BAMBARA GROUNDNUT RESPONSE TO CONTROLLED ENVIRONMENT AND PLANTING DATE ASSOCIATED WATER STRESS

Fikile Sinefu

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE (CROP SCIENCE)

Crop Science

School of Agricultural Sciences and Agribusiness

Faculty of Science and Agriculture

University of KwaZulu-Natal

Pietermaritzburg

South Africa

December, 2011

DECLARATION

I, Fikile Sinefu, certify that the material reported in this thesis represents my original work, except where acknowledged. I further declare that these results have not otherwise been submitted in any form for any degree or diploma to any university. This study was financially supported by the Water Research Commission (Project No. K5 /1771//4).

Signature _____

Fikile Sinefu

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

Signature _____

Prof. A.T. Modi

ACKNOWLEDGEMENTS

My special and most sincere gratitude go to the following:

- My supervisor Prof. A.T. Modi for his guidance, support, spirit of *ubuntu* and for financial assistance in helping me to get drivers licence during the conduct of my study
- Tafadzwanashe Mabhaudhi for patiently helping me during the conduct of my study and “for them eyes we saw”
- Water Research Commission for funding the research work
- Matt Erasmus and his team for their willingness to always assist with my field work at Ukulinga
- Asanda Mditshwa for motivating me to further my studies
- Nhlanhla "Plot-it" Mathaba for assisting me during the planting of my trials
- Dr. Samson Tesfay for assisting with my laboratory experiments
- Staff at Ukulinga Research Farm for their assistance with my field trials
- The post grads in Room 344 (Bomikazi Gqola, Siboza Xolani, Nopayi Mkhize, Quaqua Mulbah, Kayleigh Moon, Khethuxolo Mbotho, Ronelle Bosse and Sandile Hadebe) for their assistance during the course of my study,
- Lastly, my family whose support made all the difference.

DEDICATION

This thesis is dedicated to God, the Almighty, for everything I am.

ABSTRACT

Bambara groundnut is a protein-rich legume, with food security potential in drought-prone regions. It has been grown for many centuries and has remained an important crop to most African subsistence farmers. However, despite its high nutritional status and yield advantages in poor soils, it remains one of the neglected crops by science. There have now been recent efforts to study underutilised crops, with the aim of promoting them as healthy alternatives for people facing resource and environmental challenges and to contribute to food security. In order to do this, there needs to be information that can be used to advise farmers on the agronomic aspects of producing the crop. The overall aim of the study was to evaluate the response of bambara groundnut landraces to drought under controlled environment and field conditions.

Seeds were initially collected from subsistence farmers in Jozini, KwaZulu-Natal, and characterised into three seed lots distinguished by seed coat colour: red, white and brown. In the initial study (Chapter 2) seed quality of bambara groundnuts was evaluated. Seed lots were used for standard germination (SG) and cold test (CT). Seeds were germinated under two conditions, 25°C for 8 days (SG) and 4°C for 7 days followed by 8 days at 25°C (CT). Germination percentage, seedling size and mass were determined. Desiccation tolerance was evaluated by suspending 30 seeds of each seed lot over saturated salt solutions of NaCl, LiCl, KNO_3 and H_2O (control) for 0, 2, 4, 8, 24 and 48 hours. Five seeds were sampled at each interval and stored at -21°C for 7 days. Samples were ground and analysed for proline content.

In addition, early establishment performance of bambara groundnut was evaluated under controlled environment conditions in seedling trays using two water regimes (Chapter 2). The experimental design had three factors: seed lot (colour), priming (NaCl, LiCl, KNO_3, H_2O and control) and water regimes [25% and 75% Field Capacity (F.C.)]. The experiment was replicated three times. Seedling emergence was determined daily for 21 days. Seedling height and leaf number were determined weekly for three weeks, thereafter, seedling leaf area, root and shoot mass (fresh and dry), root and shoot lengths and root to shoot ratio were also

determined. Seedlings were later transplanted in 90 pots for a pot trial in order to evaluate growth responses of bambara groundnut to water stress; plant height, leaf number and yield components were determined (Chapter 3).

Lastly, the use of planting date selection as a management strategy for managing the occurrence of water stress under field conditions was evaluated in field trials. The experimental design was a split-split-plot design with planting date as main factor (early, optimum and late), irrigation and rainfed as sub-main factor, and seed colour as sub-plots (brown, red and white) arranged in a randomised complete block design (RCBD), with three replications. There were three planting dates: 7 September (early planting), 24 November (optimum planting) and 19 January (late planting).

Results from Chapter 2 showed that the brown seed lot had the highest germination across treatments, followed by red and white seeds, respectively. There were significant differences between seed lots ($P < 0.05$) and salt solutions ($P < 0.05$) with respect to proline content. Seed proline content increased from 0 to 8 hours and later declined; NaCl was associated with the highest proline accumulation. There were highly significant differences ($P < 0.001$) between seed colours, priming treatments and F.C., as well as their interaction, with respect to seedling emergence. White seeds had the highest emergence, followed by brown and red, respectively. Priming seeds improved their emergence compared to the control, with highest emergence being observed in seeds treated with LiCl. Priming also improved emergence under water stress; 25% F.C. had the highest emergence compared to 75% F.C.

Results from Chapter 3 showed that, seeds primed with NaCl and KNO_3 resulted in tallest plants with the highest number of leaves per plant. However, NaCl and KNO_3 were also the most affected under water stress. Priming was shown to improve germination and early crop establishment of bambara groundnut landraces under water stress. However, yield per plant did not improve in response to either halo- or hydro-priming.

Results from field trials showed that in terms of the measured plant growth parameters (plant height, leaf number and LAI), bambara groundnut landraces were sensitive water stress. Water

stress decreased yield components, and hence yield. However, selection of planting dates was shown to be a useful management tool for managing water stress under water limited field conditions. Choice of planting date significantly affected both plant growth and yield. The optimum planting date resulted in the best crop growth for all measured plant growth parameters followed by late and early planting dates, respectively.

Seed quality was shown to be associated with seed lot colour. Darker coloured (red and brown) seeds performed better than light (white) seeds with respect to germination. Priming was also shown to improve germination and early crop establishment of bambara groundnut landraces under water stress. However, yield per plant did not improve following priming. Growth of bambara groundnut landraces was shown to be sensitive to water stress. Water stress decreased yield components and hence yield under both controlled and field conditions. Choice of planting date significantly affected both plant growth and yield. The optimum planting date was shown to be the best performing planting date.

The findings of this study suggest that bambara groundnut seed performance in terms of germination, stand establishment and productivity is associated with seed lot colour. Seed priming improves seed performance and enhances crop capacity to withstand water stress. If the optimum planting date for groundnuts (late spring to early summer) is missed, better crop performance and yield are obtained from late planting (late summer to early spring) compared with early planting (early spring). Bambara groundnut has a potential for production under water stress conditions in controlled and field environments.

Table of Contents

List of Figures

List of Tables

CHAPTER 1

LITERATURE REVIEW

1.0 Introduction

South Africa's population is increasing, thus intensifying the need to produce more food. This has increased the pressure on the country's already scarce water resources. South Africa is a water-stressed country located in a semi-arid part of the world (Walker *et al*., 1995; DWAF, 2002; Mukheibir, 2007). It receives an annual average rainfall of about 450 mm per year, most of which (80%) is received only in 5 months. This is far less than the world average of 860 mm per year; although South Africa's evaporation (ranging from 1100 to 3000 mm annually) is comparatively higher than the worldwide average. Consequently, South Africa's water resources are, in global terms, scarce and extremely limited in extent.

The demand for water already far exceeds the natural availability of water in several river basins. There are no truly large rivers in South Africa. The Zambezi River (88 000 million cubic meters) which is the largest river closest to South Africa generates more water than the total flow of all the rivers in the country combined which amounts, approximately, to a mere 53 500 million cubic meters per year (DWAF, 1986; 2002).

Furthermore, due to the predominantly hard rock nature of the South African geology, there are few major groundwater aquifers that exist with capacity for large scale utilisation. Hence, South Africa is mainly dependent on surface water resources for most of its urban, industrial and irrigation water supplies. Agriculture represents approximately 62% of the total water requirements of the country, with urban use (23%), rural (4%), mining/industrial (6%), forestry (3%) and power generation (2%) accounting for the remainder (Fig 1.1) (DWAF, 2002). This situation is not stagnant, as there is increasing demand and competition for water amongst the sectors. As such, there is now increased pressure on farmers, to utilise water more efficiently.

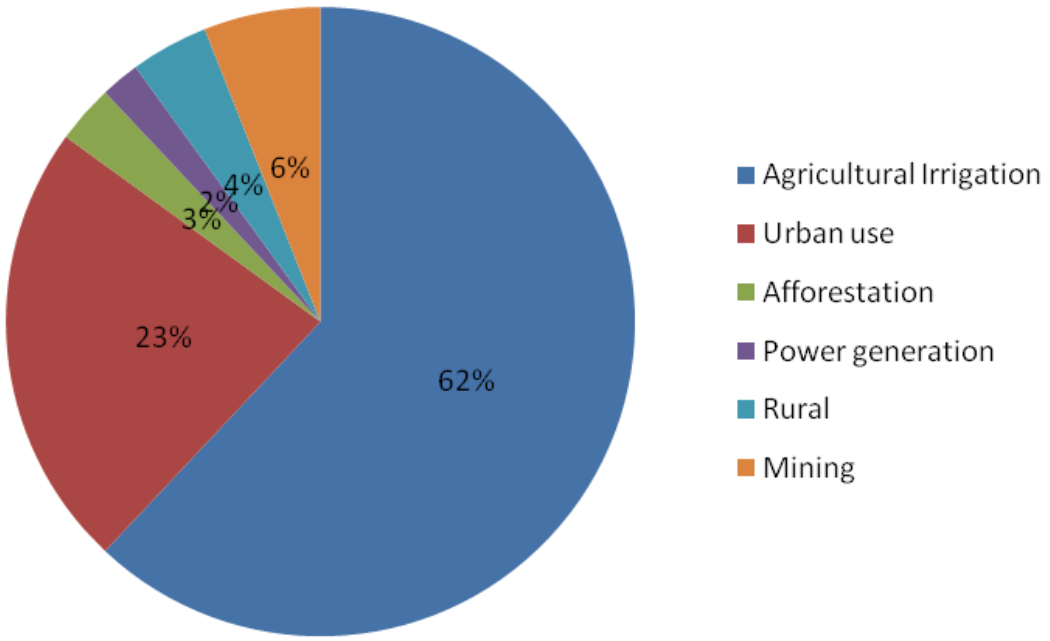


Figure 1.1: Water usage by different sectors in SA.

1.1 Water-use in agriculture

In arid and semi-arid areas, water is an important limiting factor to crop production, often resulting in low crop yields (Reij *et al*., 1991; Patil, 2010). It remains an ever-growing problem, limiting crop production worldwide and causing significant agricultural losses, particularly in arid and semi arid areas (Fischer and Turner, 1978; Boyer, 1982). It is important therefore, that the available water is used to the best advantage. According to Collinson *et al*. (1996, 1997), Craufurd and Wheeler (1999) and Mwale *et al*. (2007 b), water stress is the main limiting factor to crop production in contrast to the non-limiting amount of radiation received in the tropics. As a result, water driven crop growth generally dominates and crop growth is only driven by radiation in the absence of water stress. However, intercepted radiation and absorption of nutrients (Mohammadkhani and Heidari, 2008) can be reduced significantly under water stress. Water stress reduces plant growth, development and production. Given the above scenario, it is clear that water scarcity poses a major threat to South African agriculture and food security, especially in the arid and semi arid areas of the country (Boyer, 1982).

According to Johari-Pireivatlou *et al.* (2010), the best option in crop production is to introduce water–use-efficient crop varieties. Water use efficiency (WUE) is the ratio of biomass produced to water used (WU). Biomass can be the total biomass or a part of it such as above ground biomass or yield. Swanevelder (1998) and Massawe *et al.* (2005) stated that bambara was a suitable crop for production under traditional low input agricultural systems in Africa because of its drought tolerance and ability to produce reasonable yields when grown on poor soils. Thus, bambara is a legume crop with high potential for enhancing food security, especially in drought-prone regions (Toungos *et al.,* 2009). Its drought tolerance makes it best suited for production by resource-poor farmers. It can grow well in communal areas.

1.2 Bambara crop origin and importance

Bambara groundnut (*Vigna subterranea),* which is also known as *Nyimo* in Zimbabwe and *Jugo* beans in South Africa, originated in North Africa and migrated with indigenous people to South Africa. Their name originates from Bambara, a district on the Upper Niger near Timbuctoo. Due to the expansion of groundnut production, Bambara groundnut has been relegated to the status of an underutilized crop in most parts of Africa (Swanevelder, 1998). However, Bambara has been grown for centuries and has in the past contributed to the food security of Africa's poorest people (Swanevelder, 1998; FAO, 2001; Azam-Ali *et al*., 2001; Mwale *et al*., 2007a).

Traditionally, it has been cultivated in extreme, tropical environments by small-scale farmers without access to irrigation and/or fertilizers and with little guidance on improved practices. It is mainly grown by women for the sustenance of their families. Interestingly, its protein content (16–25%) is comparable or superior to other established legumes (Table 1.1), making it a good complement for cereal-based diets (Linnemann and Azam-Ali, 1993; Mwale *et al*., 2007a). As an underutilized crop, its germplasm improvement and management practices depend on local experience and resources (indigenous knowledge).

Table 1.1: Comparison of Bambara groundnut with other commonly used legume grain crops in terms of major nutrients (Linnemann and Azam-Ali, 1993).

	Bambara	**Soya**	**Cowpea**	**Kidney**	**Broad bean**	**Chickpea**
Calories (kCal/100g)	390	416	343	333	341	364
Protein (g/100g)	20.8	36.5	23.8	23.6	26.1	19.3
Carbohydrates (g/100g)	61.9	30.2	59.6	60	58.3	60.6
Fat (g/100g)	6.55	19.9.	2.1	0.8	5.7	6

Bambara groundnut is grown in the semi-arid tropics where water is usually in short supply (Mwale *et.al* 2007b). According to Linnemann and Azam-Ali (1993) Bambara groundnut plays an important role as a protein source. It also replenishes nitrogen in the soil through nitrogen fixation. This is important for resource-poor farmers, who cannot afford inorganic fertilizers. As a leguminous crop, bambara groundnut is useful in crop rotation; it acts as a source of residual nitrogen for the following cropping season.

1.2.1 Crop description

Bambara groundnut is an annual legume with a strong well-developed tap root and a short lateral stem on which the leaves are borne. Growth of Bambara groundnut can be divided into distinct vegetative and reproductive phases. The vegetative stage involves emergence and continuous production of leaves and roots. The leaves are trifoliate (± 5 cm long) with the petiole approximately 15 cm long. Leaves are stiff and grooved, and the base is green or purple in colour. The flowers are typically papilionaceous and borne in a raceme on long, hairy peduncles which arise from the nodes on the stem. The reproductive phase begins at flowering. Bambara has two types of flowers, the first branches and spreads and is usually self-pollinated, while the second is cross-pollinated by ants. It forms pods and seeds on or just below the soil surface. The pod is small (1.5 cm long), round or slightly oval shaped and

wrinkled with mostly one or sometimes two seeds. The unripe pod is yellowish green while mature pods are yellowish green or purple. After fertilisation the flower stem elongates while the sepal enlarges and the fruit develops above or just below the soil surface. Pod colour varies according to ripeness from light yellow to black, purple and other shades. The seeds are round with a diameter of about 1.5 cm and are smooth and when dried, very hard. Seeds also vary in colour with cream, brown, red, mottled and white being dominant (Swanevelder, 1998).

1.2.2 Agronomic requirements

Bambara groundnut takes 7 to 15 days to germinate; this, however, depends on the seed quality. Seed stored for about 12 months germinates well, but prolonged storage may result in loss of viability. Flowering starts 30 to 35 days after sowing and may continue until the plant senesces. It is a typical short day plant but fruit setting is delayed by long days. Delayed flowering and no fruit set was observed under long-day conditions. Vegetative growth takes place in spring and early summer and pods form only in late summer. Pod and seed development take place approximately 30 to 40 days after fertilisation. The pod develops first and this takes up to 30 days after fertilization.

The seed develops 10 days after the pod has developed. Seeds are mature when the parenchyma layer surrounding the embryo has disappeared and the pods become light brown (Swanevelder, 1998).

Bambara beans will grow on any well-drained soil, but light, sandy loams with a pH of 5.0 to 6.5 are most suitable. The crop does well on poor soils which are low in nutrients. Abundance of nitrogen favours vegetative growth. Bambara groundnuts grow poorly in calcareous soil. Bambara gives the best yields on a deeply ploughed field with a fine seedbed. A level seedbed is best, but it can be planted on ridges when very wet conditions prevail. Johnson (1968) found that fertilizer can be applied before planting while nitrogen can also be applied at three weeks after planting as top-dressing to stimulate growth. The number of pods/per plant and number of seeds/pod of bambara groundnut are both influenced by soil fertility

Bambara is a legume crop and it fixes nitrogen from the atmosphere, therefore phosphorus becomes the most limiting element that has to be applied on the crop (Tanimu, 1996). Phosphorus stimulates early growth and root formation. It also promotes seed production, hastens maturity, stabilises stem growth of the crop and is an active ingredient of protoplasm.

In South Africa, Bambara is sown during October and November after good rains. Later plantings produce lower yields. Seed size varies and therefore seeding rate can vary from 25 to 75 kg/ha. The average 1 000 seed mass is about 500 to 750 g. The recommended spacing is 10 to 15 cm in single rows 45 to 90 cm apart. In Swaziland the highest yield was obtained with 50 cm row spacing. Planters with the correct plates can be used. In Africa, a hand hoe is used to plant seed. It is usually sown and covered with a harrow. In conditions of high moisture levels and in heavy soils (which cannot be recommended) seed can be planted 2.5 to 3.0 cm deep and 5.0 to 7.5 cm deep in sandy soil. Large seeds are recommended. The seed should be treated with a fungicide. Seed vigour deteriorates after shelling, and shelling should therefore be done prior to planting (Swanevelder, 1998).

1.2.3 Food security

Immature seeds of bambara are normally boiled and eaten as an early harvested source of food while fully-matured seeds are cooked or made into flour. The high carbohydrate (65%) and relatively high protein (18%) content of bambara groundnut makes it a wholesome food (Linnemann, 1990; Brough and Azam-Ali, 1992). In northern Ghana, the fresh immature beans are boiled and consumed after adding a little salt. The dry beans are also boiled, crushed and made into cakes or balls, which are then fried and used to prepare stews. In southern Ghana, the beans are usually soaked overnight, after which they are boiled until soft, to produce a kind of porridge/blancmange. Capsicum pepper and salt may be added during the boiling process. This preparation, called ‘aboboi’, is served with ‘gari’ (roasted, grated cassava) or with mashed, fried, ripe plantain.

In Ghana, during the early 1960s, Bambara groundnut was canned in tomato sauce with pieces of meat, in brine, or as ‘aboboi’. Canned bambara groundnut was very popular, and competed

favourably with Heinz baked beans; however, the state-owned cannery has since collapsed. In restaurants in Angola and Mozambique, boiled salted seeds are often served as appetisers. Bambara has the potential for providing a balanced diet in areas where animal protein is expensive and where cultivation of other legumes is risky due to unfavourable rainfall (Coudert, 1982).

In South Africa, Bambara groundnut production is largely confined to the Northern Provinces (North West and Limpopo), Mpumalanga and KwaZulu-Natal. In the North it is grown in the Letaba and Louis Trichardt districts (Limpopo), and in the Baberton (Mpumalanga), Pietersburg (Polokwane), Pilgrims Rest (Mpumalanga) and Potgietersrust (Mokopane) districts. In KwaZulu-Natal, it is mainly grown in the Greytown, Msinga, Nkandla, Nquthu, Makhatini and Kosibaai areas. It is also grown on a small scale in Ixopo and Maphumulo areas of KwaZulu-Natal. Somewhat unsuccessful attempts have been made by agricultural cooperatives to grow Bambara groundnut commercially as well as for export (Swanevelder, 1998). In these areas, it is cultivated both as a sole crop and as an intercrop (with maize, cowpeas and melons).

In Africa, Bambara groundnut is generally cultivated by women on small plots**.** The size of bambara plots ranges from 300 to 2 500 m^2/farmer (Swanevelder, 1998). Production is primarily at subsistence level, and only surplus is sold. Yields are usually low because the production environments are characterized by various abiotic and biotic stresses. However, even under optimal conditions the yields are variable and unpredictable; this is partly due to variability in growth and development of individual plants within landraces.

Globally, in 1982, Bambara groundnut production was around 330 000 t, this is before expansion of groundnut production (Swanevelder, 1998; Linnemann 1994). Out of this, about 150 000-160 000 t, representing 45-50%, came from West Africa. On average, Bambara yields vary from 50 kg to 4 t/ha; yields of over 3 t/ha were reported in a cultivar trial conducted by Agricultural Research Council (ARC) at Potchefstroom, South Africa (Swanevelder, 1998). Previous predictions indicated that bambara groundnut may be unproductive in some areas of

southern Africa. For example all of Lesotho was classified as unsuitable due to low temperatures. In contrast, most of Botswana (90%), South Africa (57%) and Namibia (70%) have been classified as being suitable for bambara production (FAO, 2001).

Bambara groundnut is widely considered to be drought tolerant and was reported to out-perform groundnut *(Arachis hypogaea* L.) (a morphologically similar species) under dry environments (Collinson *et al*., 1996). Previous controlled environment experiments showed that bambara groundnut was capable of producing reasonable yields under conditions where groundnut may fail completely (Collinson *et al*., 1997; Babekir, 1989). However, there is very little evidence on how its growth, development, resource capture and conversion and yield are affected by drought (Mwale *et al*., 2007b).

1.3 Water use

1.3.1 General crop response to water stress

Mwale *et al.* (2007b) investigated on the effects of soil water on resource capture and conversion of three landraces (DipC, S19-3 and UN-from Botswana, Namibia and Swaziland, respectively) of Bambara groundnut. The study was conducted under two soil water regimes, irrigated and non-irrigated treatments, where irrigation was withheld from flowering to harvest. They found that drought stress reduced total transpiration for DipC but not for the other two landraces. While this may indicate variations in the response of the three landraces to drought, it was not clear why drought did not affect the total transpiration of S19-3 and UN.

Across landraces, crops in the non-irrigated treatments effectively extracted water up to 90 cm, with a very small decline with depth, while the irrigated treatments extracted most of the water from the top 50 cm of the profile. This represented a major difference in the water extraction pattern between the crops in the drought treatment and those that were regularly irrigated. The major extractions of water from the upper layers of the profile found in the study are a common phenomenon in non-stressed crops (Pannu and Singh, 1993; Johnson and Henderson, 2002). There was evidence that the crops rooted beyond 100 cm as shown by the presence of roots at this depth both in the irrigated and non-irrigated treatments and the

extraction of water at 90 cm in the non-irrigated treatments. Maximum rooting depth could not be established in the study since root sampling and water measurements were restricted to within 100 cm of the profile due to the design of the Tropical Crops Research Unit (TCRU) glasshouses at the University of Nottingham, UK.

However, the fact that the plants in the study extracted water at 90 cm and roots were found at 100 cm indicates that the maximum rooting depth of Bambara groundnut is possibly well beyond 100 cm. Hence, there appears to be a strong possibility that the crop could be deeper rooting than some genotypes of chickpea, 90–120 cm (Zhang *et al*., 2000; Anwar *et al*., 2003b) and lentil, 90 cm (Zhang *et al*., 2000).

In water-limited environments, the efficiency with which water is converted to dry matter is very important to crop productivity. In the study, drought reduced dry matter by 20%. This was similar to earlier reports on the effects of drought on grain amaranth (*Amaranth* spp.) (Johnson and Henderson, 2002). However, the response of dry matter to drought is quite variable, as some studies have reported an increase in dry matter (Pannu and Singh, 1993; Foulkes *et al*., 2001) while others have found no effect of soil water content on dry matter (Liu and Stutzel, 2004). The dry matter of Bambara groundnut under adequate soil water in this study (2.05 g kg^{-1}) was within the range of 1.3–2.6 g kg^{-1} reported for several grain legumes (Siddique *et al*., 2001), but was higher than that reported for mungbean (Pannu and Singh, 1993) and slightly lower than the values for faba bean (Mwanamwenge *et al*., 1998) and chickpea (Anwar *et al*., 2003b).

However, under drought, dry matter (1.65 g kg−1) was higher than for various grain legumes examined by (Siddique *et al*., 2001), mungbean (Pannu and Singh, 1993) and lentil (Zhang *et al*., 2000), all of which were grown under more favourable soil moisture conditions. This suggests that the crop maintains some buffering on its dry matter and may maintain relatively higher productivity under drought in comparison to irrigated crops or other legumes. Certainly, with progressive soil water stress in the drought treatments of this study, a mean pod yield of 0.0165 g cm^{-1} was obtained (Mwale *et al*., 2007b). The variations in dry matter

between landraces and water regimes in this study demonstrated the lack of conventional behaviour of this parameter in Bambara groundnut (Azam-Ali *et al*., 1994).

The authors concluded that the study showed that soil water content had a significant impact on resource capture (water, CO_2 and light) and conversion (carbohydrates) in bambara groundnut. The ability of the crop to capture light significantly diminished in response to drought stress, resulting in lower intercepted radiation while water extraction increased to deeper layers of the profile. In addition, drought reduced radiation conversion coefficient and dry matter by 32% and 20%, respectively. Variations in the response to drought were observed among the landraces.

1.3.2 Effect of water stress on growth and development

Crop growth is susceptible to abiotic stress and any limitation at this stage affects the reproductive stage. The expansion of leaf cells is regulated by turgor pressure within cells and reduction of turgor potential will result in reduced leaf expansion (Squire, 1990; Turner, 1997). Water stress mostly affects growth of leaves and roots, stomatal conductance, photosynthesis and dry matter accumulation (Blum, 1996). Plants close stomata in response to water stress and this optimizes the water use efficiency of the plant on a daily basis, yet resulting in a reduction in CO_2 assimilation at times when peak irradiances are commonly encountered. Stomatal closure results in CO_2 fixation being low while photosynthetic electron transport is operating at normal rates.

Most plants are exposed to water stress due to extreme soil water deficits in arid and semi arid environments (Morgan, 1984). Large areas of the earth's surface where temperature would permit plant growth are arid or semi arid deserts. The survival of land plants in such areas relies on the availability of water and their adaptation under stress (Kramer, 1984). Adaptation to water stress in plants involves the reduction of cell dehydration by avoidance (leaf shedding, leaf rolling and low stomatal conductance) or tolerance through osmotic adjustment (Turner, 1979). Osmotic adjustment refers to the lowering of osmotic potential due to the net accumulation of solutes in response to water deficits or salinity (Munns and King, 1988).

According to Wyn Jones and Gorham (1983), they reported that osmotic adjustment is an important mechanism in drought tolerance as it enables a continuation of cell expansion, stomatal and photosynthetic adjustments and better plant growth by lowering their water potential in response to decreasing soil water

One of the initial responses of plants to water stress is the decrease of leaf expansion rate and closing of stomata in order to reduce water loss through transpiration. Stomatal limitation as a response to water stress attributes to decrease in both photosynthetic rate and internal carbon dioxide concentration (Reddy *et al.,* 2004). Water stress during vegetative growth affects leaf expansion and root elongation. Thus resulting in less radiation received by the crop and consequently reduces photosynthesis (Gardner *et al.,* 1985). According to Mwale *et al*. (2007a) Bambara reduced leaf area index (LAI) under soil moisture stress conditions for different landraces. Collinson *et al.* (1996; 1999) reported that leaf number decreased by up to 60% in drought treatments resulting in reduced LAI. Mwale *et al.* (2007a) reported that soil moisture stress reduced both leaf number and LAI of Bambara crop. However, leaf expansion was affected or reduced more than root growth and photosynthesis altered to increase root to shoot ratio (Bradford and Hsiao 1982; Sharpe and Davies, 1979; Allen *et al.*, 1990).

According to Azam-Ali, (1998) Bambara yields under severe water stress were extremely small or negligible. Drought increased the relative allocation of dry matter to roots and there were variations in this trait between landraces. Roots play an important role in crop growth by water uptake from the soil. The comparable range in the dry matter or water use ratio was 1.8 to 3 g kg^{-1}. However, this variation was largely accounted for by measurements of the actual leaf to air vapour pressure difference and, when these were taken into account, the resultant transpiration equivalents varied between 4.2 and 4.6 kPa kg^{-1}. Under irrigation, pod yields varied between 2.2 and 3.5 t/ha (Azam-Ali, 1998).

Many researchers, including Gardner *et al.* (1985); Hoogenboom *et al.* (1987); Sharpe and Davies, (1985) have found that plants grown in pots or in small soil volumes have rapid reduction in leaf expansion due to water stress compared to plants grown in the field. Plants

grown in pots have their root concentration throughout the entire pot and water absorption is uniform, which results in fast drying of the plant when subjected to water stress. In contrast, field grown plants have their root concentration in the upper horizons of the soil profile where most water is extracted. With an increase in water stress severity, roots grow deeper to extract water in wetter zones of the soil profile and the drying of the plant is gradual, increasing chances of plant recovery from stress. Roots are not affected equally; growth of shallow roots is affected more than that of deep roots.

Root system morphology and fine root distributions are cardinal factors in determining the magnitude of below-ground interspecific competition in mixed species systems. To improve the utilization efficiency of soil nutrient resources by intercropping systems, the spatial distribution and activities of roots requires elucidation. Estimating root growth dynamics and biomass is also important for understanding nutrient cycling (Andika *et al.,* 2010).

1.3.3 Dry matter production

Water stress influences several plant processes, from the individual cell to the whole canopy. Cell biochemistry, division and expansion are all very sensitive to water stress. Consequently, the growth rate of a crop under water stress may be severely restricted, resulting in reduced total dry matter and smaller leaf area than where water is unlimited (Mwale *et. al.*, 2007a). Reduction in both leaf area and dry matter production have been reported in many crops including legumes, such as groundnut (*Arachis hypogaea*) (Collino *et al*., 2001), faba bean (*Vicia faba*) (Mwanamwenge *et al*., 1999), cowpea (*Vign*a *unguiculata*) (Anyia and Herzog, 2004) and chickpea (*Cicer arietinum*) (Singh, 1991). Similar results were reported in Bambara groundnut where total dry matter and leaf area index (LAI) were reduced by drought (Collinson *et al*., 1996, 1997).

Reduction of biomass in response to drought is partly a consequence of restricted plant leaf area, which in turn reduces light interception (Singh, 1991), and partly a direct effect of low net photosynthesis due to stomatal closure (Mwanamwenge *et al*., 1999; Anyia and Herzog, 2004). The result is reduced biomass production, hence reduction in dry matter.

Another factor affecting dry matter in relation to water stress is the pattern of dry matter partitioning in water stressed plants, depending on the stage at which the stress occurs. During the reproductive phase, drought can seriously affect dry matter allocation to yield components (Mwale *et. al.*, 2007a). In chickpea, for example, drought reduced both seed number and size, which led to a yield loss of up to 80% (Leport *et al*., 1999). In bambara groundnut, Collinson *et al*. (1996) reported a significant reduction in pod number per plant, harvest index (HI) and final yield due to drought.

Although drought generally has a negative impact on crop growth and development, inter- and intra-species differences are common. Variations among species determine the survival and productivity of particular species in different environments while genotypic variations within a species are essential in breeding programmes (Mwale *et al.*, 2007a). Genotypic differences have been reported in many crops, including chickpea (Leport *et al.*, 1999), peas (*Pisum sativum*) (Baigorri *et al*., 1999), groundnut (Collino *et al*., 2000) and maize (*Zea mays*) (Kamara *et al*., 2003) with respect to biomass production, resource use efficiency, HI and other growth parameters. Tatar and Gevrek (2008) found that dry matter production was significantly reduced by water stress in wheat. Stomatal closure and decrease in CO_2 concentration as an initial response to water stress inhibited dry matter production due to limitation of photosynthesis (Reddy *et al.*, 2004)

1.4 Physiological responses to water stress

1.4.1 Proline

Proline accumulates in many plant species in response to a broad range of stress conditions such as water shortage, salinity, extreme temperatures and high light intensity. It is considered to be a compatible solute. It protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source. In some plant species, proline plays a major role in osmotic adjustment (e.g. in potato), while in others, such as in tomato, proline accounts for

only a small fraction of the total concentration of osmotically active solutes (Claussen, 2005). The precise role of proline accumulation is still elusive. Whether it is to act as an osmo-regulator, an osmo-protector, or a regulator of the redox potential of cells has not been decided (Mohammadkhani and Heidari, 2008).

Proline is suggested to play a crucial role in plant cytoplasmic osmotic adjustment in response to osmotic stresses (Wyn Jones *et al.*, 1977). Proline accumulation is a common metabolic response of plants to water stress. This highly water soluble amino acid is accumulated by leaf tissues and shoot apical meristems of plants experiencing water stress (Barnett and Naylor, 1966; Boggess *et al.*, 1976; Jones *et al.*, 1980), in root apical regions growing at low water potentials (Voetberg and Sharp, 1991), and in suspension cultured plant cells adapted to water stress (Katz and Tal, 1980; Handa *et al.*, 1986; Rhodes *et al.*, 1986). According to Leigh *et al.* (1981); Ketchum *et al.* (1991); Pahlich *et al.* (1983) proline accumulated in response to water stress in plants is primarily localized in the cytosol. Many researchers Pollard and Wyn Jones, (1979); Paleg *et al.* (1981); Nash *et al.* (1982); Paleg *et al.* (1984); Brady *et al.* (1984); Gibson *et al.* (1984); Santarius, (1992); Santoro *et al.* (1992) have found that proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions and temperature extremes.

Zlatev and Stoyanov (2005) suggested that proline accumulation in water stressed plants could be only useful as possible water stress injury sensor than instead of its role as stress tolerance response mechanism. Furthermore, Vendruscolo *et al.* (2007) found that proline was involved in tolerance mechanisms against oxidative stress (active oxygen which was caused by reduction in CO_2 concentration in the tissue of water stressed leaves and dissipation of excess light energy in PS II core and antenna). This was the main strategy of plants to evade detrimental effects of water stress. Tatar and Gevrek (2008) demonstrated that wheat dry matter production and relative water content (RWC) decreased while proline content increased under drought stress. Vendruscolo *et al.* (2007) found that wheat that was subjected to water stress increased its proline content.

Proline content of two varieties of maize (*Zea mays* L.- var.704 and var.301) increased linearly with increasing water stress. Proline concentration was observed to increase in roots of stressed–(1.76 MPa in PEG 40%). Shoot proline content also increased compared to the control plants (Mohammadkhani and Heidari, 2008). In this instance, proline was higher in the shoot than in the roots. In the maize primary root, for example, the proline level increased by as much as a hundred fold under low water potential.

1.5 Conclusions and study objectives

Bambara groundnut still remains an important crop in several African countries, where resource-poor farmers produce and consume it. Its importance as a source of dietary protein and its success in drought prone regions calls for more attention on the crop. Yields ranging from as little as 50 kg/ha up to 4 000 kg/ha were reported in the literature, suggesting huge yield gaps, the causes of which were not as clearly stated in the literature. Although bambara groundnut produces a nutritious food and is cultivated throughout Africa, it remains one of the crops most neglected by science, yet empirical evidence and fragmentary research results suggest that it is a crop with great potential.

In recent years, due to climate change and increased frequency of drought, there has been renewed interest in the crop for cultivation in drought-prone regions. Bambara is believed to be tolerant to drought and capable of producing reasonable yields when grown on poor soils. Bambara groundnut is a promising commodity which needs more publicity, both as a agronomic crop and as a protein source food. Even in tropical Africa, only a few people in the forest zones are aware of its existence. It should be emphasized that it is a low-cost, dependable crop that grows under harsh environments where many other crops fail. Bambara is a legume crop with high potential in enhancing food security, especially in drought-prone regions (Toungos *et al.,* 2009). Bambara is a legume crop and it fixes nitrogen from atmosphere and its drought tolerance makes it best suited for production by resource poor farmers.

