

THE MORPHO-PHYSIOLOGICAL EFFECT OF DROUGHT AND DROUGHT QTL ANALYSIS ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA(L)* VERDC)

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Abstract

Global warming threatens global food production with rising temperatures and increasing periods of drought. Bambara groundnut with its relatively high percentage of seed protein and its ability to produce some yield where other crops fail seems to fit the characteristics of an alternative crop for the future. Although Bambara groundnut is said to be drought tolerant and is more resilient than many other crops, few studies have been carried out on this crop in comparison to other crops, especially in terms of drought tolerance. The aim of the study is to evaluate the different genotypes and a segregating population under drought and perform QTL analysis of agronomic and drought related traits for the mapped populations.

A field experiment was conducted at Crop for the Future Research Centre (Field Research Centre) in Malaysia from January 2016 to May 2016 to evaluate two genotypes from dry (S19-3 and DipC) and two from wet (Uniswa Red and Gresik) countries. The plants were harvested starting from 120 DAS due to rain daily starting from 110 DAS which could damage the pods. In this experiment, S19-3 recorded the highest seed yield (Irrigated=10.26g/plant, Rainfed=10.03g/plant) in both treatments as it has completed its lifecycle (>100 days) whereas the other genotypes were still in pod

filling stage. Under water stressed conditions, the crops complete their lifecycle earlier which may explain the higher number of seeds in DipC and Uniswa Red under rainfed conditions (DipC: Irrigated= 3.07g/plant, Rainfed= 5.30g/plant; Uniswa Red: Irrigated= 2.89g/plant, Rainfed= 7.53g/plant). The result from this experiment gives an insight into the suitable genotypes to be considered when planting the crop in Malaysia under rainfed conditions.

The second experiment was set up in a controlled environment glasshouse at the Future Crop Glasshouses, Sutton Bonington Campus, the University of Nottingham, UK. 105 F_3 lines from a segregating population derived from the cross between IITA (maternal) and LunT (paternal) was evaluated in a Randomised Complete Block Design with three replicates. For the droughted plot, irrigation was terminated for five weeks at 56 DAS, after 50% flowering was observed across all the lines. Irrigation was resumed at 91 DAS and the ability of the plant to recover was observed. The total soil moisture content based on PR2 readings during the drought treatment was reduced by an average of 22.3% and the mean value for stomatal conductance reduced from 150.45 mmol m^{-2} s^{-1} at 62 DAS to 48.6 mmol m^{-2} s^{-1} at 90 DAS. When observed, the plants were wilted, and leaves were drying off indicating severe water stress. As drought was introduced at 50% flowering, the pod filling stage was affected by drought stress. *Pod number*, *pod weight*, *no of seed*, *seed*

weight, and *harvest index* were reduced significantly in the drought treatment of the F_3 population. Rewatering at 91 DAS resulted in significant increases in both stomatal conductance and SPAD readings and it continued to increase until 104 DAS.

A group of 682 markers were subjected to linkage analysis. A genetic map comprised of 161 markers with an average spacing of 9.0 cM covering 1447.8 cM was constructed for the IITA x LunT F_2 population for QTL analysis. Morphological and physiological differences observed within the F_3 segregating population in the glasshouse experiment provided trait data for a QTL analysis. The Interval Mapping (IM) results produced a total of 23 QTLs for drought and 15 QTLs for irrigated, with 5 significant QTLs and 33 putative QTLs, associated with 14 studied traits including *double-seeded pods*, *biomass*, *shelling percentage*, *stomatal conductance*, *SPAD readings*, *leaf width*, *seed length*, *days to podding*, *days to flowering*, *pod width*, *pod length*, *seed width*, *seed length*, *single seed weight* distributed over 9 linkage groups: LG1, LG2, LG3, LG4, LG5, LG6, LG7, LG9 and LG10. Overlapping QTLs were found on LG 3, LG 4, LG 7 and LG 9. The results from this study will help to further evaluate and understand the drought responses in Bambara groundnut which could be an important for agricultural biodiversity and food security challenges

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"Death is not the greatest loss. The greatest loss is what dies inside while still alive. Never surrender." (Tupac Shak)

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

The world is currently faced with a crisis in terms of food security. Food security is defined as a state whereby all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (*AQUASTAT - FAO's Information System on Water and Agriculture*, 2016). In 2009, the World Summit on Food Security stated that the four pillars of food security are availability, access, utilization, and stability.

The growing population places enormous pressure on food security as more mouths needs to be fed. The current world population of 7.4 billion is expected to reach 9.8 billion in 2050 with the highest rate of population growth in Africa, which accounts for more than half of the world's population growth (UN, 2017). The majority of the food insecure population are farmers or farm labourers living in rural areas where they are not able to produce enough food or make adequate income to purchase food. As an addition to this, resource constraints and climate change is likely to have a stronger impact in coming years where rising temperatures, floods and droughts are predicted to reduce crop yields.

Drought in Sub- Saharan Africa and South Asia from year 2003 and 2015, resulted in losses amounting to USD 23.5 billion which represents 77% of all production losses due to drought worldwide during the same period (*AQUASTAT - FAO's Information System on Water and Agriculture*, 2016). These losses will only increase in the future following global warming. In analysing how precipitation and soil moisture content changes due to rising greenhouse gases in global climate models, Wang (2015) reported that the predicted soil moisture changes suggest that there will be a worldwide agricultural drought.

World agriculture is dependent on the four largest commodity crops; wheat, rice, maize and soybeans which represent about 75% of the calories that humans directly or indirectly consume. A 6% fall in wheat production is expected for each °C of increase in temperature (Asseng *et al.*, 2015). Similar to that, (David Pimentel, 1993) notes that global warming is likely to alter production of rice, wheat, corn, beans, and potatoes. High prices of fertilisers, fragile soils, and changes in the growing environment are all challenges to agriculture. There is therefore an increasing trend to search for options to improve agricultural biodiversity (Kahane *et al.* 2013).

1.2 Bambara Groundnut

Bambara groundnut (*Vigna subterranea(L)* Verdc.) is a grain legume indigenous to sub- Saharan Africa where it is widely cultivated. It is also cultivated in some Asian countries such as Thailand, Philippines, Malaysia and India. Very similar to peanuts, the crop is a prostrate annual with short lateral stems bearing compound leaves consisting of three leaflets. It has a well-developed taproot system and may grow as a bunch, semi-spreading or spreading type. The crop is grown for its pods which are approximately 1.5 cm long and may be wrinkled and slightly oval or round. Each pod contains one or two seeds and is usually buried in the ground for protection from pests and herbivores. The colour of the seeds varies depending on its landrace.

Bambara groundnut is regarded as the third most important legume in much of Africa after peanuts and cowpeas (Vietmeyer, 1986; S. Basu *et al.*, 2007). Though it is a very important crop, it is regarded as a 'poor-man crop' as it is a not a very lucrative cash crop and is seen more as a snack or food supplement. It is grown traditionally by women and small-scale rural farmers, giving it less value and less priority in land allocation. As it is difficult to harvest mechanically, it discourages large-scale commercial farmers from growing them.

The edible seeds of Bambara groundnut can be eaten fresh as snacks but the majority of them are consumed after they are dried. The seeds contain 18 – 24% of protein with high lysine and methionine contents and are rich in iron (4.9- 48 mg/100 g) compared to the trait range of 2.0-10.0 mg/100 g, for most food legumes (Rowland, 1993). The ground seeds of Bambara groundnuts are reported to be comparable to or even able to replace other conventional flours (Poulter and Caygill 1980; Brought et al. 1993) and the vegetable milk is comparable with soy milk. In fact, the Bambara milk is preferred because of its flavour and colour (Goli, 1997). The seeds are also used to feed poultry and livestock.

1.3 Drought tolerance in Bambara groundnut

The availability of water to plants is determined by the soil water concentration and the volume of soil explored by the roots. Stimulation of crop growth can be achieved by moderate levels of soil moisture and inhibited by either excessive or deficit levels (Israelsen & Hansen, 1962). Several studies have been carried out on Bambara groundnut under water deficit conditions in controlled environments and the field.

Leaf growth and development are usually affected by water stress and this is usually expressed as leaf area index for growth and total leaf number for development. A decreasing trend in plant height

and leaf number was observed in response to increasing water stress (T. Mabhaudhi, Modi and Beletse, 2013). A reduction in plant height and leaf number due to drought corresponds to a reduction in LAI. Leaf area of S-19-3 and Uniswa Red under drought was significantly reduced by 43% and 54% respectively 13 days after onset of drought treatment at 38 days after sowing (Jørgensen *et al.*, 2010) . Similar results were also reported by Mabhaudhi *et al* (2013) and Mwale *et al* (2007) where a decrease in LAI was observed as water stress increased and a 36% reduction in LAI in drought treatments were observed, respectively. Al Shareef *et al* (2014) reported that the decline of LAI in drought differed between temperatures and severity of drought. In the experiment carried out in this study, temperatures and landraces were the same across three years of planting. In year one, soil moisture was non-limiting and the plots were irrigated weekly to field capacity. In year two, late season drought was imposed at podding (77 DAS), and in year three at flowering (30 DAS). It was observed that as the severity of drought in Al Shareef *et al* (2013) was increased across the planting years, LAI of S19-3 decreased from 2.7 in 2006 to 1.2 in 2008 at 33 ◦C and from 1.2 in 2006 to 0.6 in 2008 at 23 ◦C.

As water stress restricts several plant processes from the cell to the canopy, plants under drought produce lower total dry matter (TDM) compared to plants with unlimited access to water. An experiment

carried out with two soil moisture treatments imposed: irrigated to 90% field capacity each week and irrigated to 60% field capacity until establishment (27 days after sowing) with no further irrigation by Collinson *et al*. (1999) reported a TDM of 5.8 t/ha for the irrigated treatments compared to 1.1 t/ha for the stress treatment. Similarly, Mwale *et al*. (2007) reported a 40% reduction in accumulated total dry matter under drought. Pod yield also decreased with drought from 2.7 t/ha to 0.05 t/ha (Collinson, Berchie and Azam-Ali, 1999) and 298 gm^{-2} to 165 gm^{-2} (Mwale, Azam-Ali and Massawe, 2007b). Pod number per plant was the yield component most severely affected by drought with a reduction of 43% (Mwale, Azam-Ali and Massawe, 2007b). On the other hand, a field trial carried out in Ghana reported that Burkina had some pods under drought whereas NAV4, Black eye, NAV Red and Tom produced no pods at all (Berchie *et al*., 2012). The ability of a plant to partition dry matter into harvestable yield under limited water supply is an important trait for drought tolerant crops. Seed weight and harvest index (HI) were reduced by 16% and 15%, respectively, in drought treatment plants (Mwale, Azam-Ali and Massawe, 2007b). Similar results have been reported in mung bean, cowpea, soybean and peanut where HI decreased linearly with increasing levels of drought (Pandey *et al.*, 1984).

Time to floral initiation and the duration from floral initiation to flowering decreases under drought. The impact of drought on

flowering is due to abscisic acid accumulation under the effect of water stress (Blum, 1996). Bambara groundnut landraces were shown to flower early, have a reduced flowering duration and mature early in response to decreasing soil water availability (Mabhaudhi *et al*., 2013). A study by Collinson *et al*. 1999 reported first flower appearance on DodR, LunT and DipC at 40 days after sowing in both drought and irrigated treatments. However, drought reduced cumulative flower production by almost 50% in the DodR and LunT landraces but by only 7% in DipC. This shows that there is a genotype/landrace effect in response to drought. Jørgensen *et al*., 2011 reported that there was genotypic diversity in performance under drought stress, with LunT and Ramayana more sensitive to drought than S19-3 and Uniswa Red. The different responses of the landraces to drought may reflect their adaptation to the local climates of collection. There were significant differences between soil moisture and landrace types on days to flowering, pod and seed production, dry weights and harvest indices (Laary, Ofori and Kumaga, 2012). Depending on soil moisture levels, about 50% of the plants in a Cream-seeded landrace flowered between 32-37 days, and in a Mottled-seeded landrace, between 40-51 days after planting, but after at least five days of first flower appearance in Cream-seeded landrace, and about 10 days in Mottled-seeded landrace. Karikari (2000) reported that the landrace with grouped flowering could have sufficient time for the maturation of the pods and seeds before the

development of significant drought stress. Later flower induction in a synchronised reproductive development stage and hence pod maturity gives higher yields.

Stomatal control of water stress is an avoidance measure and is among the plant's first line of defence to water stress. The closure of stomata reduces transpiration losses, thus minimising water losses through transpiration. Stomatal conductance decreased as water stress increased in Bambara (Mabhaudhi *et al*., 2011; Mabhaudhi *et al*., 2013; Vurayai *et al*., 2011; Jørgensen *et al*., 2010; Jørgensen *et al*., 2011; Collinson *et al*., 1997). Mean daily values declined gradually in all wet, intermediate and dry treatments, but were consistently higher in the wet treatment (0.46-0.79 cm s^{-1}) than in the intermediate and dry treatments (0.13-0.48 cm s^{-1}) (Collinson *et al*., 1997). Plants that were stressed during vegetative and flowering stages completely recovered their stomatal conductance after rewatering compared to the plants that were stressed during pod filling stage (Vurayai *et al*., 2011). The plants were at the final stage of their development and thus appear to have lower regenerative capacity.

Chlorophyll content of leaf is an indicator of the photosynthetic capacity of plant tissues (Nageswara Rao *et al*., 2001). Leaf chlorophyll content in Bambara did not reduce with water stress

(Vurayai *et al*., 2011). Similarly Mabhaudhi *et al*., 2013 reported that there were no significant difference in chlorophyll content between landraces, water regimes or the interaction between these. In contrast, drought caused a gradual decrease in levels of chlorophyll content in pea leaves (Karataş *et al*., 2014) and had a significant effect in wheat genotypes (Arjenaki *et al*., 2012). This shows that Bambara groundnut plants maintain high amounts of chlorophyll content despite the development of moisture deficit stress. This trait can be considered to be a line of defence against drought which can result in drought resistance (Vurayai *et al*., 2011).

1.4 Genetic Variation of Bambara groundnut and the marker assisted approach

Bambara groundnut originated in West Africa. It has considerable genetic diversity and is grown by small scale farmers as heterogeneous landraces. The International Institute of Tropical Agriculture (IITA) in Nigeria currently holds the major germplasm collection of Bambara groundnut however, the genetic resources of this crop have not been fully exploited. Evaluation of genetic diversity is a pre-requisite for genetic improvement in crop plants particularly in orphan crops such as Bambara groundnut (Olukolu *et al*., 2012). The genetic potential of the crop could be enhanced through targeted breeding to boost productivity, product quality and improve food security. Several analyses of the genetic diversity of the available

germplasm for Bambara using phenotypic descriptors and molecular marker technologies have been reported. Most of the studies lack adequate representation of the global germplasm (Aliyu *et al*., 2016).

Polygenic characters which were previously very difficult to analyse using traditional plant breeding methods, can now be easily tagged using molecular markers. A molecular marker is a DNA sequence that can be detected and whose inheritance can be easily monitored. These are identifiable DNA sequences that are found at specific locations of a genome, and are transmitted by the law of Mendelian inheritance from one generation to the next. Molecular markers have many advantages over phenotype-based markers that were previously used. They are stable, not environmentally regulated which means that they are unaffected by the conditions in which plants are grown and are detectable in all tissues regardless of plant growth (Mohan *et al*., 1997; Agarwal *et al*., 2008).

One of the early characterization studies on Bambara groundnut accessions carried out by Goli *et al*. (1997) using phenotypic descriptors and evaluated the frequency of seed-pattern states of 1384 accessions from the IITA collection. Pasquet *et al*. (1999) then carried out a study to investigate Bambara groundnut population structure and the partitioning of genetic diversity between domesticated and wild accessions. 41 isozyme loci were scored to

evaluate the population structure of 100 Bambara accessions and the study concluded that overall isozyme diversity was low and wild Bambara groundnut is the progenitor of domesticated Bambara groundnut. On the other hand, Amadou *et al*. (2001) and Massawe *et al*. (2002) using Random Amplified Polymorphic DNA (RAPD) analysis and Amplified Fragment Length Polymorphism (AFLP) markers respectively, reported considerable genetic diversity among bambara groundnut accessions. Small population sizes (25 and 16 accessions respectively) however, limits the conclusion drawn from their investigations. Genetic relationships in Bambara landraces from different regions in Africa were related to their place of collection (Massawe *et a*l., 2003).

Diversity Arrays Technique (DArT) markers provide better validation at a purely genetic level due to their wide genome coverage, phenotypic neutrality and reproducibility. Olukolu *et al*. (2012) included 124 accessions collected from 25 African countries and carried out an assessment of the genetic diversity within Bambara groundnut germplasm based on phenotypic descriptors and DArT markers. This study supported Nigeria and Cameroon as the likely centre of origin of Bambara groundnut as well as showing the highest phenotypic diversity among accessions. Molecular marker analysis of polymorphism in Bambara groundnut (Molosiwa, 2012) involving 20 SSR and 201 DArT Array markers, evaluated 123 single genotype

accessions from diverse landraces and found that, for the genotypes tested, landraces are essentially a series of highly inbred lines (polymorphic information content of 0.66 for the SSR markers, an expected heterozygosity of 0.69, an observed heterozygosity of 0.02), with substantial genetic variation between lines, within landrace and also between landraces.

Construction of linkage maps in crop species is one of the main uses of DNA markers. It helps to identify chromosomal regions that contain genes controlling simple traits and quantitative traits using QTL analysis (Mohan *et al*., 1997). Many QTLs have been identified by using DNA markers across various crops such as tomato, rice, maize and barley. Ideally, all the QTLs identified for marker assisted selection (MAS) should be stable across environments and be adequately tested for prediction of phenotype before used in MAS. The ability to develop genetic maps that could aid MAS in bambara groundnut breeding programmes is a strategic objective.

A first linkage map was constructed based on a cross between a domesticated landrace and a wild ancestor (DipC and VSSP11), spanning a total of 901cM and expected genome coverage of 79.6% (Ahmad, 2012). Ahmad *et al*., (2013) published the first genetic linkage map in Bambara based on 73 individuals of F_3 population derived from a cross between genotypes drawn from the DipC and

Tiga necaru landraces. The genetic map of 498cM was predicted to cover 44% of the Bambara groundnut genome and identified a major QTL for internode length on LG3. However, the population was too small to construct a high resolution map for QTL studies. The combination of both maps through 32 common markers allowed a common QTL for *days to emergence* to be detected in both populations. Combining dominant DArT and co- dominant SNPs, DArT Seq approach by Chai et al. (2014) with pre-existing microarray-based DArT and SSR markers (Ahmad, 2012) generated the first high density DNA- marker genetic map of 1250.7 cM with 212 spaced markers. Genetic mapping for photoperiod responses in Bambara identified a major QTL for photoperiod control of pod number which was identified in an F_2 population of cross IITA-686 x Ankpa4 consisting of 158 lines evaluated in a 16 hours photoperiod (Kendabie, 2014). More efforts should be made towards the development of large number of progenies in a set of structured segregating populations to improve the understanding of Bambara groundnut genome and focus on complex traits in breeding programs. Ho *et al.* (2017) used two individual intraspecific linkage maps consisting of DArTseq markers, the F_2 population from IITA686 x Ankpa4 cross and the F_3 population of the Tiga Nicuru x DipC cross to demonstrate the use of limited genetic information and resources developed within species to identify conserved synteny blocks in other related legume species which corresponded to the Linkage

Groups (LGs) in bambara groundnut genetic maps. A total of 96 DArTseq markers that were polymorphic in both populations and could be located through the sequence in the marker Tag to syntenic locations shared between the genomes of common bean (*Phaseolus vulgaris*), adzuki bean (*Vigna angularis*) and mung bean (*Vigna radiata*) were used. More than 45% of the bambara groundnut DArTseq markers were locatable on the adzuki bean and mung bean genomes and the QTL location of an agronomic trait in one of the bambara groundnut maps identified a corresponding flanking position in common bean, mung bean and adzuki bean (Ho *et al.*, 2017).

The use of controlled greenhouse or growth room experiments along with field evaluations under relevant conditions enables identification of QTLs for drought resistance mechanisms which are of value for breeding (Price and Courtois, 1999). Several QTL identified for drought have been reported in soybean and common beans. In soybean, several QTLs have been identified using Recombinant Inbred Lines (RILs) to evaluate the genetic component of the soybean response to water (Du *et al*., 2009; Specht *et al*., 2001; Vu *et al*., 2015). Du *et al*., 2009 reported a consistency of QTLs for *seed yield per plant* across different water regimes and *drought susceptibility index* in the field and greenhouse which showed the presence of genes that were stable across different environments. An integrated map using three RIL population comprising 759 DArT markers and 27

linkage groups found linkage groups associated with drought tolerance including *Relative Water Content*, *Epidermal Conductance* and *recovery ability* (Vu *et al*., 2015). The comparison of sequenced DArT markers with drought–related QTLs from previous studies confirmed that five of them overlapped whereas 13 were new. In common bean, Blair *et al*., (2012) found a high number of QTL for *seed weight* under drought. Similar findings were reported previously by Schneider *et al*. (1997). In Faba beans, Khazaei *et al*., (2014) identified 15 putative QTLs of which eight affecting *stomatal densi*ty, *length* and *conductance* and *canopy temperature* were detected. For drought related traits in Bambara groundnut, Chai, (2014) observed significant QTLs for *stomatal conductance*, *carbon isotope discrimination analysis*, and *stomatal density*, and a putative QTL of 3.04 (GW = 3.8) on LG2A was identified for *stomatal conductance* in analysis using Interval Mapping. This was explained to be due to the effect of the mild drought introduced in the study that resulted in a relatively weak association between the loci and the traits.

Better understanding of genotype-by-environment interaction (GEI) is expected to provide a solid foundation for genetic improvement of crop productivity especially under drought-prone environments (Y. Zhang *et al.*, 2010). In earlier studies, genotype × environment interaction (GEI) was considered to occur when there is a change in the relative performance of genotypes for given traits between

different environmental conditions. From a breeding standpoint, GEI must be considered to identify superior and stable genotypes when crops are planted across different environments. This is because, when marker assisted approach (MAS) is to be applied to the crop improvement programs, the target QTL should have a large and consistent effect (Salekdeh et al. 2002). Hence, it is of great importance to understand the nature of these complicated quantitative traits under different environments and in different genetic backgrounds.

Drought responses are controlled by several genes. Therefore, mapping of QTLs is of great importance to understand the genetic architecture underlying these complex traits to better understand the genetic control for their drought responses.

1.5 Aim and objectives of study

Global warming threatens global food production with rising temperatures and drought. Bambara groundnut with its relatively high percentage of protein and its ability to produce some yield where other crops has failed seems to fit the characteristics of an alternative crop for the future. Although Bambara groundnut is said to be drought tolerant and is more resilient than many other crops, few studies have been carried out on this crop in comparison to other

crops especially in terms of drought tolerance. Breeding programs that focus on adaptation traits to drought can lead to genetic improvement and development of new cultivars that will be beneficial to farmers in the future. Firstly, four genotypes (DipC, Uniswa Red, S19-3 and Gresik) were screened under field conditions in Malaysia allowing the responses of the crop under irrigation and rainfed conditions to be studied. Then, a F_3 segregating population derived from the cross between IITA and LunT was subjected to drought in a controlled glasshouse. The initial plan was to use a cross from the genotype used in the field trial in Malaysia, to be planted in the glasshouses in the UK. However, the population was too small (>50 lines) and therefore it was not ideal for mapping and QTL studies purposes. Responses of Bambara groundnut to drought stress were studied also providing phenotypic trait data for QTL mapping. This will help identify some traits or QTLs for marker assisted selection from the existing F_3 line.

The objectives of the study are;

1. To evaluate the different genotypes under irrigated and rainfed treatments in field conditions.
2. To evaluate the effect of drought on the Bambara groundnut segregating population.

3. To perform QTL analysis of agronomic and drought related traits for the mapped populations.

CHAPTER 2: GENERAL MATERIALS AND METHODS

Materials and methods that were commonly used throughout the study are presented. The protocols and procedures which were specifically conducted in some experiments are explained in respective chapters.

2.1 Standard Solution

A list of standard solution used for molecular biology experiments are listed as below:

5X TBE DNA electrophoresis buffer: 54 g Tris base, 27.5 g boric acid and 20 ml 0.5 M EDTA pH 8.0 were added together with 800 ml H_2O. After stiring the final volume was adjusted to 1 L for use.

6X loading buffer (LB): 30% glycerol was prepared using 35 ml molecular grade water. Together with 0.25 g 0.25% (w/v) bromophenol blue, 0.25 g 0.25% (w/v) xylene cyanol and 30 ml 30% glycerol. The solution was topped up with 70 ml H_2O to a final volume of 100 ml.

Lambda DNA (50 ng µl-1): 200 µl uncut Lambda DNA (500 ng µl-1) was mixed with 1,400 µl TE buffer and 400 µl 6X LB for a final volume of 2,000 µl.

2-log DNA ladder (200 ng µl-1): 100 µl 1 µg µl-1 2-log DNA ladder was mixed with 80 µl 6X LB and 320 µl TE buffer.

2.2 Gel Electrophoresis

To make a gel, agarose (Bioline, UK) was dissolved in 0.5X TBE buffer and heated in a microwave with occasional swirling until a clear solution was observed. After cooling, either SYBR® Safe or Ethidium bromide (0.5 µl; 10 mg ml^{-1} stock; per 50 ml gel) was added. The gel was then poured into an appropriate sized gel cast tray. DNA products were quantitated and/or checked by running them respectively on a 1% (w/v), 1.5% (w/v) and 2% (w/v) stained agarose gel at 80 V for 60 min alongside lambda DNA with two concentrations 50 ng $µl^{-1}$ and 10 ng $µl^{-1}$. The gel was then visualised under UV light using the Gel Doc 2000 Gel Documentation System and associated Quantify One 1-D Analysis Software (Biorad, California, US).

