RESPONSES OF BAMBARA GROUNDNUT (*VIGNA SUBTERANNEA* L. VERDC) LANDRACES TO FIELD AND CONTROLLED ENVIRONMENT CONDITIONS OF WATER STRESS

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DECLARATION

I, Lungelwa Zondi, 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, Water Use of Drought Tolerant Food Crops (WRC K5/1771//4).

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

Signature ______________________

Prof. A.T. Modi

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- Last, but not least, my Almighty God.

DEDICATION

This thesis is dedicated to my loving child Sisanda Melokuhle Fundile Zondi.

ABSTRACT

Bambara groundnut (*Vigna subterranea* L. Verdc) is a drought tolerant African legume capable of producing reasonable yields where other crops may fail. However, it remains an underutilised crop, owing to limited research, cultivated using landraces, of which scant information is available describing their agronomy and genetic diversity. The aim of this study was to evaluate the response of bambara landraces from different geographical locations to water stress under controlled and field conditions. Seeds were sourced from subsistence farmers of Tugela Ferry and Deepdale in KwaZulu-Natal (South Africa) and Zimbabwe, and characterised into three seed coat colours: light-brown, brown and red. Seed quality was assessed using the standard germination test. Vigour indices of germination velocity index and mean germination time were determined. Seedling establishment was evaluated using seedling trays using a factorial experiment, with four factors: 1. provenance – (Tugela Ferry and Deepdale), 2. seed colour – (red, light-brown and brown), 3. water regimes – (30%, 60% and 100% field capacity), and 4. soil media – (clay, sand and clay + sand). Seedling leaf samples were used to evaluate proline accumulation as an indicator of stress tolerance. A field trial was used to evaluate productivity of bambara landraces under rainfed and irrigated conditions. A pot trial was conducted under controlled environment conditions with three factors: temperature (33/27°C and 21/15°C), water regimes (30% and 100% of crop water requirement) and bambara landrace selections. Results showed no significant differences in germination capacity between bambara landrace selections. Germination time differed significantly ($P<0.001$) between bambara landrace selections. The Jozini provenance was shown to perform best, followed by Zimbabwe, Tugela Ferry and Deepdale. Brown landrace selections had higher ($P<0.001$) germination compared with red and light-brown selections, respectively. Seedling establishment showed that emergence was higher (P<0.001) at 100% FC compared with 60% FC and 30% FC. Emergence was higher (P<0.001) in the Sand+Clay mixture compared with Clay and Sand media. Dark-coloured selections had higher (P<0.001) emergence compared with light-coloured selections. Results from the field trial showed that the red landrace selections emerged better (P<0.001) than the light-brown and brown landrace selections, respectively. Plant growth was lower under irrigated compared with rainfed conditions. Stomatal conductance was higher (P<0.001) under irrigated compared with rainfed conditions, whereas chlorophyll content index was higher (P<0.05) under rainfed compared with irrigated conditions. Results of the pot trial showed that emergence was significantly (P<0.001) affected by temperature. It was higher at 33/27˚C compared with 21/15˚C (P<0.001). Dark-coloured landraces had higher emergence compared with the light-brown landraces. Stomatal conductance was lower at 30% ET relative to 100% ETc. There were no significant differences between water regimes with respect to biomass, pod number per plant, pod mass per plant, seed number per pod, seed mass per plant and harvest index. It is concluded that seed colour is an important variable in the identity of bambara landraces. Provenance plays a significant role in seed performance and there is a significant interaction between provenance and seed coat colour. This study could be expanded to obtain more data for crop improvement through inclusion of many sites and seasons for better agronomic advice to farmers.

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Chapter 1

LITERATURE REVIEW

1.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.)Verdc), formerly known as *Voandzeia subterranea* (L.), is an indigenous African legume which plays an important socio-economic role in semi-arid regions of Africa (Massawe *et al.,* 2005). It serves as a cheap source of protein to a large proportion of the population in poor countries of the tropics (Linnemann and Azam-Ali, 1993; Azam-Ali *et al*., 2001). It is ranked the third most important legume in many parts of Africa after groundnut (*Arachis hypogea*) and cowpea (*Vignau nguiculata*) (Rachie and Silvestre, 1977). In most places in Africa, bambara groundnut is produced by female subsistence farmers (Linnemann and Azam–Ali, 1993). As a result its germplasm preservation has been mostly left to women. The fact that its production is mostly done by women means that very few resources are allocated to its production. This has resulted in the crop being classified as a neglected and underutilised crop due to limited research available on it as well as the fact that its production is limited. This is despite the fact that bambara has been reported to be a drought tolerant crop (Massawe *et al*., 2005), with much potential for enriching diets of people living in marginal areas.

Water is an important limiting factor to crop production in South Africa. The country receives an average rainfall of about 500 mm per year (DWAF, 2002). The challenge is also that the rainfall is unevenly distributed both temporally and spatially (DWAF, 2002). The problem of water scarcity in South Africa has emphasized the need to develop and identify drought tolerant crops. The challenge for farmers and researchers is to find ways to increase the crop output per unit of water and overall crop production in order to meet the requirements of a growing population. It is within this context that previously neglected underutilised crops such as bambara fit. In South Africa, the species has never been allocated a large-scale research programme.

Bambara groundnut has become popular in Africa because of its ability to tolerate drought and produce reasonable yields under poor soil fertility conditions (Onwubiko *et al*., 2011; Baryeh, 2001). The ability of bambara to tolerate water stress has been attributed to osmotic adjustment and effective stomatal regulation (Collinson *et al*., 1996; 1997; Onwubiko *et al*., 2011).

Bambara groundnut is also an ideal crop for small-scale farmers because it can produce reasonable yields with low inputs. However, yields are low because production and improvement of bambara groundnut has been neglected by researchers (Onwubiko *et al*., 2011). Although the crop is important for small scale farmers due to its considerable commercial potential, there is little information describing production levels (Ntundu *et al*., 2004), agronomy, physiology and water-use of bambara groundnuts in South Africa. Recent studies have made progress in describing the agronomy of bambara groundnut (Mabhaudhi *et al*., 2011; Sinefu, 2011); however, more still needs to be done to elucidate the underlying physiology responsible for its drought tolerance.

1.2 History and origin of bambara groundnut

Bambara groundnut (*Vigna subterranea* L. Verdc) is an indigenous African legume. Its centre of origin is believed to be in Bambara in the Timbuctoo region of Niger (Masindeni, 2006). It is cultivated from Senegal to Kenya and from the Sahara to South Africa and Madagascar. The name Bambara was taken from the district of its origin. Bambara belongs to the *Idoacene* family whose members also include, but are not limited to, soybean (*Glycine max*), cowpea (*Vignau nguiculata*), dry beans (*Phaseolus vulgaris*) and mungbean (*Vigna radiata*) (Goli, 1997).

There is still a debate between the Bolobedu and Venda people about who brought bambara to South Africa (Masindeni, 2006). Vendas have a strong belief that it is them, because of the name Ndluhu-mvenda' for bambara groundnut. Farmers in the Mpumalanga province believe that bambara groundnut was possibly first introduced during dry periods when popular crops such as maize could not produce better yields (Alshareef, 2010). In

South Africa, it is cultivated in the Limpopo, KwaZulu-Natal, Mpumalanga, Eastern Cape and Northwest provinces by few smallholder farmers (Masindeni, 2006). Rural women mainly grow bambara groundnut in their home gardens for consumption or as a cash crop for their own economic benefit (Masindeni, 2006).

1.2.1 Botany

Bambara groundnut is an annual legume with a solid well-developed tap root. It consists of short (up to 20 cm long) lateral stems on which the leaves are borne. Bambara groundnut leaves are trifoliate about 5 cm long, and the petiole up to 15 cm long, stiff and grooved. The base is green or purple in colour. The flowers are typically papilionaceous and are borne in a raceme on long, hairy peduncles, which arise from the nodes on the stem. Bambara groundnuts consist of two growth patterns, namely, branching and spreading types. The branching types are usually self-pollinated while the spreading types are usually cross-pollinated by ants (Swanevelder, 1998). Yield is in the form of pods that are borne underground; however, some varieties are surface bearers (Swanevelder, 1998). The pods are about 1.5 cm long, while the seeds are round and/or oval shaped with wrinkled seeds. Mature leaves are light green or in some cases purple, whereas immature pods are light green. Usually after fertilisation has occurred the stem elongates and the sepals become large, thus fruits develop above or below the soil surface. Bambara groundnut seeds are roundish in shape with the size of about 1.5cm in diameter. They are smooth and soft when immature, but as they become dry they become hard. Swanevelder (1998) described the colours of Bambara groundnut seeds as light brown (A), red (B), and brown (C) and black (D) (Figure 1.1).

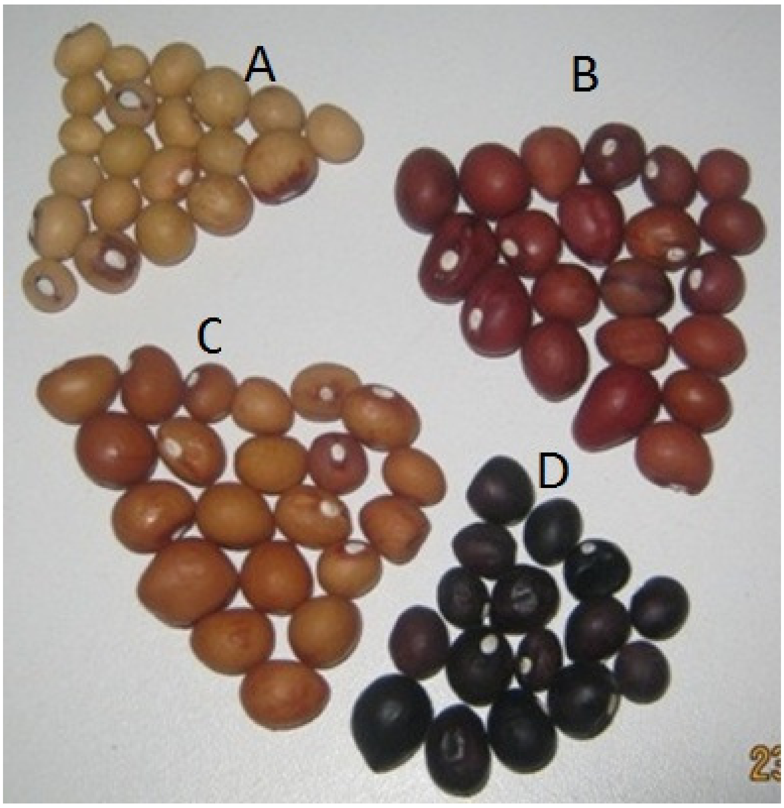

Figure 1.1: Different seed colours occurring in local bambara groundnuts landraces; A – Light brown, B - Red , C- Brown, and D –Black (Swanevelder, 1998).

1.2.2 Food security

Given reports that bambara groundnut is drought tolerant (Linneman and Azam-Ali, 1993), has high nutritional value (Swanevelder, 1998), it has the potential to provide food security in the dry and marginal areas of Africa. The crop is rich in protein (16-25%), carbohydrates (42-60%), fat (5-6%) and other essential nutrients (Linnemann, 1988; Akani *et al*., 2000; Atiku *et al.,* 2004). Its neglect has been suggested to may have been due to its low lipid concentration (Massawe *et al.,* 2005).

Bambara groundnut is mainly grown for human consumption. The seed is considered as a complete food because of its high nutritional value. The seeds can be consumed in different ways. They can be grilled, boiled or eaten fresh or they can be ground into flour (Masindeni, 2006). Immature seeds are consumed fresh or grilled. They can also be boiled, either shelled or unshelled, and eaten as a meal or mixed with immature groundnuts or green maize. In Botswana for example, they are boiled with salt and eaten as a snack. In restaurants in Angola and Mozambique, boiled salted seeds are often served as appetisers. Commercial canning of bambara groundnut in gravy is a successful industry in Ghana. Bambara groundnut can also be grown for animal consumption. Initially, bambara groundnut was used for animal feed where seeds were fed to chicks (Masindeni, 2006). The leaves are suitable for animal feed, because they are rich in nitrogen and phosphorus (Masindeni, 2006).

The study conducted by (Heller and Mushonga, 1997) also showed that bambara groundnuts can be consumed as milk. A trial of bambara groundnut milk was carried out which compared its flavour and composition with those of milk prepared from cowpea, pigeon pea and soybean. Bambara groundnut was ranked first, and while all milks were found to be acceptable, the lighter colour of the bambara groundnut milk was preferred. In South Africa, Swanevelder (1998) reported that 'Sekome' (Sesotho), 'tihove' (Shangaan) or 'tshidzimba' (Venda) is prepared by adding 'njugo' beans and peanuts, or just one of the two, to maize or millet-meal and boiling the mixture until it forms stiff dough. This is salted and pounded into a ball, and will often keep fresh for several days.

Additionally, bambara groundnut can also contribute towards food security indirectly. Bambara groundnut is a legume, which has a symbiotic relationship with bacteria (rhizobia) that form root nodules. Rhizobia can make use of free nitrogen from the air, incorporating it in the plant root tissue (Masindeni, 2006), hence increasing amount of nitrogen in the soil which in turn may be beneficial to subsequent crops. Consequently, farmers may end up applying less fertiliser thus saving on much needed and scarce resources.

1.2.3 Genetic diversity

Bambara groundnut germplasm is abundant in Sub-Saharan Africa, as the crop is grown in every tropical region of the continent. So far, wild relatives of cultivated bambara groundnut have only been found in north-eastern Nigeria and northern Cameroon, where it is believed to have originated (Heller and Mushonga, 1997). There are 327 known accessions of bambara groundnut in South Africa, with a wide range of differences in seed coat colour, seed size, pigmentation around the eye, pod shape, growth habit, yield, shelling percentage and time to maturity (Masindeni, 2006). It is important to know about genetic variation of bambara groundnuts accessions for their efficient use in breeding programs and for studies on crop evolution. Bambara groundnut shows a considerable amount of variability for various morphological, physiological and agronomic traits (Ntundu *et al*., 2004).

Despite the importance of bambara groundnut as a food source with potential to alleviate hunger, limited breeding efforts have been made to improve this crop (Ntundu *et al*., 2004). Little information is available about the extent of genetic diversity among bambara groundnut landraces, for long term conservation and improvement (Ntundu *et al*., 2004). There were few studies reported in the literature on assessment of bambara groundnut diversity using different molecular techniques. They were mainly on population structure and genetic diversity among farmers' cultivars using isozymes and Random Amplified Polymorphic DNA (RAPD) markers. Amplified fragment length polymorphism (AFLP) is one of the techniques that have been previously used to assess levels of genetic diversity among bambara groundnut accessions from diverse geographic locations in Africa (Massawe *et al*., 2005; Ntundu *et al*., 2004). Ntundu *et al*. (2004) used AFLP to assess genetic diversity among 100 bambara groundnut landraces from diverse geographical locations in Tanzania. Their results showed that bambara landraces from Tanzania consisted of much variability and that AFLP could be used to effectively study genetic diversity in bambara groundnut landraces.

Massawe *et al.* (2005) found that there was variation between and within landraces, although the variation within a landrace was lower than that between them. With regards to variations within landraces, they stated that each of the 263 landraces assessed in the study consisted of three to eight distinct genotypes. They concluded that observed genetic relationships among bambara groundnut landraces from different regions of Africa were more related to place of collection rather than phenotypic similarities. Similarly, Ntundu *et* *al.* (2004) also reported considerable genetic diversity among 25 African bambara groundnut accessions from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, using Random Amplified Polymorphic DNA (RAPD) markers, and demonstrated that there existed two main groups of accessions, based mainly along the lines of their geographic origin.

Currently, there are no cultivars of bambara groundnut available on the market, which means that cultivation still relies on landraces, which have huge variation often resulting in low yields. Such variability has been reported to be a possible reason for low farmer uptake (Ntundu *et al.*, 2004). Since there exists much variation in bambara landraces, it is important to assess bambara landraces from different geographical locations in South Africa and identify landraces that may have drought tolerance. This would allow plant breeders to easily select and develop drought tolerant cultivars.

1.3 Drought

Drought stress has a tremendous negative effect on agriculture (Sazares *et al.*, 2011). Drought in plants occurs when there is insufficient soil moisture to meet the needs of a particular crop at a particular time. This may be as a result of meteorological drought, uneven rainfall distribution, mid-season drought, inefficient irrigation and/or poor crop husbandry (Mabhaudhi, 2009). With the potential risk of increased frequency and intensity of drought associated with climate change (Hassan, 2006); drought tolerant crops are likely to become even more important in African agriculture (Berchie *et al.*, 2012). Neglected underutilised crops have been reported to have possibly evolved to become drought tolerant due to years of cultivation under often severe conditions. Bambara groundnut is one such crop that has been reported to be drought tolerant (Harris and Azam-Ali, 1993).). However, due to the noted variability that exists between and within bambara landraces (Massawe *et al.*, 2005), it is important to assess local germplasm for drought tolerance. One key step to achieving this is by understanding the mechanisms and plant adaptations to drought.

1.4 Drought tolerance mechanisms

As a way of adaptation, bambara groundnuts have developed mechanisms to withstand drought. There are three strategies that plants use to adapt to drought or to tolerate drought, namely escape, avoidance and tolerance.

1.4.1 Escape

Drought escape usually occurs in short season, dry land crops that complete their life cycle during the rainy season and reach maturity before terminal drought commences. This allows plants to reproduce before the environment becomes dry (Farooq *et al*., 2009). Flowering time becomes an important trait related to drought adaptation where a short life cycle can lead to drought escape (Araus *et al*., 2002). Drought escape takes place when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates. Farooq *et al*. (2009) concluded that developing short duration varieties was helpful as it minimises yield losses hence early maturity helps the crop to avoid stress.

1.4.2 Avoidance

Drought avoidance occurs when plants are able to avoid water deficits by reducing leaf transpiration while increasing root water uptake. It is defined as the plant capacity to sustain high plant water status or cellular hydration under drought (Blum, 2005). In this mechanism, a plant avoids water stress through enhanced capture of soil moisture as well as by limiting crop water losses while retaining cellular hydration. Drought avoidance strategy can be achieved by restricting leaf area expansion, lowering stomatal conductance, and increasing root depth and density or enhancing root hydraulic conductivity (Farooq *et al*., 2009). Previous studies have shown that bambara avoids drought by maintaining leaf turgor pressure through a combination of osmotic adjustment, reduction in leaf area and effective stomatal regulation (Berchie *et al*., 2012). Root characteristics' such as biomass, length, depth and density are the main drought avoidance traits.

1.4.3 Tolerance

Drought tolerance is frequently considered as a survival strategy under severe water stress and has been suggested to be of little relevance to crop production (Gholamin and Khayatnezhad, 2011). Blum (2005) defined drought tolerance as a plant's relative capacity to sustain normal function in a dehydrated state. In essence, drought tolerance, as drought adaptation mechanism is rare in plants, mostly occurring in seed embryo and lost after germination (Blum, 2005). As a way of adaptation, bambara groundnuts have developed the mechanisms to withstand drought. Previous studies have shown that the crop maintain leaf turgor pressure through a combination of osmotic adjustment, reduction in leaf area and effective stomatal regulation.

1.5 Crop responses to drought stress

Bambara groundnut is sensitive to severe water stress. However, at moderate stress condition, the plant can thrive (Marino *et al*., 2007). Although bambara groundnuts have a reputation of being more drought tolerant than other legume crops, the mechanisms underlying adaptation to drought are still poorly understood (Ntundu *et al.,* 2006),). Strategies of crop adaptation to drought stress have been discussed in relation to phenological, morphological and physiological aspects (Gholamin and Khayatnezhad, 2011). Most literature on bambara groundnut describes the crop's responses to water stress based on phenological and morphological aspects; there were few reports describing the crop's physiological responses to water stress.

1.5.1 Physiological responses to water stress

Water stress affects several plant processes, from the individual cell to the whole canopy, such as leaf expansion and leaf production rate. Plants have developed several adaptations, morphological and physiological, in order to cope with water stress. Plant responses to water stress are multi-faceted and consist of morphological and physiological adaptations. At the physiological level, these alterations are related to photosynthesis and include stomatal regulation and regulation of pigments (chlorophylls and carotenoids). At the molecular level, such adaptations include alterations in the levels of bioactive compounds such as proline, proteins and antioxidants.

1.5.1.1 Stomatal conductance

Stomatal conductance refers to the rate of passage of carbon dioxide entering or water vapour exiting through the stomata of the leaf (Whitehead *et al*., 2004; Whitehead, 1997). Drought tolerance in bambara is a result of osmotic adjustment and reduced water loss through stomatal closure (Collinson *et al*., 1997). Stomatal closure can result in decreased diffusion and fixation of CO_2 hence reduction in photosynthesis (Vurayai *et al*., 2011). Stomatal closure is widely believed to be the major limitation to photosynthesis, and consequently crop growth under water stress (Farquhar & Sharkey., 1987; Whitehead *et al*., 2004; Hubbard *et al*., 2001).

In a study done by Vurayai *et al*. (2011), it was shown that plants that were stressed during the pod filling stage had the greatest reduction in stomatal conductance of 90% while plants stressed during the vegetative stage had the least reduction in stomatal conductance compared with non-stressed plants. This also shows that the reproductive stage in bambara is more sensitive to water stress than the vegetative stages. Decreased stomatal conductance results in lower net carbon dioxide assimilation rate, lower intercellular carbon dioxide and lower chloroplastic carbon dioxide tension. Reduced intracellular carbon dioxide will inevitably reduce photosynthetic efficiency and dry matter production; this may have a negative impact on plant growth and yield (Vurayai *et al*., 2011). The study that was done by Collinson *et al*. (1997) showed that in potted experiments stomatal conductance was significantly decreased under conditions of water-deficit stress, whereas field experiments have shown cotton stomatal conductance to be adaptable to water stress. Quarie and Jones (1979) made similar observation on wheat.

1.5.1.2 Chlorophyll content

Pigments are very crucial for light harvesting and also act as reducing powers during photosynthesis (Jaleel *et al*., 2009). Chlorophylls (a and b) are primarily responsible for harvesting light which is then used as energy in photosynthesis (Ashraf *et al*., 2007). Chlorophylls are the most important pigments for photosynthesis. The amount of chlorophyll per unit leaf area in a plant is an important indicator of the overall condition of the plant. Healthy plants capable of maximum growth are generally expected to have larger amounts of chlorophyll than unhealthy ones. Therefore, determination of the chlorophyll content of a leaf can be used to detect and study plant water status (Wu *et al*., 2008; Anjum *et al*., 2011).

In a study by Arunyanark *et al*. (2008), drought stress was shown to greatly reduce leaf chlorophyll content, resulting in reduced photosynthetic activity as well as strong reduction of membrane-bound chloroplast antioxidants. This was indicative of oxidative stress in chloroplasts could have damaged photosynthetic apparatus. Hence, chlorophyll loss is always associated with reduction in photosynthesis (Arunyanark *et al*., 2008: Keyran, 2010). In a separate study conducted by Pirzad *et al*. (2010), it was shown that total chlorophyll content was reduced by 55% in response to drought.

Chlorophyll content in leaves has been shown to be closely linked with photosynthetic capacity in many crop plants. Drought is known to affect chlorophyll content in many crops including, wheat (Talebi, 2011), grass *Eragrostis curvula*, cattail *Typhalati folia,* turf grasses and maize (Khayatnezhad *et al*., 2011; Gholamin and Khayatnezhad, 2011) thereby inhibiting photosynthetic capacity (Arunyanark *et al*., 2008). Talebi (2011) found that drought tolerance genotypes have high chlorophyll content and low canopy temperature compared with other genotypes under different moisture conditions. The genotypes with high yield also had high chlorophyll content in well-watered and drought-stressed conditions. However, there is limited literature describing the relationship between leaf chlorophyll status and drought tolerance in bambara groundnuts. Vurayai *et al*. (2011) showed that water stress did not significantly reduce leaf chlorophyll content in bambara groundnut plants. However, chlorophyll content was observed to decrease in response to stress during flowering. Ashraf *et al.,* (2007) did a study on maize and found that diverse set of maize cultivars, exhibited a considerable variation for water stress tolerance at early growth stages, on the basis of growth under water stress conditions cultivars Sahiwal-2002 and EV-1098 proved to be more tolerant to drought, while Pak-Agfoee the most sensitive being the least biomass producer. This was consistent with previously observed decrease in stomatal conductance during the reproductive stage. This further suggests that bambara is sensitive to water stress during the reproductive stage.

1.5.1.3 Accumulation of metabolites

Drought tolerance has previously been associated with a plant's ability to maintain high water content in the tissue, thus avoiding desiccation. Maintenance of high tissue water content can be achieved, in part, by accumulation of compatible solutes such as proline and soluble sugars. During environmental stress conditions, plants show adaptations at the physiological level. Such adaptations go together with changes in the expression of different genes (Caballero *et al*., 1987; Raymond *et al*., 2002; Shtereva *et al*., 2008).

Proline is a well-known metabolite that is accumulated in plants in response to water stress (Chaves *et al*., 2003). It is defined as an amino acid with exceptional conformational rigidity, and is essential for primary metabolism (Szabados and Savoure, 2009). Proline has a clear role as an osmoticum. In particular, because of its zwitterionic, high hydrophilic characteristics, it acts as a compatible solute (Kapitan *et al*., 2006), i.e. one that can accumulate to high concentrations in the cell cytoplasm without interfering with cellular structure (Mohammadkhani and Heidari, 2008). Several roles have been ascribed to proline such as stabilization of macromolecules, a sink for excess reluctance and a store of carbon and nitrogen for use after relief of water deficit (Raymond and Smirnoff, 2002). However, there is still lack of clarity on whether proline accumulation is a sign of adaptation or rather a symptom of stress. What is clear is the fact that its accumulation is a widespread plant response to drought stress.

Vurayai *et al*. (2011) showed that water stress increased proline concentration in bambara groundnut plants, compared with non-stressed plants. They found that, depending on the stage of development, water stressed plants produced about four times the amount of proline compared with non-stressed plants. Sinefu *et al*. (2011) evaluated proline accumulation in bambara groundnut seeds subjected to desiccation and found that stress resulted in proline accumulation in the seed. Proline accumulation has also been observed in maize landrace seedlings (Mabhaudhi, 2009) and cowpea. Proline is reported to result in drought tolerance and changes in proline content have been correlated with capacity to tolerate and adapt to arid environments (Vurayai *et al.*, 2011; Anjum *et al.*, 2011).The study that was done on wheat by Tatar and Gevrek (2008) showed that wheat dry matter production and relative water content decreased as proline content was increasing under water stress. Keyran (2010) reported that proline content in resistant wheat cultivars was more than in sensitive cultivar under the drought and salinity stress. Furthermore Vendruscolo *et al.* (2007) observed that when wheat was subjected to water stress proline increased. Veeranjaneyulu and Kumari (1989) observed proline accumulation in response to stress; they found that proline accumulation was greater in the roots than leaves.

1.5.1.4 Antioxidants

In agriculture it is important for the crop to develop mechanisms to survive drought stress. Therefore protective responses at the leaf must be triggered to prevent the photosynthetic machinery from being irreversibly damaged. When plants are subjected to stress, they tend to accumulate reactive oxygen species (ROS) (Anjum *et al.*, 2011; Cazares *et al.*, 2011) such as superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radicals, which may lead to photo-oxidation if the plant is not efficient in scavenging these molecules (Van staden and Jager, 1998; *Loka et al*, 1994). This is due to an imbalance in the redox reactions resulting in oxidative damage. To counter the negative effect of ROS accumulation, plants have developed systems that actively scavenge (Van staden and Jager, 1998) for ROS – this system includes antioxidants. The antioxidant defence mechanism provides a strategy to enhance drought tolerance by scavenging reactive oxygen species and preventing metabolic impairment caused by enhanced electrolyte leakage in chloroplast and mitochondria (Kumar *et al.*, 2011:Vanstaden and Jager, 1998;Cazares *et al.*, 2011). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage.

Reactive oxygen species produced during water stress can damage many cellular components including lipids, proteins, carbohydrates and nucleic acids (*Loka et al.*, 1994; Anjum *et al.*, 2011). Membrane lipid peroxidation and protein oxidation constitute the simplest criteria of assessing the extent of oxidative damage in plant tissue. Efficient antioxidant systems in the plant can minimize the level of oxidative stress and protect the tissue. Such antioxidant systems can be enzymatic or non-enzymatic. The major antioxidant species in the plants are superoxide dismutase (SOD) (Loka *et al*., 1994). Superoxide dismutase (SOD) is a major scavenger of O_2^-. Catalases and peroxidases are major enzymatic cellular scavengers of H_2O_2. Catalase, which is present in peroxisomes, dismutates H_2O_2 into water and molecular O_2.

1.6 Seed quality

Seed is a primary input for crop production and it is the embodiment of past harvests as well as the promise of future ones (Martinson, 2009). This is particularly true of landraces where farmers keep seed from previous harvests for planting in the next season (Limbani, 2006). In addition, drought, through the interaction of a dry seedbed and seed quality can affect crop production (Mabhaudhi and Modi, 2010). Under such conditions, of an unfavourable seedbed, seed quality becomes critical to successful crop production. While seed quality of released varieties of exotic crops has been established, lack of qualitative information describing seed quality of landraces remains a hindrance to their successful utilisation by communities.

Seed quality is defined in terms of viability and vigour (Coolbear and Hill, 1988). Seed viability and vigour are the set of characteristics that determine the activity and behaviour of commercially acceptable seed lots with regards to germination under different environmental conditions (Milosevic *et al*., 2010; van der Burg, 2004). Seed viability is defined as the ability or capacity of seed to germinate under standard (optimum) conditions (Clerkx, 2004). Seed vigour refers to those properties in a given seed lot that will allow it to germinate and produce normal seedlings as well as its expected field emergence and uniformity (Hampton, 1995). Poor seed vigour results in poor seedling establishment as a result of weak seedlings that are susceptible to environmental stresses. Whereas, a high level of vigour in seeds can be expected to provide for early and uniform stands which give the growing seedlings the competitive advantage against various environmental stresses. Together, the terms viability and vigour may be used to describe the physiological characteristics of seeds that control its ability to germinate rapidly in the soil and to tolerate various, mostly negative environmental factors.

Water stress acts by decreasing the percentage and rate of germination (Delachiave and Pinho, 2003). In order for seed to geminate, it is important that they reach an adequate level of hydration and that depends on the chemical composition and the permeability of the tegument (Mohammadkhani and Heidari, 2008). Enough moisture will permit the reactivation of metabolic processes. According to Rauf *et al*. (2007), availability of soil water has a major effect on germination and emergence; they reported decreased germination and seedling growth in response to water deficit in various crop species and cultivars. Zulu (1989) reported that bambara groundnuts seeds were more sensitive to water deficit than groundnuts (*Arachis hypogea*) due to their hard seed coat. Sinefu (2011) concurred with these findings and went on to suggest a possible relationship between seed coat thickness, viability and vigour and seed coat colour.

Seed coat colour may be a useful indicator of seed quality (Anuradha *et al*., 2009). This may be especially true for landraces which typically exhibit large variations in seed coat colour and of which little is known of their seed quality. Bambara groundnut seeds exhibit high phenotypic diversity and varieties selected for cultivation are dependent on seed coat colour. Seed coat colour is attributed to the presence and amounts of phenolic derivatives (Pillay, 2003). Darker seeds are known to have high phenolic derivatives; however, this also implies poor digestibility as well as low nutritive protein. On the other hand, light coloured seeds, containing low phenolic derivatives, are usually desirable because of their reputation of being highly palatable and easy to digest. High phenolic content in darker coloured seeds may be the reason for the association between dark seed colour and seed quality as well as disease resistance (Abu and Buah, 2011). The study that was done on cowpea (known to be closely related to bambara) showed increase disease resistance on dark coloured seeds compare to white (Martinson, 2009).

There are various contradicting reports in literature describing effect of seed coat colour on seed quality. Anuradha *et al*. (2009) showed a significant variation due to the seed coat colour in seeds of bambara groundnut. Germination potential was high in light-brown seeds followed by brown seeds. Vigour parameters like seedling length, dry matter production and vigour index values also showed a similar trend as that of the germination in which the light brown colour seeds were superior to other colour grades. Contrary to reports by Anuradha *et al*. (2009), there have been studies suggesting that darker coloured seeds may have better seed quality (viability and vigour) compared with lighter coloured seeds in several landraces – maize (Mabhaudhi and Modi, 2010, 2011); wild mustard (Mbatha and Modi, 2010) and wild water melon (Zulu and Modi, 2010). More recently, Sinefu (2011) also showed evidence of an association between darker coloured landraces of bambara groundnut and seed quality. However, there is still scarce information describing whether these observed relationship hold true for other landraces originating from diverse geographical locations. Assuming that there exists much variation in landraces from different locations (Massawe *et al*., 2005), it is necessary to assess if seed coat colour can be used as an index of seed quality for diverse landraces of bambara.

1.7 Conclusion

South Africa is a water scarce country with uneven rainfall distribution. As such, water is the main limiting factor to crop production. The threat caused by predicted climate change, with regards to increased frequency and severity of drought, may only exacerbate the situation. The review of literature also showed that the current major staple crops will not be able to feed the growing population under these conditions of climate change. In addition, there is also the added threat of malnutrition. Therefore, there is need to identify “new crops” that are drought tolerant as well as nutritious – bambara groundnut is one such crop. The crop has been reported to be highly nutritious in addition to being drought tolerant. As such, bambara groundnut is a possible future crop with potential for improving human diets and providing food security in semi-arid, arid and marginal areas of production. However, there has been limited research describing drought tolerance of bambara landraces, especially locally. The fact that the crop is cultivated from landraces with no improved cultivars sums it up. The genetic diversity that exists between and within landraces necessitates a study that would evaluate different bambara provenances for drought tolerance.

The purpose of crop production is to increase and stabilise yield in order to meet man's various needs. Consequently, research on ways to improve crop production has been increasing, especially now that global food crisis is threatening food security. About 1.2 million people in the world do not have enough food to meet their daily requirements and about 2 million people in the world do not receive enough nutrients from their diets. There is a threatening challenge to successful crop production of crop failure as a result of predicted climate change. Climate change is forecast to increase the frequency and severity of droughts with an accompanying increase in average temperatures. Therefore crops like bambara groundnut that are known to be possibly drought tolerant, as well as being adapted to high temperatures are suitable.

However, previous research on bambara groundnut has been limited, mainly because its commercial value is restricted. Recently, bambara groundnut has gained a renewed interest by researchers as a food crop. It is widely regarded as drought tolerant crop and can grow where groundnuts cannot grow. The crop can produce high yield levels with low input and is an ideal crop for most farmers. Since it falls under legumes it is capable of increasing the level of soil nitrogen, thus giving acceptable grain yields where other crops usually fail. Therefore it would be of use in low-input agricultural production.

Bambara groundnut is still cultivated using landraces, with no cultivars available. Previous work on several landraces has indicated that seed colour may be a useful initial criterion for selection for drought tolerance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu, 2011). However, it has not yet been established if this suggestion holds true for other landraces originating from different geo-climatic locations. Therefore, the aim of this study was to evaluate physiological responses of different bambara landraces, obtained from different locations in South Africa and characterised according to seed colour, to water stress under controlled and field conditions. It is hypothesised that seed coat colour is associated with (i) seed performance in terms of germination, vigour and stand establishment and (ii) there is a significant effect of seed coat colour and provenance on water stress tolerance.

The objectives of this study were to:

(i) determine the effect of seed coat colour and provenance on seed performance in terms of germination capacity and vigour,

(ii) determine the effect of selected seed coat colours and provenances on seedling establishment,

(iii) determine the effect of selected provenances and seed coat colour on water stress tolerance under controlled – environment and field conditions.