The exceptionally high nutritive value of bambara groundnut should also be made known to the general public, and, in particular, to the rural poor. However, in order to ensure the wider adoption of bambara groundnut, the general mode of consumption of the crop needs improving. Modern processing methods would enable distribution of bambara groundnut to non-producing areas. However, the successful promotion of bambara as an alternative and drought tolerant crop hinges on the availability of information. Information on its agronomy, cultivation practices and water use should be made available to policy makers as well as to extension officers. Unfortunately, as an underutilised crop, little information currently exists

describing the agronomy and water use of bambara in several areas of South Africa. Such lack of information has seen the crop being overtaken by the exotic groundnut crop, even in areas where bambara was originally grown and favoured. There is thus need, as well as a sense of urgency, to collect such scientific information. The general aim of this study was to characterise bambara groundnut landraces of KwaZulu-Natal, with respect to crop growth in response to water availability and use findings to promote Bambara cultivation as an alternative legume crop under water-scarce conditions. It is hypothesised that the KwaZulu-Natal bambara groundnut landraces, which differ in seed colour, do not differ in seed quality and response to water stress under controlled and field conditions. Hence, the specific objectives of the study were:

- To compare three landraces of bambara, which differ in seed coat colour with respect to seed quality for crop establishment under different water regimes;
- To determine the effect of water stress on the growth, development and yield of the three landraces under controlled environment and field conditions; and
- To determine the effect of planting date, as a management component, on growth, development and yield of bambara groundnut.

References

ALLEN, N.S., CUMMING, J.R. & ALSCHER, R.G., 1990. Stress Responses in Plants: Adaptation and Acclimation Mechanisms.

ANDIKA, D.O., ONYANGO, A.M.O., ONYANGO, J.C. & STUTZEL, H., 2010. Roots spatial distribution and growth in bambara groundnuts (*Vigna subterranea*) and nerica rice (*Oryza sativa*) intercrop system. *ARPN J. Agricultural and Biological Science*, 5(2), 39-50

ANWAR, M.R., MCKENZIE, B.A. & HILL, G.D., 2003. Water-use efficiency and the effect of water deficits on crop growth and yield of Kabuli chickpea (*Cicer arietinum* L.) in a cool temperate sub-humid climate. *J. Agric. Sci.* 141, 285–301.

ANYIA, A.O. & HERZOG, H., 2004. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Eur. J. Agron*. 20, 327-339.

AZAM-ALI, S.N., 1998. Evaluating the potential for Bambara groundnut as a food crop in semi-arid Africa. An approach for assessing the yield potential and ecological requirements of an underutilized crop. Summary reports of European Commission supported STD-3 projects (1992-1995), published by *CTA1998.*

AZAM-ALI, S.N., SESAY, A., KARIKARI, S., MASSAWE, F.J., AGUILAR-MANJARREZ, J., BANNAYAN, M. & HAMPSON, K.J., 2001. Assessing the potential of an underutilized crop – a case study using bambara groundnut. *Expl. Agric*. 37, 433-472.

BABEKIR, A.M., 1989. Growth, dry matter and yield of bambara groundnut (*V. subterranea*) and groundnut (*A. hypogaea*) under irrigated and droughted conditions. MSc thesis, University of Nottingham, UK

BAIGORRI, H., ANTOLIN, M.C. & SANCHEZ-DIAZ, M., 1999. Reproductive response of two morphologically different pea cultivars to drought. *Eur. J Agron*. 12, 119 -128

BARNETT, N.M. & NAYLOR, A.W., 1966. Amino acid and protein metabolism in bermuda grass during water stress. *Plant Physiol.* 41, 1222-1230.

BLUM, A., 1996. Crop response to drought and the interpretation of adaptation. *J. Plant Growth Reg.* 20, 135-148.

BOGGESS, S.F., ASPINALL, D. & PALEG, L.G., 1976 .Stress Metabolism XI. The significance of end-product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. *Aust. J. Plant Physiol.* 3, 513-525.

BOYER, J.S., 1982. Plant productivity and environment. *Science* 218, 443-448.

BRADFORD, K.J. & HSIAO, T.C., 1982. Physiological responses to moderate water stress. Encyclopedia Plant Physiology. New Series, Berlin: *Springer-Verlag.*12B. pp 264-323.

BRADY, C.J., GIBSON, T.S., BARLOW, E.W.R., SPIERS, J. & WYN JONES, R.G., 1984. Salt tolerance in plants. I. Ions, compatible solutes and the stability of plant ribosomes. *Plant, Cell And Environ.* 7, 571-578.

BROUGH, S.H. & AZAM-ALI, S.N., 1992. The effect of soil moisture on the proximate composition of bambara groundnut (*Vigna subterranea* L. Verdc). *J. Sci. Food & Agric.* 60, 197-203.

CLAUSSEN, W., 2005. Proline as a measure of stress in tomato plants. *Plant Science,* 168**,** 241-248

COLLINO, D.J., DARDANELLI, J.L., SERENO, R. & RACCA, R.W., 2000. Physiological responses of argentine peanut varieties to water stress. Water uptake and water use efficiency. *Field Crops Res.* 68, 133– 142.

COLLINSON, S.T., AZAM-ALI, S.N., CHAVULA, K.M. & HODSON, D.A., 1996. Growth, development and yield of bambara groundnut (*Vigna subterranea*) in response to soil moisture. *J. Agric. Sci*. 126, 307–318.

COLLINSON, S.T., BERCHIE, J. & AZAM-ALI, S.N., 1999. The effect of soil moisture on light interception and the conversion coefficient for three landraces of bambara groundnut (*Vigna subterranea*). *J. Agric. Sci*. 133, 151–157.

COLLINSON, S.T., CLAWSON, E.J., AZAM-ALI, S.N. & BLACK, C.R., 1997. Effects of soil moisture deficits on the water relations of bambara groundnut (*Vigna subterranea* L. Verdc.). *J. Exp. Bot*. 48, 877–884.

COLLINSON, S. T., SIBUGA, K. P., TARIMO, A. J. P. & AZAM-ALI, S. N., 2000. Influence of sowing date on the growth and yield of Bambara groundnut (*Vigna subterranea* L.*)* landraces in Tanzania. *Expl. Agric*. 36, 1-13.

COUDERT, M.J., 1982. Market openings in West Africa for cowpeas and bambara groundnuts. International Trade Technology, X, 147-241.

CRAUFURD, P.Q. & WHEELER, T.R., 1999. Effect of drought and plant density on radiation interception, radiation-use efficiency and partitioning of dry matter to seeds in cowpea. *Exp. Agric*. 35, 309–325.

DEPARTMENT OF WATER AFFAIRS (DWAF), 1986. Management of the water resources of the Republic of South Africa. Cape Town: Department Of Water Affairs.

DEPARTMENT OF WATER AFFAIRS (DWAF), 2002. South Africa's water situation, and strategies to balance supply and demand. Proposed First Edition National Water Resource Strategy.

DOORENBOS, J. & KASSAM, A.H., 1979. Yield response to water. FAO Irrigation and Drainage Paper No. 33, FAO, Rome, Italy.

ESFANDIARI, E., SHAKIBA, M.R, MAHBOOB, S.A., ALYARI, H. & SHAHABIVAND, S., 2008. The effect of water stress on the antioxidant content, protective enzyme activities, proline content and lipid peroxidation in wheat seedling. *Pakistan J. of Biological Sciences* 11(5), 1916-1922

FOOD AND AGRICULTURE ORGANIZATION, 2001. A global mapping system for bambara groundnut production.

FISCHER, R.A. & TURNER, N.C., 1978. Plant productivity in the arid and semiarid zone. *Ann. Rev. Plant Physiol.* 29, 277-317.

FOULKES, J.M., SCOTT, R.K., & SYLVESTER-BRADLEY, R., 2001. The ability of wheat cultivars to withstand drought in UK conditions: resource capture. *J. Agric. Sci.* 137, 1–16.

GARDNER, F.P., PEARCE, R.B. & MITCHELL, R.L., 1985. Physiology of crop plants. Iowa State University Press. pp 87-91.

GIBSON, T.S., SPIERS, J. & BRADY, C.J., 1984. Salt tolerance in plants. II. *In vitro* translation of m-RNAs from salt-tolerant and salt-sensitive plants on wheat germ ribosomes: responses to ions and compatible solutes. *Plant Cell Environ*. 7, 579-587

HANDA, S., HANDA, A.K., HASEGAWA, P.M. & BRESSAN, R.A., 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiol*. 80, 938-945.

HOOGENBOOM, G., HUCK, M.G. & PETERSON, C.M., 1987. Root growth rate of soyabean as affected by drought stress. *Agron. J.* 79, 607-614.

JOHARI-PIREIVATLOU1, M., QASIMOV, N. & MARALIAN, H., 2010. Effect of soil water stress on yield and proline content of four wheat lines. *Afri. J. Biotechnol.* 9 (1), 36-40.

JOHNSON, B.L. & HENDERSON, T.L., 2002. Water use patterns of grain amaranth in the northern Great Plains. *Agron. J*. 94, 1437–1443.

JOHNSON, D.T., 1968. The bambara groundnut: a review. *Rhodesia Agricultural Journal* 65, 1-4.

JONES, M.M., OSMOND, C.B. & TURNER, N.C., 1980. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. *Aust. J. Plant Physiol.* 7, 193-205.

KAMARA, A.Y., MENKIR, A., BADU-APRAKU, B. & IBIKUNLE, O., 2003. The influence of drought stress on growth, yield and yield components of selected maize genotypes. *J. Agric. Sci.*, 141, 43–50

KATZ, A. & TAL, M., 1980. Salt tolerance in the wild relatives of cultivated tomato: proline accumulation in callus tissue of *Lycopersicon esculentum, L. Peruvianum*. and *Z. P. flanzen. Plant physiol. Bd*. 98, 429-435.

KETCHUM, R.E.B., WARREN, R.C., KLIMA, L.J., LOPEZ-GUTIERREZ, F. & NABORS, M.W., 1991. The mechanism and regulation of proline accumulation in suspension cultures of the halophytic grass *Distichlis Spicata* L. *Plant Physiol*. 137, 368-374.

KRAMER, P.J., 1984. Problems in water relations of plants and cells. In: International Review of cytology., (Ed.): P.J. Kramer. pp. 254-286.

LEIGH, R.A., AHMAD, N. & WYN JONES, R.G., 1981. Assessment of glycine, betaine and proline compartmentation by analysis of isolated beet vacuoles. *Planta* 153, 34-41

LEPORT, L., TURNER, N.C., FRENCH, R.J, BARR, M.D., DUDA, R., DAVIES, S.L., TENNANT, D. & SIDDIQUE, K.H.M., 1999. Physiological responses of chickpea

genotypes to terminal drought in a Mediterranean-type environment. *Euro. J. Agron*. 11, 279-291.

LINNEMANN, A. R., 1994. Photothermal regulation of phenological development and growth in bambara groundnut (*Vigna subterranea* L. Verdc.). PhD Thesis, Wageningen Agricultural University, The Netherlands.

LINNEMANN, A.R. & AZAM-ALI, S.N., 1993. Bambara groundnut (*Vigna subterranea* L. Verdc). *In*: Williams, J.T. (Ed.), Under-utilised Crops Series. II. Vegetable and Pulses. Chapman and Hall, London, UK. pp 13-58.

LINNEMANN, A.R., 1990. Cultivation of bambara groundnut (*Vigna subterranea L.* Verdc.) in Western province, Zambia. Report of a field study. Tropical Crops Communication No. 16. Department of Tropical Crop Science. Wageningen Agricultural University

LIU, F. & STUTZEL, H., 2004. Biomass partitioning, specific leaf area and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Sci. Hortic.* 102, 15–27.

MAKANDA, I., TONGOONA, P., MADAMBA, R., ICISHAHAYO, D. & DERERA, J., 2009. Evaluation of bambara groundnut varieties for off-season production in Zimbabwe. *Afri. Crop Sci. J*. 16, 175 – 183.

MASSAWE, F.J. MWALE, S.S. AZAM-ALI, S.N. & ROBERTS, J.A., 2005. Breeding in bambara groundnut (*Vigna subterranea* L. Verdc.): Strategic Considerations. *Afri. J. Biotechnol.* 4 (6), 463-471.

MOHAMMADKHANI, N. & HEIDARI, R., 2008. Drought-induced accumulation of soluble sugars and proline in two maize varieties. *World Applied Sciences Journal* 3 (3), 448-453.

MORGAN, J.M., 1984. Osmoregulation and water stress in higher plants. *Ann. Rev. Plant Physiol*. 35, 299-348.

MUKHEIBIR, P., 2007. Qualitative assessment of municipal water resource management strategies under climate impacts: The case of the Northern Cape, South Africa. *Water S. A.* 33, 575-581.

MUNNS, R. & KING, R.W., 1988. Abscisic acid is not only stomatal inhibitor in the transpiration stream of wheat plants. *Plant Physiol.* 88,703-708.

MWALE, S.S., AZAM-ALI, S.N. & MASSAWE, F.J., 2007a. Growth and development of bambara groundnut (*Vigna subterranea*) in response to soil moisture. 1. Dry matter and yield. *Euro. J. Agron*. 26, 345-353.

MWALE, S.S., AZAM-ALI, S.N. & MASSAWE, F.J., 2007b. Growth and development of bambara groundnut (*Vigna subterranea*) in response to soil moisture: 2. Resource capture and conversion. *Euro. J. Agron*. 26, 354-362.

MWALE, S.S., HAMUSIMBI, S. & MWANSA, K., 2003. Germination, emergence and growth of sunflower (*Helianthus annus* L.) in response to osmotic seed priming. *Seed Sci. Technol*. 31, 199-206.

MWANAMWENGE, J., LOSS, S.P., SIDDIQUE, K.H.M. & COCKS, P.S., 1998. Growth, seed yield and water use of faba bean (*Vicia faba* L.) in a short-season Mediterranean-type environment. *Aust. J. Exp. Agric*. 38, 171–180.

NASH, D., PALEG, L.G. & WISKICH, J.T., 1982. Effect of proline, betane and some other solutes on the heat stability of mitochondrial enzymes. *Aust. J. Plant Physiol*. 9, 47-57.

PAHLICH, E., KERRES, R. & JAGER, H.J., 1983. Influence of water stress on the vacuole/extra vacuole distribution of proline in protoplasts of *Nicotiana rustica*. *Plant Physiol*. 72, 590-591.

PALEG, L.G., DOUGLAS, T.J., VAN DAAL, A. & KEECH, D.B., 1981. Proline, betane and other organic solutes protect enzymes against heat inactivation. *Aust. J. Plant Physiol*. 8, 107-114.

PALEG, L.G., STEWART, G.R. & BRADBEER, J.W., 1984. Proline and glycine betane influence protein solvation. *Plant Physiol*. 75, 974-978.

PANNU, R.K. & SINGH, D.P., 1993. Effect of irrigation on water use, water use efficiency, growth and yield of mungbean. *Field Crops Res*. 31, 87–100.

PATIL, A.G., 2010. Water stress induced changes in proline content in *Vigna unguiculata* (Linn) var. Konkan Sadabahar at various stages of growth. (*Accessed from www.faqs.org. on 10-08-2011*).

POLLARD, A. & WYN JONES, R.G., 1979. Enzyme activities in concentrated solutions of glycine, betaine and other solutes. *Planta* 144, 291-298.

REDDY, A.R., CHAITANYA K.V. & VIVEKANANDAN, M., 2004. Drought-induced responses and antioxidant metabolism in higher plants. *J. Plant Physiol*. 161 (11), 1189-1202.

REIJ, C., MULDER, P. & BEGEMANN, L., 1991. Water harvesting for plant production. World Bank Technical Paper Number 91, Washington, D.C.

RHODES, D., HANDA, S. & BRESSAN, R.A., 1986. Metabolic changes associated with adaptation of plant cells to water stress. *Plant Physiol*. 82, 890-903.

SANTARIUS, K.A., 1992. Freezing of isolated thylakoid membranes in complex media. viii. differential cryoprotection by sucrose, proline and glycerol. *Physiol. Plant*. 84, 87-93.

SANTORO, M.M., LIU, Y., KHAN, S.M.A., HOU, L.X. & BOLEN, D.W., 1992. Increased thermal stability of proteins in the presence of naturally occurring osmolytes. B*iochemistry* 31, 5278-5283.

SESAY, A., MAGAGULA, C. N. & MANSUETUS, A. B., 2008. Influence of sowing date and environmental factors on the development and yield of Bambara groundnut (*Vigna subterranea* L.*)* landraces in a Sub-tropical region. *Expl. Agric*. 44, 167–183.

SHARPE, R.E. & DAVIES, W.J., 1979. Solute regulation and growth by roots and shoots of Water Stressed Maize plants. *Planta.* 147, 43-49.

SHARPE, R.E. & DAVIES, W.J., 1985. Root growth and water uptake by maize plants in drying soil. *J. Exp. Bot.* 36, 1441-1456.

SIDDIQUE, K.H.M., REGAN, K.L., TENNANT, D. & THOMSON, B.D., 2001. Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. *Euro. J. Agron*. 15, 267– 280.

SINGH, P., 1991. Influence of water-deficits on phenology, growth and dry matter allocation in chickpea. *Field Crops Res.* 28, 1-15.

SMITH, B., 2006. The farming handbook. University of KwaZulu-Natal Press.

SQUIRE, G.R., 1990. The physiology of tropical crop production. CAB. International, Wallingford, UK.

SWANEVELDER, C.J., 1998. Bambara food for Africa. National Department of Agriculture, Government Printer, Republic of South Africa.

TANIMU, B., 1996. Effects of sowing dates, fertilizer level and Intra-row spacing on the Agronomic Characters and yield of Bambara groundnut *Vigna subterranea* L. Vercourt. A. PhD. Thesis.

TATAR, O. & GEVREK M.N., 2008. Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. *Asian J. Plant Sci.* 7 (4), 409-412

TOUNGOS, M., SAJO, A. & GUNGULA, D., 2009. Recommended fertilizer levels on bambara groundnut (*Vigna subterranea* L. Verdc) in Yola Adamawa State, Nigeria. *Agric. J.* 4, 14-21.

TURNER, N.C., 1979. Drought resistance and adaptation to water deficits in crop plants. In: Stress physiology in Crop Plants, (Eds.): H. Mussell and R.C. Staples. pp. 181-194.

TURNER, N.C., 1997. Further progress in crop water relations. AdvancesIn Agronomy 58, 293–338

VENDRUSCOLO, A.C.G., SCHUSTER, I., PILEGGI, M., SCAPIM, C.A., MOLINAR H.B.C., MARUR, C.J. & VIEIRA L.G.C., 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant Physiol*. 164 (10), 1367-1376.

VOETBERG, G.S. & SHARP, R.E., 1991. Growth of the maize primary root tip at low water potentials. III. Role of increased proline deposition in osmotic adjustment. *Plant Physiol.* 96, 1125-1130.

WALKER, S., FYFIELD, T.P., MCDONALD, J.P.A & THACKRAH, A., 1995. The influence of different water and nitrogen levels on crop growth, water use and yield, and the validation of crop models. Water Research Commission Report No. 307/1/95

WYN JONES, R.G., STOREY, R., LEIGH, R.A., AHMAD, N. & POLLARD, A., 1977. A hypothesis on cytoplasmic osmoregulation. *In*: E. Marre & O. Cifferi (Eds.) Regulation of cell membrane activities in plants. Elsevier, Amsterdam. pp 121-136.

Wyn Jones, R.G. and J. Gorham.1983. Aspects of salt and drought tolerance in higher plants. In: Genetic engineering of plants; An agricultural perspective, (Eds.): T. Kosuge, C.P. Meredith and A. Hollander. pp. 355-370

ZHANG, H., PALA, M., OWEIS, T. & HARRIS, H., 2000. Water use and water-use efficiency of chickpea and lentil in a Mediterranean environment. *Aust. J. Agric. Res*. 51, 295–304.

ZLATEV, Z. & STOYANOV, Z., 2005. Effects of water stress on leaf water relations of young bean plants. *Journal of central Europe Agriculture*. 6 (1), 5-14.

CHAPTER 2

SEED QUALITY AND SEEDLING GROWTH IN RESPONSE TO VARYING WATER STRESS TREATMENTS

2.1 Introduction

Bambara (*Vigna subterranea* L. Verdc.) is a legume crop with high potential for enhancing food security, especially in drought-prone regions (Toungos *et al*., 2009). As an underutilized crop, its germplasm improvement and management practices depend on local experience and resources (indigenous knowledge). The crop is cultivated from landraces and farm yields are usually low and unpredictable (Linnemann and Azam-Ali, 1993; Sessay *et al*., 1999). Zeven (1998) defined a landrace as a variety with a high capacity to tolerate abiotic and biotic stresses, resulting in high yield stability and intermediate yield levels under low input agricultural systems. Furthermore, landraces are a mixture of genotypes with highly diverse populations both between and within them. In South Africa, Bambara production mainly relies on the use of landraces. However, there is little information describing seed quality of local landraces.

McDonald and Copeland (1997) defined seed quality as the overall value/suitability of seed lot for its intended use; in this case it is defined in terms of physiological quality (viability, germination and vigour). Basu (1995) defined viability as the property of the seed that allows it to germinate under optimum conditions. On the other hand, vigour refers to that aspect of the seed responsible for rapid, uniform germination, increased storability, good field emergence and an ability to perform well under field conditions (Perry, 1978; McDonald, 1980). In practice, the relationship between seed viability and vigour is an intricate one. High seed quality (viability, germination and vigour) is essential for good crop establishment and how the crop will perform under field conditions. In general, poor quality seed may result in

reduced germination and emergence rates, poor tolerance to sub-optimal conditions and low seedling growth rates (Powell *et al.*, 1984).

Germination (viability) and emergence (vigour) of Bambara groundnut is often erratic, variable and slow in the field; it has been reported to take up to 21 days after sowing (DAS) (Sessay and Yarmah, 1996). The problem of unpredictable Bambara yields has been attributed, at least in part, to variable or poor field establishment due to poor germination and/or seedling emergence (Linnemann and Azam-Ali, 1993). Bambara groundnut is often grown in areas where water supply is usually a limiting factor (Mwale *et al*., 2007). The crop is thus often exposed to a wide range of field conditions. Seedbed conditions and water stress are important factors affecting the emergence and development of seedlings (Pollock, 1972). In the semi-arid regions soil water stress, associated with high temperatures, is probably the most important factor affecting seed germination and emergence. Sessay *et al*. (2004) reported delayed and prolonged seedling emergence in Bambara groundnut trials conducted in Luve and Malkernes in Swaziland in 2001 and 2002 respectively, due to water stress (drought). Zulu (1989) observed that seed germination in Bambara groundnut appeared to be more sensitive to water stress than groundnut. He ascribed this to the restrictive water uptake by Bambara groundnut due to hard seed coat.

Seed quality has a direct implication on the capacity of a seed lot to emerge into seedlings capable of efficiently capturing and using resources such as light through early canopy development, nutrient uptake, weed control and hence final pod yield at harvest. Different landraces have been reported to exhibit different germination responses to factors such as temperature, soil water stress and pre-sowing hydration (Zulu, 1989; Kocabas *et al*., 1999; Massawe *et al*., 1999). Previous research (Zulu and Modi, 2010) indicated that seed colour was associated with seed quality. This study aimed to evaluate three colour variations of a Bambara landrace, with respect to seed quality and crop establishment under different water regimes.

2.2 Material and Methods

2.2.1 Plant material

Seeds of a Bambara landrace were collected from subsistence farmers in Jozini, KwaZulu-Natal, and sorted into three distinct colours: red, white and brown. Previous research (Zulu and Modi, 2010) indicated that seed colour was associated with seed quality. To confirm this observation a number of viability and vigour tests were carried out.

2.2.2 Standard germination test (SG)

Different colours seeds of the landrace were assessed for viability using a standard germination (SG) test. 25 seeds of each colour were arranged in 4 rows and were germinated between moistened double-layered, paper towels. The experiment was replicated four times. The paper towels were rolled, tied with plastic bands on either side, put in sealed plastic bags and germinated in a growth chamber at 25°C for 8 days (ISTA, 1999). Germination was defined as radical protrusion. Germination was recorded daily for 7 days and final germination (%) was counted on the 8^{th} day. Seedling root and shoot lengths were determined. Seedling vigour was determined based on normal, weak and abnormal seedlings (AOSA, 1996). Seedling fresh and dry mass were determined. Germination velocity index was calculated according to Maguire's (1962) formulae:

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

Where:

GVI = germination velocity index

G1, G2…Gn = number of germinated seeds in first, second… last count.

N1, N2…Nn = number of sowing days at the first, second… last count.

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

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

Where:

MGT= mean germination time,

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

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

2.2.3 Cold Test (CT)

Seed of the different colours were assessed for vigour using a cold germination test (CT). 12 plastic trays were used; each tray was covered with 2 moistened paper towels. 25 seeds of each colour were arranged in 4 rows and covered with soil and placed in a cold room at -4°C for 7 days. The experiment was replicated four times. Thereafter, the trays were removed from the cold room and placed in a growth chamber set at 25°C for 8 days. Daily germination was recorded up to 8 days. Final germination was observed on the 8^{th} day and seedling root and shoot lengths were determined. Seedling vigour was determined based on normal, weak and abnormal seedlings (AOSA, 1996). Seedling fresh and dry mass were determined.

2.2.4 Electrolyte leakage (EC)

Electrolyte leakage or conductivity ($\mu S\ cm^{-1}\ s^{-1}$) measures the amount of solute leakage in seeds. The EC test is used to measure seed vigour. 50 seeds of each colour, replicated four times, were used for the conductivity test. 50 seeds were placed in 200 ml beakers filled with distilled water and stored for 0 h, 1 h, 3 h, 6 h, 12 h, 36 h and 84 hours. Seed mass (g), water activity (a_w) and electrolyte conductivity were measured at each interval.

2.2.5 Seed coat thickness

Effects of seed coat thickness on seed germination and seed viability were evaluated using a Zeiss EVO Scanning Electron Microscope (SME). 3 seeds of each Bambara seed colour were cryo-fractured in liquid nitrogen and split into halves. Seeds were then mounted onto stubs and stuck with a two way insulating tape. Stubs containing seed sample or seed coat were then viewed under the Zeiss EVO Scanning Electron Microscope (SME) in a vapour pressure

mode. The experiment was conducted at the University of KwaZulu-Natal's Centre for Electron Microscope.

2.2.6 Desiccation test and Proline Accumulation

Changes in seed proline content in response to desiccation were determined using three salts (NaCl, LiCl and KNO_3) and water (H_2O), using sealable containers and 30 seeds of each seed colour replicated three times. Salt concentrations were prepared and 30 seeds were suspended over the three salt concentrations for 0, 2, 4, 8, 24 and 48 hours and stored in a germination chamber at 20°C. The same number of seeds was placed in a beaker filled with 200 ml of water for the same time interval and stored in a germination chamber at 20°C. Five seeds were sampled at each interval and stored at -21°C for 7 days. Seeds were then ground into a fine powder using liquid nitrogen with a mortar and pestle. 300 mg of seed sample from each seed colour was then placed into test tubes, to which 5 ml of 3% sulfusalicyclic was added. The mixture was then centrifuged at 11 000 rpm for 5 min. Thereafter, the extract was placed in glass test tubes sealed with foil and kept in a hot water bath (80°C) for 1 h. The reaction was terminated in a water bath at room temperature (21°C) for 5 min. 1 ml was then extracted from the solution and mixed with 1 ml of acidic ninhydrin reagent (2.5 g ninhydrin/100 mL of a solution containing glacial acetic acid, distilled water and ortho-phosphoric acid 85% at a ratio of 6:3:1). Absorbance was taken immediately using a spectrophotometer at a wavelength of 546 nm. The proline concentration was determined from a standard curve and calculated on a fresh mass basis (µmol g^{-1} DW) (Appendix 5).

2.2.7Seedling Establishment Under different water stress conditions

Seedling emergence was carried out under controlled environment conditions (27/15°C day/night; 65%RH & natural day length) in seedling trays using pine bark as growing media. The field capacity (FC) of the pine bark had been previously determined. The experimental design was a factorial experiment, with three factors, seed colour (red, white & brown), priming (NaCl, LiCl, KNO_3, H_2O & control or dry seeds) and water regimes (25% and 75% FC). The experiment was replicated three times. Salt concentrations, were prepared and 50 seeds of each colour were suspended over each of the three salt concentrations and distilled

water and stored in a germination chamber at 20°C for 8 hours. Thereafter, for each treatment, 30 seeds were sampled and planted in seedling trays using pine bark wetted to 25% and 75% FC, respectively, over 22 days. The trays were weighed and watered at two day intervals to maintain field capacity. Daily seedling emergence was measured for 21 days. Seedling height and leaf number were measured weekly. The experiment was terminated after 22 days. Thereafter, seedling leaf area, root and shoot mass (fresh and dry), root and shoot lengths and root: shoot ratios were determined. Leaf area was measured using Portable Area Meter LI-3000C and root and shoot lengths were measured by 30 cm ruler. Mean time to emergence was calculated using the formulae by Bewley and Black (1994):

$$MET = \frac{\sum(fx)}{\sum f} \qquad \textbf{Equation 2.3}$$

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.

2.2.7 Data Analysis

Data were analysed using ANOVA from GenStat® Version 12 (VSN International, UK). Means were separated using Duncan's Multiple Range Test in GenStat® at the 5% level of significance (Appendix 1).

2.3 Results

2.3.1 Germination

There were significant differences ($P<0.05$) between the SG and CT, with the SG showing higher (>39%) germination than CT (Fig 2.1&2.2). Brown showed the highest germination (83.6%) across treatments, followed by red (83.5%) and white (61.6%) seeds, respectively. The interaction between seed colour and treatment was not significant. There were no differences between seed colours and between the interaction between seed colour and treatment with respect to germination velocity index (Table 2.1). However, there were highly significant differences ($P<0.001$) between the treatments, with SG showing faster germination (>76.3%) than CT. With respect to mean germination time (MGT) (Table 2.1), there were no significant differences between the seed colours as well as the interaction between seed colour and treatment. However, there were highly significant differences ($P<0.001$) between treatments with SG germinating earlier than CT.

With respect to abnormal seedlings there were no significant differences between seed colours. There were no significant differences between the treatments (SG and CT). The interaction between the treatments and seed colours was not significant ($P>0.05$). White seeds had the highest (25%) percentage abnormality followed by brown (18.5%) and red (16.5%), respectively (Table 2.1). There were no significant differences between seed colours with respect to dry mass and also between treatments (Table 2.1). There were no differences between seed colours with respect to fresh mass. There was significant difference ($P> 0.05$) between SG and CT treatments with respect to fresh mass (Table 2.1). There were no significant differences between seed colours with respect to shoot length and root:shoot ratio. However, there were significant differences ($P<0.05$) in shoot length and root:shoot ratio between treatments with CT having the longest shoots (4.65 cm) followed by SG with (1.10 cm). Overall, white had tallest seedlings (3.34 cm) followed by brown (2.92 cm) and red (2.36 cm), respectively. There were no significant differences ($P>0.05$) in root length between seed colours and between the treatments.

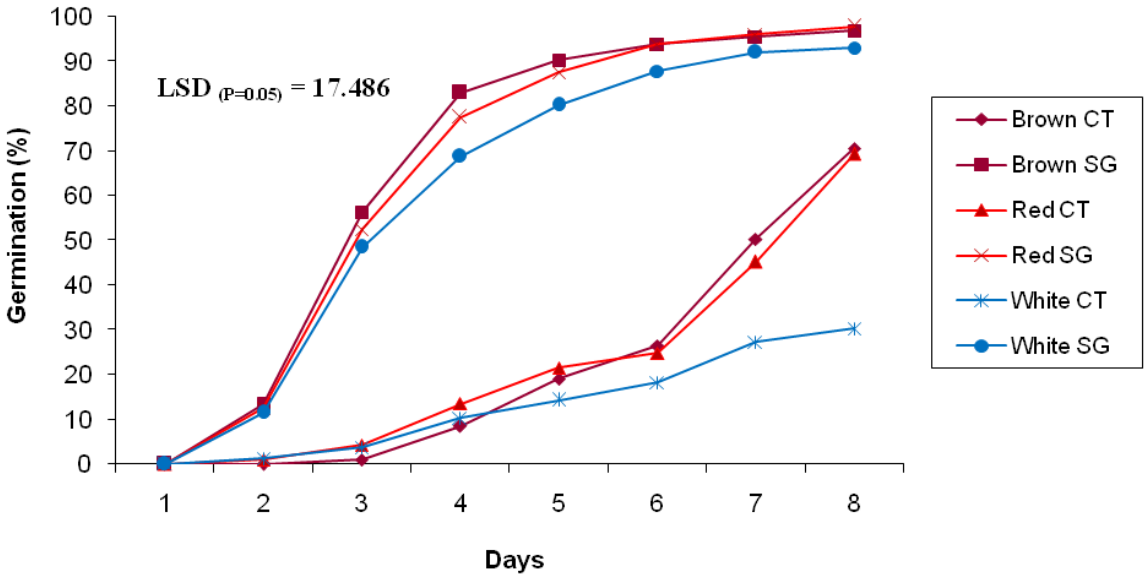


Figure 2.1: Daily germination of different Bambara seed colours (red, brown and white) as observed in the standard germination (SG) and cold tests (CT).

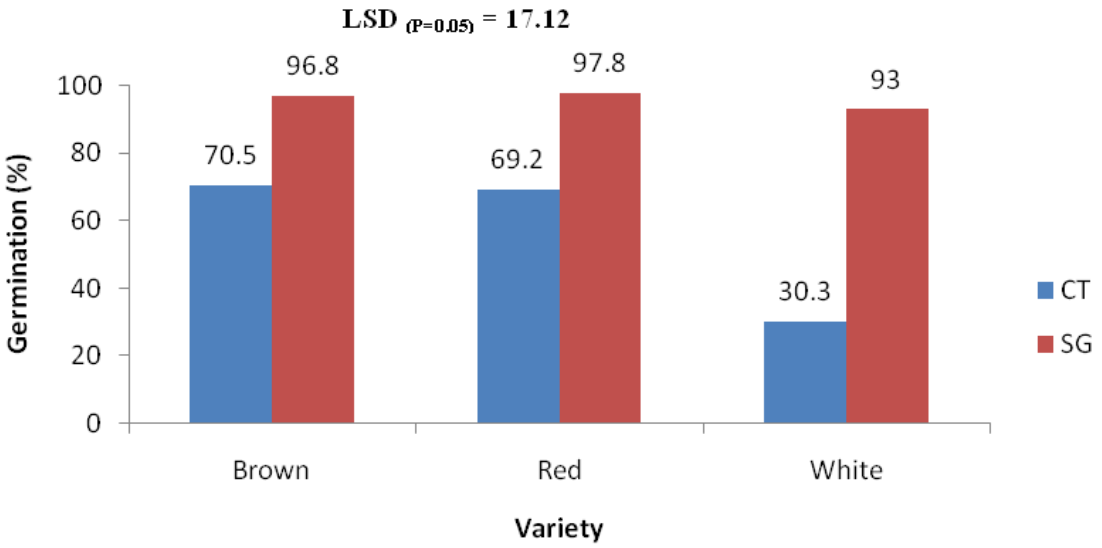


Figure 2.2: Effect of seed colour (red, brown and white) on final germination under standard germination (SG) and cold test (CT) germination.

Table 2.1: Performance of different seed colours under standard germination (SG) and cold test (CT).