2.3 Standard Measurements

A list of standard measurements used for field experiments are listed as below:

Trait	Description
Days to emergence	Number of days from sowing to discovering two fully expanded leaf
Days to flowering	Number of days from emergence to appearance of first flower(s)
Estimated days to podding	Number of days from emergence to discovering first pod
Terminal leaflet length	Recorded at 10 weeks after emergence; Average length of three leaves at the fourth node of a plant
Terminal leaflet width	Recorded at 10 weeks after emergence; Average width of three leaves at the fourth node of a plant
Plant height	Recorded at 10 weeks after emergence; Measured from the ground level (at the base of the plant) to the tip of the highest point of the plant
Plant spread	Recorded at 10 weeks after emergence; Widest length between two opposite points of a plant
Internode length	Recorded at 10 weeks after emergence; Average length of fourth internode of three longest stems
Petiole length	Recorded at 10 weeks after emergence; Average length of three petiole at the fourth node of a plant
Peduncle length	Recorded at harvest; average length of three peduncle of a plant
Number of pods	Number of pods per plant
Pod length	Average length of 10 pods per plant

Trait	Description
Pod width	Average width of 10 pods per plant
Pod dry weight	Weight of pods per plant after incubating at 37 °C for three weeks
Number of seeds per plant	Number of seeds per plant after removing pods
Seed weight per plant	Weight of seeds per plant after incubating at 37 °C for three weeks
Seed width	Average width of 10 seeds per plant
Seed length	Average length of 10 seeds per plant
Above ground biomass	Weight of above ground material after drying at 80°C for 48 hours
Harvest Index	Fraction of pod weight to above ground plant weight
100 seed weight	Weight of 100 seeds per plant
Shelling %	Measured as an average of all pods/plant, based on the weight of matured dried seeds compared to the weight of dried pods.

Table 2. 2: The morpho-physiological traits that were examined and their brief description based on Bambara groundnut descriptors list (IPGRI, IITA and BAMNET, 2000)

CHAPTER 3: FIELD WORK IN MALAYSIA

3.1 Introduction

There are approximately 2000 accessions of Bambara groundnuts in gene banks held by the International Institute of Tropical Agriculture (IITA) which exist in the form of landraces (Massawe *et al.*, 2005). A landrace of a seed propagated crop is defined as a variable population with enough characteristics in common to be recognised as a group which has similar morphology, geographical distribution, agro-ecological adaptation and breeding behaviour but has not been selected or maintained for uniformity (Saxena and Singh, 2006). As Bambara groundnut is an inbreeding crop, seed derived from a single plant is likely to be true breeding. Seeds derived from a single plant from a landrace is multiplied as a group of plants that are genetically the same which are known as genotypes.

In this study, genotypes that include Gresik from Indonesia, DipC from Botswana, Uniswa Red from Swaziland and S19-3 from Namibia were used to evaluate the effect of drought stress on Bambara groundnuts.

3.1.1 Bambara groundnut genotypes: Gresik, DipC, Uniswa Red and S19-3

Gresik takes its name after a place in Indonesia where Bambara groundnut is mostly planted. Indonesia has a tropical climate that varies across its region. Like Malaysia, the eastern monsoon brings the driest weather from June to September while the western monsoon brings the main rains from December to March (Nitis, 2006). The weather is very warm throughout the year and rainstorms can occur all year. The air temperature ranges from 14.8 °C to 35 °C with rainfall ranging from 1000 mm to 3500 mm (Nitis, 2006). In Gresik, the average temperature is 27.5 °C whereas the average rainfall is 1686 mm. Swaziland, where Uniswa Red originates from, is the smallest country in Africa. The main district that favours cropping is the Middleveld. The Middleveld climate is warm and dry with temperatures between 2.5 and 37.2 °C and the annual rainfall ranges from 762 mm to 1,143 mm (Dlamini and Lupupa, 1996).

DipC originates from Botswana which is very prone to drought. The mean rainfall ranges from 250 mm in the extreme south-west to 650 mm in the extreme north-east (Burgess, 2002). The temperature ranges from 12 - 15 °C during the early mornings to 30 - 40 °C by late afternoon in the dry season (mid-September to late October) but maximum temperatures remain 25 - 30 °C during the rainy seasons (Burgess, 2002). Namibia, the origin of S19-3 is also drought prone

as only 5% of the country receives more than 500 mm rainfall annually. In most areas of the country, there is a single wet season in summer and most rain falls between month of November and March. There is a variation in annual rainfall between the different regions with less than 20 mm in the western and coastal zones to more than 700 mm at the eastern end (Sweet and Burke, 2002). The annual potential evapotranspiration exceeds annual precipitation by ratios of up to 30:1 hence drought conditions are a common phenomenon throughout most of the country (Sweet, 1999).

GENOTYPE	ORIGIN	RAINFALL
Gresik	Indonesia	1000 mm to 3500 mm
Uniswa Red	Swazilan	250 mm in the extreme south-west to 650 mm in the extreme north-east
DipC	Botswana	762 mm to 1,143 mm
S-19	Namibia	20 mm in the western and coastal zones to more than 700 mm at the eastern end

Table 3. 2: Comparison of the four genotypes used in the experiment (Gresik, Uniswa Red, DipC and S-19 in terms of origin and rainfall.

3.2 Material and methods

3.2.1 Field site, experiment preparation and sowing

The field experiment was conducted at Crop for the Future Research

Centre (Field Research Centre) (latitute 2.94674819; longitude 101.87261581) in Malaysia from January 2016 to May 2016. The experiment was designed as a split plot; with irrigation (rainfed and irrigated) as the main treatment and genotypes as subplot treatments.

After tractor ploughing and harrowing each plot was hand harrowed to make a fine raised seedbed. Two PR2 profile access tubes (Delta-T devices, UK) were inserted into each subplot 1m apart. Sprinklers spaced at 3m between each other was placed between every two seedbeds. Seed were sown directly on the seedbed with a spacing of 40cm within rows and 50cm between rows to make a total of 40 plants per subplot.



Figure 3. 3: Experimental site, Field Research Centre, Crops for the Future Research Centre, Malaysia.

3.2.2 Plant materials

Four genotypes were tested in the field experiment. Gresik and Uniswa Red from wet conditions and DipC and S19-3 from dry conditions. The seeds used in this experiment were collected from previous experiments carried out in the FutureCrop glasshouses in University of Nottingham, UK and has been purified to a single genotype.

3.2.3 Crop protection

To control insect pest and foliar pathogens, plants were sprayed with systemic insecticide (cypermethrin) and systemic fungicide Benomyl (benzimidazole) using a knapsack sprayer once every three weeks. Plots were hand weeded throughout the experiment and earthing up was done after 100% flowering.

3.2.4 Irrigation

The watering regime for the experiment was synchronised with the existing BAMYIELD project, which had the same planting date and connected through the same source of water supply. A sprinkler irrigation system was used to irrigate the plants. For the rainfed treatment, irrigation was terminated at 30 DAS.

DAS	Irrigation
32	24mm
39	24mm
46	24mm
53	24mm
60	24mm
67	24mm
74	24mm
81	12mm
88	12mm
95	12mm
102	12mm
109	12mm

Table 3. 2: Total amounts of irrigation (mm) applied weekly to the irrigated treatment in the field experiment

3.2.5 Measurements

Soil moisture content

Soil moisture content was monitored using the PR2 probe (Delta-T Devices, UK). Three readings were taken, in each access tube (two access tubes per subplot) weekly at 1000 hours starting from 22 DAS until 105 DAS at soil depths of 10cm, 20cm, 30cm, 40cm, 60cm and 100cm. Each subplot had two access tubes. The sum of the readings represents the total amount of water in each location and the average of readings represent the mean amount of water in each subplot.

Climatic factors

Air temperature, relative humidity, rainfall, wind speed and solar radiation were recorded hourly by an automatic weather station installed at the experiment site.

Emergence

A seedling was considered emerged when the first fully expanded leaves were visible on the soil surface.

Days to flowering

The days to flowering were recorded from the emergence date to the discovery of the first flower

Normalized Difference Vegetative Index (NDVI)

A Trimble GreenSeeker handheld crop sensor was used to measure the NDV index. The NDV index The GreenSeeker was held at about 60 - 120cm above ground and three measurements were taken on each subplot. The average reading represents the NDV index of each subplot. Measurements were done weekly from 19 DAS to 110 DAS.

Continuous fluorescence yield (F_T) and light adapted quantum yield ($F_{V'}/F_{M'}$)

Continuous fluorescence yield in non-actinic light (F_T) and light adapted quantum yield ($F_{V'}/F_{M'}$) was measured starting from 34 DAS and 47 DAS respectively using the Fluorpen FP-100 MAX-D (Photon Systems Instruments), a portable battery operated fluorometer. Five plants were tagged in each subplot and the measurements were done weekly from 9000 hrs to 1100 hrs on a terminal leaflet of a fully expanded leaf on each plant. The measured leaves were at the top of the canopy and fully exposed to sunlight.

Chlorophyll content

A chlorophyll meter SPAD-50DL PLUS was used to measure the chlorophyll content of the leaves from 23 DAS. Five tagged plants from each subplot were used for measurements weekly from 9000 hrs to 1200 hrs. Measurements were taken on three terminal leaflet of fully expanded leaf on each plant. Three measurements were taken

per leaf and then averaged to represent the chlorophyll reading of the leaf. The average reading of all three leaves represents the chlorophyll reading of the plant.

Leaf area (cm^2)

Leaf area was measured on five tagged plants per subplot. Leaf area was estimated based on the central leaflet length and width using the method of Cornelissen *et al*. (2002) following the equation:

A_{plant} = 0.86 * Leaf number [0.91 * 3 (0.95 * Length * Width * π/4)]

Where leaf number = leaf number/ plant; length and width being mean length and width of the terminal leaflet of three leaves/ plant, and π=3.1416

Yield measurements

For the yield measurements, 12 plants were harvested from each subplot starting from 120 DAS due to rain daily starting from 100 DAS. Pods were oven dried at 37 °C for three weeks and measured. List of yield measurements are as in Table 3.2 below

Yield measurement	Description
Pod number/ plant	Number of pods per plant.
Pod weight/ plant	Weight of pods per plant after incubating for 3 weeks at 37°C.
Seed number/ plant	Counted after removing the shell of all pods.
Seed weight/ plant	Weight of seeds per plant after incubating for 3 weeks at 37°C.
100- seed weight	Average weight of 100 seeds after incubating for 3 weeks at 37°C.
Shelling percentage	Obtained using equation: (seed weight/pod weight) x 100

Table 3. 3: List of yield measurements taken in the field experiment

3.2.6 Data Analysis

All the results were statistically analysed using the software package Genstat 17th edition (Lawes Agriculture Trust, Rothamsted Experimental Station, UK) using repeated measures ANOVA, the difference was considered significant when probability was equal or less than 0.05. All data was also tested for normality.

3.3 Results
3.3.1 Temperature and rainfall

Figure 3.2 shows the maximum, the minimum and the daily mean of temperature at Field Research Centre. The maximum temperature ranged between 28°C and 38°C, and the minimum between 21°C and 26°C. The daily mean ranged between 25°C and 30°C. Total amount of rain was 250.4 mm with the highest recorded at 89.6mm per day. It was raining every day from 100 DAS onwards. Figure 3.3 shows the distribution of rainfall during the experiment.

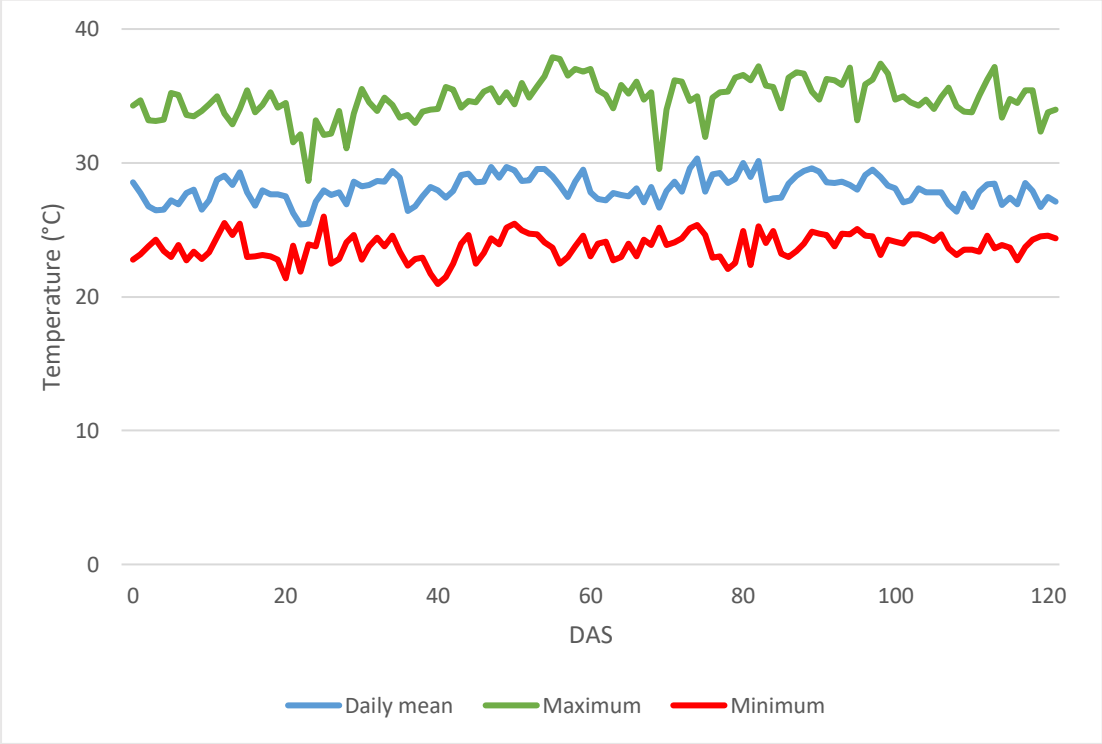


Figure 3. 4: Air temperature at Field Research Centre, Crop for the Future Research Centre during the experiment.

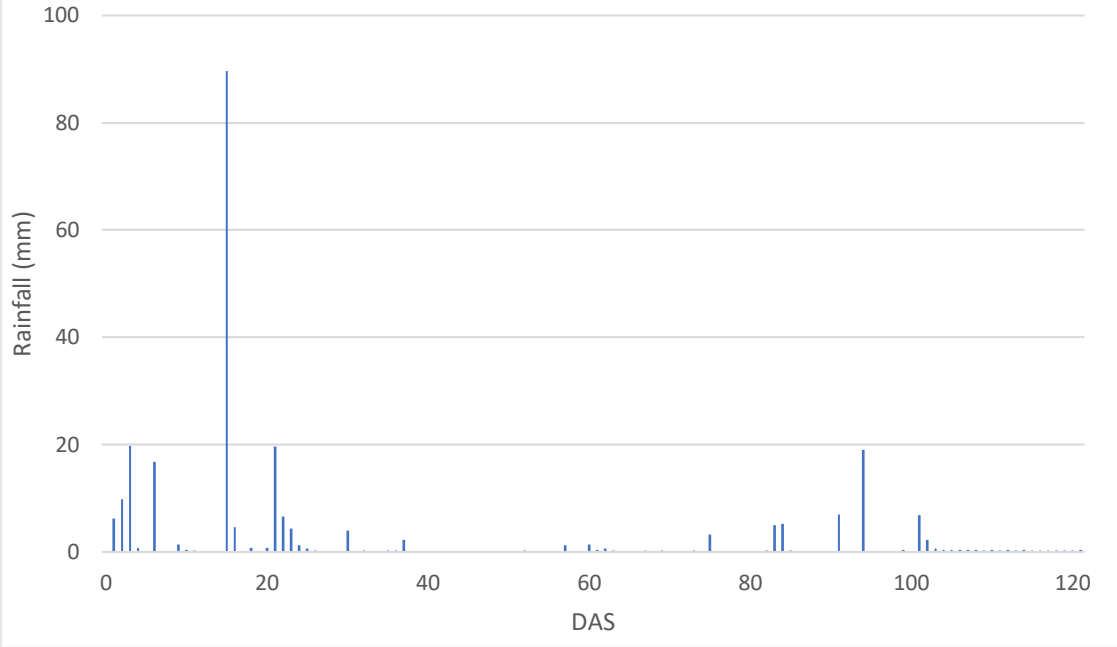
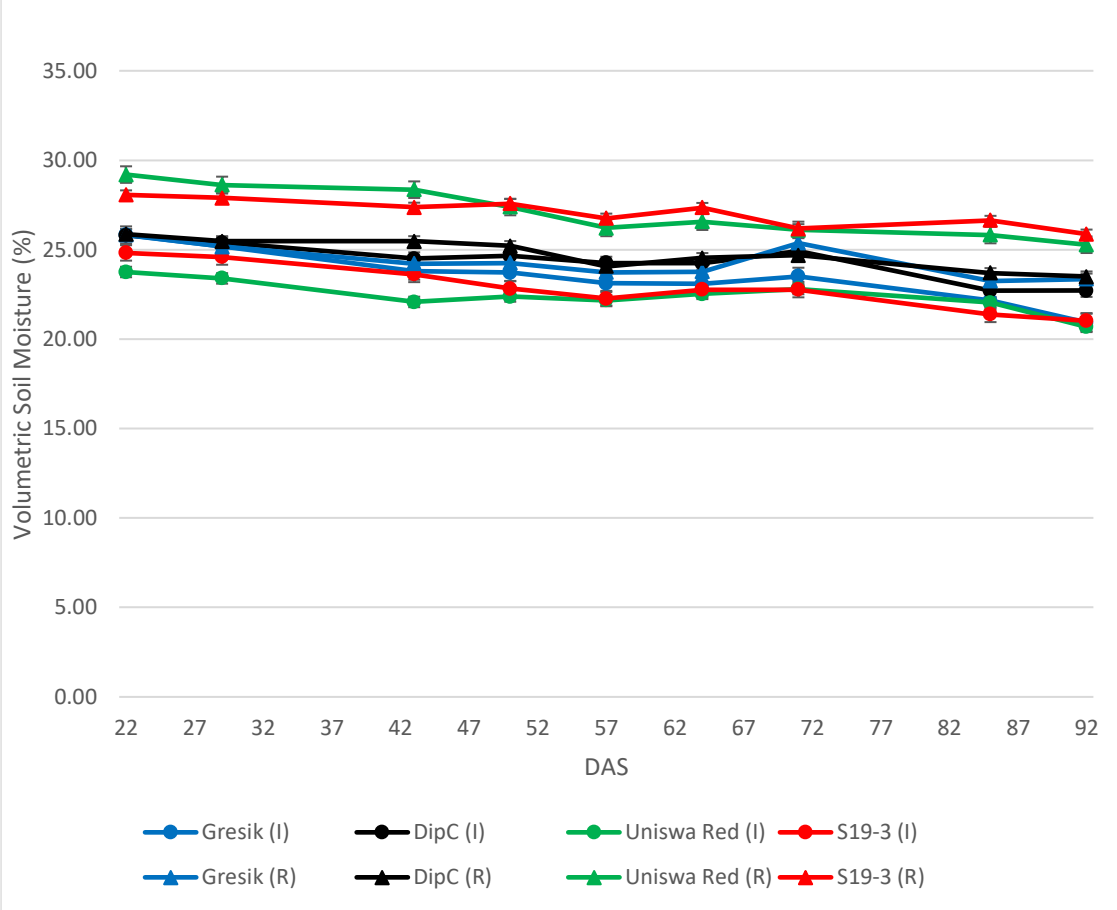


Figure 3.3: Amounts of rain per day in Field Research Centre, Crops for the Future Research Centre during the experiment.

3.3.2 Soil moisture

Figure 3.4 shows the trend of total soil moisture per treatment and figure 3.5 shows the profile soil moisture of both the irrigated and rainfed treatment. No clear trend was observed in the soil moisture content. There were some fluctuations in the soil moisture content but the differences remain small. Repeated measures ANOVA showed no significant difference between the treatments and between different genotypes for the soil moisture $P>0.05$.



n=12
$SD_{irrigated}$ = 1.35
$SD_{rainfed}$ = 1.62

Figure 3.4: Changes in the mean soil moisture content based on PR2 reading (%vol) per treatment throughout the soil profile with time during the experiment with four Bambara groundnut genotypes (Gresik, DipC, Uniswa Red and S19-3) grown in the Field Research Centre, Crops for the Future Research Centre.

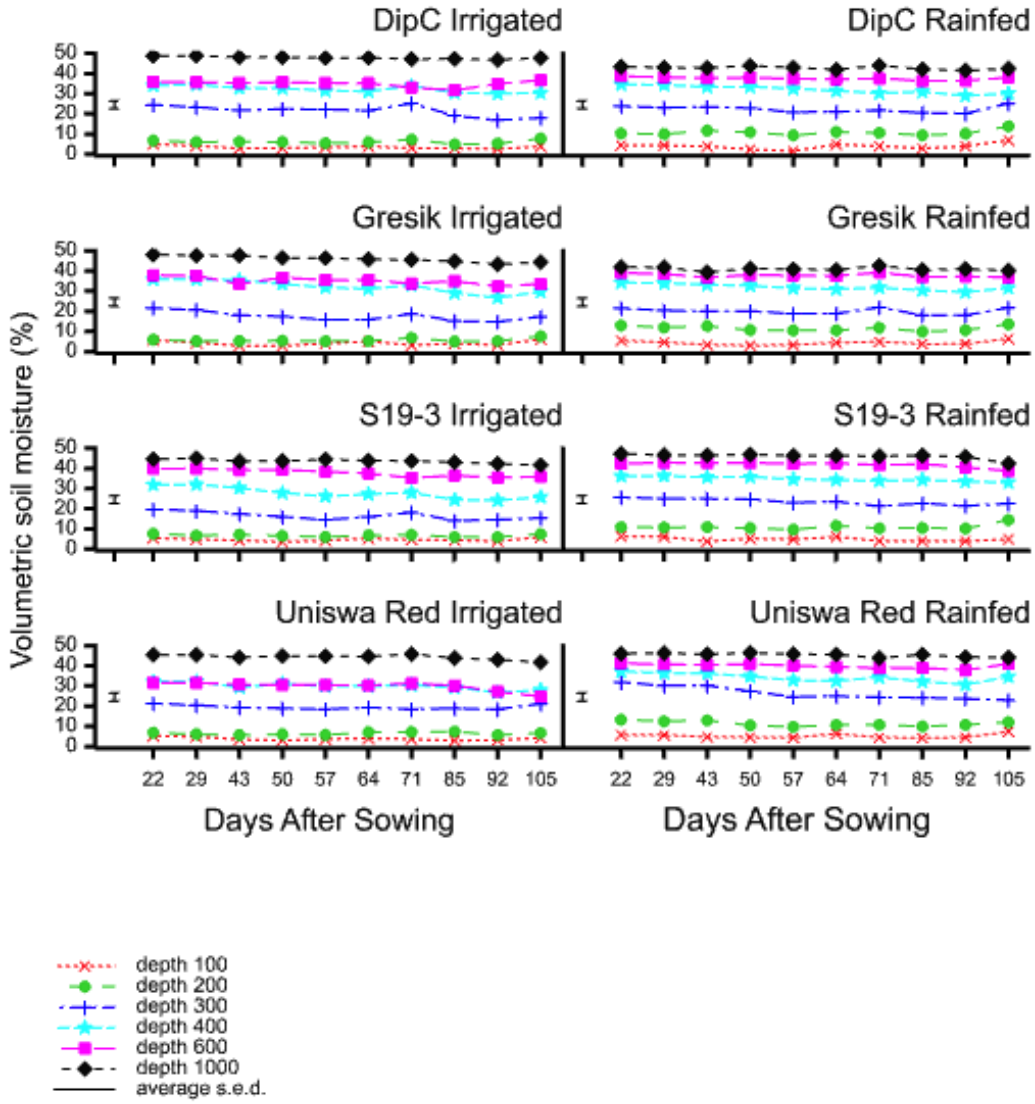


Figure 3.5: Profile soil moisture content (% volumetric) at two water treatment of four Bambara groundnut genotypes (Gresik, DipC, Uniswa Red and S19-3) grown in the Field Research Centre, Crops for the Future Research Centre (n=12).

3.3.3 Growth and development

Emergence

All four genotypes in both treatments started emergence at the same

time (7 DAS). This is because at the time of emergence, both treatments were irrigated until 30 DAS where the irrigation was terminated in the rainfed treatment. The result is presented as a mean of each genotype in the irrigation and rainfed treatment. There was no significant difference between the genotypes for emergence $P>0.05$.

Leaf appearance

The number of leaves for each genotype in both treatments are shown in Figure 3.6. Gresik gave the highest number of leaves in the irrigated treatment whereas Uniswa Red recorded the highest number of leaves in the rainfed treatment. S19-3 had the lowest number of leaves in both irrigated and rainfed treatments. The number of leaves per plant in the rainfed treatment remained lower than the irrigated treatment throughout the experiment. A repeated measures ANOVA showed a significant difference among the two treatments ($F_{1,3}= 337.32$, $P <0.001$). No significant differences were observed between the different genotypes and their interactions.

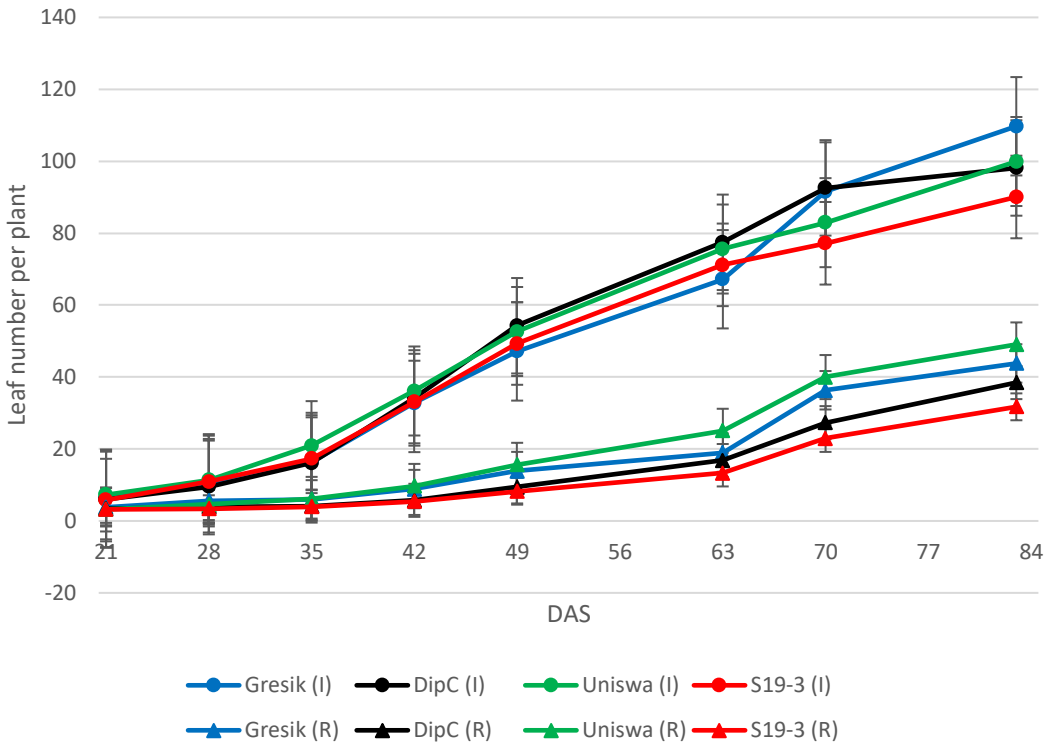


Figure 3.6: The effect of soil moisture on leaf number of four Bambara groundnut genotypes (Gresik, DipC, Uniswa Red and S19-3) grown in the Field Research Centre, Crops for the Future Research Centre (n=120).