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Chapter 2

Seed quality of bambara groundnut (*Vigna subterranea* L. Verdc) landraces based on provenances and seed coat colour

2.1 Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc) is an indigenous African leguminous crop grown primarily for its seeds. It is increasingly becoming popular as a food source in rural areas across the African continent (Abu and Buah, 2011). The crop has potential to provide food security in dry areas of Africa and has been identified as a drought tolerant crop that can produce reasonable yields where other crops, such as groundnut, fail (Harris and Azam-Ali, 1993). It can also be used in various formulations and can play an important role in protein supply to rural populations, hence alleviating malnutrition (Nti and Plahar, 1995; Massawe *et al.*, 2002). As such, bambara groundnut has a potential to provide food security in dry areas of Africa. With the potential risk of drought associated with climate change, drought tolerance is likely to become even more important in African agriculture. However, despite it being a drought tolerant crop, bambara groundnut remains an underutilised crop owing to limited research that has been done on the crop.

In South Africa, and elsewhere, the crop is still cultivated from landraces which often show great variability (Ntundu *et al.*, 2004); there are currently no improved varieties available on the market. Yields, often under low-input farming systems, are low and unpredictable. Low yields have also been associated with poor seed quality in terms of germination and emergence, which often lead to poor crop establishment (Linnemann and Azam-Ali, 1993; Sinefu, 2011). Therefore high-quality seed becomes an important prerequisite for achieving high crop yields. Only high quality seed will produce strong plants which are resistant to disease and adverse conditions (FAO, 1981).

McDonald and Copeland (1997) defined seed quality as the overall value (suitability) of a seed lot for its intended use, in which case it is defined in terms of physiological quality (viability, germination and vigour). Seed quality is affected by several factors and seed germination and vigour have an important role in determination of seed quality (Perry, 1980). Seed viability refers to the potential germination and subsequent production of a seedling of the stated cultivar (Basu, 1995). Although the viability of an individual seed can be determined, it is more usual to refer to the germination potential of a seed lot. Seed vigour, as defined by the International Seed Testing Association (ISTA) (1987), is the sum total of those properties of the seed that determine the level of activity and performance of the seed during germination and seedling emergence. The Association of Official Seed Analysts (AOSA) (1983) defined seed vigour as comprising the properties that determine a seedlot's potential for rapid and uniform emergence as well as development of normal seedlings under a wide range of field conditions. Seed vigour is an important factor in that it affects seedling establishment and crop growth and ultimately yield. Seed vigour and germination ability directly affect yield through reduced crop stand owing to poor seedling emergence (Tekrony and Egli, 1991). Seed lots with high vigour show high final emergence compared to seed lots with low vigour (Johnson and Wax, 1981). High levels of vigour in seeds can be expected to provide for early and uniform stands which give the growing seedlings the competitive advantage against various environmental stresses.

According to Anuradha *et al.* (2009), seed coat colour may be a useful indicator of seed quality. Seed colour has been reported to play a role in seed dormancy and germination, as seeds attain their specific colour at physiological maturity (Powell, 1989; Ochuodho, 2005). Seed coat colour and structure have been proven to have an influence on germination (Debeaujon and Koornneef, 2000; Debeaujon *et al*., 2000). Dharmalingam and Basu (1993) in greengram (*Vigna radiata*) indicated that off-coloured seeds had poor quality. Kozlowski (1972) reported low vigour in off-coloured seeds of alfalfa (*Medicago sativa*). Chachalis and Smith (2000), working on dark soybean cultivars, observed a greater rate of imbibitions and fast germination which was correlated with fast germination, indicating high seed quality.

Seed quality in bambara has previously been associated with seed coat thickness. Zulu (1989) observed that seed germination in bambara groundnut was more sensitive to water stress. This was due to the hard seed coat nature of bambara seed that restricts the uptake of water. Sinefu (2011) also reported a thick seed coat in red coloured seed of bambara while light coloured seeds were observed to have a thin seed coat. However, contrary to Zulu's (1989) suggestion, Sinefu (2011) found that the red coloured seeds with the thick seed coat had better emergence. A thin seed coat was shown to result in high electrolyte leakage leading to loss of quality. The differences observed by the two authors may be because of the huge variability that exists between and within landraces (Ntundu *et al*., 2004). Therefore, there is still a need to characterise the various landraces originating from different locations with regards to seed quality. The objective of this study was to determine the relationship between seed coat colour and seed quality in terms of seed performance during germination.

2.2 Materials and Methods

2.2.1 Plant material

Bambara seeds were sourced from subsistence farmers of Tugela Ferry (28˚45' S; 30˚27' E) (KwaZulu-Natal), Deepdale (30°33' S; 29°54' E) (KwaZulu-Natal) and Zimbabwe (coordinates unknown) during February 2012. Jozini seeds were obtained from experiments conducted during 2011/12 planting season (Mabhaudhi, 2012) where they had been subjected to different treatments (30, 60 and 100% of crop water requirement as well as irrigated and rainfed conditions). With regards to seed collected from these experiments, the objective was to evaluate the effect of water stress on the maternal plant on seed quality. Another batch of seed from Jozini (JMULT) comprised of seed from multiplication trials where conditions during seed production were optimum. Seed obtained from the other provenances represented material that had been produced under rainfed conditions during the previous growing season (2011/12).

Table 2.1: Background information of the geographical locations (provenances) from South Africa from which bambara landraces were obtained.

Source	Location	Provenance	Climate	Soil type	Average Temperature (˚C)	Total Rainfall (mm)
Pretoria	25º60´S; 28º35´E; 1168 masl	Jozini (J)	Semi-arid	Sandy-loam	21	466
Tugela Ferry	28˚45'S; 30˚27'E; 543 masl	Tugela Ferry (TF)	Semi-arid	Loam	19.9	191.9
Deepdale	30°33'S; 29°54'E; 1370 masl	Deepdale (DD)	Semi-arid	Clay	16.5	455.1

*masl = meters above sea level

2.2.1.1 Seed characterisation

The seeds were sorted into different colours such as light brown, brown and red seeds based on visual observation (Figure 2.1). Some studies have reported that seed quality is determined by seed colour. Seeds from the different provenances were initially characterised according to seed coat colour. Based on this initial characterisation, three distinct colours (red, brown and light-brown) were observed to be dominant in all provenances and selected for this study. For the Zimbabwe provenance, this also included black coloured seeds. For each provenance and seed colour, the average 100 grain mass was also recorded.

Figure 2.1: Different colours of bambara groundnuts before they were sorted and after they were sorted (A = Black, B = Light brown, C = Red, D = Brown).

2.2.2 Standard germination test (SG)

The standard germination test was used to evaluate germination capacity of bambara groundnut seed using the paper towel method (ISTA, 1999) in an experiment that was replicated three times. Five seeds were used per replicate. The rolled paper towels were put in sealed zip-lock bags to avoid losing moisture and incubated at 20/30°C (16 hr day/ 8hr night) for eight days. Daily measurements of germination were taken by counting the number of seeds that had geminated; the criterion for germination was at least 2 mm radicle protrusion. Final germination was determined on day eight (8) based on observations of normal seedlings. Following this, seedling root and shoot length using a ruler in (mm), fresh mass and dry mass using a scale balance in (g) as well as root: shoot ratio were determined.

Germination speed, as defined by the germination velocity index (GVI) was calculated according to the formula by Maguire (1969);

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

where:

GVI = germination velocity index,

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

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

The mean germination time was also calculated as described 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 Data analysis

Data were analysed using analysis of variance (ANOVA) from GenStat® (VSN International, UK). The means were separated using Tukey's test in GenStat® at the 5% level of significance (Appendix 2).

2.3 Results

2.3.1 Seed germination trend

There were highly significant differences (P<0.001) between seed colours in each provenance with respect to the number of seeds geminated (Figure 2.2, 2.3, 2.4, 2.5, 2.6 and 2.7). There were also highly significant differences (P<0.001) with respect to germination over time (Figure 2.2, 2.3, 2.4, 2.5, 2.6 and 2.7). On average, for all seed colours in each provenance, bambara seeds were slow to germinate in the first 4 days (Figure 2.2, 2.3, 2.4, 2.5, 2.6 and 2.7). However, once germinated, most seed colours in each provenance were able to attain 100% germination by the seventh day. Based on means for the seed colours for each provenance, the Jozini provenance was shown to perform best, followed by Zimbabwe, Tugela Ferry and Deepdale, respectively. Within the provenance Jozini, on the third day J30LB selection had the highest germination (69.52%) (Figure 2.2). For the Deepdale provenance, the brown coloured selection had the highest germination percentage (62.86%), followed by the red (60.00%) and light-brown selections (59.05%). The Tugela Ferry provenance (Figure 2.6) also showed that, over time, the brown coloured selection germinated better than light-brown and red coloured selections, respectively. On average, results showed that for all provenances, the brown coloured selections had higher germination compared to red and light-coloured selections, respectively.

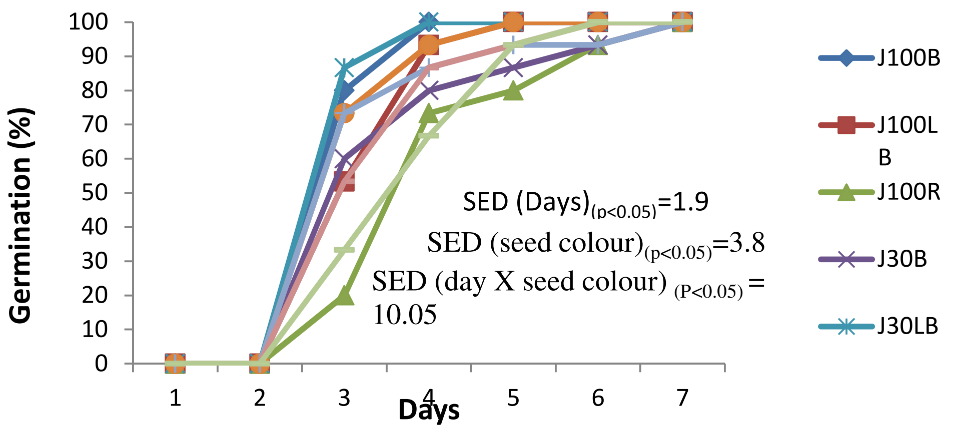

Figure 2.2: Daily germination of different Bambara seed colours (red, brown and light brown) in the Jozini provenance (J30, J60, J100). NB: J100 = Jozini from 100% treatment, J60 = Jozini from 60% treatment, J30 = Jozini from 30% treatment, B = brown colour, LB = light-brown, R = red.

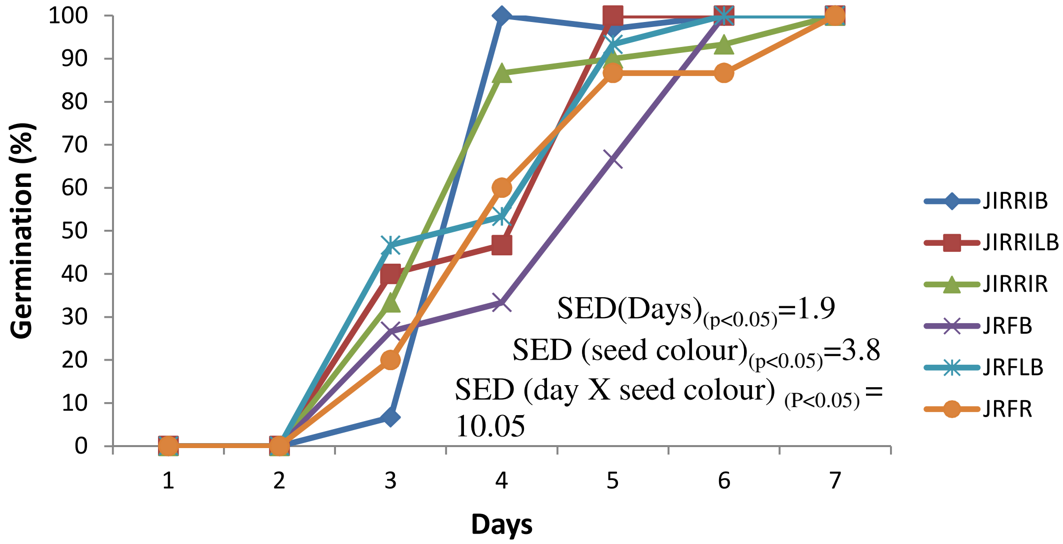

Figure 2.3: Daily germination of different Bambara seed colours (red, brown and light brown) in the Jozini provenance (Irrigated and Rainfed). NB: JIRRI = Jozini from irrigated treatment, JRF = Jozini from rainfed treatment, B = brown colour, LB = light-brown colour, R = red colour.

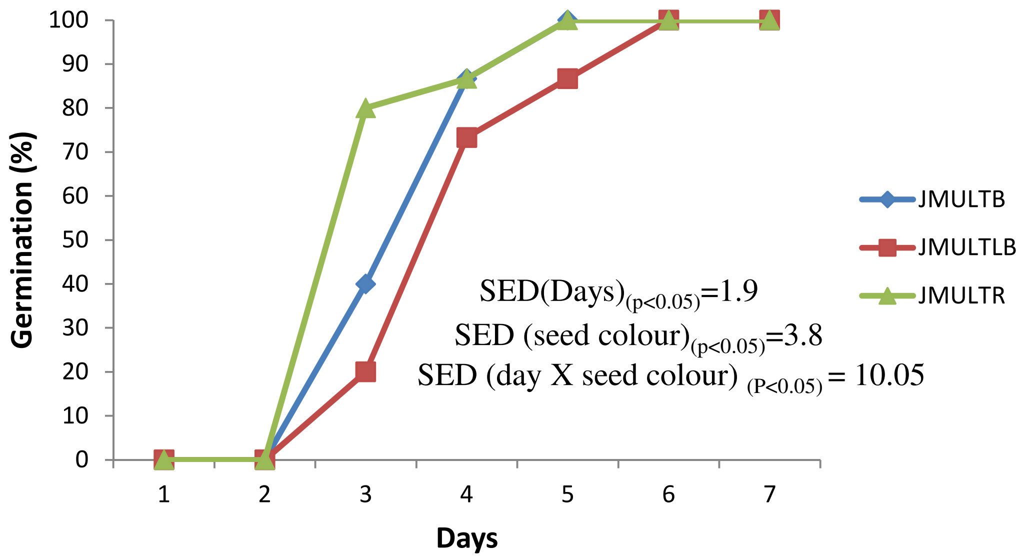

Figure 2.4: Daily germination of different Bambara seed colours (red, brown and light brown) in the Jozini provenance (Multiplication seeds).NB: JMULT = Jozini from multiplication seeds, B = brown, LB = light brown, R = red.

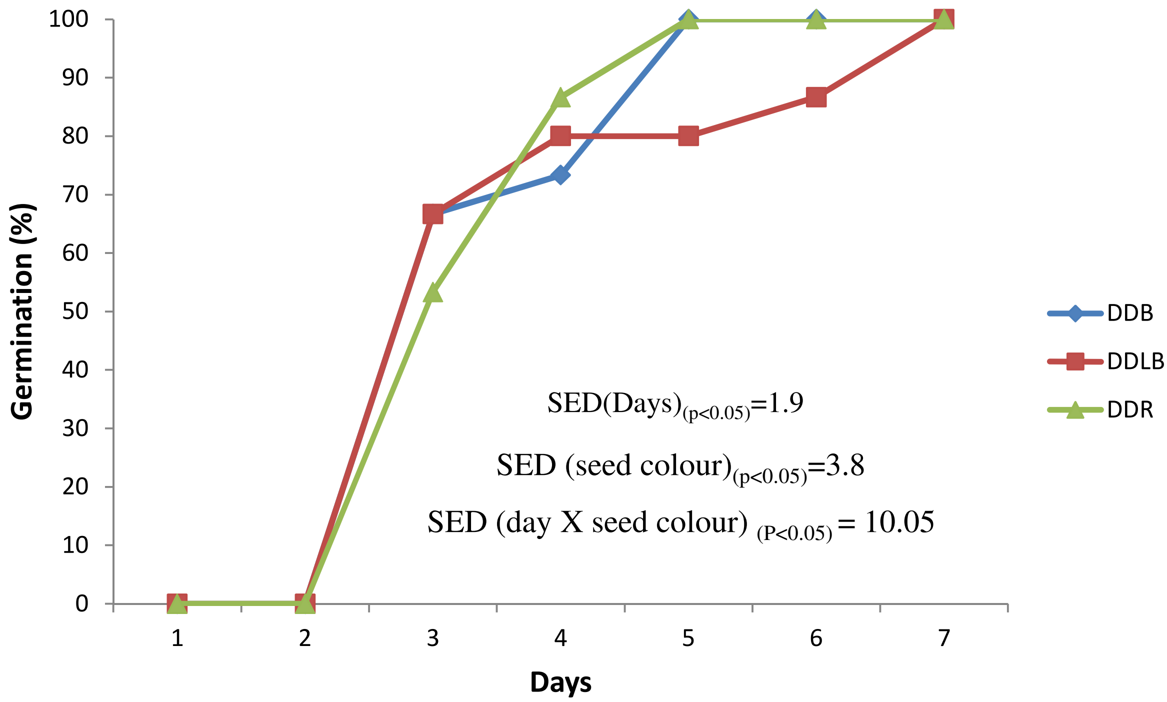

Figure 2.5: Daily germination of different Bambara seed colours (red, brown and light brown) in the Deepdale provenance. NB: DD = Deepdale provenance, B = brown colour, LB = light brown colour, R = red colour.

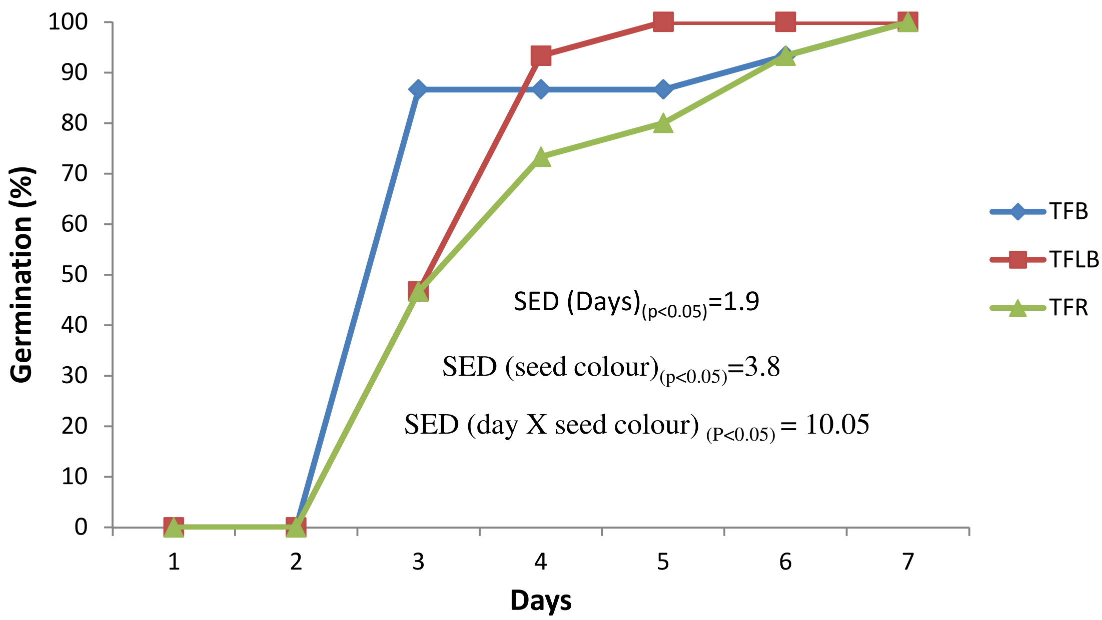

Figure 2.6: Daily germination of different Bambara seed colours (red, brown and light brown) in the Tugela Ferry provenance. NB: TF = Tugela Ferry provenance, B = brown colour, LB = light brown colour, R = red colour.

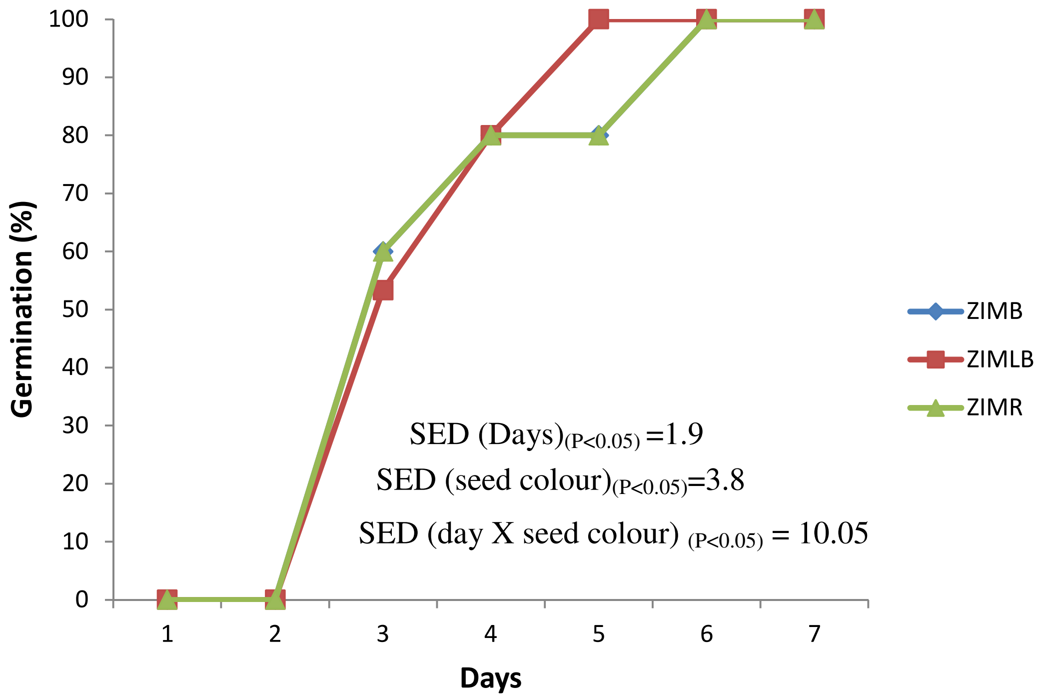

Figure 2.7: Daily germination of different Bambara seed colours (red, brown and light brown) in the Zimbabwe provenance. NB: ZIM = Zimbabwe provenance, B = brown colour, LB = light brown colour, R = red colour.

The preceding data (Figures 2.2 to 2.7) focused on the comparison of seed colour per provenance with respect to germination trend. It is important to note that the Jozini provenance was split into six groups on the basis of seed origin as influenced by treatments in the previous season in Pretoria (Figures 2.8 and 2.9). In order to compare seed colours across provenances and the provenances themselves, the mean seed germination percentages are shown in Figures 2.8 and 2.9.

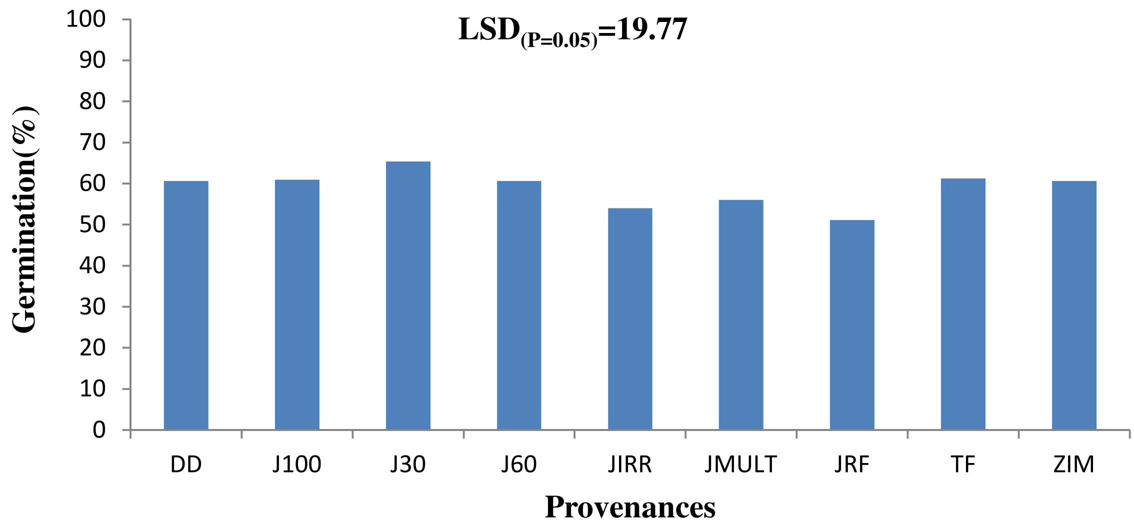

Figure 2.8: Daily germination of different Bambara groundnuts provenances. NB: DD= Deepdale provenance, J100 = Jozini from 100% treatment, J30 = Jozini from 30% treatment, J60 = Jozini from 60% treatment, JIRR = Jozini from irrigated treatment, JRF = Jozini from rainfed treatment, TF = Tugela Ferry provenance, Zim = Zimbabwe provenance.

From Figure 2.8, it is evident that the best performing provenance was Jozini 30 (J30), which had an average germination percent of 65.4% compared with the lowest performing provenance, Jozini Rainfed (JRF) with an average germination of 51.1%. A comparison of all the provenances revealed the following trend J30 > TF > J100 > J60 = JZIM > DD > JMULT > JIRR > JIRF (Figure 2.8).

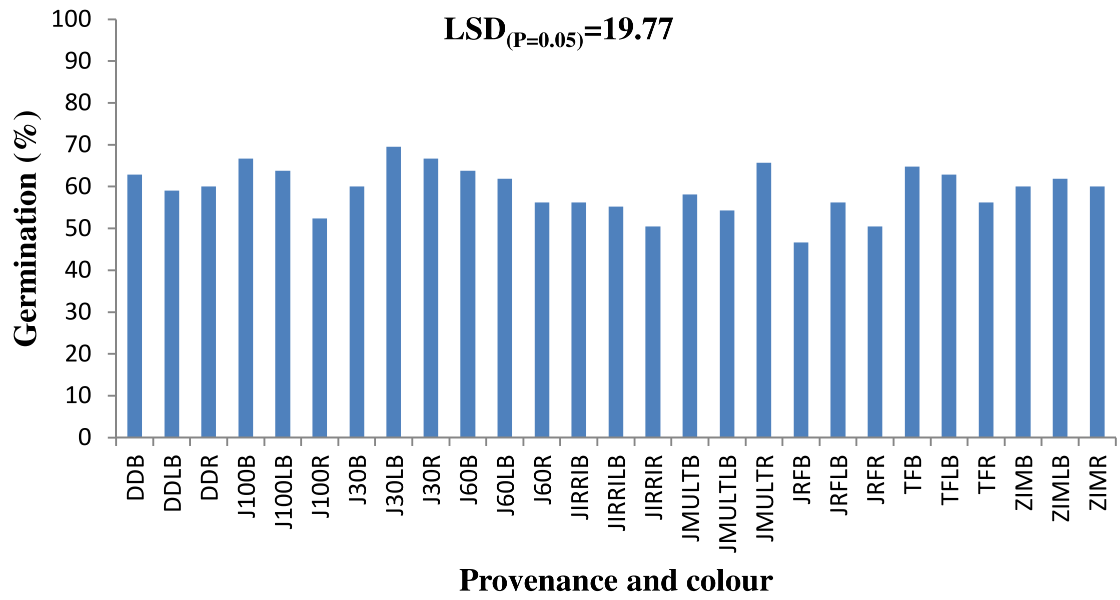

Figure 2.9: Daily germination of different Bambara groundnut provenances and colours. NB: J100 = Jozini from 100% treatment, J60 = Jozini from 60% treatment, J30 = Jozini from 30% treatment, JIRRI = Jozini from irrigated treatment, JRF = Jozini from rainfed treatment, JMULT = Jozini from multiplication seeds, DD = Deepdale provenance, TF = Tugela Ferry provenance, ZIM = Zimbabwe provenance B = brown colour, LB = light-brown, R = red.

From Figure 2.9, it is evident that within provenances (i) For Deepdale, the brown seeds (DDB) showed the highest average germination than the red and light brown seeds (i.e. DDB > DDR > DDLB), (ii) in Jozini 100, the brown seeds showed the highest germination than the light brown and red (i.e. J100B > J100LB > J100R), (iii) for Jozini 30, light brown seeds showed the highest germination followed by red and brown, respectively (J30LB > J30R > J30B), (iv) for Jozini 60, the brown seeds showed the highest germination than red and light brown, respectively (J60B > J60LB > J60R), (v) for Jozini Irrigated, the brown colour showed the highest germination, followed by light brown and red, respectively (i.e. JIRRB > JIRRLB > JIRRR), (vi) for Jozini Multiplication, red seeds showed the highest germination than brown and light brown, respectively (i.e. JMULTR > LMULTB > JMULTLB), (vii) for Jozini Rainfed, the light brown seeds had the highest germination followed by red and brown, respectively (i.e. JRFLB > JRFR > JRFB), (viii) for Tugela Ferry, the brown seeds showed the highest germination, followed by light brown and red seeds, respectively (i.e. TFB > TFLB > TFR), and (ix) for Zimbabwe, light brown seeds had the highest germination, followed by red and brown (i.e. ZIMLB > ZIMB = ZIMR). From these results brown (B) and light brown (LB) seeds generally performed better than the other seeds across the provenances.

2.3.2 Seed vigour

Germination speed (GVI) was shown to differ significantly ($P<0.05$) between provenances (Table 2.2). On average, the GVI was highest for the Deepdale provenance followed by Jozini, Tugela Ferry then Zimbabwe provenance (Table 2.2). Within the Deepdale provenance, the light brown selection had a higher GVI (5.402) compared with brown (4.514) and red (4.364) selections (Table 2.2). This trend was also observed for the Jozini (J30, J100, and JRF) provenance whereby the light-brown selection had higher GVI than brown (4.814) and red (4.52) selections. A similar trend for GVI was observed for the Tugela Ferry and Zimbabwe provenances (Table 2.2). However, three Jozini provenance selections (J IRR, J60 and JMLT) did not show this trend (Table 2.2). On average, for all provenances, the light-brown selections had the highest GVI (Table 2.2).

Table 2.2: Germination vigour parameters of different bambara groundnut provenances and seed colours under Standard Germination (SG).

^x^Prov ^y^S.C.	GVI	MGT	Shoot Length --------(mm)--------	Root Length	Root: Shoot Ratio	Fresh mass -------(g)------	Dry mass
DD B	4.51ab	5.23ab	13.92abcd	36.58ab	2.46e	1.36a	0.46a
DD LB	5.40ab	5.29ab					
DDR	4.36ab	5.12a	2.30d	28.76b	10.60ab	1.48ab	0.51a
J 100 B	4.18ab	5.09ab	12.78abcd	34.67ab	3.39de	1.41ab	0.45a
J 100 LB	4.76ab	5.23ab	18.58abc	69a	4.06cde	1.54ab	0.51a
J 100R	3.96ab	5.49ab	16.13abcd	44.67ab	2.703e	1.51ab	0.44a
J 30 B	4.81b	5.23a	8.11 bcd	52ab	7.34bcd	1.39ab	0.52a
J 30 LB	5.24ab	5.06ab	9.69abcd	44.33ab	4.71cde	1.58ab	0.51a
J 30R	4.52ab	5.13ab	13.51abcd	44ab	3.66cde	1.25ab	0.44a
J 60B	4.24ab	5.16ab	14.20abcd	43ab	3.05e	1.44ab	0.44a
J 60 LB	4.39ab	5.32ab	13.33abcd	53ab	3.96cde	1.23ab	0.43a
J 60R	4.56a	5.44b	9.47abcd	52.47ab	5.64cde	1.68ab	0.58a
J IRR B	4.26ab	5.46ab	10.13abcd	47ab	4.64cde	1.50ab	0.58a
J IRR LB	3.43ab	5.54b	5.25 bcd	65.4ab	12.99a	1.62ab	0.61a
J IRR R	3.33ab	5.33ab	10.17abcd	42.8ab	4.10cde	1.14b	0.36a
J MLT B	4.38ab	5.42ab	7.58 bcd	41.4ab	5.60cde	1.19b	0.40a
J MLT LB	3.46ab	5.47ab	9.25abcd	46.27ab	4.99cde	1.62ab	0.53a
J MLT R	4.69ab	5.32ab	4.00 cd	31.55b	8.03bc	1.44ab	0.50a
J RF B	3.08ab	5.67ab	13.61abcd	35.47ab	2.9de	1.61ab	0.52a
J RF LB	3.93ab	5.39ab	7.44 bcd	45.35ab	6.82bcde	1.60ab	0.55a
J RF R	3.39ab	5.53ab	6.42 bcd	33.08ab	4.97cde	1.57ab	0.55a
TF B	4.49ab	5.24a	6.75 bcd	37.80ab	5.40cde	1.54ab	0.56a
TF LB	4.71ab	5.09ab	10.60abcd	42.13ab	4.02cde	1.10b	0.51a
TF R	4.28ab	5.11ab	14.75abcd	50.33ab	3.61cde	1.11b	0.36a
ZIM B	4.19ab	5.24ab	23.33a	52.00ab	2.28e	1.46ab	0.48a
ZIM LB	4.35ab	5.22ab	17.22abc	51.42ab	2.94de	1.82ab	0.50a
ZIM R	4.35	5.24	18.83ab	52.92ab	3.04de	1.40ab	0.52a
P	**Ns**	**P<0.05**	**P < 0.05**	**Ns***	**P < 0.05**	**P < 0.05**	**Ns**
cv%	**15.40**	**0.28**	**40.00**	**25.70**	**29.10**	**13.10**	**17.90**
$LSD_{(P=0.05)}$	**1.08**	**3.20**	**7.53**	**19.08**	**2.35**	**0.31**	**0.15**

^x^Prov = provenance (J = Jozini, DD = Deepdale; TF = Tugela Ferry and Zim = Zimbabwe); ^y^S.C. = seed colour (B = brown, B = brown, LB = light-brown and R = Red). *Ns = not significant at P=0.05. Numbers in the same column with different letters differ significantly at LSD (P=0.05). GVI = Germination velocity index; MGT = Mean germination time.

Mean germination time (MGT) was shown to differ significantly (P<0.001) between provenances (Table 2.2). Mean germination time was highest for the Jozini provenance followed by Zimbabwe, Deepdale and Tugela Ferry. Within the Jozini provenance, there were no common trends with respect to seed colours. The Jozini (J100) selection had a higher MGT for the red selection (5.49) than light brown and brown selections, respectively (Table 2.2). Whereas, Jozini (J30 and JRF) selections had a higher MGT for brown colour (5.23 and 5.67, respectively). Jozini (JIRR) selection had a higher MGT for light-brown colour (5.54). Within the Deepdale provenance, MGT was high for the light-brown selection (5.29) and Tugela Ferry for the brown selection (5.53). There were no common trends in all provenances. The germination speed was higher mostly in light brown selections in almost all provenances which corresponded with observations of GVI (Table 2.2). For the Tugela Ferry provenance, GVI was high for the light-brown selection while MGT was correspondingly low for the light-brown selections (Table 2.2).

There were highly significant differences (P<0.001) between provenances with respect to shoot length (Table 2.2). The Zimbabwe provenance had the highest shoot length followed by Jozini, Tugela Ferry and Deepdale provenances, respectively. Results showed that there was great variation between seed colour selections for shoot length (Table 2.2). For the Zimbabwe provenance, it was observed that the brown coloured selection had a higher shoot length (23.33 mm) than the red (18.83 mm) and light-brown (17.22 mm) selections. There was no observable trend for all provenances with respect to seed colour; some provenances had higher shoot length for red colour selections while for some it was the brown colour or light-brown colour selections. There were no significant differences (P>0.05) between provenances with respect to root length (Table 2.2). Jozini landrace selections from the 100% Eta (J100) and irrigated (JIRRI) treatments had the highest root length whereas the rest of provenances had similar root length (Table 2.2). For each provenance, there were no differences in colours; similar to shoot length, there was great variability within and between provenances.

There were highly significant differences (P<0.001) between provenances with respect to root: shoot ratio (Table 2.2). The root: shoot ratio was higher for Jozini, followed by

Deepdale, Tugela Ferry then Zimbabwe provenances, respectively (Table 2.2). For most provenances, the dark coloured seed selections such as red and brown had a higher root: shoot ratio compared to light-brown selections (Table 2.2).