Treatment	Seed Colour	GVI[X]	MGT[y] (days)	Abnormal Seedlings (%)	Fresh mass(g)	Dry mass(g)	Shoot length(mm)	Root length(mm)	Root: Shoot ratio
	Brown	105.7a	5.646b	22ab	15b	10ab	12.2b	56a	5.68ab
SG-Test	Red	102.2a	5.695b	21ab	15b	11.75a	11.8b	57.2a	4.98abc
	White	94.5a	5.731b	15b	15b	10ab	9b	49.8a	6.87a
	Mean	**100.8[a]**	**5.691[a]**	**19.3[a]**	**15[b]**	**10.58[a]**	**1.10[b]**	**5.43[a]**	**5.84[a]**
	Brown	26.7b	7.023a	15b	19.75a	11.75a	46.2ab	60.2a	1.5cd
CT	Red	28.8b	6.962a	12b	16.5b	10.5ab	35.5ab	61.8a	2.33bcd
	White	18.0b	693a	35a	17.5ab	9.5b	57.8a	45a	0.9d
	Mean	**24.5[b]**	**6.892[a]**	**20.7[a]**	**17.92[a]**	**10.58[a]**	**4.65[a]**	**5.57[a]**	**1.58[b]**
LSD (Trt.) (P=0.05)		13.27	0.344	10.26	1.749	1.116	2.019	0.979	2.119
LSD (Trt*SC) (P=0.05)		22.99	0.596	17.77	3.030	1.933	3.498	1.696	3.671

Note: [X]GVI = Germination velocity index; [y]MGT = Mean germination time; Trt = Treatment; SC = seed colour. Values not sharing the same letter in the same column differ significantly at P = 0.05.

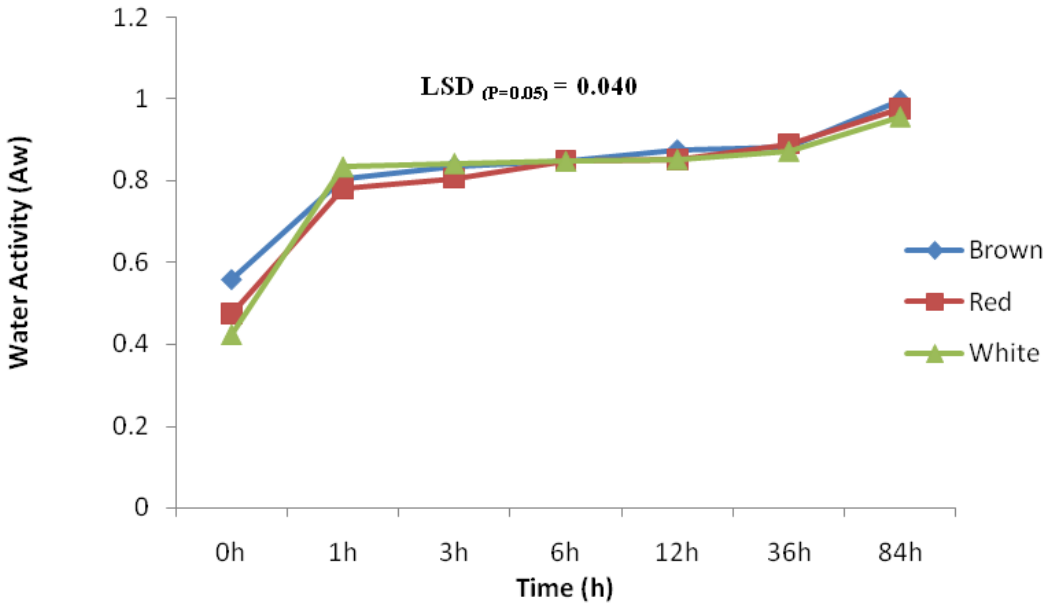


Figure 2. 3: Effect of seed colour on water activity of the seeds during imbibition.

With respect to water activity, there were highly significant differences ($P<0.001$) between seed colours as well as between treatments (time in particular) (Fig 2.3). The interaction between treatments and seed colours was also highly significant ($P<0.001$). Brown had the highest water activity followed by red and white, respectively. There were highly significant differences ($P<0.001$) between seed colours as well as between treatments, with respect to electrolyte leakage (EC) (Fig 2.4). The interaction between treatment and seed colour was also highly significant ($P<0.001$). White had the highest electrolyte leakage followed by red and brown seeds, respectively. Seed mass increased following imbibition; however, there were no significant differences ($P>0.05$) between seed colours (Fig 2.5); although there were significant differences ($P<0.05$) between treatments. The interaction between treatments and seed colours was not significant ($P>0.05$). Based on percentage mass gain, white seeds had the fastest rate of imbibition, followed by red and brown seeds, respectively.

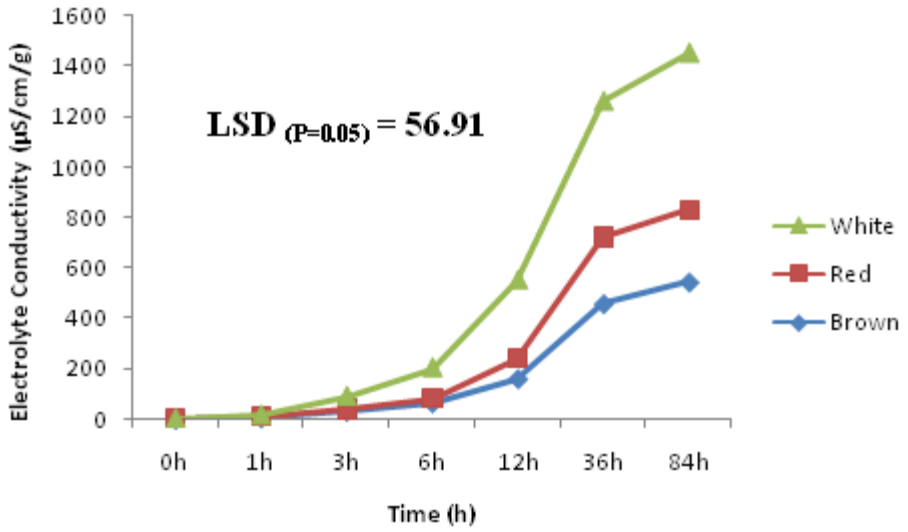


Figure 2.4: Effect of seed colour on electrolyte leakage of the seeds during imbibition.

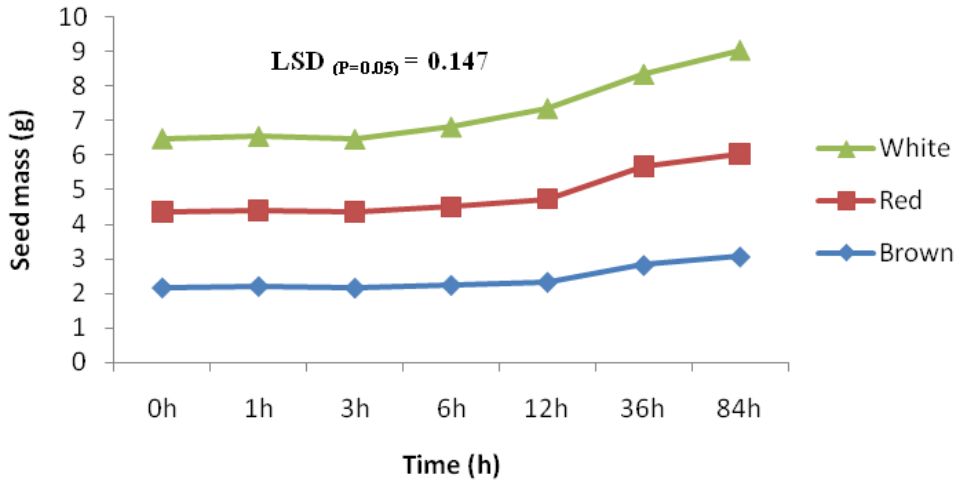


Figure 2.5: Effect of seed colour on seed mass of the seeds during imbibition.

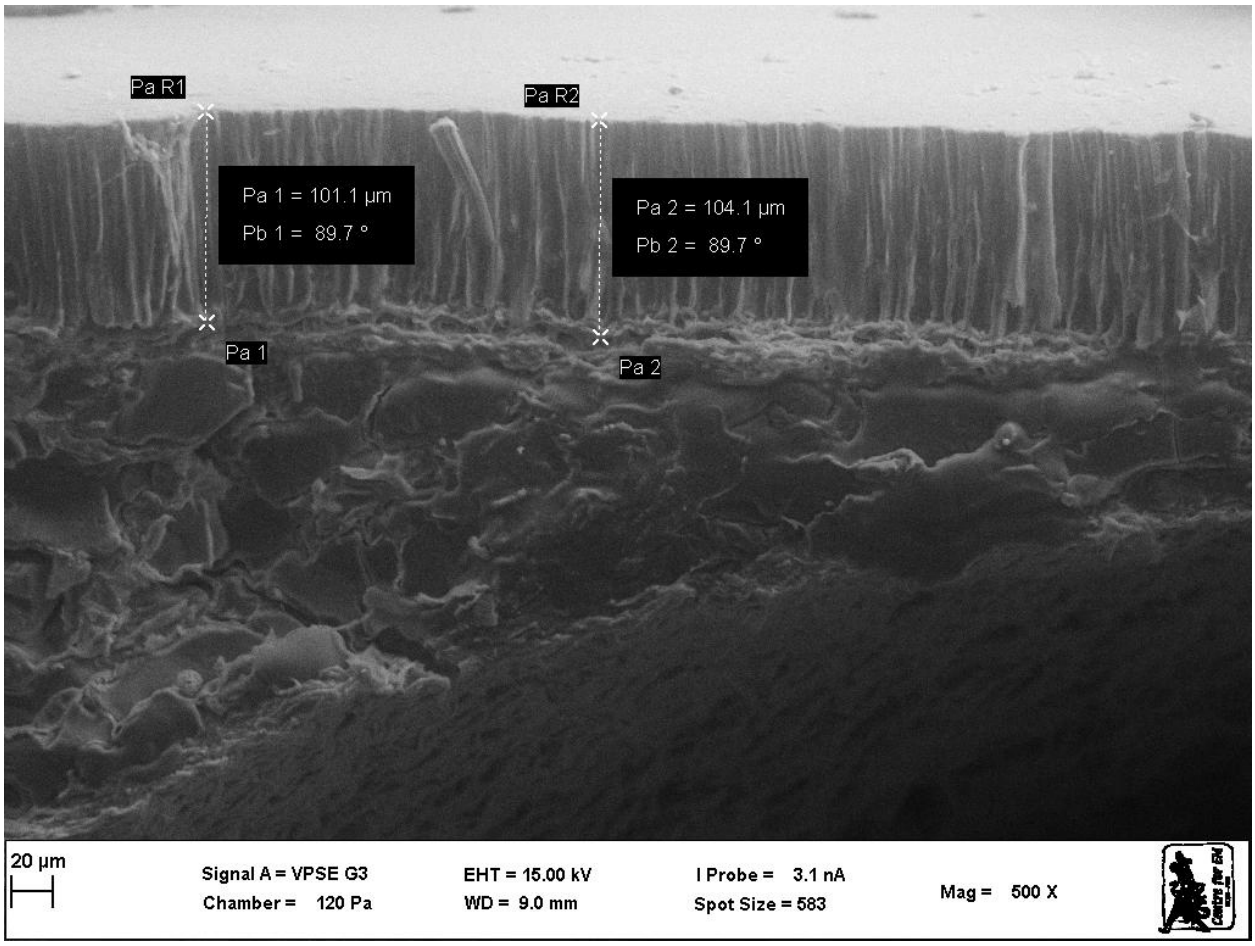


Figure 2.6: Picture Scan of brown seed coat of Bambara groundnut landrace viewed under scanning electron microscope (SME) at 500 x magnification.

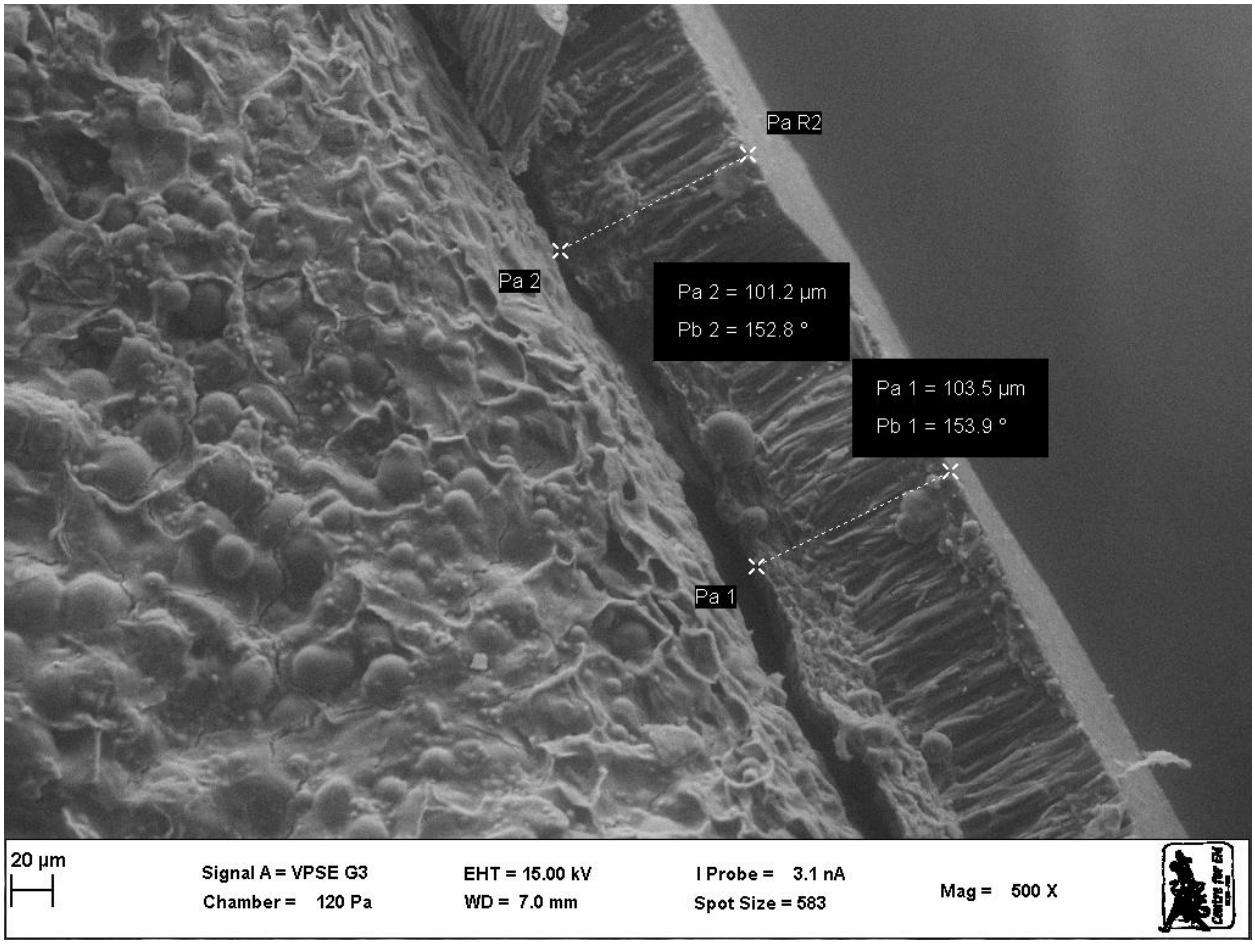


Figure 2.7: Picture Scan of red seed coat of bambara groundnut landrace viewed under scanning electron microscope (SME) at 500 x magnification.

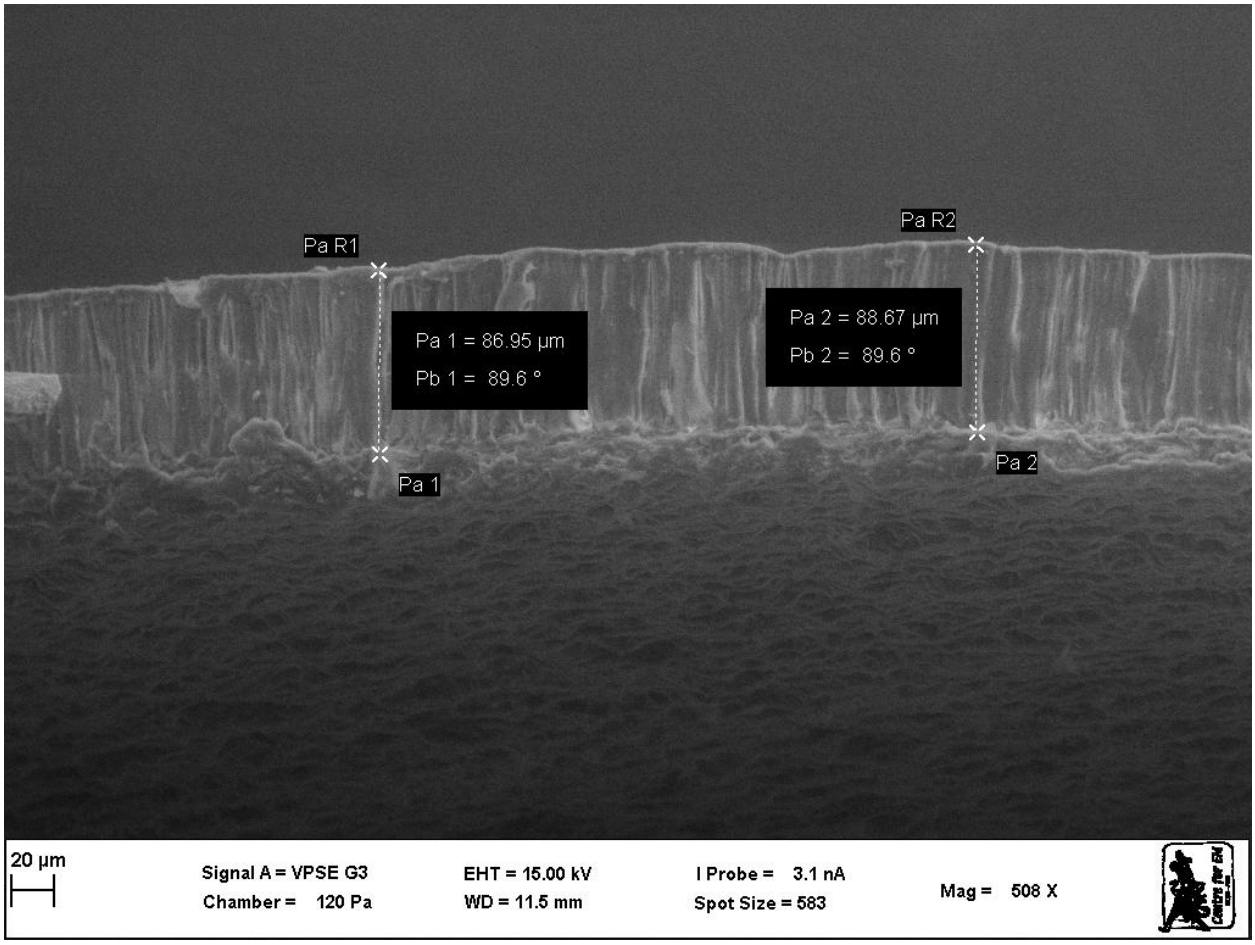


Figure 2. 8: Picture Scan of white seed coat of a Bambara groundnut landrace viewed under scanning electron microscope (SME) at 500 x magnification.

Picture scans of the seed coat of the three Bambara groundnut seed colours showed that brown and red had almost the same seed coat thickness, while white seeds had the thinnest seed coat (Figs 2.6, 2.7 & 2.8). Differences in seed coat thickness may explain observations of EC and imbibition in the three seed colours (Fig 2.4 & 2.5).

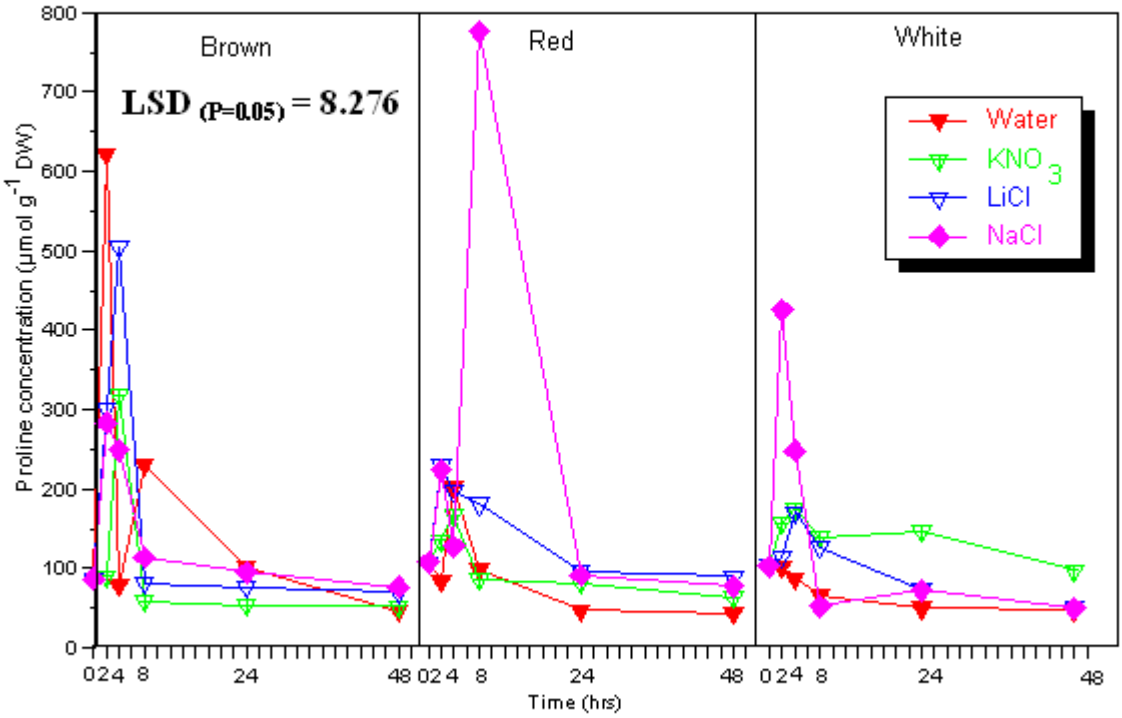


Figure 2.9: Changes in seed proline content of seed colour in response to desiccation over time.

There were highly significant differences (P<0.001) between seed colours with respect to proline content. Over-all, based on mean values brown had the highest (160.44 µmol g^{-1} DW) proline content followed by red (147.30 µmol g^{-1} DW) and white (119.74 µmol g^{-1} DW), respectively (Fig 2.9). There were highly significant differences (P<0.001) between salts with NaCl having highest proline concentration (181.33 µmol g^{-1} DW), followed by LiCl (147.93 µmol g^{-1} DW), KNO_3 (117.92 µmol g^{-1} DW) and H_20 (122.80 µmol g^{-1} DW), respectively. There were also highly significant differences (P<0.001) between the interaction between seed colour, priming and time with respect to proline accumulation. Sodium chloride was associated with the highest proline accumulation. Seed proline content increased from 0 hour to 8 hour and later declined.

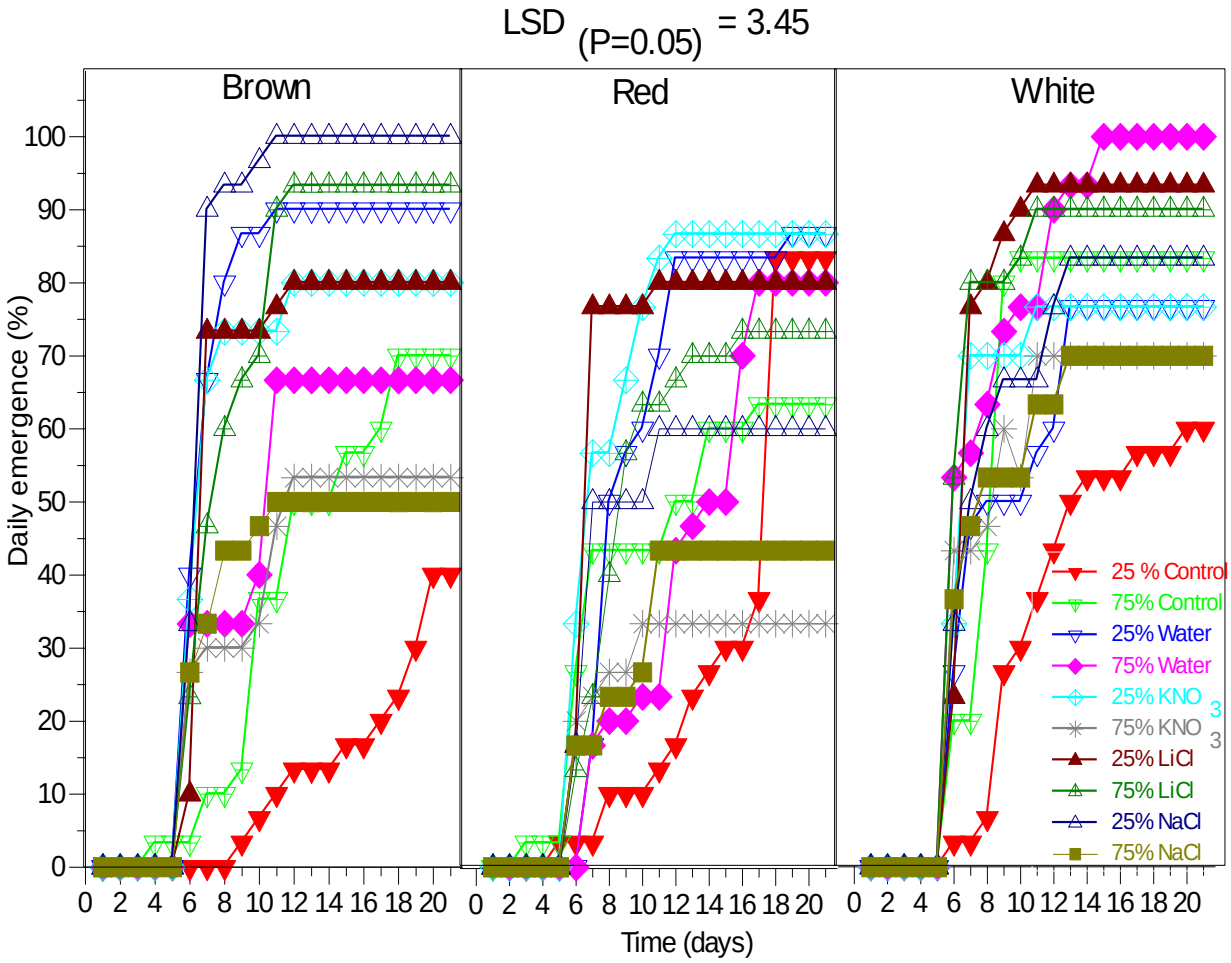


Figure 2.10: Effect of seed colour and salt solution on daily seedling emergence under two water regimes (25% & 75% FC).

There were highly significant differences (P<0.001) between seed colours with respect to daily emergence. Over-all, white had the highest (53.87%) final emergence followed by brown (47%) and red (41.10%), respectively (Fig 2.10 & 2.11). There were highly significant differences (P<0.001) between FC; 25% FC had 5.35% higher emergence than 75% FC. There were highly significant differences (P<0.001) between priming treatments with LiCl having highest emergence (58.44%), followed by H_2O (53.02%), NaCl (46.83%), KNO_3 (46.11%) and Control (32.22%), respectively. With respect to seedling emergence, the interaction between seed colour, priming and field capacity was highly significant (P<0.001). Priming improved emergence under water stress (25% FC) with brown NaCl showing highest germination (71.75%).

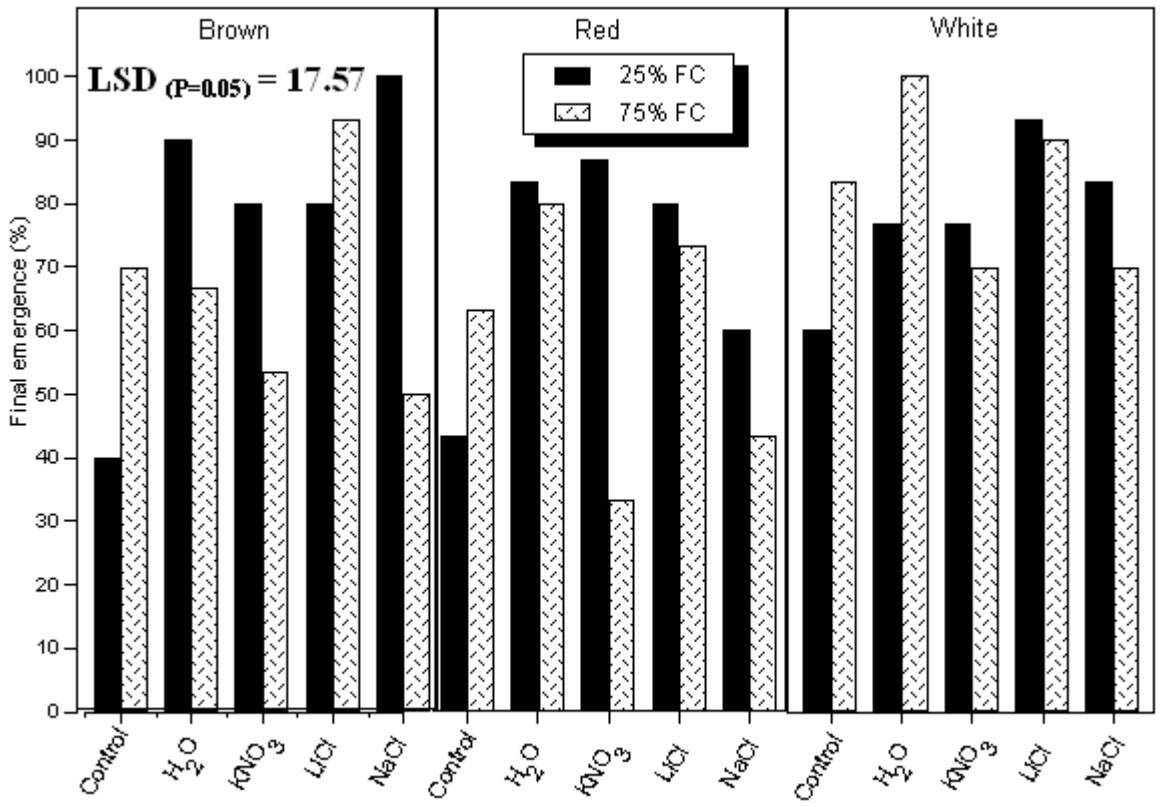


Figure 2.11: Effect of seed colour and salt solution on final seedling emergence under two water regimes (25% & 75% FC).

There were highly significant differences ($P<0.001$) between seed colours with respect to final emergence. Over-all, white had the highest (80.3%) final emergence followed by brown (72.3%) and red (64.7%), respectively. There were no significant differences ($P<0.05$) between FC with respect to final seedling emergence; although 25% FC had the highest final emergence. There were highly significant differences ($P<0.001$) between treatments with LiCl having higher (85.0%), followed by H_2O (82.8%), NaCl (67.8%), KNO_3 (66.7%) and Control (60.0%), respectively. There were also highly significant differences ($P<0.001$) for the interaction between seed colour, priming and field capacity with respect to final seedling emergence.

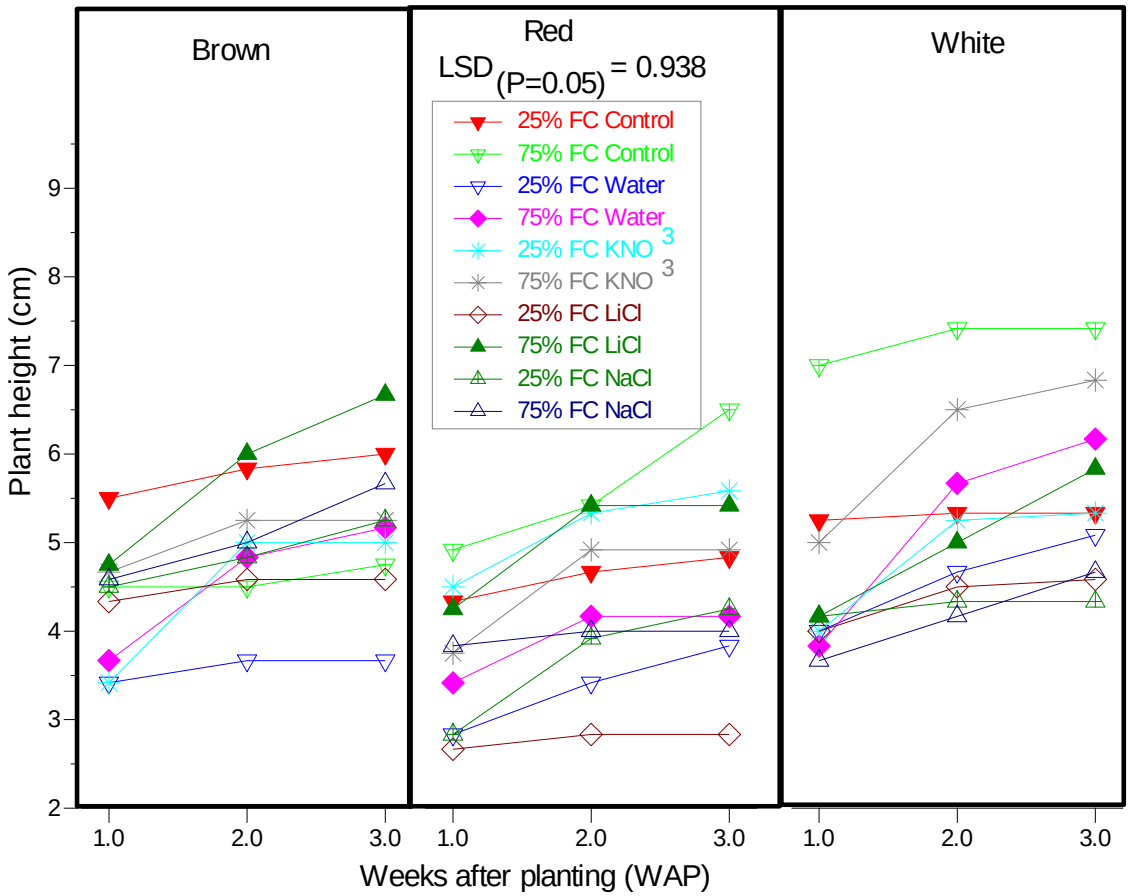


Figure 2.12: Effect of seed colour and salt solution on plant height under two water regimes (25% & 75% FC).

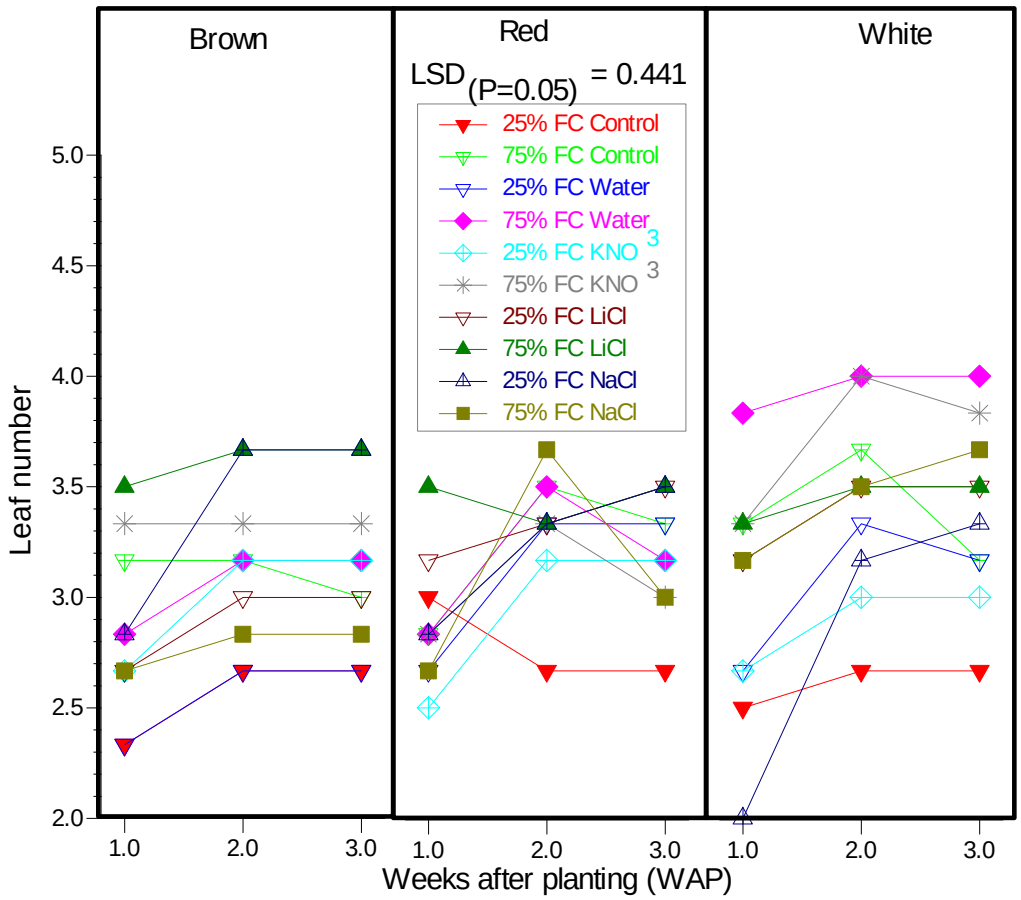


Figure 2.13: Effect of seed colour and salt solution on leaf number under two water regimes (25% & 75% FC).

