15	0.41	0.18
	Silicon	0.14	-	0.11	-	0.17	0.17	-	-
	Iron	-	-	-	-	-	-	-	-
	Sodium	-	-	-	-	-	-	-	-
	Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Rainfed	Carbon	58.38	57.22	54.83	57.86	58.25	58.44	57.52	56.64
	Oxygen	39.71	40.00	43.63	39.91	40.38	39.60	41.94	38.22
	Magnesium	0.12	0.31	0.28	0.40	0.27	0.29	0.10	0.30
	Phosphorous	-	0.27	-	0.28	0.13	0.23	-	0.50
	Sulphur	0.07	0.28	0.12	0.28	0.18	0.29	-	0.45
	Chlorine	-	-	0.09	-	-	-	-	-
	Potassium	0.37	1.46	0.33	1.03	0.60	1.00	0.31	3.54
	Calcium	0.30	0.46	0.32	0.22	0.19	0.15	0.13	0.48
	Silicon	0.68	-	0.17	-	-	-	-	-
	Iron	0.37	-	-	-	-	-	-	-
	Sodium	-	-	0.11	-	-	-	-	-
	Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

[y]= Seed coat; [z] = Cotyledon

6.3 Discussion

Bambara groundnut is a drought tolerant crop, but severe drought reduces yield or leads to crop failure (Mwale *et al*. 2007, Madukwe *et al*. 2011, Sinefu 2011, Mabhaudhi and Modi 2013). Sinefu (2011) and Mabhaudhi and Modi (2013) found that yield and yield components of bambara groundnut was lower under rainfed than irrigated conditions. In the current study, biomass was more sensitive to water stress than pod mass. The decline in pod mass was not as pronounced as that for biomass. The relative stability of pod mass under rainfed conditions resulted in a positive effect on harvest index. Under rainfed conditions, HI increased by, on average, 31% compared to under irrigated conditions. The positive effect of water stress on HI implies that, under water limited conditions, biomass partitioning will favour the yield component and not vegetative growth. This is an important attribute in that yield is guaranteed even under adverse conditions. The positive effect of water stress on HI may also explain why bambara groundnut is such a resilient crop reported to produce reasonable yields under the widest of conditions.

Furthermore, the ratio between pod and seed number, showed that grain filling was not affected by stress. Interestingly, although seed number was lower under rainfed conditions, the seeds were larger and weighed more, on average, than under rainfed conditions. This could be also related to the positive effect of stress on HI which ensured that, under water limited conditions, dry matter partitioning favoured yield at the expense of vegetative growth (Table 6.1). This is an important adaptation which further confirms the suitability of bambara groundnut for production under water limited conditions. According to Vurayai *et al*. (2011b), the severity of yield reduction is dependent on the growth and development stage at which drought was imposed. They found water stress during flowering to cause the largest yield reduction. In this study a substantial dry period was experienced during the pod filling stage and according to Vurayai *et al*. (2011b), during pod filling most of the pegs have been formed and seeds will be formed due to partitioning of assimilates. Differences in pod mass and seed mass could also have been influenced by time of harvest. Both rainfed and irrigated plants were harvested at the same time based on maturity in rainfed plants. As a result, irrigated plants could have been harvested earlier before grain filling had been completed. This could also explain the lack of significant differences between seed mass under rainfed and irrigated conditions.

The effect of water stress on subsequent seed quality of bambara groundnut is not well understood. Zondi (2013) suggested that seeds produced under water stress performed better than seeds produced under full irrigation. However, in this current study progeny from irrigated plots performed better than progeny from rainfed plots with respect to germination and germination vigour indices. Plants respond to water stress physiologically and metabolically in order to survive. Water stress alters normal plant processes and could consequently affect subsequent seed quality as processes that occur during seed development heavily rely on normal plant metabolism. A possible explanation to the inferior seed quality of seeds produced under rainfed conditions could be the changes in metabolic reactions including hormone signalling pathways caused by water stress, affecting reserve deposition to the developing embryo and hence compromising subsequent seed quality (Black *et al*. 2006).

However, EC was higher in seeds produced under irrigated conditions compared to seeds produced under rainfed conditions. In chapter four we found a relationship between high calcium content in seed coat and lower EC due to seed coat integrity. With respect to landrace selections this was the case for the brown speckled and black speckled landrace selection produced under irrigated conditions and the plain red landrace selection under rainfed conditions. Higher calcium content was observed in their seed coats and consequently reduced electrolyte leakage. Seed coat thickness also contributed to the degree of electrolyte leakage as advanced by Sinefu (2011); this was particularly evident in the low EC (857 $\mu s.g^{-1}$) of the brown speckled that had the thickest seed coat (93.19 µm) under both production environments and the high EC (2253 $\mu s.g^{-1}$) of the plain cream that had the thinnest seed coat (68.99 µm) under the irrigated environment. As previously mentioned, the rainfed and irrigated plants were harvested at the same time based on maturity in rainfed plants. If the irrigated progeny had not reached maximum dry mass (completion of reserve deposition), and there is rapid drying it disrupts cellular membranes and internal structures. A high influx of water disposed seeds to imbibitional injury as indicated by increased rates of solute leakage, a symptom of cellular membrane disruption (Black *et al*. 2006). A period of slow dehydration should be allowed at the end of maturation when the seeds are still attached to the parent plant for the acquisition of desiccation tolerance. The latter might not have been allowed in the irrigated plants, hence the high EC observed.