Leaf Area

Figure 3.7 shows the plant leaf area (cm_2) of each genotype (Gresik, DipC, Uniswa Red and S19-3) in both irrigated and rainfed treatments. The highest peak in leaf area was in Gresik (3996.85cm_2) in the irrigated treatment and Uniswa Red (1987.86cm_2) in the rainfed treatment. The repeated measures ANOVA showed a significant difference among the two treatments ($F_{1,3}$= 187.54, P <0.001). No significant differences were observed between the

different genotypes and their interactions.

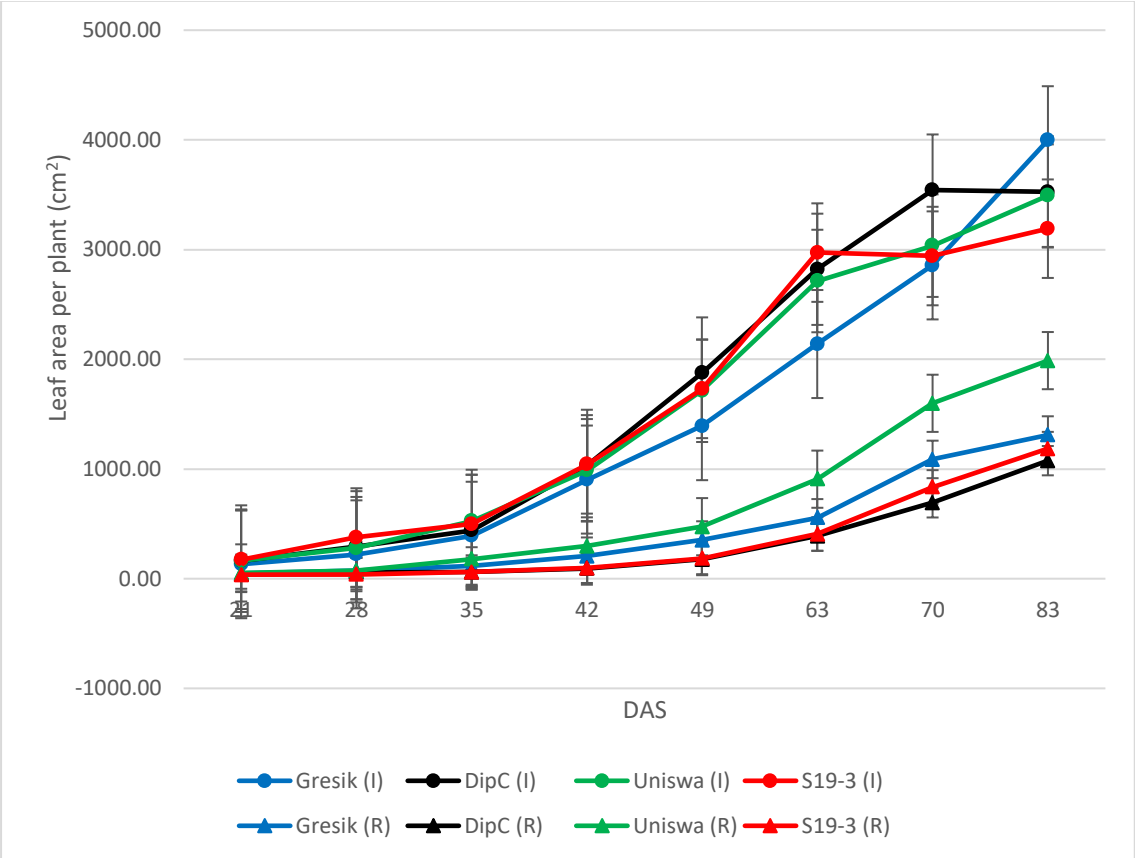


Figure 3.7: The effect of soil moisture on Plant Leaf Area of four Bambara groundnut genotypes (Gresik, DipC, Uniswa Red and S19-3) grown in the Field Research Centre, Crops for the Future Research Centre (n=120).

Normalized Difference Vegetation Index (NDVI)

The changes in NDV index during the experiment are shown in Figure 3.8. S19-3 in the irrigated treatment constantly recorded the highest NDVI index throughout the experiment and gave the highest peak (0.77) at 85 DAS. Repeated measures ANOVA showed no significant difference among genotypes and its interactions but showed a

significant difference between the treatments ($F_{1,3}$= 95.79, P <0.001).

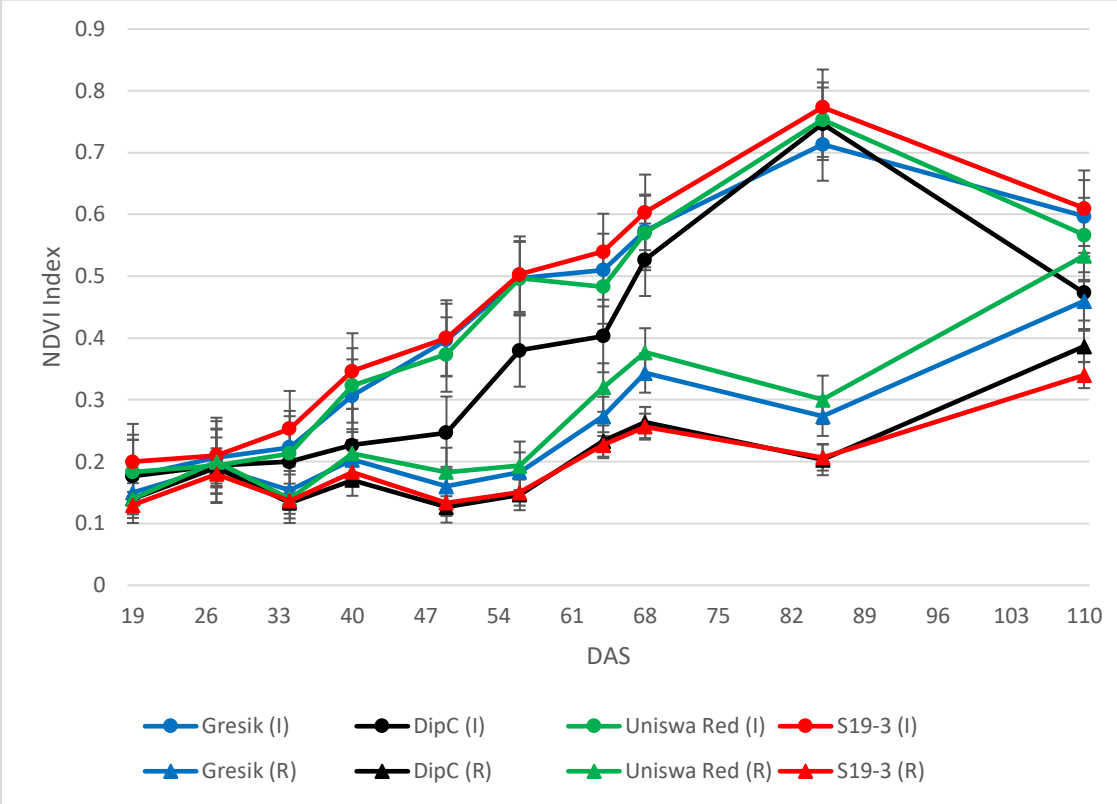


Figure 3.8: Changes in the NDV Index during the experiment with four Bambara groundnut genotypes (Gresik, DipC, Uniswa Red and S19-3) grown in the Field Research Centre, Crops for the Future Research Centre (n=24).

Chlorophyll Content

Figure 3.9 represents the mean chlorophyll content per plant in the irrigated and rainfed plot. The highest mean in both the irrigated and rainfed plot was DipC at 95 DAS with SPAD measurements of 57.14 and 55.79 respectively. Though repeated measures ANOVA showed a significant difference between the treatments ($F_{1,3}$= 95.79, P

<0.001), no significant differences were observed among different genotypes and their interactions.

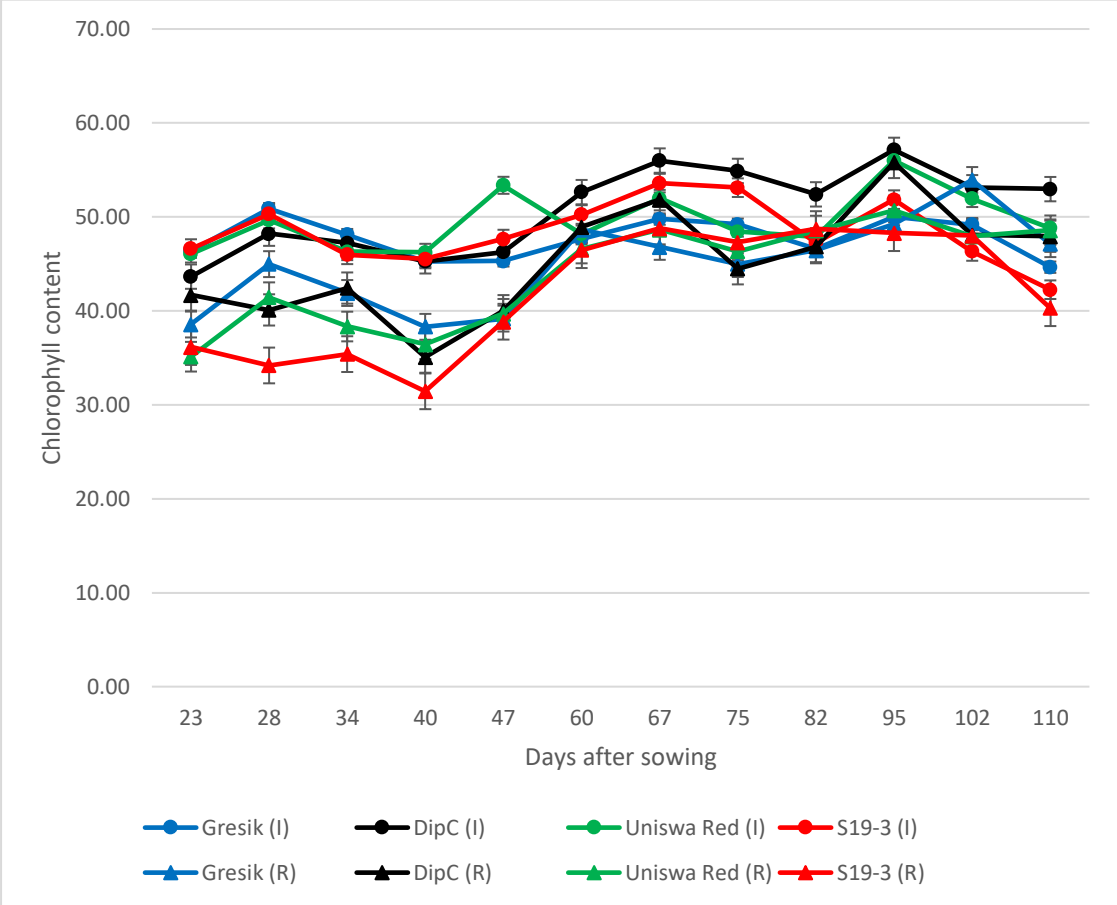


Figure 3.9: Mean Chlorophyll content of four Bambara groundnut genotypes (Gresik, DipC, Uniswa Red and S19-3) in the irrigated and rainfed treatment grown in the Field Research Centre, Crops for the Future Research Centre.

Continuous fluorescence yield (F_T) and light adapted quantum yield ($F_{v'}/F_{m'}$)

Results from the continuous fluorescence yield and quantum yield ($F_{v'}/F_{m'}$) are presented in Figure 3.10 and Figure 3.11 respectively. A repeated measures ANOVA showed no significant differences for

continuous fluorescence yield (P>0.05) among genotypes and treatments. However, a significant difference was observed in the continuous fluorescence yield with time (P<0.001) and the interaction between time and treatment (P<0.001). A significant difference (P<0.05) in the continuous fluorescence yield was also observed in the interaction between genotype, time and treatment.

A repeated measures ANOVA showed significant differences in the quantum yield ($F_{v'}/F_{m'}$) between the two treatments ($F_{1,3}$= 40.98, P <0.001) with a significant interaction between the genotype and treatment (P<0.01). A significant difference was also observed in quantum yield ($F_{v'}/F_{m'}$) measurements across time (P<0.001) and the interaction between time and treatment (P<0.001).

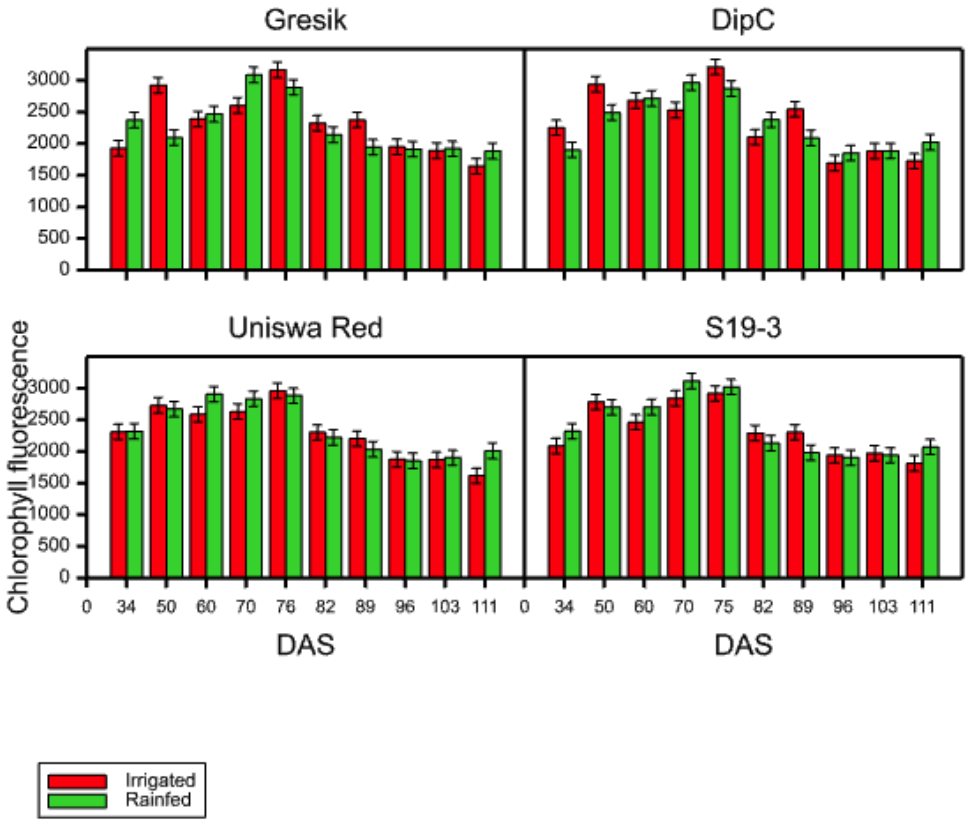


Figure 3.10: Continuous fluorescence yield (F_T) in four different genotypes in irrigated and rainfed treatments

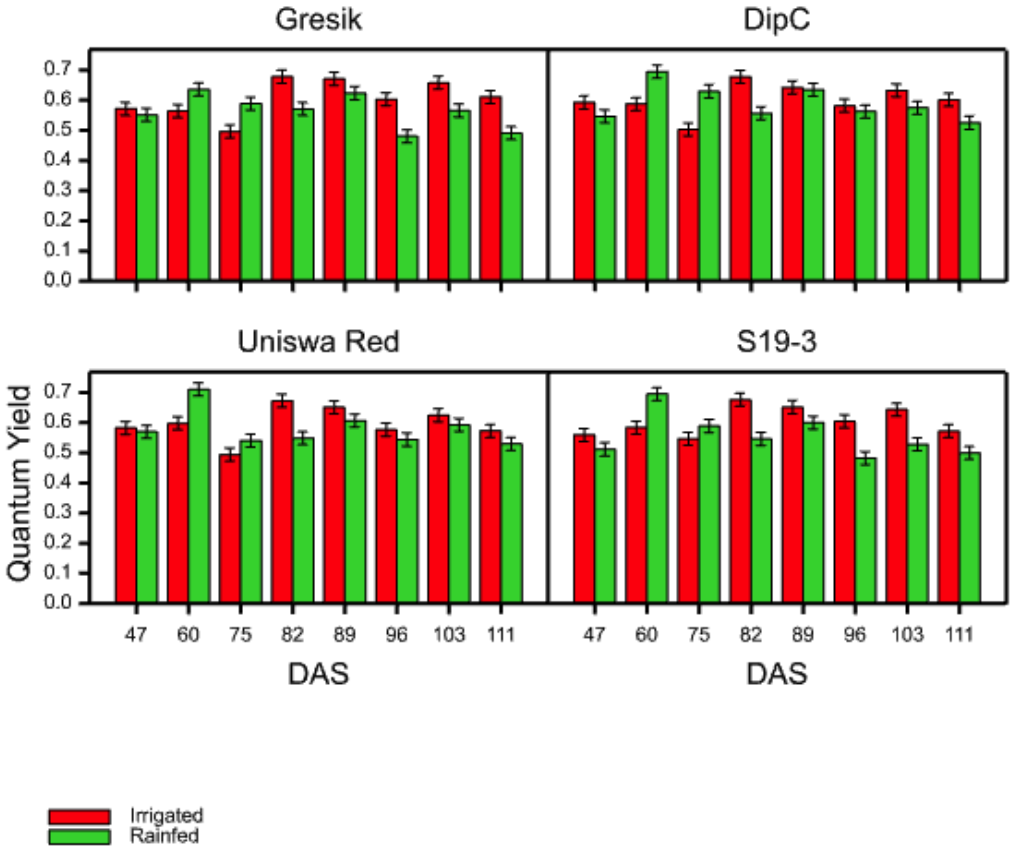


Figure 3.11: Light adapted Quantum Yield ($F_{V'}/F_{M'}$) in four different genotypes in irrigated and rainfed treatments

Yield and Yield components

Table 3.3 shows pod yield, seed yield, shelling percentage and 100 seed weight of the two different treatments and four different genotypes. S19-3 produced most yields in both irrigated (14.29 g/plant) and rainfed (11.97 g/plant) treatment. The lowest yield in the irrigated treatment was produced by DipC (4.01 g/plant) and

Gresik (5.22 g/plant) in the rainfed treatment.

Genotype	Treatment	Pod Yield (g/plant)	Seed Yield (g/plant)	Shelling %	100 seed weight (g)
Gresik	Irrigated	6.7	4.15	26.89	29.25
	Rainfed	5.22	4.10	19.17	39.21
DipC	Irrigated	4.01	3.07	21.60	41.60
	Rainfed	6.19	5.30	15.48	33.31
Uniswa	Irrigated	5.01	2.89	33.23	29.82
Red	Rainfed	10.19	7.53	24.31	52.09
S19-3	Irrigated	14.29	10.26	23.13	35.25
	Rainfed	11.97	10.03	16.42	39.96

	Pod Yield df	*SED*	*Seed Yield df*	*SED*	*Shelling % df*	*SED*	*100 Seed weight df*	*SED*
Genotype	6	3.348^{ns}	6	2.60^{ns}	6	1.73^{**}	6	4.95^{ns}
Irrigation	226	0.805^{ns}	257	0.65^{*}	226	0.79^{***}	272	1.78^{***}
G*I	7.46	3.536^{**}	7.56	2.76^{*}	11.97	2.06^{ns}	9.47	5.56^{***}

ns for not significant, * for P< 0.05, ** for P<0.01 and *** for P<0.001

Table 3.4: Yield components and yield (g/plant) (from final harvest) among four genotypes of bambara groundnut (Gresik, DipC, Uniswa Red and S19-3) under two water regimes grown in the Field Research Centre, Crops for the Future Research Centre.

Seed number per plant produced by Gresik, DipC, Uniswa Red and S19-3 in the two different treatments is presented in Table 3.4. No significant effect was observed among the genotype and between the treatments (P>0.05).

GENOTYPE	TREATMENT	SEED NUMBER
Gresik	Irrigated	14.14
	Rainfed	10.42
DipC	Irrigated	7.75
	Rainfed	11.13
Uniswa Red	Irrigated	9.22
	Rainfed	14.44
S19-3	Irrigated	24.97
	Rainfed	24.19

Table 3.5: Seed number per plant of four genotypes of Bambara groundnuts under two water regimes grown in the Field Research Centre, Crops for the Future Research Centre.

3.4 Discussion

3.4.1 Environmental conditions

Most studies of Bambara groundnuts have been carried out under controlled environmental conditions. Therefore, conducting a field

experiment gives a chance to test crop growth and productivity under natural environment. However, it is difficult to control environmental factors under field conditions, in this case amounts of water.

If water stress is significant for the study, the stress should be enough to cause some physiological response in the crop (Mwale *et al.*, 2003). As Malaysia is a tropical country, there is rainfall throughout the year and even when rainfall is more limited, the air is humid, so vapour pressure deficit is relatively mild compared to truly arid countries, such as Namibia. In this experiment, there was a total of 47.6mm of rain from 30 DAS to 100 DAS with 49 days of no rain. The amount of rainfall coupled with daily mean temperatures of 25-30°C provided some water stress to the plants.

3.4.2 Growth and development

Seedling establishment is a critical stage where stable rooting and initial green leaf area is attained (Blum, 1996). Among factors that affect crop establishment and emergence are crop type, variety, seed quality, depth and method of planting, seedbed preparation and soil moisture, aeration and temperature (Rowland and Whitman, 1993). In drought prone areas, it is very important for the crops to establish quickly. The faster they develop, the duration of time that they are susceptible to stress is shortened. Therefore, rapid root development

can be a strategy for young crops to withstand drought (Al shareef, 2010).

The genotypes started emergence at the same time (7 DAS) in the present study which indicates that genotype is not an important factor in determining crop establishment when soil moisture is non-limiting. Similar results were obtained by Alshareef (2010) in a field experiment carried out in Botswana using the DipC and Uniswa Red landraces, where he found no significant differences in establishment between the two landraces under non-limiting soil moisture.

Water stress has a significant effect on growth, yield and plant quality, in most species. The most visible effect of stress in plants is the loss of turgor that affects the rate of cell expansion and ultimate cell size (Hale & Orcutt, 1987). The statistical analysis showed a significant effect of treatment (irrigated and rainfed) on leaf number in the field experiment. The rate of leaf appearance started to cease after 70 DAS in the rainfed treatment. In the irrigated treatment, leaf production for Gresik and DipC ceased after 70 DAS indicating a switch into pod filling. However, S19-3 and Uniswa Red continued leaf production at a reduced rate. This result contradicts the findings of Mwale *et al*. (2007) who reported that the irrigated treatments of DipC and Uniswa Red still continued to produce leaves after 73 DAS, albeit at a lower rate than before, while leaf production completely ceased in the irrigated treatment of S19-3. S19-3 showed the highest

reduction in leaf number (64.77%) between the two treatments followed by DipC (60.8%), Gresik (60.09%) and Uniswa Red (50.9%).

A reduction in the leaf number resulted in significant reductions in the plant leaf area. At 83 DAS, DipC gave the highest reduction in terms of leaf area (69.49%) followed by Gresik (67.24%), S19-3 (62.84%), and Uniswa Red (43.07%) when compared between treatments. The leaf area reduction in the rainfed treatment is more noticeable than can be explained by the leaf number alone. The loss of turgor due to limited water availability results in reduced cell size accompanied by a reduction in leaf area. As reported by Collinson *et al*. (1997), drought-induced reductions in leaf area are common and may improve foliar water status.

Specific pigments in plants contained within the chloroplasts captures light energy. The major pigments are chlorophyll. The chlorophyll absorbs light mainly at the blue and red ends of the visible spectrum of light and reflects mainly green wavelengths of light (Scott, 2008). Significant differences were observed in leaf chlorophyll content between the irrigated and rainfed treatment in this experiment. These findings contradict the findings from Vurayai *et al.* (2011) where there were no significant effect ($P<0.05$) found in chlorophyll content index using both destructive and non-destructive methods

after water stress was induced during the vegetative, flowering and pod filling stages. The values recorded with portable chlorophyll meter provide an indication of the relative amount of total chlorophyll content of the leaves. Reduction or no-change in chlorophyll contents of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration (Kyparissis *et al*., 1995). Water shortage gradually decreased the levels of chlorophyll content in pea leaves (Karataş *et al.*, 2014). Soybean plants that were subjected to continuous water stress from early seed filling until maturity lost chlorophyll from their leaves more rapidly than non-stressed plants (De Souza *et al*., 1997).

Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as light—chlorophyll fluorescence (Maxwell and Johnson, 2000). The products of the light-dependent phase of xanthophyll cycles play an important role in the protection against oxidative stress generated not only by an excess of light but also by other factors such as drought, chilling, heat, senescence, or salinity stress (Latowski, Kuczyńska and Strzałka, 2011). Four mechanisms explaining the protective role of xanthophyll cycles in oxidative stress are direct quenching of overexcitation by products of the light phase of xanthophyll cycles, indirect quenching of overexcitation by

aggregation-dependent light-harvesting complexes quenching, light-driven mechanisms in light-harvesting complexes and a model based on charge transfer quenching between Chlorophyll a and Zeaxanthin (Latowski, Kuczyńska and Strzałka, 2011). Environmental stresses can reduce the ability of a plant to metabolise normally. The imbalance between the absorption of light energy by chlorophyll and use of energy in photosynthesis makes chlorophyll fluorescence measurements useful as a proxy for plant stress. Though no significant differences were observed in the continuous fluorescence yield (F_T), there was a significant effect of quantum yield ($F_{V'}/F_{M'}$) between treatments and the interaction between genotype and treatment. Quantum Yield (dark adapted) decreased significantly in stressed plants compared to control plants in Bambara groundnuts (Vurayai *et al.*, 2011; Muhammad *et al*., 2015) and cowpea (Souza *et al.*, 2004). A study carried out by Jefferies (1994) in field grown potato (*Solanum tuberosum*) found no significant effect on the quantum yield of PSII and concluded that in leaves of droughted plants, excess energy as a result of stomatal limitation of photosynthesis is dissipated by photoprotection. Drought resistant varieties dissipates the excess energy more efficiently via the xanthophyll cycle whereas the more susceptible varieties photoprotection is mainly based on photorespiration (Beis and Patakas, 2012).