There were highly significant differences (P<0.001) between seed colours with respect to plant height (Fig 2.12). Over-all, white had the tallest plants (5.12 cm), followed by brown (4.83 cm) and red seeds (4.26 cm), respectively. There were highly significant differences (P<0.001) between treatments with the control having the tallest plants (5.53 cm) followed by KNO_3 (5.01 cm), LiCl (4.58 cm), NaCl (4.33 cm) and H_2O (4.2 cm), respectively. There were also highly significant differences (P<0.001) in the interaction between seed colour, priming and field capacity, with respect to plant height. With respect to leaf number, there were significant differences (P<0.05) between seed colours (Fig 2.13). Over-all, white had the highest number of leaves followed by red and brown, respectively.

Table 2.2: **Effects of different priming treatments and water regimes on Bambara seedling emergence**.

Water regime	Priming	Seed colour	MET[x] (days)	Leaf Area (cm^2/plant)	Fresh mass(g)	Dry mass(g)	Shoot length(mm)	Root length(mm)	Root: Shoot ratio
25% FC	**Control**	Red	16.20b	8.83f	0.86ab	0.22a	5.67cdefgh	4.00b	0.71bcd
		Brown	17.17a	12.06def	0.92ab	0.21a	7.00abcde	4.83ab	0.74bcd
		White	15.59c	17.36bcdef	1.11ab	0.29a	5.58cdefgh	5.00ab	0.95bc
	LiCl	Red	13.83e	26.51abcd	1.34ab	0.29a	4.83egh	5.00ab	1.04b
		Brown	14.08de	19.10bcdef	1.07ab	0.25a	6.50abcdefgh	4.67ab	0.75bcd
		White	14.04de	20.44bcdef	1.18ab	0.27a	7.83ab	4.83ab	0.64cd
	NaCl	Red	14.30de	13.15def	0.76b	0.19a	5.83bcdefgh	5.17ab	0.89bcd
		Brown	13.93de	19.16bcdef	1.03ab	0.24a	6.17bcdefgh	5.83a	0.95bc
		White	14.32de	22.08abcdef	1.26ab	0.31a	5.33defgh	5.17b	0.92bc
	KNO_3	Red	14.16de	16.24bcdef	0.94ab	0.22a	7.00abcdef	5.50ab	0.78bcd
		Brown	13.93de	17.76bcdef	1.010ab	0.22a	5.83bcdefgh	5.50ab	0.95bc
		White	13.90de	17.78bcdef	0.91ab	0.21a	6.00bcdefgh	5.50ab	1.03b
	Water	Red	14.40de	12.65def	0.86ab	0.22a	4.50h	6.00a	1.35a
		Brown	13.95de	12.40def	0.761b	0.19a	5.17defgh	5.33ab	1.03ab
		White	14.48de	12.00ef	0.83ab	0.21a	5.83bcdefgh	5.50ab	0.94bc
75% FC	**Control**	Red	14.29de	34.55a	1.72a	0.42a	6.33abcdefgh	4.83ab	0.77bcd
		Brown	15.70bc	17.03bcdef	1.34ab	0.34a	5.58cdefgh	5.33ab	0.97bc
		White	14.46de	21.79abcdef	1.42ab	0.27a	8.42a	4.50ab	0.54 d
	LiCl	Red	14.63d	29.89ab	1.35ab	0.33a	6.50abcdefgh	5.50ab	0.85bcd
		Brown	14.45de	18.76bcdef	1.34ab	0.31a	7.67abc	5.67ab	0.74bcd
		White	13.84e	15.27cdef	0.93ab	0.26a	7.00abcdef	5.33ab	0.76bcd
	NaCl	Red	14.57de	24.71abcde	1.25ab	0.37a	5.17defgh	5.00ab	0.99bc
		Brown	13.99de	28.35abc	0.87ab	0.23a	6.33abcdefgh	5.50ab	0.88bcd
		White	14.13de	15.96bcdef	1.06ab	0.27a	6.83abcdefg	6.17a	0.92bc
	KNO_3	Red	13.99de	28.88abc	1.49ab	0.37a	6.17bcdefgh	5.00ab	0.82bcd
		Brown	14.49de	23.62abcde	1.39ab	0.33a	7.33abcd	5.50ab	0.76bcd
		White	14.10de	21.00abcdef	1.22ab	0.30a	7.67abc	5.83a	0.76bcd
	Water	Red	15.57c	16.41bcdef	1.24ab	0.16a	6.17bcdefgh	5.67ab	0.92bc
		Brown	14.51de	19.48bcdef	1.16ab	0.30a	7.17abcd	5.33ab	0.75bcd
		White	14.31de	19.37bcdef	1.28ab	0.29a	6.67abcdefg	5.33ab	0.80bcd
LSD (Trt.) (P=0.05)			0.2480	4.792	0.2979	0.1487	0.719	0.5522	0.1252
LSD (SC*Prm*FC) (P=0.05)			**0**.6074	11.739	0.7296	0.3641	1.761	1.3526	0.3067

Note: MET = mean emergence time; SC = seed colour; Trt = treatment; Prm = Priming; FC = Field Capacity

With respect to mean emergence time (MET), there were significant differences ($P<0.05$) between seed colours and the interaction between seed colour and water regimes (Table 2.2). Over-all, white emerged faster (14.318 days) followed by red (14.595 days) and brown seeds (14.621 days), respectively. There were no significant differences ($P>0.05$) between FC with respect to MET. There were highly significant differences ($P>0.001$) between the treatments; seeds treated with KNO_3 showed the fastest (14.094 days) emergence time followed by LiCl (14.145 days), NaCl (14.206 days), H_2O (14.538 days) and control (15.575 days), respectively. White seeds responded best to priming with NaCl; they emerged fast (14.318 days) under both 75% FC (14.134 days) & 25% FC (14.322 days). There were no significant differences ($P>0.05$) between seed colours and also between the salts, with respect to shoot length; however, there were highly significant difference between 25% and 75% FC, with respect to shoot length. There were no significant differences ($P>0.05$) between treatments with respect to root length. There were no significant differences ($P>0.05$) between seed colours and between the priming treatments, with respect to root length. There were no significant differences ($P>0.05$) between seed colours and between the treatments with respect to root:shoot ratio, while there were no significant differences ($P<0.05$) between priming treatments. There were no significant differences between seed colours with respect to fresh mass. There was significant difference ($P> 0.05$) between 25% FC and 75% FC treatments with respect to fresh mass. There were no significant differences between seed colours with respect to dry mass and also between treatments. There were highly significant differences ($P<0.01$) between 25% FC and 75% FC treatments with respect to leaf area with 75% FC having larger (22.34 cm^2/plant) leaf area followed by 25% FC (16.50 cm^2/plant), respectively.

2.4 Discussion

Seed quality refers to the suitability of a seed lot for its intended purpose; in this context it is defined in terms of physiological quality (viability and vigour) (McDonald & Copeland, 1997). Basu (1995) defined viability as the property of the seed that allows it to germinate under optimum conditions. Previous research (Powell, 1989; Zulu & Modi, 2010) indicated that seed colour was associated with seed quality. The current study sought to evaluate seed quality components of a local Bambara landrace, based on seed colour.

The standard germination test is the most common measure of seed viability (Peñaloza, 2005**)**. However, the test is conducted under ideal laboratory conditions seldom encountered in the field. Thus, germination results do not necessarily correlate with field performance. Ideally, a viability test should differentiate between poor and good seed lots (Trawatha *et al*., 1990). Brown seeds were viable (>80%) and showed the highest (83.6%), germination across treatments, followed by red (83.5%) and white (61.6%) seeds, respectively.

On the other hand, the cold germination test is used to evaluate seed vigour. Vigour refers to that aspect of the seed responsible for rapid, uniform germination, increased storability, good field emergence and an ability to perform well under field conditions (Perry, 1978; McDonald, 1980). Results showed significant differences between SG and CT, with SG showing higher germination than CT. This result supports previous findings on small grain legumes, such as soybean (Khan *et al*., 2007) and forage species, such as *Lolium multiflorum* (Marshall & Naylor, 1995), *Bromus biebersteinii* (Hall & Wiesner, 1990), *Lotus corniculatus* L., and alfalfa (Wang *et al*., 1996) where SG showed higher germination than field emergence. Brown seeds showed the highest germination across treatments, followed by red and white, respectively. Even though brown had the highest germination across the treatments, there were no significant differences ($P>0.05$) between the seed colours with respect to germination. The interaction between seed colours and treatments was not significant effect ($P>0.05$).

The electrical conductivity (EC) test is used to measure seed vigour. It has previously been used for the selection of highly vigorous pea seed lots for sowing under unfavourable stress

conditions (Matthews & Powell, 1981). A positive relationship was found between EC measurements and germination capacity of pea seeds (Taweekul *et al*., 1998; Vieira *et al*., 1999; Siddique *et al*., 2002). Some seeds leak (ions, amino acids, sugars and inorganic compounds) during imbibition, leakage increases as seeds age or are damaged. This is due to a loss of membrane integrity in the dry seed. The amount of solute leakage can be diagnostic for seed quality e.g. peas. However, it does not work for many seeds that have an impermeable barrier around the embryo e.g. lettuce, melons and tomato. Higher conductivity suggests more deterioration. White had the highest electrolyte leakage followed by red and brown seeds, respectively. This implies that, based on results of electrolyte leakage, white seeds had lesser vigour than red and brown seeds, respectively.

The results of EC concurred with previous reports on cowpea (*Vigna unguiculata* (L) Walp) (Asiedu and Powell, 1998), long bean (*Vigna sesquipedalis*) (Abdullah *et al*., 1993), soybean (*Glycine max*) (Mugnisjah *et al*., 1987), and radicchio (*Cichorium intybus*) (Pimpini *et al*., 2002), who reported that solute leakage, seed germination and vigour were associated with seed coat colour. Pigmented seeds showed lower solute leakage, slower decline in seed vigour and good storage potential, whereas unpigmented seeds showed higher solute leakage, rapid decline in vigour and poor storage potential. However, Hamman *et al*. (2001) failed to establish a relationship between conductivity of individual soybean seeds and their emergence performance; they observed that seeds with low conductivity performed poorly while those with high values performed well. Seed coat plays a role in the control of water absorption hence on the germination.

Several researchers - Duran & Retamal (1989); Wyatt (1977); Powell (1989); Kantar *et al*. (1996); Bewley, (1997) reported that seed coat colour was an important external factor which contributed significantly in water uptake and early protein synthesis of seeds. Zulu (1989) observed that seed germination in Bambara groundnut appeared to be more sensitive to water stress than groundnut. He ascribed this to the restrictive water uptake by Bambara groundnut due to hard seed coat. Effects of seed coat thickness on seed germination and seed viability were determined using Zeiss EVO Scanning Electron Microscope (SME). Picture scans of seed coat of three bambara groundnut seeds which differed in colour showed that brown and red had almost the same seed coat size followed by white respectively. The fact that white

seeds had the thinnest seed coat, as compared to brown and red seeds, may explain the higher EC and seed mass gain during imbibition. Therefore, poor germination observed in the white seeds may have been the result of increased leaking of solutes, and imbibitional injury caused by the rapid uptake of water during imbibition as a result of a thin seed coat.

The ability of cells to withstand stress imposed by an almost complete loss of cellular water during desiccation and to resume normal metabolic activities upon imbibition is described as desiccation tolerance (Kermode, 1995; Vertucci and Farrant, 1995; Hoekstra *et al.*, 2002). Proline accumulation is a common metabolic response of plants to abiotic and biotic stresses. It accumulates in plants experiencing water stress (Barnett & Naylor, 1966; Boggess *et al.*, 1976; Jones *et al.*, 1980). Results from the study showed that there were significant differences between seed lots and salt solutions, with respect to proline accumulation. Brown seeds had the highest (160.44 µmol g^{-1} DW) proline content, followed by red (147.30 µmol g^{-1} DW) and white (119.74 µmol g^{-1} DW), respectively. Sodium chloride was associated with the highest proline accumulation. Seed proline content increased from 0 hour to 8 hour and later declined. Brown seeds showed desiccation tolerance by accumulating high proline concentrations followed by red and white, respectively.

Crop establishment depends on an interaction between seedbed environment and seed quality (Khajeh-Hosseini *et al.*, 2003). Seedbed conditions and water stress are important factors affecting emergence and development of seedlings (Pollock, 1972). Bambara groundnut is often grown in areas where water supply is usually a limiting factor (Mwale *et al.*, 2007). The crop is thus often exposed to a wide range of field conditions. However, if the water stress effect can be alleviated at the germination stage, chances for attaining a good crop with economic yield production would be high (Ashraf & Rauf, 2001). Sivritepe and Dourado (1995) reported that seed priming improved seed germination.

Seed priming is a pre-sowing strategy for enhancing seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance (Bradford, 1986; Taylor and Harman, 1990).

Seed priming experiment was conducted to determine the effect halo-priming (NaCl, LiCl & KNO_3), hydro-priming (H_2O) on seedling emergence of Bambara groundnut. With respect to daily emergence, results from the study showed that, over-all, white had the highest (53.87%) emergence followed by brown (47%) and red (41.10%), respectively. 25 % FC had 5.35% higher emergence than 75% FC. LiCl had the highest emergence (58.44%), followed by H_2O (53.02%), NaCl (46.83%), KNO_3 (46.11%) and Control (32.22%), respectively. Under water stress (25% FC) LiCl continued having higher (85.0%), followed by H_2O (82.8%), NaCl (67.8%), KNO_3 (66.7%) and Control (60.0%) respectively. Over-all, emergence was higher at 25% FC than 75% FC. Priming improved emergence under water stress with brown NaCl showing highest germination (100%). Results showed that seed colours had significant differences with respect to plant height. Over-all, white had the tallest plants (5.12 cm) followed by brown (4.83 cm) and red seeds (4.26 cm), respectively. With respect to priming, control (dry seeds) had the tallest plants (5.53 cm) followed by KNO_3 (5.01 cm), LiCl (4.58 cm), NaCl (4.33 cm) and H_2O (4.2 cm), respectively. With respect to leaf number, white (3.272) had the highest number of leaves followed by red (3.139) and brown (3.028), respectively. Water stress reduced seedling growth but it had no effect on seedling vigour of the three seed colours.

2.5 Conclusions

Indications that brown seed germinated better than white or red seed deserve more research. However, white performed better than brown and red under both water regimes in seedling establishment. Water stress reduced seedling growth but it had no effect on seedling vigour of the three seed colours. Bambara seed germination is associated with seed colour. Seed colour can be used for germplasm selection to grow the crop under various conditions. Darker coloured seed were more vigorous than light coloured seed, therefore farmers may use darker coloured seeds for fast emergence which directly proportional to the higher yields. Brief exposure of the seed to saturated salt solutions resulted in measurable increases in the proline concentration, but with longer exposure the proline concentration declined. Sodium chloride (NaCl) was associated with the highest proline accumulation. Priming improved seedling emergence under water stress (25% FC) with brown seeds primed with NaCl showing highest germination (100%). Furthermore, white seeds primed with NaCl emerged fast under both 75% FC & 25% FC. Information from this study will be useful for future germplasm studies of Bambara groundnuts.

References

ABDULLAH, W.D., POWELL A.A. & MATHEWS S., 1993. Association of differences in seed vigour in long bean (*Vigna sesquipedalis*) with testa colour and imbibition damage. *J. Agric. Sci.* 116, 259-264.

ASSOCIATION OF OFFICIAL SEED ANALYSTS (AOSA), 1996. Rules for testing seeds. Journal of Seed Technology 16(3), 1B113.

ASHRAF, M. & RAUF, H., 2001. Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts, growth and ion transport at early growth stages. *Acta Physiol. Plant* 23, 407-414.

ASIEDU E.A. & POWELL A.A., 1998. Comparison of storage potential of cultivars of cowpea (*Vigna unguiculata* L.) differing in seed coat pigmentation. *Seed Sci. Technol.* 26, 211-221.

BARNETT, N.M. & NAYLOR, A.W., 1966. Amino acid and protein metabolism in bermuda grass during water stress. *Plant Physiol*. 41, 1222-1230.

BASU, R.N., 1995. Seed viability. *In*: Basra, A.S. (Ed.), Seed Quality: Basic mechanisms and agricultural implications, Food Products Press.

BEWLEY, J.D. & BLACK, M., 1994. Seeds: Physiology of development and germination, Plenum, New York.

BEWLEY, J.D., 1997. Seed germination and dormancy. *Plant Cell* 9, 1055-1066

BOGGESS, S.F., ASPINALL, D. & PALEG, L.G., 1976. Stress Metabolism. IX. The significance of end-product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. *Aust. J. Plant Physiol*. 3, 513-525.

BRADFORD, K.J., 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hort. Sci*. 21, 1105–1112.

CLAUSSEN, W., 2005. Proline as a measure of stress in tomato plants. *J. Plant Sci.* 168, 241–248.

DURAN, J.M. & RETAMAL, N., 1989. Coat structure and regulation of dormancy in (*Sinapis arvensis* L.) seeds. *J. Plant Physiol*. 135, 218-222.

ELLIS, R.A. & ROBERTS, E.H., 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. & Technol.* 9, 373-409.

HALL, R.D. & WIESNER, L.E., 1990. Relationship between seed vigour tests and field performance of Regar meadow bromegrass. *Crop Sci*. 30, 967-970.

HAMMAN, B., HALMAJAN H. & EGLI D, B., 2001. Single seed conductivity and seedling emergence in soybean. *Seed Sci. Technol*. 29, 575-586.

HOEKSTRA, F.A., GOLOVINA, E.A. & NIJSE, J., 2002. What do we really know about desiccation tolerance mechanisms? *In*: Nicholas G., Bradford K.J., Come D. and Pritchard H.W. (Eds.) *The Biology of Seeds*: CAB International.

JONES, M.M., OSMOND, C.B., & TURNER, N.C., 1980. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. *Aust. J. Plant Physiol*. 7, 193-205.

KANTAR, F., PILBEAM, C.J. & HEBBLETHWAITE, P.D., 1996. Effect of tannin content of faba bean (*Vicia faba*) seed on seed vigour, germination and field emergence. *Ann. App. Biol.*, 128, 85-93.

KERMODE, AR., 1995. Regulatory mechanisms in the transition from seed development to germination: Interactions between the embryo and the seed environment. *In*: Kigel, J. and Galili, D. (Eds.) Seed Development and Germination. New York: Marcel Dekker Inc.

KHAJEH-HOSSEINI, M., POWELL, A.A. & BINGHAM, I.J., 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. Technol.*, 31, 715-725.

KHAN, A. Z., KHAN, H. GHONEIM, A. & KHAN, R., 2007. Seed quality and vigour of soybean as influenced by planting date, density and cultivar under temperate environment. *Int. J. Agric. Res*. 2(4), 368-376.

KOCABAS, Z., CRAIGON J. & AZAM-ALI, S.N., 1999. The germination response of Bambara groundnut (*Vigna subterranea* L. Verdc.) to temperature. *Seed Sci. Technol.* 27, 303-313.

LINNEMANN, A.R. & AZAM-ALI, S.N., 1993. Bambara groundnut (*Vigna subterranea* L. Verdc). *In*: Williams, J.T. (Ed.) Under-utilised Crops Series. II. Vegetable and Pulses. Chapman and Hall, London, UK. Pp 13-58.

MAGUIRE, J.D., 1962. Speed of germination - aid in selection and evaluation for seedling emergence and vigour. *Crop Sci.* 2, 176-177.

MARSHALL, A.H. & NAYLOR, R.E.L., 1995. Seed vigour and field establishment in Italian ryegrass. *Seed Sci. Technol*. 13, 781-794.

MASSAWE, F.J., COLLINSON, S.T., ROBERTS, J.A. & AZAM-ALI, S.N., 1999. Effect of pre-sowing hydration treatment on germination, emergence and early seedling growth of Bambara groundnut (*Vigna subterranea* (L.) Verdc) landraces. *Seed Sci. Technol*. 27, 893-905.

MATTHEWS, S. & POWELL, A.A. , 1981. The electrical conductivity test. *In*: D.A. Perry (Ed.) Handbook of vigour test Methods. ISTA, Zurich. Pp 49-56.

MCDONALD, M.B. 1980. Assessment of seed quality. *Hort. Sci.* 15, 784-788.

McDONALD., M.B. & COPELAND, L., 1997. Seed production: principles and practices. International Thomson Publishing, New York.

MUGNISJAH, W.Q., SHIMANOH, I. & MATSUMOTO, S., 1987.Studies on the vigour of soybean seeds. I Varietal differences in seed vigour. *Journal of the Faculty of Agriculture*, Kyushu University 31, 213-226.

MWALE, S.S., AZAM-ALI, S.N. & MASSAWE, F.J., 2007a. Growth and development of bambara groundnut (*Vigna subterranea*) in response to soil moisture. 1. Dry matter and yield. *Euro. J. Agron*. 26, 345-353.

PEÑALOZA, P., 2005. Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. *J. Biotechnol.* 8, 3.

PERRY, D.A., 1978. Report of the vigour test committee, 1974-1977. *Seed Sci. Technol*. 6, 159–181.

PIMPINI, F., FILIPINI, M.F., SAMBO, P. & GIANQUINTO, G., 2002.The effect of seed quality (seed colour variation) on storage, germination temperature and field performance of radicchio. *Seed Sci. Technol.* 30, 393-402.

POLLOCK, B.M., 1972. Effects of environment after sowing on viability. 150-171. *In* Roberts, E. H. (Ed.) Viability of seeds. Syracuse University Press, Syracuse, N.Y.

POWELL, A. A. & MATTHEWS, S., 1984. Prediction of the storage potential of onion seed under commercial storage conditions. *Seed Sci. Technol*., 12, 641-647.

POWELL, A.A., 1989. The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Ann. Bot.* 63, 169-175.

SESSAY A., KUNENE I.S. & EARNSHAW, D.M., 1999. Farmer's knowledge and cultivation of Bambara groundnut (*Vigna subterranea* L.) in Swaziland. UNISWA Research *J. Agric. Sci. Technol.* 3(1), 27-37.

SESSAY, A. & YARMAH, A., 1996. Field studies of Bambara groundnut in Sierra Leone. Proceedings of the International Bambara Groundnut Symposium, July 23-25, 1996, University of Nottingham, UK. Pp 45-60.

SESSAY, A., EDJE, O.T. & MAGAGULA, C.N., 2004. Agronomic performance and morphological traits of field grown Bambara groundnut in Swaziland. Proceedings of the International Bambara Groundnut Symposium, Aug. 8-12, 2003, Botswana College of Agriculture, Botswana. pp 47-63.

SIDDIQUE, A.B., WRIGHT, D. & MAHUM ALI, S.M., 2002. Effects of time of sowing on the quality of pea seeds. *J. Biol. Sci.*, 2 (6), 380-383.

SIVRITEPE, H.O. & DOURADO, A.M., 1995. The effect of priming treatment on the viability and accumulation of chromosomal damage in aged pea seeds. *Ann. Bot.*, 75, 165-171.

TAWEEKUL, N., HILL, G.D., MCKENZIE, B.A. & HILL, M.J., 1998. Field performance of field pea seeds with varying vigour levels. *Proc. of Agronomy Soc. of New Zealand*, 28, 99-105.

TAYLOR, A.G. & HARMAN, G.E., 1990. Concepts and technologies of selected seed treatments. *Ann. Rev. Phytopathol*. 28, 321– 339.

TOUNGOS, M., SAJO, A. & GUNGULA, D., 2009. Recommended Fertilizer Levels on Bambara Groundnut (*Vigna subterranea* L. Verdc) in Yola Adamawa State, Nigeria. *Agricultural Journal* 4, 14-21.

TRAWATHA, S., STEINER, J. & BRADFORD, K., 1990. Laboratory vigour tests to predict pepper seedling field emergence performance. *Crop Sci.* 30, (3), 713-717.

VERTUCCI, C.W. &FARRANT, J.M., 1995. Acquisition of desiccation tolerance. *In*: Kigel, J. and Galili, D. (Eds.) Seed Development and Germination. New York: Marcel Dekker Inc.

VIEIRA, R.D., PAIVA-AGUERO, J.A. & PERECIN, D., 1999. Electrical conductivity and field performance of soybean seeds. *Seed Sci. Technol*. 21, 15-24.

WANG, Y.R., YU, L. & NAN, Z.B., 1996. Use of seed vigour tests to predict field emergence of Lucerne (*Medicago sativa*). *N.Z. J. Agric. Res*. 39, 255-262.

WYATT, J.E., 1977. Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat colour. *J. Am. Soc. Hort. Sci*., 102, 478-480.

ZEVEN, A. C., 1998. Landraces: A review of definitions and classifications. *Euphytica* 104:127-139.

ZULU, D., 1989. Germination and establishment of Bambara groundnut (*Vigna Subterranea* L. Verdc) and groundnut (*Arachis hypogeae*) in response to temperature, moisture, sowing depths and seed size. MSc. Thesis, University of Nottingham, UK.

ZULU, N.S. & MODI, A.T., 2010. A preliminary study to determine water stress tolerance in wild melon (*Citrillus lanatus* L.). *S. Afr. J. Plant & Soil 27,* 334-336.

CHAPTER 3

GROWTH, DEVELOPMENT AND YIELD RESPONSES OF A BAMBARA GROUNDNUT LANDRACES TO SEED PRIMING AND WATER STRESS

3.1 Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc) is an indigenous African legume. The crop is usually grown under water limiting conditions and has high potential for enhancing food security in rural areas across the African continent (Toungos *et al.*, 2009). Bambara groundnut is widely regarded as drought tolerant (Linnemann and Azam-Ali, 1993). Collinson *et al*. (1997) suggested that drought tolerance of bambara groundnut was a result of osmotic adjustment and low water loss through stomatal closure. However, despite its potential, bambara groundnut is still cultivated from local landraces rather than from varieties suitable for particular environments hence farm yields are still low. Unpredictable Bambara yields have been attributed, but not exclusively, to variable or poor field establishment due to poor crop establishment (Linnemann and Azam-Ali, 1993).

Zulu (1989) reported that seed germination in Bambara groundnut was sensitive to water stress. He ascribed this to the restrictive water uptake by Bambara groundnut due to a hard seed coat. Furthermore, Bambara groundnut is often grown in areas where water supply is usually a limiting factor (Mwale *et al*., 2007). The crop is thus often exposed to a wide range of field conditions. Water stress can affect crops at almost any stage of growth and development, such as early establishment, vegetative growth, flowering and yield formation, resulting in low economic yield and poor quality produce. However, if water stress effect can be alleviated at the germination stage, chances for attaining a good crop with reasonable economic yield production would be high (Ashraf and Rauf, 2001). Sivritepe and Dourado

(1995) reported that seed priming improved seed germination, resulting in rapid seedling emergence. This enabled seedlings to efficiently capture and use resources such as light through early canopy development and nutrient uptake.

Seed priming is a pre-sowing strategy for enhancing seedling development by modulating pre-germination metabolic activity prior to emergence of the radical. The objective of priming seeds is to enhance germination capacity and vigour (Bradford, 1986; Taylor and Harman, 1990). Seed priming has been reported to have improved germination and emergence of many crops, particularly vegetables and small seed grasses (Heydecker and Coolbear, 1977; Bradford, 1986). Priming has also been reported to improve early establishment performance of many field crops such as wheat, sugar beet, maize, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini *et al.*, 2003; Sadeghian and Yavari, 2004).

Physiological treatments to improve seed germination and seedling emergence under various stress conditions have been previously investigated (Bradford, 1986). Several methods have been used and described in the literature to prime seeds. Two of these methods are hydro-priming and halo-priming. Hydro-priming has been described as a low-cost method of seed priming, requiring little sophistication and equipment, if any, and giving good results consistently (Foti *et al*., 2008). This may explain why it has been traditionally practiced by farmers; Harris *et al*. (1999) reported a positive impact on the farming system and livelihoods of communities in response to a hydro-priming intervention on several crops. Hydro-priming maize resulted in improved field emergence and seedling performance (Nagar *et al*., 1998). Mabhaudhi and Modi (2011) also reported improved germination speed, vigour and emergence in maize landraces in response to hydro-priming. The technique has been successfully used to improve establishment, vigour and yield of upland rice and chick-pea (Harris *et al*., 1999).

Halo-priming involves soaking seeds in salt solutions, as an alternative to hydro-priming, which enhances germination and seedling emergence uniformity under adverse environmental conditions (Cantliffe, 1997). Seeds with more rapid germination under salt stress may be expected to achieve high final germination as well as rapid and vigorous seedling

establishment (Rogers *et al.*, 1995). Plant responses to salt and water stress have much in common. Salinity causes reductions in growth rate, along with a host of metabolic changes identical to those caused by water stress. A number of salts have been used for soaking seeds (Raul, 1992; Bose and Mishra, 1999). Sung and Chiu (1995) found that halo-primed watermelon seeds had higher seedling emergence.

Although Bambara groundnut is a crop with much potential, it is still cultivated using landraces as opposed to improved varieties. Landraces exhibit a huge variation within and without a particular landrace. One of the primary limitations to higher yields is poor crop establish; in most cases farmers are unable to recover from the effects of poor crop establishment. The fact that the crop is often cultivated under water limited conditions does not do much to improve the situation. Under such circumstances, priming has been suggested to enhance germination speed, vigour and emergence. This study investigated the effects of priming bambara groundnut landrace seeds, using halo- and hydro-priming, on germination and subsequent growth under water stress conditions.

3.2 Material and Methods

3.2.1 Plant material

Seeds of Bambara landraces were collected from subsistence farmers in Jozini, KwaZulu-Natal, and sorted into three distinct colours: red, white and brown. Previous research (Zulu and Modi, 2010) indicated that seed colour was associated with seed quality. To confirm this observation a number of viability and vigour tests were carried out.

3.2.2 Glass house environment

Pot trials were conducted under controlled environment conditions (27/15°C day/night; 65% Relative Humidity and natural day length) at the University of KwaZulu-Natal's Controlled Environment Research Unit (CERU), Pietermaritzburg. The experiments were conducted under simulated drought conditions were temperature and relative humidity were monitored electronically using a HOBO 2K logger (Onset Computer Corporation, Bourne, USA).

3.2.3 Experimental design and layout

The experimental design was a factorial experiment, with three factors, seed colour (red, white & brown), priming (NaCl, LiCl, KNO_3, H_2O & control or dry seeds) and water regimes (25% and 75% FC). The experiment was replicated three times. Salt concentrations, were prepared and 50 seeds of each colour were suspended over each of the three salt concentrations and distilled water and sealed in a small box and stored in a germination chamber at 20°C for 8 hours. Thereafter, for each treatment, 30 seeds were sampled and planted in seedling trays using pine bark wetted to 25% and 75% FC, respectively, over 22 days. The trays were weighed and watered at two day intervals to maintain field capacity. After 22 days seedlings were transplanted into undrained pots (17 cm diameter and 15 cm depth in size). 90 pots were each filled with 2 kg of soil whose field capacity had previously been determined in situ. Soil water content in the pots was monitored gravimetrically. Individual pots were placed on a balance and weighed at two-day intervals. Water was then added to the individual pots until the required soil water content of 75% and 25% FC was attained. In order to account and make corrections for plant mass when watering, a few extra pots with plants separate from the

experiment were used to verify calculations and estimates. The experimental pots were randomly rotated at every watering interval.

3.2.4 Fertilization and pests & disease management

Fertilizer application was based on a soil analysis report of the soil used in this study under field conditions since used the same soil in the pot experiment. 2 ml of Funginex (triforine 190 g/l) and Mikal (fosetyl-Al/Alky/phosphate 440 g/kg) per litre of water plus 40 g (mancozeb/dithiocarbonate 260 g/kg) plus 2ℓ of milk per 10 litre of water were used to spray plants for powdery mildew and red spider mites.

3.2.5 Data collection

Plant height and leaf number were determined weekly. Plant height was measured from the soil surface to the base of the leaf. Leaf number was counted for leaves with at least 50% green area up till flowering and each trifoliate leaf was counted as one leaf. Yield components were measured at harvest.

3.2.6 Data analysis

Data were analysed using ANOVA from GenStat® Version 12 (VSN International, UK). Means were separated using Duncan's Multiple Range Test in GenStat® at the 5% level of significance (Appendix 2).

3.3 Results

There was a highly significant interaction (P<0.001) between seed colour, priming and field capacity, with respect to plant height. There were significant differences (P<0.05) between priming treatments with the KNO_3 having the tallest plants (9.82 cm) followed by NaCl (9.74 cm), Control (9.36 cm), LiCl (8.87 cm) and H_2O (8.87 cm), respectively. Priming reduced plant height under water stress (25% FC) by (5.84%) using KNO_3, (11.27%) when primed by LiCl and seeds primed with NaCl showing highest reduction of (19.63%). However, when seeds primed with water and Control (non-primed) seeds resulted in increased plant height by (3.22%) and (11.57%) respectively. There were no significant differences (P>0.05) between seed colours with respect to plant height. Based on means, over-all, red had the tallest plants (9.45 cm), followed by white (9.39 cm) and brown seeds (9.16 cm), respectively.

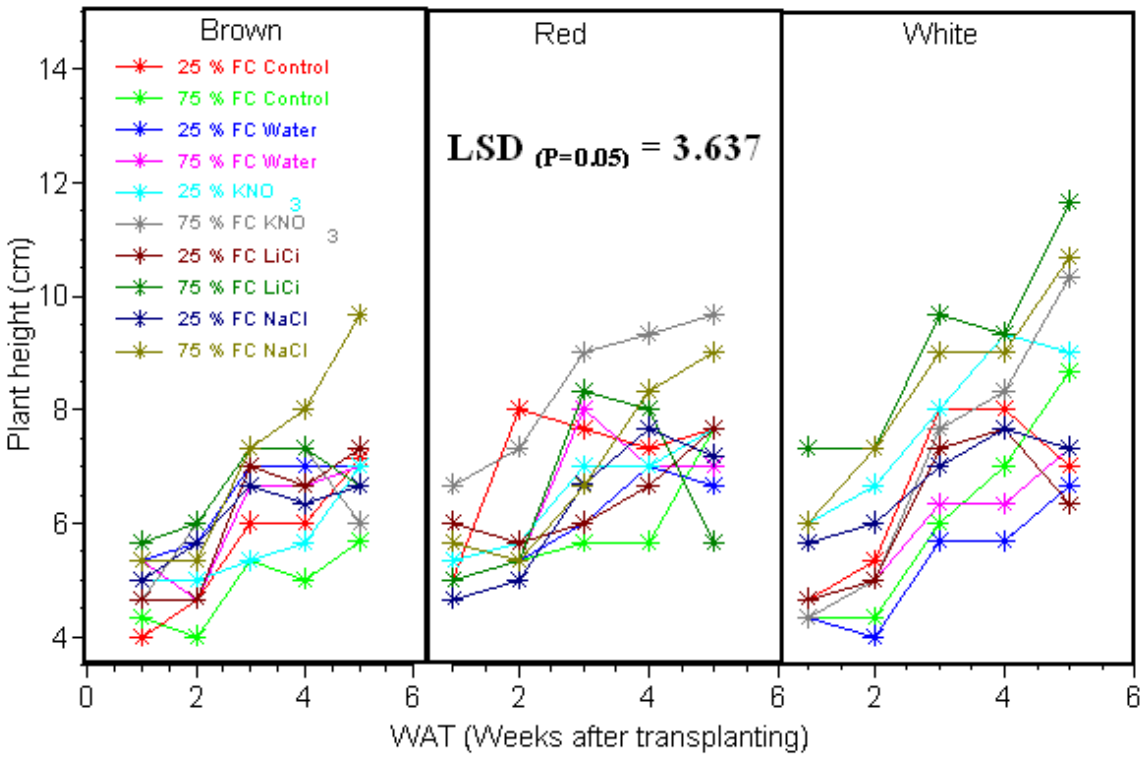


Figure 3.1: Plant height of bambara landraces (brown, red & white) planted under two water regimes (25% & 75% FC) in response to hydro- and halo-priming.