It is important to note that the seeds used in the current study had previously had their seed quality evaluated prior to planting (Chapter 4). At harvest, we harvested progeny that was true to type with respect to phenotypic seed colour. Interestingly, initial seed quality was

inconsistent with that in the current study, regardless of production environments. The initial seedlot was of inferior germinability and superior vigour as compared to the subsequent generation. The initial seedlot was also bigger and heavier, accompanied by a thicker seed coat compared to the successive generation. Seed mineral composition also varied significantly and according to Roach and Wulff (1987), seed mineral composition is largely influenced by environmental conditions the seed is produced under. The only consistent finding with respect seed coat mineral composition was that iron was only present in the seed coat of the plain red seeds. The initial seedlot was sourced from a local seed company; information describing production environment and management conditions was unavailable. Differences in production environments may possibly explain our observed differences in seed quality of the two generations. However, Odindo (2007) found no differences in seed quality (germinability) of cowpea produced under different environments. This suggests that there may have been other reasons for our observations. Another possibility is that ageing and storage conditions may also have contributed to observed differences in seed quality (Sultana 1994, Kapoor *et al.* 2011, Moncaleano-Escandon *et al.* 2013, Shaban 2013). Nevertheless, similar to the previous generation we found the dark coloured and similarly speckled landrace selections (plain red and black speckled) to perform better in terms of germinability than the light coloured landrace selection regardless of production environment.

Yield and subsequent seed quality of bambara groundnut are negatively affected by water stress. Yield of bambara groundnut was lower under rainfed than irrigated conditions. This implies that where irrigation is available, higher yields could be achieved. Under rainfed conditions, a positive effect on harvest index accounted for yield stability. This implies that under water limited conditions dry matter partitioning will favour yield over vegetative growth thus ensuring attainment of some yield. This makes bambara groundnut suitable for rainfed cropping systems characterised by drought. However, sub-optimum growing conditions (rainfed) resulted in progeny of inferior seed quality. Seed quality (viability and vigour) was lower in seed produced under rainfed than irrigated conditions. This suggests optimum conditions for growing bambara groundnut for purposes of seed production. In the absence of this, farmers may be risking recycling seed with inferior seed quality; this may negatively affect yields in the short to medium term. We found the plain cream landrace selection to yield better compared to other landrace selections under both production environments (rainfed and irrigated). Compared to other landrace selections, the plain red landrace selection performed better with respect to seed quality. Future studies should focus

on strategies aimed at enhancing seed quality of cultivated landraces, short term, and establishing proper seed systems (long term).

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

Drought and climate change are phenomena introducing new dynamics and further threatening agriculture in Africa, especially in semi– and arid areas (Rijsberman 2006). Changes in seasonal timing of rainfall as well as higher incidence and severity of drought are expected (OECD 2011). This will affect crop production and major crops are likely to fail. Scientists have identified bambara groundnut (*Vigna subterranea* L.), an indigenous African grain legume as a possible future crop because of its drought tolerance (Collinson 1997, Azam-Ali *et al*. 2001, Sinefu 2011, Vurayai *et al*. 2011ab, Mabhaudhi and Modi 2013, Zondi 2013), heat tolerance (Berchie 2012) and high water use efficiency (WUE) (Azam–Ali *et al*. 2004, Mabhaudhi *et al*. 2013), especially when compared to its exotic counterpart *Arachis hypogaea*. Although common among subsistence farmers in rural areas of sub-Saharan Africa, the crop still remains underutilized (Azam-Ali *et al*. 2011, Bamshiye *et al*. 2011). Efforts to promote bambara groundnuts include identifying possible reasons for poor uptake by farmers, conducting research in order to address these problems and possibly devising strategies to improve its production. Among the demerits of bambara groundnut is the reported poor seed quality and consequently poor and slow emergence under field conditions.

Bambara groundnut is cultivated using landraces and little is known about their seed quality. Studies have suggested using seed coat colour as a selection criterion for seed colour (Mabhaudhi 2012), while another study found that provenance also had an effect on seed quality (Zondi 2013). This study sought to contribute to these efforts on using seed coat colour as a criterion for seed quality by exploring speckling in bambara groundnut. The study also went further to determine seed quality characteristics of bambara groundnut and how they affect seed germination and vigour. Seed quality is intricately related with emergence and early crop establishment. The reported poor and slow establishment of bambara groundnut could lead to unproductive water loss to evaporation, negatively affecting total water use efficiency (Mabhaudhi *et al*. 2013). Understanding seedling establishment of bambara groundnut could aid in identifying solutions to improve seed performance under field conditions. Seedling establishment is an important phenological event as it influences crop stand and ultimately crop yield. Furthermore, it was hypothesised that if bambara

groundnut is produced under marginal conditions and farmers use seed retained from previous harvests this could be the cause of inferior seed quality; farmers continuously recycling seed of inferior quality.