3.4.3 Yield and yield components

Drought stress is known to affect translocation by altering the source to sink relationship for assimilates. When cell expansion is reduced, smaller leaves are produced (source), and less photosynthates are available for translocation to fruits (sink) resulting in reduced fruit size (Hale and Orcutt, 1987). Reduction in yield in Bambara groundnut due to water stress has been reported by several studies (Collinson, *et al*., 1999; Mwale *et al*., 2007; Jørgensen *et al.*, 2010)

The treatment had a significant effect in seed yield, shelling% and 100 seed weight of the plants whereas genotype only had a significant effect on the shelling%. A glasshouse experiment conducted by Mwale *et al*. (2007) found that landrace had a significant influence ($p<0.05$) on the shelling percentage, 100-seed weight and yield. DipC and Uniswa Red produced higher seed yields (g/plant) in the rainfed treatment compared to the irrigated treatment. Similar observation was made by Alshareef (2010) in a field experiment carried out in Botswana. Significant yield reductions in drought treatment were observed in DipC and Uniswa Red under glasshouse conditions (Jørgensen *et al.*, 2010). Although there were significant reductions in leaf number and leaf area, S19-3 and Gresik maintained similar seed yields under irrigated and rainfed treatments.

The short life cycle of S19-3 (>100 days) with compressed phenology with some reduction in leaf area and stomata closure makes S19-3 a combination of drought escaping and avoiding genotype (Jørgensen *et al.*, 2011). The plants were harvested starting from 120 DAS due to rain daily starting from 110 DAS which could damage the pods. It is possible that S19-3 recorded the highest yield in both treatments as it has completed its lifecycle (>100 days) whereas the other genotypes were still in pod filling stage. Under water stressed conditions, the crops complete their lifecycle earlier which may explain the higher number of seeds in DipC and Uniswa Red under rainfed conditions. The crops under irrigation were harvested earlier due to heavy rain that may cause damage to pods, therefore did not complete their lifecycle. The experiment should be repeated in drier months to allow all genotypes to complete their lifecycle to make a better comparison of yield and yield components of rainfed and irrigated treatments under field conditions.

CHAPTER 4: GLASSHOUSE EXPERIMENT

4.1 Introduction

4.1.1 Bambara groundnut: IITA-686 and LunT

In the present study, a segregating population derived from a controlled cross hybridization between two genotypes, IITA-686 from Tanzania and LunT from Lungi, Sierra Leone was used to study the effect of drought stress in Bambara groundnut.

The United Republic of Tanzania consists of the mainland and Zanzibar, which is made up of islands. Rainfall varies from 500mm to 1,000mm over most of the country and temperatures are hot and humid with an average around 30 °C (AQUASTAT, 2016). An experiment carried out in Morogoru and Miwaleni with three sowing dates carried out between 30th June and 30th August 1993 showed that pod production declined with later sowing (Heller *et al.*, 1997). Dodoma is thought to be more representative of the Bambara groundnut growing regions in Tanzania. Planting in the reported experiment was done under rain-fed conditions with three sowing dates from January to March 1994. The first sowing produced higher total dry matter and pod yield compared to the two later sowings due to little rain from April onwards.

Sierra Leone has a tropical weather with two pronounced seasons: Wet season from May to October and, a dry season from November

to April. Rainfall is highest in the coastal areas (3,000 to 5,000mm per annum), decreasing in the inland and the eastern borders (2,000–2,500mm per annum) and in the north of the country (2,930–2,540mm) (Larbi, 2012). The average temperature is 26 °C and varies around 26 °C to 33 °C during the year with high relative humidity with an average of 80% most of the year (Larbi, 2012).

Climatic conditions in Tanzania and Sierra Leone suggests that both IITA-686 and LunT are likely to be less tolerant to drought. However, drought tolerance is a complex trait that is controlled by numerous genes and their expressions are influenced by various environmental elements (Nezhadahmadi, Prodhan and Faruq, 2013). Broad avoidance/tolerance to mild-moderate stresses can be given by constitutive traits that are also expressed under stress-free conditions (Araus *et al.*, 2002). By using molecular markers for genome mapping and tagging of different drought related traits in this cross, the marker-assisted breeding (MAS) approach can be used to develop drought tolerant lines in Bambara groundnut.

IITA-686 and LunT have very different physiological characteristics in terms of peduncle length and growth habit. IITA-686 is a spreading type and has short peduncles, therefore seed are visible above ground. On the other hand, LunT is a bunched type and produces longer peduncles, burying its pods below ground (Figure 4.1). IITA-

686 starts flowering earlier (36-40 DAS) compared to LunT (45-50 DAS) and produces more seeds and pods (Kendabie, 2014). The weight of pods and seeds of IITA-686 is also higher compared to LunT (Kendabie, 2014).



Figure 4.1: The comparison between IITA-686 (left) 'spreading type' and LunT (right) 'bunched type' (Kendabie, 2014).

4.2 Materials and methods

4.2.1 Glasshouse constitution and location

The experiment was set up in controlled environment glasshouse at the FutureCrop Glasshouses, Sutton Bonington Campus, the University of Nottingham, UK. The dimension of the glasshouse is approximately 9m x 12m. The glasshouse has climate-control facilities with automatic blackout screens. The glasshouses are also located in an open space to avoid local shading and are aligned in parallel with a spacing of 15m apart to minimise mutual shading. The cropping area in the glasshouse is divided into two pits measuring 5m x 5m each. The soil is a sandy loam type in 1.2m deep pits with a heavy-duty butyl liner installed to prevent vertical and lateral

infiltration of water. A central computer located in the first glasshouse site controls blackout screen, ventilation, irrigation, temperature, humidity and CO_2 concentration of each glasshouse.

4.2.2 Plant Material

The F_3 segregating population derived from the cross between genotypes drawn from IITA (maternal) and LunT (paternal) was evaluated. Plant material, consisting of two parental lines (IITA-686 and LunT) and 103 F_3 individual lines were planted.

4.2.3 Experimental Design and crop management

The experiment was carried out from late April to late September 2016. The experimental design was a split plot with a complete randomised design (CRD) with three plant replicates.

The soil pits were prepared by digging, raking and levelling to remove unwanted plants and ensure a good soil structure. The soil was then covered with black plastic for two weeks to supress growth of weeds.

12 seeds from each parent and 6 seeds from individual lines were sown in trays on 22nd April, 2016. They were then transplanted in the soil pits on 11th May, 2016 at a spacing of 25cm between plants and 25cm between rows giving 20 plants per row.

4.2.4 Photoperiod control and crop protection

The photoperiod in the glasshouse was set to 12 hours using an automated blackout system (Cambridge Glasshouses, Newport, UK) with day temperature set at 28°C and night temperature set at 23°C.

Control of red spider mite (*Tetranychus urticae*) was done using a biological control agent, *phytoseilus persimilis* which was applied once in every two weeks. Savona (soap) for Aphids and Thiovit (sulphur) for mildew and fungal diseases were applied as needed.

4.2.5 Irrigation

A trickle irrigation system was used which consists of PVC micro-porous tubing placed next to each row. Crops received irrigation at 0600 hrs and 1800 hrs for 10 minutes, twice per day with a measured flow rate of 1L/hr per tube.

For the drought plot, irrigation was terminated for five weeks at 56 DAS after 50% flowering was observed across all the lines. Irrigation was resumed at 91 DAS and the ability of the plant to recover was observed.

4.2.6 Measurements

Soil moisture content

Two PR2 profile tubes (Delta-T devices, UK) were inserted into each soil pit diagonally from the irrigation source towards the end of the trickle tape. PR2 readings, which are displayed in unit of Volumetric Moisture ($m^3 m^{-3}$), were taken twice a week at 1000hrs starting from 55 DAS to 104 DAS at soil depths 10cm, 20cm, 30cm, 50cm and 90cm.

Environmental factors

The environmental conditions in the glasshouse was monitored using an automated record system (Cambridge Glasshouses, Newport, UK) which was placed in the glasshouse. Photosynthetically active radiation (PAR), humidity and temperature was recorded every eight minutes throughout the experiment.

Stomatal conductance

Stomatal conductance readings were taken using an AP4 leaf porometer (Delta-T devices, UK) on the middle leaflet of one fully expanded leaf per plant. Measurements were taken between 0900 hrs to 1500 hrs weekly starting from 55 DAS until two weeks after drought recovery. Calibration of the porometer was done whenever there was a change of cup temperature or humidity registered on the porometer.

Chlorophyll content

Chlorophyll content measurements were done as explained in Chapter 3.

Morpho-physiological traits

Morphological and physiological traits were measured based on the Bambara groundnut descriptor list (IPGRI, IITA, BAMNET, 2000). Traits measured throughout the growing period and its descriptions are stated in Chapter 2.

4.3 Results

4.3.1 Environmental conditions

Figure 4.2 shows the maximum, minimum and average temperatures during the experiment. The maximum temperature ranged between 28°C to 50°C whereas the minimum temperatures ranged between 16°C to 27°C. Average temperatures were between 21°C to 36°C

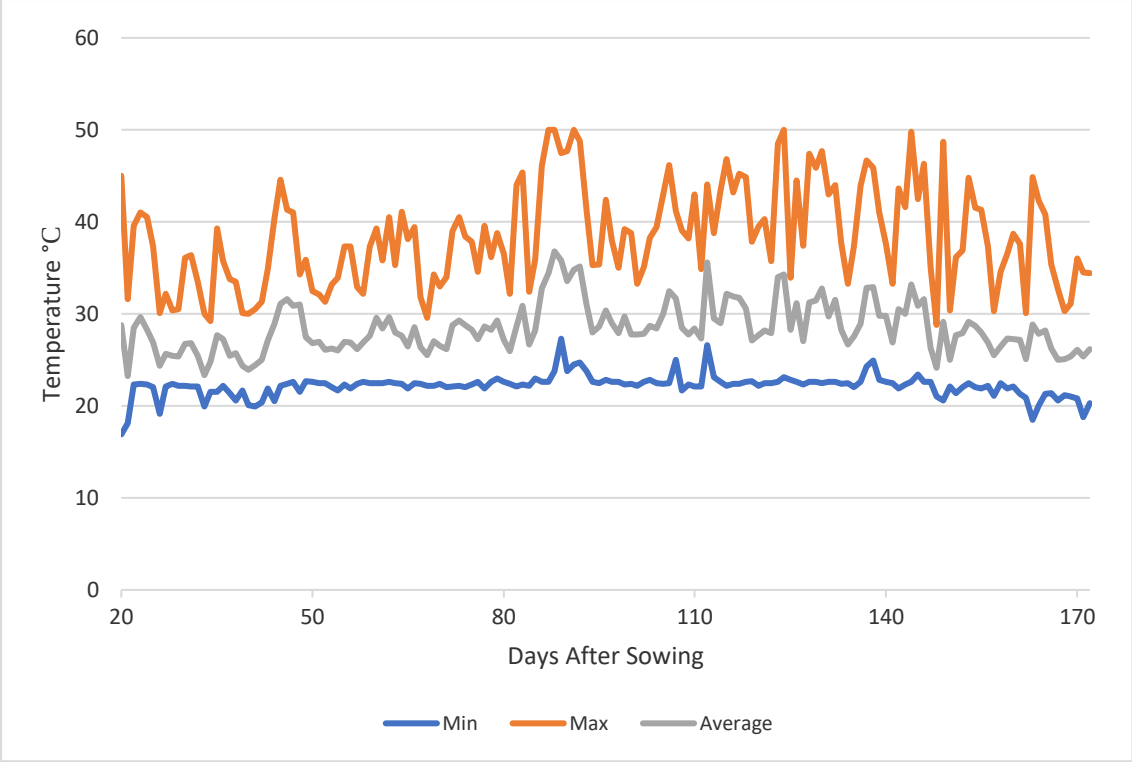


Figure 4.2: Maximum, minimum and average temperatures during the experiment with the F_3 population of IITA x LunT grown in the Future Crop Glasshouse.

4.3.2 Soil moisture

The total soil moisture content based on the PR2 readings during the drought treatment was reduced by an average of 22.3%. The highest reduction in soil moisture content was observed at 100mm depth with a reduction of 49.5% from 55 DAS to 90 DAS. The average soil moisture loss across all depths during the treatment was 0.6% per day. Rapid loss of soil moisture was observed at the depth of 100mm and 200mm with a rate of 1.4% per day and 1% per day respectively. The soil moisture in the drought plot was consistently lower compared to the irrigated plot during drought treatment for all depths.

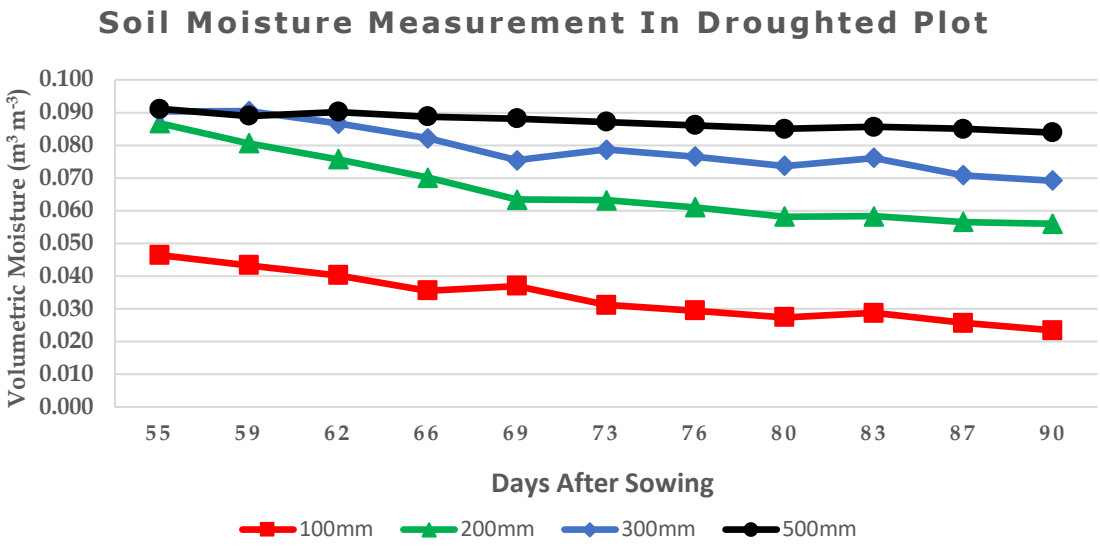


Figure 4.3: Soil moisture content (Volumetric moisture) in the drought plot during treatment from 55 DAS to 90 DAS (n=16)

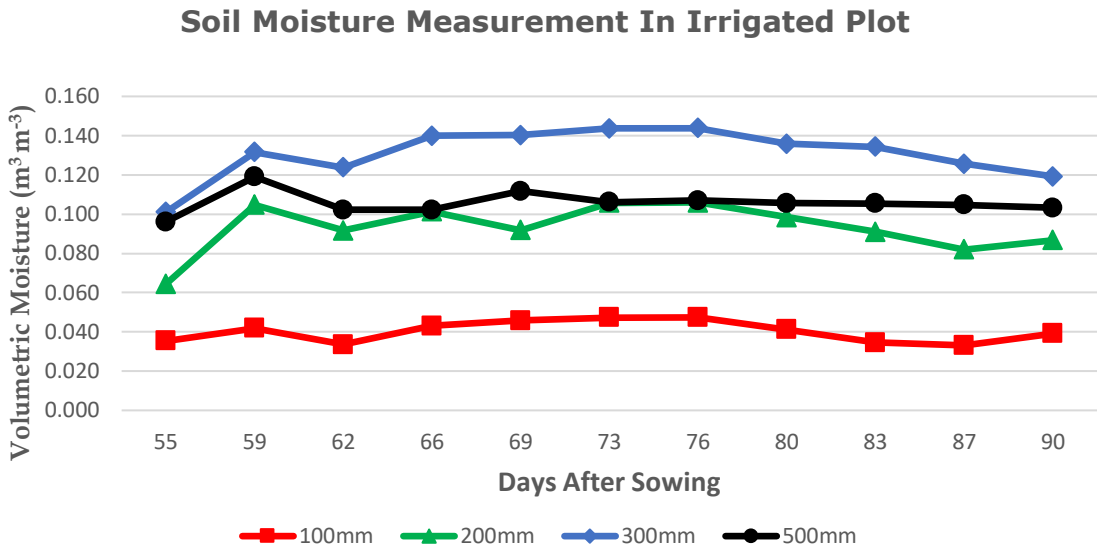


Figure 4.4: Soil moisture content (Volumetric moisture) in the irrigated plot during the treatment from 55 DAS to 90 DAS (n=16; SD_i= 0.07; SD_d=0.06)

4.3.3 Stomatal conductance

The mean values of stomatal conductance for the irrigated and drought treatment is shown in Figure 4.5 below. Drought was introduced at 56 DAS and stomatal conductance (g_s) was measured at 55 DAS before the treatment was introduced. Higher values of g_s were consistently observed in the irrigated plot (150-400 mmol m^{-2} s^{-1}) although there are some missing values due to the recording priority given to drought plot. The grand mean values for stomatal conductance declined from 150.45 mmol m^{-2} s^{-1} at 62 DAS to 48.6 mmol m^{-2} s^{-1} at 90 DAS in the droughted plot. Re-watering at 91 DAS after the drought treatment showed a significant increase in g_s ($P<0.001$).

The data analysis using repeated measures ANOVA showed significant differences between the treatments ($F_{(1,420)}= 46.73$, $P<0.001$) however, no significant differences were observed within the lines. A significant difference was also observed in the stomatal conductance with time ($P<0.001$) and the interaction between time and treatment ($P<0.01$). A few lines were observed to have shown high stomatal conductance under both drought and irrigated conditions, such as, L131 (D: 136.2 mmol m^{-2} s^{-1}; IR: 242.1 mmol m^{-2} s^{-1}) L171 (D107.8 mmol m^{-2} s^{-1}; IR 156.9 mmol m^{-2} s^{-1}); L193 (D:212.0 mmol m^{-2} s^{-1}; IR 426.2 mmol m^{-2} s^{-1}); L209 (D:111.6 mmol m^{-2} s^{-1}; IR:103.2 mmol m^{-2} s^{-1}) at 90 DAS. However, lines such as

L64 (D: 1.1 mmol m^{-2} s^{-1}; IR:321.3 mmol m^{-2} s^{-1}); L57 (D:6.2 mmol m^{-2} s^{-1}; IR:546.4 mmol m^{-2} s^{-1}); L54 (D: 9.2 mmol m^{-2} s^{-1}; IR: 395.8 mmol m^{-2} s^{-1}); L47 (D:5.4 mmol m^{-2} s^{-1}; IR:349.0 mmol m^{-2} s^{-1}); L241 (D:0.6 mmol m^{-2} s^{-1}; IR:398.5 mmol m^{-2} s^{-1}) showed very low stomatal conductance at 90 DAS.

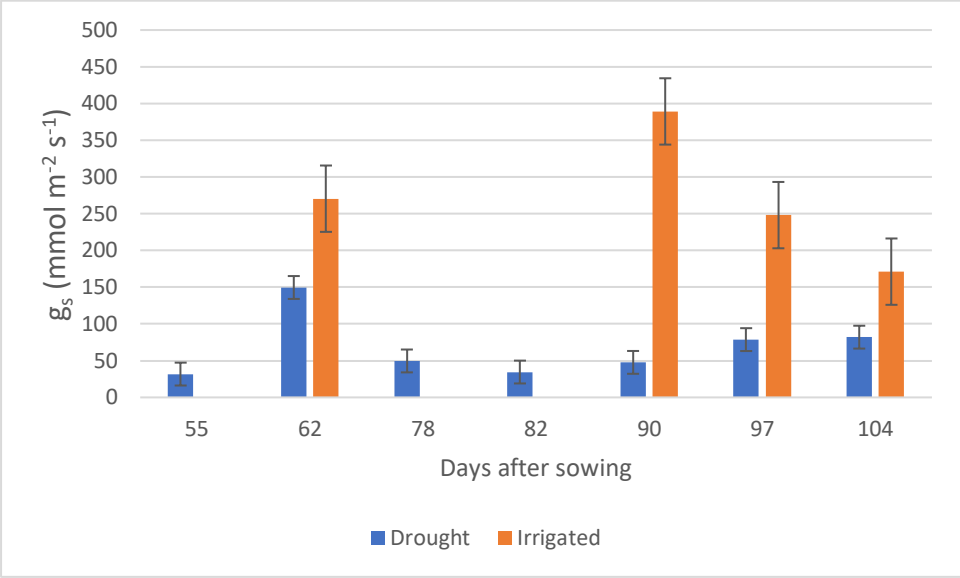


Figure 4.5: Changes in the stomatal conductance g_s as an effect of drought stress in the irrigated and drought plot with time during the experiment with the F_3 population of IITA x LunT grown in the Future Crop Glasshouse (DAS 55: n=320, SD_d=0.106; DAS 62: n=320, SD_i =0.27, SD_d= 0.4; DAS 78: n=160, SD_d=0.12; DAS 82: n=160, SD_d = 0.12; DAS 90: n=640, $SD_{i=}$ 1.2, SD_d= 0.17; DAS 97: n=640, $SD_{i=}$ 0.6, SD_d= 0.15; DAS 104: n=640, $SD_{i=}$ 0.6, SD_d= 0.17).

4.3.4 Chlorophyll content

The grand mean values of chlorophyll content (as indicated by SPAD readings) in the irrigated and drought treatment is shown in Figure 4.6. Higher values of chlorophyll content were consistently observed in the irrigated plot (45-55). The lowest mean value for drought treatment was 89 DAS with SPAD measurements of 36.76 whereas the irrigated treatment had the lowest SPAD value of 44.4 at 47 DAS. The SPAD measurement readings in the drought plot were maintained during drought treatment around before it dropped to 36.76 at 89 DAS. There was a significant increase in chlorophyll content ($P<0.001$) after rewatering at 91 DAS. The highest mean in both irrigated and drought treatment was at 96 DAS and 103 DAS respectively with SPAD measurements of 51.1 and 42.94.

Repeated measures ANOVA showed significant differences between the treatments ($F_{(104,420)}= 187.41$, $P<0.001$) however, no significant differences were observed among the lines. A significant difference was also observed in the chlorophyll content with time ($P<0.001$) and the interaction between time and treatment ($P<0.001$) and the interaction between time, treatment and different lines ($P<0.001$). Rewatering at 91 DAS produced a significant increase (>50) in chlorophyll content within the treatments ($F_{(1,343)}= 176.25$, $P<0.001$) and also within the lines ($F_{(104,343)}= 2.44$, $P<0.001$). There was also

a significant interaction between the treatment and lines (P<0.001). In the drought treatment, lines L54 (51.85); L103 (48); L11 (47.2); L116 (45.2); L131 (46.3); L151 (45.6); L160 (46.2); L168 (47.1); L171 (45.3); L2 (48.9) maintained their SPAD readings above 40 at 89 DAS. However, SPAD readings of lines L132 (17.9); L152 (23.21); L187(25.0); L4 (19.1); L89 (20.2) dropped below 25 on 89 DAS in the drought plot.

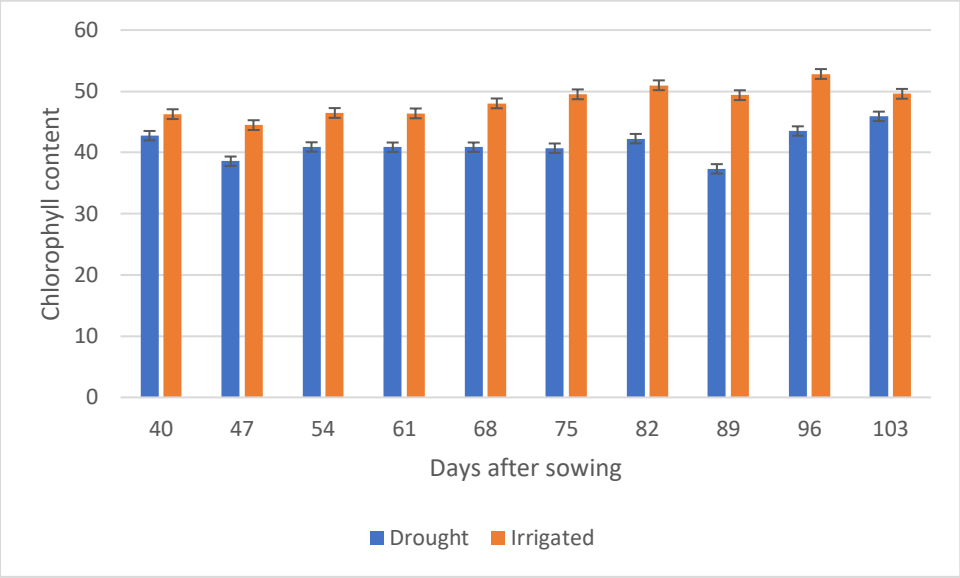


Figure 4.6: Changes in the chlorophyll content as an effect of drought stress in the irrigated and drought plot with time during the experiment with the F_3 population of IITA x LunT grown in the Future Crop Glasshouse (n=640)

4.3.5 Morpho-physiological traits

Table 4.1 presents the results from the analysis of all the morpho-physiological traits measured of the two parental lines and the F_3 population. The results are obtained from three replicates of

each sample. Most of the traits were non-normally distributed except for *petiole length* (drought), *pod length* (drought) and 100-seed weight (irrigated and drought). This observation is due to the F3 population still segregating.