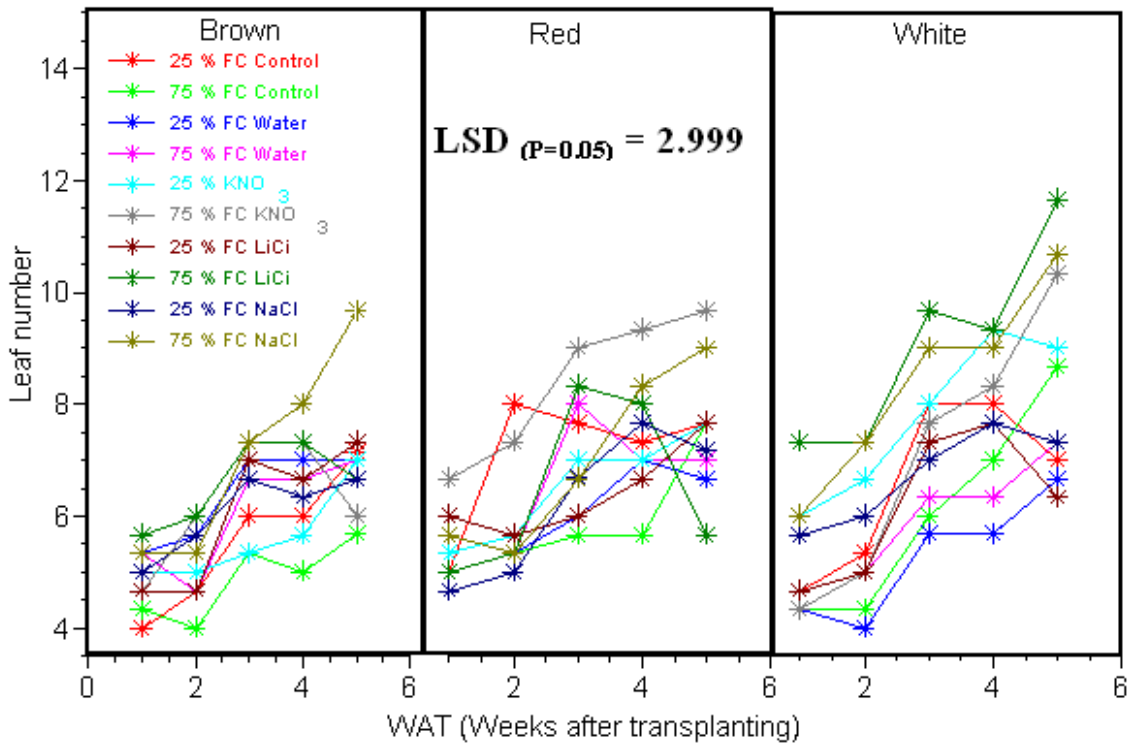


Figure 3.2: Leaf number of bambara landraces (brown, red & white) planted under two water regimes (25% & 75% FC) in response to hydro- and halo-priming.

There was significant interaction ($P<0.05$) between seed colour, priming and field capacity, with respect to leaf number. There were also highly significant differences ($P<0.001$) between priming treatments. Plants which were primed using KNO_3 had the most leaves (6.96) followed by NaCl (6.93), LiCl (6.8), Control (6.02) and H_2O (6.01), respectively. Priming reduced leaf number under water stress (25% FC) by (8.57%) using KNO_3, water (3.95%), when primed by NaCl (15.49%) and plants primed with LiCl showing highest reduction of (15.67%). However, unprimed (Control) plants resulted in increased leaf number by (12.94%). There were highly significant differences ($P<0.001$) between seed colours. Based on means, over-all, white had the highest number of leaves (6.9) followed by red (6.6) and brown (6.1), respectively. Same trend was also observed in seedling establishment (Chapter 2) using similar priming treatments.

Table 3.1: Yield components of a bambara landrace (red, brown and white) grown under two water regimes (25% & 75% FC).

Water regime	Priming	Seed colour	Total biomass (g)	Harvest index (%)	Pod mass(g)	Pod No./Plant	Grain No./Pod	Total grain mass/plant	Yield (kg/ha)
25% FC	Control	Red	1.57ab	42.48abcd	0.84abc	2.54abc	0.98cd	0.68abc	113.92abc
		Brown	1.09b	16.78ghi	0.14cd	0.54bc	0.98cd	0.07de	11.09de
		White	1.06b	17.38fghi	0.25cd	0.91bc	1.01bcd	0.16cde	25.90cde
	LiCl	Red	1.18b	48.33ab	0.85abc	2.17abc	0.95d	0.67abc	112.05abc
		Brown	0.93b	31.02bcdefgh	0.41abcd	1.17abc	1.45a	0.36abcd	60.55abcd
		White	1.05b	36.42abcdefg	0.52abc	2.17abc	0.95d	0.40abcd	67.05abcd
	NaCl	Red	1.03b	24.00cdefgh	0.52abc	2.06abc	0.88d	0.42abcd	69.35abcd
		Brown	1.24b	34.03abcdefgh	0.83abc	1.92abc	1.02bcd	0.45abcd	75.18abcd
		White	1.02b	33.00abcdefgh	0.43abcd	1.93abc	1.04bcd	0.36abcd	60.05abcd
	KNO_3	Red	1.18b	25.60cdefgh	0.36abcd	1.92abc	1.02bcd	0.27bcde	45.01bcde
		Brown	1.04b	41.61abcde	0.55abc	1.91abc	1.01bcd	0.42abcd	70.56abcd
		White	2.12ab	26.28bcdefgh	0.50abc	2.00abc	1.17b	0.41abcd	69.00abcd
	Water	Red	1.26b	31.86bcdefgh	0.43abcd	1.93abc	1.04bcd	0.37abcd	61.39abcd
		Brown	1.17b	22.59cdefgh	0.28cd	1.67abc	1.00bcd	0.21cde	34.56cde
		White	1.27b	0.00i	0.00d	0.00d	0.00bcd	0.00cde	0.00e
75% FC	Control	Red	1.34b	39.59abcdef	0.53abc	2.54abc	0.98cd	0.41abcd	68.92abcd
		Brown	1.24b	41.12abcde	0.52abc	2.92abc	1.02bcd	0.43abcd	70.84abcd
		White	1.19b	20.17defgh	0.17cd	0.17c	0.95d	0.14cde	22.72cde
	LiCl	Red	1.61ab	55.09a	1.079a	3.05abc	1.16bc	0.89a	148.32a
		Brown	0.91b	19.72efgh	0.32bcd	0.91bc	1.01bcd	0.22cde	36.40cde
		White	2.65a	34.27abcdefgh	1.08ab	4.33a	1.00bcd	0.84ab	139.44ab
	NaCl	Red	1.48ab	35.60abcdefg	0.69abc	2.54abc	0.98cd	0.53abcd	88.17abcd
		Brown	1.80ab	44.87abc	0.55abc	3.33abc	1.00bcd	0.70abc	117.39abc
		White	1.19b	36.25abcdefg	0.42abcd	1.33abc	1.02bcd	0.35abcd	58.28abcd
	KNO_3	Red	1.98ab	42.65abcd	0.89abc	3.67ab	1.02bcd	0.71abc	117.89abc
		Brown	1.24b	17.14fghi	0.38abcd	2.42abc	1.02bcd	0.63abcd	105.22abcd
		White	1.50ab	30.23bcdefgh	0.48abcd	2.67abc	1.00bcd	0.38abcd	62.72abcd
	Water	Red	1.47ab	43.38abc	0.79abc	2.92abc	1.02bcd	0.68abc	113.26abc
		Brown	1.21b	44.09abc	0.72abc	3.04abc	0.98cd	0.59abcd	97.67abcd
		White	1.10b	12.41hi	0.13cd	1.04bc	0.98cd	0.05de	9.09de
LSD (Trt.) (P=0.05)			0.433	7.79	0.261	1.123	0.062	0.205	34.15
LSD (SC*Prm*FC) (P=0.05)			1.061	19.07	0.640	2.752	0.151	0.502	83.65

There was no significant interaction (P>0.05) between seed colours, priming treatments and water regimes with respect to total biomass; same trend was observed for the interaction between treatments and water regimes. There were no significant differences (P>0.05) between water regimes. Over-all, 75% field capacity (FC) had highest total biomass (1.462g) followed by 25% FC (1.214g). There were no significant differences (P>0.05) between priming treatments based on mean values, over-all, KNO_3 had higher total biomass (1.512g) followed by LiCl (1.390g), NaCl (1.292), control (1.248g) and H_2O (1.248g), respectively. There were no significant differences (P>0.05) between seed colours. Based on means, over-all, white had higher total biomass (1.415g), followed by red (1.411g) and brown seeds (1.188g), respectively. Priming reduced total biomass under water stress by (8.31%) using KNO_3, when primed by NaCl (26.63%), seeds primed with LiCl showing highest reduction of, (38.93%) and when seeds were primed with water and Control (non-primed) seeds reduced total biomass by (2.29%) and (1.12%), respectively. Brown seeds decreased in total biomass by (16.6%) under water stress when primed with KNO_3, (31.5%) when primed with NaCl, when seeds were primed with water and control or non-primed seeds, plants reduced total biomass by (3.71%) and (12.6%) respectively. However, total biomass increased by (2.1%) when primed with LiCl.

Red seeds decreased in total biomass under water stress when primed with KNO_3 by (40.5%), when primed with NaCl (18.9%), control (non-primed) increased by (14.9%) and when seeds were primed with water and LiCl seeds reduced total biomass by (14.3%) and (27.1%), respectively. Seeds with white seed coat colour decreased in total biomass under water stress by (10.2%) when not-primed, (13.9%) when primed with NaCl, (14.3%) when primed with LiCl. However, when seeds were primed with KNO_3 and water, they increased total biomass by (28.9%) and (13.3%), respectively.

There were significant interaction (P<0.05) between seed colours, priming treatments and water regimes with respect to harvest index; however, there was significant interaction (P<0.05) for the interaction between treatments and water regimes. There were significant differences (P<0.05) between water regimes. Over-all, 75% field capacity (FC) had highest HI

(34.4%) followed by 25% FC (28.6%). There were significant differences ($P<0.05$) between priming treatments based on mean values, LiCl had higher total biomass (37.5%) followed by NaCl (34.6%), KNO_3 (30.6%), control (29.6%) and H_2O (25.4%), respectively. There were highly significant differences ($P<0.001$) between seed colours. Based on means, over-all, red had optimum HI (38.9%), followed by brown (31.3%) and white seeds (24.4%), respectively. Priming increased HI by (3.9%) under water stress using KNO_3, by (5.7%) when primed with LiCl; however, it decreased by (22.1%) when primed with NaCl, by (47.4%) and (24.1%) when primed with water and Control (non-primed) respectively.

Brown seeds increased HI by (30.7%) under water stress when primed using KNO_3 by (23.7%) primed by LiCl, however, HI decreased by (32.9%) when seeds were primed with NaCl, by (60.6%) and (74.1%) when seeds were primed with water and control (non-primed), respectively. Red seeds decreased in HI by (59.5%) under water stress when primed using KNO_3, by (24.6%) when primed with NaCl. However, non-primed seeds increased HI by (37.2%) and plants primed with water and LiCl reduced HI by (45.9%) and (25.5%) respectively. White seeds increased HI under water stress when not-primed by (28.7%), by (2.1%) when primed with NaCl, by (3.8%) when primed with KNO_3, however, HI decreased by (53.1%) and (100%) when seeds were primed with LiCl and water, respectively.

There were significant interaction ($P<0.05$) between seed colours, priming treatments and water regimes with respect to grain mass per plant; same trend was observed for the interaction between treatments and water regimes. There were significant differences ($P<0.05$) between water regimes. Over-all, 75% field capacity (FC) had highest grain mass per plant (0.503 g) followed by 25% FC (0.334 g). There were significant differences ($P<0.05$) between priming treatments based on mean values, LiCl had higher grain mass per plant (0.564g) followed by KNO_3 (0.470g), NaCl (0.468g), control (0.313g) and H_2O (0.277g), respectively. There were significant differences ($P<0.05$) between seed colours. Based on means, over-all, red had higher grain mass per plant (0.563g), followed by brown (0.408g) and white (0.285g), respectively. Priming decreased grain mass per plant under water stress by (35.49%) using KNO_3, by (22.54%) when primed with NaCl, by (26.1%) when primed with LiCl and by

(74.32%) and (7.1%) when primed with water and control (non-primed), respectively. Brown seeds increased grain mass per plant by (39.94%) under water stress when primed using LiCl, however, decreased by (35.9%) when primed with NaCl, by (32.96%) using KNO_3, by (64.7%) and (84.2%) when primed with water and control (non-primed), respectively. Red seeds decreased in grain mass per plant by (61.8%) under water stress when primed using KNO_3, by (21.4%) when primed with NaCl, however, grain mass per plant increased by (39.5%) when seeds were not primed and when seeds were primed with water and LiCl seeds reduced grain mass per plant by (45.9%) and (24.5%) respectively. White seeds increased grain mass per plant under water stress when not-primed by (12.3%), (2.8%) when primed with NaCl, (9.2%) when primed with KNO_3, however, when seeds were primed with LiCl and water grain mass per plant reduced by (51.97%) and no yield (100% reduction), respectively.

There were highly significant interaction ($P<0.001$) between seed colours, priming treatments and water regimes with respect to grains per pod; however, there was no significant interaction ($P>0.05$) for the interaction between treatments and water regimes. There were no significant differences ($P>0.05$) between water regimes. Over-all, 25% field capacity (FC) had highest grains per pod (1.04) followed by 75% FC (1.01). There were significant differences ($P<0.05$) between priming treatments based on mean values, LiCl had higher grains per pod (1.09) followed by KNO_3 (1.04), H_2O (1.01), control (0.99) and NaCl (0.98), respectively. There were no significant differences ($P>0.05$) between seed colours. Based on means, over-all, brown had higher grains per pod (1.05), followed by white (1.01) and red (1.00), respectively. Priming decreased grains per pod by (1.34%) under water stress using NaCl, however, it increased by (4.38%) when seeds were primed using KNO_3, by (5.63%) when primed with LiCl, water and Control (non-primed) seeds increased grains per pod by (2.8%) and (0.6%) respectively. Brown seeds increased grains per pod by (30.4%) under water stress when primed using LiCl, by (2.3%) when primed with NaCl, by (1.8%) when primed with H_2O.However, grains per pod decreased when seeds were primed with KNO_3 and control (non-primed) by (1.2%) and (4%) respectively.

Red seeds decreased grains per pod by (10.1%) under water stress when primed using NaCl and by (17.8%) when primed with LiCl. However, grains per pod increased by (1.8%) when

seeds were primed with water and there was no change between water regimes when seeds were not-primed and also when primed by KNO_3, (50%) and (50%), respectively. White seeds increased grains per pod by (5.7%) under water stress when not-primed, by (3.4%) when primed NaCl, by (14.3%) and (5.3%) when primed with KNO_3 and water, respectively, however, grains per pod decreased by (4.7%) when seeds were primed with LiCl.

There were no significant interaction ($P>0.05$) between seed colours, priming treatments and water regimes with respect to pod yield per plant; same trend was observed for the interaction between treatments and water regimes. There were significant differences ($P<0.05$) between water regimes. Over-all, 75% field capacity (FC) had highest pod yield per plant (2.46) followed by 25% FC (1.68). There were no significant differences ($P>0.05$) between priming treatments based on mean values, KNO_3 had higher pod yield per plant (2.43) followed by LiCl (2.30), NaCl (2.18), H_2O (1.82) and control (1.60), respectively. There were no significant differences ($P<0.05$) between seed colours. Based on means, over-all, red had higher pod yield per plant (2.53), followed by brown (1.98) and white (1.69), respectively. Priming decreased pod yield per plant under water stress by (17.9%) using NaCl by (33.6%) when seeds were primed using KNO_3, LiCl (33.3%), water and Control (non-primed) seeds decreased pod yield per plant by (43.4%) and (28.9%) respectively. Brown seeds increased pod yield per plant by (22.2%) under water stress when primed using LiCl, however, decreased by (42.3%) when seeds were primed with NaCl, by (45.1%) when primed with H_2O, by (21.1%) and (81.5%) when primed with KNO_3 and control (non-primed) respectively. Red seeds decreased pod yield per plant by (18.9%) under water stress when primed with NaCl, by (28.9%) when primed with LiCl and by (47.7%) when primed with KNO_3. When seeds were primed with water, pod yield per plant decreased by (33.9%), however, there was no change when seeds were not-primed (50%). White seeds increased pod yield per plant by (81.3%) under water stress when not-primed, by (31.1%) and (64.4%) when primed with NaCl and water, respectively. However, when seeds were primed with LiCl and KNO_3 pod yield per plant reduced by (49.9%) and (25.1%), respectively.

There were significant interaction ($P<0.05$) between seed colours, priming treatments and water regimes with respect to yield; same trend was observed for the interaction between treatments and water regimes. There were significant differences ($P<0.05$) between water regimes. Over-all, 75% field capacity (FC) had highest yield (83.8 kg/ha) followed by 25% FC (55.7 kg/ha). There were significant differences ($P<0.05$) between priming treatments based on mean values, LiCl had higher yield (94 kg/ha) followed by KNO_3 (78.4 kg/ha), NaCl (78.1 kg/ha), control (52.2 kg/ha) and H_2O (46.1 kg/ha), respectively. There were significant differences ($P<0.05$) between seed colours. Based on means, over-all, red had higher yield (93.8 kg/ha), followed by brown (67.9 kg/ha) and white (47.5 kg/ha), respectively. Priming decreased yield by (35.5%) under water stress using KNO_3, by (22.4%) when primed with NaCl, by (26.1%) when primed with LiCl. When seeds were primed with water and control (non-primed), seeds reduced yield by (74.4%) and (7.2%) respectively. When brown seeds were primed using LiCl they increased yield by (39.94%) under water stress conditions. However, yield decreased by (36%) when primed with NaCl, by (32.9%) when primed with KNO_3. When seeds were primed with water and control (non-primed), plants reduced yield by (64.6%) and (84.3%) respectively. Red seeds decreased yield by (61.8%) under water stress when primed using KNO_3, by (21.3%) when primed with NaCl, by (45.8%) and (24.4%) when primed with water and LiCl respectively. However, non-primed seeds, plants increased yield by (39.5%). White seeds increased yield by (12.4%) under water stress when not-primed, by (3%) and (9.1%) when primed by NaCl and KNO_3 salts, respectively. However, when seeds were primed with LiCl and water yield reduced by (51.9%) and no yield (100% reduction), respectively.

3.4 Discussion

The study investigated the effects of priming bambara groundnut landrace seeds, using halo- and hydro-priming, on germination and subsequent growth under water stress conditions. bambara groundnut is a crop with much potential; despite its potential, it is still cultivated using landraces as opposed to improved varieties. Landraces exhibit a huge variation within and without a particular landrace. One of the primary limitations to successful yields is poor crop establish; in most cases farmers are unable to recover from the effects of poor crop establishment. The fact that the crop is often cultivated under water limited conditions does not do much to improve the situation. Under such circumstances, priming has been suggested to enhance germination speed, vigour and emergence.

Halo-priming is a pre-sowing soaking of seeds in salt solutions for enhancing germination and seedling emergence uniformity under adverse environmental conditions (Cantliffe, 1997). Hydro-priming has been described as a low-cost method of seed priming, requiring little sophistication and equipment, if any, and giving good results consistently (Foti *et al*., 2008). According to Heydecker and Coolbaer (1977); Bradford (1986) reported that seed priming improved germination and emergence of vegetables and small seed grasses. Priming has also been reported to improve germination and emergence in wheat, sugar beet, maize, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini *et al*., 2003; Sadeghian and Yavari, 2004).

Seeds with more rapid germination under salt stress may be expected to achieve a high final germination percentage and rapid and vigorous seedling establishment (Rogers *et al.,* 1995). According to Demir and Van de Venter (1999) reported that Osmo-priming with KNO_3 has effectively improved germination in watermelons at low temperature. Results from the study showed that priming reduced plant height under water stress (25% FC) by (5.84%) using KNO_3, (11.27%) when primed by LiCl and seeds primed with NaCl showing highest reduction of (19.63%). However, when seeds primed with water and control (non-primed) seeds resulted in increased plant height by (3.22%) and (11.57%) respectively. Priming reduced leaf number under water stress (25% FC) by (8.57%) using KNO_3, water (3.95%), when primed by NaCl

(15.49%) and plants primed with LiCl showing highest reduction of (15.67%). However, unprimed (control) plants resulted in increased leaf number by (12.94%). Consequently, priming decreased yield by (35.5%) under water stress using KNO_3, by (22.4%) when primed with NaCl, by (26.1%) when primed with LiCl. When seeds were primed with water and control (non-primed), seeds reduced yield by (74.4%) and (7.2%) respectively. The results were in contrary with reports by Farooq *et al*., (2005) that priming with NaCl and KNO_3 resulted in more vigorous seedlings than untreated seeds. Also in contrary with reports by Cano *et al*., (1991) that priming with NaCl had positive results on growth and yield of mature tomato plants when salt treatments were applied with seed sowing. Priming seeds with NaCl, LiCl and KNO_3 resulted in tallest plants and highest yields than hydro-priming but they were the most affected by water stress.

3.5 Conclusions

During the course of the study, seeds primed with NaCl and KNO_3 resulted in tallest plants and many leaves. However, with respect to yield components LiCl had optimum yield and overlapping with KNO_3 with yield components. Little information found in literature describing effect of seed priming on seedling growth and yield. Although seeds primed with LiCl, NaCl and KNO_3 had tallest plants and many leaves hence highest yields; they were the most affected under water stress. Priming had shown to improve germination and early crop establishment of bambara groundnut landraces under water stress. Yield per plant had not improved by either halo or hydro-priming. Famers who plant early before onset of the rains may prime their seeds for fast emergence. Further research is needed on priming of bambara groundnut landrace seeds, using halo- and hydro-priming on proline accumulation of seedlings under water stress.

References

ASHRAF, M., & RAUF, H., 2001. Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts, growth and ion transport at early growth stages. *Acta Physiol. Plant.* 23, 407-414.

BOSE, B., & MISHRA, T., 1999. Influence of pre-sowing soaking treatment in *Brassica juncea* seeds with Mg-salts on growth, nitrate reductase activity, total protein content and yield responses. *Physio/. Mol. BioI. Plants*. 5, 83-88.

BRADFORD, K.J., 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hort. Sci.* 21, 1105–1112.

CANO, E.A., BOLARIN, M.C., PEREZ-ALFOCEA, F., & CARO, M., 1991. Effect of NaCl priming on increased salt tolerance in tomato. *J. Hort. Sci*., 66, 621-628.

CANTLIFFE, D.G., 1997. Industrial processing of vegetable seeds. *J. Korean Soc. Hort. Sci.* 38, 441-445.

COLLINSON, S.T., CLAWSON, E.J., AZAM-ALI, S.N., & BLACK, C.R., 1997. Effects of soil moisture deficits on the water relations of bambara groundnut (*Vigna subterranea* L. Verdc.). *J. Exp. Bot*. 48, 877–884.

DEMIR I., & VAN DE VENTER, H.A., 1999. The effect of priming treatments on the performance of watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) seeds under temperature and osmotic stress. *Seed Sci. & Technol., 27,* 871–875.

FAROOQ, M., BASRA, S.M.A., HAFEEZ, K., & AHMAD, N., 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *J. Integr. Plant Biol.*, 47, 187-193.

FOTI, R., ABURENI, K., TIGERE, A., GOTOSA, J., & GERE, J., 2008. The efficacy of different seed priming osmotica on the establishment of maize (*Zea mays* L.) caryopses. *J. Arid Environ*. 72, 1127-1130.

HARRIS, D., JOSHI, A., KHAN, P.A., GOTHKAR, P., & SODHI, P.S., 1999. On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. *Exp. Agric*. 35, 15-29.

HEYDECKER, W., & COOLBAER, P., 1977. Seed treatments for improved performance survey and attempted prognosis. *Seed Sci. & Technol*. 5, 353-425.

KHAJEH-HOSSEINI, M., POWELL, A.A., & BINGHAM, I.J., 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. & Technol*. 31, 715-725.

LINNEMANN, A.R., & AZAM-ALI, S.N., 1993. Bambara groundnut (*Vigna subterranea* L. Verdc). In: Williams, J.T. (Ed.), pp. 13-58. Under-utilised Crops Series. II. Vegetable and Pulses. Chapman and Hall, London, UK.

MABHAUDHI, T., & MODI, A.T., 2011. Can hydro-priming improve germination speed, vigour and emergence of maize landraces under water stress? *J. Agric. Sci. Technol. B1*, 20-28.

MWALE, S.S., AZAM-ALI, S.N., & MASSAWE, F.J., 2007a. Growth and development of bambara groundnut (*Vigna subterranea*) in response to soil moisture. 1. Dry matter and yield. *Euro. J. Agron*. 26, 345-353.

NAGAR, R.P., DADLAN, M.I., & SHARAMA, S.P., 1998. Effect of hydro-priming on field emergence and crop growth of maize genotypes. *Seed Res*. 26, 1-5.

PARERA, C. A., & CANTLIFFE, D. J., 1994. Presowing seed priming. *Hort. Rev*. 16, 109-141.

RAUL, S.R., 1992. Effect of pre-sowing seed treatment, seed rates, fertility levels and surface compaction on growth and yield of late sown rainfed wheat. Assam. *Ann. Agric. Res.* 13,410-411.

ROGERS, M.E., NOBLE, G.M., HALLORAN, G.I., & NICOLAS, M.E., 1995. The effect of NaCI on the germination and early seedling growth of white clover population selected for high and low salinity tolerance. *Seed Sci. & Technol.* 23, 277-287.

SADEGHIAN, S. Y., & YAVARI, N., 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. *J. Agron. Crop Sci*. 190, 138-144.

SINGH, B. G., 1995. Effect of hydration-dehydration seed treatments on vigour and yield of sunflower. *Indian J. Plant Physiol*. 38, 66-68.

SIVRITEPE, H.O., & DOURADO, A.M., 1995. The effect of priming treatment on the viability and accumulation of chromosomal damage in aged pea seeds. *Ann. Bot.*, 75, 165-171.

SUNG, J.M., & CHIU, K.Y., 1995. Hydration effects on seedling emergence strength of watermelon seed differing in ploidy. *Plant Sci*. 110, 21-26.

TAYLOR, A.G., & HARMAN, G.E., 1990. Concepts and technologies of selected seed treatments. *Ann. Rev. Phytopathol*. 28, 321– 339.

TOUNGOS, M., SAJO, A. & GUNGULA, D. 2009., Recommended Fertilizer Levels on Bambara Groundnut (*Vigna subterranea* L. Verdc) in Yola Adamawa State, Nigeria. *Agric. J.* 4, 14-21.

ZULU, D., 1989. Germination and establishment of Bambara groundnut (Vigna Subterranea (L.) Verdc) and groundnut (*Arachis hypogeae*) in response to temperature, moisture, sowing depths and seed size. MSc. Thesis, University of Nottingham, UK.

ZULU, N.S., & MODI, A.T., 2010. A preliminary study to determine water stress tolerance in wild melon (*Citrillus lanatus* L.). *S. Afr. J. Plant & Soil* 27, 334-336.

CHAPTER 4

GROWTH, DEVELOPMENT AND YIELD RESPONSES OF A BAMBARA GROUNDNUT LANDRACES TO PLANING DATE AND WATER STRESS

4.1 Introduction

Bambara groundnut (*Vigna subterranean* L. Verdc) is a legume crop with high potential for enhancing food security, especially in drought-prone regions (Toungos *et al.*, 2009). Bambara has been grown for centuries and has in the past contributed to the food security of Africa's poorest people (Swanevelder, 1998; FAO, 2001; Azam-Ali, 2001; Mwale *et al*., 2007b). It is grown in the semi-arid tropics where water is usually in short supply (Mwale *et al.*, 2007a). Under these conditions, water is an important factor limiting production, often resulting in low crop yields (Reij *et al*., 1991; Patil, 2010). Water scarcity remains an ever-growing problem, limiting crop production worldwide and causing important agricultural losses, particularly in arid and semi arid areas (Fischer and Turner, 1978; Boyer, 1982).

South Africa remains a water scarce country characterised by uneven rainfall distribution. Most plants are exposed to water stress due to extreme soil water deficits in arid and semi-arid environments (Morgan, 1984). Sensitivity to water stress varies according to development stage of the plant (Doorenbos and Kassam, 1979). Many aspects of crop growth have been reported to be affected by water stress including emergence, growth of leaves and roots, stomatal conductance, photosynthesis and dry matter accumulation (Blum, 1996). Water stress can occur at any time during the cropping season; thus, affecting plant efficiency to capture and utilise resources such as light, nutrient uptake and hence final yield at harvest.

The early crop is usually planted at the onset of the rainy season before the rains are fully established, and is therefore often exposed to water stress during the establishment stage due to a dry seedbed. Water stress during emergence has a negative impact on the capacity of a seed lot to emerge into seedlings capable of efficiently capturing and utilising resources such as light through early canopy development, nutrient uptake, weed control and hence final pod yield at harvest. Seedbed conditions and water stress are important factors affecting the emergence and development of seedlings (Pollock, 1972). In the semi-arid regions, soil water stress associated with high temperatures, is probably the most important factor affecting seed germination and emergence. Sesay *et al*. (2004) reported delayed and prolonged seedling emergence in Bambara groundnut due to water stress (drought) in trials conducted at Luve and Malkernes in Swaziland during 2001 and 2002, respectively. Zulu (1989) also reported that seed germination in Bambara groundnut appeared to be sensitive to water stress.

Water stress during vegetative growth affects leaf expansion and root elongation, resulting in reduced radiation interception by the crop and consequently reduced photosynthesis (Gardner *et al.,* 1985). In water-limited environments, the efficiency with which water is converted to dry matter is very important to crop productivity. Several authors (Collinson *et al*., 1999; Mwale *et al*., 2007a) observed reduced leaf area index (LAI) in Bambara in response to soil water stress conditions for different landraces. Collinson *et al.* (1996; 1999) reported that leaf number decreased by up to 60% in drought treatments resulting in reduced LAI. Mwale *et al.* (2007a) reported that soil water stress reduced both leaf number and LAI of Bambara crop. Similar results were reported elsewhere that total dry matter and leaf area index (LAI) were reduced by drought (Collinson *et al*., 1996, 1997).

Water stress during the reproductive phase can seriously affect dry matter allocation to yield components. In chickpea, for example, drought reduced both seed number and size, which led to yield losses of up to 80% (Leport *et al*., 1999). Collinson *et al*. (1996) reported a significant reduction in pod number per plant, harvest index (HI) and final yield due to drought in bambara groundnut. Thus, when planting, it is important to know whether the rains are continuous and sufficient in order to ensure optimal soil water availability during planting and

whether the soil water will be maintained or increased during the growing period to avoid total crop failure and to attain optimum yields (Walter, 1967).

Furthermore, it is important to select the best planting date where critical growth stages will coincide with favourable field conditions. According to Kucharik (2006), changes in climate as well as changes in technological and socio-economic factors usually result in variations in planting dates over time. For example, farmers may have to plant later than the climatically optimal time due to unavailability or shortages of machinery and or labour. While there are variations in planting dates; environmental factors, such as day length, temperature and rainfall also vary, thus limiting the time available for growth, reproductive development and yield formation. Planting too early might lead to poor crop stands and crop failure due to low soil temperatures and or frost damage while planting too late might reduce valuable growing time and crop yield. The late season crop is planted during the short second cycle of rains, a planting which terminates in terminal drought.

In South Africa, Bambara production is carried out in the KwaZulu-Natal, Mpumalanga, Limpopo and North West Provinces. Rainfall amount and distribution throughout the year vary considerably among these provinces. The crop is normally planted during October and November after good rains. Elsewhere, it is planted during October to January and planting dates vary from year to year (Swanevelder, 1998; Sesay *et al*., 1999). In Swaziland, observations suggested that bambara groundnut production by subsistence farmers was characterized by low and unpredictable yields (Linnemann and Azam-Ali, 1993; Sesay *et al*., 1999); lower yields have been associated with late plantings (Johnson, 1968; Swanevelder, 1998; Harris and Azam Ali, 1993). The aim of the study was to evaluate the selection of planting dates as a management factor for managing water stress in bambara groundnut landraces under field conditions. Furthermore, information generated from the study would be useful to promote Bambara cultivation as an alternative legume crop under water-scarce conditions.

4.2 Material and Methods

4.2.1Planting material

Seeds were collected from subsistence farmers in Jozini (27°26'S; 32°4'E), KwaZulu-Natal, and sorted into three distinct colours: red, white and brown. Seed colour was used to characterise the landrace since it has been reported to affect seed viability, vigour and possibility water stress tolerance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010). The three seed colours were used in the study to evaluate the effect of planting date on growth and yield components of local Bambara landraces under irrigated and non-irrigated conditions.

4.2.2Field layout

Three field experiments were planted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 845 m asl) during the 2010/11 season under irrigated and rainfed conditions. Ukulinga has a warm subtropical climate with an average annual rainfall of about 694 mm received mainly during the summer months.

The experimental design was a split-split-plot design with planting date as main factor (early, optimum and late), irrigation and rainfed as sub-main factor, and seed colour as sub-plots (Brown, red and white) arranged in a completely randomised block design (CRD), with three replications. There were three planting dates: 7 September 2010 (early planting), 24 November 2010 (optimum planting) and 19 January 2011 (late planting). The trials were planted on an area of 144 m^2. The main plots were 6.5 x 8 m each with a spacing of 5 m between them, and sub-plots measuring 1.5 x 2 m. Initial plant spacing at planting was 0.3 x 0.1 m; plants were later thinned to a spacing of 0.3 x 0.2 m after emergence, leaving a plant density of 15 plants per square meter.

4.2.3 Data collection

Plant emergence was measured weekly, up to when 90% of plants had emerged. Plant height and leaf number were determined weekly. Leaf number was counted for leaves with at least 50% green area up till flowering and each trifoliate leaf was counted as one leaf. Destructive

sampling was done weekly to determine leaf area per plant, fresh and dry mass; leaf area was measured using a Portable Leaf Area Meter (LI-3000C, Li-Cor, USA). Yield components were measured at harvest.

4.2.4 Crop management

Weeding was done mechanically. Fertiliser application was based on soil analysis recommendations; 20 kg phosphorus (P) per hectare and 20 kg nitrogen (N) per hectare. Fertiliser application was split into two, half at planting and the balance 28 days after planting.

4.2.5 Weather and soil water content

Weather data for the duration of the study (August 2010 to May 2011) was obtained from measurements collected by an automatic weather station (AWS) located about 100 m from the study site. Measurements shown are monthly averages compiled from hourly readings. Three samples for soil water content were taken weekly from the 30 cm profile throughout the duration of the study from both the irrigated and rainfed plots. Soil samples were weighed to obtain mass of wet soil and thereafter dried at 80°C until they had reached constant mass. Gravimetric soil water content (SWC) was then calculated using the following formula;

$$SWC\ (\theta_m) = \left(\frac{\theta_w - \theta_d}{\theta_d}\right) \qquad \textbf{Equation 4.1}$$

Where θ_m = gravimetric soil water content

θ_w = mass of wet soil, and

θ_d = mass of oven dried soil

4.2.6 Data Analysis

Data were analysed using ANOVA from GenStat® Version 12 (VSN International, UK). Means were separated using Duncan's Multiple Range Test in GenStat® at the 5% level of significance (Appendix 3).