7.2 Aims and Objectives

The aim of this study was to evaluate seed quality of selected seed coat colours of bambara groundnut, determine water use efficiency during seedling establishment and to determine the effect of water stress on maternal plants on subsequent seed quality of bambara groundnut. It was hypothesised that seed coat colour had no effect on (i) seed performance in terms of germination, vigour and (ii) seedling water use efficiency of bambara groundnut.

The specific objectives of the study were to:

1. determine the effect of seed coat colour of bambara groundnut on seed performance, i.e. germination capacity and vigour,
2. to evaluate water use efficiency and water use characteristics at the seedling establishment of selected seed coat colours of bambara groundnut under varying water regimes in a controlled environment facility,
3. to evaluate yield and yield components of selected bambara groundnut seed coat colours under rainfed and irrigated field conditions, and
4. to evaluate subsequent seed quality of selected bambara groundnut seed coat colours in response to imposed stress on maternal plants under field conditions.

7.3 Challenges

- Seed of bambara groundnut was not easily available. We finally sourced it from a local seed company and information regarding the production environment and age of the seed was not available to us.
- Bambara groundnut is susceptible to sclerotia root rot. We experienced two crop failures under controlled environment conditions due to this. The plant pathologists advised that it was difficult to control; we had to change the soil and growth tunnel.
- We experienced erratic emergence in our first and second field trial attempts, hence the decision to establish seedlings under controlled environment and transplant into the field.
- We experienced extreme weather conditions under field conditions. The continuous showers during 73 DAT to 85 DAT followed by high temperatures led to fungal diseases. A heavy storm (72 mm) was accompanied by strong winds and this led to damage to the crop and possibly yield loss.

7.4 Future Teaching, Learning and Research Possibilities

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

- Seed physiology may prove to be vital in grasping a better understanding in the relationship between seed morphology and seed quality. These measurements may also be useful for breeding purposes and selecting particular traits.
- Scientists should expand their knowledge on seed mineral composition in different structures of the seed and how it affects seed performance.
- Future studies should evaluate the genetics of seed coat colour and its variations if this characteristic is to be exploited for future crop improvement.
- Extension officers should be encouraged to obtain seed from farmers and submit it for seed quality testing. Better selection of material to be used for seed in the next season should also be provided to farmers. The use of good quality seeds may result in improved yields for farmers.
- Farmers should be encouraged to plant dark coloured seeds for the purposes of seed production and if possible irrigate the plot intended for seed production/multiplication to ensure good quality seed.

- In the case where seed quality is not known and rainfall pattern is uncertain, farmers and extension workers should explore transplanting seedlings as opposed to direct seeding in order to ensure maximum crop stand.
- Researchers and extension officers should be encouraged to use decision support tools such as crop models to aid in strategic, tactical and operational decision making for yield maximisation.

7.5 Final Comments and Summary Conclusions

The review of literature showed that bambara groundnut is an important African indigenous legume with high nutritional value, drought tolerance characteristics and N-fixation properties. This combination makes it a possible multi-use crop in marginal areas of agricultural production with great potential to contribute to food and nutritional security in Africa, more so with predicted climate change. However, despite showing much potential, the crop remains relatively underutilised.

Seed viability did not entail good seed vigour. While the dark coloured and similarly speckled landrace selections were more viable, the plain cream landrace selection was more vigorous. However, during seedling establishment the plain red landrace selection performed better than light coloured seeds especially under stress conditions. We found that the superior performance of dark coloured seeds was associated with high phenolic compounds. In addition to proline that has been shown by Vurayai *et al*. (2011a) and Zondi (2013) to accumulate under water stress, this study showed that total soluble sugars and antioxidants also accumulate under water stress. This contributed to drought avoidance strategies and acclimation to water stress present in bambara groundnut seedlings. Withdrawing irrigation led to changes in physiological and metabolic responses, with previously stressed seedlings showing better acclimation to terminal stress compared to previously non–stressed seedlings. Water use efficiency of bambara groundnut seedlings increased with decreasing water supply and this was mainly a function of reduced water use (denominator) and not biomass (numerator). Improved WUE under water limited conditions was associated with smaller seedling canopy size, longer roots, and higher root: shoot ratio as well as accumulation of solutes which allowed for maintenance of high tissue water potential.

Yield and subsequent seed quality of bambara groundnut were negatively affected by water stress. Yield of bambara groundnut was lower under rainfed than irrigated conditions.

This implies that where irrigation is available, higher yields could be achieved. Bambara groundnut was able to produce reasonable yields under rainfed conditions owing to its ability to favour dry matter partitioning to yield over vegetative growth under water stress conditions. However, sub-optimum growing conditions (rainfed) resulted in progeny of inferior seed viability. Viability was lower in seed produced under rainfed than irrigated conditions. This suggests that optimum conditions are needed for bambara groundnut seed production. In the absence of this, farmers may be risking recycling seed with inferior seed quality; this may negatively affect yields in the short to medium term. The plain cream landrace selection was the highest yielding landrace selection under both rainfed and irrigated conditions. Although not consistent, in general we observed the plain red landrace selection to perform better with respect to seed quality, compared to the other landrace selections.