Traits	Treatment	Mean	SD	Min	Max	SE	IITA		LunT		SD (Parental)
							Min	Max	Min	Max	
Days to emergence	-	11.42	1.69	8	17		9	9	8	9	0.45
Days to flowering	-	47.74	10.58	29	92	0.440***	40	54	41	55	5.27
Estimated days to podding	Irrigated	58.30	7.083	47	90	0.415***	56	61	57	59	2.06
	Drought	64.21	11.27	48	102	0.703***	56	62	60	64	3.19
Internode length	Irrigated	1.541	0.92	0.33	5.4	0.06***	1	2.27	0.7	1.43	0.53
	Drought	1.336	0.845	0.433	5.9	0.05***	0.533	2.26	0.5	1.73	0.66
Petiole length	Irrigated	13.57	3.321	1.8	27.5	0.2**	12.4	16.3	13.5	17.1	1.74
	Drought	14.15	3.33	2.8	24.9	0.2ns	13.3	16.6	8.9	15.6	2.59
Leaf width	Irrigated	2.767	0.776	1.4	5.8	0.04**	2.4	2.9	2.1	3.1	0.31
	Drought	2.543	0.435	1.6	4.2	0.02**	2.2	3.2	2.7	2.9	0.35
Leaf length	Irrigated	7.796	1.241	3.1	11.2	0.07*	7.1	9	7.9	9.9	0.95
	Drought	7.701	1.053	3	10.4	0.06*	6.8	8.1	7.1	8.5	0.59
Plant height	Irrigated	19.11	3.804	10.9	34.7	0.22**	16.7	25.7	16.4	20.7	3.19
	Drought	19.89	3.488	10.2	32.9	0.2*	16.7	30	13.7	24.7	5.31
Plant spread	Irrigated	9.29	9.778	2	64.5	0.56**	3	14.7	3.4	4.8	4.06
	Drought	7.23	8.878	1.5	59.2	0.51**	3	28.4	2.9	19.6	10.16
Pod length (mm)	Irrigated	15.28	2.294	1.77	23.78	0.05**	9.88	17.87	8.9	15.34	1.94
	Drought	14.61	2.236	5.68	23.83	0.05ns	9.22	13.94	9.67	16.26	1.72

Traits	Treatment	Mean	SD	Min	Max	SE	IITA		LunT		SD (Parental)
							Min	Max	Min	Max	
Pod Width (mm)	Irrigated	11.65	1.578	1.19	19.4	0.04**	7.94	13.29	6.85	11.82	1.44
	Drought	10.98	1.698	5.32	18.78	0.04**	7.61	11.1	7.97	12.39	1.18
Single Seed Weight (g)	Irrigated	0.474	0.193	0.01	1.15	0.003**	0.1	0.63	0.06	0.36	0.13
	Drought	0.393	0.187	0.01	1.35	0.004**	0.1	0.4	0.09	0.63	1.05
Seed length (mm)	Irrigated	10.37	1.642	4.29	16.23	0.03**	7.03	11.73	4.95	10.91	1.4
	Drought	9.815	1.645	3.61	16.1	0.04*	5.35	9.27	5.58	11.42	1.28
Seed width (mm)	Irrigated	8.110	1.129	0.77	11.31	0.02**	5.45	8.62	4.13	9.13	1.0
	Drought	7.637	1.222	0.4	12.11	0.03**	5.34	8.07	5.12	8.8	0.77
Peduncle length (cm)	Irrigated	3.073	1.585	0.4	12	0.07**	0.5	1	2	2.2	0.77
	Drought	3.027	1.704	0.1	11.5	0.07**	0.5	1.5	-	-	0.40
No of pods	Irrigated	19.58	20.29	0	191	1.16**	7	31	1	12	9.13
	Drought	8.660	11.35	0	108	0.64**	0	15	3	7	4.32
No of seeds	Irrigated	20.27	21.08	0	193	1.2**	7	34	1	12	9.89
	Drought	8.867	11.52	0	111	0.66**	0	15	3	9	4.45
Pod weight (g)	Irrigated	12.72	14.39	0.16	136.0	0.84**	1.2	4.5	0.21	4.33	1.66
	Drought	4.950	7.295	0.08	69.92	0.44**	1.61	4.07	0.83	5.18	1.57
Seed weight (g)	Irrigated	9.782	11.19	0.06	106.2	0.66**	2.13	7.4	0.15	3.25	2.15
	Drought	3.877	5.688	0.06	51.6	0.34**	1.29	3.38	0.58	4.22	1.31
Biomass (g)	Irrigated	9.050	10.41	0.5	66.9	0.6**	2.4	9.6	3	3.5	1.53
	Drought	5.402	6.922	0.6	52.7	0.4**	1.6	6	2.1	5.4	9.13

Traits	Treatment	Mean	SD	Min	Max	SE	IITA		LunT		SD (Parental)
							Min	Max	Min	Max	
Harvest Index (HI)	Irrigated	0.405	0.16	0	0.76	0.09**	0.29	0.68	0.04	0.44	0.20
	Drought	0.295	0.16	0	0.73	0.01**	0	0.34	0.15	0.34	0.13
100 seed weight (g)	Irrigated	41.7	14.11	6	78.62	0.82ns	21.76	48.57	15	33.33	11.04
	Drought	34.41	12.77	4.5	71.18	0.76ns	21.5	25.17	19.33	46.89	10.92
Shelling percentage (%)	Irrigated	76.31	9.216	0	88.89	0.538**	76.07	83.13	71.43	75.06	5.12
	Drought	76.55	8.52	8.18	91.67	0.51**	80.12	83.89	69.88	81.47	5.98

ns not significant

Level of significance * p<0.05; ** p<0.01; *** p<0.001

Table 4.1: Descriptive statistics for morphological and physiological traits measured in two parental lines and the F_3 segregation population under both drought and irrigated conditions.

Anderson Darling normality test was done to test if the trait data was normally distributed. P*etiole length* (Drought), l*eaf length* (Drought), *pod Length* (Drought) and 100 seed weight (irrigated and drought) were normally distributed while other traits were transformed with a Box-Cox transformation to correct to a normal distribution. Traits such as *seed weight* (Drought), *No of seeds*, *seed width*, *seed length* and *single seed weight* were not distributed normally even after transformation analysis.

Plant Height (Irrigation)

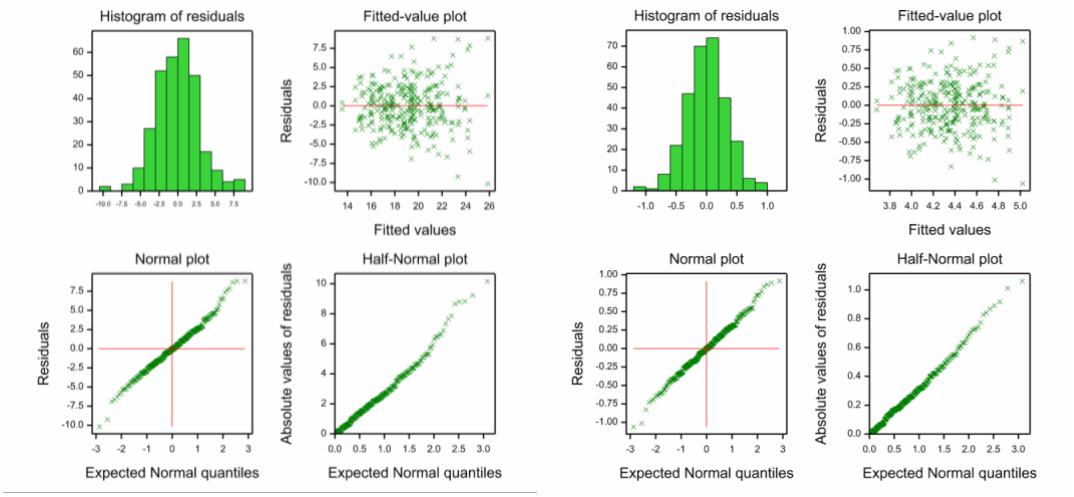


Figure 4.7: The histogram, fitted-value plot, normal plot and half normal plot of distribution for plant height (irrigation) before (left) and after (right) transformation using Box-Cox transformation.

The parental lines IITA-696 and LunT differ in terms of growth habit, peduncle length, pod number per plant, seed number per plant and also seed weight per plant. As the F_3 population used in this experiment is derived from these parents, some genetic

variability between the lines would be expected. Each trait that has been measured is explained below:

Days to emergence: All IITA-686 emerged at 9 DAS whereas LunT emerged at 8 to 9 DAS. No significant differences where observed in days to emergence between the two parents. However, ANOVA showed significant differences ($F_{(1,348)}$= 50.95, P<0.001) in emergence of the F_3 population where the plants emerged between 8 to 15 DAS.

Days to flowering: The Bambara groundnut F_3 population took a minimum of 29 days to start flowering although some took up to 92 days to start flowering. IITA-686 started flowering between 40 - 54 days. Similarly, LunT took 41-55 days to flower. No significant differences were observed in days to flowering among both parents.

Estimated days to podding: The estimated days to podding was recorded when the first visible pod was observed on the surface of the soil. However, the seed of LunT are normally buried under the soil. Therefore, the recording must be taken with caution. Similar characteristics will also be observed in the individual lines of the F_3 population. ANOVA analysis showed no significant differences between the two parental lines. For the F_3 lines, a minimum estimated day to podding of 47 and maximum of 90 were recorded in the irrigated treatment and minimum value of 48 and maximum

value of 102 were recorded for the drought treatment. Significant differences were observed in estimated days to podding between treatment within the F_3 population ($F_{(1,348)}$= 50.95, P<0.001).

Internode length: Although high genetic variability was expected due to IITA-686 being a spreading type, expected to have longer internode length in comparison to LunT which is a bunched type, ANOVA results of measurements of internode length at 10 weeks after emergence showed no significant differences between the two parents. In the F_3 segregating population, internode length ranged from 0.3 cm to 5.4 cm in the irrigated plot whereas internode length varied from 0.4 cm to 5.9 cm in the droughted plot. ANOVA results showed significant differences between treatment ($F_{(1,325)}$= 6.26, P<0.05) and between lines ($F_{(102,325)}$= 1.37, P<0.05).

Petiole length: The petiole length of the F_3 lines were between 1.8-27.5cm in the irrigated plot and 2.8-24.9cm in the drought plot. IITA-686 had a minimum petiole length of 12.4cm and a maximum of 16.3cm in the irrigated plot and minimum 13.3cm and maximum 16.6cm in the drought plot. LunT had a minimum of 13.5cm and maximum 17.1cm in the irrigated plot and minimum 8.9cm and maximum 15.6cm in drought plot.

Leaf width and leaf length: The mean terminal leaf width and terminal leaf length of IITA-686 was 2.65cm and 8.02cm in the irrigated plot

and 2.52cm and 7.48cm in droughted plot, respectively. LunT had a mean terminal leaflet width of 2.75cm in irrigated plot and 2.76cm in droughted plot and mean length of 9.05cm in irrigated and 7.86cm in droughted plot. The F_3 population had a mean terminal leaf length of 7.79cm in the irrigated plot and 7.7cm in the droughted plot and a mean terminal leaf width of 2.76cm in the irrigated plot and 2.54cm in the drought plot. Terminal leaf width was significantly higher ($P<0.001$) in the irrigated plot compared to the droughted plot.

Plant height : The F_3 population in the irrigated plot were significantly higher ($P<0.01$) in the irrigated treatment (10.9cm to 34.7cm) compared to the drought treatment (10.2cm to 32.9cm). There were no significant differences between the parents across treatments.

Plant spread: In both parents, the mean values for plant spread was higher in drought treatment (IITA-686=9.57cm; LunT=10.16cm) compared to irrigated treatment (IITA-686=9.1cm; LunT= 4.32cm). However, ANOVA showed no significant differences between parents nor between treatments. The mean value for plant spread of the F_3 lines in irrigated treatment was significantly higher ($P<0.01$) in the irrigated treatment (9.29cm) compared to the drought treatment (7.23cm)

Pod width and pod length: Pod width and pod length in the irrigated plot for IITA-686 ranged from 7.9mm to 13.2mm and 9.8mm to

17.8mm respectively, which was higher than the droughted plot which ranged from 7.6mm to 11.1mm for pod width and 9.2mm to 13.9mm for pod length. In LunT, the pod width and pod length in the drought treatment ranged from 7.9 to 12.3mm and 9.6 to 16.2mm, respectively, which was higher than the irrigated plot which ranged from 6.8mm to 11.8mm for pod width and 8.9mm to 15.3 mm for pod length. ANOVA showed significant differences in pod width ($P<0.001$) (SD_i = 1.44; SD_d= 1.18) and pod length ($P<0.05$) (SD_i = 1.94; SD_d= 1.72) of the parents in different treatments. In the F_3 lines, pod width was significantly higher ($F_{(1,4055)}$= 408.93, $P<0.001$) in the irrigated treatment (11.6cm) compared to the droughted treatment (10.9cm). Significant differences were also observed between the F_3 population ($F_{(102,325)}$= 17.68, $P<0.001$). Similar observations were also made in pod length (IR=15.2mm; DR=14.6mm) ($F_{(1,4059)}$= 235.13, $P<0.001$), with significant differences between the F_3 lines ($F_{(102,4059)}$= 23.88, $P<0.001$)

Seed width and seed length: Mean seed width and seed length were significantly higher [SW:($F_{(1,4073)}$= 448.27, $P<0.001$); SL:($F_{(1,4072)}$= 344.31, $P<0.001$)] in the irrigated plot (SW: 8.11mm; SL: 10.3mm) compared to the droughted plot (SW:7.63mm; SL:9.81mm) in the F_3 population. Significant differences were also observed between the F_3 population for both traits [SW: ($F_{(102,4073)}$= 16.66, $P<0.001$);SL:

($F_{(102,4072)}$= 18.33, P<0.001)]. No significant differences were observed in seed width and seed length between the parents.

Peduncle length: A significant difference between the two parental lines was obtained (p<0.001). A wide range of peduncle length was observed in both drought plot (0.1cm to 11.5cm) and the irrigated plot (0.4cm to 12cm) with a significant difference between the F_3 population lines at (P<0.001).

Pod number per plant: The F_3 population plants produced higher numbers of pods per plant in the irrigated plot (19.58 pods) compared to the drought plot (8.6 pod) ($F_{(1,412)}$= 71.20, P<0.001). Significant differences were observed between the F_3 population lines ($F_{(102,412)}$=1.29, P<0.05).

Pod weight per plant: Pod weight per plant ranged from 0.16g to 136g and 0.08g to 69.92g, respectively, under irrigated and drought conditions. ANOVA showed a significant reduction in pod weight with drought ($F_{(1,365)}$=70.56, P<0.001). Differences between the F_3 lines were observed at a significant level of P<0.05.

No of seeds per plant: After removal of the pod shells, 0 to 193 seeds per plant were observed in the irrigated F_3 plot and 0 to 111 seeds per plant were observed in the drought F_3 plot. ANOVA showed significantly higher number of seeds in the irrigated treatment ($F_{(1,412)}$=73.38, P<0.001). Some pods contain double seeds which

helps to explain the higher number of seed per plant. Similarly, some pods are empty due to seeds being aborted which explains the decrease in number of seeds compared to the number of pods.

Seed weight and seed weight per plant: The F_3 population plants produced single seeds with a minimum and maximum weight of 0.01g and 1.15g in the irrigated plot which is significantly higher ($F_{(1,4084)}$=476.45, P<0.001) than 0.01g and 1.35g in the droughted plot . ANOVA also showed significant differences in single seed weight between the lines ($F_{(102,4084)}$= 17.18, P<0.001). Seed weight per plant was significantly higher ($F_{(1,365)}$= 66.53, P<0.001) in the irrigated plot ranging from 0.06g to 106.2 g compared to the droughted plot ranging from 0.06g to 51.6g. No significant differences were observed in single seed weight and seed weight per plants in the parental lines between treatment and within treatment.

Biomass: A higher biomass was observed in the irrigated plot ranging from 0.5g to 66.9g compared to the drought plot ranging from 0.6g to 52.7g at a significant level ($F_{(1,411)}$= 30.64, P<0.001). ANOVA also showed significant differences between the lines ($F_{(102,411)}$= 1.90, P<0.001). IITA-686 generally produced more biomass (IR:2.4-9.6g; DR:1.6-6g) than LunT (IR:3-3.5g; DR:2.1-5.4g) in both treatment although no significant differences were observed between both the parental lines as well as between treatments.

Harvest index: No significant difference was observed between the two treatments and between both parents for the parental lines. However, for the population a significantly higher mean value ($F_{(1,412)}$=66.65, p<0.001) of 0.405 was obtained in the irrigated plot as compared to the droughted plot which had a harvest index mean value of 0.295. No significant differences were observed between the population for harvest index.

100-seed weight: Significantly lower 100-seed weight ($F_{(1,365)}$=50, p<0.001) was obtained when the F_3 population was placed under drought stress. When plants were stressed, a minimum value of 4.5g and maximum value of 71.18g for 100- seed weight was recorded with a mean value of 34.41g. The irrigated plot had a higher 100-seed weight ranging from 6g to 78.62g with a mean value of 41.7g. Significant differences were also observed within the population at a significant level of P<0.001. No significant difference was observed between the drought and irrigated treatments for the two parental lines and also within the lines.

Shelling percentage: IITA-686 had a significantly higher shelling percentage ($F_{(1,6)}$=17.11, p<0.01) with a grand mean value of 83% compared to LunT which had a grand mean value of 73.5%. No significant difference was observed between the treatments in the parental lines. Similarly, no significant differences were observed

between the drought and irrigated treatment in the F_3 population as well as within the population.

Generally, wide variation in trait values was observed between the parents and also within the F_3 population. Although ANOVA analysis shows significant differences for only two traits (*peduncle length* and shelling percentage) for the two parents (IITA-686 and LunT), the F_3 population lines were significantly segregating for traits such as *days to emergence*, *internode length*, *pod width*, *pod length*, *seed width*, *seed length*, *peduncle length*, *pod no per plant*, *pod weight per plant*, *single seed weight per plant*, biomass, harvest index and 100-seed weight. Drought significantly influenced *days to podding*, *internode length*, *plant height*, *leaf width*, *plant spread*, *pod width*, *pod length*, *seed width*, *seed length*, *pod number per plant*, *pod weight per plant*, *number of seeds*, *seed weight per plant*, *single seed weight per plant*, *biomass*, *harvest index* and *100-seed weight*. There were lines that produced more than 20% higher total seed weight per plant compared to irrigated conditions under drought such as L2 (D: 12.15g; IR: 7.31g), L82 (D: 6.42g; IR:1.63g), L121 (D: 4.96g; IR: 1.85 g), L124 (D:4.28g; IR: 3.32g), L136 (D: 15.51g; IR: 10.39g), L137 (D: 13.53g; IR: 9.40g), L131 (D: 4.75g; IR: 2.51g), L77 (D: 14.28g; IR: 3.96g), L53 (D: 2.39g; IR: 1.91) and L34 (D: 26.73g; IR: 10.14g). Lowest seed weight in the drought plot was observed in L152 (D: 0.4g; IR: 29.52g). Other lines with more than 90% lower

seed weight per plant compared to the irrigated plot include L16 (D: 1.38g; IR: 16.36g); L152 (D:0.41g; IR: 29.52g), L132 (D:0.19g; IR:3.47g), L64 (D:11.21g; IR: 0.95g), L120 (D:0.98g; IR:10.16g), L89 (D: 0.68g; IR: 7.64g); L92 (D:1.14g; IR: 11.62g), L141 (D:2.57g; IR:27.22g), L213 (D:1.53g; IR: 28.817g)and L229 (D:1.19g IR: 17.91g).

4.4 Discussion

The stomata are an important portal for gas and water exchange in plants and strongly influence characteristics associated with photosynthesis and transpiration. Stomatal conductance (g_s) is a measure of the degree of stomatal opening and can be used as an indicator of plant water status. The total soil moisture content based on PR2 readings during the drought treatment was reduced by an average of 22.3% and the mean value for stomatal conductance reduced from 150.45 mmol m^{-2} s^{-1} at 62 DAS to 48.6 mmol m^{-2} s^{-1} at 90 DAS. When observed, the plants were wilted, and leaves were drying off indicating severe water stress. Most of the Abscisic acid in non-stressed leaves is in the chloroplasts, and since the chloroplast envelope is leaky when water stressed, Abscisic acid may be responsible for the rapid reduction in stomatal conductance for the longer term correlation between reduced conductance and assimilation rate (Farquhar and Sharkey, 1982). Under conditions of

prolonged water deficit, plants frequently also produce leaves with reduced maximum stomatal conductance resulting from altered stomatal density and/or size (Franks *et al.*, 2015) .Rapid decline in stomatal conductance of Bambara groundnut under water stress were also observed by Collinson *et al.*, 1997, Vurayai *et al.*, 2011 and Chai, Massawe and Mayes, 2016.

Early leaf senescence was observed in the drought plot and 15% (96 plants) from the drought plot were dead by the end of the drought treatment. Leaf senescence is a physiological process induced by plants aging and environmental stresses, that leads to leaf death and is the last developmental stage of the leaf. Drought induces leaf senescence that enables nutrient remobilisation during stress, thus allowing the rest of the plant (i.e. the youngest leaves, fruits or flowers) to benefit from the nutrients accumulated during the life span of the leaf (Munné-Bosch and Alegre, 2004). Leaf senescence accompanied by leaf abscission contributes to the maintenance of water balance of the whole plant by avoiding water loss through transpiration.

Chlorophyll is one of the major chloroplast components for photosynthesis and the decrease in chlorophyll content under drought stress has been considered a typical symptom of pigment photo oxidation and chlorophyll degradation (Anjum *et al.*, 2011). Reduction of total chlorophyll content during water stress implies a

lowered capacity for light harvesting. In this study, the plants maintained SPAD measurement readings (as a surrogate for chlorophyll content) around 40 during drought treatment before dropping to 36.76 at 89 DAS. Reduction and maintenance of chlorophyll levels during drought stress has been reported in many species, depending on the duration and severity of drought (Fanizza, Ricciardi and Bagnulo, 1991; Naderikharaji, Pakniyat and Biabani, 2008).

SPAD index declined progressively with exposure to drought but the decline was more severe in sugarcane for the susceptible genotypes with TCP02-4587 having the highest SPAD index values under drought conditions and TCP02-4624 having the lowest ones (Silva *et al.*, 2007). Similarly, in Bambara groundnut, water stressed plants were observed to have lower chlorophyll readings (T Mabhaudhi, Modi and Beletse, 2013; Muhammad and Massawe, 2015) although Vurayai *et al.*, (2011) and Mabhaudhi, Modi and Beletse, (2013) reported otherwise. Loss of chlorophyll is one of the consequences of drought stress which may result from sustained photoinhibition and photo-bleaching.

Rewatering at 91 DAS resulted in significant increases in both stomatal conductance and SPAD readings and it continued to increase until 104 DAS. It was observed that Flexas *et al.*, (2008) and Galmés, Medrano and Flexas, (2007) observed an incomplete photosynthetic

recovery from drought when stomatal conductance dropped below 0.15 mol m^{-2} s^{-1} across a range of phylogenetically and functionally diverse plant species. However, no reduction in the leaf biochemical capacity was observed until stomatal conductance was lower than 0.05 mol m^{-2} s^{-1}, and stomatal limitation seemed to prevail after re-watering. Kirschbaum, (1987) showed that recovery after a severe drought was a two-stage process: a first stage occurred during the first days upon re-watering, and consisted basically in leaf re-watering and stomata re-opening; and a second stage lasted several days and was supposed to require *de novo* synthesis of photosynthetic proteins. Generally, plants subjected to mild stress recover fast (within 1 or 2 d) after stress is alleviated, but plants subjected to severe water stress recover only 40–60 % of the maximum photosynthesis rate during the day after re-watering, and recovery continues during the next days, but maximum photosynthesis rates are not always recovered (Kirschbaum, 1987; Miyashita *et al.*, 2005).

Optimizing plant performance and crop sustainability under different environmental stresses depends on the degree to which plant vegetative and reproductive growth patterns are regulated. A plant can be divided into source and sink with sources being the parts where net fixation of carbon dioxide occurs and sinks being the site where assimilates are stored or used. Daughter-cell production by

meristematic cell divisions and subsequent massive expansion of the young cells results in growth of plants. Permanent or temporary water deficit hampers plant growth severely, more than any other environmental factor (Anjum *et al.*, 2011). *Plant height*, *plant spread*, *peduncle length*, *leaf width* and *petiole length* were significantly reduced in the drought treatment compared to the irrigated treatment. This may be due to drought impaired mitosis which reduces cell elongation and expansion, resulting in reduced growth (Hussain *et al.*, 2008). Internode length was significantly higher in drought plants compared to irrigated plants. Similar observations were made by Chai, Massawe and Mayes (2016) where the F_5 population of Tiga Nicuru x DipC in the drought treatment had higher internode lengths compared to the irrigated treatment.

As drought was introduced at 50% flowering, the pod filling stage was affected by drought stress. *Pod number*, *pod weight*, *no of seed*, *seed weight*, and *harvest index* were reduced significantly in the drought treatment of the F_3 population. Although not significant, a reduction in 100 seed weight was also observed. Similar observations of a reduction of 100 seed weight in Bambara groundnuts with drought were made by Mwale, Azam-Ali and Massawe (2007b); Vurayai *et al.* (2011); Chai, Massawe and Mayes (2016). Significant differences observed within the population ($P<0.001$) for 100 seed weight were also reported by Mwale, Azam-Ali and Massawe (2007)

and Chai, Massawe and Mayes (2016) indicating differences in response to drought of the individual plants in terms of 100 seed weight. In terms of harvest index, the values obtained in the present study (0.405) in the irrigation treatment agree with the value obtained by Mwale, Azam-Ali and Massawe, (2007) of 0.46. Water stress leads to severe decline in yield traits of crop plants by disrupting leaf gas exchange properties which not only limits the size of the source and sink tissues but also impairs the phloem loading, assimilate translocation and dry matter portioning (Farooq *et al.*, 2009).

Chapter 5: CONSTRUCTION OF A DARTSEQ GENETIC LINKAGE MAP IN BAMBARA GROUNDNUT

5.1 Introduction

5.1.1 Genetic linkage maps

Genetic linkage maps are the foundation to understanding which genes contribute to important plant traits such as yield, drought resistance, disease resistance and seed quality (Hyten and Lee, 2016). Calculation of recombination frequencies in genetic crosses enable us to determine if two genes are linked, and how tightly they are linked. By calculating the recombination frequency for many gene pairs, a genetic linkage map that shows the relative order and relative distances of the genes on the chromosome is obtained.

Genetic linkage maps are constructed based on mapping populations derived from controlled crosses that are segregating for traits. Genetic mapping has been studied using various population types including in Backcross, Doubled Haploid, and Recombinant Inbred Line (RIL) (Wu *et al*., 2008) and are useful for key applications such as gene mapping, quantitative trait loci (QTL) mapping, marker-assisted selection (MAS) and comparative genome studies (Hyten and Lee, 2016).