Results of fresh mass showed highly significant differences ($P<0.001$) between provenances (Table 2.2). The Zimbabwe provenance had the highest fresh mass relative to Jozini, Deepdale and Tugela Ferry provenances, respectively (Table 2.2). For the Deepdale provenance, the red colour selection had higher fresh mass compared to brown and light-brown selections (Table 2.2). While for the Jozini provenance, there were no differences between seed colour selections; some selections had higher fresh mass for red colour (J60) and some had higher fresh mass for the light brown (J100) while others had higher fresh mass on brown colour selection (JIRRI) (Table 2.2), again indicating huge within landrace (provenance) variability. There were no significant differences ($P>0.05$) between landraces selections with respect to dry mass (Table 2.2).

2.4 Discussion

The aim of this study was to evaluate the relationship between seed coat colour and seed quality in terms of seed performance during germination and to determine if such a relationship is influenced by different provenances. Seed quality is defined in terms of viability, germination and vigour (Coolbear and Hill, 1988). However, germination is an indication of viability in that viability is defined as the property of the seed that allows it to germinate under optimum conditions (Basu, 1995). Seed vigour is defined as the sum total of those properties of the seed that determine the level of activity and performance of the seed or seed lot during germination and seedling emergence (ISTA, 1995). Good seed quality will result in good crop establishment. Low quality seed will result in poor crop stand hence low yields. As such, seed quality is essential for successful crop production (Mabhaudhi and Modi, 2011). According to several authors (Powell, 1989; Zulu and Modi, 2010; Sinefu, 2011) seed colour has an influence on seed quality.

The results of this study showed no significant differences in the total germination between the seed coat colours within a provenance since they all attained 100% on the seventh day. However, the fact that germination time differed significantly between seed colours could be an indication of the effect of seed coat colour on seed quality (Figures 2.2 to 2.7). Since germination is conducted under ideal conditions, it does not necessarily reflect the performance potential of that seed lot under field conditions. There are significant differences between standard germination and actual field emergence (Munn, 1926). High standard germination does not necessarily result in rapid and uniform emergence or vigorous stand under actual planting conditions (Delouche and Baskin, 1973). It is in this context that time to final germination is relevant in a germination test.

Germination proceeded slowly during the first two days for all provenances. Differences in germination between provenances started to show by the third day. By the seventh day all provenances were able to attain 100% germination. The difference in germination speed within provenances could be due to the great amount of variation that normally exists in landraces from different geographical locations (Ntundu *et al*., 2004). Seed coat colour selections also showed significant variation in germination speed; however, there was no observable trend. Nonetheless, on average, a trend emerged showing the dark colours (brown and red) performing better than the light coloured seed selection. On average, for most provenances, the brown coloured selection germinated faster. This might be due to the fact that dark coloured seeds are more vigorous than light coloured seeds (Mabhaudhi, 2009; Mbatha, 2010; Zulu, 2010; Sinefu, 2011).

Examination of seed performance in the context of a provenance per se, showed that the Jozini 30 seeds, showed the best performance. These seeds were derived from conditions of water stress. It was interesting to observe their performance being better than the seeds derived from irrigated conditions (Figure 2.8).

Sinefu (2011), working on bambara groundnut, associated the better quality of dark coloured seeds to a relatively thick seed coat compared to light coloured seeds. A thin seed coat in light coloured seeds was shown to result in high electrolyte leakage hence loss of quality (Sinefu, 2011). Zhang *et al*. (2006,. 2008) working on watermelon also observed that red and black (dark) coloured seed had higher melanin pigment with slow water uptake, low electrical conductivity value and high tolerance to slow water uptake. While in yellow-coloured (light) seeds lower melanin content and faster water uptake were observed. However, there have been reports contradicting this hypothesis. Anuradha *et al.* (2009) found that germination percentage in bengalgram cv. co 4 (*Cicer arietinum*) was high in light brown coloured seeds compared to brown colour seeds and red coloured seeds.

Vigour parameters such as shoot length, root length, dry mass and fresh mass also showed a similar trend to that of the germination in which the Zimbabwe and Jozini provenances were superior to other provenances. There was no observable trend for all provenances with respect to seed coat colour. Some provenances had longer shoot length for red colour selections while for some it was the brown colour or light-brown colour selections that had longer shoots. For each provenance in terms of root length, there were no differences in colours; similar to shoot length, there was great variability within and between provenances. This shows the great amount of variation that normally exists in landraces from different geographical locations (Ntundu *et al*., 2004). For most provenances, the dark coloured seed selections such as red and brown had a higher root: shoot ratio compared with light-brown selections. The increase of these parameters in dark coloured seeds might be due to the direct relation with seed germination capacity and vigour. In terms of fresh and dry mass, results of this study showed no observable trends for both seed colour selections and provenances. Although this study showed no clear trend, closer analysis revealed that the dark colour seed selections often performed better than the light coloured selections for most provenances. Previous studies (Mavi, 2010) on watermelon also reported higher seedling fresh and dry mass for brown seed lots compared with light-coloured seed.

Moreover, differences in seed colour of bambara groundnut can be used as a marker of seed quality. Farmers can be advised based on seed colour. Light coloured seeds are usually desirable because of their reputation of being highly palatable and easy to digest, whilst dark coloured seeds are promoted for their disease resistance. Studies on cowpea (Pakela, 2003) which is closely related to bambara groundnut, have reported increased disease resistance for dark coloured and patterned seeds relative to lighter ones. For this reason farmers are encouraged to plant dark coloured seeds for better crop performance. However, darker seeds have higher phenolic derivatives especially in the seed coats, as reported for cowpeas (Pakela, 2003), dry beans (Beninger and Hosfield, 1999), legumes species in general (Sosulski and Dabrowski, 1984), and selected *Brassica* seeds (Simbaya *et al*., 1995). These phenolic derivatives contribute to adverse tastes, poor digestibility (Aw and Swanson, 1985) and decreased nutritive protein value (Krygier *et al*., 1982).

2.5 Conclusions

This study revealed that: (i) it is difficult to separate the performance of bambara landrace seeds with respect to standard germination (Figures 2.2 to 2.7). Therefore, germination rate and vigour are useful to achieve separation. Clearly, seed colour is an important variable in the identity of bambara landraces. (ii) Provenance plays a significant role in seed performance and there is a significant interaction between provenance and seed coat colour (Figures 2.8 and 2.9). This argument was indicated by the differences between Jozini seeds, which mainly differed in terms of environmental growth conditions for the mother plant or previous generation. In the context of bambara groundnut being an underutilised indigenous crop in South Africa, this study provided an opportunity to identify landraces with potential for germplasm collection and improvement. Further studies should, among others, focus on the performance of selected landraces under field or controlled environments to determine agronomic characters such as yield.

2.6 Reference

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Chapter 3

Seedling growth in response to different water treatments under three different soil media

3.1. Introduction

Currently, world food supply relies on a few major crops – wheat, rice, maize and potatoes (Kouassi and Zorobi, 2011). In terms of protein crops, there are a few major legumes such as dry bean, soybean and groundnut. Historically, man's food basket was diverse, consisting of well over 7 000 edible crops (Collins and Hawtin, 1999). The shrinking of man's food basket has led to loss of agro-biodiversity and a reliance on a few major crops. There is now a new threat that these few major crops may not be able to cope with predicted future climate (Petit *et al*., 1999; Hassan, 2006). As such, there is a need to identify crops that were previously neglected and evaluate them for drought tolerance and possible crop improvement. Bambara groundnut (*Vigna subterranea* (L.) Verdc) is one such indigenous African legume with significance as a source of protein in sub-Saharan Africa where it is mainly grown by women farmers in subsistence agricultural systems (Mukurumbira, 1985). There is a need to generate basic information on the crop's responses to drought at different stages of growth, starting from early establishment.

The period from germination leading up to seedling emergence is perhaps the most vulnerable stage in a crop's life. It is hardly possible to attain maximum yield without successful seedling establishment (Mabhaudhi, 2009). Poor seedling emergence results in low crop stands which ultimately translate into low yields. Besides seed quality related reasons, the reasons for poor seedling emergence range from low soil water content (Forbes and Watson, 1992), poor seed-soil contact (Stewart *et al*., 1999) to inaccurate seed placement as well as low and high soil temperatures (Forbes and Watson, 1992). Exposure of seeds to unfavourable seedbed conditions like water stress may lead to poor seedling establishment (Albuquerque and Carvalho, 2003). Optimal seedbed conditions are therefore needed for successful seedling emergence; however, given the conditions often experienced in areas where bambara is cultivated, seedbed conditions are hardly ever optimum. Other than having a deleterious effect on emergence, water stress can affect stand establishment by inhibiting growth, leaf extension and leaf area thus affecting the seedling's ability to grow into a healthy plant capable of utilising resources (water, light and nutrients) efficiently. As such, the ability to tolerate drought or water stress at the seedling stage is critical.

Under water stress conditions, plants show physiological adaptations to cope with water stress. Such adaptations include alterations in levels of bioactive compounds such as proline, proteins and antioxidants. Of particular interest is proline, which is known to accumulate in many plant species under various stress conditions (Delaunay and Verma, 1993). High proline content in plants under water stress is frequently observed in several species (Clifford *et al*., 1998; Bajji *et al*., 2001) and may act as a regulatory or signalling molecule to activate multiple responses that are part of the adaptation process (Maggio *et al*., 2002; Claussen, 2005). There are several reports of proline accumulation in seedlings of maize landraces (Mabhaudhi, 2009), wild mustard (*Brassica juncea* and *B. Nigra*) (Mbatha, 2010), and wild water melon (*Citrullus lanatus*) (Zulu, 2010); these authors found that proline accumulation was related to stress tolerance. As such, proline accumulation could be used as a measure of stress acclimation during the early establishment stage.

Water stress remains a major problem in arid and semi-arid areas where bambara is cultivated. Most rural farmers in sub-Saharan Africa reside in marginal areas of agricultural production whereby both water availability and soil type interact to affect seedling establishment thus effectively lowering their yields. The problem of unpredictable yields in bambara has been attributed, at least in part, to variable or poor field establishment due to poor seedling emergence (Linnemann and Azam-Ali, 1993); this may be linked to water stress and soil types. It has been reported that bambara can perform successfully in a variety of soil conditions (Uguru and Ezeh, 1997). A study that was done by Pillay (2003) showed that bambara varieties emerged well in both sand and clay. The objective of the present study was to evaluate seedling establishment of bambara groundnut landraces, within the context of provenance and seed coat colour, in response to different water levels and different soil media.

3.2 Materials and methods

3.2.1 Plant material

Bambara seeds were sourced from subsistence farmers of Tugela Ferry (28˚45'S; 30˚27'E; 543 masl) and Deepdale (30°33'S; 29°54'E; 1370 masl) both in KwaZulu–Natal, South Africa, during February 2012. In addition, Jozini seeds were obtained from experiments conducted during the 2011/12 planting season (Mabhaudhi, 2012) where they had been subjected to different water treatments (30% and 100% of crop water requirement). Bambara landrace seeds were then sorted into three distinct colours: red, light-brown and brown. Seeds were characterised according to seed coat colour based on previous studies that have suggested that seed coat colour may have an effect on early establishment performance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu 2011).

3.2.2 Experimental design

A seedling establishment trial was conducted using 200 units seedling trays (Figure 3.1) at the University of KwaZulu–Natal's Controlled Environment Research Unit (CERU), Pietermaritzburg. Throughout the experiment, the tray were kept in a tunnels (27°C/21°C day/night; 65% RH and natural day length), an environment considered to be typically warm sub-tropical (Modi, 2007).

Figure 3. 1: Seedling tray used for seedling establishment

The experimental design was a factorial experiment, with four factors: 1. provenance – with two levels (Tugela Ferry and Deepdale), 2. seed colour – with three levels (red, light-brown and brown), 3. water regimes – with three levels (30%FC, 60% FC and 100% FC), and 4. Soil media – with three levels (clay, sand and clay + sand). The experiment was replicated three times. Bambara seeds were planted in trays filled with the different soil media whose field capacity had been previously determined. The physical properties of the three soil media are shown in Table 3.1 The trays were weighed and watered every two days to maintain field capacity. Water was then added to the individual trays until the required soil water content of 100%, 60% and 30% FC was attained.

Table 3.1: Soil physical characteristics of the three media (Clay, Sand and Clay+Sand) used in the seedling establishment experiment.

Media	**Clay**	**Sand**	**Silt**	***Field Capacity**
	------------------------------%------------------------------			
Clay	43.5	24	32.5	40.6
Sand	11	6	83	20.8
Clay+Sand	27	16	57	37.8

*Field capacity represents gravimetric field capacity.

3.2.4 Emergence data collection

Measurements of plant emergence were taken daily for 28 days. Seedling height and leaf number were determined weekly for fully emerged seedlings. Seedling height was measured from the soil surface to the base of the leaf. Leaf number was counted for fully unfolded leaves. The experiment was terminated at 35 days after planting at which point shoot length, root length, root volume, root: shoot ratio, dry mass and wet mass were determined.

3.2.4 Proline determination

Due to limitations in seedlings, proline accumulation was only evaluated for the 30% and 100% FC regimes and only Tugela Ferry and Jozini seeds were used. Proline content was determined according to the method of Bates *et al*. (1973). Leaf samples were ground into fine powder under liquid nitrogen using mortar and pestile. Subsequently, 0.5 g of ground leaf material was homogenized in 10 ml of 3% aqueous sulphosalicyclic acid. The homogenate was then filtered through Whatman® No. 2 filter paper. 2 ml of the filtrate was added to a test tube to which 2 ml of glacial acetic acid and acid ninhydrin were added, respectively. The solution was then heated in a boiling (100°C) water bath for 1 hour. The reaction was then terminated in an ice water bath. The reaction mixture was extracted with 4 ml toluene and vortexed for 15 – 20 sec. The chromosphere containing toluene was aspirated from the aqueous phase, warmed to room temperature and absorbance read at 520 nm using toluene as a blank. Proline concentration was calculated using the standard curve on a dry weight basis. The following equation was used to calculate proline.

[(µg proline/ml x ml toluene)/ (115 µg/µmole)]/ [(g sample)/5] = µmoles proline/g of dry weight material.

3.2.5 Data analyses

Data were analysed using analysis of variance (ANOVA) from GenStat® Version 14 (VSN International, UK). Thereafter, means were separated using Duncan's Multiple Range Test in GenStat® at the 5% level of significance (Appendix 3).

3.3. Results

3.3.1 Emergence

There were significant differences (P<0.001) between water regimes with respect to final emergence 28 days after planting (DAP) (Figure 3.2). Emergence was respectively 21% and 50% higher at 100% FC relative to 60% FC and 30% FC. There were highly significant differences (P<0.001) between soil media with respect to emergence (Figure 3.2). Based on mean values for media, emergence of bambara landraces was respectively 13% and 2% higher in the Sand+Clay mixture compared with Clay and Sand media.

Results of emergence also showed highly significant (P<0.001) differences between provenances and seed coat colours; however, there was no clear pattern with regards to provenance or seed coat colour (Figure 3.2). Nonetheless, the Tugela Ferry provenance exhibited a trend whereby the darker coloured landrace selections (brown and red, respectively) performed better than the light-brown landrace selection.

The interaction between soil media, water regimes and bambara landrace selections was shown to be highly significant (P<0.001).There was huge variation with regards to performance of landrace selections in different soil media and water regimes. The Tugela Ferry red (TFR) landrace selection achieved 100% emergence under all water regimes and in all soil media. In addition, the Tugela Ferry red landrace selection performed better than other landraces under the varying water regimes. Furthermore, at 30% FC in clay media, the TFR (100%) landrace selection had the highest emergence followed by TFB (70%), J30R (60%) = J30B (60%), TFLB (50%) = J100LB (50%), J30LB (40%) = J100R (40%), J100B (30%), respectively. the trend was similar at 60% FC in clay media, although percentage emergence was higher relative to 30% FC. All landraces were able to attain 100% emergence at 100% FC in clay media. The same trend was observed for sand as well as the mixture of Sand+Clay.

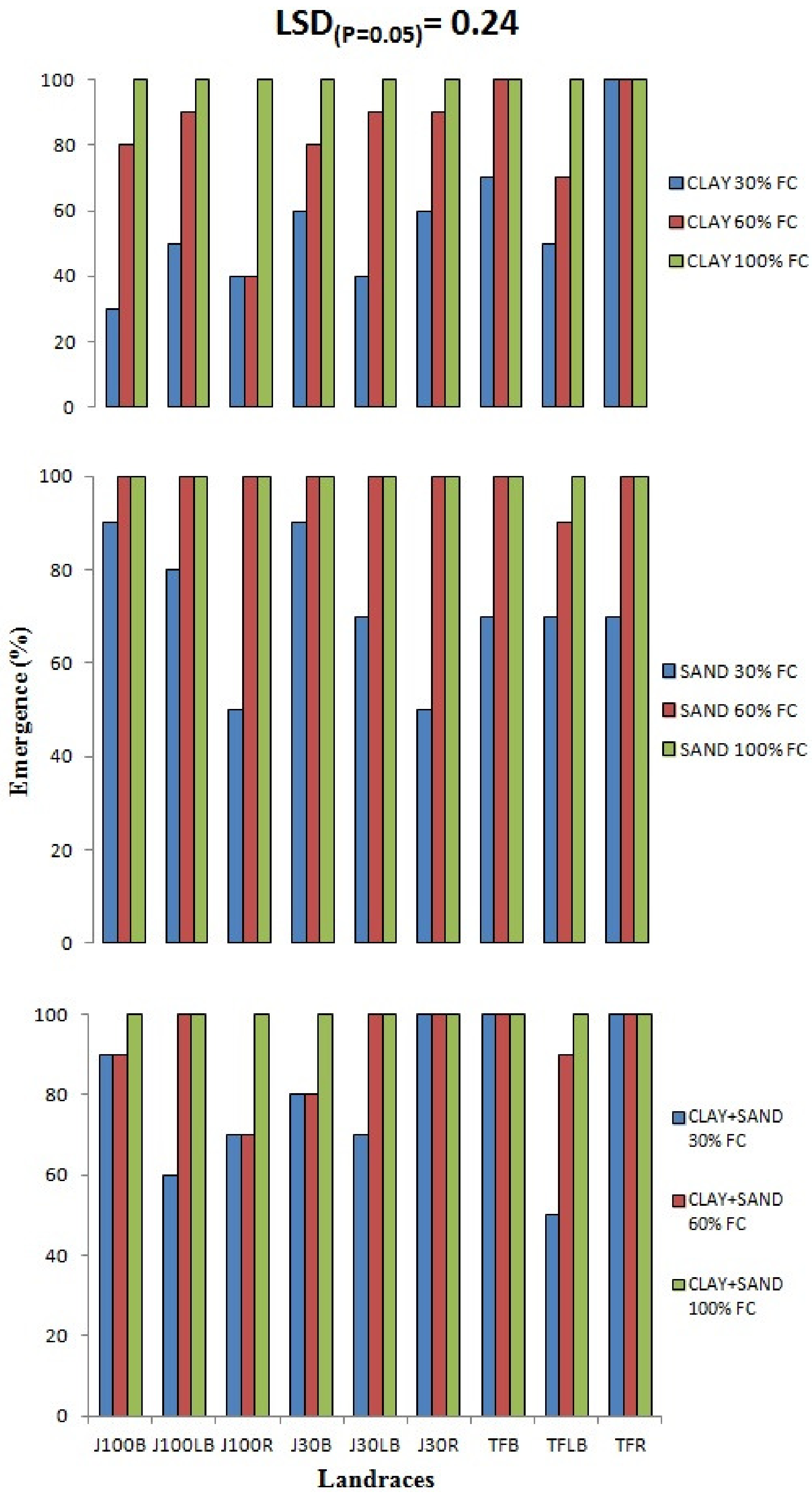

Figure 3.2: Final emergence of different bambara landraces grown in different soil media (Clay, Sand and Sand+ Clay mixture) at different water levels (30, 60 and 100% field capacity). J100= Jozini from 100% treatments, J30= Jozini from 30% treatment, TF Tugela Ferry, LB=Light brown, B=brown, R=red.

3.3.2 Plant growth

There were highly significant differences ($P<0.001$) between water regimes with respect to plant height. Plant height was, on average, 48% and 70% lower at 60% FC and 30% FC, respectively, relative to 100% FC (Figure 3.3, 3.4 and 3.5). There were highly significant differences ($P< 0.001$) between soil media with respect to plant height. Plant height was, on average 22% and 20% lower for sand and clay, respectively, relative to the Sand+Clay mixture (Figure 3.3, 3.4, 3.5). There was a highly significant ($P<0.001$) interaction between media, field capacities and time (DAP), with respect to plant height (Figure 3.3, 3.4, 3.5). At 30% FC in clay media, there was still no emergence at 2 weeks after planting (WAP); hence no plant height was measured (Figure 3.3). In addition, due to unevenness of emergence, plant height measurements for certain treatments were delayed. As such, results of plant height mainly focus on measurements taken at 4 WAP when all plants had emerged. At 4 WAP, the TFR landrace selection had the tallest plants (62 mm) followed by TFB (48 mm), TFLB (38 mm), J30B (35 mm), J30R (32 mm), J30LB (27 mm), J100LB (18 mm), J100R (15 mm) and J100B (13 mm), respectively (Figure 3.3). On average, the Tugela Ferry provenance had the tallest plants compared with the Jozini provenance. Within the Jozini provenance, Jozini from 30% treatment had the tallest plants compared to Jozini from 100% treatments. For both provenances, the red colour was shown to perform better than brown and light-coloured seeds.

At 60% FC in clay media, only J30B, J30R and J30LB had emerged by 1 WAP, with J30B having the tallest plants compared with J30R and J30LB. By 4 WAP, J30R had the tallest plants (52 mm), followed by J30LB (47 mm), TFB (44 mm), TFR (42 mm) = J30B (42 mm) = J100LB (42 mm), TFLB (39 mm), J100B (38 mm) and J100R (36 mm), respectively (Figure 3.3). On average, the Jozini provenance performed better than Tugela Ferry provenance. Within the Jozini provenances, Jozini from 30% treatment performed better than Jozini from 100% treatment. For both provenances, the dark coloured (red and brown) seeds performed better than light-coloured seeds.

All plants emerged by 1 WAP at 100% FC in clay media; hence plant height was taken from the first week (Figure 3.3). By 4 WAP, J30R had the tallest plants (119 mm), followed by J100B (113 mm), J100R (105 mm), J30B (103 mm), TFLB (100 mm), J30LB (98 mm) = J100LB (98 mm), TFR (92 mm) and TFB (82 mm), respectively (Figure 3.3). On average, the Jozini provenance had the tallest plants compared with Tugela Ferry. A closer analysis of the Jozini provenance showed that dark coloured landrace selections had taller seedlings than light-coloured landrace selections; however, for Tugela Ferry provenance, light-brown had taller seedlings compared with red and brown. Over- all, the Tugela Ferry provenance performed well under 30% FC, while Jozini provenance performed well under 60% and 100% FC. Dark coloured seeds performed well for all provenances and under all water regimes.

At 30% FC in sand media, plants had not emerged at 1 WAP hence there was no plant height; by 2 WAP all landraces had emerged. At 4 WAP, TFR landrace selection had the tallest plants (64 mm), followed by TFB (63 mm), J100R (52 mm), J30B (44 mm), TFLB (40 mm), J100B (40 mm), J30R (39 mm), J100LB (31 mm) and J30LB (28 mm), respectively (Figure 3.4). Over-all, Tugela Ferry provenance had the tallest plants compared with Jozini provenance, and dark coloured seeds performed better than light coloured seeds in all provenances.

At 60% FC in sand media, plant height was measured starting from 1 WAP while in clay plant height was measured from 2 WAP onwards. At 4 WAP, J100LB had the tallest plants (58 mm), followed by J30R (57 mm), J30B (51 mm) = J30LB (51 mm), J100B (50 mm), TFLB (48 mm) = TFB (48 mm), J100R (47 mm) and TFR (46 mm), respectively (Figure 3.4). The Jozini provenance had taller plants than Tugela Ferry. Within the Jozini provenance, Jozini from 30% treatment had taller plants than Jozini from 100% treatment. At 100% FC in sand media, at 4 WAP, J100R had the tallest plants (106 mm), followed by J100B (105 mm), J100LB (81 mm), TFLB (75 mm), TFB (73 mm), J30LB (72 mm), J30R (66 mm), TFR (57 mm) and J30B (54 mm), respectively. On average, the Jozini provenance had the highest plant height compared with Tugela Ferry provenance. Within the Jozini provenance, Jozini from 100% treatment performed better than Jozini from 30% treatment. There was no observable trend in terms of colours.

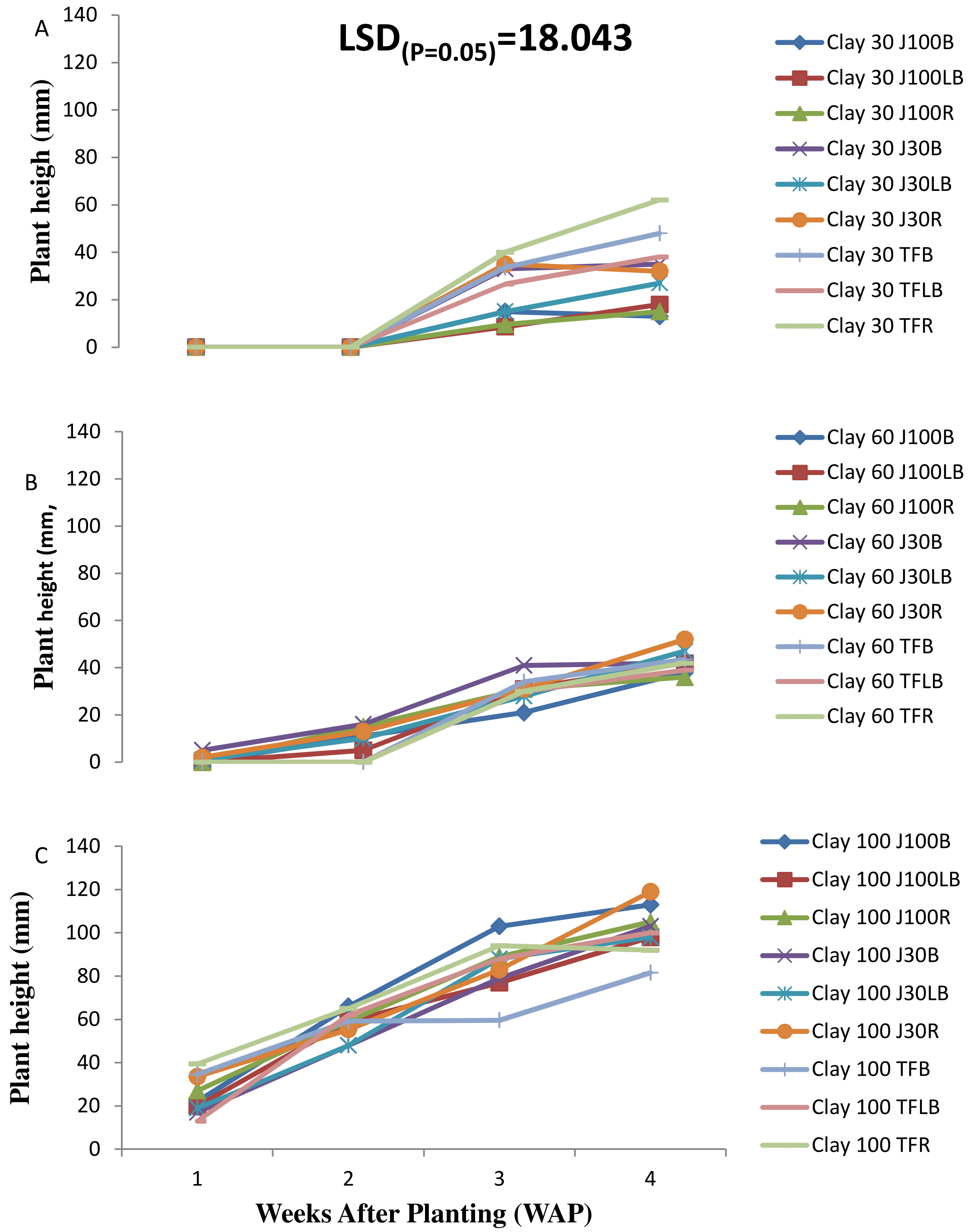

Figure 3. 3: Plant height of Bambara groundnuts landraces subjected to different water regimes and soil media (A= plant height at 30%FC in clay media B= plant height at 60%FC in clay media and C= plant height at 100%FC in clay media). Note: J100B= Jozini brown from 100% treatment , J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment. J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment, J30R= Jozini red from 30% treatment, TFB= Tugela ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

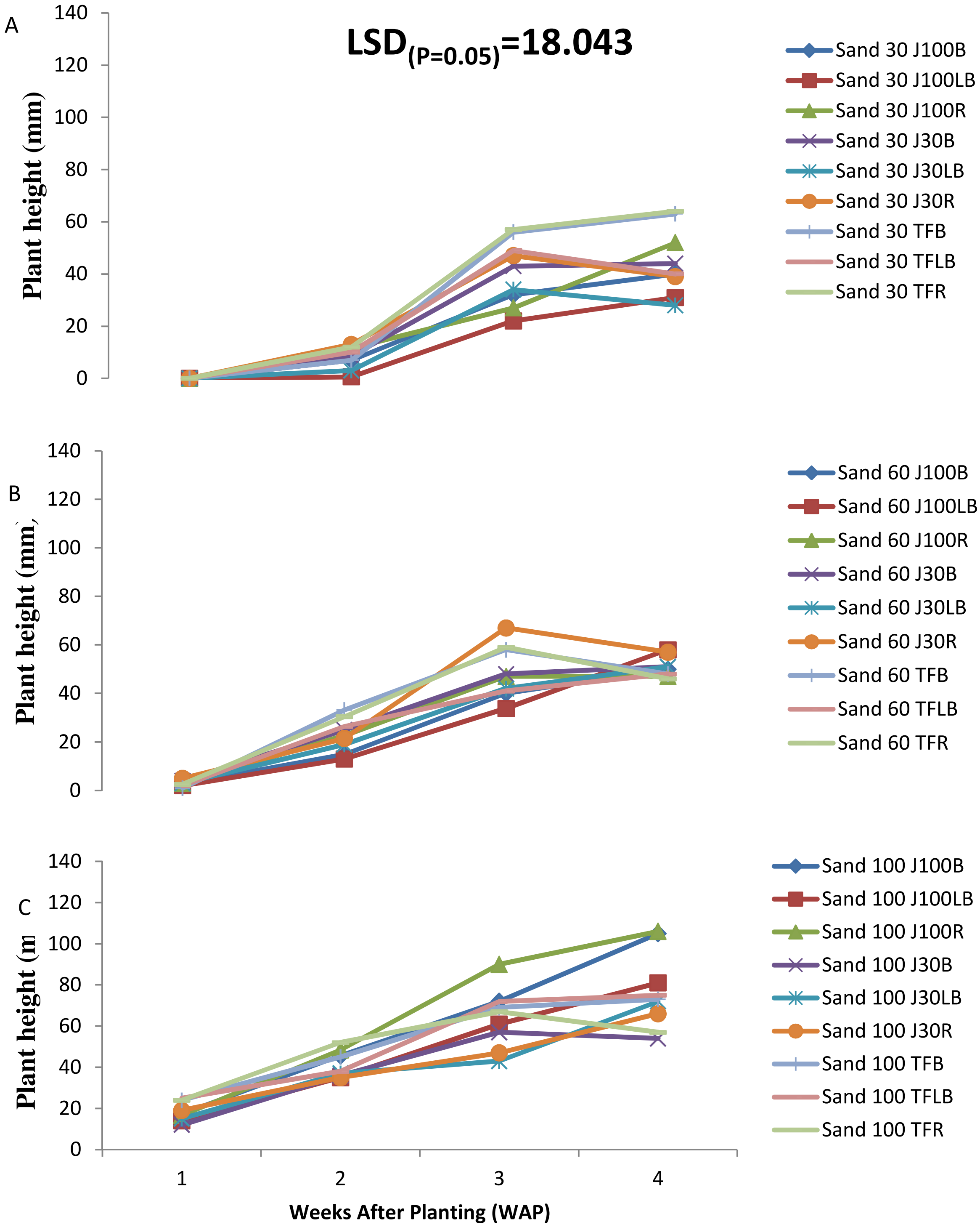

Figure 3.4: Plant height of Bambara groundnuts landraces subjected to different water regimes and planting media (A= plant height at 30%FC in sand media B= plant height at 60%FC in sand media and C= plant height at 100%FC in sand media). Note: J100B= Jozini brown from 100% treatment , J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment. J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment, J30R= Jozini red from 30% treatment, TFB= Tugela ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

At 30% FC in the Mixture (sand + clay), plants only emerged 2 WAP. A similar trend was observed in the sand media (Figure 3.5). However, by 4 WAP, TFR had the tallest plants (68 mm), followed by TFB (62 mm), J30B (49 mm), J30LB (39 mm) = J100R (39 mm), TFLB (35 mm) = J100B (35 mm), J30R (32 mm) and J100LB (26 mm), respectively (Figure 3.5). On average, the Tugela Ferry provenance performed better than Jozini provenance. At 60% FC, plants had emerged by 1 WAP in the Mixture (sand + clay) and sand media; however, only few plants had emerged in clay media. At 4 WAP, J30B had the tallest plants (82 mm), followed by J30LB (62 mm), J100B (61 mm), TFB (55 mm), J30R (54 mm), J100R (52 mm), J100LB (51 mm), TFLB (48 mm) and TFR (36 mm), respectively (Figure 3.5). On average, the Jozini provenance had taller plants than Tugela Ferry provenance. Based on mean values, dark coloured landrace selections had taller plants compared with light-brown landrace selections. By 4 WAP at 100% FC in the mixture (clay + sand) media, J100B had the tallest plants (124 mm), followed by J100LB (112 mm), TFB (111 mm), J30R (101 mm), J100R (94 mm) = J30LB (94 mm), J30B (93 mm), TFLB (88 mm) and TFR (78 mm), respectively (Figure 3.5). The Jozini provenance had the tallest plants compared with Tugela Ferry. Within the Jozini provenance, Jozini from 100% treatment performed better than Jozini from 30% treatment.

Over–all, plant height of bambara seedlings was shown to be affected by water availability and media. It was observed that, for both provenances, plants were generally taller at 100% FC compared with 60% FC and 30% FC, respectively. The Sand+Clay media produced taller seedlings compared with sand and clay media, respectively. In addition, differences in responses to field capacity and soil media were observed within and between provenances. Tugela Ferry performed well at 30% FC, whereas Jozini performed better at 60% and 100% FC, respectively. The Jozini 30 landrace selection performed better at 60% FC while Jozini 100 landrace selection performed better at 100% FC in all soil media. Dark coloured landrace selections were found to perform better than light-coloured landrace selections under all field capacities and in all soil media.