4.3 Results

For all planting dates, crops were established under irrigation to allow for optimum crop stand. Irrigation was then withdrawn from the non-irrigated trial after 90% emergence was attained; therefore results reported are of planting date and seed colour. Results showed significant differences ($P<0.05$) between planting dates; while there were no differences ($P>0.05$) between seed colours (Fig 4.1). There was no significant interaction ($P>0.05$) between seed colours and planting dates as well as the interaction between seed colours, water regimes and planting dates (Fig 4.1). Based on mean values, planting at the optimum date resulted in the highest final emergence (82.1%), followed by late (68.9%) and early (68.5%) planting dates, respectively (Fig 4.1). Brown had the highest emergence for both early (74.5%) and late planting dates (78.9%). Darker coloured seeds (brown and red) emerged better than the white coloured seeds (Fig 4.1).

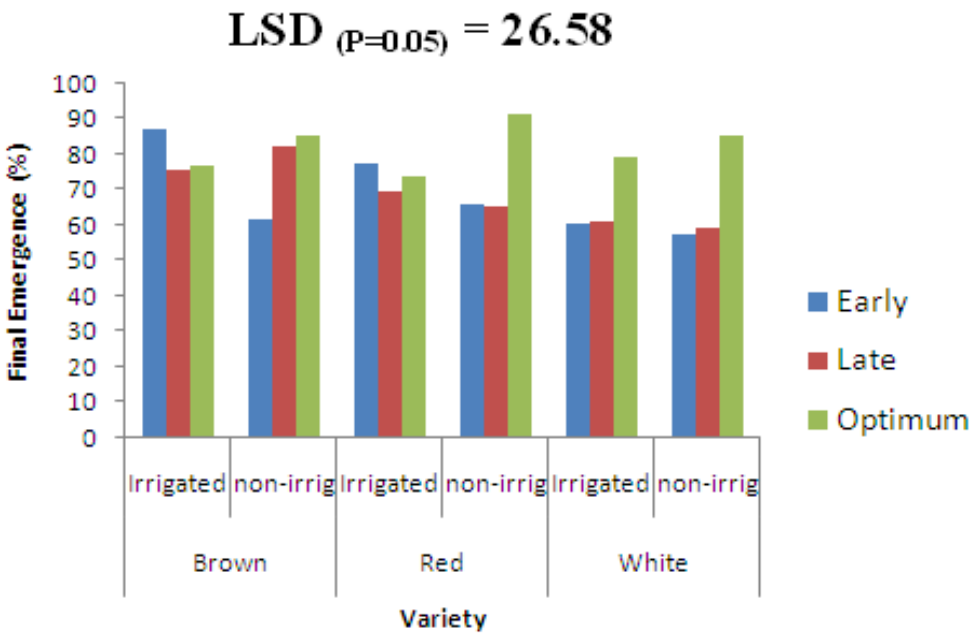


Figure 4.1: Final emergence of red, brown and white coloured bambara landraces over three planting dates (early, optimum and late).

Results of plant height for the early planting date showed highly significant differences ($P<0.001$) between irrigation treatments as well as between seed colours; although the interaction between the two factors was not significant ($P>0.05$) (Fig 4.2A). Results showed that plants grew taller under irrigated conditions as opposed to non-irrigated conditions (Fig 4.2A). Darker coloured seeds had taller plants than white coloured seeds. Based on mean values, over-all, brown had the tallest plants (9.87 cm), followed by red (9.38 cm) and white (8.47 cm), respectively. Brown had the tallest plants (10.40 cm) under irrigated conditions, followed by red (10.17 cm) and white (8.66 cm) respectively; the same trend was observed under non-irrigated conditions, with brown (9.35 cm), followed by red (8.59) and white (8.27). Over-all, plant height decreased in response to non-irrigated conditions; plant height decreased by 16% in red, 10% in brown and 5% in white. Interestingly, despite the decrease in plant height caused by limited water under non-irrigated conditions, brown still performed better than white under irrigated conditions.

Results from the optimum planting date showed significant differences ($P<0.05$) between irrigation treatments and also between seed colours, although their interaction was not significant ($P>0.05$) over time (seed colour*treatment*time) (Fig 4.2B). Darker coloured seeds had taller plants than white coloured seeds. Over-all, red had the tallest plants (17.20 cm), followed by brown (16.67 cm) and white (16.02 cm), respectively. Under irrigated conditions, red had the tallest plants (17.97 cm), followed by brown (17.18 cm) and white (16.39 cm), respectively. Plant height decreased in response to limited water availability under non-irrigated conditions; red, brown and white each decreased by 9%, 6% and 4%, respectively. Similar to the early planting, the largest decrease in response to water stress was observed in the red landrace.

Unlike the early and optimum planting dates, results from the late planting date showed no significant differences ($P>0.05$) between seed colours as well as between irrigation treatments. There was no significant interaction ($P>0.05$) between seed colours and treatments (Fig 4.2C). Darker coloured seeds had taller plants than white coloured seeds. Brown had the tallest plants (12.40 cm) under irrigated conditions, followed by red (12.34 cm) and white (12.30 cm),

respectively (Fig 4.2). Under non-irrigated conditions, plant height only decreased in brown (10%) and white (6%); it however increased in red by 3% compared to irrigated conditions.

Results of leaf number from the early planting date showed no significant differences ($P>0.05$) between irrigation treatments while there were significant differences ($P<0.05$) between seed colours. However, their interaction over time was not significant ($P>0.05$) (seed colour*treatment*time) (Fig 4.3A). Darker coloured seeds had more leaves than white coloured seeds. Under irrigated conditions, red had the most leaves (15.01), followed by brown (13.84) and white (12.30), respectively. Leaf number decreased in response to non-irrigated conditions by 12% in red, 3% in brown and 7% in white. Interestingly, despite the decrease in leaf number caused by limited water under non-irrigated conditions, red still performed better than white under irrigated conditions.

For the optimum planting date, there were highly significant differences ($P<0.001$) between irrigation treatments; however, there were no differences ($P>0.05$) between seed colours as well as in the interaction between seed colour and treatment (Fig 4.3B). Darker coloured seeds had more leaves than white coloured seeds. Under irrigated conditions, white had the most leaves (16.68), followed by brown (16.52) and red (15.01), respectively. Leaf number decreased under non-irrigated conditions by 31% in red, 38% in brown and 39% in white. In this instance, the greatest decrease was in the white landrace.

Results from the late planting date showed no significant differences ($P>0.05$) between irrigation treatments and seed colours, as well as in their interaction ($P>0.05$) (Fig 4.3C). Under irrigated conditions, white had the most leaves (24.10), followed by red (23) and brown (19.17), respectively (Fig 4.3C). Leaf number decreased for the red (8.1%) and white (12.3%) landraces under non-irrigated conditions; however, an increase (3.5%) was observed for the brown landrace (Fig 4.3A). Interestingly, despite the decrease in leaf number caused by limited water under non-irrigated conditions, brown under irrigated conditions performed better compared to brown under irrigated conditions.

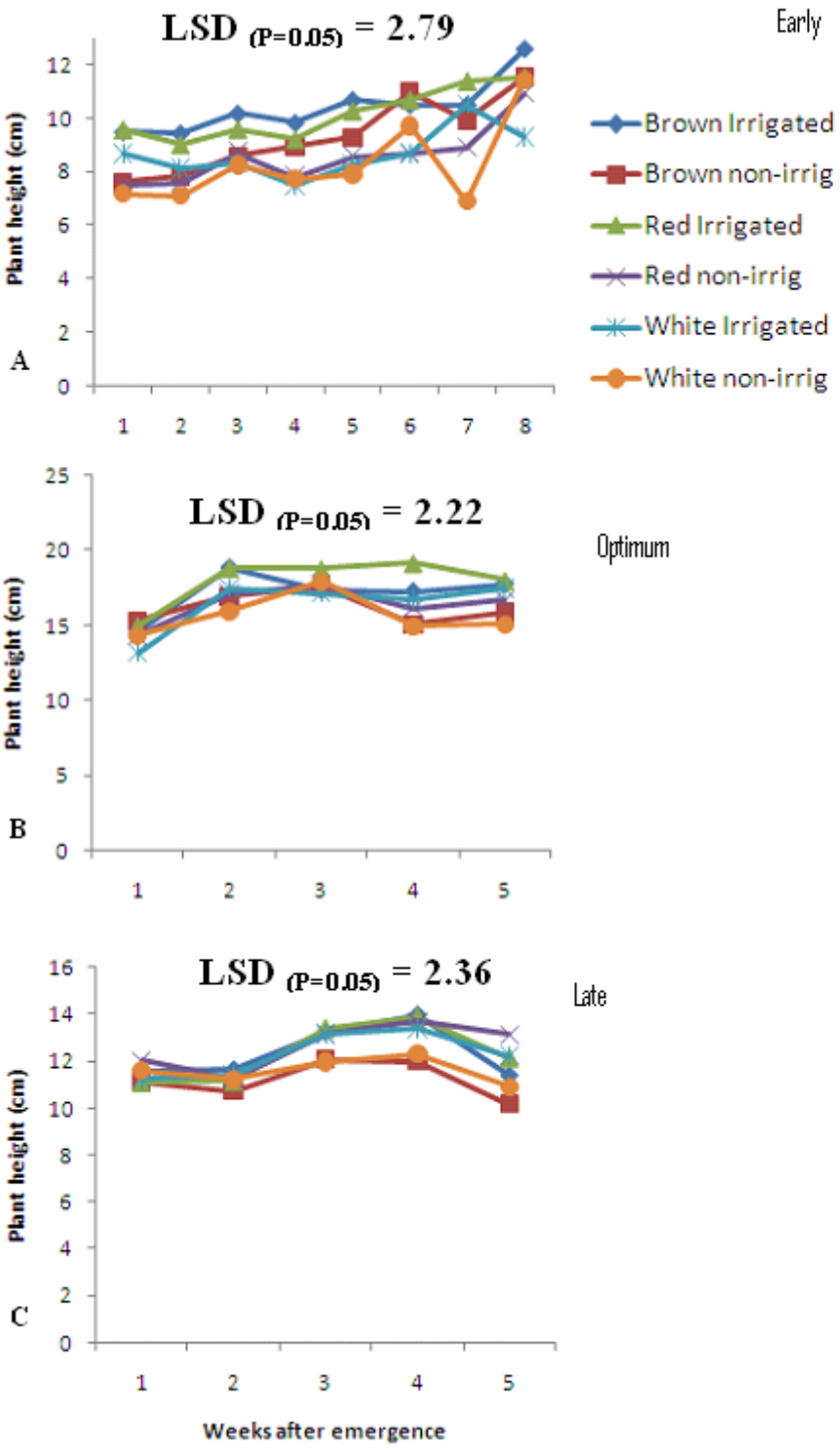


Figure 4.2: Effect of planting date (A-early, B-optimum, C-late) and seed colour (red, brown and white) on plant height in response to irrigated and non-irrigated conditions.

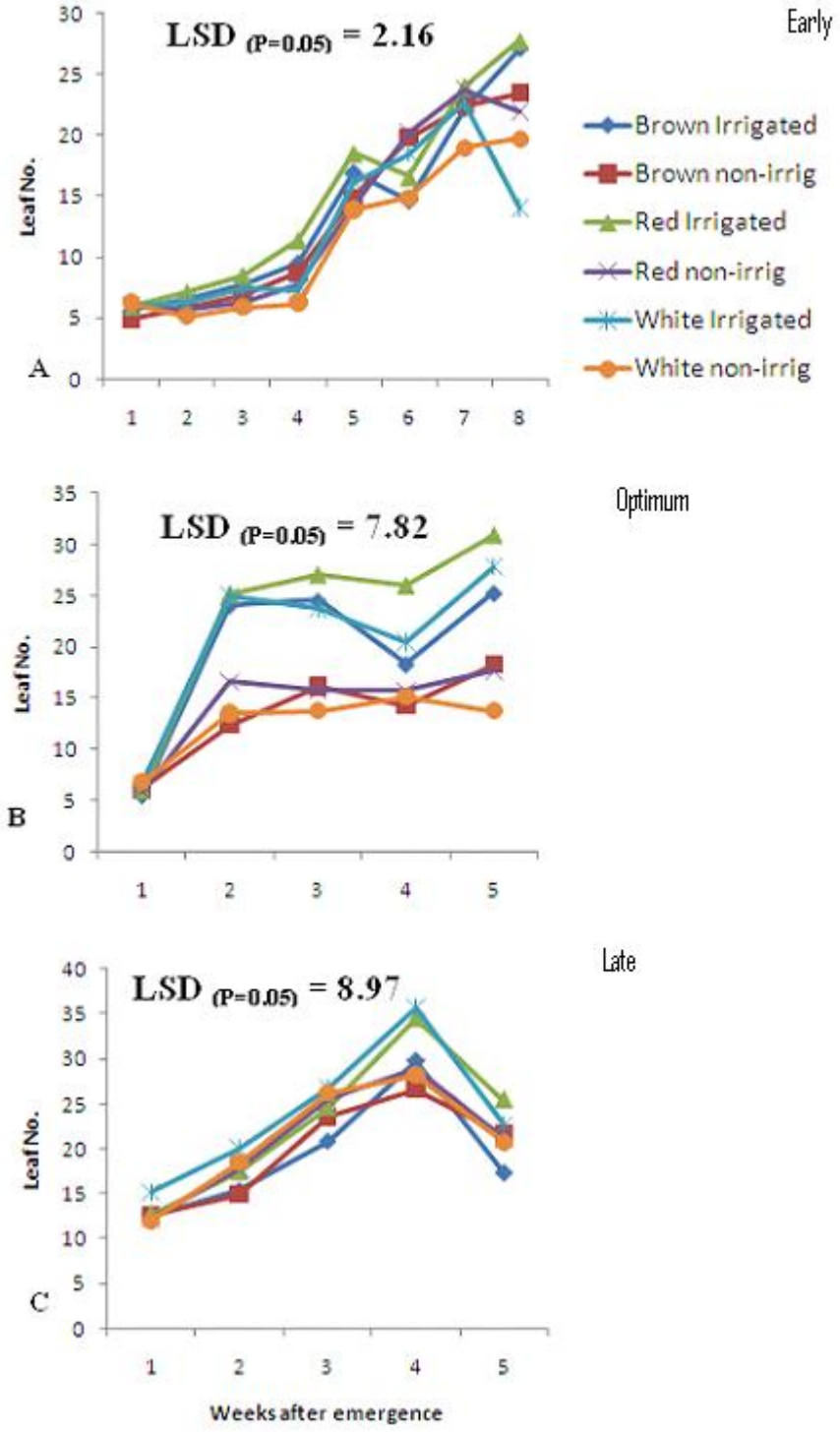


Figure 4.3: Effect of planting date (A-early, B-optimum, C-late) and seed colour (red, brown and white) on leaf number in response to irrigated and non-irrigated conditions.

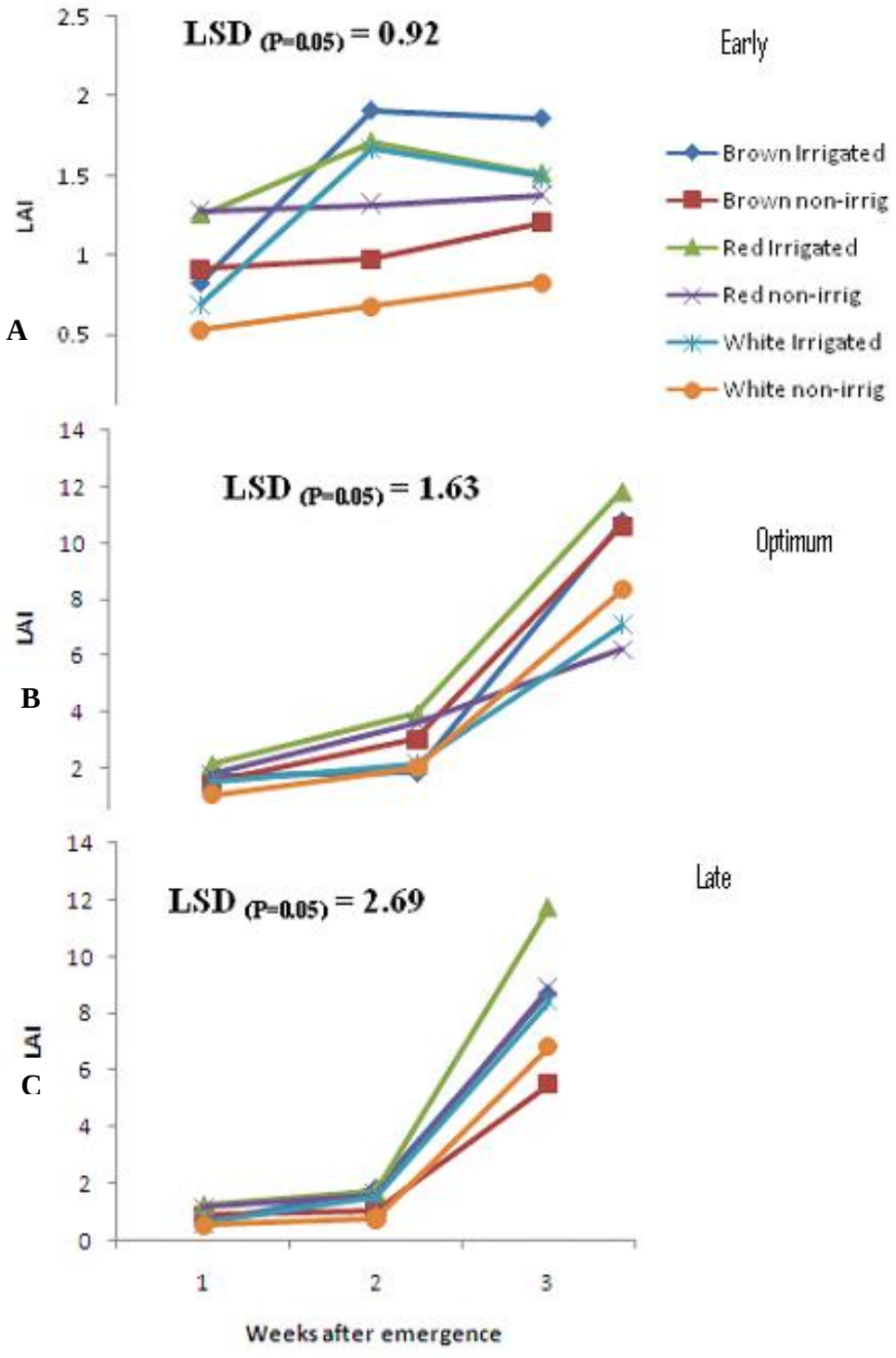


Figure 4.4: Effect of planting date (A-early, B-optimum, C-late) and seed colour (red, brown and white) on leaf area index in response to irrigated and non-irrigated conditions.

Results from the early planting date showed significant differences ($P<0.05$) between irrigation treatments. However, there were no significant differences ($P>0.05$) between seed colours, with respect to leaf area index (LAI) (Fig 4.4A). The trend observed was consistent with observations for leaf number; red had the highest LAI (1.410), followed by brown (1.282) and white (0.980), respectively. Under irrigated conditions, brown had the highest LAI (1.535), followed by red (1.496) and white (1.283), respectively (Fig 4.4A). However, LAI decreased under non-irrigated conditions; the greatest decrease in LAI was observed in white (47.3%), followed by brown (32.9%) and red (11.4%), respectively. The decreases in LAI correspond with observations of decreasing plant height (Fig 4.2) and leaf number (Fig 4.3) observed under non-irrigated as compared to irrigated conditions.

For the optimum planting date, results of LAI showed significant differences ($P<0.05$) between treatments and seed colours; while the interaction of the two factors was shown to be highly significant ($P<0.001$) (Fig 4.4B). The darker seed colours continued to perform well under irrigated conditions; red (5.97), brown (5.06) and white (3.79). Similar to the early planting date, LAI decreased under non-irrigated conditions (brown - 4.77; red - 3.87; white - 3.58). The dark colours (brown and red) performed better than the light colour (white) under both irrigated and non-irrigated conditions. Brown was shown to be most sensitive to limited water availability; LAI decreased by 5.7% in brown, 5.5% in white and 3.5% in red.

Results of LAI measured from the late planting date showed significant differences ($P<0.05$) between treatments and seed colours (Fig 4.4C). Over-all, for both irrigated and non-irrigated conditions, red had the highest LAI (4.40) followed by brown (3.10) and white (3.10). Under irrigated conditions, red (4.90) and brown (3.72) performed better than white (3.50). Similar to the previous planting dates, LAI decreased under non-irrigated conditions; LAI decreased by 33% in brown, 22.9% in white and 20.6% in red.

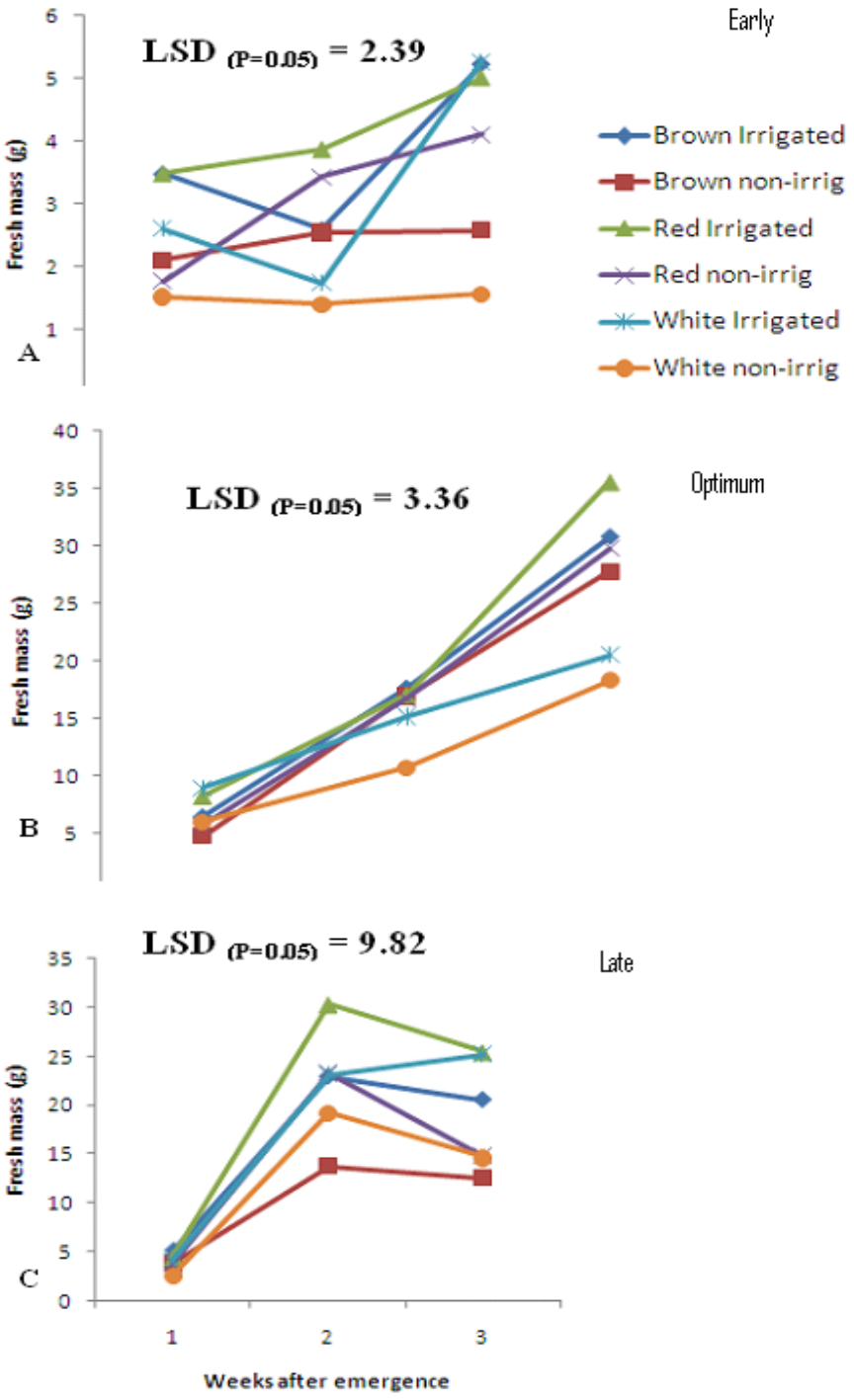


Figure 4.5: Effect of planting date (A-early, B-optimum, C-late) and seed colour (red, brown and white) on biomass accumulation (fresh mass) in response to irrigated and non-irrigated field conditions.

Results of fresh mass from the early planting date showed highly significant differences ($P<0.001$) between treatments and seed colours ($P<0.05$) (Fig 4.5A). There was no significant interaction ($P>0.05$) between seed colour and treatment (Fig 4.5A). Mean values, for both irrigated and non-irrigated conditions, showed that red had the highest fresh mass (3.61 g), followed by brown (3.08 g) and white (2.35 g), respectively. Fresh mass was higher under irrigated as compared to non-irrigated conditions. Under irrigated conditions, red had the highest fresh mass (4.12 g), followed by brown (3.76) and white (3.21 g), respectively (Fig 4.5A). Fresh mass decreased in response to limited water under non-irrigated conditions; fresh mass of red, brown and white decreased by 25%, 36% and 54%, respectively under non-irrigated conditions.

Planting at the optimum date resulted in highly significant differences ($P<0.001$) between treatments and seed colour variations of the landrace (Fig 4.5B). The interaction, over time, between seed colour and treatment was not significant ($P>0.05$). Based on mean values, irrigated plants had the highest fresh mass (17.81 g) compared to non-irrigated (15.19 g). The dark colours (brown and red) performed better than the light colour (white) under both irrigated and non-irrigated conditions (Fig 4.5B). Fresh mass decreased in response to limited water under non-irrigated conditions; fresh mass of red, brown and white decreased by 14%, 10% and 22%, respectively under non-irrigated conditions.

Results from the late planting date showed highly significant differences ($P<0.001$) between treatments as well as significant differences ($P<0.05$) between seed colour variations of the landrace (Fig 4.5C). The interaction between seed colour and treatment, over time, was not significant ($P>0.05$) (Fig 4.5C). The dark colours (brown and red) continued to out-perform the white landrace under both irrigated and non-irrigated conditions. Under irrigated conditions, red had the highest fresh mass (20.9 g), followed by white (17.50) and brown (16.16 g), respectively. Fresh mass decreased in response to non-irrigated conditions; fresh mass of red, brown and white decreased by 31%, 38% and 31%, respectively under non-irrigated conditions.

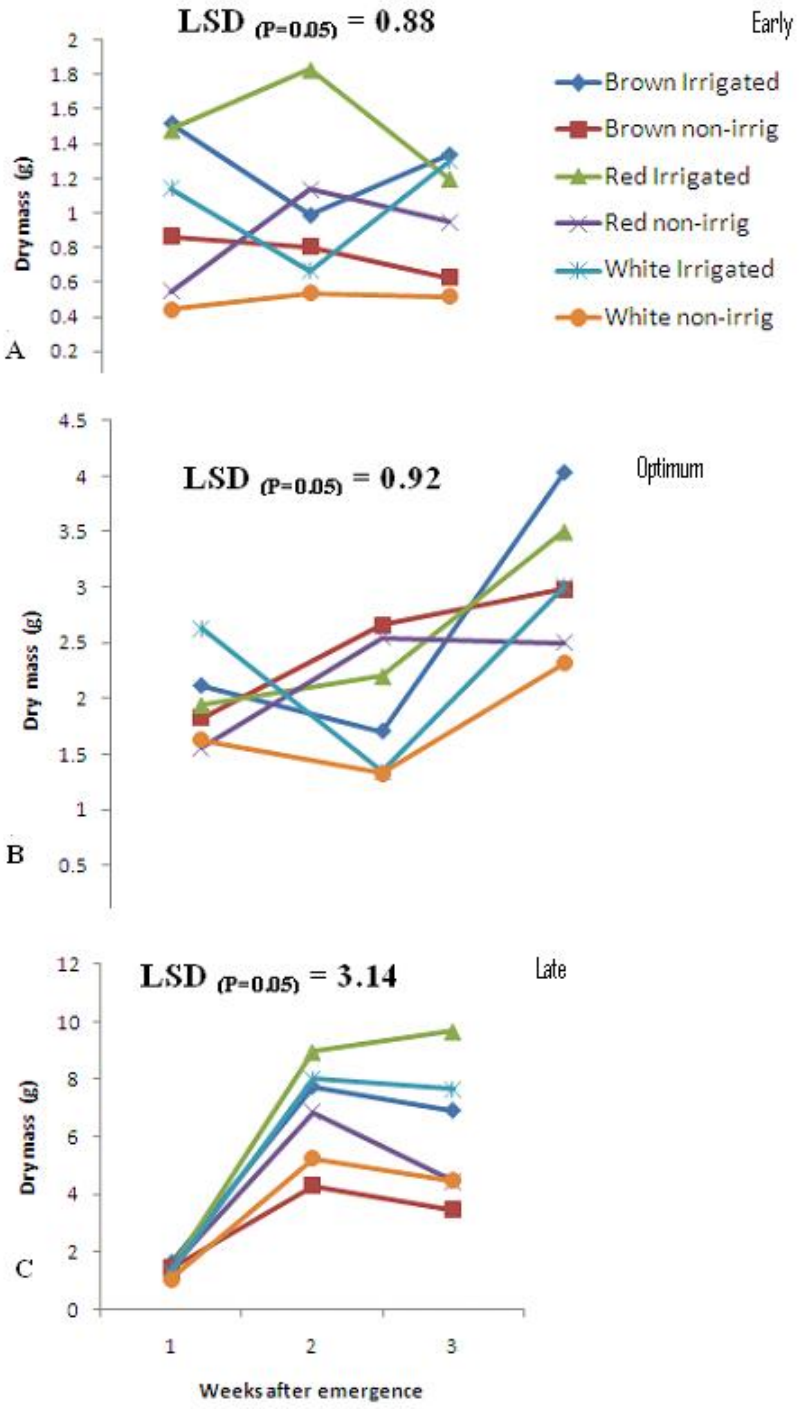


Figure 4.6: Effect of planting date (A-early, B-optimum, C-late) and seed colour (red, brown and white) on biomass accumulation (dry mass) in response to irrigated and non-irrigated field conditions.

Results from early planting date showed highly significant differences ($P<0.001$) between treatments with respect to dry mass; however, there were no significant differences ($P>0.05$) between seed colour variations of the landrace (Fig 4.6A). The interaction, over time, between seed colour and treatment was not significant ($P>0.05$). Over-all, irrigated plants had higher dry mass compared to non-irrigated plants. The dark colours (brown and red) performed better than the light colour (white) under both irrigated and non-irrigated conditions. Red had the highest dry mass (1.504 g) under irrigated conditions, followed by brown (1.281) and white (1.039 g), respectively (Fig 4.6A). Dry mass decreased in response to non-irrigated conditions; dry mass of red, brown and white decreased by 42%, 40% and 52%, respectively.

Results from the optimum planting date showed significant differences ($P<0.05$) between irrigation treatments and between seed colour variations of the landrace, with respect to dry mass (Fig 4.6B). The interaction between seed colour and treatment was shown to be significant ($P<0.05$). The dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions. Brown had the highest dry mass (2.617 g) under irrigated conditions, followed by red (2.552 g) and white (2.328 g) respectively. Dry mass decreased in response to limited water under non-irrigated conditions; dry mass of red, brown and white decreased by 13.8%, 4.9% and 24.6%, respectively under non-irrigated conditions.

The late planting date showed no significant differences ($P>0.05$) between seed colours, with respect to dry mass. However, there were highly significant differences ($P<0.001$) between treatments (Fig 4.6C). Red had the highest dry mass (6.66 g) under irrigated conditions, followed by white (5.65 g) and brown (5.43 g) respectively (Fig 4.6C). Dry mass decreased in response to limited water under non-irrigated conditions; dry mass of red, brown and white decreased by 36.5%, 43.5% and 36.3%, respectively under non-irrigated conditions. Consistent with the early and optimum plantings, the dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions.

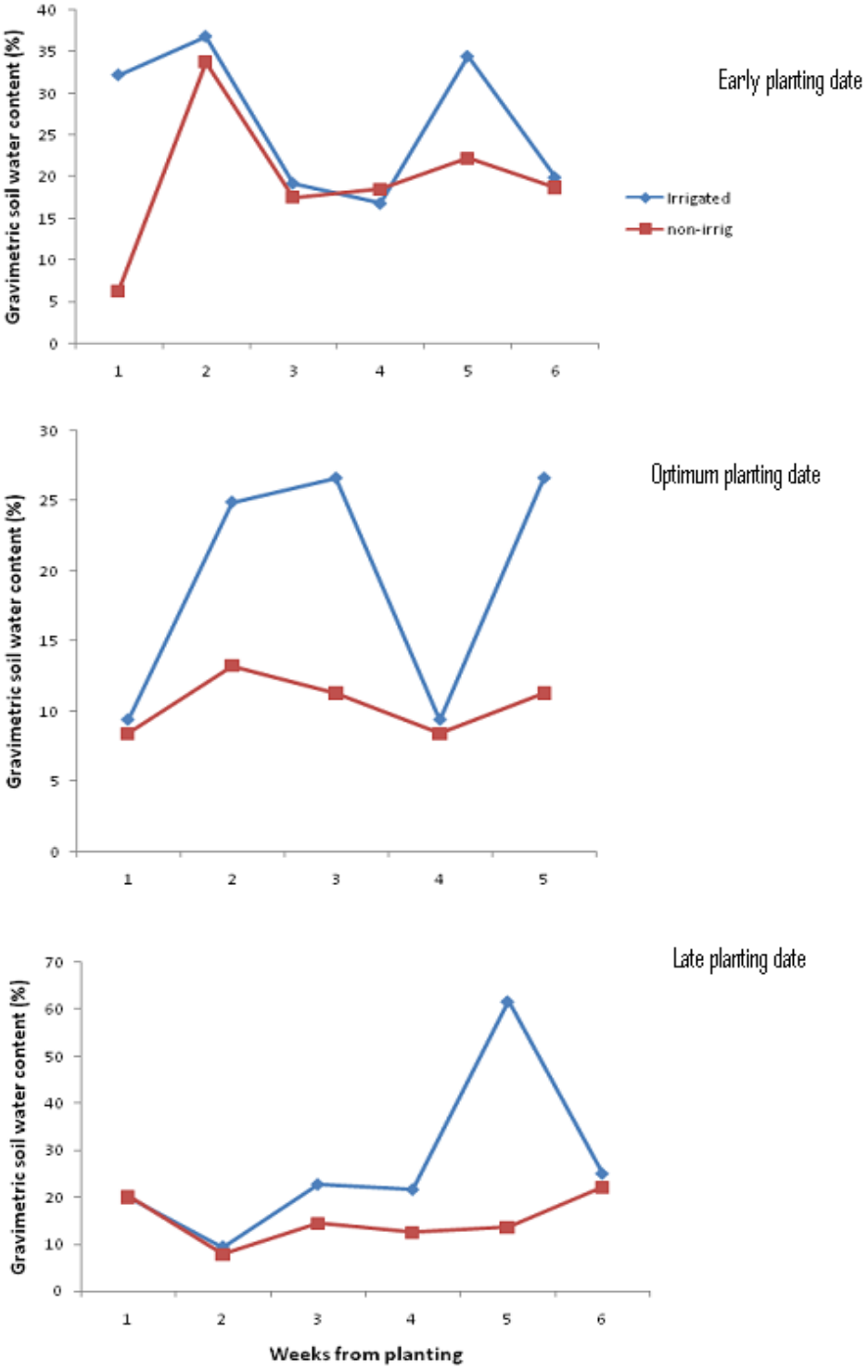


Figure 4.7: Changes in soil water content during the first 5 weeks of bambara groundnut growth.