5.1.2 DArT molecular marker

Diversity Array Technology (DArT) markers were developed in the early 2000s and are relatively new compared to other molecular

marker systems. The basis of the method is to use restriction endonucleases (REs) to produce a 'representation' of the complex genome. By using the *Pst*I RE (or other rare cutting RE), coupled with a frequent cutting RE (four base-pair recognition sequence), the genome can be reproducibly cut into a series of fragments and adaptors allow a subsection to be amplified. The RE focuses this subsection towards the more expressed regions in the genome. Initially developed using hybridization- based methods, based on nucleic acids immobilised on solid-state surfaces, genetic marker analysis through Diversity Array offered high throughput at low cost (Jaccoud *et al*., 2001). More recently, the Next Generation Sequencing (NGS) has allowed the array hybridisation detection system to be replaced with direct illumina sequencing of the representation (bar-coded and pooled). DArT markers have been successfully utilized in genetic diversity analysis, linkage mapping and to determine population structure in various crop species with many publications (DArT, 2014).

The advantages of using DArT markers over other marker systems include high throughput allowing rapid germplasm characterization in a single experiment, independence of sequence data and the ability to detect single base changes/indels within the sequence tag site of individual markers and the lack of a need for detailed prior sequence data from the species of study (Jones *et al.*, 2009). Due to its ability

to provide comprehensive genome coverage even in organisms without any DNA sequence information, the application of DArT markers is accelerating gene discovery in minor crops (Howard *et al*. 2011; Varshney *et al*., 2017). DArT has been used for genotyping and developing genetic maps in many crops such as wheat (Cui *et al.*, 2014), barley (Jing *et al.*, 2009), Arabidopsis (Wittenberg *et al.*, 2005) , pigeonpea (Yang *et al.*, 2006), capsicum (Naresh *et al.*, 2018) melon (Abu Zaitoun *et al.*, 2018), einkorn (Marino *et al.*, 2018) and pineapple (Sanewski, 2020).

5.1.3 Genetic mapping in Bambara Groundnut

The first map in Bambara groundnut was constructed by Basu *et al.* (2007a) using a set of 98 individuals from a F_2 population of a controlled cross between an ancestral wild type (VSSP11) and the domesticated form of DipC. Differences in growth habit, maturity and yield were observed in both parents (Basu *et al.*, 2007b). Basu *et al.*, (2007b) reported the identification of 20 linkage groups using 67 AFLP markers and one SSR marker covering a total length of 516 cM, with inter-markers distances varying from 4.7 cM to 32 cM.

The construction of the first intraspecific genetic map was reported by Ahmad (2012) using an F_3 segregating population derived from the self-pollination of a single F_1 genotype derived from the landraces

DipC and Tiga Nicuru. Twenty-one linkage groups were identified using 29 SSR and 209 microarray-based DArT markers, covering 608.6 cM with marker-marker distances ranging from 0 cM and 10.1 cM. The initial map by Ahmad (2012) was improved by including a total of 3,670 classical dominant DArT markers and 2993 bi-allelic SNP markers developed by Diversity Arrays Technology Pty. Ltd, using DArT Seq (Yarralumla, Australia) for construction of a higher density genetic map (Chai, 2014). This resulted in 11 linkage groups with 171 spaced markers covering 1,341.3 cM with an average distance of 7.8 cM between two adjacent markers across all linkage groups. This map was derived from a far higher density map, which was spaced before QTL analysis.

With the aim of demonstrating the value of identifying photoperiod-related trait QTLs Kendabie, (2014) reported three maps;

a) Using 263 F_2 lines derived from an IITA-686 x Ankpa4 cross that produced 11 linkage groups with 836 markers covering 1,192 cM with an average distance of 1.4 cM

b) Using a subset of 105 lines from the 263 F_2 lines in (a) which was previously evaluated under 12 hours of daylight and produced a map with 11 linkage groups covering 1,188 cM with 746 markers with an average distance of 1.6 cM

c) Using the remaining 158 lines from the 263 F_2 lines in (a) which was previously evaluated under 16 hours of daylight which produced

a map with 11 linkage groups covering 1,197 cM with 821 markers with an average distance of 1.5 cM.

Lower density (spaced) genetic maps were produced for all three maps with total map lengths of 1,135 cM, 1,134 cM and 1,120 cM for the 263 lines, 158 lines and 105 lines, respectively (Kendabie, 2014).

An expression- based genetic map (GEM map) was constructed following cross-hybridisation of RNA from Bambara groundnut to the Affymetrix Soybean Genome Genechip using a F_5 segregating population of a controlled cross between Tiga Nicuru (maternal) and DipC (paternal) landraces (Chai *et al.*, 2017). A spaced marker Linkage analysis generated 13 linkage groups with 218 GEMs, spanning 982.7 cM. There was an average of 16.8 marker per linkage group reported with the average distance of 4.2 cM between two neighbouring markers across all linkage groups (Chai *et al.*, 2017).

Recently, Ho *et al.* (2017) demonstrated the use of limited genetic information and resources developed within species to identify conserved synteny blocks in other related legume species which corresponded to the Linkage Groups (LGs) in bambara groundnut genetic maps. Two individual intraspecific linkage maps consisting of DArTseq markers were used in this study, the F_2 population from IITA686 x Ankpa4 cross and the F_3 population of the Tiga Nicuru x

DipC cross. A total of 96 DArTseq selected markers that were polymorphic in both populations could be located through the sequence in the marker Tag to syntenic locations shared between the genomes of common bean (*Phaseolus vulgaris*), adzuki bean (*Vigna angularis*) and mung bean (*Vigna radiata*). A good correspondence was observed at the macro synteny level, with more than 45% of the total bambara groundnut DArTseq markers locatable on the adzuki bean and mung bean genomes and the QTL location of an agronomic trait in one of the bambara groundnut maps identified a corresponding flanking position in common bean, mung bean and adzuki bean (Ho *et al.*, 2017). The putative synthetic blocks identified across legume genomes should facilitate marker-assisted selection, with effective comparison of gene order and identification of the location of candidate genes underlying QTL involved in controlling agronomic and yield traits in bambara groundnut, based on better studied genomes (Ho *et al.*, 2017).

In the present study, construction of a genetic linkage map using DArT markers is attempted with a F_2 population derived from a cross between IITA-686 and LunT. This map will be used to identify the genomic regions (QTLs) associated with drought response in Bambara groundnut.

5.2 Materials and methods

5.2.1 List of molecular markers

The genomic representations were prepared using 184 individuals from the F_2 segregating population derived from a cross between genotypes of IITA-686 (maternal) and LunT (paternal). A total of 6,514 bi-allelic SNP markers were developed by Diversity Arrrays Technology Pty. Ltd (Yarralumla, Australia) in the current study using the DArTseq platform.

5.2.2 Coding of markers

The SNP markers developed through DArTseq are reported in an Excel sheet with each marker reported as two lines: 'variant' line and 'reference' line. 'a', 'h' and 'b' were assigned to each individual with data depending on the scoring pattern in both parental lines for each co-dominant marker (Table 5.1). SNP markers that had identical scores in both parental lines (non-polymorphic) were eliminated, whether there was segregation in the offspring or not.

	IITA-686	***LunT***	*Conversion code*		
			Progeny 1	Progeny 2	Progeny 3
Variant	1	0	1	0	1
Reference	0	1	0	1	1
Genotype code			**a**	**b**	**h**
Variant	0	1	1	0	1
Reference	1	0	0	1	1
Genotype code			**a**	**b**	**h**

Table 5.1: Conversion of genotype code in SNP markers

5.2.3 Selection of markers

The 6,514 bi-allelic SNP markers were first screened for polymorphism. Polymorphic markers with 0 missing values across the individuals in the F_2 population were selected for linkage analysis. After that, markers with average count less that 25% were excluded from the analysis in order to remove poorly scored markers. In addition to this, 162 pre-selected common 'link' DArTseq markers were also selected. These common markers were selected based on two framework genetic maps from (Ho et al., 2017), the genetic map of IITA686 x Ankpa4 from 263 F_2 lines and the genetic map of Tiga Nicuru x DipC from 71 F_3 lines. They were screened and markers which were polymorphic and had missing values $\leq$5 and added into the list of markers selected for linkage analysis to allow cross map comparisons. All markers were then screened to remove redundant markers.

5.2.4 Preparation of data files

A plain text 'Locus genotype file; .loc' was prepared using Wordpad. The locus genotype file (loc-file) contains information on the marker segregation patterns within the population. It contains a four lines of header instruction which are; name of the population(NAME), the type of the population(POP), the number of loci, and the number of individuals as shown in 5.2 below. A total of 682 markers were used to construct the initial linkage map using JoinMap v4.1.

5.2.5 Genetic linkage mapping

The population type was entered as 'F_2'. The grouping markers was set from LOD 2.0 and 10.0 with a step of 1.0, with the independence LOD option was adopted. After generating the grouping trees, the groups were manually selected for grouping at LOD 3 based on the initial split of markers into linkage groups. Initial Maximum Likelihood (ML) map and Regression map with the Haldane mapping function were generated. Markers were removed manually when two adjacent markers were too closely located (1-3 cM) and segregation distortion through a chi-square test for goodness of fit and the maps were calculated again. This process of markers removal was repeated until a spaced map was obtained from the initial dense map, cycling through ML mapping and Regression mapping to try to ensure consistency across both.

5.3 Results

5.3.1 Polymorphic markers in a segregating F_2 population

Out of the 6,514 SNP markers developed through DArT, only 1,555 polymorphic markers had 0 missing values (23.87%). These markers were then screened further for average count of SNP with ratio of less that 25% which resulted in 656 markers (10.07%). 162 common markers (see Ho et al., 2017) were selected and added into the list.

After removing 136 duplicate markers, a total of 682 markers were analysed using JoinMap4.1 for the construction of the genetic map.

5.3.2 Segregation distortion of markers

The segregation patterns of markers are automatically detected by JoinMap4.1 by performing a Chi-square test against expected segregation patterns ($p<0.05$). 464 markers segregated in the expected Mendelian ratio of 1:2:1 whereas 218 markers showed segregation distortion and did not segregate according to the expected Mendelian inheritance as they were dominant markers and were expected to segregate 3:1.

5.3.3 Linkage group and markers distribution

A group of 682 markers were subjected to linkage analysis. 185 markers could not be mapped and grouping analysis at LOD 3 resulted into 11 linkage groups (LG). An initial regression map with the Haldane mapping function resulted in a high-density map with a length of 1682 cM and average distance between markers of 3.5 cM (Table 5.2). An average of 45.2 markers were placed into each LGs and the highest number of markers were observed in LG3 (67).

The initial ML map resulted in a high-density map measuring 2641.1 cM with an average distance between two adjacent markers of 5.1 cM (Table 5.3). An average of 48.3 markers were obtained in each

LG with the highest number of markers observed in LG3 (71) and the lowest number of markers in LG 11 (32).

Linkage group (LG)	Map length (cM)	Number of loci	Average marker interval (cM)
1	185.5	48	3.9
2	205.6	36	5.7
3	146.8	67	2.2
4	110.2	39	2.8
5	179.3	42	4.3
6	231.1	54	4.3
7	81.4	43	1.9
8	138.4	51	2.7
9	137.2	46	3.0
10	140.1	41	3.4
11	126.2	30	4.2
Total	1682	497	38.4
Mean	152.9	45.2	3.5

Table 5.2: The distribution of markers across 11 LGs for genetic linkage analysis in the F_2 population of Bambara groundnut (regression mapping)

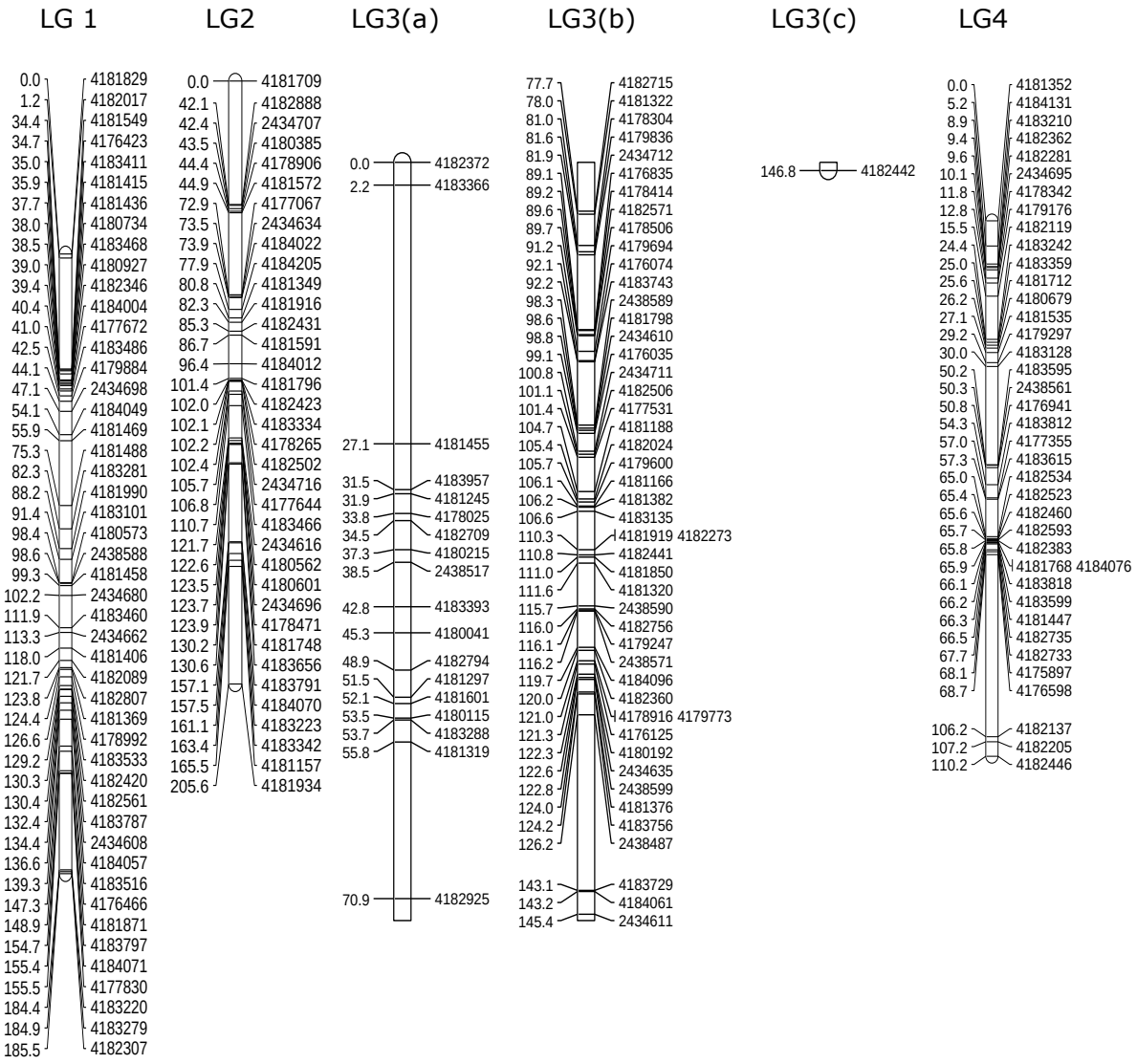


Figure 5.1: High density genetic linkage map of LG1, LG2, LG3(Section a), LG3(Section b), LG3(Section c) and LG4 constructed with the regression mapping option using SNP markers from F_2 lines derived from the IITA-686 x LunT cross. Positions are given to the left of the linkage groups in centimorgan (Kosambi units) and the names of the markers to the right.

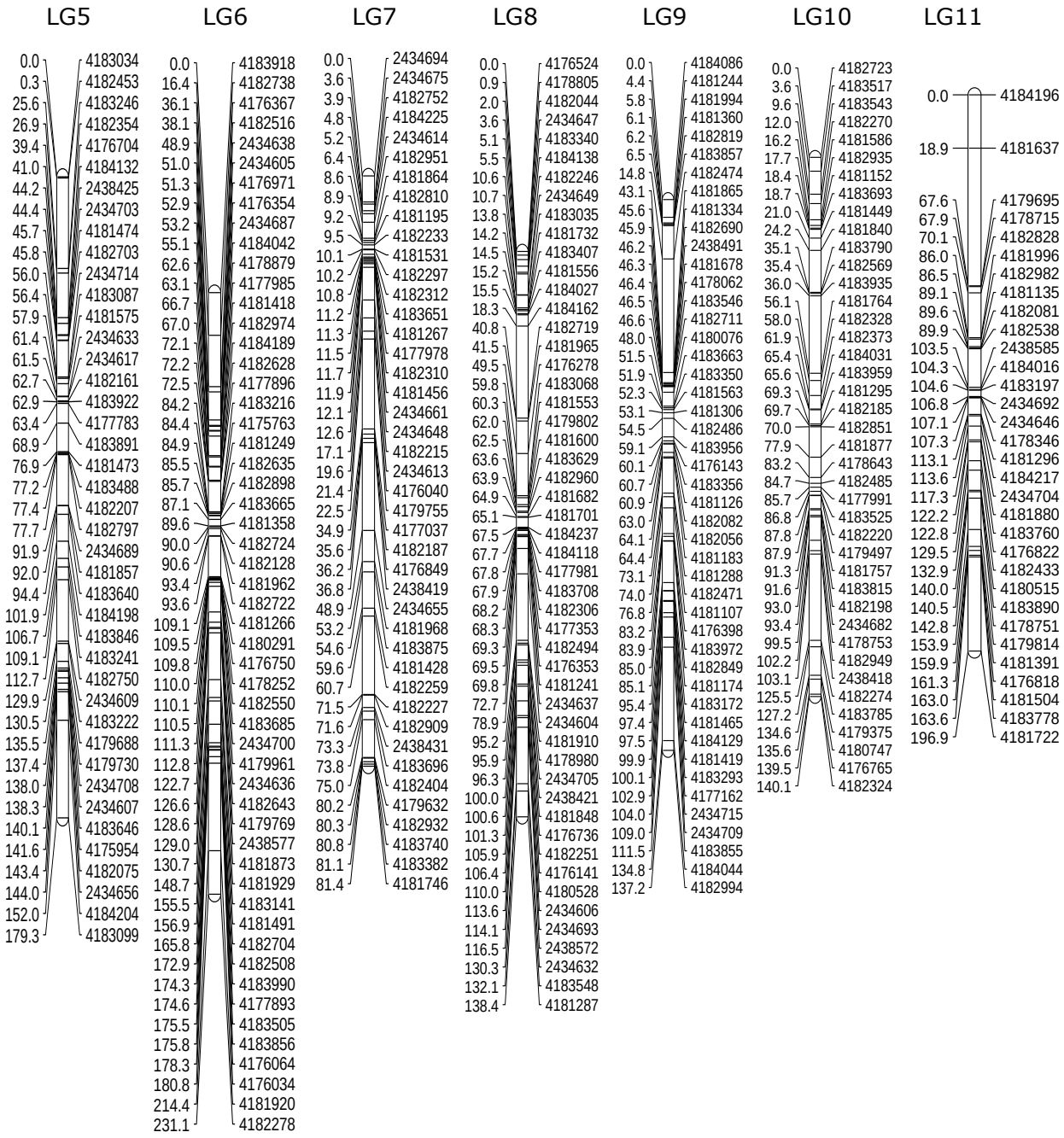


Figure 5.2: High density genetic linkage map of LG5, LG6, LG7, LG8, LG9, LG10 and LG11 constructed with regression mapping using SNP markers from F_2 lines derived from IITA-686 x LunT cross. Positions are given to the left of the linkage groups in centimorgan (Kosambi units) and the names of the markers to the right.

Linkage group (LG)	Map length (cM)	Number of loci	Average marker interval (cM)
1	272.5	52	5.3
2	227.9	37	6.2
3	257.9	71	3.6
4	194.6	44	4.4
5	277.7	47	5.9
6	282.0	54	5.2
7	215.1	44	4.9
8	262.2	57	4.6
9	204.9	50	4.1
10	249.4	43	5.8
11	196.9	32	6.2
Total	2641.1	531	56.2
Mean	240.1	48.3	5.1

Table 5.3: The distribution of markers across 11 LGs for genetic linkage analysis in the F_2 population of Bambara groundnut (ML mapping)

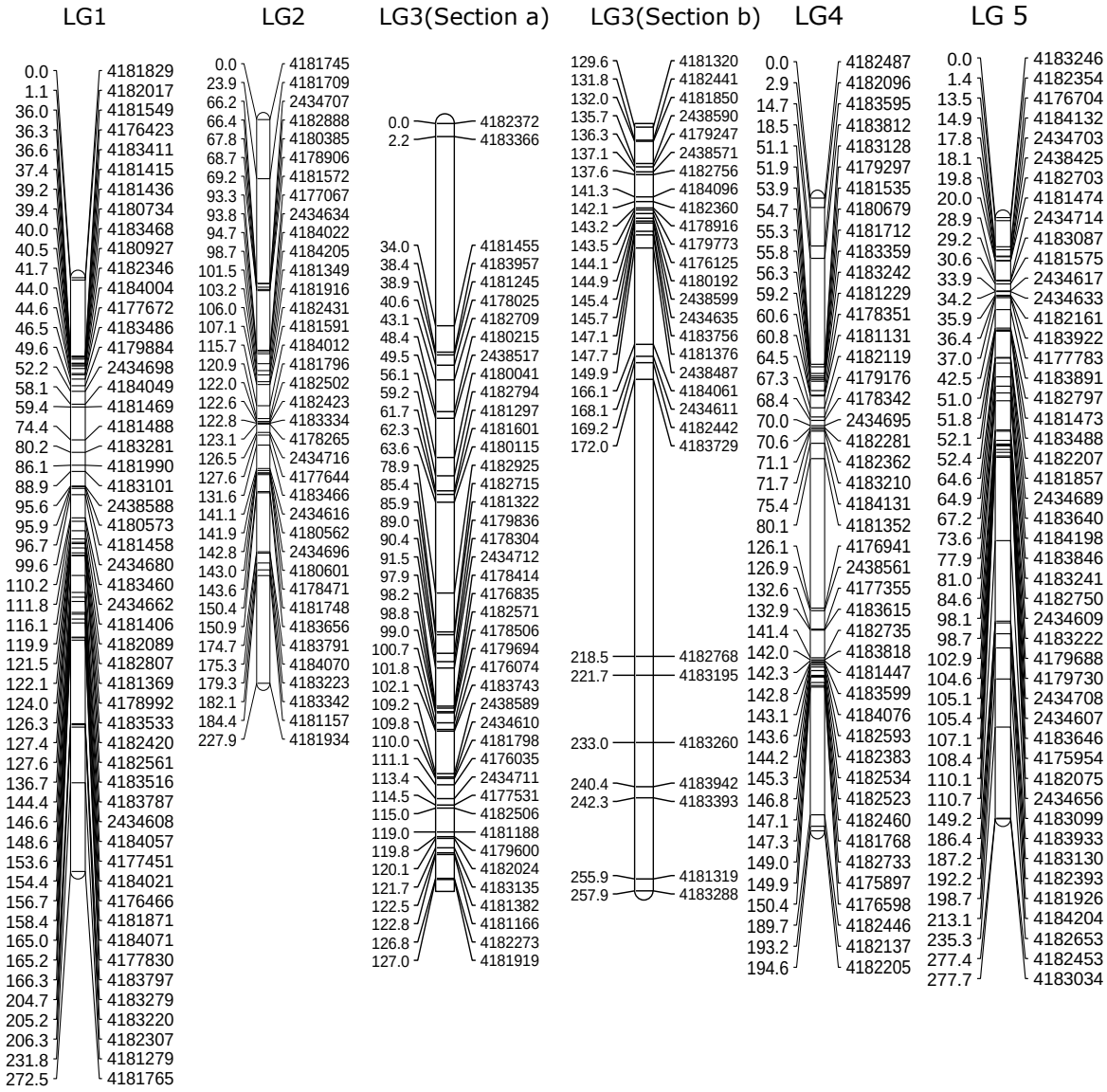


Figure 5.3: High density genetic linkage map of LG1, LG2, LG3(Section a), LG3(Section b), LG4 and LG5 constructed with ML mapping option using SNP markers from F_2 lines derived from IITA-686 x LunT cross. Positions are given to the left of the linkage groups in centimorgan (Kosambi units) and the names of the markers to the right.

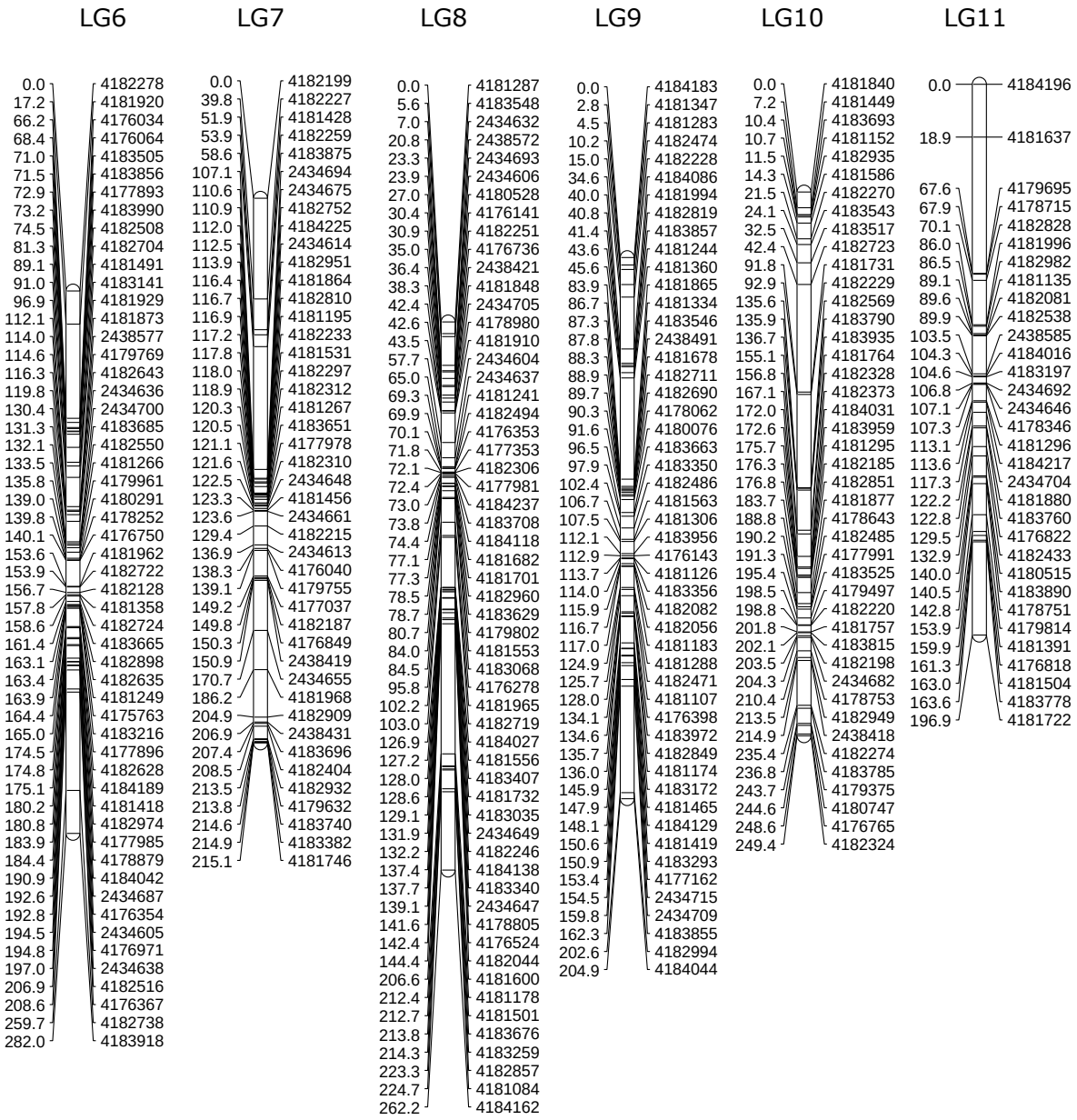


Figure 5.4: High density genetic linkage map of LG6, LG7, LG8, LG9, LG10 and LG11 constructed with ML mapping option using SNP markers from F_2 lines derived from IITA-686 x LunT cross. Positions are given to the left of the linkage groups in centimorgan (Kosambi units) and the names of the markers to the right.