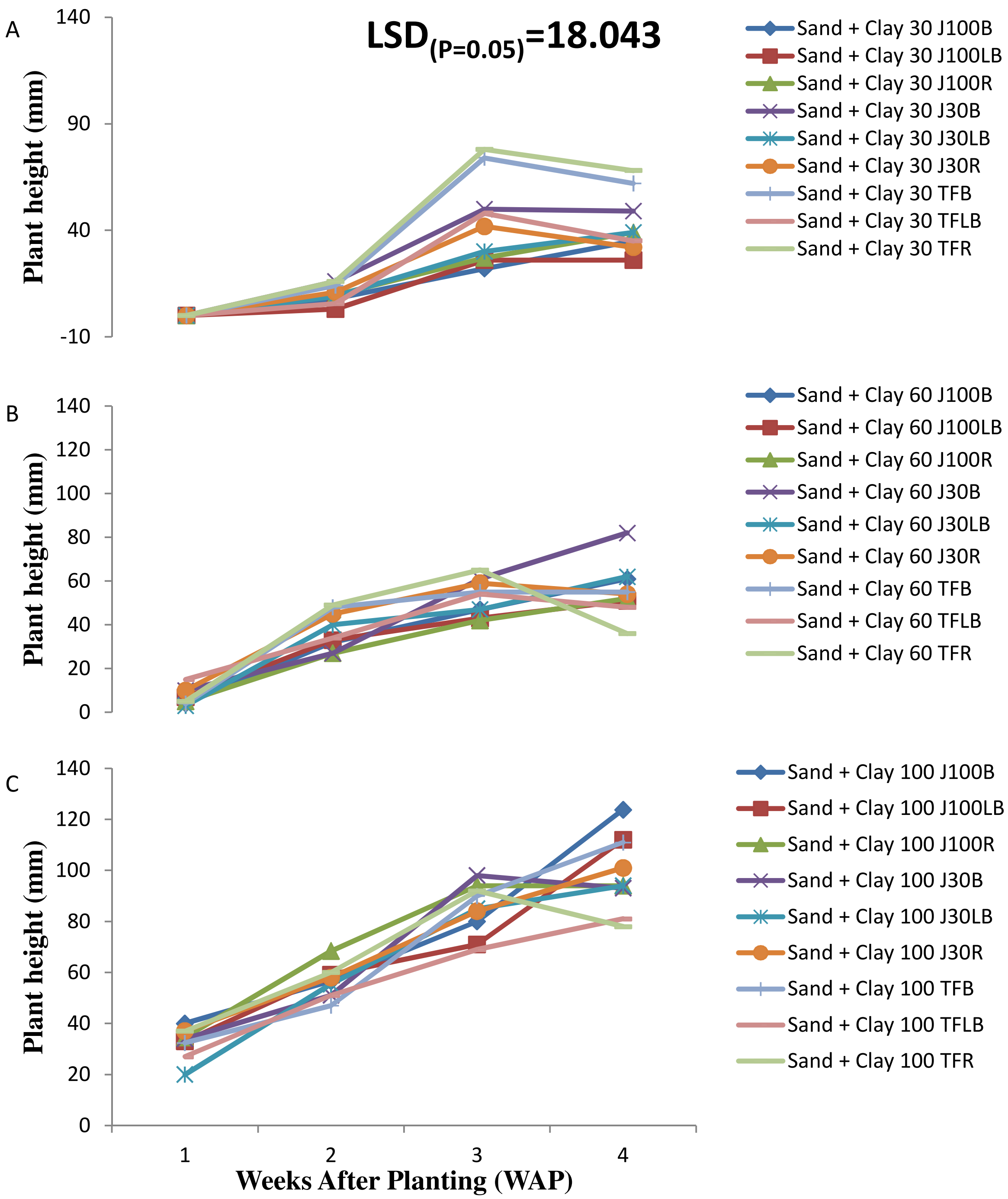

Figure 3.5: Plant height of Bambara groundnuts landraces subjected to different water levels (A= plant height at 30%FC in clay+sand media B= plant height at 60%FC in clay+sand media and C= plant height at 100%FC in clay+sand media). Note: J100B= Jozini brown from 100% treatment , J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment. J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment, J30R= Jozini red from 30% treatment, TFB= Tugela ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

There were highly significant differences (P<0.001) between water regimes, media and landrace selections, with respect to leaf number. At 30% FC in clay, leaf number measurements started at 3 WAP (Figure 3.6). J100B had the highest leaf number (2.4) at 4 WAP; J100LB, J30B, J30R, TFB and TFR had similar leaf number (2) as well as J100R, J30LB and TFLB (1.6) (Figure 3.6). There was no observable trend between provenances and seed colour. At 60% FC in clay media, by 1 WAP only three landraces (J30B, J30LB, and J30R) had emerged. J30B had the highest leaf number compared with J30LB and J30R. At 4 WAP, J30R had the highest leaf number (2.8); followed by TFB (2.77), J100R and J100B were similar (2.6), J100LB (2.4), J30B, J30B, and TFLB were also similar (2.2) while TFR (2) had the least number of leaves (Figure 3.6). Jozini provenance had, on average, more leaves than Tugela Ferry provenance; however there was no observable trend for seed colour. At 100% FC, leaf number was high in all provenances; all landrace selections, with the exception of TFB and TFLB, had 4 leaves.

At 30% FC in sand media, there was still 0% emergence 1 WAP hence measurements of leaf number are from 2 WAP. At 4 WAP, the trend in leaf number was such that TFLB, TFR, J100B, J100R, J30B and TFLB were all similar and had the highest leaf number (≈3) (Figure 3.6). On average, at 30% FC, Tugela Ferry provenance landrace selections had about 12% more leaves than Jozini provenance landrace selections; there was no observable trend with respect to differences between seed colours. At 60% FC in sand media, at 4 WAP, J100R had the highest leaf number (4) followed by J100B (3.8), TFLB (3.4), TFB (3.2), J100LB (3), J30LB (2.8), TFR (2.6) and J30B (2.4), respectively (Figure 3.7). There was no observable trend between provenances and seed colours. At 100% FC, at 4 WAP, J100B, J100LB, J100R, TFB and TFLB had the highest leaf number (4) followed by J30R = J30LB (3.7) and J30B = TFR (3), respectively (Figure 3.7). Under optimum conditions of 100% FC, Jozini from 100% treatment performed better than Jozini from 30% treatment and Tugela Ferry landrace selections. There were no observable trends with respect to differences between seed colours.

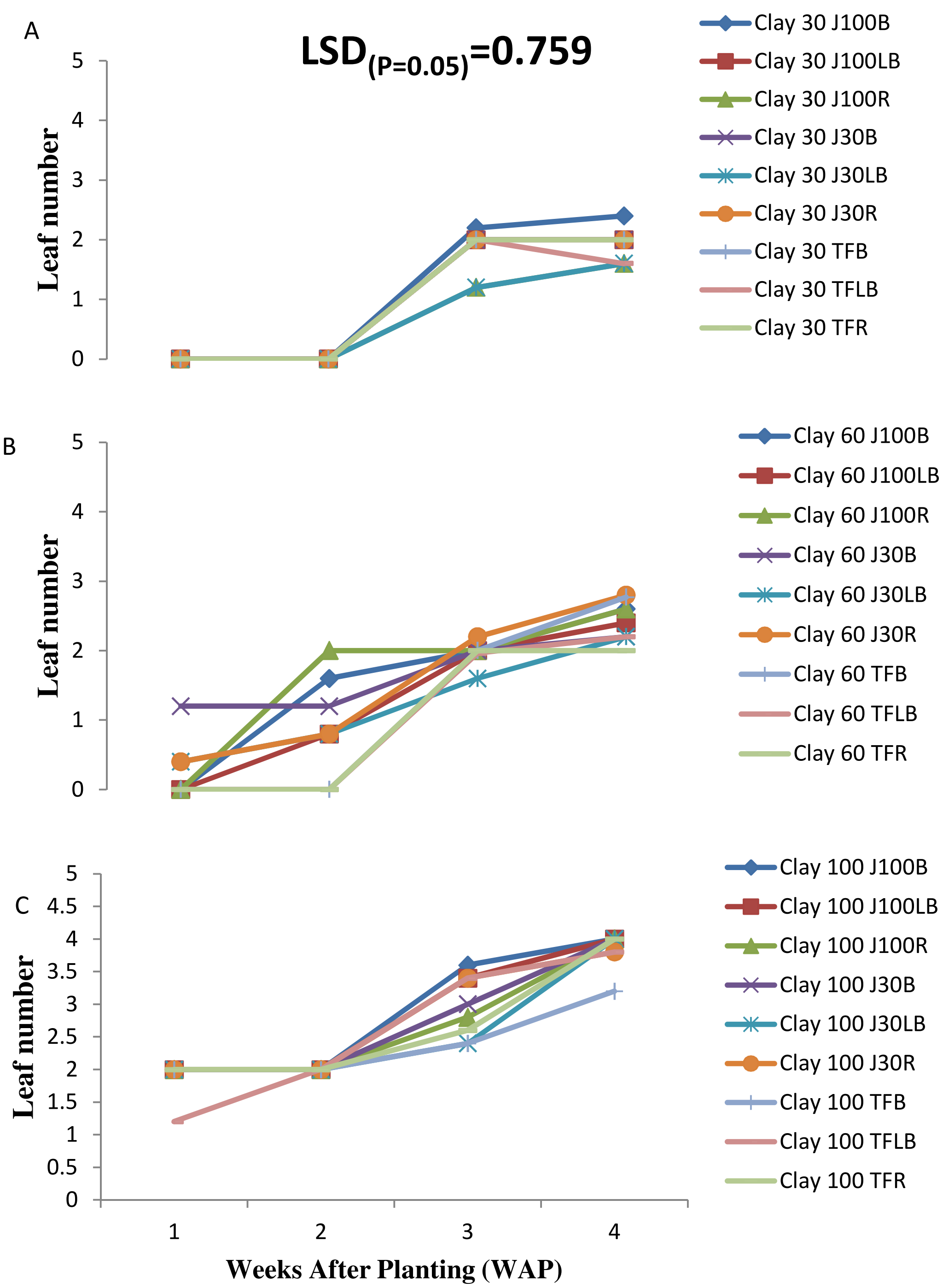

Figure 3. 6: Leaf number of Bambara groundnuts landraces subjected to different water levels under Clay soil media. (A= Leaf number at 30%FC in clay media B= Leaf number at 60%FC in clay media and C= leaf number at 100%FC in clay media). Note: J100B= Jozini brown from 100% treatment, J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment. J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment, J30R= Jozini red from 30% treatment, TFB= Tugela ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

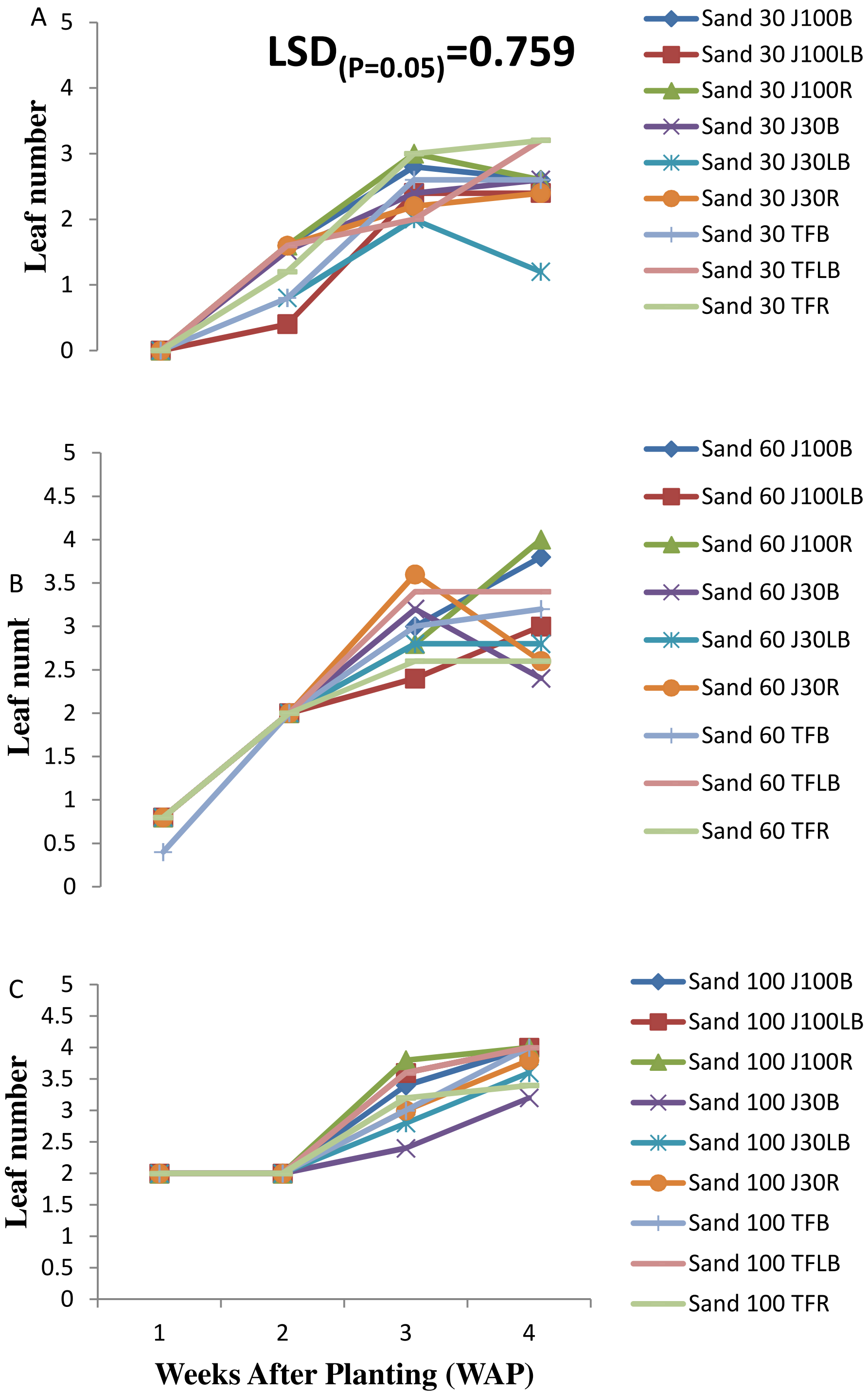

Figure 3.7: Leaf number of Bambara groundnuts landraces subjected to different water levels. (A= Leaf number at 30%FC in sand media B= Leaf number at 60%FC in sand media and C= leaf number at 100%FC in sand media). Note: J100B= Jozini brown from 100% treatment, J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment. J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment, J30R= Jozini red from 30% treatment, TFB= Tugela ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

At 30% FC in the mixture of clay + sand, there was no emergence 1 WAP; hence leaf number was measured from 2 WAP. At 4 WAP, TFB had the highest leaf number followed by J30LB = J100B > TFR = J100R = J100LB > J30B = J30R > TFLB, respectively (Figure 3.8). There were no observable trends on the provenance and seed colours. At 60% FC, leaf number was measured starting from 1 WAP. At 4 WAP J100B had the highest leaf number followed by J100LB > J100R > J30B = J30LB = TFB > J30R = TFLB > TFR, respectively (Figure 3.8). On average, Jozini provenance had more leaves than Tugela Ferry provenance. There were no observable trends in seed colour. At 100% FC, at 4 WAP, almost all landraces had obtained 4 numbers of leaves except TFB and TFR. Therefore Jozini provenance performed better than Tugela Ferry provenance; however, there were no observable trends on seed colour.

Over-all, differences were observed between water regimes; 100% FC had more leaves than at 60% FC and 30% FC, respectively. Differences were observed for soil media, the mixture of sand + clay had more leaves than in sand and clay, respectively. On average, differences were also observed between provenances in response to water field capacity and soil media. Tugela Ferry was observed to have more leaves at 30% FC, whereas Jozini provenance had more leaves at 60% and 100% FC. There were no observable trends in seed colour.

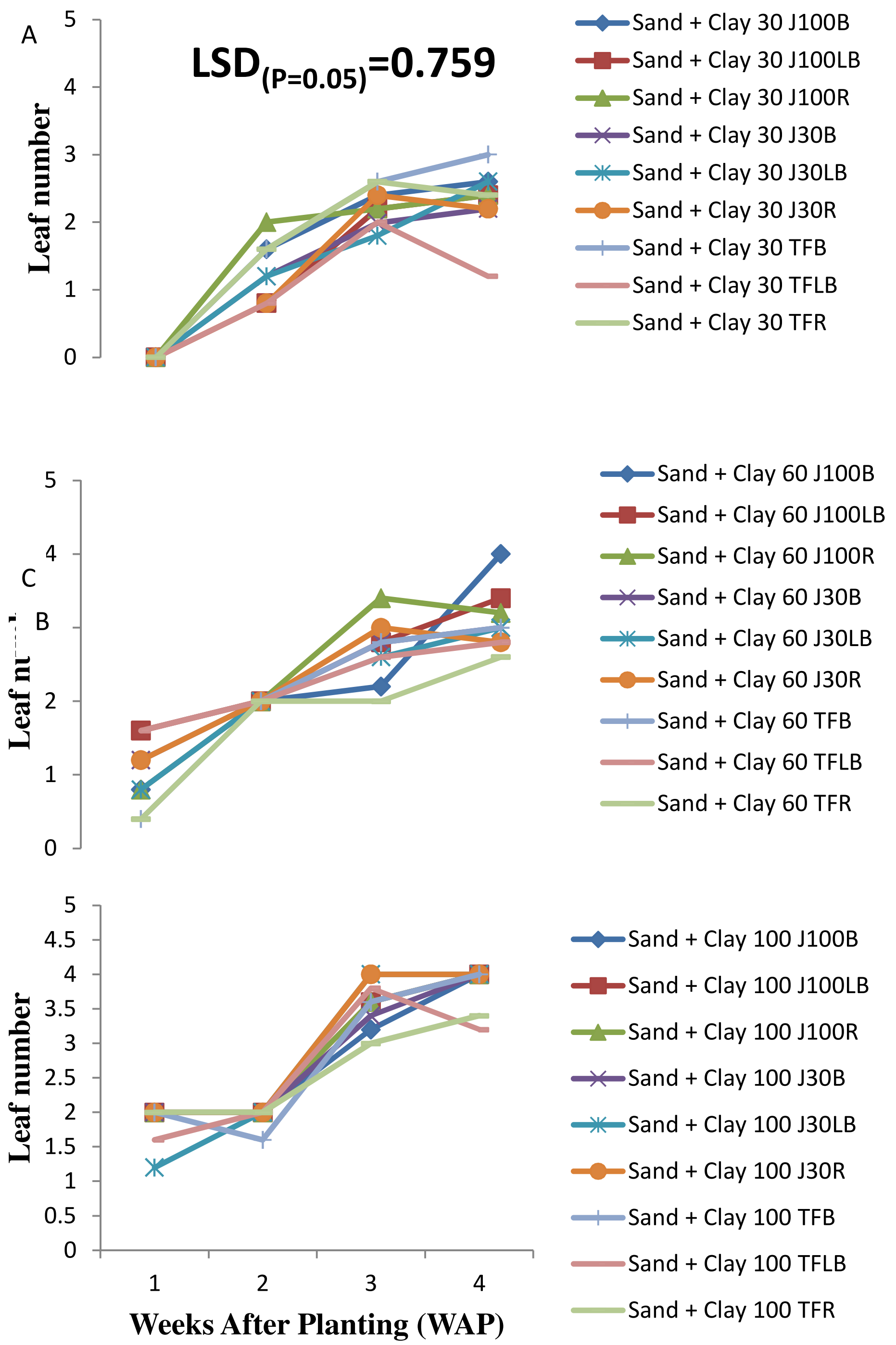

Figure 3. 8: Leaf number of Bambara groundnuts landraces subjected to different water levels under different soil media. (A= Leaf number at 30%FC in clay+sand media B= Leaf number at 60%FC in clay+sand media and C= leaf number at 100%FC in clay+sand media). Note: J100B= Jozini brown from 100% treatment, J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment. J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment, J30R= Jozini red from 30% treatment, TFB= Tugela ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

3.3.3 Seedling vigour indices

Results of all measured seedling vigour indices (root length, shoot length, root: shoot ratio, root volume, dry mass and fresh mass) showed no significant (P>0.05) interaction between media, soil water content and bambara landrace selections (Tables 3.2, 3.3 and 3.4). However, with the exception of shoot length and root: shoot ratio, all other measured parameters showed significant differences (P<0.05) between soil media (Table 3.2, 3.3, 3.4). The trend observed for root length, root volume, fresh and dry mass was such that Sand > Mixture > Clay, respectively. For shoot length the trend was such that Mixture > Sand > Clay while for root: shoot ratio it was such that Clay = Sand > Mixture. Therefore, on average, the sand and Clay+Sand media were respectively shown to be better suited for bambara seedling establishment compared with clay media (Tables 3.2, 3.3 and, 3.4).

With the exception of fresh mass, all other seedling vigour indices (shoot length, root length, dry mass, root volume, root: shoot ratio) were shown to significantly differ (P<0.05) in response to the varying water regimes. The trend observed for shoot and root length as well as fresh and dry mass was such that 100% FC > 60% FC > 30% FC. The observed trend was different for root volume which showed that 60% FC > 30% FC > 100% FC and for root: shoot ratio which was 30% FC > 60% FC > 100% FC.

Almost all vigour indices (root length, shoot length, root volume, root: shoot ratio and dry mass), with the exception of fresh mass, showed significant differences (P<0.05) between landraces (Tables 3.2, 3.3 and, 3.4). The trend observed for shoot length, root volume, fresh mass and dry mass with respect to differences between varieties, was such that J100R > J30R > TFR > J100B > J30B > TFB > J30LB > J100LB > TFLB, respectively (Table 3.2, 3.3, 3.4). Jozini provenance performed better than Tugela Ferry provenance. On average, for both provenances, the red landrace selection was shown to perform than the brown and light-brown landrace selections, respectively. Results of root length and root; shoot ratio showed that J30B had the longest roots followed by J100B > J30R > TFLB > TFB > J100R > J30LB > J100LB > TFR, respectively (Tables 3.2, 3.3 and, 3.4). Similar to observations of shoot length, the Jozini provenance had longer roots than Tugela Ferry provenance hence high root; shoot ratio. There was no clear trend with respect to seed colour. Over-all, Jozini provenances performed well, for all parameters, compared with Tugela Ferry provenance landrace selections, while dark coloured landrace selections (red and brown) performed better than light-brown landrace selections.

The interaction between soil media and water field capacity was significant ($P<0.05$) for shoot length, root: shoot ratio, root volume and dry mass, however there was no significant ($P>0.05$) interaction between soil media and water field capacity with respect to fresh mass and root length (Tables 3.2, 3.3 and, 3.4). In clay soil media, shoot length, root length, fresh mass and dry mass was high under 100%FC followed by 60%FC and 30%FC respectively, while root: shoot ratio and root volume was high at 30% FC > 60% FC > 100% FC. In sand media, shoot length, root length and root; shoot ratio was high at 100% FC > 30% FC > 60% FC, whereas root volume fresh mass and dry mass was high at 60% FC > 30% FC > 100% FC. In Clay+Sand mixture media, shoot length, root length, fresh mass and dry mass was shown to perform better at 100% and 60% FC while root: shoot ratio and root volume performed better at 60% and 30% FC.

The interaction between soil media and variety was significant ($P<0.05$) for shoot length, root length, and root: shoot ratio, however, the interaction between soil media and variety was not significantly different ($P>0.05$) with respect to root volume, fresh mass and dry mass. In clay media, shoot length, root; shoot ratio, fresh mass and dry mass was shown to be high in Jozini provenance compared with Tugela Ferry provenance. The trend was such that J30R > J100R > J100B > TFB > TFR > J100LB > J30LB > J30B > TFLB and all these vigour indices were high in dark coloured landrace selection compared with light-brown selection. For root length, in clay soil media, the trend was such that TFB > J100B > J30B > TFLB > TFR > J100LB > J30LB > J30R > J100R, respectively. There was no observed trend between provenances, however, the trend was observed for seed colour that dark coloured seeds (red and brown) performed better than light-brown seeds. In sand media, the results showed that shoot length, fresh mass and dry mass had performed similar. The trend was such that J100R > TFR > J30R > J100B > J30B > TFB > J30LB > TFLB > J100LB, while root length and root; shoot ratio also performed similar, J30LB > J30B > J30R > TFR = TFLB > J100LB > J100B > J100R > TFB. In Clay+Sand mixture media, shoot length, fresh mass and dry mass had almost performed the same. Jozini provenance performed better than Tugela ferry and dark colour landrace selection performed better than light-colour landrace selection. Root length and root; shoot ratio were also observed to have similar trend. On average, Jozini provenance was shown to perform better than Tugela ferry provenance in all soil media. Dark colour selection also out-performed light-colour selections in all soil media.

The interaction between water field capacity and landraces was significant ($P<0.05$) with respect to shoot length, root length and root: shoot ratio (Tables 3.2, 3.3 and, 3.4). However, the interaction between water field capacity and landraces was not significantly ($P>0.05$) with respect to root volume, fresh mass and dry mass (Tables 3.2, 3.3 and, 3.4). At 30% FC, it was observed that, shoot length, fresh mass and dry mass had similar trend, TFR and J100R was shown to be higher compared to other landraces. While for root length, volume and root; shoot ratio the Jozini 30 landrace was shown to perform better than other landraces. At 60% FC, the results showed that for shoot length, fresh mass and dry mass, Jozini 100 landrace performed better than other landraces, and dark colour was also observe to outperform the light-brown selection. Root length and root volume was high in Jozini 30 landrace compared to other landraces. At 100% FC, results observed show that shoot length and root length was respectively higher in J100 and J30 landraces compared with other landraces. While root volume and root; shoot ratio were respectively higher in TF and J30 landraces compared with other landraces. Dry mass and fresh mass was observed to be respectively higher in TFR, J100R and J30 landrace compared with other landraces.

Table 3.2: Vigour indices of different Bambara groundnut landraces grown at different water levels (30, 60 and 100% field capacity) in clay media.

Media	Water	Landrace	Shoot length	Root length	Root volume	Root :Shoot Ratio	Dry Mass	Fresh Mass
			-----(mm)----		(ml)		------(g)-----	
CLAY	30% FC	J100B	40.00	35.00	1.67	1.49	0.36	0.86
		J100LB	36.67	28.33	1.50	1.01	0.27	1.15
		J100R	46.67	28.33	1.33	0.64	0.32	1.08
		J30B	35.00	36.67	1.50	1.07	0.16	0.72
		J30LB	31.27	30.30	1.95	0.99	0.11	1.74
		J30R	38.33	30.00	1.07	0.8	0.36	1.05
		TFB	58.33	46.67	1.50	0.67	0.20	1.38
		TFLB	53.33	43.33	1.33	0.74	0.20	1.08
		TFR	70.00	36.67	1.67	0.75	0.35	1.35
	60% FC	J100B	58.33	33.33	1.33	0.62	0.34	1.41
		J100LB	50.00	38.33	1.67	0.52	0.19	1.63
		J100R	76.67	26.67	1.67	0.60	0.33	1.32
		J30B	63.33	33.33	1.50	0.53	0.35	1.49
		J30LB	53.33	33.33	1.17	0.51	0.26	1.18
		J30R	65.00	30.00	1.50	0.64	0.36	1.40
		TFB	50.00	38.33	1.50	0.71	0.29	1.45
		TFLB	45.00	30.00	1.50	0.67	0.18	1.22
		TFR	55.00	35.00	1.50	0.70	0.21	0.91
	100% FC	J100B	116.67	50.00	1.00	0.40	0.36	1.75
		J100LB	103.33	40.00	0.50	0.39	0.30	0.90
		J100R	130.00	41.67	1.03	0.38	0.38	1.71
		J30B	108.33	45.00	1.50	0.42	0.31	1.29
		J30LB	110.00	41.67	1.00	0.35	0.27	1.18
		J30R	120.00	38.33	0.83	0.36	0.38	2.12
		TFB	93.33	43.33	0.83	0.47	0.38	0.99
		TFLB	93.33	40.00	0.50	0.33	0.27	0.91
		TFR	120.00	36.67	1.00	0.40	0.47	1.46
P			**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**
CV%			**20.2**	**16.3**	**25.4**	**38.8**	**29.5**	**28.5**
$LSD_{(P=0.0.5)}$			**23.8**	**10.5**	**0.61**	**0.39**	**0.15**	**0.69**

NB Provenances: J100B=Jozini brown from 100% treatment, J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment, J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment and J30R= Jozini red from 30% treatment, TFB=Tugela Ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

Table 3.3: Vigour indices of different Bambara groundnut landraces grown at different water levels (30, 60 and 100% field capacity) in Sand media.

Media	Water	Landrace	Shoot length -----(mm)----	Root length	Root volume (ml)	Root :Shoot Ratio	Dry Mass ------(g)-----	Fresh Mass
SAND	30% FC	J100B	80.00	43.33	1.83	0.54	0.39	1.97
		J100LB	68.33	36.67	1.83	0.55	0.26	1.72
		J100R	95.00	40.00	2.00	0.43	0.31	2.08
		J30B	61.67	51.67	2.00	0.71	0.39	1.95
		J30LB	53.33	45.00	1.67	0.87	0.34	1.71
		J30R	73.33	46.67	2.00	0.77	0.30	2.16
		TFB	78.33	33.33	2.00	0.34	0.37	1.43
		TFLB	68.33	33.33	1.50	0.52	0.28	1.31
		TFR	98.33	31.67	1.67	0.42	0.32	2.25
	60% FC	J100B	63.33	43.33	2.33	0.56	0.37	1.99
		J100LB	46.67	36.67	1.50	0.82	0.41	1.67
		J100R	80.00	36.67	2.00	0.59	0.39	2.19
		J30B	61.67	41.67	1.67	0.70	0.32	1.91
		J30LB	58.33	36.67	1.67	0.69	0.24	1.72
		J30R	66.67	48.33	2.17	0.73	0.29	1.43
		TFB	78.33	38.33	1.67	0.41	0.28	2.00
		TFLB	60.00	38.33	1.67	0.64	0.36	1.82
		TFR	95.00	38.33	2.00	0.49	0.30	1.90
	100% FC	J100B	90.00	46.67	2.17	0.52	0.30	2.19
		J100LB	78.33	41.67	1.67	0.35	0.24	2.05
		J100R	120.00	45.00	2.00	0.59	0.38	2.42
		J30B	66.67	55.00	1.67	0.83	0.39	1.74
		J30LB	50.00	63.33	1.50	1.36	0.34	1.36
		J30R	90.00	43.33	1.50	0.50	0.31	1.57
		TFB	63.33	45.00	1.83	0.72	0.35	2.42
		TFLB	53.33	43.33	1.50	0.64	0.32	1.72
		TFR	68.33	46.67	1.69	0.89	0.32	1.87
		P	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**
		CV%	**20.2**	**16.3**	**25.4**	**38.8**	**29.5**	**28.5**
		LSD$_{(P=0.0.5)}$	**23.8**	**10.5**	**0.61**	**0.39**	**0.15**	**0.69**

NB Provenances: J100B=Jozini brown from 100% treatment, J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment, J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment and J30R= Jozini red from 30% treatment, TFB=Tugela Ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

Table 3.4: Vigour indices of different Bambara groundnut landraces grown at different water levels (30, 60 and 100% field capacity) in Clay + Sand media.

Media	Water	Landrace	Shoot length -----(mm)----	Root length	Root volume (ml)	Root :Shoot Ratio	Dry Mass ------(g)-----	Fresh Mass
CLAY + SAND	30% FC	J100B	53.33	35.00	1.17	0.67	0.26	1.44
		J100LB	45.00	35.00	1.19	0.79	0.26	1.48
		J100R	68.33	40.00	1.67	0.59	0.32	1.82
		J30B	55.00	40.00	1.69	0.67	0.27	1.50
		J30LB	43.33	33.33	2.00	0.81	0.23	2.01
		J30R	66.67	40.00	1.67	0.76	0.26	1.64
		TFB	43.77	30.30	1.19	0.69	0.23	1.47
		TFLB	43.70	45.13	1.09	0.80	0.26	1.66
		TFR	46.67	31.67	1.50	0.69	0.24	1.57
	60% FC	J100B	81.67	45.00	1.33	0.42	0.29	1.53
		J100LB	63.33	36.67	1.33	0.64	0.31	1.48
		J100R	108.33	43.33	1.50	0.56	0.24	1.46
		J30B	100.00	38.33	1.50	0.37	0.29	1.07
		J30LB	73.33	26.67	1.50	0.44	0.31	1.33
		J30R	105.00	45.00	1.69	0.45	0.28	1.57
		TFB	76.67	35.00	1.50	0.35	0.37	1.46
		TFLB	65.00	45.00	1.17	0.71	0.30	1.13
		TFR	100.00	36.67	1.67	0.53	0.21	1.30
	100% FC	J100B	95.00	50.00	1.33	0.43	0.28	1.73
		J100LB	76.67	40.00	1.33	0.53	0.27	1.49
		J100R	116.67	51.67	1.33	0.55	0.48	1.48
		J30B	103.33	31.67	1.50	0.31	0.31	1.46
		J30LB	98.33	41.67	1.17	0.43	0.33	1.41
		J30R	120.00	45.00	1.00	0.38	0.40	1.41
		TFB	51.67	43.33	1.00	0.59	0.29	1.04
		TFLB	63.45	47.39	0.78	0.61	0.34	0.62
		TFR	81.67	38.33	1.19	0.74	0.39	1.73
		P	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**
		CV%	**20.2**	**16.3**	**25.4**	**38.8**	**29.5**	**28.5**
		LSD$_{(P=0.0.5)}$	**23.8**	**10.5**	**0.61**	**0.39**	**0.15**	**0.69**

NB Provenances: J100B=Jozini brown from 100% treatment, J100LB= Jozini light-brown from 100% treatment, J100R= Jozini red from 100% treatment, J30B= Jozini brown from 30% treatment, J30LB= Jozini light-brown from 30% treatment and J30R= Jozini red from 30% treatment, TFB=Tugela Ferry brown, TFLB= Tugela Ferry light-brown and TFR= Tugela Ferry red.

3.3.4 Seedling proline content

Due to limitations in seedlings, proline accumulation was only evaluated for the 30% and 100% FC regimes and only Tugela Ferry and Jozini seeds were used. Proline content showed highly significant ($P<0.001$) differences between water regimes and varieties (Figure 3.9).

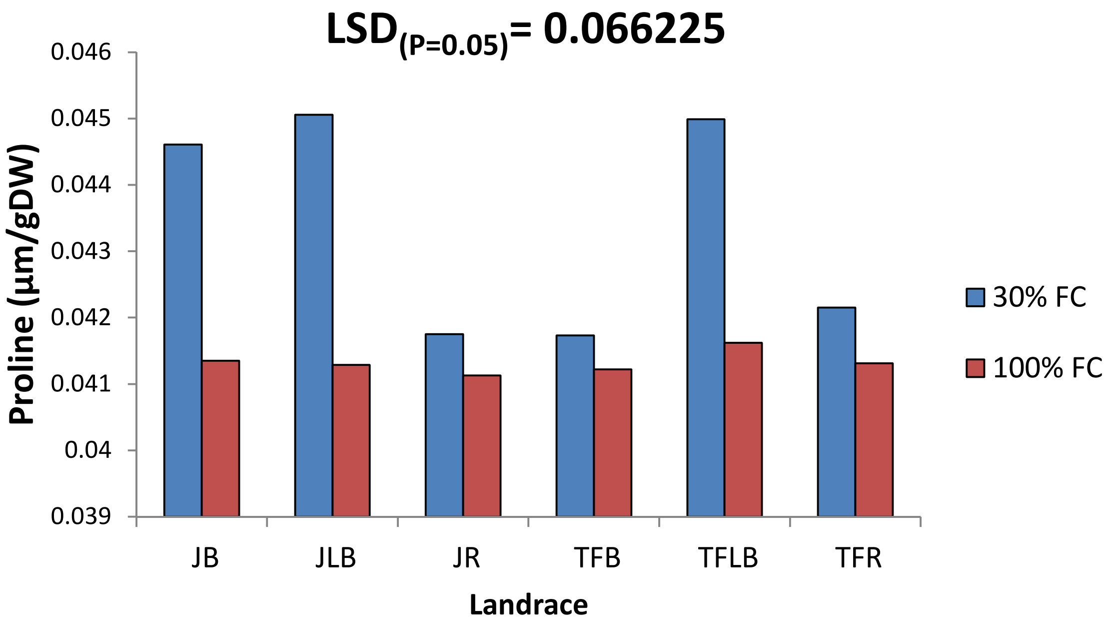

Figure 3.9: Proline content of Bambara groundnuts landrace selections in response to different water levels (30% & 100% field capacity).