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APPENDICES

Appendix 1: List of ANOVAS for Chapter 4

Variate: %_Germ

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	140.00	70.00	1.53	
Rep.*Units* stratum					
Time	9	133835.87	14870.65	324.72	<.001
Landrace selection	3	2886.80	962.27	21.01	<.001
Time.Landrace selection	27	1490.53	55.20	1.21	0.258
Residual	78	3572.00	45.79		
Total	119	141925.20			

CV (%) = 15.8

Variate: Seedling_length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	930.	465.	0.30	
Rep.*Units* stratum					
Landrace selection	3	6338.	2113.	1.35	0.343
Residual	6	9363.	1560.		
Total	11	16631.			

CV (%) = 40.4

Variate: Shoot_length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	12.64	6.32	0.41	
Rep.*Units* stratum					
Landrace selection	3	313.17	104.39	6.83	0.023
Residual	6	91.66	15.28		
Total	11	417.47			

CV (%) = 13.6

Variate: Root_length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1157.	578.	0.43	
Rep.*Units* stratum					
Landrace selection	3	4384.	1461.	1.08	0.426
Residual	6	8111.	1352.		
Total	11	13653.			

CV (%) = 53.2

Variate: Root_shoot_Ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.0958	1.0479	1.18	
Rep.*Units* stratum					
Landrace selection	3	2.1177	0.7059	0.79	0.541
Residual	6	5.3443	0.8907		
Total	11	9.5578			

CV (%) = 39.2

Variate: Dry_mass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.034698	0.017349	5.72	
Rep.*Units* stratum					
Landrace selection	3	1.217241	0.405747	133.69	<.001
Residual	6	0.018210	0.003035		
Total	11	1.270148			

CV (%) = 2.8

Variate: Fresh_mass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.31308	0.15654	5.25	
Rep.*Units* stratum					
Landrace selection	3	0.99278	0.33093	11.09	0.007
Residual	6	0.17903	0.02984		
Total	11	1.48489			

CV (%) = 4.8

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.892	1.946	0.46	
Rep.*Units* stratum					
Landrace selection	3	43.519	14.506	3.41	0.094
Residual	6	25.541	4.257		
Total	11	72.951			

CV (%) =14.0

Variate: MGT_Days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00022	0.00011	0.01	
Rep.*Units* stratum					
Landrace selection	3	0.13482	0.04494	3.03	0.115
Residual	6	0.08905	0.01484		
Total	11	0.22409			

CV (%) = 1.6

Variate: Ec_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	27437169.	3048574.	18.08	
Rep.*Units* stratum					
Landrace selection	3	42035449.	14011816.	83.11	<.001
Time_hrs	23	52220635.	2270462.	13.47	<.001
Landrace selection.Time_hrs	69	24269187.	351727.	2.09	<.001
Residual	855	144143479.	168589.		
Total	959	290105920.			

CV (%) = 121.1

Variate: %_change (seed testing water bath)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.489E-02	7.444E-03	0.90	
Rep.*Units* stratum					
Landrace selection	3	1.936E+01	6.455E+00	779.00	<.001
Time_Hrs	9	2.645E+04	2.939E+03	3.547E+05	<.001
Landrace selection.Time_Hrs	27	6.452E+02	2.390E+01	2883.82	<.001
Residual	78	6.463E-01	8.286E-03		

Total	119	2.712E+04			

CV (%) = 1

Variate: Aw

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.067E-06	5.333E-07	0.50	
Rep.*Units* stratum					
Landrace selection	3	4.938E-03	1.646E-03	1548.00	<.001
Time_Hrs	9	2.975E+00	3.306E-01	3.109E+05	<.001
Landrace selection.Time_Hrs	27	3.365E-01	1.246E-02	11722.01	<.001
Residual	78	8.293E-05	1.063E-06		

CV (%) = 0.1

Variate: %_change (seed soaking method)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.2744	0.6372	0.99	
Rep.*Units* stratum					
Landrace selection	3	534.0979	178.0326	277.71	<.001
Time_hrs	12	36711.6679	3059.3057	4772.15	<.001
Landrace selection.Time_hrs	36	12263.1194	340.6422	531.36	<.001
Residual	102	65.3896	0.6411		
Total	155	49575.5493			

CV (%) = 3.3

Variate: Aw

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.205E-07	1.603E-07	3.76	
Rep.*Units* stratum					
Landrace selection	3	3.364E-03	1.121E-03	26313.59	<.001
Time_hrs	12	3.044E+00	2.537E-01	5.954E+06	<.001
Landrace selection.Time_hrs	36	2.876E-02	7.988E-04	18746.79	<.001
Residual	102	4.346E-06	4.261E-08		
Total	155	3.076E+00			

Variate: Thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	15.229	7.614	2.22	
Rep.*Units* stratum					
Landrace selection	3	109.666	36.555	10.66	0.008
Residual	6	20.573	3.429		