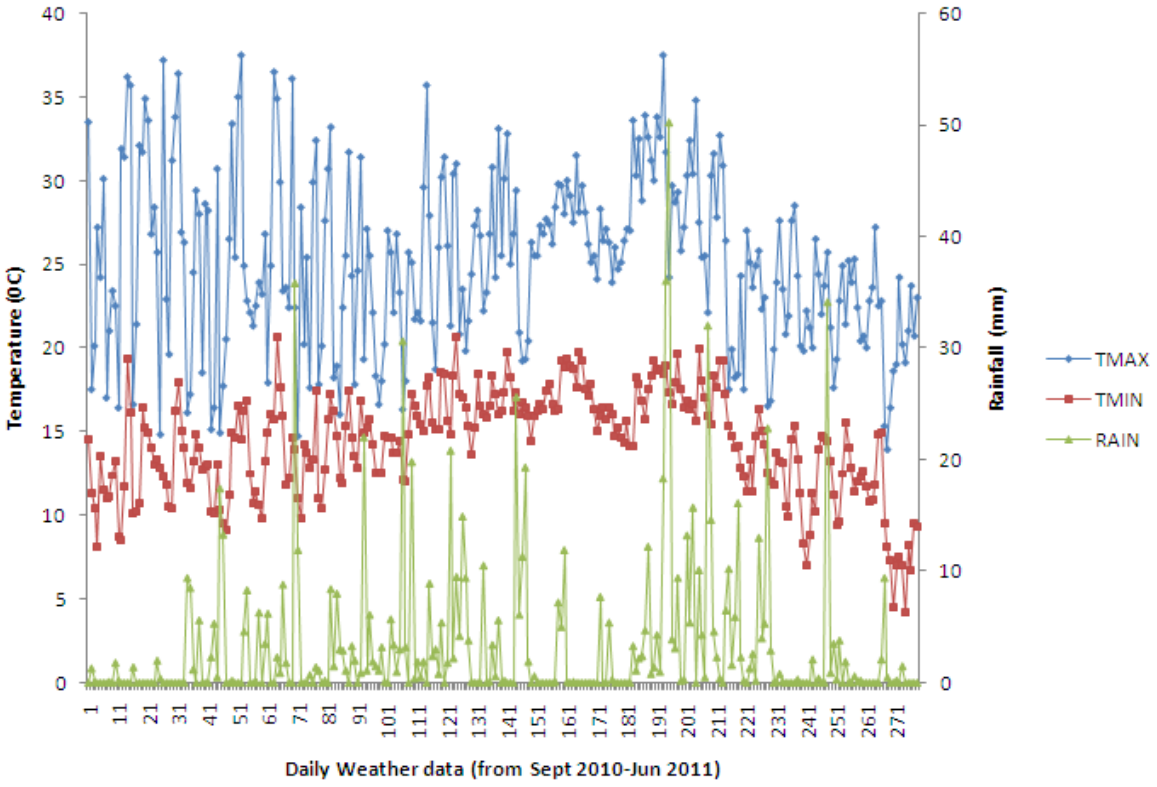


Figure 4.8: Changes in daily weather patterns measured during the growing period of bambara groundnut for all three planting dates.

Daily maximum and minimum air temperature, in addition to rainfall, were measured from a nearby automatic weather station located about 100 m from the study site (<100 m from trial site) (Fig 4.8). The pattern in weather showed low minimum air temperature and rainfall during the month of September when the early crop was planted. Therefore, there were less than optimum temperatures, resulting in delayed accumulation of thermal units, thus leading to the slow and low emergence observed in the early planting. Temperatures warmed up, while rainfall increased during the month of November, hence it was termed the optimum planting date. The warmer temperatures at the start of the optimum and late plantings resulted in faster seedling growth hence yield. Crop growth for the optimum planting date was sustained well by more rainfall and higher soil water content during the period when plants were growing faster in response to increasing leaf number and plant height.

Table 4.1: Yield components of a bambara landrace (red, brown and white) grown under irrigated and non-irrigated field conditions at Ukulinga Research Farm.

Treatment	Planting date	Seed colour	Total biomass(g)	HI (%)	Pod mass (g)	Pod No./Plant	Grain No./Pod	Total grain mass/Plant	Yield (kg/ha)
Irrigated	Early	Red	4.82b	50.14abcd	2.79b	6.00bcd	1.00b	1.94c	323.1c
		Brown	6.11b b	32.72cd	2.16b	4.33cd	1.08b	1.43c	239.0c
		White	0.10b	45.86abcd	0.17b	2.20d	0.97b	0.27c	46.7c
		Mean	3.68[b]	42.9[c]	1.61[b]	4.18[b]	1.017[b]	1.21[b]	203[b]
	Optimum	Red	29.82a	70.96a	20.74a	14.33a	1.73a	10.57a	1761.2a
		Brown	21.36 a	66.59ab	14.61a	11.67abc	1.17b	8.13ab	1354.6ab
		White	22.239a	64.36ab	15.05a	12.00ab	1.18b	6.86b	1143.4b
		Mean	24.48[a]	67.3[a]	16.80[a]	12.67[a]	1.361[a]	8.52[a]	1420[a]
	Late	Red	10.82b	37.16bcd	4.29b	11.93ab	1.20b	2.89c	481.4c
		Brown	4.88b	26.35d	2.30b	8.11abcd	0.98b	1.19 c	129.2c
		White	6.70b	25.66d	2.04b	7.00abcd	1.09b	1.37c	228.8c
		Mean	7.47[a]	29.7[c]	2.88[b]	9.01[b]	1.091[b]	1.82[a]	280[b]
Non-irrigated	Early	Red	7.21b b	59.60abc	4.24b	7.67abcd	1.07b	2.64c	440.7c
		Brown	3.99b	37.10bcd	1.14b	3.33d	1.02b	0.92c	152.8c
		White	3.47b	23.07d	-0.12b	3.20d	0.97b	0.16c	28.4c
		Mean	4.89[b]	39.9[d]	1.85[b]	4.73[b]	1.019[b]	1.24[b]	207[b]
	Optimum	Red	8.00b	56.61abc	4.34b	7.67abcd	1.25b	3.34c	556.2c
		Brown	6.32b	57.41abc	3.69b	6.00bcd	1.37b	2.88c	479.9c
		White	7.19b	49.13abcd	3.65b	6.33bcd	1.00b	2.69c	449.4c
		Mean	7.17[b]	54.4[b]	3.90[a]	6.67[b]	1.206[b]	2.97[b]	495[a]
	Late	Red	8.54b	26.85d	2.54b	8.69abcd	1.05b	1.79c	298.2c
		Brown	3.64b	25.41d	0.93b	4.47bcd	1.00b	0.59c	98.4c
		White	5.17b	22.86d	1.27b	5.39bcd	1.00b	0.89c	149.4c
		Mean	5.79[b]	25.0[d]	1.58[b]	6.19[b]	1.017[b]	1.09[b]	182[b]
LSD (Trt.Date) (P=0.05)			5.59	14.83	4.17	3.69	0.21	1.96	322.6
LSD (SC*Trt*Date) (P=0.05)			9.68	25.68	7.22	6.40	0.35	3.39	558.7

There were highly significant differences ($P<0.001$) between planting dates, with respect to total biomass (Table 4.1). Over-all, the optimum planting date had the highest total biomass followed by the late and early planting dates, respectively. There was a highly significant interaction ($P<0.001$) between planting dates and treatments with respect to total biomass. Over-all, under irrigated conditions, the optimum planting date had the highest total biomass followed by late and early planting dates, respectively (Table 4.1). The same trend was observed when plants were grown under non-irrigated conditions. Under non-irrigated conditions, optimum and late planting dates performed better than early planting date under irrigated conditions. There were no significant differences ($P>0.05$) between seed colour variations of the landrace, with respect to total biomass (Table 4.1). Over-all, red had the highest total biomass (11.54 g), followed by brown (7.72 g) and white (7.48 g), respectively. There were highly significant differences ($P<0.001$) between treatments with respect to total biomass. Over-all, irrigated plants had higher total biomass than non-irrigated plants (Table 4.1). Total biomass decreased in response to limited water under non-irrigated conditions; total biomass of red, brown and white decreased by 48%, 57% and 46%, respectively under non-irrigated conditions. The dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions (Table 4.1).

There was no significant interaction ($P>0.05$) between seed colours, treatment and planting date, with respect to pod mass (Table 4.1). However, there were highly significant differences ($P<0.001$) between planting dates as well as in the interaction between planting dates and treatment. Over-all, the optimum planting date had the highest pod mass followed by the late and early planting dates, respectively (Table 4.1). Under irrigated conditions, the optimum planting date had the highest pod mass, followed by the late and early planting dates, respectively. Under non-irrigated conditions, the optimum planting date had the highest pod mass, followed by early and late planting dates, respectively. Even under non-irrigated conditions, the optimum planting date performed better than the early and late planting date under irrigated conditions. Although results showed no significant differences ($P>0.05$) between seed colour variations of the landrace, the dark colours (brown and red) performed better than the white landrace, under both irrigated and non-irrigated conditions. There were highly significant differences ($P<0.001$) between treatments with respect to pod mass. Over-all, irrigated plants had the highest pod mass

compared to non-irrigated plants. Pod mass decreased in response to limited water under non-irrigated conditions; pod mass of red, brown and white decreased by 60%, 69% and 70%, respectively.

There was no significant interaction ($P>0.05$) between seed colour, treatments and planting dates, with respect to harvest index (HI); the same was true for the interaction between treatment and planting dates (Table 4.1). However, there were highly significant differences ($P<0.001$) between planting dates (Table 4.1). Planting at the optimum date resulted in the highest HI, followed by early and late planting, respectively. Over-all, for both irrigated and non-irrigated conditions, the optimum planting date had the highest HI, followed by early and late planting dates, respectively. Interestingly, even under non-irrigated conditions, the optimum planting date performed better than the early and late planting dates under irrigated conditions. There were no significant differences ($P>0.05$) between treatments (Table 4.1); although irrigated plants had higher HI than non-irrigated plants (Table 4.1). Results showed no significant differences ($P>0.05$) between seed colour variations of the landrace (Table 4.1). Over-all, red had the highest HI under irrigated conditions, followed by white and brown respectively; however, under non-irrigated red was followed by brown and white respectively. HI decreased in response to limited water under non-irrigated conditions; HI of red, brown and white decreased by 10 %, 5% and 30%, respectively under non-irrigated conditions. The dark colours (red and brown) performed better than the white landrace under non-irrigated conditions.

There was no significant interaction ($P>0.05$) between seed colours, treatments and planting dates with respect to pod yield per plant; the same was true for the interaction between irrigation treatments and planting date (Table 4.1). However, there were highly significant differences ($P<0.001$) between planting dates (Table 4.1). The optimum planting date had the highest pod yield, followed by the late and early planting dates, respectively (Table 4.1). There were no significant ($P>0.05$) between irrigation treatments (Table 4.1). Under both irrigated and non-conditions, the optimum planting date had the highest pod yield, followed by the late and early planting dates, respectively. The same trend was observed under non-irrigated conditions. Red had the highest pod yield per plant under irrigated conditions, followed by brown and white, respectively. Pod yield decreased under non-irrigated conditions; pods yield of red, brown and white decreased by 26%, 43%

and 30%, respectively. Although results showed no significant differences (P>0.05) between seed colour variations of the landrace, brown and red performed better than the white landrace under both irrigated and non-irrigated conditions (Table 4.1).

There was no significant interaction (P>0.05) between seed colour, treatments and planting dates with respect to grains per pod; similar trend was observed for the interaction between irrigation treatments and planting dates (Table 4.1). There were significant differences (P<0.05) between planting dates; the optimum planting date had the highest grain number per pod, followed by the late and early planting dates, respectively. Under irrigated conditions, the optimum planting date had the most grains per pod, followed by the late and early planting dates, respectively (Table 4.1). However, under non-irrigated conditions, the optimum planting date had the most grains per pod, followed by the early and late planting dates, respectively. Interestingly, the optimum planting date, under non-irrigated conditions, performed better than the early and late planting dates under irrigated conditions. There were no significant differences (P>0.05) between irrigation treatments; however, irrigated plants had more grains per pod than non-irrigated (Table 4.1). Red had more grains per pod under irrigated conditions, followed by white and brown (1.075 g) respectively. Grain number per pod decreased under non-irrigated conditions; red and white decreased by 14% and 9%, respectively, however, brown increased by 5%. Results showed no significant differences (P>0.05) between seed colour variations of the landrace (Table 4.1). The dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions (Table 4.1).

Results of grain yield per plant were consistent with those of grain number and pod yield. There was no significant interaction (P>0.05) between seed colours, treatments and planting dates with respect to grain mass per plant; however, there was a highly significant interaction (P<0.001) between treatments and planting dates (Table 4.1). The optimum planting date had the highest grain yield per plant followed by the late and early planting dates, respectively. There were highly significant differences (P<0.001) between treatments; irrigated plants had more grain yield per plant than non-irrigated plants (Table 4.1). Red had more grain mass per plant (5.13 g) under irrigated conditions, followed by brown (3.58 g) and white (2.83 g) respectively; same trend was observed under non-irrigated conditions (Table 4.1). Grain yield per plant decreased in under non-irrigated

conditions; red, brown and white decreased by 50%, 59% and 56%, respectively. Results showed significant differences ($P<0.05$) between seed colour variations of the landrace. The dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions (Table 1).

Results of the final yield followed a similar pattern as results of pod and grain number and grain yield per plant; indicating that pod yield and grain number are closely correlated to final yield. There was no significant interaction ($P>0.05$) between seed colours, irrigation treatments and planting date with respect to yield (kg/ha); however, there were highly significant ($P<0.001$) between treatments and planting dates (Table 4.1). There were highly significant differences ($P<0.001$) between planting dates with respect to final yield (kg/ha). Based on mean values, the highest yields were obtained from the optimum planting date (1420 kg/ha), followed by the late (280 kg/ha) and early planting dates (203 kg/ha), respectively, under irrigated conditions (Table 4.1). However, under non-irrigated conditions, optimum planting date had the yield (495kg/ha), followed by the early (207 kg/ha) and late planting dates (182 kg/ha), respectively. Even under non-irrigated conditions, the optimum planting date still yielded better than crops from the irrigated early planted crop. There were highly significant differences ($P<0.001$) between treatments. Based on mean values, irrigated plants had more yield (634.0 kg/ha) than non-irrigated plants (295.0 kg/ha). Results showed significant differences ($P<0.05$) between seed colour variations of the landrace (Table 4.1). Brown and red performed better than the white landrace under both irrigated and non-irrigated conditions. Red had more yield (855.0 kg/ha) under irrigated conditions, followed by brown (574.0 kg/ha) and white (473.0 kg/ha), respectively. Yield decreased in response to limited water availability under non-irrigated conditions; red, brown and white decreased by 50%, 58% and 56%, respectively.

4.4 Discussion

The objective of the study was to determine the effect of planting date, as a management component, on growth, development and yield of bambara groundnut in response to water stress. According to Walter (1967), when planting, it is important to know whether the rains are continuous and sufficient to ensure optimal soil water during planting and whether the soil water will be maintained or increased during the growing period in order to avoid total crop failure and achieve optimum yields. Appropriate planting date selection aids in the attainment of good crop yield; hence the need for detailed investigation on optimum planting dates.

Seedbed conditions and water stress are important factors affecting emergence and development of seedlings (Pollock, 1972). However, in the study, seedbed conditions were made optimum, with regards to soil water availability. Thus observed differences were related to seed colour or planting and/or their interaction. Contrary to reports by Sesay *et al*. (2008) that planting date had no effect on final seedling emergence, results showed that maximum emergence was related with the optimum planting date; emergence decreased with late and early planting, respectively. Previous research (Powell, 1989; Zulu & Modi, 2010) indicated that seed colour may be associated with seed quality and that darker coloured seeds may have more vigour than light coloured seeds. Brown had the highest emergence for both early and late planting dates. Darker coloured seeds emerged better than the lighter coloured seeds; over-all, brown and red seeds performed better than white seeds, with respect to early establishment performance.

Results from the study were in agreement with reports by Sesay *et al*. (2008) that plant height and leaf number per plant were significantly affected by sowing date. They found that the optimum planting date had taller plants and most leaves compared to the late and early planting dates, respectively; the same trend was observed for LAI. Results of plant growth parameters, plant height, leaf number and LAI, showed that plants attained their optimum growth for the measured parameters when planted at the optimum planting date. Plant growth generally decreased with late and early planting. The poor growth observed in late planting date may have been the result of reduced season duration and limited water availability, especially in the non-irrigated crops. Mwale *et al*. (2003) and Makanda *et al*.

(2009) reported that optimum and later plantings had higher vegetative growth than early planting due to high levels of soil water received later in the cropping season. Results from the study showed that water stress limited plant growth; early planting showed greater reduction for all plant growth parameters followed by the late planting date. Planting at the optimum planting date resulted in plants not being stressed as there was sufficient soil water. It was evident (Fig 4.8) that the early crop experienced low soil water immediately after withdrawal of irrigation. Optimum planting date emerged better than late and early planting, respectively. Thus, giving the optimum planted crop an advantage with regards to capture of resources; even after irrigation had been withdrawn there was sufficient soil water.

Bambara groundnut is a warm temperature crop originated from North Africa (Swanevelder, 1998). Its growth during the optimum planting date was optimised by both more rainfall and higher temperatures resulting in plants growing faster as observed by increasing leaf number and plant height. The early crop was affected by water stress under non-irrigated conditions during early vegetative growth; crop growth steadily improved as soil water content increased with increasing rainfall from October to January (Fig 4.8). The late planted crop had better soil water conditions during early vegetative growth; however SWC later declined during February when the crop was flowering as the rains started to fade.

Seed colour has previously been associated with seedling vigour (Powell, 1989; Zulu & Modi, 2010). It is also well established that vigorous seedling are better able to capture light, develop roots and access water, and thus photosynthesise much earlier than less vigorous seedlings (Perry, 1978; McDonald, 1980). True to these assumptions, darker coloured seeds performed better than light coloured seeds under non-irrigated conditions. Of the three landraces, red performed best, with most measured plant growth parameters, followed by brown and white, respectively.

Results from the study were in agreement with reports by Sesay *et al*. (2008), that planting date significantly affected pod yield and dry matter production. They were, however, in contradiction to earlier reports by Collinson *et al*. (2000) that planting date had no significant effect on yield. Under non-irrigated conditions, the optimum planting date

resulted in the highest HI, followed by late and early planting dates, respectively. However, with respect to final yield, the early planted crop yielded more than the late planted crop, under non-irrigated conditions. The late planted crop flowered in February when rainfall was low and temperatures were higher (Fig 4.8), thus, resulting in decreased pod filling. Results were in agreement with reports from Johnson, (1968), Swanevelder, (1998), Harris and Azam Ali, (1993) that lower yields were associated with later plantings.

Azam-Ali *et al*. (2001) and Collinson *et al*. (1996, 2000) reported that Bambara productivity was adversely affected by limited soil water. Results from the study showed that water stress affected yield; final yield decreased in response to limited water under non-irrigated conditions; yield of red, brown and white decreased by 50%, 58% and 56%, respectively. The dark colours (brown and red) still out-performed the white landraces under both irrigated and non-irrigated conditions; even though brown was more sensitive to water stress than white, in terms of percentage yield reduction, it still had higher yield than the white landrace.

4.5 Conclusions

Plant growth (plant height, leaf number and LAI) of Bambara groundnut landraces was shown to be sensitive water stress. Water stress decreased yield components and hence yield. However, selection of planting dates was shown to be a useful management tool for managing water stress under water limited conditions. Choice of planting date significantly affected both plant growth and yield. The optimum planting date resulted in the best crop growth for all measured plant growth parameters followed by late and early planting dates, respectively. With respect to yield and yield components, planting at the optimum planting date. Under non-irrigated conditions optimum planting resulted in higher yield followed by early and late planting dates respectively. Seed colour may be used as a selection criterion when planting bambara groundnut landraces under rainfed field conditions. The dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions. Red and brown managed to use water efficiently and were able to partition more assimilates towards yield when grown under water limiting conditions. Further research is also needed to focus on water stress imposed at specific growth stages. In addition, plant nutrition may also be affected by water stress; hence research is needed to evaluate effect of water stress on nitrogen fixation of bambara groundnut landraces.

References

AZAM-ALI, S.N., SESAY, A., KARIKARI, S., MASSAWE, F.J., AGUILAR-MANJARREZ, J., BANNAYAN, M. & HAMPSON, K.J., 2001. Assessing the potential of an underutilized crop – a case study using bambara groundnut. *Expl. Agric.* 37, 433-472.

BLUM, A., 1996. Crop response to drought and the interpretation of adaptation. *J. Plant Growth Reg* 20 : 135-148.

BOYER, J.S., 1982. Plant Productivity and Environment. *Science* 218, 443-448.

COLLINSON, S. T., SIBUGA, K. P., TARIMO, A. J. P., & AZAM-ALI, S. N., 2000. Influence of sowing date on the growt and yield of Bambara groundnut (*Vigna Subterranean* L.*)* landraces in Tanzania. *Expl. Agric*. 36, 1-13.

COLLINSON, S.T., AZAM-ALI, S.N., CHAVULA, K.M., & HODSON, D.A., 1996. Growth, development and yield of bambara groundnut (*Vigna subterranea*) in response to soil moisture. *J. Agric. Sci.* 126, 307–318.

COLLINSON, S.T., BERCHIE, J., & AZAM-ALI, S.N., 1999. The effect of soil moisture on light interception and the conversion coefficient for three landraces of bambara groundnut (*Vigna subterranea*). *J. Agric. Sci*. 133, 151–157.

COLLINSON, S.T., CLAWSON, E.J., AZAM-ALI, S.N., & BLACK, C.R., 1997. Effects of soil moisture deficits on the water relations of bambara groundnut (*Vigna subterranea* L. Verdc.). *J. Exp. Bot*. 48, 877–884.

DOORENBOS, J. & KASSAM, A.H., 1979. Yield Response to Water. FAO Irrigation and Drainage paper No. 33, FAO, Rome, Italy, p. 193.

FOOD AND AGRICULTURE ORGANIZATION, 2001. A Global Mapping System for Bambara Groundnut Production.

FISCHER, R.A. & TURNER, N.C., 1978. Plant Productivity in the arid and semiarid zone. *Ann. Rev. Plant Physiol.* 29, 277-317.

GARDNER, F.P., PEARCE, R.B., & MITCHELL, R.L., 1985. Physiology of Crop Plants. Iowa State University Press. pp. 87-91.

HARRIS, D. & AZAM-ALI, S.N., 1993. Implications of daylength sensitivity in bambara groundnut (*Vigna subterranea* L. Verdc) on production in Botswana. *J. Agric. Sci.*, 120, 75-78.

JOHNSON, D.T., 1968. The bambara groundnut: a review. *Rhodesia Agricultural Journal* 65, 1-4.

KUCHARIK, C.J., 2006. A multidecadal trend of earlier corn planting in the central USA. *Agron. J.*, 98, 1544–1550.

LEPORT, L., TURNER, N.C., FRENCH, R.J, BARR, M.D., DUDA, R., DAVIES, S.L., TENNANT, D., & SIDDIQUE, K.H.M., 1999. Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *Euro. J. Agron*., 11, 279-291.

LINNEMANN, A.R. & AZAM-ALI, S.N., 1993. Bambara groundnut (*Vigna subterranea* L. Verdc). In: Williams, J.T. (Ed.), pp. 13-58. Under-utilised Crops Series. II. Vegetable and Pulses. Chapman and Hall, London, UK.

MABHAUDHI, T. & MODI, A.T., 2011. Can hydro-priming improve germination speed, vigour and emergence of maize landraces under water stress? J. Agric. Sci. Technol. B1, 20-28.

MABHAUDHI, T. & MODI, A.T., 2010. Early establishment performance of local and hybrid maize under two water stress regimes. *S. Afr. J. Plant & Soil* 27, 299-304.

MAKANDA, I., TONGOONA, P., MADAMBA, R., ICISHAHAYO, D. & DERERA, J., 2009. Evaluation of bambara groundnut varieties for off-season production in Zimbabwe *Afri. Crop Sci. J*. 16, 175 – 183.

MBATHA, T.P. & MODI, A.T., 2010. Response of local mustard germplasm to water stress. *S. Afr. J. Plant & Soil* 27, 328-330.

MCDONALD, M.B. 1980. Assessment of seed quality. HortScience 15:784-788.

MORGAN, J.M., 1984. Osmoregulation and water stress in higher plants. *Ann. Rev. Plant Physiol.*, 35: 299-348.

MWALE, S.S., AZAM-ALI, S.N. & MASSAWE, F.J., 2007a. Growth and development of bambara groundnut (*Vigna subterranea*) in response to soil moisture: 2. Resource capture and conversion. *Euro. J. Agron*. 26, 354-362.

MWALE, S.S., AZAM-ALI, S.N. & MASSAWE, F.J., 2007b. Growth and development of bambara groundnut (*Vigna subterranea*) in response to soil moisture. 1. Dry matter and yield. *Euro. J. Agron*. 26, 345-353.

MWALE, S.S., HAMUSIMBI, S. & MWANSA, K., 2003. Germination, emergence and growth of sunflower (*Helianthus annus* L.) in response to osmotic seed priming. *Seed Sci. Technol*. 31, 199-206.

PATIL, A.G., 2010. Water Stress Induced Changes in Proline Content in *Vigna unguiculata* (Linn) var. Konkan Sadabahar at Various Stages of Growth. (*Accessed from www.faqs.org. on 10-08-2011*).

PERRY, D.A., 1978. Report on the vigour test committee. P. 1974-1977. *Seed Sci.*

POLLOCK, B.M., 1972. Effects of environment after sowing on viability. P. 150-171. *In*: E. H. Roberts (ed.) Viability of seeds. Syracuse University Press, Syracuse, N.Y.

POWELL, A.A., 1989. The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Ann. Bot*. 63, 169-175.

REIJ, C., MULDER, P. & BEGEMANN, L., 1991. Water harvesting for plant production. World Bank Technical Paper Number 91, Washington, D.C.

SESAY, A., MAGAGULA, C. N. & MANSUETUS, A. B., 2008. Influence of sowing date and environmental factors on the development and yield of Bambara groundnut (*Vigna Subterranean* L.*)* landraces in a Sub-tropical region. *Expl. Agric*. 44, 167–183.

SESSAY A., KUNENE I.S. & EARNSHAW, D.M., 1999. Farmer's knowledge and cultivation of Bambara groundnut (*Vigna subterranea* L.) in Swaziland. UNISWA Research *J. Agric. Sci. Technol.* 3(1), 27-37.

SESSAY, A., EDJE, O.T. & MAGAGULA, C.N., 2004. Agronomic performance and morphological traits of field grown Bambara groundnut in Swaziland. Proceedings of the International Bambara Groundnut Symposium, Aug. 8-12, 2003, Botswana College of Agriculture, Botswana. pp 47-63.

SWANEVELDER, C.J., 1998. Bambara – food for Africa. National Department of Agriculture, Government Printer, Republic of South Africa
Technol. 6, 159-181.

TOUNGOS, M., SAJO, A. & GUNGULA, D. 2009., Recommended Fertilizer Levels on Bambara Groundnut (*Vigna subterranea* L. Verdc) in Yola Adamawa State, Nigeria. *Agric. J.* 4, 14-21.

WALTER, M.W., 1967. Length of the rainy season in Nigeria. *Nigerian Geographical Journal of Agricultural Science* 10, 123–128.

ZULU, D., 1989. Germination and establishment of Bambara groundnut (Vigna Subterranea (L.) Verdc) and groundnut (*Arachis hypogeae*) in response to temperature, moisture, sowing depths and seed size. MSc. Thesis, University of Nottingham, UK.

ZULU, N.S. & MODI, A.T., 2010. A preliminary study to determine water stress tolerance in wild melon (*Citrillus lanatus* L.). *S. Afr. J. Plant & Soil* 27, 334-336.

CHAPTER 5

GENERAL DISCUSSION

It was clear from the literature that Bambara groundnut still remains an important crop in several African countries, where resource-poor farmers produce and consume it. Its importance as a source of dietary protein, as a legume able to fix nitrogen as well as its success in poor soils and drought prone regions makes it best suited for production by resource-poor farmers. Although bambara groundnut produces a nutritious food and is cultivated throughout Africa, it remains one of the crops most neglected by science, yet empirical evidence and fragmentary research results suggest that it is a crop with great potential. The general aim of the study was to characterise bambara groundnut landraces of KwaZulu-Natal, with respect to seed colour and responses to water availability and use the findings to promote Bambara cultivation as an alternative legume crop under water-scarce conditions. The specific objectives of the study were:

- To compare three landraces of bambara, which differ in seed coat colour (red, brown and white) with respect to seed quality for crop establishment under different water regimes;
- To determine the effect of water stress on the growth, development and yield of the three landraces under controlled environment and field conditions; and
- To determine the effect of planting date, as a management component, on growth, development and of yield bambara groundnut.

As an underutilized crop, bambara groundnut's germplasm improvement and management practices depend on local experiences and resources (indigenous knowledge). Bambara groundnut is still cultivated from local landraces rather than from improved varieties suitable for particular environments, hence farm yields are still low. Unpredictable bambara yields have been attributed, but not exclusively, to variable or poor field establishment due to poor crop establishment (Linnemann and Azam-Ali, 1993). Previous research (Zulu and Modi, 2010) also indicated that seed colour was associated with seed quality.

In the initial study (Chapter 2), we evaluated seed quality components of a local bambara landrace, based on seed colour. Results showed that darker colours germinated better that light coloured seeds. Brown seeds had the highest percentage germination followed by red and white seeds, respectively. Thus, dark coloured seeds were more viable than the white landrace. This led to evaluation of seedling establishment of these landraces under varying water stress regimes. Interestingly, white seeds performed better than brown and red seeds, under both water regimes, with respect to seedling establishment; although darker coloured seeds (brown and red) performed better than white in subsequent experiments (Chapters 3 & 4). Water stress reduced seedling growth but it had no effect on seedling vigour of the three seed colours.

Zulu (1989) observed that seed germination in Bambara groundnut was sensitive to water stress. He ascribed this to the restrictive water uptake by Bambara groundnut due to hard seed coat. Consequently, there was a need for assessing seed coat thickness of the three seed colours using Zeiss EVO Scanning Electron Microscope (SME). Picture scans showed that brown and red had almost the same seed coat thickness while white had the thinnest seed coat. The fact that white seeds had the thinnest seed coat, compared to brown and red seeds, may explain the higher electrolyte leakage and seed mass gain observed during imbibitions (Chapter 2). Therefore, poor germination observed in the white seeds may have been the result of increased leaking of solutes, and imbibitional injury caused by the rapid uptake of water during imbibition as a result of a thin seed coat.

The fact that the crop is usually planted in dry harsh conditions necessitated that we also evaluate desiccation tolerance (Chapter 2). Desiccation tolerance is defined as the ability of cells to withstand stress imposed due to an almost complete loss of cellular water and the ability to resume normal metabolic activities upon imbibition (Vertucci and Farrant, 1995; Hoekstra *et al*., 2002). Proline accumulation has been found to be a common metabolic response of plants to abiotic and biotic stresses. Thus, we evaluated changes in seed proline content over time in response to desiccation Chapter 2). Desiccation was imposed on the seeds by priming them with salts (NaCl, LiCl and KNO_3) and water (H_2O). Brief exposure of the seeds to saturated salt solutions resulted in measurable increases in the proline concentration, but with longer exposure the proline concentration declined. Sodium

chloride (NaCl) was associated with the highest proline accumulation indicating desiccation tolerance by the seeds.

Priming improved seedling emergence under water stress (25% FC) with brown seeds primed with NaCl showing highest germination (100%). Furthermore, white seeds primed with NaCl emerged fast under both 75% FC & 25% FC. When seedlings were transplanted into pots under controlled conditions at 75% FC & 25% FC, results showed that seeds primed with LiCl, NaCl and KNO_3 had tallest plants and many leaves hence highest yields than unprimed and hydro-primed seeds under water stress. However, halo-primed seeds showed the highest reduction in plant height and leaf number under water stress. Priming was shown to improve germination and early crop establishment of bambara groundnut landraces under water stress. However, yield per plant was not improved by either halo- or hydro-priming.

Plant responses to water stress depend greatly upon management prior to and during the cropping season. The selection of planting dates as a management factor for managing water stress in bambara groundnut landraces under field conditions was evaluated in Chapter 4. In South Africa, Bambara production is carried out in the KwaZulu-Natal, Mpumalanga, Limpopo and North West Provinces. Rainfall amount and distribution throughout the year varies considerably among these provinces. According to Kucharik (2006), changes in climate as well as changes in technological and socio-economic factors usually result in variations in planting dates over time. Water stress can occur at any time during the cropping season; thus, affecting plant efficiency to capture and utilise resources such as light, nutrient uptake and hence final yield at harvest. Thus, when planting, it is important to know whether the rains are continuous and sufficient in order to ensure optimal soil water availability during planting and whether the soil water will be maintained or increased during the growing period to avoid total crop failure and to attain optimum yields (Walter, 1967). It is important to select the best planting date where critical growth stages will coincide with favourable field conditions.

For all planting dates in the study, crops were established under irrigation to allow for optimum crop stand. Darker coloured seeds (brown and red) emerged better than the white coloured seeds (Fig 4.1). A similar trend was observed in early crop establishment under

varying water stress treatments under controlled environments (Chapter 2). Selection of planting dates was shown to be a useful management tool for managing water stress under water limited conditions. The optimum planting date resulted in the best crop growth for all measured plant growth parameters followed by late and early planting dates, respectively. Plant growth (plant height, leaf number and LAI) of Bambara groundnut landraces was shown to be sensitive to water stress. Water stress, decreased yield components and yield. The dark colours (brown and red) performed better than the white landrace under both irrigated and non-irrigated conditions.

CONCLUSIONS

Choice of planting dates was shown to be a useful management tool for managing water stress under water limited conditions. Bambara landraces demonstrated the measure of water stress tolerance during crop establishment and vegetative growth both under controlled and field conditions. Bambara landraces yield were affected by water stress under field conditions. Seed coat colour was shown to be associated with seed quality. Red and brown managed to use water efficiently and were able to partition more assimilates towards yield when grown under water limiting conditions.

RECOMMENDATIONS

- The fact that red seeds showed highest viability and vigour warrants further research.
- Priming was shown to improve seedling establishment, however, it did not improve yield. Since seeds primed with salts accumulated high proline, it would be of interest to know if proline continues to accumulate in seedlings under water stress conditions and if the relative higher yield obtained from halo-primed seeds as compared unprimed and hydro-primed seeds is the result of high proline levels observed in halo-primed seeds (NaCl). Roots of water stressed plants are known to grow deep in search of water as compared to roots of irrigated plants. Therefore further research is needed to evaluate halo- and hydro-priming of bambara groundnut landrace seeds, on proline accumulation in seedlings and root behaviour under water stress.
- Farmers in drought prone areas may use hydro-priming to improve seedling emergence and seedling establishment.
- Further research is also needed to focus on water stress imposed at specific growth stages (vegetative, at flowering and after flowering).
- In addition, plant nutrition may also be affected by water stress; hence research is needed to evaluate effect of water stress on nitrogen fixation of bambara groundnut landraces.
- Data obtained from the study will be valuable for modelling crop responses to different water regimes and to contribute to the establishment of a valid basis for advice on crop management.

References

HOEKSTRA, F.A., GOLOVINA, E.A., & NIJSE, J., 2002. What do we really know about desiccation tolerance mechanisms? *In*: Nicholas G, Bradford KJ, Come D, Pritchard HW, (Eds.) The Biology of Seeds, CAB International.

KUCHARIK, C.J., 2006. A multidecadal trend of earlier corn planting in the central USA. *Agron. J.*, 98, 1544–1550.

LINNEMANN, A.R., & AZAM-ALI, S.N., 1993. Bambara groundnut (*Vigna subterranea* L. Verdc). *In*: Williams, J.T. (Ed.), Under-utilised Crops Series. II. Vegetable and Pulses. Chapman and Hall, London, UK. pp. 13-58.

VERTUCCI, C.W. & FARRANT, J.M., 1995. Acquisition of desiccation tolerance. *In*: J. Kigel, D. Galili, (Eds.) Seed Development and Germination. New York: Marcel Dekker Inc.