The interaction between water regimes and varieties was also highly significant ($P<0.001$). Proline content was higher at 30% FC relative to 100% FC (Figure 3.9). At 100% FC, TFLB had the highest proline content followed by JB > JLB > TFR > TFB > JR, respectively. At 30% FC, JLB had the highest proline content followed by TFLB > JB > TFR > JR > TFB, respectively. Jozini provenance showed higher levels of proline at 30% FC compared with Tugela Ferry provenance. Interestingly, light-brown landrace selections were shown to accumulate more proline content under water stress compared with dark coloured landrace selections.

3.4 Discussion

The objective of this study was to evaluate seedling establishment of bambara groundnut, within the context of provenance and seed coat colour, in response to different water regimes and different soil media under controlled environment conditions. According to Hartmann *et al.* (2007), seedling establishment is influenced by many factors such as type of growth media and environmental factors such as oxygen, water and temperature. Growth media and water are generally believed to be the most critical factors determining seedling quality in the nursery (Baiyeri and Mbah, 2006); this is because growth media act as a reservoir of nutrients and water (Grower, 1987). As such, physical properties of growth media can have a profound effect on the availability of water and air to the growing plant (Baiyeri, 2005).

The results of this study showed that emergence was high at 100% FC compared with 60% and 30% FC, respectively, as expected. The failure of bambara landraces to establish successfully at 60% and 30% FC suggests that seedling establishment in bambara is sensitive to water availability. This concurs with reports by Sesay (2009) that bambara groundnut emergence was higher at high levels of water compared with low water level. This was also similar with the results that were reported for bambara groundnut by (Massawe *et al*., 1999), and a range of other crop species, including tea (*Camellia sinensis* L.) (Habib *et al.,* 1990), sunflower (*Helianthus annus* L.) (Singh and Vig, 1981), maize (*Zea mays* L.), rice (*Oryza sativa* L.) and chickpea (*Cicer arietinum* L.) (Harris *et al*., 1999). However, the great variability that exists within and between bambara landraces (Massawe *et al*., 1999) makes it difficult to conclude definitively on their response to water. In certain instances, certain landraces emerged well under water stress while other failed to emerge. This suggests that there may be landraces capable of emerging under conditions of limited water availability – an adaptation that may be hugely beneficial in dryland farming.

Results of the current study showed that dark coloured selections had better emergence compared with light-brown selections. This may be due to the fact that dark coloured seeds are more vigorous than light-brown seeds. Seed colour is thought to be associated with water uptake; several studies have reported a relationship between seed coat colour and water uptake (McDonald *et al*., 1988; Hou and Thseng, 1991). Sinefu (2011) observed that dark coloured seeds had a thick seed coat and low electrolyte leakage whereas light-coloured seeds had a relatively thin seed coat and higher electrolyte leakage. This relationship explained the low seed quality and emergence of light-coloured bambara landraces. To the contrary, there have been other authors working on bambara groundnuts who, reported that cream coloured seeds emerged faster because they were able to imbibe water faster than dark coloured seeds (McDonald *et al*., 1988; Hou and Thseng, 1991). However, rapid imbibition in light-coloured seeds may result in imbibitional injury leading to poor emergence (Mabhaudhi, 2009; Sinefu, 2011).

The results of this study showed that emergence was respectively higher in Clay+Sand and sand media compared with clay media. Emergence of bambara landrace selections was slow and erratic in clay media. This may have been due to the fact that emergence is greatly affected by the aggregate size of soil particles in which they are sown (Idu *et al*., 2003). Poor aeration, water logging and an impervious layer formed by the compact structure of clay may have accounted for the low emergence (Idu *et al*., 2003). Large particle size of sand allows for good drainage and aeration (Idu *et al*., 2003). Small particle size in clay makes it compact and this may have impeded emergence. Large particle size in clay may have meant that seeds required less energy to emerge hence high emergence observed in clay. Pandaya and Bighela (1973); Boada (1976) and Anoliefo and Gill (1992) using seeds of *Celosia argentea*, *Eucalyptus degulta* and *Bauhinia monandra*, respectively, observed high seedling emergence in sand medium. The high emergence obtained for the Sand+Clay mixture may have been due to its lack of such chemical and physical properties as with clay. Another reason for good emergence in the Sand+Clay mixture could be that, in theory, the Sand+Clay mixture would have good aeration and drainage as well as good water holding capacity. It does appear from the results of this study that Sand+Clay and sand may be the most ideal media for raising seedlings of bambara. Under field conditions, fine particles such as clay soil often result in an impervious crust due to raindrop impact hence deterring seedling emergence.

Growth of bambara seedlings, as measured by seedling height and leaf number, was shown to be affected by both water availability and soil media type. Seedling height and leaf number were higher at 100% FC and lower at 60% and 30% FC, respectively. Reduced seedling growth, especially at 30% FC, may be attributed to impairment of cell division and expansion caused by water stress (Hussain *et al*., 2008). Growth is a turgor driven process, hence a limitation on water availability will naturally retard growth (Taiz and Zeiger, 2006). Water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq *et al*., 2009). Another explanation for low seedling height and leaf number observed at 30% FC could be linked to poor and slow emergence; this will affect seedling establishment and plant population.

Seedling growth (plant height and leaf number) was higher in Sand+Clay mixture and sand media compared with clay media which showed the least growth. Reduced plant height and leaf number in clay media maybe attributed to the physical properties of clay soil. The high water holding capacity and poor aeration of clay could have resulted in brief periods of water logging; this could have resulted in oxidative stress in the roots resulting in poor seedling growth. Plant height and leaf number was shown to perform best in the clay+sand mixture; this is because the mixture was an ideal media with fairly balanced proportions of sand and clay.

Results of seedling vigour characteristics showed that shoot length, root length, fresh and dry mass were respectively lower at 60% and 30% FC, relative to 100% FC. Reductions in root and shoot lengths could be due to impairment of cell division and enlargement caused by water stress (Bahrami *et al*., 2012). This was in accordance with reports by Khalil and Grace (1992) in sycamore; Ibrahim (1995) in poplar and Pokhriyal *et al*. (1997) in *Acacia nilotica*. Contrary to this, Osonubi *et al*. (1992) reported that Faidherbia albida (*A. albida*) tolerated drought stress by producing long taproots whereas *A. nilotica* tolerated drought stress by developing larger root systems able to explore a greater volume of soil. Seiler and Gazell (1990) concluded that extreme soil drying ultimately reduced root growth. This was supported by results of the present study observed at 30% FC which showed low root growth in bambara seedlings. However, root volume and root: shoot ratio were shown to increase in response to decreasing water availability. Increase in root: shoot ratio may be due to the fact that when water supply is limiting allocation of assimilates tends to be modified in favour of root growth (Hsiao and Acevedo, 1974, Ibrahim, 1995). In addition, shoot growth is more affected by water stress than root growth (Bongarten and Teskey 1987: Wilson, 1988). Joly *et al*. (1989) considered this as an adaptation that restricts transpiration surface area while increasing soil water capture from the soil. These findings were in harmony with reports by Barrose and Barbose (1995) for *Acacia farnesiana*. Low fresh and dry mass could be due to reductions in root and shoot lengths

There was an interaction between media and field capacity. In clay media, the seedling vigour parameters were highest at 100% and 60% FC, respectively relative to 30% FC; this was contrary to our expectation. Our expectation was that, in clay, these parameters would perform better at 60% and 30%FC due to problems of water logging and poor drainage. The results showed that in sand media, the pattern of seedling vigour indices was such that 100% FC > 30% FC > 60% FC, respectively. The good performance at 100%FC was due to the fact that sand has low water holding capacity; hence water drains easily therefore no water logging. Interestingly, seedling vigour indices were higher at 30% FC relative to 60% FC. This was somewhat contrary to our expectation that stress in sand would be severe at 30% FC due to rapid loss of water from the sand media.

Bambara seedlings were able to accumulate proline in response to water stress at 30% FC. Jozini provenance accumulated more proline under water stress compared with Tugela Ferry provenance. Light-brown landrace selections were shown to have higher levels of proline under water stress compared with dark coloured landrace selections. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Kishor *et al*., 1995). Vurayai *et al* (2011) observed higher proline concentration in leaves of stressed bambara groundnut than non-stressed plants. The increase in proline concentration under water stress has been observed in other crops like maize (Mohammadkhan and Heidari, 2008), wheat (Johari and Pireivatlou *et al*., 2010) and cowpeas (Patil, 2010). These results were also in harmony with the finding of Azza *et al.* (2006) on *Taxodium distichum.* The precise physiological significance of proline in stressed plants has yet to be fully elucidated. Many researchers have ascribed a positive role to proline under water stress, suggesting that proline is a source of energy, carbon and nitrogen alleviating stress shock (Delauney and Verna, 1993; Hare *et al.*, 1998). In contrast, Hanson *et al.* (1977) considered proline accumulation to be a symptom of damage rather than tolerance. In the current study, basing on results of seedling growth and vigour indices, proline accumulation in the Jozini provenance as well as light-coloured landrace selections, may be taken to imply that it was an indicator of damage as opposed to tolerance. Other parameters measured in the study suggested that Tugela Ferry provenance and darker coloured landrace selections were better adapted to water stress. Our observations, with respect to seed colour, concur with reports by Mabhaudhi (2009) who also observed low proline content in leaves of a dark coloured maize landrace that was drought tolerant at the seedling stage.

The two bambara provenances used in this study showed differences with regards to their responses to varying water regimes and different media. Tugela Ferry provenance showed resilience to water stress by performing well at 30% FC, whereas Jozini performed better at 60% and 100% FC, respectively. The Jozini 30 landrace selections performed better at 60% FC while Jozini 100 landrace selections performed better at 100% FC in all soil media. This was very interesting, since our expectation was that seeds from the previously stressed treatments would perform well under stress while the J100 would perform well at 100% FC and poorly at water stress. Dark coloured landrace selections were found to perform better under all field capacities and in all soil media.

3.5. Conclusion

The results in this study indicated that different soil media at different water regimes have an impact in seedling growth and establishment in bambara. Sand and the mixture of sand+clay is best suited for the establishment of bambara groundnut seedlings. It is concluded that bambara can be affected by water availability. Tugela Ferry provenance seeds were more drought tolerant compared to Jozini provenance seeds and the dark coloured selection is more drought tolerant compared with light-brown one.

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Chapter 4

Field performance of bambara groundnut landraces under irrigated and rainfed conditions

4.1 Introduction

Previously, most investment in agricultural research has primarily focused on a few major crops such as wheat, rice, maize, and cotton (Shewry, 2009; Dawe, 2008; Mabhaudhi, 2009; Smale, 2012). However, owing to the challenges posed by climate change (Petit *et al*., 1999) and increasing food insecurity, interest in minor crop species has now increased throughout the world (Mabhaudhi, 2009). The aim is to identify and develop new crops (Kouassi and Zorobi, 2011) that will be suited to the predicted future conditions, mainly drought. Bambara groundnut (*Vigna subterranea* L. Verdc) is one of Africa's minor crops that has recently received interest from governments, research institutions and other researchers. This is because of its several promising agronomic characteristics, such as yield, resistance to diseases as well as adaptability to poor soils and drought (Elia and Mwandemele, 1986; Collinson *et al*., 1997; Brink, 1999; Massawe *et al*., 2003; Mwale *et al*., 2007).

Bambara groundnut is consumed in different forms and is highly nutritious, consisting of about 63% carbohydrate, 19% protein, and 6.5% oil (Goli, 1997). Several researchers (Linnemann, 1988; Akani *et al*., 2000; Atiku *et al.,* 2004) reported that bambara groundnut can be used for baby food, human consumption and industrial products as well as for animal feed. In countries like Zambia, bambara groundnuts are ground into a flour and used for baking bread (Linnemann, 1990). Brough *et al*. (1993) highlighted that milk prepared from bambara groundnut gave a flavour that was more preferable to that of milk from other legumes such as cowpea, pigeon pea and soybean. In other countries, it is used as a snack, relish and medicine. The crop also has high ceremonial value in certain cultures (Atiku, 2000). In addition, bambara plays a big role as a legume crop capable of fixing atmospheric nitrogen and is beneficial in crop rotation (Gueye *et al*., 1998). Despite having much potential and a variety of uses, bambara groundnut has received scant research attention compared to other legumes (Mabhaudhi *et al*., 2011). In much of Africa, including South Africa, the crop is still produced by subsistence farmers who rely on landraces (Doku, 1997).

Drought is one of the most important environmental stresses limiting global crop production (Bohnert *et al.,* 1995). It affects crop growth and physiology at any stage limiting yields (Liu *et al*., 2003). The study done by Collinson *et al*. (1996) in Zimbabwe on a bambara groundnut landrace reported significant reductions in pod number per plant, harvest index and final seed yield due to terminal drought. Inter-and intra-species differences with respect to plant responses to drought are frequently observed (Leport *et al*., 1999; Baigorri *et al*., 1999; Collino *et al*., 2000; Liu and Stutzel, 2002a; Liu and Stutzel, 2002b; Mwale *et al*., 2007). This may provide a basis for selecting landraces suitable for production in drought-prone regions. In addition, crop physiologists have identified a range of physiological and biochemical traits that contribute to drought tolerance (Turner *et al*., 2001). These can be used to explore genotypic diversity of landraces when selecting for drought tolerance.

Drought tolerance in bambara groundnut has previously been ascribed to an ability to maintain leaf turgor pressure through a combination of osmotic adjustment, reduction in leaf area and effective stomatal regulation (Collinson *et al*., 1997). Plants grown under drought conditions normally exhibit lower stomatal conductance relative to plants grown under non-stress conditions (Turner, 1986; Hirayama *et al*., 2006), as a mechanism to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for biomass and yield (Vurayai *et al,* 2011). Diffusive resistance of stomata to CO_2 entry is probably the main factor limiting photosynthesis under drought (Boyer, 1970). Severe drought stress also inhibits photosynthesis by causing changes in chlorophyll content, and by damaging photosynthetic apparatus (IturbeOrmaetxe *et al*., 1998). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995).

Given its drought tolerance and high nutritional value, bambara groundnut has a potential to reduce food insecurity in dry areas of Africa where most crops would fail. Unfortunately, until recently, very little research has been done on the crop, leaving the crop to be described as underutilized. The crop is still cultivated using landraces of which little information is available describing their agronomy. The extent of genetic diversity of local bambara groundnut landraces is still unknown. As such, there is a need to characterise landraces from different geographical locations in South Africa for drought tolerance. In this regard, seed colour may also be a useful tool for selection. Previous research (Powell, 1989; Zulu and Modi, 2010; Sinefu, 2011) has suggested a link between seed colour, seed quality and possible drought tolerance. The aim of this study was to evaluate the performance of two different bambara groundnut provenances under irrigated and rainfed conditions during winter. Secondary to this, the use of seed colour as a possible selection criterion for drought tolerance was also evaluated.

4.2 Materials and methods

4.2.1 Planting material

Two bambara groundnut landraces (provenances) were obtained from Tugela Ferry (28˚45',S; 30˚27' E, <543 masl) and Jozini (27°26 S; 32°4' E; <500 masl). Seeds from Tugela Ferry were obtained from smallholder farmers during February 2012. The seed had been produced under rainfed conditions during the 2011/12 season. Although seed from Jozini were initially sourced from subsistence farmers in Jozini, seeds used in this study were obtained from experiments conducted in Pretoria, whereby seeds had been subjected to different water stress treatments. Bambara landrace seeds were then sorted into three distinct colours: red, light brown and brown. Seeds were characterised according to seed coat colour based on previous studies that have suggested that seed coat colour may have an effect on early establishment performance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu 2011).

4.2.2 Description of experimental site

A field experiment was planted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 845 masl) during the 2012 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 (November – December and February-March). The long-term mean rainfall and temperatures for Ukulinga are presented in Table 4.1.

Table 4.1: Long-term mean rainfall and temperatures (maximum and minimum) for Ukulinga.

	Feb	**Mar**	**April**	**May**	**June**	**Jul**	**Aug**
Tmax (°C)	27.2	26.6	24.5	23.5	20.7	20.3	22.7
Tmin (°C)	17.0	16.4	13.7	11.7	8.7	7.9	9.5
Rainfall (mm)	72	103	49.1	20.8	11.1	6.1	41.8

4.2.3 Field layout and experimental design

The experimental design was a split- plot design with irrigation (full irrigation and rainfed) as a main factor. Landrace provenance and seed colour (brown, red and light brown) was the sub- factor. The experiment was arranged in a randomised complete block design (RCBD), with three replications. The size of the whole field trial was 150.4 m^2. The size of main plots (IRR and RF) measured 51.7 m^2 each, with 10 m spacing between them. The sprinklers were designed to have a 6 m spray radius in order to prevent water sprays from irrigated plots from reaching the RF plots. Sub-plot size was 0.9 m^2, and plant spacing was 0.3 m x 0.2 m, translating to 24 plants per plots. Two seeds per station were sown directly at a depth of 20 mm and later thinned to one seedling per station at the first leaf-stage. The rainfall that was received during the planting season was 120.4 mm and the supplemented irrigation was 264 mm.

4.2.4 Data collection

Plant emergence was measured weekly starting from seven days after planting (DAP), until full emergence. Full emergence was defined as when plants had attained 90% emergence. Following this, plant height, leaf number, leaf area index (LAI), stomatal conductance and chlorophyll content index (CCI) were measured weekly. Leaf number was counted for leaves with at least 50% green area; each trifoliate was counted as one leaf. Plant height was measured from the base of the plant to the base of the longest stem. Stomatal conductance and chlorophyll content index were measured on the abaxial and adaxial surfaces, respectively, using a steady state leaf promoter (Model SC-1, Decagon Devices, USA) and CCM-200 *Plus* chlorophyll content meter (OPTI-SCIENCES, USA). Leaf area index was measured using the LAI 2200 canopy analyser (Li-Cor, USA & Canada). Yield and yield components such as biomass, harvest index, pod number, pod mass, grains per pod and seed mass were measured at harvest.

4.2.5 Crop management

Prior to planting, soil samples were taken for fertility and textural analyses. Land preparation involved ploughing, disking and rotovating to achieve fine tilth. Using results of soil fertility analysis, an organic fertiliser, Gromor Accelerator® (30 g kg^{-1} N, 15 g kg^{-1} P, and 15 g kg^{-1} K) was applied immediately after planting to meet crop nutritional requirements (Swanevelder, 1998). Weeding was done using hand-hoes and hands to avoid damaging roots of the crop.

4.2.6 Weather and soil water content

Weather data (maximum and minimum temperature, maximum and minimum relative humidity, reference evapotranspiration and rainfall) were obtained from an automatic weather station located within 50 m radius from the experimental site. Soil water content was measured using a PR2/6 profile probe connected to an HH-2 moisture meter (Delta-T Devices, UK) at depths of 100, 200, 300, 400, 600 and 1000 mm. Access tubes were inserted in both irrigated and rainfed plots to measure soil water content

4.2.7 Data analysis

Data were analysed using analysis of variance (ANOVA) from GenStat® Version 14 (VSN International, UK). Means were separated using Tukey Test in GenStat® at the 5% level of significance (Appendix 4).

5.3 Results

5.3.1 Weather data and soil water content

The respective average minimum (Tmin) and maximum (Tmax) temperatures for the duration of the trial were 13.7°C and 24.3°C respectively. The total rainfall received was 120.4 mm against an evaporation demand of 243.2 mm therefore there was a deficit of 122.8 mm. During the growing season rainfall was very limited (Figure 4.1). At the beginning of the growing season rainfall and temperatures were relatively high; thereafter, rainfall and temperature decreased with the onset of winter. The base temperature for bambara groundnuts is 10^{o}C which means that below this temperature this crop cannot survive. It is shown in the graph (figure 4.1) that as winter was setting in, there are days where the temperature was found to be below 10^{o}C.

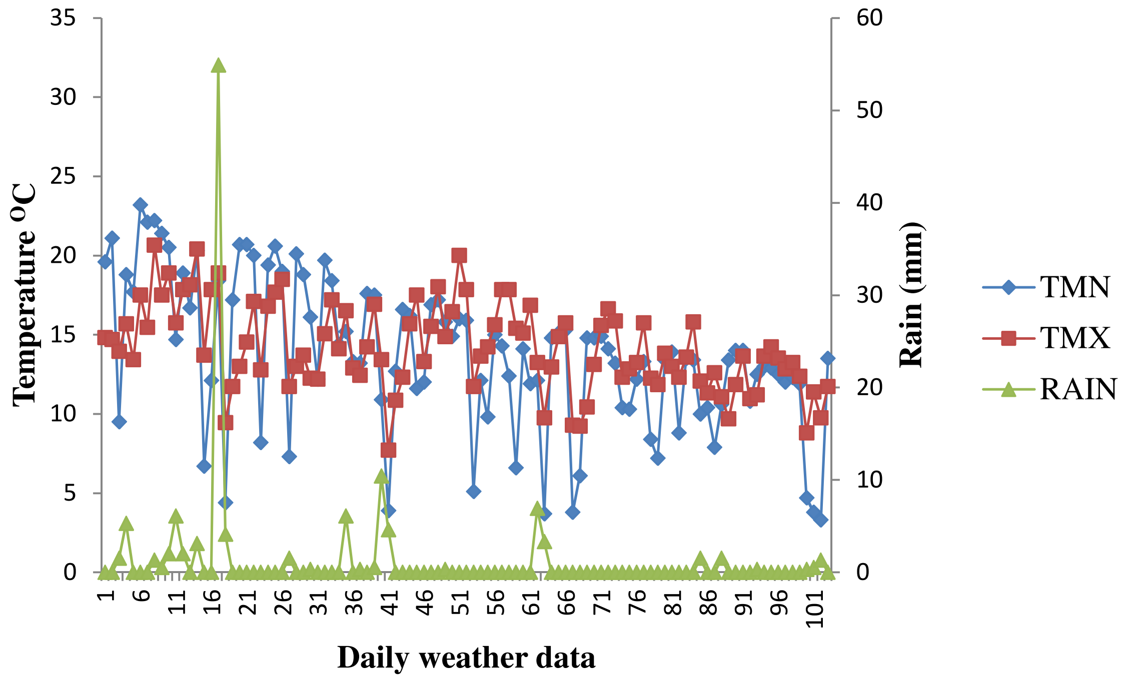

Figure 4.1: The weather condition for Ukulinga during the growing season of bambara groundnut.

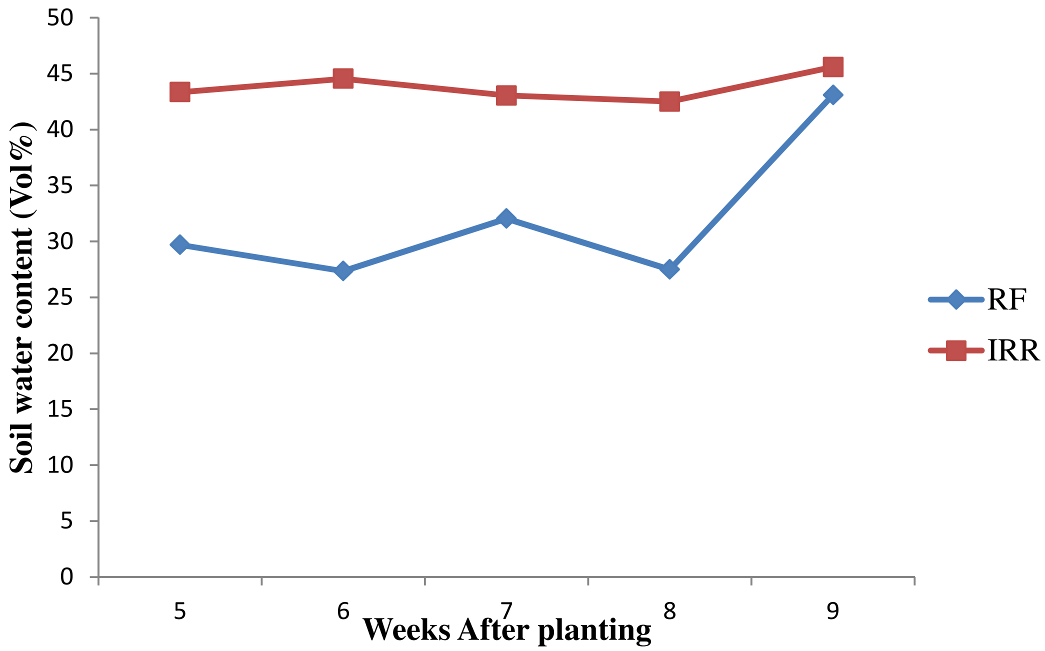

Figure 4. 2 : Soil water content that was measured during planting for both irrigated and rainfed condition.

Figure 4.2 shows the trends of soil water content observed in the irrigated and rainfed trials. Soil water content was higher under irrigated than rainfed conditions for the duration of the trials (Figure 4.2). The field capacity of the soil was 36.3%, the permanent wilting point was 23.5 and saturation point was 46.7%. As such, SWC in the irrigated trial was above FC and closer to saturation for most of the time. This suggests that they may have been brief periods of water-logging in the rooting zone thus causing aeration stress in plants under irrigated conditions. Soil water content under rainfed conditions from (5-8 WAP) was higher than wilting point and lower than field capacity.

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4.3.2 Crop establishment

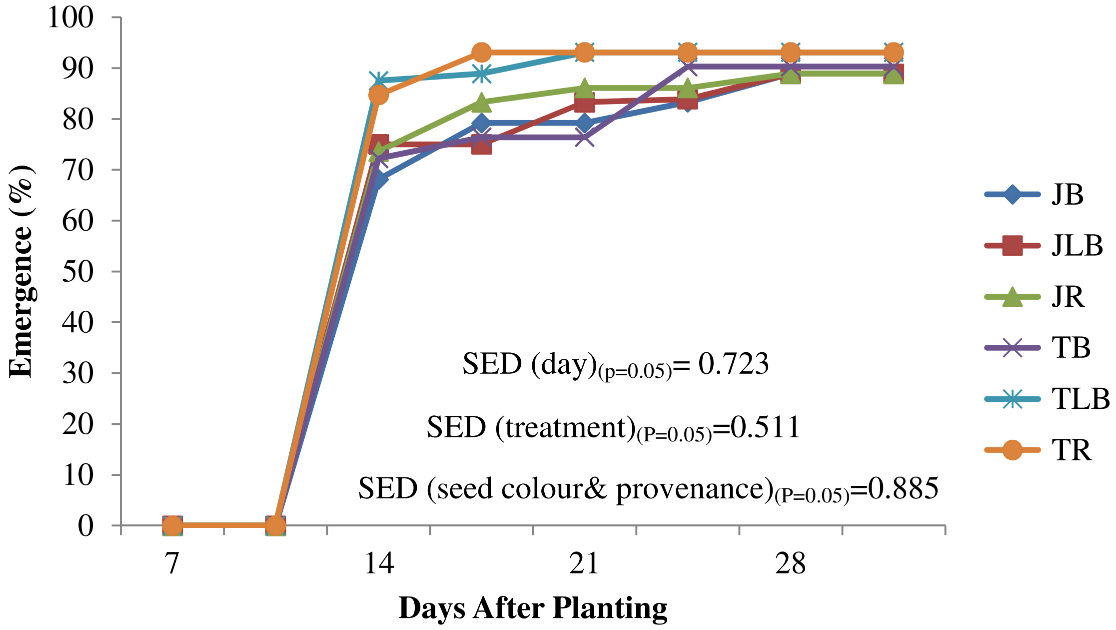

Figure 4. 3: Final emergence of Bambara groundnut in two different provenances with three different colours. NB: RF=rainfed, IRRI= irrigated, J= Jozini provenance, T= Tugela Ferry provenance, B=brown colour, LB=light brown colour, R=red colour.

Both rainfed and irrigated trials were established under full irrigation; thereafter, irrigation was from the irrigated trial. As such, results reported here are for differences between provenances and seed colours. There were no significant differences between bambara provenances and seed colours (Figure 4.3). However, there were highly significant differences ($P<0.001$) between days after planting, although the interaction between DAP and provenances was not significant. Results showed that, for all provenances and seed colours, emergence was fast during 7-14 DAP, reaching a maximum at 21 DAP (Figure 4.3). Tugela Ferry red had the highest emergence percentage on the 14th day followed by Tugela Ferry light brown, Jozini red, Jozini light brown, Tugela Ferry brown then Jozini brown respectively. There was no significant difference between the two provenances. For both provenances the red colour performed well followed by light brown then brown respectively.

4.3.3 Growth and development

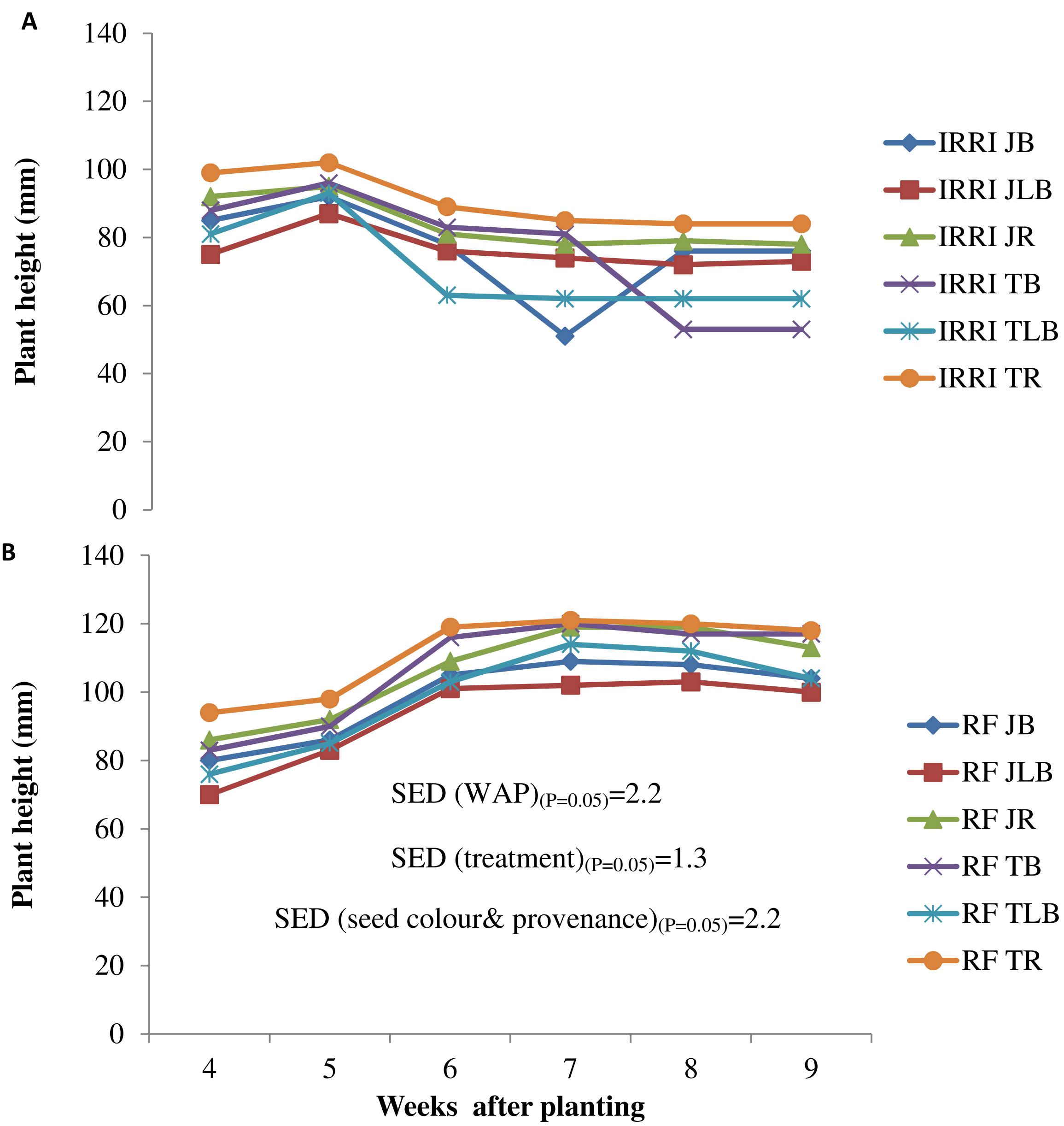

Figure 4. 4: The effect of seed coat colour and landraces on plant growth under (A) irrigated and (B) rainfed field conditions. NB: RF=rainfed, IRRI= irrigated, J= Jozini provenance, T= Tugela Ferry provenance, B=brown colour, LB=light brown colour, R=red colour.

Measurement of plant height and leaf number started 4 weeks after planting (WAP) when plants had fully established. There were highly significant differences ($P<0.001$) between water treatments, landraces as well time (WAP), with respect to plant height (Figure 4.4). Although plants had been receiving equal amounts of irrigation during establishment, results showed that, on average, at 4 WAP plant height was higher under irrigated conditions compared with rainfed (Figure 4.4). At 4 WAP, the Tugela Ferry red landrace selection had the highest plant height (99 mm) followed by Jozini red (92 mm), Tugela Ferry brown (88 mm), Jozini brown (85 mm), Tugela Ferry light brown (81 mm) and Jozini light-brown (75 mm) landrace selections, respectively. A similar trend was observed under rainfed conditions during the corresponding period. At 6 WAP, plant height under irrigated conditions started to decrease while plants under rainfed conditions continued to grow (Figure 4.4). By 9 WAP, plant height was higher under rainfed compared with irrigated conditions (Figure 4.4). Under irrigated conditions, the Tugela Ferry red landrace selection had the highest plant height (84 mm) followed by Jozini red (78 mm), Jozini brown (76 mm), Jozini light brown (73 mm), Tugela Ferry light brown (62mm) and Tugela Ferry brown (53 mm) landrace selections, respectively. Under rainfed conditions, the trend was such that Tugela Ferry red (118 mm) > Tugela Ferry brown (117 mm) > Jozini red (113 mm) > Jozini brown (104 mm) > Tugela Ferry light-brown (104 mm) > Jozini light-brown (100 mm) landrace selections, respectively. Over-all, plant height decreased significantly under irrigated conditions relative to rainfed conditions. A comparison of landrace provenances showed that, based on mean values, the Tugela Ferry provenance generally performed better than the Jozini provenance with regards to plant height. For both provenances and under both irrigated and rainfed conditions, the red landrace selections had taller plants relative to the brown and light-brown selections.