Total	11	145.468

CV (%) = 1.7

Appendix 2: List of ANOVAs for Chapter 5

Variate: %_Emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	225.77	75.26	1.05	
Rep.*Units* stratum					
DAP	9	376815.05	41868.34	582.92	<.001
Landrace selection	3	4535.29	1511.76	21.05	<.001
Treatment	2	1308.67	654.34	9.11	<.001
DAP.Landrace selection	27	7305.70	270.58	3.77	<.001
DAP.Treatment	18	3206.63	178.15	2.48	<.001
Landrace selection.Treatment	6	1443.03	240.50	3.35	0.003
DAP.Landrace selection.Treatment	54	3973.64	73.59	1.02	0.433
Residual	357	25641.58	71.83		
Total	479	424455.36			

CV (%) = 39.1

Variate: MET_Days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	0.30073	0.10024	2.49	
REP.*Units* stratum					
Landrace_selection	3	0.40710	0.13570	3.37	0.068
Residual	9	0.36212	0.04024		
Total	15	1.06995			

CV (%) = 2.3

Variate: Plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	82.659	27.553	6.97	
Rep.Treatment stratum					
Treatment	2	163.978	81.989	20.75	0.002
Residual	6	23.703	3.950	2.49	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	6.332	2.111	1.33	0.285
Treatment.Seed_colour	6	11.106	1.851	1.17	0.353
Residual	27	42.871	1.588	0.63	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	2	2404.257	1202.128	475.83	<.001
Treatment.DAP	4	130.565	32.641	12.92	<.001
Seed_colour.DAP	6	24.202	4.034	1.60	0.161
Treatment.Seed_colour.DAP	12	9.130	0.761	0.30	0.987
Residual	72	181.900	2.526		

Total	143	3080.702			

Variate: CCI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	50.95	16.98	0.78	
Rep.Treatment stratum					
Treatment	2	109.18	54.59	2.52	0.161
Residual	6	130.02	21.67	0.80	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	87.36	29.12	1.07	0.377
Treatment.Seed_colour	6	124.27	20.71	0.76	0.605
Residual	27	732.36	27.12	1.30	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	2	49.86	24.93	1.19	0.310
Treatment.DAP	4	44.49	11.12	0.53	0.713
Seed_colour.DAP	6	94.83	15.80	0.76	0.607
Treatment.Seed_colour.DAP	12	166.30	13.86	0.66	0.781
Residual	72	1506.34	20.92		
Total	143	3095.94			

Variate: PEA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	133.42	44.47	0.99	
Rep.Treatment stratum					
Treatment	2	89.35	44.67	1.00	0.423
Residual	6	268.75	44.79	1.00	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	132.67	44.22	0.99	0.413
Treatment.Seed_colour	6	267.10	44.52	1.00	0.448
Residual	27	1207.45	44.72	1.00	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	2	88.05	44.02	0.98	0.379
Treatment.DAP	4	178.76	44.69	1.00	0.414
Seed_colour.DAP	6	266.94	44.49	1.00	0.435
Treatment.Seed_colour.DAP	12	535.60	44.63	1.00	0.460
Residual	72	3219.24	44.71		
Total	143	6387.33			

Variate: Leaf_number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.1250	0.3750	0.77	

Rep.Treatment stratum					
Treatment	2	3.5625	1.7812	3.64	0.092
Residual	6	2.9375	0.4896	3.16	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	0.8750	0.2917	1.88	0.157
Treatment.Seed_colour	6	1.9375	0.3229	2.08	0.089
Residual	27	4.1875	0.1551	0.35	
Rep.Treatment.Seed_colour.*Units* stratum					
	48	21.0000	0.4375		
Total	95	35.6250			

Variate: leaf_area_cm2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.2	0.7	0.07	
Rep.Treatment stratum					
Treatment	2	5286.9	2643.4	257.02	<.001
Residual	6	61.7	10.3	0.44	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	12.2	4.1	0.17	0.913
Treatment.Seed_colour	6	18.2	3.0	0.13	0.991
Residual	27	630.9	23.4	0.09	
Rep.Treatment.Seed_colour.*Units* stratum					
	48	12549.8	261.5		
Total	95	18562.0			

Variate: PEA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1116.45	372.15	10.34	
Rep.Treatment stratum					
Treatment	3	125.64	41.88	1.16	0.376
Residual	9	323.93	35.99	0.43	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	42.18	14.06	0.17	0.917
Treatment.Seed_colour	9	948.61	105.40	1.27	0.288
Residual	36	2996.89	83.25	1.35	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	6	512.64	85.44	1.39	0.219
Treatment.DAP	18	873.39	48.52	0.79	0.714
Seed_colour.DAP	18	957.44	53.19	0.86	0.623
Treatment.Seed_colour.DAP					
	54	3579.30	66.28	1.08	0.343
Residual	288	17723.61	61.54		
Total	447	29200.09			

Variate: CCI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	298.66	99.55	4.65	
Rep.Treatment stratum					
Treatment	3	6921.93	2307.31	107.79	<.001
Residual	9	192.66	21.41	0.55	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	197.35	65.78	1.69	0.186
Treatment.Seed_colour	9	125.75	13.97	0.36	0.947
Residual	36	1399.03	38.86	2.94	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	6	5773.83	962.31	72.89	<.001
Treatment.DAP	18	3835.35	213.08	16.14	<.001
Seed_colour.DAP	18	207.69	11.54	0.87	0.611
Treatment.Seed_colour.DAP	54	428.23	7.93	0.60	0.987
Residual	288	3802.19	13.20		
Total	447	23182.67			