WALTER, M.W., 1967. Length of the rainy season in Nigeria. *Nigerian Geographical Journal of Agricultural Science* 10, 123–128.

ZULU, D., 1989. Germination and establishment of Bambara groundnut (Vigna Subterranea (L.) Verdc) and groundnut (*Arachis hypogea*) in response to temperature, moisture, sowing depths and seed size. MSc. Thesis, University of Nottingham, UK.

ZULU, N.S., & MODI, A.T., 2010. A preliminary study to determine water stress tolerance in wild melon (*Citrillus lanatus* L.). *S. Afr. J. Plant & Soil* 27, 334-336.

APPENDICES

Appendix 1: List of ANOVAs for Early Establishment Trial

Variate: Daily Germination

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1743.3	581.1	3.71	
Rep.*Units* stratum					
Variety	2	2168.8	1084.4	6.93	0.001
Treatment	1	95007.5	95007.5	607.21	<.001
Day	7	129480.7	18497.2	118.22	<.001
Variety.Treatment	2	187.9	94.0	0.60	0.550
Variety.Day	14	1960.6	140.0	0.90	0.566
Treatment.Day	7	28539.2	4077.0	26.06	<.001
Variety.Treatment.Day	14	2321.7	165.8	1.06	0.399
Residual	141	22061.5	156.5		
Total	191	283471.1			

Variate: % Final Germination

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	317.8	105.9	0.82	
Rep.*Units* stratum					
Variety	2	2566.8	1283.4	9.94	0.002
Treatment	1	9204.2	9204.2	71.31	<.001
Variety.Treatment	2	1673.6	836.8	6.48	0.009
Residual	15	1936.2	129.1		
Total	23	15698.5			

Variate: Water Activity (Aw)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0032243	0.0010748	1.33	
Rep.*Units* stratum					
Variety	2	0.0124640	0.0062320	7.69	0.001
Treatment	6	1.7085136	0.2847523	351.29	<.001
Variety.Treatment	12	0.0397393	0.0033116	4.09	<.001
Residual	60	0.0486354	0.0008106		
Total	83	1.8125767			

Variate: EC (us/cm/g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	19078.	6359.	3.93	
Rep.*Units* stratum					
Variety	2	294324.	147162.	90.91	<.001
Treatment	6	2979414.	496569.	306.77	<.001
Variety.Treatment	12	256137.	21345.	13.19	<.001
Residual	60	97121.	1619.		
Total	83	3646074.			

Variate: Seed_Mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.05856	0.01952	1.81	
Rep.*Units* stratum					
Variety	2	0.00006	0.00003	0.00	0.997
Treatment	6	8.50245	1.41707	131.25	<.001
Variety.Treatment	12	0.31375	0.02615	2.42	0.012
Residual	60	0.64779	0.01080		
Total	83	9.52261			

Variate: Abnormality (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	634.7	211.6	1.52	
Rep.*Units* stratum					
Variety	2	316.0	158.0	1.14	0.347
Treatment	1	10.7	10.7	0.08	0.786
Variety.Treatment	2	1049.3	524.7	3.77	0.047
Residual	15	2085.3	139.0		
Total	23	4096.0			

Variate: Dry_Mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	10.833	3.611	2.20	
Rep.*Units* stratum					
Variety	2	8.583	4.292	2.61	0.107
Treatment	1	0.000	0.000	0.00	1.000
Variety.Treatment	2	9.750	4.875	2.96	0.082
Residual	15	24.667	1.644		

Total 23 53.833

Variate: Fresh_Mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.125	1.375	0.34	
Rep.*Units* stratum					
Variety	2	11.083	5.542	1.37	0.284
Treatment	1	51.042	51.042	12.63	0.003
Variety.Treatment	2	11.083	5.542	1.37	0.284
Residual	15	60.625	4.042		
Total	23	137.958			

Variate: GVI (days)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	490.5	163.5	0.70	
Rep.*Units* stratum					
Variety	2	489.3	244.7	1.05	0.374
Treatment	1	34925.6	34925.6	150.08	<.001
Variety.Treatment	2	32.1	16.1	0.07	0.934
Residual	15	3490.8	232.7		
Total	23	39428.4			

Variate: MGT (days)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.7886	0.2629	1.68	
Rep.*Units* stratum					
Seed_Colour	2	0.0765	0.0382	0.24	0.786
Treatment	1	8.6637	8.6637	55.46	<.001
Seed_Colour.Treatment	2	0.1838	0.0919	0.59	0.568
Residual	15	2.3432	0.1562		
Total	23	12.0557			

Variate: Daily_Emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9327.09	4663.54	47.93	
Rep.*Units* stratum					
Variety	2	51528.99	25764.50	264.79	<.001
Trt	4	145804.97	36451.24	374.62	<.001
FC	1	13546.88	13546.88	139.22	<.001
DAP	20	1563462.01	78173.10	803.40	<.001
Variety.Trt	8	26630.26	3328.78	34.21	<.001
Variety.FC	2	25730.90	12865.45	132.22	<.001
Trt.FC	4	116821.90	29205.48	300.15	<.001
Variety.DAP	40	18755.45	468.89	4.82	<.001
Trt.DAP	80	78675.03	983.44	10.11	<.001
FC.DAP	20	13213.12	660.66	6.79	<.001
Variety.Trt.FC	8	29528.89	3691.11	37.93	<.001
Variety.Trt.DAP	160	29996.40	187.48	1.93	<.001
Variety.FC.DAP	40	12642.43	316.06	3.25	<.001
Trt.FC.DAP	80	48329.21	604.12	6.21	<.001
Variety.Trt.FC.DAP	160	27453.33	171.58	1.76	<.001
Residual	1258	122406.24	97.30		
Total	1889	2333853.12			

Variate: % Final Emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	895.6	447.8	3.87	
Rep.*Units* stratum					
Variety	2	3682.2	1841.1	15.93	<.001
Trt	4	8540.0	2135.0	18.47	<.001
FC	1	871.1	871.1	7.54	0.008
Variety.Trt	8	1440.0	180.0	1.56	0.158
Variety.FC	2	1335.6	667.8	5.78	0.005
Trt.FC	4	8784.4	2196.1	19.00	<.001
Variety.Trt.FC	8	3608.9	451.1	3.90	<.001
Residual	58	6704.4	115.6		
Total	89	35862.2			

Variate: Met (days)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0513	0.0257	0.19	
Rep.*Units* stratum					
Seed_Colour	2	1.6909	0.8454	6.12	0.004
Trt	4	27.5998	6.9000	49.96	<.001
FC	1	0.1591	0.1591	1.15	0.288
Seed_Colour.Trt	8	8.2083	1.0260	7.43	<.001
Seed_Colour.FC	2	0.5119	0.2560	1.85	0.166
Trt.FC	4	12.0404	3.0101	21.79	<.001
Seed_Colour.Trt.FC	8	2.6694	0.3337	2.42	0.025
Residual	58	8.0106	0.1381		

Total 89 60.9418

Variate: Plant_Height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	17.378	8.689	8.55	
Rep.*Units* stratum					
Seed_Colour	2	34.334	17.167	16.90	<.001
Trt	4	63.842	15.960	15.71	<.001
FC	1	28.519	28.519	28.07	<.001
WAP	2	42.557	21.279	20.95	<.001
Seed_Colour.Trt	8	28.969	3.621	3.56	<.001
Seed_Colour.FC	2	2.879	1.440	1.42	0.245
Trt.FC	4	12.121	3.030	2.98	0.020
Seed_Colour.WAP	4	0.331	0.083	0.08	0.988
Trt.WAP	8	5.003	0.625	0.62	0.764
FC.WAP	2	2.239	1.119	1.10	0.334
Seed_Colour.Trt.FC	8	35.540	4.442	4.37	<.001
Seed_Colour.Trt.WAP	16	3.678	0.230	0.23	0.999
Seed_Colour.FC.WAP	4	0.919	0.230	0.23	0.923
Trt.FC.WAP	8	3.562	0.445	0.44	0.897
Seed_Colour.Trt.FC.WAP	16	4.544	0.284	0.28	0.997
Residual	178	180.830	1.016		

Total 269 467.246

Variate: Leaf_No.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1352	0.0676	0.30	
Rep.*Units* stratum					
Seed_Colour	2	2.6963	1.3481	5.99	0.003
Trt	4	4.5037	1.1259	5.01	<.001
FC	1	8.3565	8.3565	37.16	<.001
WAP	2	7.9630	3.9815	17.70	<.001
Seed_Colour.Trt	8	3.1741	0.3968	1.76	0.087
Seed_Colour.FC	2	3.0296	1.5148	6.74	0.002
Trt.FC	4	3.1667	0.7917	3.52	0.009
Seed_Colour.WAP	4	0.2037	0.0509	0.23	0.923
Trt.WAP	8	2.2963	0.2870	1.28	0.258
FC.WAP	2	0.8074	0.4037	1.80	0.169
Seed_Colour.Trt.FC	8	4.6556	0.5819	2.59	0.011
Seed_Colour.Trt.WAP	16	0.8426	0.0527	0.23	0.999
Seed_Colour.FC.WAP	4	0.5815	0.1454	0.65	0.630
Trt.FC.WAP	8	0.7111	0.0889	0.40	0.922
Seed_Colour.Trt.FC.WAP	16	1.8167	0.1135	0.50	0.942
Residual	178	40.0315	0.2249		

Total 269 84.9713

Variate: Root_Length (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.5681	0.7840	1.14	
Rep.*Units* stratum					
Seed_Colour	2	0.5056	0.2528	0.37	0.693
Trt	4	7.2389	1.8097	2.64	0.043
FC	1	1.0028	1.0028	1.46	0.231
Seed_Colour.Trt	8	3.3278	0.4160	0.61	0.768
Seed_Colour.FC	2	0.2722	0.1361	0.20	0.820
Trt.FC	4	1.9833	0.4958	0.72	0.579
Seed_Colour.Trt.FC	8	5.1167	0.6396	0.93	0.496
Residual	58	39.7236	0.6849		
Total	89	60.7389			

Variate: Root_Shoot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.12574	0.06287	1.79	
Rep.*Units* stratum					
Seed_Colour	2	0.12180	0.06090	1.73	0.186
Trt	4	0.47134	0.11784	3.35	0.016
FC	1	0.20542	0.20542	5.83	0.019
Seed_Colour.Trt	8	0.43329	0.05416	1.54	0.164
Seed_Colour.FC	2	0.02062	0.01031	0.29	0.747
Trt.FC	4	0.25514	0.06378	1.81	0.139
Seed_Colour.Trt.FC	8	0.54422	0.06803	1.93	0.072
Residual	58	2.04246	0.03521		
Total	89	4.22003			

Variate: Leaf_Area/plant (cm^2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	189.47	94.74	1.84	
Rep.*Units* stratum					
Seed_Colour	2	142.96	71.48	1.39	0.258
Trt	4	457.52	114.38	2.22	0.078
FC	1	766.41	766.41	14.86	<.001
Seed_Colour.Trt	8	535.48	66.93	1.30	0.263
Seed_Colour.FC	2	429.09	214.54	4.16	0.021
Trt.FC	4	360.83	90.21	1.75	0.152
Seed_Colour.Trt.FC	8	427.35	53.42	1.04	0.420
Residual	58	2991.93	51.59		
Total	89	6301.05			

Variate: Fresh_mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.8112	0.4056	2.04	
Rep.*Units* stratum					
Seed_Colour	2	0.1473	0.0737	0.37	0.693
Trt	4	0.6219	0.1555	0.78	0.543
FC	1	1.7651	1.7651	8.86	0.004
Seed_Colour.Trt	8	0.4556	0.0569	0.29	0.968
Seed_Colour.FC	2	0.4013	0.2006	1.01	0.372
Trt.FC	4	1.0019	0.2505	1.26	0.297
Seed_Colour.Trt.FC	8	0.5580	0.0697	0.35	0.942
Residual	58	11.5579	0.1993		
Total	89	17.3202			

Variate: Dry_mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.05391	0.02695	0.54	
Rep.*Units* stratum					
Seed_Colour	2	0.00348	0.00174	0.04	0.966
Trt	4	0.04387	0.01097	0.22	0.926
FC	1	0.10100	0.10100	2.03	0.159
Seed_Colour.Trt	8	0.03835	0.00479	0.10	0.999
Seed_Colour.FC	2	0.02638	0.01319	0.27	0.768
Trt.FC	4	0.02453	0.00613	0.12	0.973
Seed_Colour.Trt.FC	8	0.08321	0.01040	0.21	0.988
Residual	58	2.87903	0.04964		
Total	89	3.25377			

Variate: Shoot_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.385	3.192	2.75	
Rep.*Units* stratum					
Seed_Colour	2	13.018	6.509	5.61	0.006
Trt	4	10.739	2.685	2.31	0.068
FC	1	14.201	14.201	12.23	<.001
Seed_Colour.Trt	8	5.711	0.714	0.62	0.762
Seed_Colour.FC	2	1.976	0.988	0.85	0.432
Trt.FC	4	3.317	0.829	0.71	0.586
Seed_Colour.Trt.FC	8	27.350	3.419	2.95	0.008
Residual	58	67.324	1.161		
Total	89	150.020			

Appendix 2: List of ANOVAs for Controlled Experiment Study

Variate: Plant_Height (cm)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		110.939	55.469	10.83	
Rep.*Units* stratum						
Seed_Colour	2		6.972	3.486	0.68	0.507
Trt	4		73.889	18.472	3.61	0.007
FC	1		24.610	24.610	4.80	0.029
WAT	4		46.000	11.500	2.24	0.064
Seed_Colour.Trt	8		199.716	24.965	4.87	<.001
Seed_Colour.FC	2		9.274	4.637	0.91	0.406
Trt.FC	4		141.156	35.289	6.89	<.001
Seed_Colour.WAT	8		5.535	0.692	0.14	0.998
Trt.WAT	16		40.264	2.516	0.49	0.951
FC.WAT	4		2.418	0.605	0.12	0.976
Seed_Colour.Trt.FC	8		414.215	51.777	10.11	<.001
Seed_Colour.Trt.WAT	32		63.295	1.978	0.39	0.999
Seed_Colour.FC.WAT	8		7.316	0.915	0.18	0.994
Trt.FC.WAT	16		30.881	1.930	0.38	0.987
Seed_Colour.Trt.FC.WAT	32		35.885	1.121	0.22	1.000
Residual	287	(11)	1470.210	5.123		
Total	438	(11)	2649.634			

Variate: Leaf_No

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		110.281	55.141	15.82	
Rep.*Units* stratum						
Seed_Colour	2		56.891	28.446	8.16	<.001
Trt	4		85.050	21.262	6.10	<.001
FC	1		24.891	24.891	7.14	0.008
WAT	4		460.692	115.173	33.05	<.001
Seed_Colour.Trt	8		77.817	9.727	2.79	0.005
Seed_Colour.FC	2		5.906	2.953	0.85	0.430
Trt.FC	4		61.241	15.310	4.39	0.002
Seed_Colour.WAT	8		20.970	2.621	0.75	0.645
Trt.WAT	16		16.979	1.061	0.30	0.996
FC.WAT	4		10.093	2.523	0.72	0.576
Seed_Colour.Trt.FC	8		61.189	7.649	2.20	0.028
Seed_Colour.Trt.WAT	32		28.630	0.895	0.26	1.000
Seed_Colour.FC.WAT	8		23.013	2.877	0.83	0.581
Trt.FC.WAT	16		26.276	1.642	0.47	0.959
Seed_Colour.Trt.FC.WAT	32		44.346	1.386	0.40	0.999
Residual	287	(11)	1000.048	3.484		
Total	438	(11)	2103.271			

Variate: Total_Biomass_(g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		2.8696	1.4348	3.43	
Rep.*Units* stratum						
Variety	2		1.0162	0.5081	1.21	0.305
Treatment	4		0.9197	0.2299	0.55	0.700
FC	1		1.3901	1.3901	3.32	0.074
Variety.Treatment	8		3.9964	0.4996	1.19	0.322
Variety.FC	2		0.0876	0.0438	0.10	0.901
Treatment.FC	4		1.4340	0.3585	0.86	0.496
Variety.Treatment.FC	8		3.8985	0.4873	1.16	0.339
Residual	50	(8)	20.9195	0.4184		
Total	81	(8)	36.0870			

Variate: Grain_Mass/plant_(g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.74942	0.37471	4.24	
Rep.*Units* stratum						
Variety	2		1.16504	0.58252	6.60	0.005
Treatment	4		1.03502	0.25876	2.93	0.043
FC	1		0.63529	0.63529	7.19	0.013
Variety.Treatment	8		1.58004	0.19750	2.24	0.063
Variety.FC	2		0.02391	0.01195	0.14	0.874
Treatment.FC	4		0.22451	0.05613	0.64	0.642
Variety.Treatment.FC	6	(2)	0.76128	0.12688	1.44	0.244
Residual	23	(35)	2.03096	0.08830		
Total	52	(37)	4.93376			

Variate: Grains/pod

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.108055	0.054028	6.76	
Rep.*Units* stratum						
Variety	2		0.035939	0.017970	2.25	0.128
Treatment	4		0.129672	0.032418	4.05	0.012
FC	1		0.016393	0.016393	2.05	0.166
Variety.Treatment	8		0.218362	0.027295	3.41	0.010
Variety.FC	2		0.086315	0.043157	5.40	0.012
Treatment.FC	4		0.017949	0.004487	0.56	0.693
Variety.Treatment.FC	6	(2)	0.311542	0.051924	6.49	<.001
Residual	23	(35)	0.183895	0.007995		
Total	52	(37)	0.575525			

Variate: Harvest_Index_HI (%)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		561.2	280.6	2.19	
Rep.*Units* stratum						
Variety	2		3119.7	1559.8	12.18	<.001
Treatment	4		1572.0	393.0	3.07	0.036
FC	1		760.5	760.5	5.94	0.023
Variety.Treatment	8		4609.0	576.1	4.50	0.002
Variety.FC	2		101.1	50.6	0.39	0.678
Treatment.FC	4		1017.1	254.3	1.99	0.129
Variety.Treatment.FC	6	(2)	2257.8	376.3	2.94	0.027
Residual	24	(34)	3073.5	128.1		
Total	53	(36)	9056.6			

Variate: Pod_No_plant

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		47.195	23.597	8.85	
Rep.*Units* stratum						
Variety	2		10.932	5.466	2.05	0.151
Treatment	4		8.560	2.140	0.80	0.536
FC	1		13.649	13.649	5.12	0.033
Variety.Treatment	8		32.490	4.061	1.52	0.201
Variety.FC	2		1.596	0.798	0.30	0.744
Treatment.FC	4		1.288	0.322	0.12	0.974
Variety.Treatment.FC	6	(2)	15.558	2.593	0.97	0.465
Residual	24	(34)	64.005	2.667		
Total	53	(36)	130.593			

Variate: Pod_Mass/plant_(g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.9155	0.4577	3.17	
Rep.*Units* stratum						
Variety	2		1.6427	0.8213	5.69	0.009
Treatment	4		1.3913	0.3478	2.41	0.077
FC	1		0.7379	0.7379	5.12	0.033
Variety.Treatment	8		2.0582	0.2573	1.78	0.130
Variety.FC	2		0.0069	0.0034	0.02	0.976
Treatment.FC	4		0.4028	0.1007	0.70	0.601
Variety.Treatment.FC	6	(2)	1.1763	0.1961	1.36	0.271
Residual	24	(34)	3.4622	0.1443		
Total	53	(36)	7.6961			

Variate: Yield (kg/ha)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		20817.	10409.	4.24	
Rep.*Units* stratum						
Variety	2		32362.	16181.	6.60	0.005
Treatment	4		28751.	7188.	2.93	0.043
FC	1		17647.	17647.	7.19	0.013
Variety.Treatment	8		43890.	5486.	2.24	0.063
Variety.FC	2		664.	332.	0.14	0.874
Treatment.FC	4		6236.	1559.	0.64	0.642
Variety.Treatment.FC	6	(2)	21147.	3524.	1.44	0.244
Residual	23	(35)	56416.	2453.		
Total	52	(37)	137049.			

Appendix 3: List of ANOVAS for field trials

Variate: % Final Emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	805.3	402.7	1.57	
Rep.*Units* stratum					
Variety	2	1096.6	548.3	2.14	0.134
Trt	1	9.3	9.3	0.04	0.850
Planting_Date	2	2145.4	1072.7	4.18	0.024
Variety.Trt	2	48.2	24.1	0.09	0.911
Variety.Planting_Date	4	788.9	197.2	0.77	0.553
Trt.Planting_Date	2	1313.7	656.9	2.56	0.092
Variety.Trt.Planting_Date	4	541.6	135.4	0.53	0.716
Residual	34	8725.8	256.6		
Total 53 15474.8					

Analysis of variance Planting Date 1

Variate: Pant Height (cm)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		29.567	14.783	4.97	
Rep.*Units* stratum						
Variety	2		48.839	24.420	8.20	<.001
Trt	1		36.329	36.329	12.20	<.001
weeks	7		125.986	17.998	6.05	<.001
Variety.Trt	2		8.471	4.236	1.42	0.246
Variety.weeks	14		6.374	0.455	0.15	1.000
Trt.weeks	7		20.394	2.913	0.98	0.451
Variety.Trt.weeks	14		20.914	1.494	0.50	0.927
Residual	93	(1)	276.821	2.977		
Total	142	(1)	571.278			

Variate: Leaf No

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		559.51	279.76	19.93	
Rep.*Units* stratum						
Variety	2		135.82	67.91	4.84	0.010
Trt	1		38.81	38.81	2.76	0.100
weeks	7		6411.99	916.00	65.25	<.001
Variety.Trt	2		10.88	5.44	0.39	0.680
Variety.weeks	14		207.09	14.79	1.05	0.410
Trt.weeks	7		57.38	8.20	0.58	0.767
Variety.Trt.weeks	14		197.12	14.08	1.00	0.457

Residual	92	(2)	1291.58	14.04	
Total	141	(2)	8877.33		

Analysis of variance Planting Date 2

Variate: Plant Height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	22.415	11.208	6.07	
Rep.*Units* stratum					
Variety	2	20.863	10.432	5.65	0.006
Trt	1	26.914	26.914	14.57	<.001
weeks	4	122.567	30.642	16.58	<.001
Variety.Trt	2	2.472	1.236	0.67	0.516
Variety.weeks	8	7.612	0.951	0.51	0.840
Trt.weeks	4	26.537	6.634	3.59	0.011
Variety.Trt.weeks	8	4.835	0.604	0.33	0.952
Residual	58	107.170	1.848		
Total	89	341.385			

Variate: Leaf_No

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	47.13	23.56	1.03	
Rep.*Units* stratum					
Variety	2	86.83	43.42	1.90	0.159
Trt	1	1316.63	1316.63	57.54	<.001
weeks	4	2902.97	725.74	31.72	<.001
Variety.Trt	2	28.86	14.43	0.63	0.536
Variety.weeks	8	60.58	7.57	0.33	0.951
Trt.weeks	4	410.49	102.62	4.49	0.003
Variety.Trt.weeks	8	64.66	8.08	0.35	0.941
Residual	58	1327.09	22.88		
Total	89	6245.25			

Analysis of variance Planting Date3

Variate: Plant Height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.474	0.237	0.11	
Rep.*Units* stratum					
Variety	2	7.991	3.995	1.92	0.156
Trt	1	6.084	6.084	2.92	0.093
weeks	4	56.437	14.109	6.77	<.001
Variety.Trt	2	8.892	4.446	2.13	0.128
Variety.weeks	8	7.478	0.935	0.45	0.886
Trt.weeks	4	4.733	1.183	0.57	0.687
Variety.Trt.weeks	8	2.267	0.283	0.14	0.997

Residual	58	120.839	2.083		
Total 89 215.195					

Variate: Leaf_No

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.05	1.52	0.05	
Rep.*Units* stratum					
Variety	2	163.35	81.68	2.71	0.075
Trt	1	42.64	42.64	1.41	0.239
weeks	4	3330.09	832.52	27.63	<.001
Variety.Trt	2	52.99	26.50	0.88	0.421
Variety.weeks	8	48.79	6.10	0.20	0.989
Trt.weeks	4	106.29	26.57	0.88	0.481
Variety.Trt.weeks	8	42.23	5.28	0.18	0.993
Residual	58	1747.90	30.14		
Total 89 5537.33					

Analysis of variance Planting Date 1

Variate: Fresh_mass_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.060	0.030	0.01	
Rep.*Units* stratum					
Variety	2	14.434	7.217	3.46	0.043
Trt	1	25.297	25.297	12.11	0.001
weeks	2	24.033	12.016	5.75	0.007
Variety.Trt	2	1.075	0.537	0.26	0.775
Variety.weeks	4	4.198	1.050	0.50	0.734
Trt.weeks	2	10.287	5.143	2.46	0.100
Variety.Trt.weeks	4	5.303	1.326	0.63	0.641
Residual	34	71.003	2.088		
Total 53 155.690					

Variate: Dry_mass_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2684	0.1342	0.48	
Rep.*Units* stratum					
Variety	2	1.6236	0.8118	2.91	0.068
Trt	1	4.2448	4.2448	15.24	<.001
weeks	2	0.0015	0.0007	0.00	0.997
Variety.Trt	2	0.0318	0.0159	0.06	0.945
Variety.weeks	4	1.3516	0.3379	1.21	0.323
Trt.weeks	2	0.4195	0.2097	0.75	0.479
Variety.Trt.weeks	4	0.5779	0.1445	0.52	0.722
Residual	34	9.4690	0.2785		

Total 53 17.9881

Analysis of variance Planting Date 2

Variate: Fresh_mass_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.497	0.749	0.18	
Rep.*Units* stratum					
Variety	2	299.119	149.560	36.42	<.001
Trt	1	92.538	92.538	22.54	<.001
weeks	2	3773.304	1886.652	459.47	<.001
Variety.Trt	2	4.690	2.345	0.57	0.570
Variety.weeks	4	350.162	87.540	21.32	<.001
Trt.weeks	2	8.286	4.143	1.01	0.375
Variety.Trt.weeks	4	23.185	5.796	1.41	0.251
Residual	34	139.610	4.106		

Total 53 4692.392

Variate: Dry_mass_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.1956	0.0978	0.33	
Rep.*Units* stratum					
Variety	2	2.4272	1.2136	4.05	0.027
Trt	1	1.6583	1.6583	5.53	0.025
weeks	2	14.5007	7.2503	24.17	<.001
Variety.Trt	2	0.4476	0.2238	0.75	0.482
Variety.weeks	4	3.8440	0.9610	3.20	0.025
Trt.weeks	2	4.3554	2.1777	7.26	0.002
Variety.Trt.weeks	4	0.8502	0.2125	0.71	0.592
Residual	34	10.1991	0.3000		

Total 53 38.4780

Analysis of variance Planting Date 3

Variate: Fresh_mass_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	153.98	76.99	2.20	
Rep.*Units* stratum					
Variety	2	138.28	69.14	1.97	0.154
Trt	1	466.99	466.99	13.34	<.001
weeks	2	3377.38	1688.69	48.22	<.001
Variety.Trt	2	1.62	0.81	0.02	0.977
Variety.weeks	4	140.02	35.00	1.00	0.421
Trt.weeks	2	170.53	85.26	2.43	0.103
Variety.Trt.weeks	4	26.50	6.62	0.19	0.942

Residual	34	1190.57	35.02		
Total 53 5665.86					

Variate: Dry_mass_(g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	22.826	11.413	3.20	
Rep.*Units* stratum					
Variety	2	13.440	6.720	1.88	0.168
Trt	1	70.475	70.475	19.74	<.001
weeks	2	319.599	159.800	44.76	<.001
Variety.Trt	2	0.365	0.183	0.05	0.950
Variety.weeks	4	8.308	2.077	0.58	0.678
Trt.weeks	2	34.870	17.435	4.88	0.014
Variety.Trt.weeks	4	4.801	1.200	0.34	0.852
Residual	34	121.377	3.570		
Total 53 596.062					

Analysis of variance of all yield dates (Split split plot arrangement)

Variate: Total_Biomass_(g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		410.97	205.48	6.06	
Rep.*Units* stratum						
Variety	2		186.47	93.23	2.75	0.079
Treatment	1		473.90	473.90	13.98	<.001
Date	2		1339.20	669.60	19.75	<.001
Variety.Treatment	2		18.32	9.16	0.27	0.765
Variety.Date	4		52.92	13.23	0.39	0.814
Treatment.Date	2		892.51	446.26	13.16	<.001
Variety.Treatment.Date	4		54.24	13.56	0.40	0.807
Residual	32	(2)	1084.98	33.91		
Total	51	(2)	4276.26			

Variate: Pod_Mass/plant_(g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		154.68	77.34	4.11	
Rep.*Units* stratum						
Variety	2		81.93	40.96	2.18	0.130
Treatment	1		292.22	292.22	15.54	<.001
Date	2		842.85	421.43	22.41	<.001
Variety.Treatment	2		6.13	3.07	0.16	0.850
Variety.Date	4		10.71	2.68	0.14	0.965
Treatment.Date	2		464.79	232.39	12.36	<.001
Variety.Treatment.Date	4		26.82	6.70	0.36	0.837
Residual	31	(3)	582.95	18.80		
Total	50	(3)	2357.69			

Variate: Harvest_Index_HI (%)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		27.8	13.9	0.06	
Rep.*Units* stratum						
Variety	2		1379.2	689.6	2.89	0.070
Treatment	1		636.1	636.1	2.67	0.112
Date	2		10164.1	5082.1	21.31	<.001
Variety.Treatment	2		329.7	164.9	0.69	0.508
Variety.Date	4		611.6	152.9	0.64	0.637
Treatment.Date	2		254.3	127.2	0.53	0.592
Variety.Treatment.Date	4		678.3	169.6	0.71	0.590
Residual	32	(2)	7631.3	238.5		
Total	51	(2)	21280.9			

Variate: Pod_No/plant (g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		75.14	37.57	2.54	
Rep.*Units* stratum						
Variety	2		124.64	62.32	4.22	0.024
Treatment	1		102.64	102.64	6.95	0.013
Date	2		247.79	123.89	8.39	0.001
Variety.Treatment	2		4.05	2.02	0.14	0.873
Variety.Date	4		12.74	3.18	0.22	0.928
Treatment.Date	2		96.73	48.36	3.27	0.051
Variety.Treatment.Date	4		6.18	1.54	0.10	0.980
Residual	31	(3)	457.86	14.77		
Total	50	(3)	1048.11			

Variate: Grains/pod (g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.34781	0.17391	3.73	
Rep.*Units* stratum						
Variety	2		0.30414	0.15207	3.26	0.052
Treatment	1		0.07747	0.07747	1.66	0.207
Date	2		0.74541	0.37271	7.98	0.002
Variety.Treatment	2		0.13607	0.06804	1.46	0.248
Variety.Date	4		0.26079	0.06520	1.40	0.258
Treatment.Date	2		0.05603	0.02802	0.60	0.555
Variety.Treatment.Date	4		0.25206	0.06302	1.35	0.274
Residual	31	(3)	1.44699	0.04668		
Total	50	(3)	3.48984			

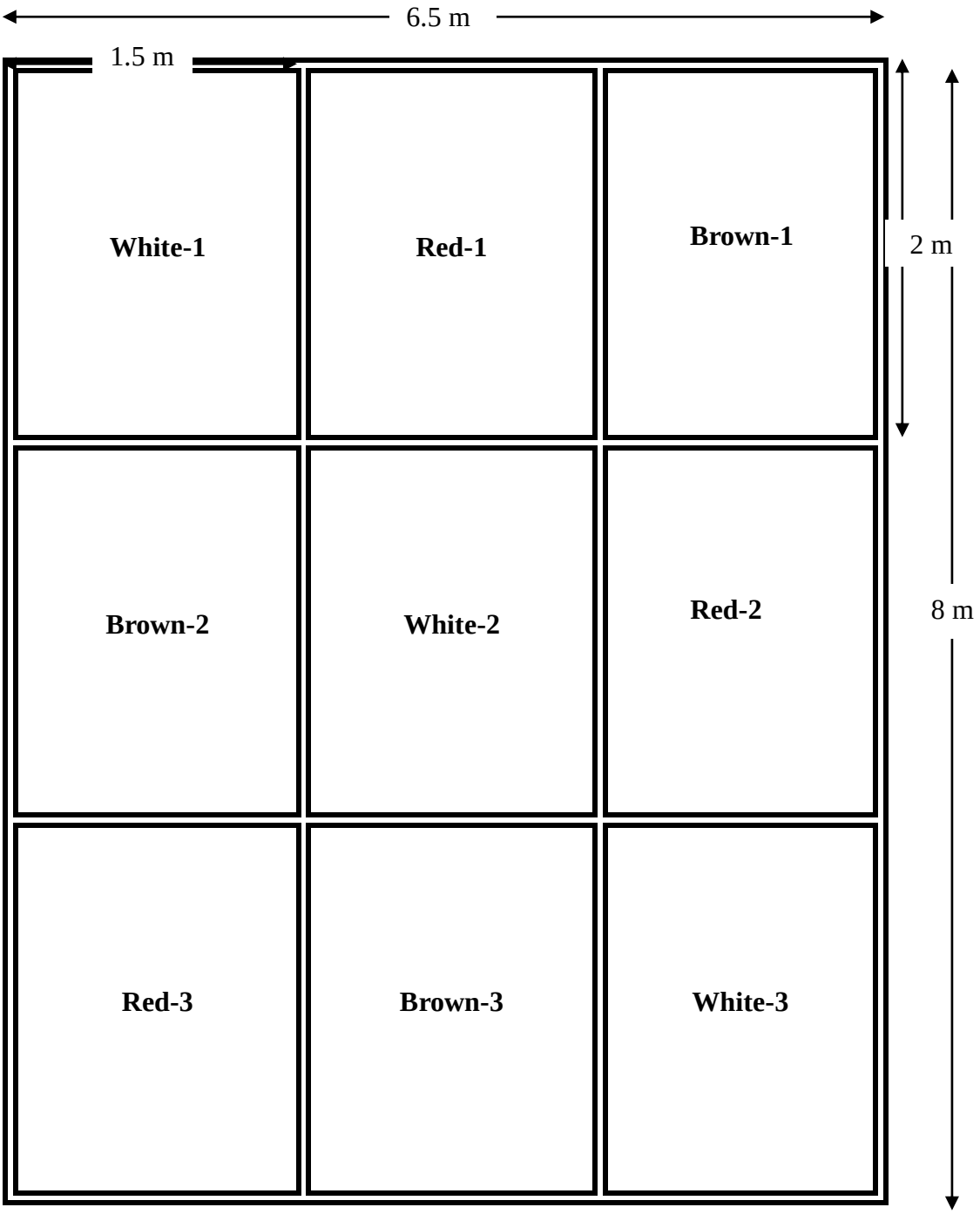
Variate: Grain_Mass/plant_(g)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		36.031	18.016	4.33	
Rep.*Units* stratum						
Variety	2		31.978	15.989	3.84	0.032
Treatment	1		58.510	58.510	14.06	<.001
Date	2		233.250	116.625	28.02	<.001
Variety.Treatment	2		2.075	1.037	0.25	0.781
Variety.Date	4		2.927	0.732	0.18	0.949
Treatment.Date	2		82.327	41.164	9.89	<.001
Variety.Treatment.Date	4		6.668	1.667	0.40	0.807
Residual	31	(3)	129.037	4.162		
Total	50	(3)	552.600			

Variate: Yield_(kg/ha)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		994632.	497316.	4.41	
Rep.*Units* stratum						
Variety	2		906386.	453193.	4.02	0.028
Treatment	1		1554383.	1554383.	13.77	<.001
Date	2		6567410.	3283705.	29.10	<.001
Variety.Treatment	2		57857.	28929.	0.26	0.775
Variety.Date	4		102558.	25640.	0.23	0.921
Treatment.Date	2		2335189.	1167595.	10.35	<.001
Variety.Treatment.Date	4		194105.	48526.	0.43	0.786
Residual	32	(2)	3611245.	112851.		
Total	51	(2)	15592189.			

Appendix 4: Field trial layout for Bambara groundnut main plot



Appendix *5:* Proline standard Curve