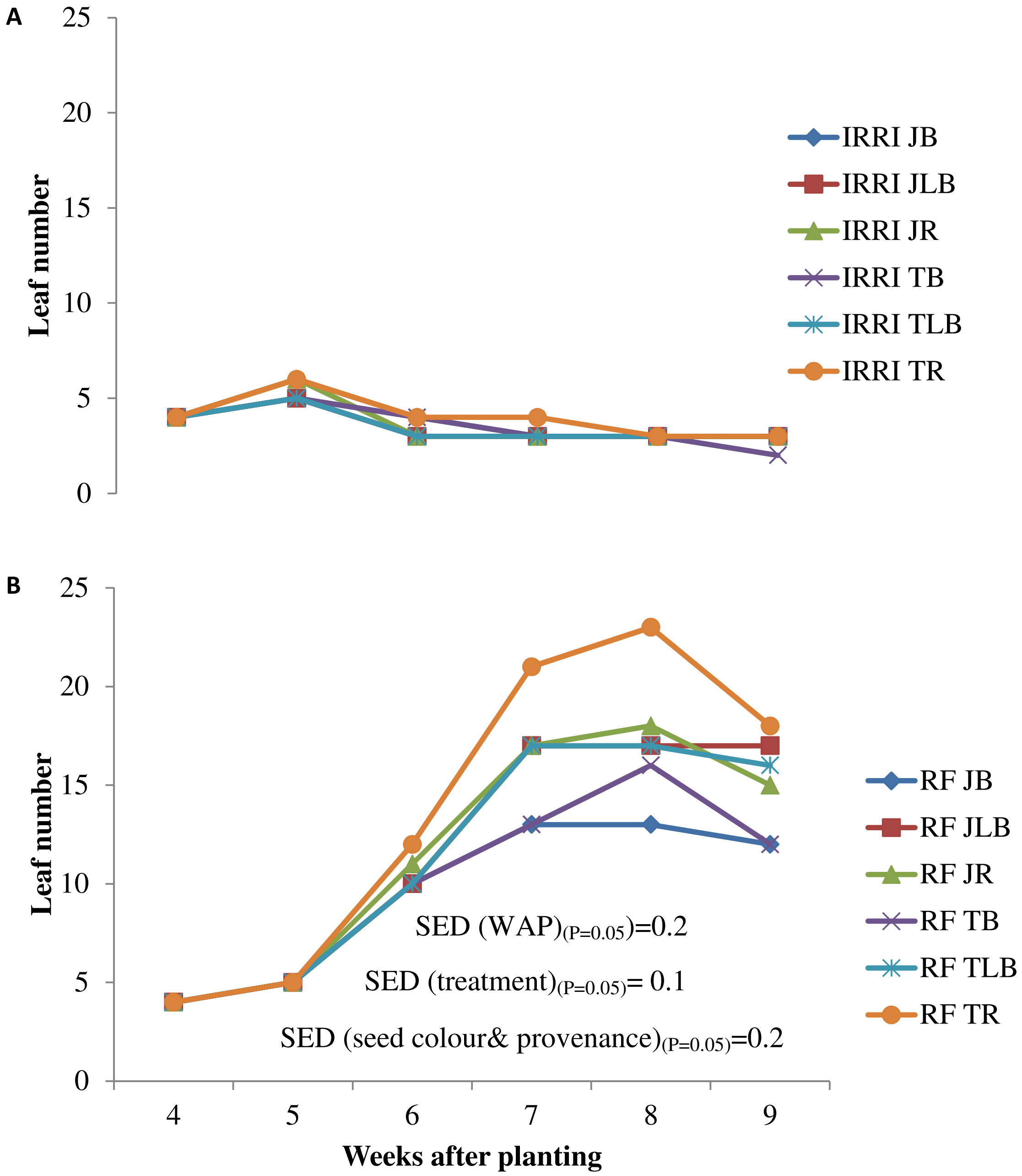

Figure 4. 5: The effect of seed coat colour and landraces on plant growth under irrigation and rainfed. NB: RF=rainfed, IRRI= irrigated, J= Jozini provenance, T= Tugela Ferry provenance, B=brown colour, LB=light brown colour, R=red colour.

There were highly significant differences ($P<0.001$) between treatments, seed colour and days with respect to leaf number (Figure 4.5). By the fourth week, leaf number was the same (4) under both irrigated and rainfed conditions. Similar to this, seed colours, for all provenances, had the same leaf number (4) under both irrigated and rainfed conditions. Leaf number continued to increase by the 5th week under both water regimes. However, at 6 WAP difference in leaf number started to show between irrigated and rainfed plants. Under irrigated conditions, leaf number was shown to decrease over time while under rainfed conditions leaf number was shown to increase over time. Under irrigated conditions, at 9 WAP, all landrace selections (brown, light-brown and red) had the same leaf number (3). Under rainfed conditions, during the same period, the Tugela Ferry red landrace selection had the highest leaf number (18) followed by Jozini light-brown (17), Tugela Ferry brown (16), Jozini red (15), Tugela Ferry light brown and Jozini brown (12) landrace selections, respectively. Despite the lower leaf number under irrigated conditions, the red colour selections, for both provenances, had the highest leaf number under both irrigated and rainfed conditions.

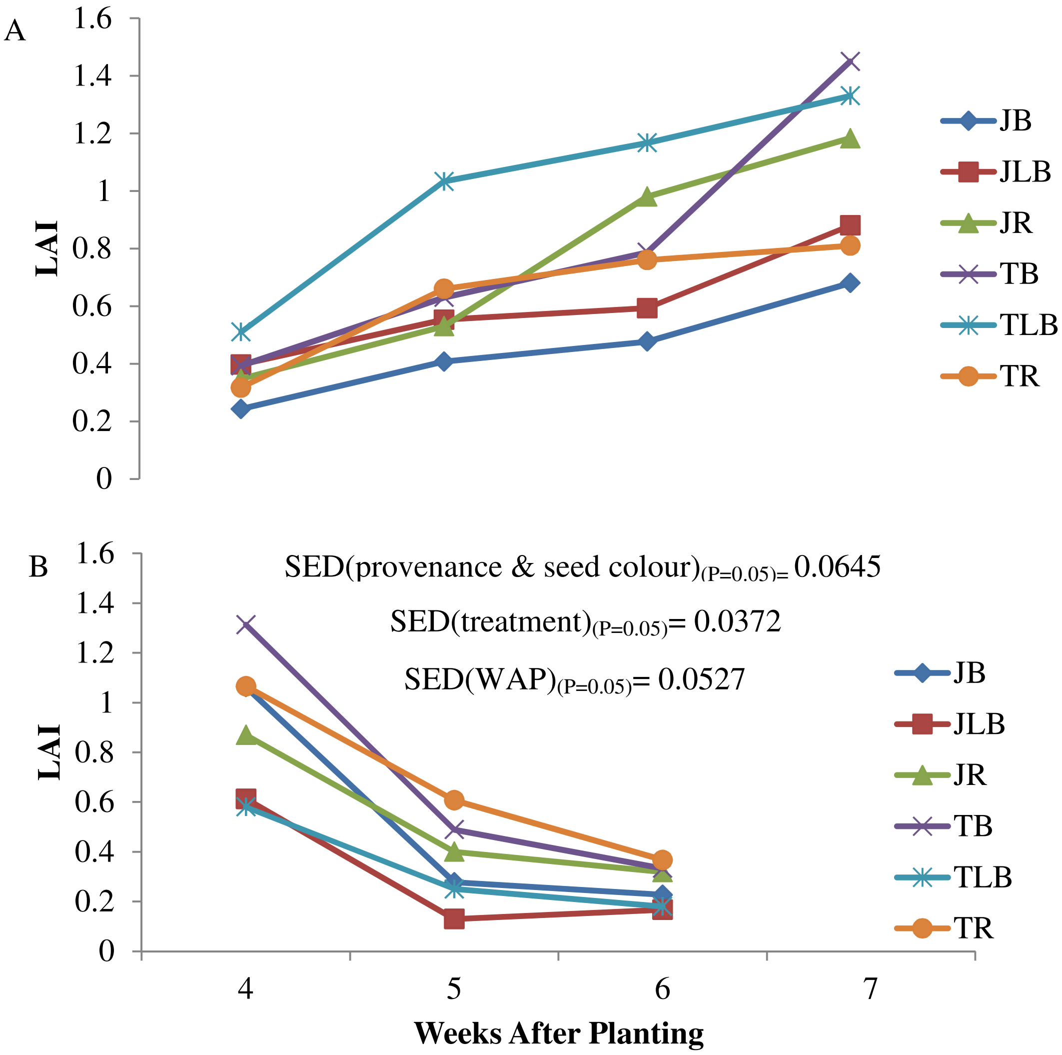

Figure 4. 6: The effect of seed coat colour and provenances on leaf area index (LAI) under two water treatments, (A) Rainfed and (B) Irrigated. NB: RF=rainfed, IRRI= irrigated, J= Jozini provenance, T= Tugela Ferry provenance, B=brown colour, LB=light brown colour, R=red colour.

There were highly significant differences ($P<0.001$) between water regimes, provenances and seed colours with respect to leaf area index (figure 4.6). There were significant differences ($P<0.05$) in leaf area index over time (Figure 4.6). The trend observed for LAI was similar to observations for leaf number (Figure 4.5). At 4 WAP, LAI was higher under irrigated than rainfed conditions (Figure 4.6). During this period (4 WAP), under irrigated conditions, the Tugela Ferry brown landrace selection had the highest LAI (1.313) followed by Tugela Ferry red (1.066), Jozini brown (1.063), Jozini red (0.870), Jozini light-brown (0.613) and Tugela Ferry light brown (0.580) landrace selections, respectively. On the other hand, under rainfed conditions, the Tugela Ferry light-brown landrace selection had the highest LAI (0.510) followed by Jozini light-brown (0.397), Tugela Ferry brown (0.393), Jozini red (0.347), Tugela Ferry red? (0.332) and Jozini brown (0.243) landrace selections, respectively. As growth a progress, LAI under irrigated conditions was observed to decline while LAI under rainfed conditions was increasing. The observed decline in LAI corresponded with observations of lower plant height (Figure 4.4) and leaf number (Figure 4.5) observed under irrigated as compared to rainfed conditions.

4.3.4 Crop Physiology

There were highly significant differences ($P<0.001$) between water regimes with respect to stomatal conductance. There were highly significant differences ($P<0.001$) in stomatal conductance measurements over time (Figure 4.7). However, stomatal conductance showed no significant differences ($P>0.05$) between seed colour selections for each provenance. Stomatal conductance under irrigated conditions show to be higher than rainfed condition (Figure 4.7). under irrigated conditions, at 6 WAP, the Jozini brown landrace selection had the highest stomatal conductance (211.3 mmol m^{-2} m^{-1}) followed by Tugela Ferry brown (200.6 mmol m^{-2} m^{-1}), Tugela Ferry red (194.2 mmol m^{-2} m^{-1}), Jozini light-brown (192.9 mmol m^{-2} m^{-1}), Jozini red (189.9 mmol m^{-2} m^{-1}) and Tugela Ferry light brown (182.5 mmol m^{-2} m^{-1}) landrace selections, respectively. Under rainfed conditions, at 6 WAP, the same trend was observed, Jozini brown landrace selection had the highest stomatal conductance (132.1 mmol m^{-2} m^{-1}) followed by Tugela Ferry brown (125.3 mmol m^{-2} m^{-1}), Tugela Ferry light-brown (120.11 mmol m^{-2} m^{-1}), Tugela Ferry red (119.4 mmol m^{-2} m^{-1}), Jozini light-brown (116.2 mmol m^{-2} m^{-1}), and Jozini red (113.6 mmol m^{-2} m^{-1}) landrace selections, respectively. There was no significant interaction ($P>0.05$) between provenances and seed colour with respect to stomatal conductance over time. There was no observed trend in seed colour and provenances over time (Figure 4.7). At 7, 8 and 9 WAP, stomatal conductance was high in the Jozini red, Tugela Ferry light-brown, and Tugela Ferry brown landrace selections, respectively.

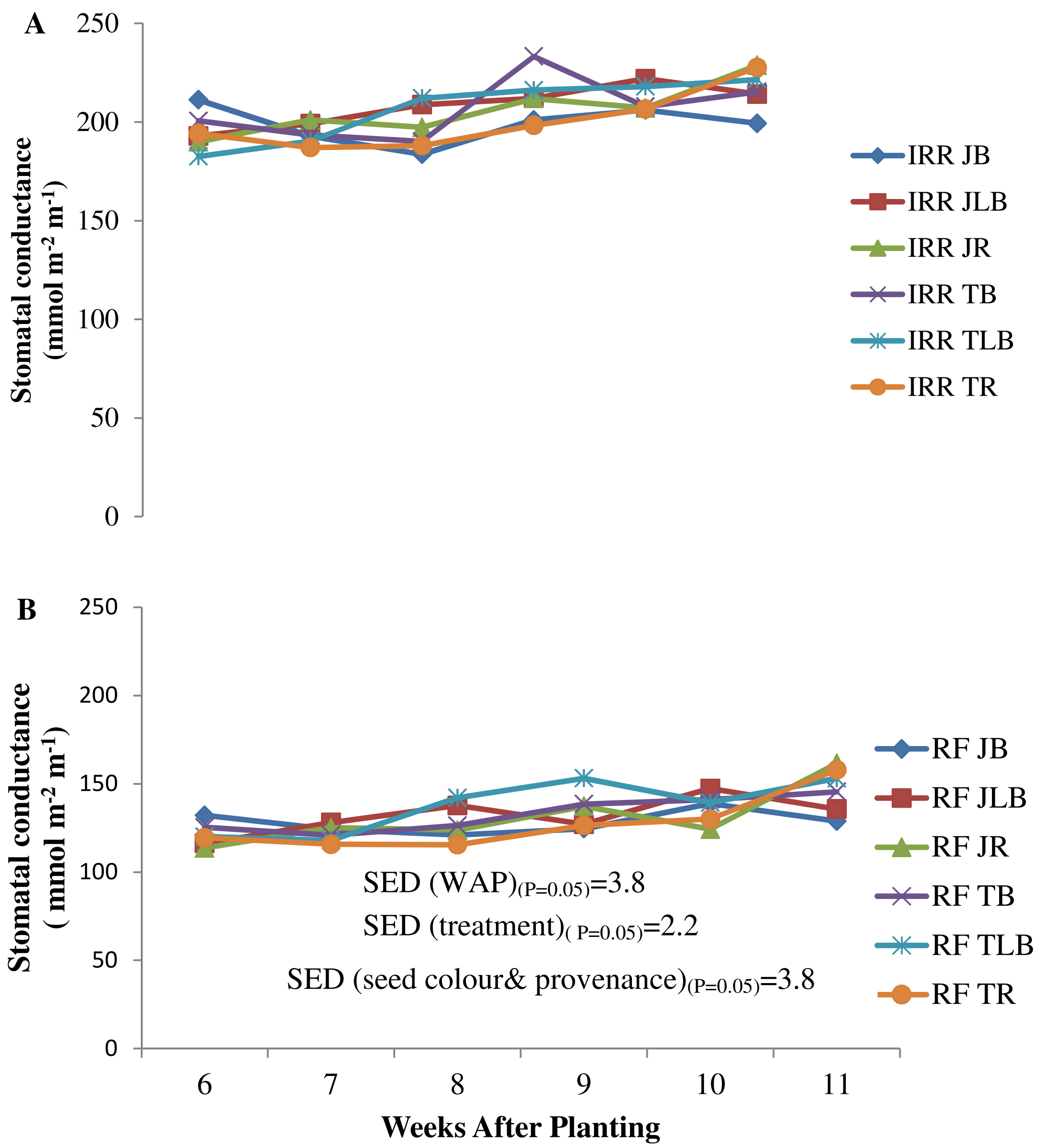

Figure 4. 7: Effect of different water regimes (A) irrigated and (B) rainfed condition on stomatal conductance of bambara groundnut landrace selections. NB: RF=rainfed, IRRI= irrigated, J= Jozini provenance, T= Tugela Ferry provenance, B=brown colour, LB=light brown colour, R=red colour.

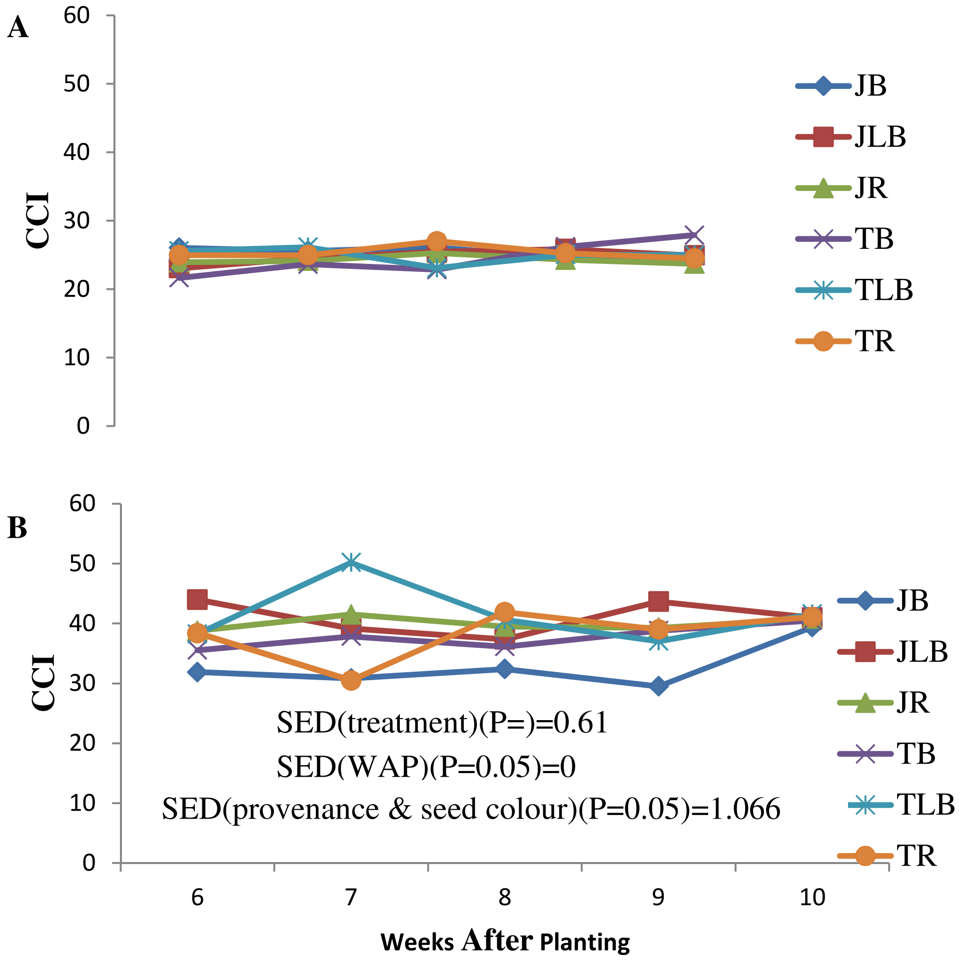

Figure 4. 8 Effect of different water regimes (A) irrigated and (B) rainfed condition on chlorophyll content index (CCI) of bambara groundnut landrace selections. NB: RF=rainfed, IRRI= irrigated, J= Jozini provenance, T= Tugela Ferry provenance, B=brown colour, LB=light brown colour, R=red colour.

Results of chlorophyll content index showed highly significant differences ($P<0.001$) between water regimes. Chlorophyll content index was higher under rainfed compared with irrigated conditions (Figure 4.8). There were significant differences ($P<0.05$) between provenances and seed colour selections with respect to chlorophyll content index. Chlorophyll content index was shown to not vary significantly over time (Figure 4.8). At 6 WAP, under irrigated conditions, the Jozini brown landrace selection had the highest chlorophyll content index, followed by Tugela Ferry light-brown, Tugela Ferry red, Jozini red, Jozini light-brown and Tugela Ferry brown landrace selections, respectively. Whereas under rainfed conditions, at 6 WAP, chlorophyll content index was highest in the Jozini light-brown landrace selection, followed by Jozini red, Tugela Ferry red, Tugela ferry light-brown, Tugela ferry brown and Jozini brown landrace selections, respectively (Figure 4.8). There was no observable trend between provenances as well as seed colour selections. Chlorophyll content index varied each and every week under both irrigated and rainfed conditions. However, on average, under both irrigated and rainfed conditions, the dark coloured landrace selections in both provenances performed better than light-coloured landrace selections (Figure 4.8).

Table 4. 2: Yield components of Bambara groundnuts landraces grown under irrigated and rainfed conditions

Treatment	Provenances	GM/P (g)	HI	GRAIN NO/P	POD NO/P	PM/P (g)	Biomass/P (g)
RAINFED	JB	0.010	0.037	0.333	0.333	0.060	3.260
	JLB	0.000	0.000	0.000	0.000	0.000	1.640
	JR	0.030	0.103	1.000	1.000	0.255	5.190
	TB	0.030	0.157	1.000	1.000	0.203	3.530
	TLB	0.000	0.002	0.015	0.015	0.009	3.170
	TR	0.150	0.298	1.000	1.000	0.502	6.260
IRRIGATED	JB	0.000	0.000	0.000	0.000	0.000	1.120
	JLB	0.000	0.000	0.000	0.000	0.000	1.070
	JR	0.000	0.000	0.000	0.000	0.000	1.160
	TB	0.000	0.000	0.000	0.000	0.000	1.490
	TLB	0.000	0.000	0.000	0.000	0.000	1.960
	TR	0.000	0.000	0.000	0.000	0.000	2.180
LSD (P=0.05)		**0.024**	**0.071**	**0.289**	**0.289**	**0.074**	**1.268**

NB GM/P= grain mass per plant, HI= harvest index, GRAIN NO/P= grain number per plant, POD NO/P= pod number per plant, PM/P= pod mass per plant, J=Jozini provenance, T=Tugela Ferry provenance, B=brown colour, LB=light-brown colour, R=red colour.

There were highly significant differences (P<0.001) between water regimes, with respect to total biomass (Table 4.2). Over-all, under rainfed conditions, total biomass was higher compared with irrigated conditions. There was a significant interaction (P<0.05) between provenances, seed colour selections and water regimes with respect to total biomass. Under rainfed conditions, the Tugela Ferry red landrace selection (6.26 g) had the highest biomass followed by Jozini red (5.19 g), Tugela Ferry brown (3.53 g), Jozini brown (3.26 g), Tugela Ferry light-brown (3.17 g) and Jozini light-brown (1.64g) landrace selections, respectively (Table 4.2). Over-all, the red colour selection showed superiority for both provenances followed by brown and light-brown selections, respectively. However, it was a different case under irrigated conditions, the Tugela Ferry red landrace selection had the highest biomass (2.18 g) followed by Tugela ferry light-brown (1.96 g), Tugela ferry brown (1.490g), Jozini red (1.16 g), Jozini brown (1.12 g), then Jozini light-brown (1.07 g) landrace selections, respectively (Table 4.2). Therefore, under irrigated conditions, the Tugela Ferry provenance had higher biomass compared with Jozini provenance. Within these two provenances the red colour selection showed superiority again followed by brown then light-brown colour selection. Tugela Ferry provenance performed better than the Jozini provenance under both irrigated and rainfed conditions. Also, the red coloured seeds performed better in both water regimes.

There were highly significant differences (P<0.001) between water regimes with respect to pod mass (Table 4.2). There were highly significant differences (P<0.001) between provenances and seed colour with respect to pod mass. There was also highly significant difference (P<0.001) interaction between water regimes, provenances and seed colour with respect to pod mass. The pod mass was high under rainfed conditions compared with irrigated conditions. Under rainfed condition, the Tugela Ferry red (0.502g) landrace selection had the highest pod mass followed by Jozini red (0.255g), Tugela Ferry brown (0.203g), Jozini brown (0.060g) the Tugela Ferry-light brown (0.009g) landrace selection respectively and Jozini light-brown selection was zero. Under rainfed condition the red colour selection for both provenances (Tugela Ferry and Jozini) performed better, followed by brown and light-brown respectively. There were highly significant differences (P<0.001) between water regimes with respect to number of grain per pod. There were highly significant differences (P<0.001) between provenances and seed colour with respect to grain number per pod (Table 4.2). There was also highly significant difference (P<0.001) interaction between water regimes, provenances and seed colour with respect to grain number per pod The grain number per pod was actually higher under rainfed conditions and it was zero under irrigated conditions (Table 4.2). Under rainfed conditions the grain number per pod was higher in dark coloured seeds (red and brown) than lighter coloured seeds. .Under irrigated conditions the grain number per pod was zero for all provenances and seed colours.

There were highly significant differences (P<0.001) between water regimes, provenances and seed colour with respect to pod number (Table 4.2). There was also highly significant difference (P<0.001) interaction between water regimes, provenances and seed colour with respect to pod number. The pod number was high under rainfed conditions compared to irrigated conditions. Under irrigated conditions pod number was zero for both provenances (Tugela Ferry and Jozini). Under rainfed conditions the pod number was high on dark coloured seeds (red and brown) compared to lighter coloured seeds for both provenances (Table 4.2). Over- all there was no observable trend between provenances both of them performed well and dark coloured seeds performed better that lighter coloured seed.

There were highly significant differences (P<0.001) between water regimes, provenances and seed colour with respect to harvest index (Table 4.2). There was also highly significant difference (P<0.001) interaction between treatments, provenances and seed colour with respect to harvest index (Table 4.2). The harvest index under irrigated conditions was zero (table 4.2). Under rainfed conditions dark coloured seeds had a higher harvest index compared to light coloured seeds for both provenances. There were highly significant differences (P<0.001) between water regimes, provenances and seed colour with respect to grain mass (Table 4.2). There was also highly significant difference (P<0.001) interaction between treatments, provenances and seed colour with respect to grain mass (Table 4.2). The grain mass under irrigated conditions was zero (table 4.2). Under rainfed conditions, the Tugela Ferry landrace selection had the highest grain mass (0.150g) followed by Tugela brown (0.030g) and Jozini (0.030g) then Jozini brown (0.030g) landrace selection respectively (Table 4.2) for the light-brown colour it was zero in both provenances. Overall dark coloured seeds (red and brown) had a higher grain mass compared to light coloured seeds for both provenances.

4.4 Discussion

The aim of this study was to evaluate the performance of two different bambara groundnut provenances under irrigated and rainfed conditions; seed colour was also evaluated as to whether it could be used to select for drought tolerance in bambara landraces. For the bambara groundnut crop, it is very important to have stable production, even in adverse, harsh growing conditions like low soil fertility, limited water availability and hot dry conditions. Yields are reduced to a lesser extent by these factors when compared to other crops (Linnemann *et al*., 1995; National Academy of Sciences, 1979). The planting dates are also important when producing bambara groundnuts. Bambara groundnut produces good yields when planted in October and November, especially after good rains (Swanevelder, 1998). If the crop is planted early or late, factors such as pod formation will be affected (Swanevelder, 1998). In this study we evaluated the possibility of growing bambara as a late summer or an early winter crop planted in March.

Both rainfed and irrigated treatments were established under full irrigation. Results showed that, for all provenances and seed colour selections, emergence was fast during 7-14 DAP, reaching a maximum at 21 DAP. In most cases, it usually takes 7 to 15 days under favourable temperatures (28.5 to 32.5°C) for bambara groundnut to emerge; but under lower temperatures, it can take up to 31 DAP, with some seeds remaining dormant indefinitely (Linnemann and Azam-Ali, 1993; Swanevelder, 1998). Our results of emergence were similar to those stated by Swanevelder (1998); however, they were contrary to recent reports by Mabhaudhi *et al*. (2011) and Sinefu (2011) that bambara emergence was slow. These authors reported that bambara landraces generally took up to 35 DAP to emerge. The difference between our results and theirs may be related to planting dates.

Although there was no significant difference between provenances, the trend observed for both provenances showed that the red colour selections of bambara performed better than light-brown and brown, respectively. This might be due to the fact that dark coloured seeds are more vigorous than light coloured seeds (Mabhaudhi, 2009; Mbatha, 2010; Zulu, 2010; Sinefu, 2011). The study that was done by (Pedersen & Toy, 2001) on grain sorghum found that seedling emergence under field conditions was higher in red than white coloured seeds. Saeidi and Mohammadi Mirik (2006) found that the effect of seed colour on emergence was significant and that yellow seeds (light colour) had significantly lower percentage emergence than brown seeds (dark colour). This was due to the fact that brown seeds were more resistant to soil borne diseases. This was associated with the presence of phenolic compounds (tannins) in dark seeds which are known to have anti-microbial properties (Scalbert, 1991). The antimicrobial properties of tannins (phenolic compounds) are therefore the likely reason of seed vigour improvement in dark coloured seeds. This may also be the case for red seed in bambara groundnuts, it is known to resist diseases in the soil compared to light coloured seeds.

It is now evident that selection using specific morpho-physiological traits is a viable way forward for crop improvement for water stress tolerance (Reynolds *et al*., 2005; Kiani *et al*., 2007; Tambussi *et al*., 2007). Stomatal conductance was shown to be higher under irrigated than rainfed conditions. Water stress experienced during the vegetative, flowering and pod filling stages of growth of bambara groundnuts significantly reduced stomatal conductance. Plants exposed to water stress close their stomata as a mechanism to maintain leaf water status; consequently, transpiration, photosynthetic rates and productivity are also decreased (Turner, 1986; Hirayama *et al*., 2006). Our observations of declining stomatal conductance under rainfed conditions was consistent with previous report on bambara groundnuts by Collinson *et al*. (1997) and Cornellisen (2005). Decreased stomatal conductance results in lower net carbon dioxide assimilation rate, lower intercellular carbon dioxide and lower chloroplast carbon dioxide tension, thus reducing carbon dioxide fixation.

Decreased chlorophyll levels during drought stress have been reported in many species (Kpyoarissis *et al*., 1995; Zhang and Kirkham, 1996). Results of chlorophyll content index showed that limited water availability under rainfed conditions did not significantly reduce chlorophyll content index of bambara groundnut. Chlorophyll content index was higher under rainfed compared with irrigated conditions. Cornellisen (2005) reported similar results on bambara groundnut. This suggests that bambara groundnut plants maintain high levels of chlorophyll content despite development of water stress. This trait can be considered to be a line of defence against drought which can result in drought tolerance (Vurayai *et al.,* 2011). However, this trait does not apply for all crops; in maize, barley and sunflower, respectively, drought stress caused a large decline in chlorophyll content (Anjum *et al.,* 2011; Kuroda *et al*., 1990; Manivannan *et al*., 2007).

There were highly significant differences between water regimes, landraces as well as time for plant height and leaf number. Plant height and leaf number were higher under irrigated conditions during the first four weeks compared to rainfed conditions. The observed decline in LAI corresponded with observations of lower plant height (Figure 5.2) and leaf number (Figure 5.5) observed under irrigated as compared to rainfed conditions However, over time the pattern changed; plant height and leaf number were now higher under rainfed compared to irrigated conditions. This was contrary to expectations; in most cases water stress reduces the number of leaves and plant height. This was also contrary to other reports in the literature that water stress reduced plant height and leaf number of bambara (Mwale *et al*., 2007; Mabhaudhi *et al*., 2011; Sinefu, 2011; Vurayai *et al*., 2011). A comparison of landrace provenances showed that, based on mean values, the Tugela Ferry provenance generally performed better than the Jozini provenance with regards to plant height and leaf number. For both provenances and under both irrigated and rainfed conditions, the red landrace selections had taller plants and more leaves relative to the brown and light-brown selections, respectively. Seed colour has previously been associated with seedling vigour (Powell, 1989; Zulu & Modi, 2010). vigorous seedling can catch light easily, develop roots and access water, and thus photosynthesise much earlier than less vigorous seedlings (Perry, 1978; McDonald, 1980) The reduction of plant growth and leaf number over time under irrigated condition could be because of sub-optimal temperatures during the growth season. Bambara cannot stand cold conditions; in South Africa, for example, it can only be grown in areas with a frost-free period of at least 3-5 months and with high temperatures during that time (Holm and Marloth, 1940). A combination of high rainfall and low temperatures has also been reported to reduce yield in bambara (Holm and Marloth 1940). In addition, bambara groundnut genotypes have been reported to exhibit variable responses to different planting dates (Linnemann, 1994: Karikari *et al*., 1995). Sesay *et al* (2008) showed that leaf number was higher in plants that were grown in

October compared to plants that were planted in February. This decrease in plant growth was due to the declining temperature which usually occurs later in the season in Southern Africa (Sesay *et al*., 2008).

Results of yield showed an interesting trend in line with observations of plant growth; yield was higher under rainfed conditions compared with irrigated conditions. This was contrary to expectations; in most cases water stress reduces yield. Azam-Ali *et al*. (2001) and Collinson *et al*. (1996, 2000) reported that bambara productivity was adversely affected by limited soil water availability. The reason for low yields under irrigated conditions could be related to selection of planting dates. The combination of water and low temperature could also be the reason of under performance in bambara groundnut yield. However, there is not enough evidence to support this.

Bambara groundnut is a warm temperature crop (Swanevelder, 1998). It performs well when conditions are favourable (28.5 to 32.5°C) (Linnemann and Azam-Ali, 1993; Swanevelder, 1998). The results obtained from this study showed that bambara groundnuts cannot be grown in winter in Pietermaritzburg. Although the crop performed better under rainfed conditions than irrigated conditions, the yield obtained was far less than yields reported in the literature for previous planting during summer at the same site. Sinefu (2011) reported yields of 182 kg/ha for bambara grown in September. In the current study planting was done in March, before the onset of winter, as a result emergence was rapid for both irrigated and rainfed conditions due to very warm conditions. Also, plant height and leaf number performed well in the beginning of planting. However as winter started to set in, the crop started to fail. Under irrigated conditions, crop failure was significant suggesting that supplementary irrigation had a negative impact on bambara groundnut growth.

Another explanation for the crop failure especially under irrigated conditions could be because of the soil type. The soils in the field trial were clay loam soils; such soils are known to have poor drainage which can result in water–logging (Figure 5.2). Bambara is very sensitive to water-logging. Water-logging reduces growth and causes chlorosis of older leaves this is a symptom of plants grown under irrigated conditions. Under water-logged conditions, aeration of the soil is reduced, and loss of nitrogen by identification and leaching is increased. The inhibition of gas exchange within the root zone also has the potential to damage plant roots, resulting in restricted water and nutrient uptake by the plant. Chlorosis of older leaves is observed due to poor root development and the consequential slow uptake of nitrogen by crop roots from the anaerobic soil.

4.5 Conclusion

The response of two provenances (landraces) with three different colours of bambara groundnuts differed significantly with respect to most growth and yield parameters. The results of the study were contrary opposite to our expectations and initial hypothesis. The irrigated trial was expected to perform better than the rainfed trial. However, bambara landraces grew better under rainfed compared with irrigated conditions. This was possibly due to the interaction between low temperatures and irrigation. Bambara groundnut is a warm temperature crop. This study showed that supplementary irrigation in winter resulted in low yield of bambara groundnuts. This study, however, did confirm that stomatal regulation is a drought tolerance mechanism used by bambara groundnuts. Leaf chlorophyll content was, however, not reduced by water stress at all stages of growth and development. Further research on the effect of temperature and water regimes on growth, development and yield of bambara groundnuts landraces is recommended. There is also need to develop efficient irrigation management options for bambara, such information is currently lacking.

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Chapter 5

The performance of Bambara groundnut landrace in controlled environment under different water regimes and different temperatures

5.1 Introduction

Agriculture is highly dependent on temperature and water availability. Changes in these factors can have major effects on crop water requirements and yields, hence world food supply. Water stress is expected to become more severe due to climate change since predicted higher temperatures coupled with higher radiation will result in increased evapotranspiration (Maqsood and Azam-ali, 2007). An adequate water supply is one of the most important factors in crop production. The amount of irrigation water needed for crops depends on rainfall, temperature and crop genetics (Maqsood and Azam-ali, 2007). Under climate change scenarios, demand for irrigation water will increase due to large decreases in summer rainfall and increases in temperature (Wigley and Raper, 2001; Chaves *et al*., 2003). Irrespective of the degree of climate change at any location, the risk of climatic variability within and between seasons is likely to be greatest in vulnerable tropical environments where the availability of soil water and high temperatures are already limitations to crop growth. One option to reduce the potential impacts of climate change on food security is to assess the contribution that previously underutilized food crops can make to agriculture in regions where limitations such as drought are likely to increase.

Bambara groundnut (*Vigna subterranea* L. Verdc) has been identified as a drought tolerant crop that can produce reasonable yields (Ntundu *et al*., 2006); however, variations exist among landraces with respect to drought tolerance. Bambara groundnut can produce relatively greater yield under drought conditions compared with other more established legumes (Babiker, 1989). Thus, extension of bambara groundnut cultivation into drought-prone regions is encouraging in that it offers prospects of sustainable food and nutrients supply to farmers. Bambara is essentially grown for human consumption with the seed being considered as a complete food because of the high nutritional value (Linnemann and Azam-Ali, 1993). Bambara groundnut seeds can be consumed in different ways; they can be grilled, boiled or eaten fresh. However, there is still a need to fully explore the potential of bambara groundnut cultivation, i.e. more knowledge describing the mechanisms underlying their adaptation to drought is required (Collinson *et al*., 1997). Investigations into landrace diversity in relation to drought tolerance are necessary for selecting and breeding high yielding landraces suited for drought-prone regions. However, there is great variability in the growth and development characteristics within and between landraces (Squire *et al*., 1997; Massawe, 2000); different landraces show different responses to factors such as temperature and water (Zulu, 1989; Kocabas *et al*., 1999; Massawe *et al.,* 1999).