Variate: Phenols_1 Phenols

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	8437.9	2812.6	0.88	
Rep.Treatment stratum					
Treatment	3	24311.6	8103.9	2.53	0.123
Residual	9	28869.0	3207.7	1.49	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	51696.8	17232.3	7.99	<.001
Treatment.Seed_colour	9	21159.5	2351.1	1.09	0.393
Residual	36	77595.5	2155.4	3.49	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	6	60142.4	10023.7	16.24	<.001
Treatment.DAP	18	148821.4	8267.9	13.40	<.001
Seed_colour.DAP	18	32212.5	1789.6	2.90	<.001
Treatment.Seed_colour.DAP	54	86627.4	1604.2	2.60	<.001
Residual	288	177757.6	617.2		
Total	447	717631.7			

Variate: TAO_mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.19568	0.06523	2.48	
Rep.Treatment stratum					

Treatment	3	2.13621	0.71207	27.03	<.001
Residual	9	0.23707	0.02634	0.36	

Rep.Treatment.Seed_colour stratum

Seed_colour	3	0.31582	0.10527	1.43	0.249
Treatment.Seed_colour	9	1.83508	0.20390	2.78	0.014
Residual	36	2.64466	0.07346	1.79	

Rep.Treatment.Seed_colour.*Units* stratum

DAP	6	16.85777	2.80963	68.55	<.001
Treatment.DAP	18	8.31755	0.46209	11.27	<.001
Seed_colour.DAP	18	1.71733	0.09541	2.33	0.002
Treatment.Seed_colour.DAP	54	2.11738	0.03921	0.96	0.564
Residual	288	11.80382	0.04099		

Total	447	48.17836

Variate: TSS_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	48.91	16.30	1.60	
Rep.Treatment stratum					
Treatment	3	460.32	153.44	15.06	<.001
Residual	9	91.70	10.19	0.42	
Rep.Treatment.Seed_colour stratum					
Seed_colour	3	55.23	18.41	0.75	0.528
Treatment.Seed_colour	9	267.40	29.71	1.21	0.317
Residual	36	880.55	24.46	0.92	
Rep.Treatment.Seed_colour.*Units* stratum					
DAP	2	10034.63	5017.31	188.41	<.001
Treatment.DAP	6	7845.02	1307.50	49.10	<.001
Seed_colour.DAP	6	110.39	18.40	0.69	0.657
Treatment.Seed_colour.DAP	18	733.29	40.74	1.53	0.096
Residual	96	2556.43	26.63		
Total	191	23083.85			

Variate: Seedling_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	44.659	14.886	8.22	
REP.*Units* stratum					
Landrace_selection	3	8.908	2.969	1.64	0.199
FC	2	91.803	45.902	25.36	<.001
Landrace_selection.FC	6	12.642	2.107	1.16	0.349
Residual	33	59.734	1.810		
Total	47	217.746			

CV (%) = 6.9

Variate: Shoot_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	19.391	6.464	4.21	
REP.*Units* stratum					
Landrace_selection	3	4.886	1.629	1.06	0.379
FC	2	196.004	98.002	63.90	<.001
Landrace_selection.FC	6	11.251	1.875	1.22	0.320
Residual	33	50.615	1.534		
Total	47	282.147			

CV (%) = 8.7

Variate: Root_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	5.3310	1.7770	4.77	
REP.*Units* stratum					
Landrace_selection	3	0.7482	0.2494	0.67	0.577
FC	2	19.7349	9.8675	26.50	<.001
Landrace_selection.FC	6	1.7209	0.2868	0.77	0.599
Residual	33	12.2873	0.3723		
Total	47	39.8224			

CV (%) = 11.5

Variate: Root__Shoot_ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	0.000512	0.000171	0.05	
REP.*Units* stratum					
Landrace_selection	3	0.001783	0.000594	0.18	0.908
FC	2	0.469979	0.234990	71.86	<.001
Landrace_selection.FC	6	0.017647	0.002941	0.90	0.507
Residual	33	0.107906	0.003270		
Total	47	0.597827			

CV (%) = 14.7

Variate: Dry_mass_g_1 Dry_mass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum					
Variety	3	0.02311	0.00770		
REP.*Units* stratum					
Variety	3	0.10566	0.03522	2.89	0.050
FC_%	2	0.00713	0.00357	0.29	0.748
Variety.FC_%	6	0.02533	0.00422	0.35	0.907
Residual	33	0.40263	0.01220		

Total	47	0.56386			

CV (%) = 22.4

Variate: Midday Stem water potential_MPa

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.005672	0.002836	0.38	
Rep.*Units* stratum					
Variety	3	0.067122	0.022374	2.99	0.053
FC_%	2	0.196739	0.098369	13.14	<.001
Variety.FC_%	6	0.105194	0.017532	2.34	0.067
Residual	22	0.164661	0.007485		
Total	35	0.539389			