The final spaced map after removal of markers resulted in 11 LGs with 161 spaced markers covering 1447.8 cM of Bambara groundnut genome. An average of 14.6 markers were obtained in each LG with the highest number of markers observed in LG 3 (19). An average distance of 9.0 cM between two adjacent markers across all LG was obtained. The shortest distance between two markers of 2.7 cM was observed in LG 10 whereas the longest distance of 41.4 cM was observed in LG 8. LG 3 with 19 markers, covering 230 cM was the longest linkage group while LG 10 with 12 markers covering 81.4 cM was the shortest linkage group.

Linkage group (LG)	Map length (cM)	Number of loci	Average marker interval (cM)
1	117.3	18	6.9
2	137.1	17	8.1
3	230.0	19	12.1
4	116.8	15	7.8
5	113.9	13	8.8
6	122.0	12	10.2
7	136.7	16	8.5
8	128.2	12	10.7
9	128.3	13	9.9
10	81.4	12	6.8
11	136.1	14	9.7
Total	1447.8	161	99.5
Mean	131.6	14.6	9.0

Table 5.4: The distribution of markers across 11 LGs for genetic linkage analysis in the F_2 population of Bambara groundnut low density

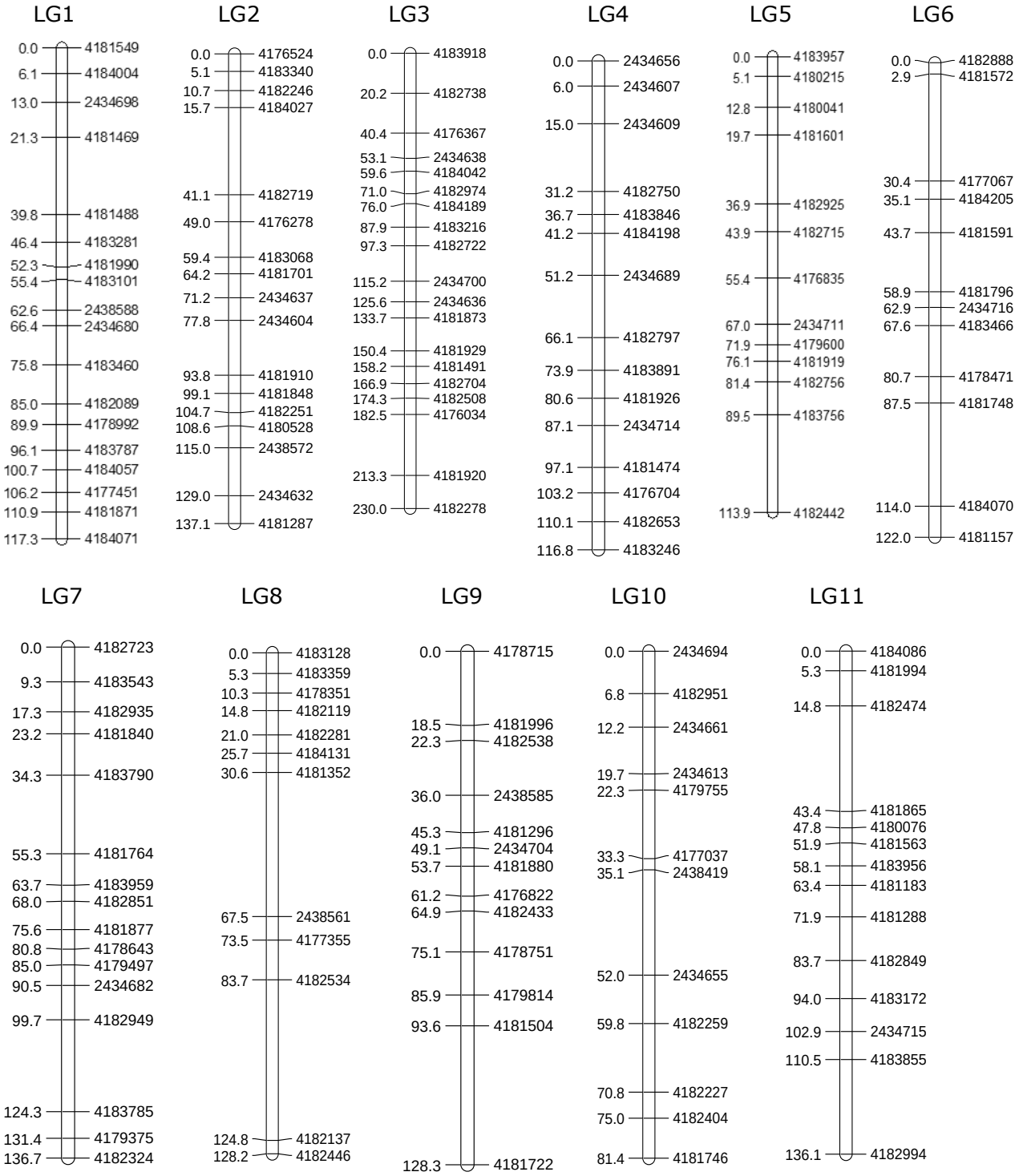


Figure 5.5: Low density genetic linkage map of 11 linkage groups using SNP markers from F_2 lines derived from IITA-686 x LunT cross. Positions are given to the left of the linkage groups in centimorgan (Kosambi units) and the names of the markers to the right. Groupings were identified through the common markers included (Ho et al., 2017)

5.4 Discussion

5.4.1 Marker selection for genetic mapping

Construction of a genetic map requires a segregating plant population generally ranging from 50 to 250 individuals (Mohan, Nair, A. Bhagwat, *et al.*, 1997) and good numbers of markers showing polymorphism between parents used in the genetic cross. In the present study, the mapping population was a cross between IITA-686 and LunT. A total of 6,514 SNP markers were developed through DArTSeq for this study. However, after removal of markers and individuals with 5% missing data points, only 682 (10.5%) markers were used for mapping. Markers with more than five missing values were removed from the marker list as individuals with too many missing data contributes very little information in the map calculations and might even cause problems (Van Ooijen, 2009). When compared to maps developed by Chai, (2014) using 2993 markers (33.9%) and Kendabie, (2014) using 3,852 markers (43.4% of SNPs and SilicoDArTs) the number of polymorphic markers available for this study was lower, perhaps due to the limited genetic differences between the two parents.

Generally, markers will segregate in a Mendelian fashion although distorted segregation ratios are often encountered (Sayed *et al.*, 2002). Segregation distortion affects the estimated recombination

fractions between marker loci (Wang *et al.*, 2005) and so the common practise in genetic mapping is to use Mendelian marker loci to construct a genetic map. However, there are various arguments as to whether to include or not include distorted markers in the construction of a genetic map.

Segregation distortion was reported to result in incorrect map orders and inflation of map lengths, particularly as marker density increases (Hackett and Broadfoot, 2003). However, only a relatively minimal effect was observed. A study by Zhang *et al.*, (2010) reported that segregation distortion does not produce more QTLs or affect QTL detection and distorted markers only affects the QTL when the QTL and the distorted marker are closely linked.

A Chi- square test against expected segregation patterns ($p<0.05$) in this study revealed 464 markers that segregated in the expected Mendelian ratio of 1:2:1 whereas 218 markers (32 %) showed segregation distortion. This finding is in agreement with Ahmad, (2012) that reported 69 out of 210 microarray-based DArT (33%) exhibited segregation distortion. Distorted markers were included in the linkage analysis to avoid losing markers that might be linked to traits of interest or creating genomic regions which were not represented.

5.4.2 Linkage group and marker distribution

A genetic map comprised of 161 markers with an average spacing of 9.0 cM covering 1447.8 cM was constructed for the IITA x LunT F_2 population. The interval between markers in this study was higher than reported by Chai, (2014) with a mean value of 7.8 cM and Ahmad, (2012) with a mean value of 3 cM. However, QTL detection was reported to be the same for a marker spacing of 10 cM as for an infinite number of markers and was only slightly decreased for marker spacing of 20 or even 50 cM (Darvasi *et al.*, 1993). In addition, this map corresponds to the 11 chromosomes number reported by Chai, (2014) and Kendabie, (2014).

There are many reasons for genotyping errors such as variation in DNA quality and quantity of DNA, potential bias with different sequencing methods and biochemical artefacts as well as human errors. Genotyping errors can mask the true segregation of alleles leading to incorrect allele identification and frequencies (Pompanon *et al.*, 2005). Using different tools with different advantages in an iterative map development and refinement process may achieve better results (Cheema and Dicks, 2009). Maximum likelihood mapping functions offers greater robustness to missing data and the ability to find the most likely group wide marker order whereas regression mapping function allows markers that are poorly fitted to be removed (De Keyser *et al.*, 2010). In the present study, the two

mapping approaches (Maximum likelihood mapping and regression mapping) were alternatively used until maps with similar marker order and position was achieved with the two approaches.

The high density map (ML: 2641.1 cM; RM: 1682 cM) provides a higher coverage of the Bambara groundnut genome in comparison to previous maps (Ahmad, 2012; Kendabie, 2014). The lower density map which was constructed based on regression mapping and the Haldane mapping function covering 1447.8 cM was also longer compared to previous maps (Hui Hui, 2014; Kendabie, 2014), although there is always a concern about incorrect marker data leading to map expansion. The maps were ordered from LG1 to LG11 based on previous integrated genetic map produced by (Ho *et al.*, 2017) to allow easier comparison and use of the map for other research work.

CHAPTER 6: QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS

6.1 Introduction

6.1.1 Quantitative Trait Loci (QTL)

A quantitative trait locus (QTL) is a specific chromosomal region or genetic locus in which particular sequences of bases in DNA markers are statistically associated with variation in a trait (Philibin and Crabbe, 2015). Many important agriculture traits such as productivity and quality, tolerance to environmental stresses, and disease resistance are quantitative (also known as polygenic, continuous, multifactorial or complex traits) in nature (Sehgal, Singh and Rajpal, 2016). Therefore, identification of the QTLs of agronomic importance for crop improvement requires mapping of these QTLs in the genome of crop species using molecular markers.

6.1.2 QTL analysis and its application

QTL analysis has been widely used to study mechanisms controlling traits of interest. In rice, a major QTL for grain yield $qDTY_{1.1}$ was identified on rice chromosome 1 which is the first reported QTL in rice with a major and consistent effect in multiple elite genetic backgrounds under both reproductive-stage drought stress and non-stress conditions (Vikram *et al.*, 2011). The effect of a QTL which is consistent across different genetic backgrounds makes it suitable as a candidate for use in marker-assisted breeding (Melchinger, Utz and Schön, 1998).

Four significant QTLs associated with different root architectural traits were identified on Gm06 and Gm07 in soybean by Prince *et al.*, (2015). Manavalan *et al.*, (2015) identified a major QTL on Gm08 controlling tap root length, lateral root number and shoot length. The identification of QTLs underlying root system architecture and canopy characteristics are crucial to develop soybeans that are suited to water- limited environments. The positive association between water use efficiency and total biomass yield in a drought environment suggests that improvement of the water use efficiency of a crop plant should result in superior yield performance under drought. QTLs associated with water use efficiency and leaf ash was studied by Mian *et al.*, (1996) using a restriction fragment length polymorphism (RFLP) map from a soybean [G. max (L.) Merr] population of 120 F4-derived lines from a cross of' Young × PI416937'. The study identified one marker locus (cr497-1), on USDA LG J, explaining 13.2% of variation in water use efficiency indicating presence of a major QTL. Two QTLs were associated with both water use efficiency and leaf ash and for each of the QTLs, the allele for increased water use efficiency was associated with reduced leaf ash.

The understanding of the genetic basis underlying domestication-related traits are important to utilise wild germplasm efficiently for improving yield, stress tolerance and quality of crops. One of the

major QTLs, qPD-J was identified as accounting for the wide variation in pod dehiscence and seed hardness in soybeans (Liu *et al.*, 2007). In a study of the domestication of mungbean using a BC_1F_1 mapping population derived from a cross between cultivated and wild mungbean, Isemura *et al.*, (2012) reported that many more linkage groups have been involved in the domestication of mungbean than in other crops: 20 major QTLs with phenotypic variation explained (PVE) ≥20% for domestication-related traits distributed on 11 linkage groups. The QTLs on LG2 was reported to have the largest effect on 100 seed weight in azuki bean making the locus to have contributed significantly to the seed size increase that occurred during *Vigna* domestication (Isemura *et al.*, 2012). In Bambara groundnut, a single F_2 population derived from a cross between a domesticated landrace (DipC) and a wild accession (VSSP11) was used to study domestication-related traits (Shravani Basu *et al.*, 2007). A limited number of genes were reported to control the major morphological differences between the spreading or bunched plant habit (Shravani Basu *et al.*, 2007). Leaf area, specific leaf area, 100 seed weight and carbon isotope discrimination (CID) were also identified by the authors as quantitative traits with significant variation observed compared to the domesticated form by wild type offspring population.

The first QTL analysis in Bambara groundnut was carried out by Ahmad (2012) using a F_3 segregating population derived from a cross

between DipC and Tiga Nicuru. A total 37 QTLs were reported for 23 morphological and agronomical traits that were mapped, and a major QTL for *internode length* was discovered on linkage group (LG) 4 with a peak LOD score of 7.9 (Ahmad, 2012). This significant QTL explained 43.5% of the total phenotypic variation and was mapped close to marker bgPabg-596988 at 3.0 cM on LG4. A major QTL related to *peduncle length* was also discovered on LG4 with a LOD score of 9.7 at 1.0 cM and the closely linked marker was bgPt-423527 located at 2.4 cM. The identification of QTLs for internode length is of great importance for breeding programs as the morphological trait offers easier management when the bunched types are planted in a mixed cropping system (Ahmad, 2012).

Using the same cross as Ahmad (2012), a total of 16 traits relating to agronomy, drought and morphology from a F_5 segregating population were analysed for QTLs by Chai (2014). The multiple QTL mapping (MQM) results produced a total of 10 QTLs (6 significant QTLs and 4 putative QTLs) associated with 10 studied traits (*days to flowering*, *internode length*, *peduncle length*, *pod number per plant*, *pod weight per plant*, *seed number per plant*, *seed weight per plant*, *100-seed weight*, *shoot dry weight* and *harvest index*) distributed over four LG (LG1, LG2B, LG8B and LG11A). A comparison of two different generations (F_3 and F_5) of the same cross (DipC x Tiga Nicuru) to verify the location of QTLs for common traits recorded and

used in both QTL analysis found that markers bgPabg-596988-4 and MM135 derived from the F_3 and F_5 segregating populations, respectively, were closely linked to both QTLs associated with *internode length* and *peduncle length*.

A study on photoperiod control of pod-set and pod-filling carried out by Kendabie (2014) based on 158 F_2 lines derived from the cross IITA-686 x Ankpa4 reported that the inheritance of photoperiod insensitivity for pod set in Bambara groundnut is determined by a major dominant gene with substantial environment effects for this cross The study identified one major QTL for photoperiod control of the yield related trait (pod number) associated with the marker s4182250 (84.3 cM position) on LG4 with a LOD score of 6.4 and total phenotypic variation of 19% (Kendabie, 2014).

Using pre-selected genotyping-by-sequencing DArTseq markers from two Bambara groundnut crosses (IITA-686 x Ankpa4 and Tiga Nicuru x DipC), linkage maps with conserved synteny links to the sequenced chromosomes of common bean (*Phaseolus vulgaris*), adzuki bean (*Vigna angularis*) and mung bean (*Vigna radiata*) were generated (Ho *et al.*, 2017). This study located a significant internode length QTL in the Tiga Nicuru x DipC F_3 population in LG2 (50.6 cM) where the nearest flanking markers from the pre-selected common marker set are SNP100025953|F|0-27 and SNP100027611|F|0-18 (47.6 – 54.4

cM) which have syntenic blocks in Pv03 (38.4 – 39.1 Mbp) in common bean, Va11 (12.5 – 17.4 Mbp) in adzuki bean and Vr07 (39.4 – 43.5 Mbp) in mung bean. It is suggested that translation of information and the generation of gene lists in the conserved synteny region of a more researched species that corresponds in location to a within species trait controlling locus can be used in an attempt to identify candidate controlling genes for further analysis within the species of interest which has no genome sequence (Ho *et al.*, 2017).

In Chapter 6, the construction of a new genetic linkage map using DArT markers was completed. Using the map created in Chapter 6, a QTL analysis in the Bambara groundnut F_3 population was carried out to study the position and magnitude of QTLs for agronomic and morphological traits to improve the understanding of the mechanisms of the underlying traits.

6.2 Materials and methods

6.2.1 Plant materials

A F_3 population of Bambara groundnut derived from a cross between IITA-686 and LunT was grown and mapped to evaluate QTLs involved in agronomic, morphological and drought-related traits. The phenotypic data for the population was adopted from Chapter 4 which consists of 25 traits recorded for the F_3 population grown in both drought and irrigated conditions. 105 individual lines were subjected

to QTL analysis using a genetic linkage map (Chapter 5) covering 1447.8 cM across 11 linkage groups. The pre-testing and transformation of trait data (where necessary) have been discussed in Chapter 4.

6.2.2 Data files for QTL analysis

Files were prepared for the analysis according to the instructions given in the software manual (Van Ooijen, 2009). Three data files were prepared as below;

a) Locus genotype file: this file (loc-file) contains the genotype codes for the loci of segregating lines being the same file used for JoinMap 4.1 software in Chapter 6 to construct the genetic linkage map

b) Map file: contains the mapped positions of all loci which are the results from grouping and mapping markers in JoinMap 4.1. The map file does not have a header and starts with the instruction of group number. The loci and their map positions are given on subsequent lines in ascending order.

c) Quantitative data file: this file (qua-file) contains all the data for quantitative traits of all individuals. The header file has three instructions followed by the names and numerical values of traits (Figure). The three instructions define the number of traits (ntrt= NTRT), number of individuals (nind= NIND) and the text that indicated a missing value (miss= MISS), here

denoted by '*'. The number and order of the individuals should correspond to the *.loc* file.

6.2.3 The analysis of QTL

Two mapping approaches, Non-parametric mapping (Kruskal-Wallis analysis; KW), and Interval mapping (IM) were done. KW analysis was done for all normally and non-normally distributed data. The Kruskal-Wallis test ranks all individuals according to the quantitative trait, while classifying them according to their marker genotype. It does not rely on the assumption of normally distributed residuals and is regarded as the nonparametric equivalent of the one-way analysis of variance. The Kruskal-Wallis statistic K* is a Chi-square like distribution, including the degrees of freedom (n-1 df for F2 and F3 populations) and the significance level in asterisks. [Significance level: *:0. 1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001]. IM was used to detect and identify QTLs with the analysis options set to default. Permutation tests using 10,000 permutation were conducted to determine the significance threshold of the LOD score. The Genome Wide (GW) threshold at $p \leq 0.05$ was used to compare with the LOD scores obtained from IM. QTLs were identified as 'significant' if LOD score was equivalent or higher than the GW threshold and 'putative' if LOD score was lower than GW threshold by up to one LOD interval. The

maximal LOD position was determined as a most likely position of QTL and a one LOD decrease on either side of LOD peak was considered as a confidence interval for the effect.

6.3 Results

A total of 25 traits relating to agronomy, morphology and drought response were analysed for QTL based on the genetic linkage map. The IM mapping results produced a total of 23 QTLs were found in the drought treatment and 15 QTLs were found in the irrigated treatment, with 5 significant QTLs and 33 putative QTLs, associated with 14 studied traits distributed over 9 linkage groups presented in Table 6.1 and Table 6.2.

Trait	LG	Position (cM)	Nearest marker/ Locus	Interval Mapping				Kruskal Wallis		
				LOD	PVE_b	Additive	PT_a	K*	Df	Significance
Double seeded pods	*9*	*113.6*	*4181722 (128.3cM)*	*2.37*	*10.1*	*-0.29992*	*3.2*			
	3	59.6	4184042					10.101	2	***
Biomass	7	25.2	4181840 (23.2cM)	4.01	16.6	2.25006	3.8			
	7	23.2	4181840					23.026	2	*******
Shelling percentage	U	*[28]*	*4181731*	*3.24*	*13.6*	*-0.9445*	*3.4*			
	3	59.6	4184042					12.241	2	****
Porometer 48 DAS	*3*	*176.3*	*4182508 (174.3cM)*	*2.8*	*11.9*	*-11.3361*	*3.8*			
	6	80.7	4178471					13.538	2	****
Porometer 62 DAS	*6*	*4.9*	*4181572 (2.9cM)*	*3.62*	*15.3*	*-50.0432*	*3.7*			
	6	2.9	4181572					15.194	2	*****
Porometer 78 DAS	*U*	*[155]*	*4179802*	*3.52*	*15*	*2.20358*	*3.8*			
	2	64.2	4181701					13.06	2	****
Porometer 90 DAS	*U*	*[25]*	*4183582*	*3.72*	*15.4*	*3.03817*	*3.9*			
	9	45.7	4181296					10.22	2	***
Porometer 104 DAS	7	23.2	4181840 (23.2cM)	3.95	16.3	19.9907	3.7			
	7	23.2	4181840					14.555	2	*****
SPAD 47 DAS	*3*	*25.2*	*4182738 (20.2cM)*	*2.77*	*11.7*	*-1.44815*	*3.7*			
	3	20.2	4182738					12.269	2	****
SPAD 61 DAS	*U*	*[20]*	*4183195*	*2.81*	*11.9*	*-1.98272*	*3.8*			
	1	75.8	4183460					9.96	2	***
SPAD 75 DAS	*U*	*[166]*	*4182932*	*3.12*	*13.1*	*1.00671*	*3.6*			
	10	12.2	2434661					11.94	2	****
SPAD 82 DAS	*U*	*[481]*	*2434675*	*3.08*	*13*	*0.226104*	*3.7*			
	10	75.0	4182404					8.869	2	**
SPAD 89 DAS	U	[9]	4183099	4.58	18.8	-3.88321	3.8			
	5	12.8	4180041					7.749	2	**
SPAD 103 DAS	*U*	*[25]*	*4183582*	*2.8*	*12*	*1.67669*	*3.7*			
	2	59.4	4183068					8.051	2	**
Days to flowering	*5*	*69.0*	*2434711 (67.0cM)*	*2.77*	*11.9*	*-1.92217*	*3.6*			
	5	67.0	2434711					11.683	2	****
Days to podding	*U*	*[323]*	*4183525*	*2.9*	*12.5*	*1.97646*	*3.7*			
	2	0	4176524					9.179	2	**
Pod width	*4*	*113.1*	*4182653 (110.0cM)*	*2.99*	*12.6*	*-0.59159*	*3.6*			
	4	110.1	4182653					9.98	2	***
Pod length	U	[45]	4181084	4.42	18.1	-0.8776	3.7			
	7	0	4182723					13.314	2	****
Seed length	*7*	*25.2*	*4181840 (23.218cM)*	*2.76*	*11.7*	*0.491211*	*3.7*			
	7	23.2	4181840					11.505	2	****
Seed width	*U*	*[343]*	*4182794*	*2.89*	*12.2*	*0.224734*	*3.7*			
	10	75.0	4182404					10.158	2	***
Single seed weight	*U*	*[447]*	*4181455*	*2.93*	*12.4*	*0.04129*	*3.7*			
	2	115.0	2438572					9.9	2	***

a : permutation-10000 times test

b : percentage of total phenotypic variation explained by the QTL

Position (cM) in brackets [ ] are marker numbers for unlinked loci

Significant level of K * values: *: 0.10, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001

Table 6.1: QTL mapping results in bambara groundnut using interval mapping and Kruskal-Wallis analysis for the F_3 generation data (Drought) of the IITA(686) x LunT cross in the FutureCrop glasshouses (significant and putative QTLs).