Drought affects crop growth at any development stage with the early establishment stage being thought to be the most susceptible (Liu *et al*., 2003). In soybean (*Glycine max* L.), loss of seed yield was found to be high when drought occurred during anthesis (Liu *et al*., 2003; Liu *et al*., 2004; Eslami *et al*., 2010). Collinson *et al*. (1996) reported significant reductions in pod number per plant, harvest index and final seed yield due to terminal drought in a Zimbabwean bambara groundnut landrace. Although drought affects plant growth and development, inter- and intra-species differences are frequently observed (Leport *et al*., 1999; Baigorri *et al*., 1999; Collino *et al*., 2000; Liu and Stutzel, 2002a; Liu and Stutzel, 2002b; Mwale *et al*., 2007). Therefore, there is a need for selecting landraces that are tolerant to drought.

Furthermore, physiological studies have shown that growth and development of bambara groundnut is greatly influenced by external factors like temperature (Linnemann, 1993; Linnemann *et al*., 1995). Similar to water stress, temperature affects crop growth at any development stage. Non-optimal temperatures slow the growth rate or stop growth altogether. Germination of bambara groundnuts was reported to increase from 16.8°C until 32.5°C, where it reached a peak and declined until 39.5°C (Karikari *et al*., 1995). Too high and too low temperatures affect crop growth. The optimum temperature for bambara groundnuts is 28.5 to 32.5°C (Linnemann and Azam-Ali, 1993; Swanevelder, 1998).

Under field conditions water and temperature stress seldom occur alone. They almost always occur together. However, most studies that have evaluated the effects of the two factors on crop growth and yield, have almost always looked at them separately. There is still limited literature describing the combined effect of temperature and water stress on crop growth and development (Rizhsky *et al.,* 2004). The objective of this study was to evaluate the performance of bambara groundnut landraces in response to different water and temperatures regimes under controlled environments conditions.

5.2 Materials and methods

5.2.1 Plant material

Two bambara groundnut landraces (provenances) were obtained from Tugela Ferry (28˚45' S; 30˚27' E, <543 masl) and Jozini (30°33' S; 29°54' E; <500 masl). Seeds from Tugela Ferry were obtained from smallholder farmers. Bambara landrace seeds were then sorted into three distinct colours: red, light brown and brown. Seeds were characterised according to seed coat colour based on previous studies that have suggested that seed coat colour may have an effect on early establishment performance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu 2011).

5.2.2 Glasshouse environment

Pot trials were conducted under controlled environment conditions at the University of KwaZulu-Natal's Controlled Environment Research Unit (CERU), Pietermaritzburg. Two glasshouses were used for the study with different temperatures set at 33/27˚C (day/night; natural day length; 65% relative humidity) and 21/15°C (day/night; natural day length; 65% relative humidity).

5.2.3 Experimental design and layout

The experimental design was a factorial with three factors, namely: temperature (33/27˚C and 21/15˚C), water regimes [30% and 100% of crop water requirement (ETc) (Allan *et al*., 1998) and bambara landrace selections. Bambara landrace selections were from two provenances (Tugela Ferry and Jozini) split into three seed colours (brown, light-brown and red). The experiment was replicated three times. Seventy-two (72), 5-litre pots were each filled with 3 kg of soil whose field capacity had previously been determined. Soil used for this study was sourced from the exact location as the field trial (Chapter 4). Soil physical and chemical properties are described in Table 5.1. Two seeds were planted per pot and later thinned to one plant per pot after establishment. At planting, all pots were watered up to field capacity. Soil water content was monitored daily using an ML-2x Theta probe connected to an HH2 handheld moisture meter (Delta-T Devices, UK). Irrigation in the pot was applied twice daily and scheduled using reference evapotranspiration (ETo) and a crop factor (Kc) as described by Allen *et al.* (1998);

$$ETc = ETo * Kc \qquad \text{Equation 5.1}$$

where: ETc = crop water requirement,

ETo = reference evapotranspiration obtained using the FAO-Penman Monteith method, and

Kc = crop factor.

Table 5. 1: Physical characteristics of the soil media used in the pot experiment.

Media	**Clay**	**Sand**	**Silt**	***Field Capacity**	**PWP**
	------------------------------		%------------------------------		
Clay	43.5	24	32.5	40.6	28.3

*Field capacity represents gravimetric field capacity.

5.2.4 Data collection

Plant emergence was measured daily up to 28 days after planting (DAP). Plant height and leaf number were measured weekly from 2 weeks after planting (WAP). Plant height was measured from the base of the plant to the tip of the upper leaf and leaf number was counted for leaves with at least 50% green area. Measurements of plant height and leaf number were taken until 50% flowering. Stomatal conductance and chlorophyll content index were measured weekly on the abaxial and adaxial surfaces, respectively, using a steady state leaf poromoter (Model SC-1, Decagon Devices, USA) and CCM-200 *Plus* chlorophyll content meter (OPTI-SCIENCES, USA). Measurements, except for plant height, leaf number and emergence, were only taken from the plants growing at 33/27˚C, as a result when they reached maturity, both trial were harvested. This was because at 21/15˚C plant growth was too slow. Yield and yield components(total biomass, pod number/plant, pod mass/plant, seed number/pod, and seed mass/plant) were determined at harvest for the 33/27˚C temperature regime only, because there was no yield for the 21/15˚C temperature regime treatment.

5.2.5 Crop management

Plants in the pot trial were managed according to best agronomic practices. Routine hand-weeding was done to ensure that there was no competition for light and water.

5.2.5 Data analyses

Data were analysed using analysis of variance (ANOVA) from GenStat® Version 14 (VSN International, UK). Means of significantly different variables were separated using least significant differences (LSD) at a probability level of 5% (Appendix 5).

5.3 Results

5.3.1. Soil water content

Soil water content (SWC) varied significantly (P<0.001) between temperature regimes (Figure 5.1). Soil water content was 34% higher at 21/15˚C compared with 33/27˚C (Figure 5.1). Soil water content was also shown to vary significantly (P<0.001) between the different water regimes (Figure 5.1). It was 9.5% higher at 100% ETc compared with 30% ETc. There was also a significant variation ($P<0.001$) in SWC over time (Figure 5.1). There was an inverse relationship between time [days after planting (DAP)] and SWC, whereby SWC decreased with time. In addition, there were significant differences ($P<0.001$) between soil water with respect to bambara landrace (Figure 5.1). However, there was no observable trend between provenances, although a trend was observed within provenances. Pots planted with the light-brown selections were shown to have higher SWC compared with pots planted with brown and red landrace selections (Figure 5.1). The interaction between temperature regimes and water regimes was significant ($P<0.001$); at 30% ETc, in the 21/15˚C temperature regime, SWC was 43% higher than at 33/27˚C, while at 100% ETc, at 21/15˚C, SWC was 26% higher compared with 33/27˚C.

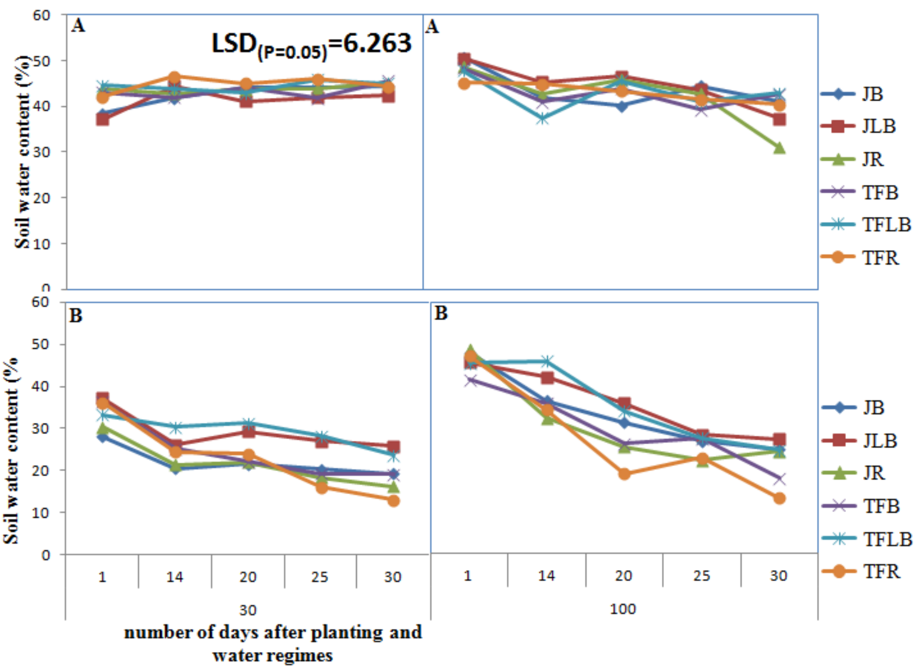

Figure 5. 1: Soil water content measured at different temperature regimes A. (21/15˚C) and B. (33/27˚C) under different water regimes (30 = 30% ETc; 100 = 100% ETc). LSD (landraces)$_{(P=0.05)}$ = 1.400, LSD (DAP)$_{(P=0.05)}$ = 1.278, LSD (water regimes)$_{(P=0.05)}$ = 0.809 and LSD (temperature regimes)$_{(P=0.05)}$ = 0.809. Note: JB = Jozini brown, JLB = Jozini Light brown, JR= Jozini red, TFB= Tugela Ferry brown, TFLB= Tugela Ferry light brown and TFR= Tugela Ferry red**.**

5.3.2 Emergence

Emergence varied significantly (P<0.001) over time (DAP), especially at 33/27˚C. There were highly significant differences (P<0.001) between temperature regimes with respect to emergence. Emergence was 90% higher at 33/27˚C compared with 21/15˚C. At 28 DAP, under 33/27˚C, JB, JR, TFB and TFR had obtained 100% emergence while at 21/15˚C maximum emergence was 70% for JR and JB. There was no observed trend on how provenances performed; however, there was a clear trend on seed colour performance. The dark coloured (red and brown) selections had higher emergence compared with light-brown selections. There were no significant differences (P>0.05) between water regimes with respect to emergence since all plants were established at 100%ETc. Landraces differed significantly (P<0.001) with respect to emergence. On average, the trend was such that JR (39.88) > TFB (38.39) > JB (37.80) > TFR (34.52) > TFLB (24.70) > JLB (19.94), respectively. The interaction between water and temperature regimes as well as landrace selections over time (DAP) was not significant (P>0.05).

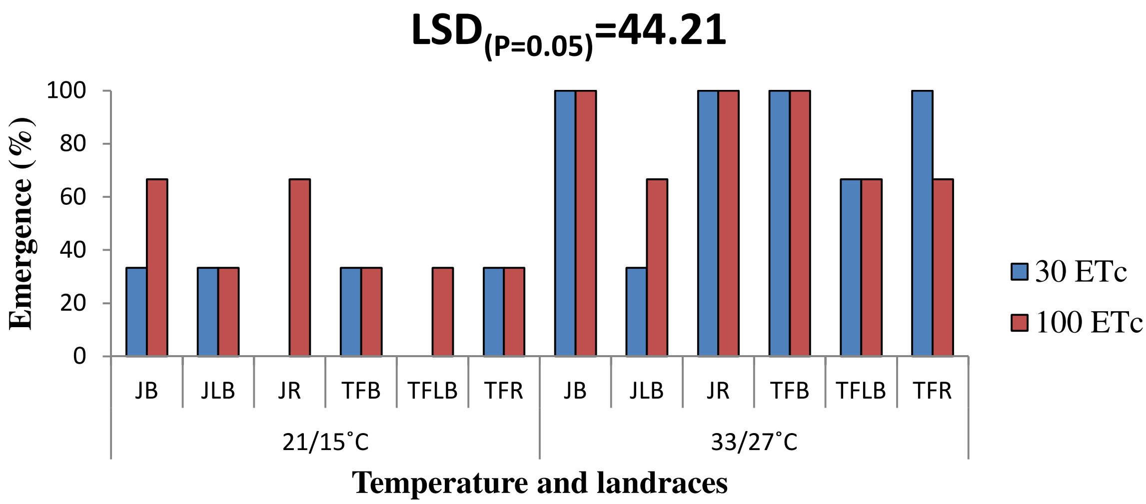

Figure 5. 2: Final emergence of different bambara landraces grown under different temperature at different water levels (30% and 100% ETc). Note: JB = Jozini brown, JLB = Jozini Light brown, JR= Jozini red, TFB= Tugela Ferry brown, TFLB= Tugela Ferry light brown and TFR= Tugela Ferry red.

Water regimes did not significantly differ (P>0.05) with respect to plant height (Figure 5.3). There were highly significant differences between landrace selections with respect to plant height, with dark coloured selections having taller plants than light-brown selections. However, within provenances, there was no observed trend: Tugela Ferry and Jozini showed similar performance. The interaction between water regimes, temperature regimes, landrace selections and time (Weeks after planting; WAP) was not significant (P>0.05). There were significant differences (P<0.001) with respect to plant height over time (WAP). At 33/27˚C, plant height was shown to increase with time. Leaf number also performed similar to plant height (Figure 5.4). Bambara groundnut growth was shown to be more affected by temperature than water availability. Dark coloured selections were shown to perform better than light-brown selections.

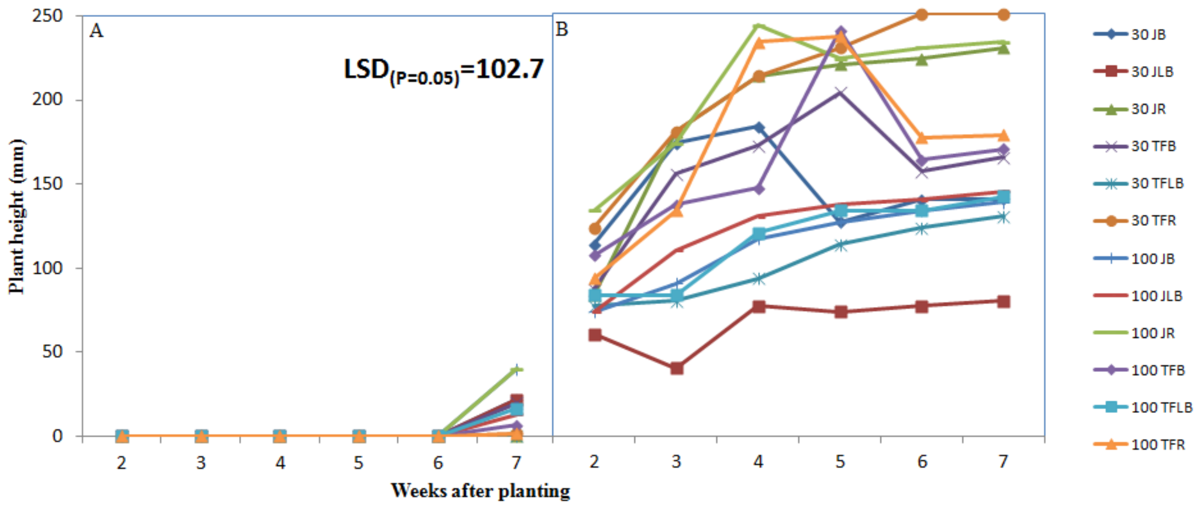

Figure 5. 3: Plant height of Bambara groundnut landraces under different temperatures (A = 21/15˚C and B = 33/27˚C) and at different water regimes (30% and 100% ETc). LSD $(landraces)_{(P=0.05)}$= 20.97, LSD $(WAP)_{(P=0.05)}$= 20.97, LSD (water $regimes)_{(P=0.05)}$= 12.10 and LSD (temperature $regimes)_{(P=0.05)}$= 12.10. Note: JB = Jozini brown, JLB = Jozini Light brown, JR= Jozini red, TFB= Tugela Ferry brown, TFLB= Tugela Ferry light brown and TFR= Tugela Ferry red.

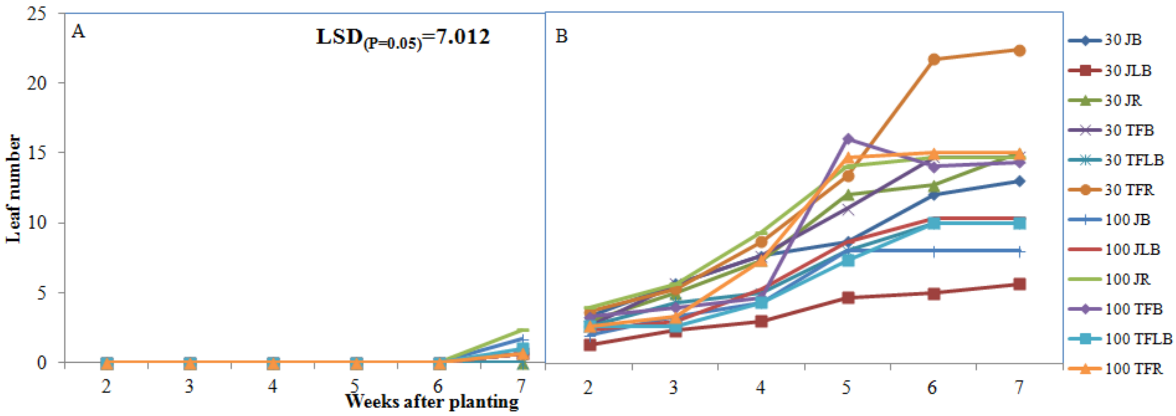

Figure 5. 4: Leaf number of Bambara groundnut landraces under different temperatures (A =21/15˚C and B=33/27˚C) and at different water regimes (30% and 100%ETc). LSD (landraces)$_{(P=0.05)}$= 1.431, LSD (WAP)$_{(P=0.05)}$= 1.431, LSD (water regimes)$_{(P=0.05)}$= 0.826 and LSD (temperature regimes)$_{(P=0.05)}$= 0.826. Note: JB = Jozini brown, JLB = Jozini Light brown, JR= Jozini red, TFB= Tugela Ferry brown, TFLB= Tugela Ferry light brown and TFR= Tugela Ferry red.

Chlorophyll content index (CCI) and stomatal conductance (SC) were only measured for 33/27˚C due to slow growth at 21/15˚C (Figure 5.5). There were no significant differences (P>0.05) between water regimes with respect to chlorophyll content index (Figure 5.5). There were highly significant differences (P<0.001) between landrace selections; TFR (30.35) landrace selection had the highest CCI followed by JR (27.57) > TFB (24.13) > TFLB (16.97) > JB (15.76) > JLB (12.33), respectively. On average, Tugela Ferry had higher CCI compared with Jozini provenance, and in each provenance the dark coloured selections were shown to have higher CCI compared with light-brown selections. The interaction between water regimes and landrace selections was significant (P<0.05). Overall, with the exception of JB and TFR, CCI was higher at 100% ETc compared with 30% ETc. The interaction between water regime, landrace selections and time (WAP) was not significant (P>0.05).

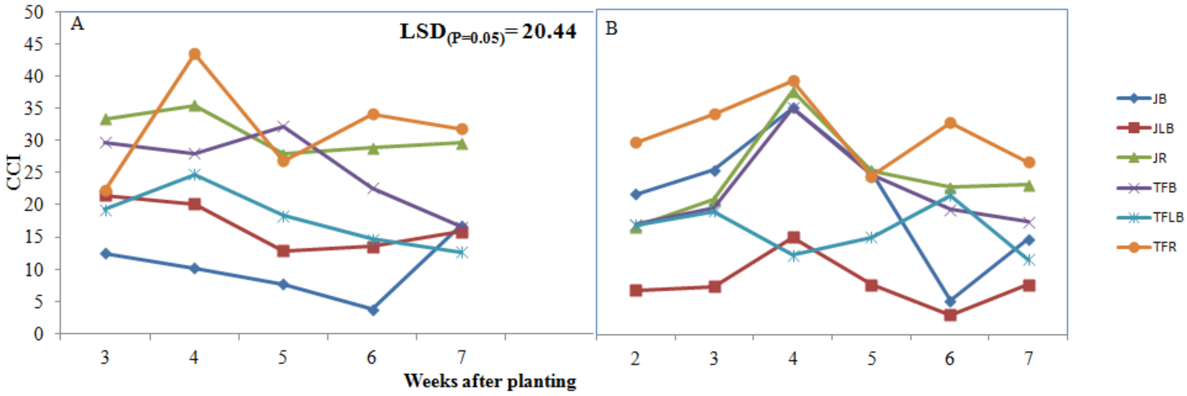

Figure 5. 5: Effect of different water regimes (A = 100% ETc) and (B = 30% ETc) on chlorophyll content index (CCI) of bambara groundnut landrace selections. LSD $(\text{landraces})_{(P=0.05)}$= 5.581, LSD $(\text{WAP})_{(P=0.05)}$= 5.581, LSD $(\text{water regimes})_{(P=0.05)}$= 3.222 and LSD $(\text{temperature regimes})_{(P=0.05)}$= 3.222. Note: JB = Jozini brown, JLB = Jozini Light brown, JR= Jozini red, TFB= Tugela Ferry brown, TFLB= Tugela Ferry light brown and TFR= Tugela Ferry red.

Stomatal conductance was only measured at 33/27˚C; plant growth was very slow at 21/15˚C. Therefore, results of stomatal conductance only show the comparison between water regimes, landrace selection and time. There were highly significant differences (P<0.001) between water regimes (Figure 5.6); stomatal conductance was 29% higher at 100% ETc compared with at 30% ETc. There were highly significant differences between (P<0.001) landrace selections (Figure 5.6); stomatal conductance was higher in the TFR (144.8) landrace selection followed by JR (138.8) > TFB (105.5) > JB (86.5) > TFLB (58.6) > JLB (42.6), respectively. The Tugela Ferry provenance was shown to have higher stomatal conductance compared with the Jozini provenance. Dark coloured selections had higher stomatal conductance compared with light-brown selections under both water regimes (30% and 100% ETc). The interaction between water regimes, landrace selection and time (WAP) was not significant (P>0.05).

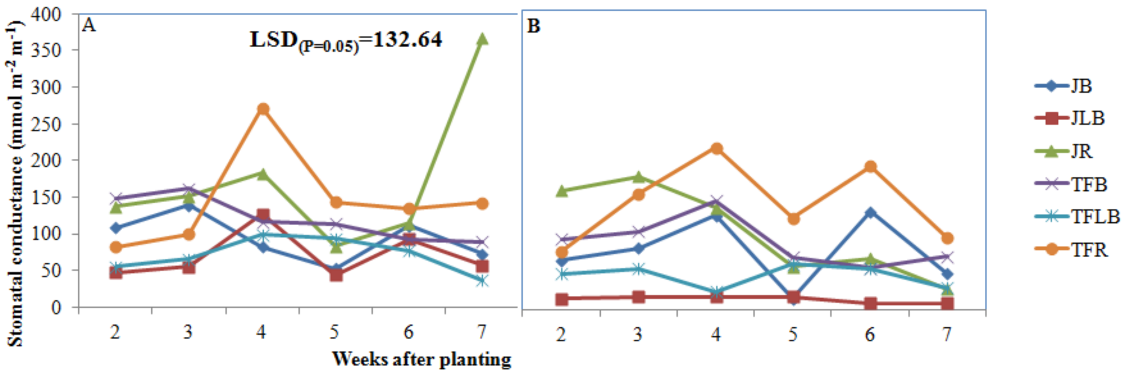

Figure 5. 6: Effect of different water regimes (A = 100%ETc) and (B = 30%ETc) on stomatal conductance of bambara groundnut landrace selections. Note: LSD (landraces)(P=0.05)= 38.29, LSD (WAP)(P=0.05)= 38.29, LSD (water regimes)(P=0.05)= 22.11 and LSD (temperature regimes)(P=0.05)= 22.11. JB = Jozini brown, JLB = Jozini Light brown, JR= Jozini red, TFB= Tugela Ferry brown, TFLB= Tugela Ferry light brown and TFR= Tugela Ferry red.

5.3.4 Yield components

The results for yield components were measured only from 33/27˚C temperature regime due to slow growth at 21/15˚C (Section 5.2.4). There were no significant differences (P>0.05) between water regimes with respect to biomass, pod number per plant, pod mass per plant, seed number per pod, seed mass per plant and HI (Table 5.2). Water stress did not affect these yield components. The interaction between water regimes and landraces was not significant (P>0.05) with respect to total biomass, pod number per plant, pod mass per plant, seed number per pod, seed mass per plant and HI (Table 5.2). However, landrace selections differed significantly (P<0.001) with respect to total biomass, pod number per plant, pod mass per plant, seed number per pod, seed mass per plant and HI (Table 5.2). The results showed that Tugela Ferry red (TFR) (15.13g) landrace selection had a higher biomass followed by TFB (7.48 g) > JB (6.61 g) > JR (6.29 g) > TFLB (3.48 g) > JLB (2.44 g), respectively. Similar results were observed for pod number per plant, pod mass per plant, seed number per pod, seed mass per plant and HI (Table 5.2). It was clear from the results that Tugela Ferry provenance had the highest seed yield compared with Jozini provenance (Table 5.2). The trend observed was such that dark coloured (brown and red) selections had higher seed yield compared with light-brown selection.

Table 5. 2: Yield components of Bambara groundnuts landraces grown under control environment, at 33/27˚C with different levels of water (30 and 100% ETc).

Water Regimes	Variety	Total Biomass(g)	Pod mass/P(g)	Pod NO/P	Seed NO/Pod	Seed mass/P(g)	HI
30%	JB	7.19	2.37	10.33	1.00	1.45	0.20
	JLB	1.46	0.13	1.00	0.33	0.11	0.02
	JR	6.74	1.52	7.00	1.00	1.26	0.19
	TFB	7.37	2.13	6.33	1.00	0.99	0.13
	TFLB	3.24	0.18	1.67	0.67	0.13	0.03
	TFR	14.34	5.30	26.67	1.00	3.75	0.26
100%	JB	6.04	3.01	8.33	1.00	1.36	0.20
	JLB	3.41	0.21	1.67	0.66	0.13	0.03
	JR	5.85	3.16	11.33	1.00	2.15	0.37
	TFB	7.59	2.33	7.33	1.00	1.25	0.16
	TFLB	3.71	0.22	1.67	0.66	0.20	0.04
	TFR	15.91	5.90	24.67	1.00	4.46	0.28
	$LSD_{(P=0.05)}$	**3.19**	**1.45**	**5.67**	**0.58**	**1.24**	**0.16**

NB. JB=Jozini brown, JLB=Jozini light brown, JR=Jozini red, TFB=Tugela Ferry brown, TFLB=Tugela Ferry light brown, TR=Tugela ferry red. P=Plant. HI=Harvest Index.

5.4 Discussion

The objective of the study was to evaluate the performance of bambara groundnut landraces under different water regimes and different temperatures under controlled environment conditions. Plant growth, development and subsequent yield are influenced by environmental conditions such as temperature and water availability. In the tropics, most crops are grown under unfavourable environmental conditions such that their genetic potential is hardly realised. Inadequate soil water content and non–optimal temperatures are the major limitations to crop production. Exploration of crops which can survive in these harsh environments is of paramount importance to sustain agricultural production in the tropics. Thus the need for increased food production in the tropics and the whole world due to population pressure calls for a new challenge to develop crops which have previously been looked at as minor crops, like bambara groundnuts.

Results of this study showed that temperature regimes had an effect on soil water content. High temperature (33/27°C) was associated with low soil water content compared with low temperature (21/15°C). Plants compete for water with the evaporative demand related to high temperatures; this competition becomes worse when there is shortage of water. There were differences between landraces; the light-brown selections were associated with higher SWC compared with pots planted with brown and red landrace selections (Figure 5.2). The trend in SWC between the landrace selections may be related to the nature of their growth, with dark coloured selections being more vigorous and having a larger canopy than the light-brown landrace selections.

Emergence under 33/27˚C temperature regime commenced at 8 DAP whereas in the 21/15˚C temperature regime it commenced at 28 DAP (Figure 5.2). This was due to the fact that bambara groundnut is sensitive to low temperatures. This was also confirmed by Linnemann and Azam-Ali (1993) and Swanevelder (1998) that, under favourable temperatures (28.5 to 32.5°C), bambara groundnut takes seven to 15 days to emerge; but under lower temperatures, it can take up to 31 days to emerge, with some seeds remaining dormant indefinitely. This was confirmed in the present study, under 21/15˚C, bambara emerged at 24 DAP (Figure 5.2) According to Massawe *et al*. (2003), optimum temperatures for bambara groundnut emergence ranged from 30.2 to 35.3˚C. Logendra (1984) also reported that emergence of soybean was low at very low and very high temperatures. Landraces were shown to differ significantly with respect to emergence. The dark colour landrace selection had the highest emergence compared with light-brown colour. The differences in performance of landraces during emergence could be associated with seed coat colour. True to our hypothesis, the dark coloured selections showed higher emergence compared with the light-brown selections. This concurs with previous findings associating dark seed coat colour with better emergence under various conditions (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu 2011).

Slow emergence under 21/15˚C temperature regime resulted in stunted plant growth. Plant height and leaf number were high at 33/27˚C. Massawe *et al*. (2003) also observed that the rate of plant growth was linearly related to temperature. Water stress under high temperatures was expected to negatively affect growth of bambara groundnuts. However, results were contrary to this expectation. At both 100% ETc and 30% ETc, bambara landrace selections showed similar growth. This may be due to the fact that all plants were established at 100% ETc to allow for optimum crop stand. The 30% ETc water treatment was only imposed thereafter. This may suggest that imposing water stress post-establishment, did not have an effect on growth of bambara landrace selections. This would imply that the vegetative stage in bambara landrace selections used in this study was not very sensitive to water stress (Mwale *et al*., 2007).

Results showed that dark coloured selections had taller plants than light-brown selections. The differences in growth observed between seed colours may be related to their establishment performance. Generally, plants that emerge faster are able to start photosynthesising earlier, thus making use of resources such as light, water and nutrients. This observation re-affirms the importance of the establishment stage.

Chlorophyll content index was not affected by water stress (Figure 5.5). This showed that bambara groundnut plants maintain high levels of chlorophyll despite the development of water stress and this trait can be considered to be a line of defence against drought which can result in drought tolerance. Cornellisen (2005) reported similar results on bambara groundnut. Unchanged chlorophyll level during water stress has been reported in many species, depending on the duration and severity of drought (Kpyoarissis *et al*., 1995; Zhang and Kirkham, 1996). Contrary to results of the present study, Manivannan *et al*. (2007) observed a decline in chlorophyll content under water stress in different sunflower varieties.

Water stress resulted in lower stomatal conductance relative to the optimum watering regime (100% ETc) (Figure 3.6). Decreased stomatal conductance results in lower net CO_2 assimilation rate, lower intercellular $CO_2$and lower chloroplastic CO_2tension. The CO_2insufficiency will reduce photosynthetic efficiency and dry matter production and may have negative impact on plant growth and yield. Collinson *et al*. (1997) and Cornellisen (2005) working on bambara groundnuts also observed similar results. The results of the current study also revealed that, under both water regimes, Tugela Ferry provenance had higher stomatal conductance compared with Jozini provenance and that dark coloured selections had higher stomatal conductance compared with light-brown selections. Previous studies have shown that seed colour may be used a good indicator for drought tolerance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu 2011). According to Ntundu *et al.* (2004), variations exist among landraces with respect to drought tolerance; in the current study, Tugela Ferry provenance demonstrated a capacity to tolerate drought stress compared with Jozini.

Due to slow and erratic emergence which resulted in stunted plant growth, there was no yield under 21/15˚C environment. However, bambara landrace selections were able to form yield under the 33/27˚C temperature regime. Results obtained showed that yield components were not affected by water regimes. At both 100% ETc and 30% ETc the yield components were similar; this was contrary to our expectation. It was expected that at 100% ETc there would be more yield compared with 30% ETc. The fact that results of the current study showed no differences between water regimes implies that there is great degree of tolerance to water stress in bambara groundnut. Other authors have, despite describing bambara groundnut as being drought tolerant, reported yield reduction in response to water stress. Collinson *et al*. (1996) working on bambara groundnut landraces reported significant reductions in pod number per plant, harvest index and final seed yield due to water stress. Pod number per plant was reduced due to drought while HI was not affected by drought (Mwale *et al*., 2007). Shamudzarira (1996) reported a high pod yield under irrigation and low yield in bambara groundnut subjected to drought stress from establishment. This suggests that the reason for high yield under water stress was due to good establishment. The results of the current study clearly showed that Tugela Ferry provenance had the highest yield compared with Jozini provenance and that dark coloured (brown and red) selections had higher yield compared with light-brown selection. This trend was consistent with trends observed from crop responses to stress which showed that the Tugela Ferry landrace selection was more capable of adapting to water stress than the Jozini landrace.

5.5 Conclusion

Soil water availability and temperature are critical for crop productivity. From the results of this study, it can be concluded that bambara groundnuts performed well under high temperature. Low temperature resulted in slow emergence and stunted plant growth, therefore zero yield. It is concluded that bambara groundnuts is mainly affected by low temperatures at an early stages of growth. Water availability did not seem to affect bambara groundnuts. High temperature resulted in low soil water content. This suggests that high temperature had an effect on water availability. Tugela Ferry landrace was shown to be the most tolerant provenance. Dark-coloured selection also performed well under stress and non stress conditions.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Predicted climate change, with regards to increased frequency and severity of drought, threatens to worsen the water scarcity situation of many sub-Saharan countries. In this study, the review of literature showed that current major staple crops will not be able to feed the growing population under these conditions of predicted climate change. There is a need to identify “new” crops that are drought tolerant and can be used as alternative future crops. Bambara groundnut (*Vigna subterranea* L. Verdc) has been identified as a potential drought tolerant crop that can produce reasonable yields under water stress (Ntundu *et al*., 2006). Despite its potential to grow in dry areas and still produce reasonable yields, there has been limited research describing drought tolerance of bambara groundnut. It is still cultivated using landraces, with no available improved varieties. In addition, the genetic diversity that exists between landraces from different origins as well as within landraces has not been fully explored. As such, there is also a need to characterise these diverse landraces for drought tolerance.

Previous work on several landraces suggested that seed colour may be a useful initial criterion for selection for crop performance (Mabhaudhi and Modi, 2010, 2011; Mbatha and Modi, 2010; Zulu and Modi, 2010; Sinefu, 2011). However, this has not yet been established for other landraces originating from different geo-climatic locations. The aim of this study was to evaluate responses of different bambara landraces, obtained from different locations in KwaZulu-Natal, South Africa (Jozini, Tugela Ferry and Deepdale) and Zimbabwe and characterised according to seed colour, to water stress under controlled and field conditions. In order to achieve this, the study took an approach that involved (i) determining seed quality in terms of germination capacity and vigour, (ii) evaluating the seedling establishment, and (iii) evaluating the responses of bambara landraces to water stress under controlled and field conditions. All this was done within the context of landrace provenance and seed colour.

An evaluation of seed quality (Chapter 2) revealed that it was difficult to distinguish the performance of bambara landrace seeds with respect to standard germination. An understanding of seed quality is critical to an understanding of how the crop may perform under field conditions. This study also showed that provenance plays a significant role in seed performance and that there is an interaction between provenance and seed coat colour. The Jozini provenance was shown to perform best, followed by Zimbabwe, Tugela Ferry and Deepdale. These results confirmed that there is a greater variation between landraces from different locations (Ntundu *et al*., 2004). Seed colour was also shown to be an important variable in the identity of bambara landraces. The brown coloured selections had higher germination capacity compared with red and light-coloured selections. Vigour parameters such as shoot length, root length, dry mass and fresh mass also showed a similar trend to that of germination.

Seedling establishment was evaluated using different growth media and varying water regimes (Chapter 3). Using different soil media at different water regimes had an impact on seedling growth and establishment of bambara landraces. Sand and the mixture of sand and clay (sand + clay) were shown to be best suited for establishment of bambara groundnut seedlings. This was due to the fact that sand has large particle size that allows for good drainage and aeration (Idu *et al*., 2003) while the clay + sand mixture was an ideal medium with fairly balanced proportions of sand and clay. It was difficult to conclude on the responses of bambara landraces to water availability during seedling establishment. Their performance showed great variability between and within landraces. However, an overall assessment of landrace performance appeared to show that the Tugela Ferry provenance performed better under water stress conditions compared with the Jozini provenance. This suggests that, despite reports in literature stating that bambara establishment was sensitive to water stress, certain landraces may be capable of emerging under conditions of limited water availability – an adaptation that may be greatly beneficial under dryland farming conditions. Consistent with observations of seed quality, dark-coloured seeds were shown to perform well, under all conditions, relative to light-coloured landrace selections. This concurred with the earlier hypothesis that dark-coloured seeds have superior seed quality and possibly drought tolerance, especially during the early establishment stage of crop growth.