CV (%) = 22.8

Variate: Predawn Leaf water potential_MPa

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02896	0.01448	0.93	
Rep.*Units* stratum					
Variety	3	0.08930	0.02977	1.91	0.157
FC_%	2	0.07287	0.03644	2.34	0.120
Variety.FC_%	6	0.06902	0.01150	0.74	0.624
Residual	22	0.34271	0.01558		
Total	35	0.60286			

CV (%) = 58.4

Variate: CCI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	298.66	99.55	6.15	
Rep.*Units* stratum					
DAP	6	5773.83	962.31	59.41	<.001
Landrace selection	3	197.35	65.78	4.06	0.007
Treatment	3	6921.93	2307.31	142.45	<.001
DAP.Landrace selection	18	207.69	11.54	0.71	0.799
DAP.Treatment	18	3835.35	213.08	13.15	<.001
Landrace selection.Treatment	9	125.75	13.97	0.86	0.559
DAP.Landrace selection.Treatment	54	428.23	7.93	0.49	0.999
Residual	333	5393.88	16.20		
Total	447	23182.67			

CV (%) =10.2

Variate: PEA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.004579	0.001526	1.35	
Rep.*Units* stratum					
Treatment	3	1.599719	0.533240	472.48	<.001
Landrace selection	3	0.019640	0.006547	5.80	<.001
DAP	6	3.222300	0.537050	475.86	<.001
Treatment.Landrace selection	9	0.129976	0.014442	12.80	<.001
Treatment.DAP	18	1.434784	0.079710	70.63	<.001
Landrace selection.DAP	18	0.064713	0.003595	3.19	<.001
Treatment.Landrace selection.DAP	54	0.219946	0.004073	3.61	<.001
Residual	333	0.375821	0.001129		
Total	447	7.071478			

CV (%) = 4.8

Total	447	48.17836			

CV (%) = 53.3

Variate: WUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	8.264E-05	2.755E-05	15.26	
Rep.*Units* stratum					
FC_%	2	6.236E-05	3.118E-05	17.27	<.001
Variety	3	1.815E-05	6.050E-06	3.35	0.031
FC_%.Variety	6	1.131E-05	1.886E-06	1.04	0.415
Residual	33	5.957E-05	1.805E-06		
Total	47	2.340E-04			

CV (%) = 28.9

Appendix 3: List of ANOVAs for Chapter 6

Variate: Biomass_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	15.3	7.7	0.37	
Rep.Treatment stratum					
Treatment	1	1300.2	1300.2	62.63	0.016
Residual	2	41.5	20.8	0.16	
Rep.Treatment.Landrace_selection stratum					
Landrace_selection	3	454.1	151.4	1.16	0.364
Treatment.Landrace_selection					
	3	169.9	56.6	0.44	0.731
Residual	12	1560.4	130.0		
Total	23	3541.5			

Variate: pod_mass_per_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	10.71	5.36	2.36	
Rep.Treatment stratum					
Treatment	1	67.60	67.60	29.83	0.032
Residual	2	4.53	2.27	0.08	
Rep.Treatment.Landrace_selection stratum					
Landrace_selection	3	4.24	1.41	0.05	0.984
Treatment.Landrace_selection					
	3	18.05	6.02	0.22	0.883
Residual	12	334.21	27.85		
Total	23	439.34			

Variate: pod_number_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.7	2.9	0.08	
Rep.Treatment stratum					
Treatment	1	283.4	283.4	7.95	0.106
Residual	2	71.3	35.7	0.34	
Rep.Treatment.Landrace_selection stratum					
Landrace_selection	3	101.3	33.8	0.32	0.808
Treatment.Landrace_selection					
	3	22.4	7.5	0.07	0.974
Residual	12	1252.7	104.4		
Total	23	1737.0			

Variate: seed_mass_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.10	4.05	3.31	
Rep.Treatment stratum					
Treatment	1	19.64	19.64	16.05	0.057
Residual	2	2.45	1.22	0.07	
Rep.Treatment.Landrace_selection stratum					
Landrace_selection	3	5.90	1.97	0.11	0.955
Treatment.Landrace_selection	3	10.01	3.34	0.18	0.908
Residual	12	222.29	18.52		
Total	23	268.38			

Variate: seed_no_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.7	1.8	0.09	
Rep.Treatment stratum					
Treatment	1	320.4	320.4	16.39	0.056
Residual	2	39.1	19.5	0.18	
Rep.Treatment.Landrace_selection stratum					
Landrace_selection	3	53.8	17.9	0.16	0.920
Treatment.Landrace_selection	3	24.0	8.0	0.07	0.974
Residual	12	1331.9	111.0		
Total	23	1772.9			

Variate: HI_ (Pod)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00267	0.00134	0.58	
Rep.Treatment stratum					
Treatment	1	0.06338	0.06338	27.48	0.035
Residual	2	0.00461	0.00231	0.13	
Rep.Treatment.Landrace_selection stratum					
Landrace_selection	3	0.05157	0.01719	0.98	0.434
Treatment.Landrace_selection	3	0.00576	0.00192	0.11	0.953
Residual	12	0.21005	0.01750		
Total	23	0.33805			