Proline accumulation has been found to be a common metabolic response of plants to abiotic and biotic stresses. Accumulation of proline in many plant species subjected to stress has previously been correlated with stress tolerance (Kishor *et al*., 1995) while others (Hanson *et al.,* 1977) considered proline accumulation to be a symptom of damage rather than tolerance. An evaluation of proline accumulation in leaves of seedlings (Chapter 3) showed that the Jozini provenance accumulated more proline under water stress compared with Tugela Ferry provenance. In addition, light-brown landrace selections had higher levels of proline under water stress compared with dark coloured landrace selections. However, an assessment of seedling vigour indices seemed to imply that Tugela Ferry and dark-coloured landrace selections, not Jozini provenance and light-brown landrace selections, were drought tolerant. Therefore, based on these observations, it was concluded that the higher proline levels in the Jozini provenance and light-brown landrace selections were more a symptom of stress rather than tolerance.

In the subsequent field trial (Chapter 4), the performance of bambara landraces was evaluated under irrigated and rainfed conditions. The expectation was that bambara landrace selections would perform better under irrigated conditions since in the seedling establishment experiment bambara landrace selections had performed better at 100% FC. However, contrary to expectation, bambara landrace selections grew better under rainfed compared with irrigated conditions. This was possibly due to the interaction between low temperatures and irrigation since the trial was conducted from March to May, an initially wet season followed by the start of winter in KwaZulu-Natal. Initially, emergence was good since average temperatures were still relatively warm; however, as winter set in, plants started dying under irrigated condition. This ultimately led to failure of yield formation under irrigated conditions. In addition, there was no clear trend with regards to performance of bambara provenances under rainfed and irrigated conditions. Nonetheless, a trend was observed within provenances with respect to seed colours which showed that the red landrace selections performed well under both rainfed and irrigated conditions. Even under irrigated conditions, the red landrace selections did not easily succumb to the low temperatures. This further concurred with results obtained in the seed quality study and the association between seed coat colour and seedling vigour (Powell, 1989; Zulu & Modi, 2010).

Results of the field trial confirmed the fact that bambara groundnut is a warm season crop and that autumn/winter production is not a viable option. Reports in the literature indicated that optimum temperatures for bambara growth were in the range of 28.5 to 32.5°C (Linnemann and Azam-Ali, 1993; Swanevelder, 1998). Although the crop performed relatively better under rainfed than irrigated conditions, the yield obtained was far less than bambara yields for previous planting during summer at the same site (Sinefu, 2011). This then necessitated a further experiment to evaluate the effect temperature and water stress on growth, development and yield of bambara landraces (Chapter 5).

Therefore, a pot trial experiment was conducted under controlled environment conditions (Chapter 5) in order to verify whether the failure of bambara groundnuts under irrigated conditions was due to an interaction of water and temperatures effects. Results showed that emergence was comparatively slower at 21/15˚C than 33/27˚C. This also caused stunted growth and yield failure at 21/15˚C. This confirmed that indeed bambara was sensitive to low temperatures and confirmed observations from the field trials (Chapter 4). Interestingly, water availability did not affect the performance of bambara, which this was contrary to expectation. Usually, under high temperatures, water stress affects plant growth. The lack of an effect of water stress may be due to the fact that bambara landraces were established at 100% ETc to allow for optimum crop stand; water stress was only imposed after full emergence. This would suggest that while bambara is sensitive to water stress during establishment, the crop is less sensitive to water stress post-establishment. Overall, these results suggested that temperature is more limiting than water stress. Dark-coloured selections were observed to perform well under both water regimes. Generally, plants that emerge well have a higher possibility of growing faster and producing yield.

Seed coat colour and provenances were shown to be a useful tool for assessing seed quality of bambara groundnuts. Dark colour selections had the best quality compared with light-brown colour selection. There was a significant variation between seeds from different locations. Bambara groundnuts seedling growth was affected by soil media and water availability. The best media suitable for bambara is the mixture of sand +clay and sand. Clay media was shown to affect the performance of bambara seedlings. Bambara groundnut was shown to perform better under rainfed conditions in the field compared with irrigated conditions. This was due to the fact that, temperatures were low. Bambara groundnut emergence was shown to be sensitive to low temperature, hence plant growth was low and therefore low yield. However, since bambara is a landrace, there is a great variability between and within them. Despite all the stresses that bambara was subjected to, Tugela Ferry provenance was still able to survive drought stress, accompanied by dark colour landrace selection. Therefore, it is advised farmers to use dark colour selection.

The future direction from this study should be a genetic study of the bambara landraces leading to selection of varieties for crop improvement. That this study was undertaken over one season was a limitation. Although a significant amount of data were obtained under controlled environment conditions to explain crop performance these data need to be expanded with more field data. In future, it should be undertaken over more than one season, preferably covering the times that mimic subtropical, tropical and winter conditions under which emerging farmers could grow the crop.

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APPENDICES

Appendix 2: List of ANOVAs for germination study (Chapter 2)

Variate: Daily Germination (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	856.4	428.2	2.82	
Rep.*Units* stratum					
Day	6	918110.8	153018.5	1009.20	<.001
Provenance	26	17021.5	654.7	4.32	<.001
Day.Provenance	156	59146.4	379.1	2.50	<.001
Residual	376	57010.2	151.6		
Total	566	1052145.3			

CV% = 20.8

Variate: Root_Length_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.4	4.7	0.03	
Rep.*Units* stratum					
Variety	25	6853.6	274.1	2.02	0.017
Residual	50	6769.1	135.4		
Total	77	13632.1			

CV% = 25.7

Variate: Shoot_Length_mm

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		24.28	12.14	0.58	
Rep.*Units* stratum						
Variety	25		1871.24	74.85	3.57	<.001
Residual	46	(4)	964.30	20.96		
Total	73	(4)	2660.78			

CV%= 40.0

Variate: Fresh_mass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.09852	0.04926	1.38	
Rep.*Units* stratum					
Variety	25	2.57344	0.10294	2.89	<.001
Residual	50	1.78174	0.03563		
Total	77	4.45370			

CV% = 13.1

Variate: Dry_mass_g

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.007988	0.003994	0.51	
Rep.*Units* stratum						
Variety	24	(1)	0.312482	0.013020	1.66	0.067
Residual	48	(2)	0.375714	0.007827		

Total 74 (3) 0.695913

CV% = 17.9

Variate: Root_Shoot_Ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		9.126	4.563	2.23	
Rep.*Units* stratum						
Variety	25		478.775	19.151	9.36	<.001
Residual	46	(4)	94.109	2.046		

Total 73 (4) 513.179

CV% = 29.1

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2789	0.1394	0.32	
Rep.*Units* stratum					
Provenance	26	24.3703	0.9373	2.18	0.009
Residual	52	22.4042	0.4308		

Total 80 47.0533

CV%= 15.4

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.02406	0.01203	0.42	

Rep.*Units* stratum

Provenance	26	2.00626	0.07716	2.66	0.001
Residual	52	1.50566	0.02895		

Total 80 3.53598

CV%= 3.2

Appendix 3: List of ANOVAs for Seedling Establishment (Chapter 3)

Variate: Emergence

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	9		3.962E+02	4.403E+01	535.10	
REP.*Units* stratum						
DAP	29		4.588E+03	1.582E+02	1923.05	<.001
MEDIA	2		4.083E+01	2.042E+01	248.14	<.001
TREATMENT	2		8.624E+02	4.312E+02	5240.76	<.001
VARIETY	14		4.821E+01	3.444E+00	41.86	<.001
DAP.MEDIA	58		4.072E+01	7.021E-01	8.53	<.001
DAP.TREATMENT	58		3.776E+02	6.511E+00	79.14	<.001
MEDIA.TREATMENT	4		2.154E+01	5.385E+00	65.45	<.001
DAP.VARIETY	406		7.890E+01	1.943E-01	2.36	<.001
MEDIA.VARIETY	28		4.999E+01	1.785E+00	21.70	<.001
TREATMENT.VARIETY	28		1.254E+02	4.478E+00	54.43	<.001
DAP.MEDIA.TREATMENT	116		5.121E+01	4.415E-01	5.37	<.001
DAP.MEDIA.VARIETY	812		5.318E+01	6.549E-02	0.80	1.000
DAP.TREATMENT.VARIETY	812		1.235E+02	1.521E-01	1.85	<.001

MEDIA.TREATMENT.VARIETY	56		7.127E+01	1.273E+00	15.47	<.001
DAP.MEDIA.TREATMENT.VARIETY						
	1624		1.098E+02	6.763E-02	0.82	1.000
Residual	36437	(4)	2.998E+03	8.228E-02		
Total	40495	(4)	1.004E+04			

CV% = 52.5

Variate: Leaf Number

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	4		18.6679	4.6670	12.45	
REP.*Units* stratum						
MEDIA	2		97.1026	48.5513	129.51	<.001
TREATMENT	2		1008.3085	504.1542	1344.86	<.001
VARIETY	14		29.0449	2.0746	5.53	<.001
WEEK	3		1830.8616	610.2872	1627.98	<.001
MEDIA.TREATMENT	4		43.7053	10.9263	29.15	<.001
MEDIA.VARIETY	28		31.7320	1.1333	3.02	<.001
TREATMENT.VARIETY	28		33.0933	1.1819	3.15	<.001
MEDIA.WEEK	6		24.7682	4.1280	11.01	<.001
TREATMENT.WEEK	6		65.3410	10.8902	29.05	<.001
VARIETY.WEEK	42		43.2719	1.0303	2.75	<.001
MEDIA.TREATMENT.VARIETY	56		36.9158	0.6592	1.76	<.001
MEDIA.TREATMENT.WEEK	12		30.7604	2.5634	6.84	<.001
MEDIA.VARIETY.WEEK	84		41.9401	0.4993	1.33	0.025
TREATMENT.VARIETY.WEEK	84		41.4010	0.4929	1.31	0.031

MEDIA.TREATMENT.VARIETY.WEEK						
	168		84.3045	0.5018	1.34	0.003
Residual	2144	(12)	803.7319	0.3749		
Total	2687	(12)	4262.8092			

CV% = 31.4

Variate: Plant height

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	4		1484.3	371.1	1.75	
REP.*Units* stratum						
MEDIA	2		46135.7	23067.9	109.00	<.001
TREATMENT	2		809386.6	404693.3	1912.34	<.001
VARIETY	14		30911.5	2208.0	10.43	<.001
WEEK	3		1031428.2	343809.4	1624.64	<.001
MEDIA.TREATMENT	4		72634.0	18158.5	85.81	<.001
MEDIA.VARIETY	28		14418.7	515.0	2.43	<.001
TREATMENT.VARIETY	28		53343.3	1905.1	9.00	<.001
MEDIA.WEEK	6		6429.9	1071.7	5.06	<.001
TREATMENT.WEEK	6		61525.4	10254.2	48.46	<.001
VARIETY.WEEK	42		20256.6	482.3	2.28	<.001
MEDIA.TREATMENT.VARIETY	56		24356.0	434.9	2.06	<.001
MEDIA.TREATMENT.WEEK	12		14469.7	1205.8	5.70	<.001
MEDIA.VARIETY.WEEK	84		25082.8	298.6	1.41	0.009
TREATMENT.VARIETY.WEEK	84		45325.7	539.6	2.55	<.001
MEDIA.TREATMENT.VARIETY.WEEK						
	168		41733.3	248.4	1.17	0.070

Residual	2144	(12)	453718.1	211.6		

Total 2687 (12) 2750209.8

CV% = 40.4

Variate: Shoot_legnth_mm

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		3060.1	1530.1	7.00	
Rep.*Units* stratum						
Media	2		843.1	421.5	1.93	0.149
Treatment	2		48689.1	24344.5	111.36	<.001
Variety	8		26838.8	3354.8	15.35	<.001
Media.Treatment	4		40374.4	10093.6	46.17	<.001
Media.Variety	16		9279.2	579.9	2.65	0.001
Treatment.Variety	16		11493.9	718.4	3.29	<.001
Media.Treatment.Variety	30	(2)	6939.7	231.3	1.06	0.396
Residual	154	(6)	33666.3	218.6		

Total 234 (8) 175709.6

CV% = 20.2

Variate: Root_length_mm

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		192.45	96.22	2.29	
Rep.*Units* stratum						
Media	2		1411.25	705.63	16.80	<.001
Treatment	2		2615.38	1307.69	31.14	<.001

Variety	8		766.13	95.77	2.28	0.025
Media.Treatment	4		194.39	48.60	1.16	0.332
Media.Variety	16		3033.34	189.58	4.51	<.001
Treatment.Variety	16		1130.73	70.67	1.68	0.055
Media.Treatment.Variety	30	(2)	1928.29	64.28	1.53	0.051
Residual	154	(6)	6467.34	42.00		

Total 234 (8) 17328.09

CV% = 16.3

<u>Variate: Root_Volume_ml</u>

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		3.1840	1.5920	11.18	
Rep.*Units* stratum						
Media	2		12.2358	6.1179	42.97	<.001
Treatment	2		5.8306	2.9153	20.48	<.001
Variety	8		3.4834	0.4354	3.06	0.003
Media.Treatment	4		1.9622	0.4906	3.45	0.010
Media.Variety	16		2.6247	0.1640	1.15	0.313
Treatment.Variety	16		2.5722	0.1608	1.13	0.333
Media.Treatment.Variety	30	(2)	4.1759	0.1392	0.98	0.506
Residual	149	(11)	21.2122	0.1424		
Total	229	(13)	53.9659			

CV% = 25.4

Variate: Root_Shoot_Ratio

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.19015	0.09507	1.67	
Rep.*Units* stratum						
Media	2		0.21674	0.10837	1.91	0.152
Treatment	2		1.71992	0.85996	15.12	<.001
Variety	8		0.53530	0.06691	1.18	0.317
Media.Treatment	4		3.04784	0.76196	13.40	<.001
Media.Variety	16		1.97856	0.12366	2.17	0.008
Treatment.Variety	16		1.81592	0.11349	2.00	0.017
Media.Treatment.Variety	30	(2)	1.70609	0.05687	1.00	0.475
Residual	154	(6)	8.75854	0.05687		

Total 234 (8) 19.75091

CV% = 38.8

<u>**Variate: Fresh_Mass_g**</u>

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		2.1646	1.0823	5.82	
Rep.*Units* stratum						
Media	2		14.8184	7.4092	39.84	<.001
Treatment	2		0.2607	0.1304	0.70	0.498
Variety	8		4.5789	0.5724	3.08	0.003
Media.Treatment	4		0.9299	0.2325	1.25	0.292
Media.Variety	16		2.5925	0.1620	0.87	0.603
Treatment.Variety	16		4.0079	0.2505	1.35	0.176

Media.Treatment.Variety	30	(2)	6.9107	0.2304	1.24	0.201
Residual	153	(7)	28.4515	0.1860		

Total 233 (9) 62.3223

CV% = 28.5

Variate: Dry_mass_g

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.023252	0.011626	1.43	
Rep.*Units* stratum						
Media	2		0.058926	0.029463	3.61	0.029
Treatment	2		0.135152	0.067576	8.29	<.001
Variety	8		0.142945	0.017868	2.19	0.031
Media.Treatment	4		0.075520	0.018880	2.32	0.060
Media.Variety	16		0.180964	0.011310	1.39	0.155
Treatment.Variety	16		0.145212	0.009076	1.11	0.348
Media.Treatment.Variety	31	(1)	0.313378	0.010109	1.24	0.198
Residual	151	(9)	1.231380	0.008155		

Total 232 (10) 2.282508

CV% = 29.5

Variate: Proline

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	4.298E-09	2.149E-09	1.40	
REP.*Units* stratum					
TREATMENT	1	3.828E-05	3.828E-05	25030.19	<.001
VARIETY	5	2.376E-05	4.752E-06	3107.30	<.001
TREATMENT.VARIETY	5	1.803E-05	3.606E-06	2357.91	<.001
Residual	22	3.365E-08	1.529E-09		
Total	35	8.011E-05			

CV% = 0.1

Appendix 4: Field trial layout for Bambara groundnut main plot (Chapter 4)

Variate: Emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	164.292	82.146	8.74	
REP.*Units* stratum					
DAP	3	11468.500	3822.833	406.63	<.001
TREATMENT	1	23.361	23.361	2.48	0.118
VARIETY	5	85.500	17.100	1.82	0.117
DAP.TREATMENT	3	10.028	3.343	0.36	0.785
DAP.VARIETY	15	42.000	2.800	0.30	0.995
TREATMENT.VARIETY	5	52.639	10.528	1.12	0.355
DAP.TREATMENT.VARIETY	15	32.972	2.198	0.23	0.999

Residual	94		883.708	9.401		
Total	143		12763.000			

CV% = 19.9

Variate: Plant height

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		178.23	89.11	1.00	
REP.*Units* stratum						
TREATMENT	1		30708.21	30708.21	344.47	<.001
VARIETY	5		7762.49	1552.50	17.42	<.001
WAP	5		2249.34	449.87	5.05	<.001
TREATMENT.VARIETY	5		1267.43	253.49	2.84	0.018
TREATMENT.WAP	5		23557.42	4711.48	52.85	<.001
VARIETY.WAP	25		2469.20	98.77	1.11	0.342
TREATMENT.VARIETY.WAP	25		2531.70	101.27	1.14	0.312
Residual	140	(2)	12480.56	89.15		
Total	213	(2)	82871.31			

CV% = 10.4

Variate: Leaf number

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		4.3437	2.1719	2.70	
REP.*Units* stratum						
TREATMENT	1		3209.4032	3209.4032	3994.80	<.001
VARIETY	5		119.3157	23.8631	29.70	<.001

WAP	5		1187.8855	237.5771	295.72	<.001
TREATMENT.VARIETY	5		91.7960	18.3592	22.85	<.001
TREATMENT.WAP	5		1968.9208	393.7842	490.15	<.001
VARIETY.WAP	25		118.3834	4.7353	5.89	<.001
TREATMENT.VARIETY.WAP	25		98.0420	3.9217	4.88	<.001
Residual	138	(4)	110.8686	0.8034		
Total	211	(4)	6832.2872			

CV% = 11.9

<u>Variate: Biomass per plant</u>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.2129	0.1065	0.19	
REP.*Units* stratum					
TREATMENT	1	49.4847	49.4847	88.24	<.001
VARIETY	5	27.9777	5.5955	9.98	<.001
TREATMENT.VARIETY	5	15.6698	3.1340	5.59	0.002
Residual	22	12.3378	0.5608		
Total	35	105.6830			

CV% = 28.1

<u>Variate: Grain mass per plant</u>

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.0003159	0.0001580	0.79	
REP.*Units* stratum						
TREATMENT	1		0.0111047	0.0111047	55.27	<.001

VARIETY	5		0.0233125	0.0046625	23.20	<.001
TREATMENT.VARIETY	5		0.0233125	0.0046625	23.20	<.001
Residual	21	(1)	0.0042195	0.0002009		
Total	34	(1)	0.0619694			

CV% = 80.7

Variate: HI

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.000487	0.000243	0.14	
REP.*Units* stratum						
TREATMENT	1		0.088604	0.088604	51.03	<.001
VARIETY	5		0.098802	0.019760	11.38	<.001
TREATMENT.VARIETY	5		0.098802	0.019760	11.38	<.001
Residual	21	(1)	0.036461	0.001736		
Total	34	(1)	0.321096			

CV% = 84

Variate: Number of grain per plant

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.05820	0.02910	1.00	
REP.*Units* stratum						
TREATMENT	1		2.75258	2.75258	94.78	<.001
VARIETY	5		1.91443	0.38289	13.18	<.001
TREATMENT.VARIETY	5		1.91443	0.38289	13.18	<.001
Residual	21	(1)	0.60985	0.02904		

Total 34 (1) 7.14286

CV% = 61.6

Variate: Pod number per plant

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.05820	0.02910	1.00	
REP.*Units* stratum						
TREATMENT	1		2.75258	2.75258	94.78	<.001
VARIETY	5		1.91443	0.38289	13.18	<.001
TREATMENT.VARIETY	5		1.91443	0.38289	13.18	<.001
Residual	21	(1)	0.60985	0.02904		

Total 34 (1) 7.14286

CV% = 61.6

Variate: Pod mass per plant

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.3367	0.1683	1.18	
REP.*Units* stratum						
TREATMENT	1		1.9482	1.9482	13.63	0.001
VARIETY	5		4.2092	0.8418	5.89	0.001
TREATMENT.VARIETY	5		4.2092	0.8418	5.89	0.001
Residual	21	(1)	3.0020	0.1430		

Total 34 (1) 13.5745

CV% = 62

Variate: Chlorophyll content index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	516.92	258.46	12.63	
Rep.*Units* stratum					
Treatment	1	9959.57	9959.57	486.62	<.001
WAP	4	71.22	17.81	0.87	0.483
variety	5	387.78	77.56	3.79	0.003
Treatment.WAP	4	53.39	13.35	0.65	0.626
Treatment.variety	5	507.40	101.48	4.96	<.001
WAP.variety	20	591.46	29.57	1.44	0.110
Treatment.WAP.variety	20	429.00	21.45	1.05	0.410
Residual	154	3151.90	20.47		

Total 215 15668.66

CV% = 14.3

Variate: Stomatal conductance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	519.6	259.8	0.98	
REP.*Units* stratum					
TREATMENT	1	287537.5	287537.5	1088.20	<.001
VARIETY	5	2198.8	439.8	1.66	0.147
WAP	5	18143.1	3628.6	13.73	<.001
TREATMENT.VARIETY	5	309.3	61.9	0.23	0.947
TREATMENT.WAP	5	426.2	85.2	0.32	0.899
VARIETY.WAP	25	12514.7	500.6	1.89	0.011

TREATMENT.VARIETY.WAP	25	1484.3	59.4	0.22	1.000
Residual	142	37520.9	264.2		
Total	215	360654.5			

CV% = 9.7

Appendix 5: Bambara groundnut under controlled environment (Chapter 5)

Variate: Soil_moisture_content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	10.95	5.48	0.36	
Reps.*Units* stratum					
Temperature_TREATMENT	1	19900.52	19900.52	1312.70	<.001
WATERTreatment	1	1154.91	1154.91	76.18	<.001
DAP	4	5134.69	1283.67	84.68	<.001
Variety	5	762.55	152.51	10.06	<.001
Temperature_TREATMENT.WATERTreatment	1	1246.94	1246.94	82.25	<.001
Temperature_TREATMENT.DAP	4	3009.45	752.36	49.63	<.001
WATERTreatment.DAP	4	1354.32	338.58	22.33	<.001
Temperature_TREATMENT.Variety	5	809.89	161.98	10.68	<.001
WATERTreatment.Variety	5	254.37	50.87	3.36	0.006
DAP.Variety	20	404.14	20.21	1.33	0.159
Temperature_TREATMENT.WATERTreatment.DAP	4	340.10	85.03	5.61	<.001

Temperature_TREATMENT.WATERTreatment.Variety					
	5	148.39	29.68	1.96	0.086
Temperature_TREATMENT.DAP.Variety					
	20	727.47	36.37	2.40	<.001
WATERTreatment.DAP.Variety					
	20	276.52	13.83	0.91	0.572
Temperature_TREATMENT.WATERTreatment.DAP.Variety					
	20	494.44	24.72	1.63	0.047
Residual	238	3608.07	15.16		

Total 359 39637.74

CV% = 10.8

Variate: %_Emegernce

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	4295.6	2147.8	2.82	
Reps.*Units* stratum					
DAP	27	889285.7	32936.5	43.23	<.001
Temperature_TREATMENT	1	1414464.3	1414464.3	1856.68	<.001
Variety	5	114206.3	22841.3	29.98	<.001
WATERTreatment	1	972.2	972.2	1.28	0.259
DAP.Temperature_TREATMENT					
	27	592757.9	21954.0	28.82	<.001
DAP.Variety	135	81904.8	606.7	0.80	0.955
Temperature_TREATMENT.Variety					
	5	99226.2	19845.2	26.05	<.001
DAP.WATERTreatment	27	13472.2	499.0	0.65	0.912
Temperature_TREATMENT.WATERTreatment					
	1	3888.9	3888.9	5.10	0.024

Variety.WATERTreatment	5	23789.7	4757.9	6.25	<.001
DAP.Temperature_TREATMENT.Variety					
	135	76884.9	569.5	0.75	0.984
DAP.Temperature_TREATMENT.WATERTreatment					
	27	10555.6	390.9	0.51	0.982
DAP.Variety.WATERTreatment					
	135	25099.2	185.9	0.24	1.000
Temperature_TREATMENT.Variety.WATERTreatment					
	5	26468.3	5293.7	6.95	<.001
DAP.Temperature_TREATMENT.Variety.WATERTreatment					
	135	25754.0	190.8	0.25	1.000
Residual	1342	1022371.0	761.8		

Total 2015 4425396.8

CV% = 84

Variate: Plant Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	3189.	1595.	0.39	
Reps.*Units* stratum					
Temperature_TREATMENT	1	2258536.	2258536.	552.96	<.001
Variety	5	163160.	32632.	7.99	<.001
WAP	5	101283.	20257.	4.96	<.001
WATER_Treatment	1	660.	660.	0.16	0.688
Temperature_TREATMENT.Variety					
	5	169247.	33849.	8.29	<.001
Temperature_TREATMENT.WAP					
	5	79640.	15928.	3.90	0.002
Variety.WAP	25	28828.	1153.	0.28	1.000

Temperature_TREATMENT.WATER_Treatment					
	1	106.	106.	0.03	0.872
Variety.WATER_Treatment	5	24109.	4822.	1.18	0.319
WAP.WATER_Treatment	5	3048.	610.	0.15	0.980
Temperature_TREATMENT.Variety.WAP					
	25	30812.	1232.	0.30	1.000
Temperature_TREATMENT.Variety.WATER_Treatment					
	5	25633.	5127.	1.26	0.284
Temperature_TREATMENT.WAP.WATER_Treatment					
	5	3300.	660.	0.16	0.976
Variety.WAP.WATER_Treatment					
	25	14752.	590.	0.14	1.000
Temperature_TREATMENT.Variety.WAP.WATER_Treatment					
	25	14482.	579.	0.14	1.000
Residual	286	1168151.	4084.		
Total	431	4088937.			

CV%= 85

Variate: Leaf_Number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	21.00	10.50	0.55	
Reps.*Units* stratum					
Temperature_TREATMENT	1	6920.00	6920.00	363.48	<.001
Variety	5	471.71	94.34	4.96	<.001
WAP	5	1803.76	360.75	18.95	<.001
WATER_Treatment	1	1.02	1.02	0.05	0.817
Temperature_TREATMENT.Variety					
	5	465.26	93.05	4.89	<.001

Temperature_TREATMENT.WAP					
	5	1536.32	307.26	16.14	<.001
Variety.WAP	25	214.39	8.58	0.45	0.990
Temperature_TREATMENT.WATER_Treatment					
	1	5.11	5.11	0.27	0.605
Variety.WATER_Treatment	5	118.47	23.69	1.24	0.288
WAP.WATER_Treatment	5	25.13	5.03	0.26	0.932
Temperature_TREATMENT.Variety.WAP					
	25	216.34	8.65	0.45	0.990
Temperature_TREATMENT.Variety.WATER_Treatment					
	5	115.65	23.13	1.21	0.302
Temperature_TREATMENT.WAP.WATER_Treatment					
	5	39.09	7.82	0.41	0.841
Variety.WAP.WATER_Treatment					
	25	73.30	2.93	0.15	1.000
Temperature_TREATMENT.Variety.WAP.WATER_Treatment					
	25	75.39	3.02	0.16	1.000
Residual	286	5445.00	19.04		

Total 431 17546.94

CV% = 100

<u>Variate: Total_Biomass</u>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.904	0.952	0.27	
Reps.*Units* stratum					
WATER_Treatment	1	1.177	1.177	0.33	0.571
Variety	5	600.970	120.194	33.75	<.001
WATER_Treatment.Variety	5	11.759	2.352	0.66	0.657

Residual	22	78.357	3.562		

Total 35 694.167

CV% = 27.3

Variate: Pod number per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	26.00	13.00	1.16	
Reps.*Units* stratum					
WATER_Treatment	1	1.00	1.00	0.09	0.768
Variety	5	2371.00	474.20	42.29	<.001
WATER_Treatment.Variety	5	41.33	8.27	0.74	0.604
Residual	22	246.67	11.21		

Total 35 2686.00

CV% = 37.2

Variate: Pod_mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.6278	0.3139	0.43	
Reps.*Units* stratum					
WATER_Treatment	1	2.5387	2.5387	3.48	0.075
Variety	5	119.5684	23.9137	32.79	<.001
WATER_Treatment.Variety	5	2.7059	0.5412	0.74	0.600
Residual	22	16.0455	0.7293		

Total 35 141.4863

CV% = 38

Variate: Seed_number per pod

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.0556	0.0278	0.23	
Reps.*Units* stratum					
WATER_Treatment	1	0.0278	0.0278	0.23	0.633
Variety	5	1.4722	0.2944	2.48	0.063
WATER_Treatment.Variety	5	0.1389	0.0278	0.23	0.943
Residual	22	2.6111	0.1187		

Total 35 4.3056

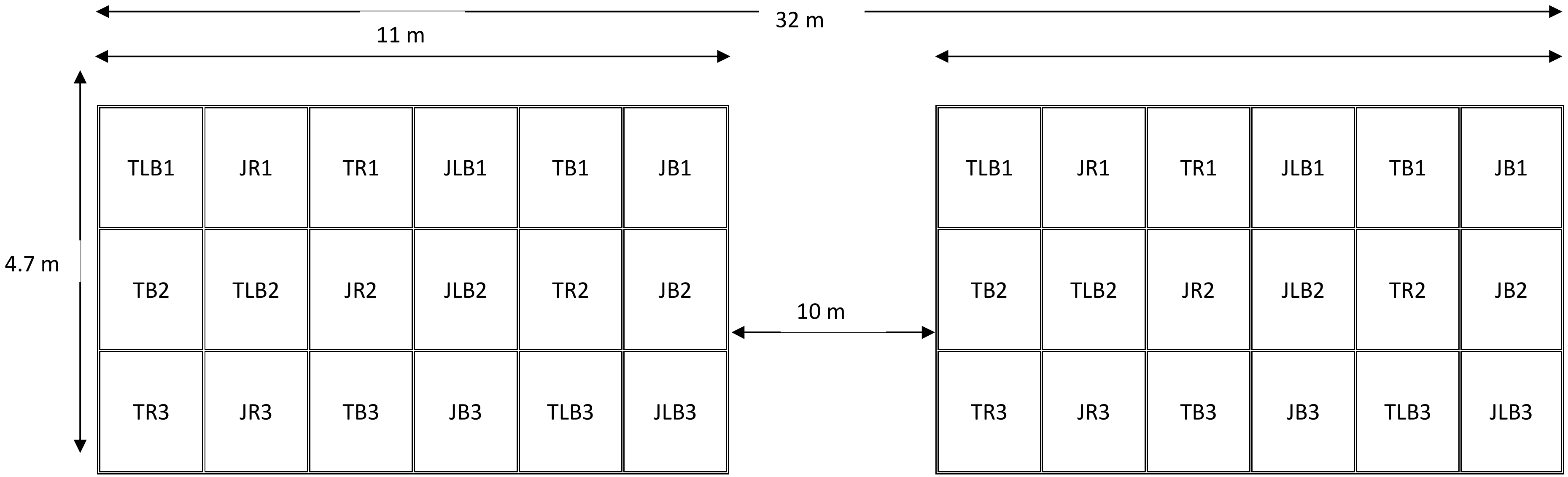
CV% = 40

Variate: Seed_mass_plant

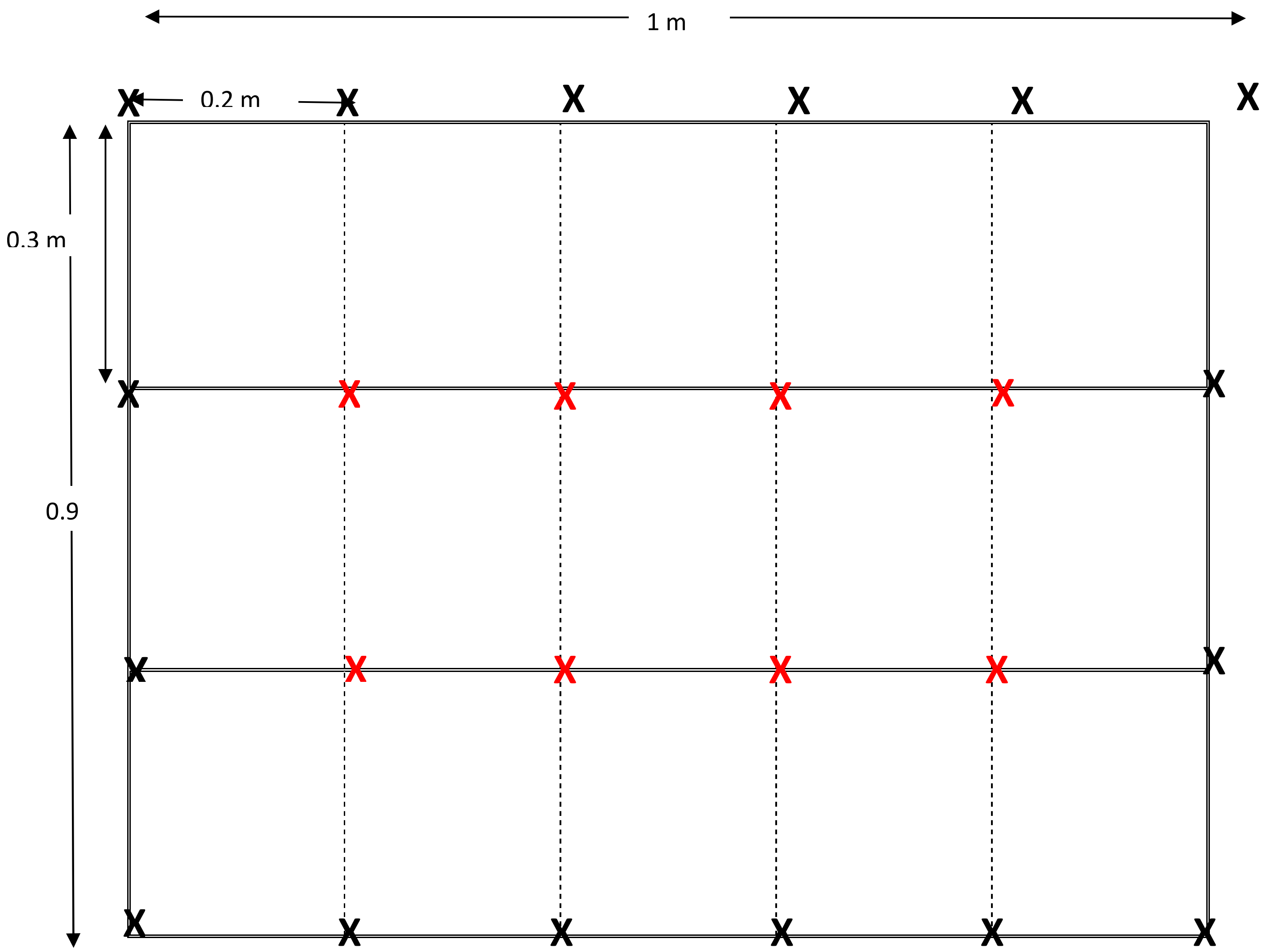
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.3843	0.1922	0.36	
Reps.*Units* stratum					
WATER_Treatment	1	0.8525	0.8525	1.59	0.221
Variety	5	63.8558	12.7712	23.77	<.001
WATER_Treatment.Variety	5	1.1935	0.2387	0.44	0.813
Residual	22	11.8197	0.5373		
Total	35	78.1059			

CV% = 51

Field layout (Main overview)



Individual plot layout



Key words

X border rows

X Experimental rows