Variate: Germination Percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	244.44	81.48	3.13	

Rep.*Units* stratum					
Landrace_selection	3	1661.11	553.70	21.24	<.001
Treatment	1	1680.56	1680.56	64.47	<.001
Time_Days	9	430072.78	47785.86	1833.22	<.001
Landrace_selection.Treatment	3	1976.11	658.70	25.27	<.001
Landrace_selection.Time_Days	27	3730.56	138.17	5.30	<.001
Treatment.Time_Days	9	855.56	95.06	3.65	<.001
Landrace_selection.Treatment.Time_Days	27	3343.33	123.83	4.75	<.001
Residual	237	6177.78	26.07		
Total	319	449742.22			

CV (%) = 7.3

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.3871	0.4624	0.75	
Rep.*Units* stratum					
Landrace_selection	3	29.1682	9.7227	15.85	<.001
Treatment	1	30.2222	30.2222	49.26	<.001
Landrace_selection.Treatment	3	3.4349	1.1450	1.87	0.166
Residual	21	12.8845	0.6135		
Total	31	77.0969			

CV (%) = 4.5

Variate: %5_seedlings_fresh_mass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1.4765	0.4922	1.06	
Rep.*Units* stratum					
Landrace_selection	3	35.0532	11.6844	25.07	<.001
Treatment	1	24.9571	24.9571	53.55	<.001
Landrace_selection.Treatment	3	28.7158	9.5719	20.54	<.001
Residual	21	9.7873	0.4661		
Total	31	99.9900			

CV (%) = 6.1

Variate: Root_length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4816.8	1605.6	1.89	
Rep.*Units* stratum					

Landrace_selection	3	2930.2	976.8	1.15	0.351
Treatment	1	4851.1	4851.1	5.73	0.026
Landrace_selection.Treatment	3	1514.1	504.7	0.60	0.625
Residual	21	17793.8	847.3		
Total	31	31906.0			

CV (%) = 26.0

Variate: Seedling_length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3576.	1192.	0.74	
Rep.*Units* stratum					
Landrace_selection	3	3732.	1244.	0.78	0.520
Treatment	1	10841.	10841.	6.76	0.017
Landrace_selection.Treatment	3	1532.	511.	0.32	0.812
Residual	21	33660.	1603.		
Total	31	53340.			

CV (%) = 19.3

Variate: Shoot_length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2277.1	759.0	1.12	
Rep.*Units* stratum					
Landrace_selection	3	420.8	140.3	0.21	0.890
Treatment	1	1188.3	1188.3	1.76	0.199
Landrace_selection.Treatment	3	94.8	31.6	0.05	0.986
Residual	21	14200.7	676.2		
Total	31	18181.7			

CV (%) = 27.3

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.31262	0.10421	2.52	
Rep.*Units* stratum					
Landrace_selection	3	0.37579	0.12526	3.03	0.052
Treatment	1	1.00779	1.00779	24.36	<.001

Landrace_selection.Treatment	3	0.23528	0.07843	1.90	0.161
Residual	21	0.86879	0.04137		
Total	31	2.80028			

CV (%) = 3.0

Variate: EC_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	5.905E+07	6.561E+06	12.42	
Rep.*Units* stratum					
Seed_colour	3	2.876E+07	9.587E+06	18.15	<.001
Treatment	1	7.676E+06	7.676E+06	14.53	<.001
Time	23	1.876E+08	8.155E+06	15.44	<.001
Seed_colour.Treatment	3	4.376E+07	1.459E+07	27.62	<.001
Seed_colour.Time	69	3.353E+07	4.860E+05	0.92	0.662
Treatment.Time	23	1.491E+07	6.484E+05	1.23	0.209
Seed_colour.Treatment.Time	69	2.063E+07	2.990E+05	0.57	0.998
Residual	1719	9.078E+08	5.281E+05		
Total	1919	1.304E+09			

CV (%) = 124.3

Variate: Root_shoot_Ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.6738	0.8913	3.35	
Rep.*Units* stratum					
Landrace_selection	3	0.3347	0.1116	0.42	0.741
Treatment	1	0.0095	0.0095	0.04	0.852
Landrace_selection.Treatment	3	0.8373	0.2791	1.05	0.392
Residual	21	5.5948	0.2664		
Total	31	9.4501			

CV (%) = 40.6

Variate: Seed_coat_thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	212.3	106.2	0.63	
Rep.*Units* stratum					
Landrace_selection	3	2485.1	828.4	4.88	0.016
Treatment	1	36.2	36.2	0.21	0.651

Landrace_selection.Treatment					
	3	70.4	23.5	0.14	0.935
Residual	14	2374.3	169.6		
Total	23	5178.4			

CV (%) = 16.3

Variate: %5_seedlings_dry_mass

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	(2)	0.01418	0.01418	0.44	
Rep.*Units* stratum						
Landrace_selection	3		2.48826	0.82942	25.60	<.001
Treatment	1		1.23486	1.23486	38.12	<.001
Landrace_selection.Treatment						
	3		1.57807	0.52602	16.24	0.003
Residual	6	(15)	0.19437	0.03240		
Total	14		(17)	2.69835		

CV (%) = 19.4