Trait	LG	Position (cM)	Nearest marker/ Locus	Interval Mapping				Kruskal Wallis		
				LOD	PVE[b]	Additive	PT[a]	K*	Df	Significance
Double seeded pods	*7*	*73.029*	*4181877 (75.595 cM)*	*2.8*	*11.9*	*-0.72511*	*3.5*			
	8	124.753	4182137					11.828	2	****
Biomass	*9*	*49.109*	*2434704 (49.109cM)*	*3.6*	*15*	*-3.3132*	*3.9*			
	9	45.263	4181296					8.433	2	**
Leaf width	*4*	*84.643*	*2434714 (87.123 cM)*	*2.82*	*11.9*	*0.226964*	*3.3*			
	4	97.062	4181474					10.354	2	***
Shelling percentage	3	24.227	4182738 (20.227 cM)	3.32	13.9	4.71529	3.7			
	3	40.421	4176367					16.414	2	******
Porometer 78 DAS	*10*	*73.791*	*4182404 (75.023 cM)*	*3.25*	*13.8*	*28.47*	*3.7*			
	10	75.023	4182404					8.684	2	**
Porometer 82 DAS	*1*	*85.996*	*4182089 (84.996 cM)*	*3.4*	*14.2*	*333.333*	*3.6*			
	11	63.439	4181183					15.089	2	*****
Porometer 90 DAS	*3*	*175.333*	*4182508 (174.33 cM)*	*2.82*	*12*	*108.912*	*3.7*			
	1	96.07	4183787					13.358	2	****
SPAD 47 DAS	*U*	*[52]*	*4181829*	*2.54*	*10.8*	*2.7096*	*3.2*			
	7	17.25	4182935					7.033	2	**
SPAD 54 DAS	*7*	*20.25*	*4181840 (23.218cM)*	*2.41*	*10.3*	*-2.53999*	*3.4*			
	4	5.979	2434607					7.502	2	**
SPAD 61 DAS	*U*	*[38]*	*4182199*	*3.18*	*13.4*	*0.835202*	*3.6*			
	10	70.791	4182227					7.329	2	**
SPAD 68 DAS	*2*	*99.084*	*4181848 (99.084 cM)*	*3.13*	*13.2*	*2.23126*	*3.7*			
	2	99.084	4181848					11.476	2	****
SPAD 75 DAS	*U*	*[51]*	*4183220*	*2.29*	*9.8*	*3.03417*	*3.2*			
	7	9.333	4183543					8.401	2	**
SPAD 96 DAS	U	[25]	4183582	3.68	15.6	4.14307	3.6			
	7	68.029	4182851					8.066	2	**
Pod length	U	[311]	4183548	2.61	11.1	-0.09147	3.6			
	2	129.036	2434632					8.922	2	**
Seed length	*10*	*0*	*2434694 (0 cM)*	*2.93*	*12.4*	*-0.13674*	*3.8*			
	10	0	2434694					11.48	2	****

a : permutation-10000 times test

b : percentage of total phenotypic variation explained by the QTL

Position (cM) in brackets [ ] are marker numbers for unlinked loci

Significant level of K * values: *: 0.10, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001

Significant and putative interval mapping QTLs are in bold

Table 6.2: QTL mapping in bambara groundnut using interval mapping and Kruskal-Wallis analysis for the F_3 generation data (Irrigated) of the IITA(686) x LunT cross in the FutureCrop glasshouses (significant and putative QTLs).

The distribution of QTLs and their effects for each trait are explained as below:

Double seed pods: Two putative QTLs for double seeded pod located at 73.0 cM on LG 7 for irrigated and at 113.6cM on LG9 for the drought treatment were discovered. The QTLs for the irrigated treatment had a LOD score (2.8) lower that the GW threshold (3.5) which explained 11.9% of the total phenotypic variation and the drought treatment QTL had a LOD score (2.37) lower than the GW threshold (3.2) which explained 10.1% of the total phenotypic variation. Markers nearest to the locus is 4181877 at 75.595 cM for irrigated and 4181722 128.296 cM for drought. Kruskal-Wallis analysis revealed potential QTL for double seed pods associated with marker 4182137 at 124.75cM (irrigated) and 4184042 at 59.6cM (drought) on different linkage groups of 8 and 3 respectively, valued K*>6 at p=0.005 (irrigated) and p= 0.01(drought).

Biomass: A significant QTL located at 25.2 cM on LG 7 for the drought treatment was discovered and a putative QTL in the irrigated treatment was located at 49.1 cM on LG 9. The QTLs for irrigated had a LOD score (3.6) lower that the GW threshold (3.9) which explained 15% of the total phenotypic variation and drought QTL had a LOD score (4.01) higher than the GW threshold (3.8) which explained

16.6% of the total phenotypic variation. Markers nearest to the QTL is 4181840 at 23.2 cM for the drought treatment and 2434704 at 49.109 cM for the irrigated treatment. Kruskal-Wallis analysis revealed potential QTL for biomass associated with marker 4181296 45.3 cM (irrigated) and 4181840 at 23.2 cM (drought) on different linkage groups of 9 and 7 respectively, valued K*>6 at p=0.05 (irrigated) and p= 0.0001(drought).

Leaf width: A single putative QTL for leaf width located at 84.6 cM on LG 4 for the irrigated treatment was discovered. It had a LOD score (2.8) lower than the GW threshold (3.3) which explained 11.9% of the total phenotypic variation. The marker 2434714 at 87.1 cM was the nearest marker to the locus. The Kruskal-Wallis analysis revealed a putative QTL for leaf width associated with marker 4181474 at 97.0 cM (irrigated) and 4184071 at 117.3 cM (drought) on linkage groups of 4 and 1 respectively, valued K*>6 at p=0.01 (irrigated) and p= 0.005(drought).

Shelling percentage: Two putative QTLs for shelling percentage located at 24.2 cM on LG 3 for the irrigated treatment and marker number 28 [unmapped] for the drought treatment were discovered. The QTLs for irrigated had a LOD score (3.3) lower that the GW threshold (3.7) which explained 13.9% of the total phenotypic variation and the drought QTL had a LOD score (3.2) lower than the

GW threshold (3.4) which explained 13.6% of the total phenotypic variation. Markers nearest to the locus is 4182738 at 20.227 cM for irrigated and 4181731 (unmapped) for drought. Kruskal-Wallis analysis revealed potential QTL for shelling percentage associated with marker 4176367 at 40.42 cM (irrigated) and 4184042 at 59.6 cM (drought) on linkage group 3 valued K*>6 at p=0.0005 (irrigated) and p= 0.005(drought).

Stomatal conductance (g_s): Readings for stomatal conductance were carried out throughout the course of the experiment. A significant QTL was identified in the drought treatment for 104 DAS located at 23.2 cM on LG 7. The QTL had a LOD score (3.95) higher that the GW threshold (3.7) which explained 16.3% of the total phenotypic variation. Marker nearest to the locus is 4181840 at 23.2 cM. A total of 7 putative QTLs were identified for stomatal conductance (g_s) using porometer readings. Four QTLs were identified in drought located at 176.3 cM (LG3) for 48 DAS and 4.9 cM (LG6) for 62 DAS, and marker number 155 (unmapped) for 78 DAS and marker number 25 (unmapped) for 90 DAS respectively. Three other QTLs were identified in irrigated located at 73.79 cM for 78 DAS, 85.9 cM for 82 DAS and 175.3 cM for 90 DAS on LG 10, LG 1 and LG3 respectively.

Chlorophyll content:

SPAD meter readings (as a surrogate for chlorophyll content) were taken throughout the course of the experiment. Two significant QTL were identified located at marker number 25 (irrigated) for 96 DAS and 9 (drought) for 89 DAS both on the unmapped markers. The QTL (drought) had a LOD score (3.95) higher that the GW threshold (3.7) which explained 16.3% of the total phenotypic variation whereas the marker (drought) had a LOD score (3.68) higher that the GW threshold (3.6) which explained 15.6% of the total phenotypic variation. Markers nearest to the locus were 4183582 (drought) and 4183099 (irrigated). A total of 10 putative QTLs, five in drought and five in irrigated were also identified. The putative QTLs in irrigated were located at 20.2 cM (LG7) for 54 DAS and 99.0 cM (LG2) for 68 DAS and marker number 38 (unmapped) for 61 DAS, 51 (unmapped) for 75 DAS and 52 (unmapped) for 47 DAS. The putative QTLs in drought were located at 25.2 cM on LG 3 for 47 DAS, and marker number 20 for 61 DAS, 25 for 103 DAS, 166 for 75 DAS and 481 for 82 DAS on ummapped.

Days to flowering: A putative QTL was identified for the drought treatment located at 69.0 cM on LG 5. The QTL had a LOD score (2.77) lower that the GW threshold (3.6) which explained 11.9% of the total phenotypic variation. Markers nearest to the locus is 2434711 at 67.0 cM. The Kruskal-Wallis analysis revealed potential QTL for days to flowering associated with marker 4181287 at 137.0

cM (irrigated) and 2434711 at 67.0 cM (drought) on different linkage groups of 2 and 5 respectively, valued K*>6 at p=0.01 (irrigated) and p= 0.005(drought).

Days to podding : A putative QTL was identified in drought located on marker 4183525 on (unmapped). The QTL had a LOD score (2.9) lower that the GW threshold (3.7) which explained 12.5% of the total phenotypic variation. The Kruskal-Wallis analysis revealed potential QTL for *days to podding* associated with marker 4182251 at 104.7cM (irrigated) and 4176524 at 0cM (drought) on LG 2 valued K*>6 at p=0.05 for both irrigated and drought.

Pod width: A putative QTL was identified in drought located at 113.1 cM on LG 4. The QTL had a LOD score (2.99) lower that the GW threshold (3.6) which explained 12.6% of the total phenotypic variation. Markers nearest to the locus is 4182653 at 110.0 cM. The Kruskal-Wallis analysis revealed potential QTL for pod width associated with marker 2434632 at 129.0 cM (irrigated) and 4182653 at 110.1cM (drought) on different linkage groups of 2 and 4 respectively, valued K*>6 at p=0.01 for both irrigated and drought.

Pod length: A single significant QTL was identified in drought on marker number 45 (unmapped). The QTL had a LOD score (4.42) higher that the GW threshold (3.7) which explained 18.1% of the

total phenotypic variation. Marker nearest to the locus is 4181084. Kruskal-Wallis analysis revealed a potential QTL for pod length associated with marker 2434632 at 129.0 cM (irrigated) and 4182723 at 0.0 cM (drought) on different linkage groups of 2 and 7 respectively, valued K*>6 at p=0.05 for irrigated and p=0.005 for drought.

Seed length: Two putative QTLs for seed length located at 0 cM on LG 10 for irrigated and at 25.2cM on LG 7 for drought were discovered. The QTLs for the irrigated treatment had a LOD score (2.93) lower that the GW threshold (3.8) which explained 12.4% of the total phenotypic variation and drought QTL had a LOD score (2.76) lower than the GW threshold (3.7) which explained 11.7% of the total phenotypic variation. Markers nearest to the locus is 2434694 at 0 cM for the irrigated treatment and 4181840 at 23.2 cM for the drought treatment. Kruskal-Wallis analysis revealed potential QTL for seed length associated with marker 2434694 at 0.0 cM (irrigated) and 4181840 at 23.2 cM (drought) on different linkage groups of 10 and 7 respectively, valued K*>6 at p=0.005 for both irrigated and drought treatments

Seed width: A putative QTL was identified in the drought treatment located on marker 343 on unmapped. The QTL had a LOD score (2.89) lower that the GW threshold (3.7) which explained 12.2% of

the total phenotypic variation. Marker nearest to the locus is 4182794. The Kruskal-Wallis analysis revealed potential QTL for seed width associated with marker 4182433 at 64.9 cM (irrigated) and 4182404 at 75.0 cM (drought) on different linkage groups of 9 and 10 respectively, valued K*>6 at p=0.005 for irrigated and p=0.01 drought.

Single seed weight: A putative QTL was identified in drought located at marker 447 (unmapped). The QTL had a LOD score (2.93) lower that the GW threshold (3.7) which explained 12.4% of the total phenotypic variation. Marker nearest to the locus is 4181455. The Kruskal-Wallis analysis revealed potential QTL for seed width associated with marker 4183956 at 58.1 cM (irrigated) and 2438572 at 115.0 cM (drought) on different linkage groups of 11 and 2 respectively, valued K*>6 at p=0.005 for irrigated and p=0.01 for drought.

Trait	LG	Position (cM)	Nearest marker/ Locus	Interval Mapping				Kruskal Wallis		
				LOD	PVE$_b$	Additive	PT$_a$	K*	Df	Significance
Single Seed Pods	3	28.2	4182738 (20.2cM)	1.71	7.4	-7.89022	3.1			
	7	23.2	4181840					8.476	2	**
Pod number	U	[34]	4183812	2.2	9.5	-0.60917	3.7			
	1	110.9	4181871					7.477	2	**
Pod weight	U	[230]	4182328	2.06	8.9	1.7501	4			
	7	23.2	4181840					8.106	2	**
Seed weight	U	[230]	4182328	2.21	9.5	1.39619	3.7			
	10	75.0	4182404					8.063	2	**
No of seed	U	[34]	4183812	2.06	8.9	-0.63917	3.9			
	1	110.9	4181871					7.216	2	**
Leaf length	U	[121]	4182310	2.04	8.8	0.331577	3.7			
	10	70.8	4182227					13.484	2	****
Leaf width	U	[329]	4183665	1.32	5.8	0.091584	3			
	1	117.3	4184071					11.82	2	****
Internode length	U	[481]	2434675	1.76	7.6	0.127296	3.8			
	10	12.2	2434661					10.158	2	***
Plant height	U	[118]	4179773	2.1	9	-0.86382	3.4			
	5	89.5	4183756					6.573	2	**
Plant spread	10	16.2	2434613 (19.7cM)	2.7	11.5	0.495144	3.8			
	6	2.9	4181572					9.784	2	***
Petiole	4	113.0	4182653 (110.1cM)	2.16	9.3	1.22717	3.7			
	5	5.1	4180215					7.407	2	**
100seed-weight	U	[209]	4181458	2.27	9.7	-0.67781	3.6			
	2	99.0	4181848					9.771	2	***
Harvest Index	3	30.2	4182738 (20.2cM)	1.38	6	-1.4282	3.5			
	4	87.1	2434714					10.256	2	***
Porometer 55 DAS	U	[267]	4181418	2.4	10.3	-11.9153	3.8			
	4	66.0	4182797					8.298	2	**
Porometer 82 DAS	6	67.6	4183466 (67.6cM)	2.58	11	5.13691	3.9			
	6	67.6	4183466					11.653	2	****
Porometer 97 DAS	U	[111]	4181391	2.62	11.2	-11.6559	3.8			
	2	104.7	4182251					8.789	2	**
SPAD 40 DAS	U	[250]	4179297	1.77	7.7	1.21983	3.8			
	8	0	4183128					8.01	2	**
SPAD 54 DAS	U	[41]	4184044	1.86	8.1	0.7605	3.7			
	4	36.7	4183846					7.693	2	**
SPAD 68 DAS	U	[166]	4182932	2.64	11.2	0.664371	3.7			
	3	213.3	4181920					9.447	2	***
SPAD 96 DAS	3	1	4183918 (0cM)	2.39	10.4	-2.49957	3.7			
	3	0	4183918					9.498	2	***

a : permutation-10000 times test

b : percentage of total phenotypic variation explained by the QTL

Position (cM) in brackets [ ] are marker numbers for unlinked loci

Significant level of K * values: *: 0.10, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001

Table 6.3: QTL mapping results in bambara groundnut using interval mapping and Kruskal-Wallis analysis for the F_3 generation data (Drought) of the IITA(686) x LunT cross in the FutureCrop glasshouses (non significant QTLs).

Trait	LG	Position (cM)	Nearest marker/ Locus	Interval Mapping				Kruskal Wallis		
				LOD	PVE$_b$	Additive	PT$_a$	K*	Df	Significance
Single Seed Pods	U	[176]	4178346	2.17	9.3	-4.3751	3.7			
	2	59.414	4183068					6.53	2	**
Pod number	U	[176]	4178346	2.32	9.9	-4.39907	3.9			
	5	19.665	4181601					7.074	2	**
Pod weight	2	77.776	2434604 (77.766 cM)	1.77	7.7	0.2475	3.8			
	7	75.595	4181877					8.169	2	**
Seed weight	2	77.776	2434604 (77.766 cM)	1.75	7.6	0.19058	3.8			
	7	75.595	4181877					8.13	2	**
No of seed	U	[176]	4178346	2.37	10.2	-4.50286	3.7			
	5	19.665	4181601					6.861	2	**
Leaf length	4	111.069	4182653 (110.069 cM)	2.59	11	-0.13735	3.6			
	8	73.482	4177355					10.684	2	****
Internode length	U	[4]	4181600	2.44	10.5	-0.21202	3.7			
	6	122.016	4181157					7.309	2	**
Plant height	U	[23]	4181745	2.5	10.7	1.07415	3.6			
	1	0	4181549					9.299	2	***
Plant spread	U	[4]	4181600	1.53	6.7	-1.53303	3.8			
	2	0	4176524					5.785	2	*
Petiole	10	9.813	2434661 (12.226 cM)	2.28	9.9	-0.95163	3.6			
	8	0	4183128					7.422	2	**
100seed-weight	U	[25]	4183582	1.98	8.6	3.25154	3.6			
	7	9.333	4183543					7.889	2	**
Harvest Index	4	106.18	4176704 (103.180 cM)	2.36	10.1	1.85132	3.5			
	8	124.753	4182137					9.084	2	**
Porometer 62 DAS	U	[102]	4181320	2.43	10.5	-68.2579	3.6			
	5	76.132	4181919					9.638	2	***
Porometer 97 DAS	3	174.333	4182508(174.333cM)	2.31	9.9	57.1375	3.8			
	3	97.296	4182722					9.536	2	***
Porometer 104 DAS	U	[27]	4181152	2.79	11.8	60.4803	3.9			
	10	12.226	2434661					9.049	2	**
SPAD 40 DAS	U	[19]	4183260	2.03	8.8	1.27296	3.7			
	3	53.148	2434638					7.672	2	**
SPAD 82 DAS	U	[418]	2434695	2.55	10.9	1.17487	3.7			
	8	0	4183128					8.691	2	**
SPAD 89 DAS	U	[342]	4183242	2.47	10.6	0.397083	3.7			
	8	5.256	4183359					8.683	2	**
SPAD 103 DAS	10	72.791	4182227 (70.791 cM)	1.41	6.3	8.82997	3.2			
	2	99.084	4181848					9.714	2	***
Days to flowering	U	[60]	4183875	2.26	9.7	1.26444	3.5			
	2	137.085	4181287					9.406	2	***
Days to podding	2	104.702	4182251 (104.702 cM)	2.36	10.1	-1.43854	3.6			
	2	104.702	4182251					9.201	2	**
Pod width	U	[7]	4184162	2.45	10.5	0.149179	3.6			
	2	129.036	2434632					9.557	2	***
Pod length	U	[311]	4183548	2.61	11.1	-0.09147	3.8			
	2	129.036	2434632					8.922	2	**
Seed width	9	68.933	4182433 (64.9cM)	2.65	11.3	-0.05929	3.7			
	9	64.933	4182433					11.636	2	****
Single seed weight	8	21.048	4182281 (21.048 cM)	2.59	11	0.005876	3.7			
	11	58.104	4183956					13.215	2	****

a : permutation-10000 times test

b : percentage of total phenotypic variation explained by the QTL

Position (cM) in brackets [ ] are marker numbers for unlinked loci

Significant level of K * values: *: 0.10, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001

Table 6.4: QTL mapping in bambara groundnut using interval mapping and Kruskal-Wallis analysis for the F_3 generation data (Irrigated) of the IITA(686) x LunT cross in the FutureCrop glasshouses (non significant QTLs).

6.4 Discussion

Based on IM analysis, four significant QTLs and 17 putative QTLs for drought conditions were identified. These QTLs were located on LG 3, LG 4, LG 5, LG 6, LG 7 and LG 9 and associated with unmapped markers. One significant QTL and 13 putative QTLs were identified in the irrigated environment and were located on LG 1, LG 2, LG 3, LG 4, LG 7, LG 9, LG 10 and unmapped. QTLs were detected on all LG except for LG 8 and LG 11. Overlapping QTLs were found on LG 3, LG 4, LG 7 and LG 9. QTLs for *double seeded pods*, biomass, shelling percentage, *stomatal conductance*, *SPAD* readings and *seed length* were identified in both treatments. Additional QTLs for *days to flowering*, *days to podding*, *pod width*, *pod length*, *seed width* and *single seed weight*, QTLs were detected only under the drought conditions as QTL associated with a response to stress are expected in the drought treatment. The drought treatment might be expected to activate gene expression in response to stress leading to identification of response-associated QTL (Chai, 2014). A QTL for leaf width was also identified under the irrigated treatment.

QTL associated with traits related to *biomass*, *stomatal conductance* (104 DAS) and *seed length* in the drought environment consistently mapped to LG7 and were associated with the same single marker

4181840 at 23.2 cM. This suggests that these traits could be effected by a single gene. Chai *et al.* (2017) hypthesised a clustered QTL could correspond to a single gene that has pleiotropic effects on different traits including seed and plant growth related traits. Similarly, QTL detected for seed traits in pea were found to be located in the genomic regions regulating traits such as plant morphology, phenology and plant biomass (Burstin *et al.*, 2007). The stomatal conductance at 104 DAS are readings during the recovery period after drought treatment (Chapter 5). This may be an interesting trait to look at for breeders when fast recovery after drought is needed to produce reasonable yield. Kruskal-Wallis analysis also confirmed QTL for biomass associated with marker 4181840 on LG 7 valued K*>6 at p= 0.0001. Ahmad *et al.*, (2016) reported a major QTL in Bambara groundnut for biomass on LG 1 which was tightly linked with the marker bgPabg-596774 located at 33.0 cM with a LOD score of 3.5 explained 22.4% of the phenotypic variation.

Days to podding, *pod width*, *pod length*, *seed width*, and *single seed weight* QTLs (1 significant and 4 putative) were observed only in the drought treatment on LG 4, LG 7 and unmapped markers with total phenotypic variation explained ranging from 12.2% to 18.1%. A putative QTL for *seed length* and *shelling percentage* was also observed in both treatments. For *seed length*, the QTL was located in LG 10 (irrigated) and LG 7 (drought) and for shelling percentage

in LG 3 (irrigated) and unmapped (drought). Kruskal-Wallis analysis also revealed potential QTL for shelling percentage associated with marker 4176367 (irrigated) and 4184042 (drought) on linkage group 3 valued K*>6 at p=0.0005 (irrigated) and p= 0.005(drought). In a study carried out by Yuste-Lisbona *et al.*, (2014) using common beans, the total phenotypic variation explained by all putative single-locus QTLs was 2.2–5.9 % for pod size traits (*pod width*, *pod thickness*, *pod length* and *pod size index*). The single-locus QTL analysis results in this study showed that the genetic basis of pod size traits seems to be controlled by a few minor QTLs. The results of the study was also in accordance with previous reports for pod traits for soybean (Liu *et al.*, 2007) and azuki bean (Isemura *et al.*, 2012). *100 seed weight*, (which is mainly determined by seed size which can be measured by seed length and seed width), *100 pod weight* and *shelling percentage* are important components of grain yield (Chen *et al.*, 2017). Therefore, identification of QTLs related to seed traits will advance the knowledge of biological pathways conditioning yield components and seed morphology.

Drought tolerance is a complex trait and its interaction with multiple environment factors makes the validation of QTLs more difficult. Molecular markers are powerful tools to better understand the molecular basis of drought tolerance in Bambara groundnut and once validated, can be used in molecular breeding. However, the

population used in the current study is not big enough detect all QTL (Collard *et al.*, 2005). A larger population is needed for a better understanding of more complex agronomical traits so that these QTL markers can be used in marker assisted selection of Bambara groundnut crop.

CHAPTER 7: GENERAL DISCUSSION

7.1 Issues and Challenges in Food Security

Food has always been linked to environmental conditions with it's production, storage, distribution and markets all sensitive to weather and climate changes. Food production and the quality of food produced is also sensitive to soil and water, presence of pest and disease, and other biophysical influences. Through the years, people have adjusted the production and consumption of food in relation to the risks of climate change, biodiversity loss and water scarcity (Ingram, Ericksen and Liverman, 2012). Even under current conditions, agricultural systems fail to address malnutrition of more than 2 billion people who suffer from micro-nutrient deficiencies (*UNIFORMITY TO DIVERSITY A paradigm shift from industrial agriculture to diversified agroecological systems*, 2016). This is partly

due to the insufficient production levels of nutrient-rich crops required for healthy human nutrition (Siegel *et al.*, 2014).

Of a total of 250 000 identified plant species, 7 000 have been used in agriculture throughout human history. However, currently, 75% of the world's food comes from just 12 plant and 5 animal species, and 60% of total worldwide caloric input comes from just three plants: rice, wheat and maize (*AQUASTAT - FAO's Information System on Water and Agriculture*, 2016). As climate change progresses, farmers will need to shift from crops with increasingly low yields and substitute them with other crops better adapted to the new conditions which are more nutrient dense. There is therefore an increasing trend to search for options to improve agricultural biodiversity and crop resilience (Kahane *et al.* 2013).

Neglected and underutilised crops could be a potential solution for a more a resilient and diversified agricultural system. Underutilised crops are predominantly climate resilient, but their yield and quality are compromised due to lack of selective breeding (Muthamilarasan, Singh and Prasad, 2019). The application of technologies in delineating the complex molecular machinery governing growth, development and stress responses, to gain insights through individual techniques is not sufficient. Integrating technologies however, could be an effective strategy to decipher gene functions,

genome structures, biological pathways, metabolic and regulatory networks underlying complex traits (Muthamilarasan, Singh and Prasad, 2019). Conservation and use of neglected and underutilized crop species has a value in themselves because it contributes to overall agrobiodiversity. However, its main value is in widening the genetic pool in crops that ensures adaptation to a changing environment and provides useful traits to be used in genetic breeding programs (Hajjar, Jarvis and Gemmill-Herren, 2008).

Selecting for drought related traits is difficult as the genetic basis of the trait is not clear and is controlled by many factors. With the existence of marker assisted selection, the pace of crop improvement has been boosted, especially for complex traits. In addition to this, the availability of advanced genomic and expression analysis techniques such as next generation sequencing (NGS) and microarrays has made it possible to carry out extensive gene expression analysis which provides a foundation to understand gene expression, biological responses and tolerance traits (Swamy and Kumar, 2013).

The present study aimed to investigate and evaluate the response of bambara groundnut under drought stress and to perform QTL analysis of agronomic and drought related traits for the mapped populations. Drought-related genes underlying quantitative trait loci

(QTL) involved in controlling drought traits in this study could build a foundation for marker-assisted selection (MAS) for breeding purposes.

7.2 Drought resistance in Bambara groundnut

Drought in agriculture is defined as the inadequacy of water availability (including precipitation and soil moisture storage capacity, in quantity and distribution) during the life cycle of a crop plant so that it restricts the expression of full genetic potential of the plant (Mitra, 2001). Drought resistance is ability of a crop to stay alive, grow, and produce seeds when part of its life cycle has been under stress. Three mechanisms are involved in drought resistance, namely drought escape, drought avoidance and drought tolerance (Collinson *et al.*, 1997). The ability of a plant to complete its life cycle before drought has a major impact is drought escape. Drought avoidance is when a plant shows high ability to reduce water loss by stomatal control, and maintaining a high plant water status by maximising water uptake through deep roots. Plants such as Bambara groundnut are classified as drought tolerant as they maintain positive turgor at low water potential (Alshareef, 2010). Crop adaptation to drought must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity. Although bambara groundnut is known as a drought resistant crop,

the shortage of quantitative evidence regarding the mechanism of its response means that it has not been classified into one of the drought resistance groups (escape, avoidance and tolerance) (Alshareef, 2010) and different genotypes may have different combinations of approaches, based upon the farmer selection environments.

In this study, Bambara groundnut crops were observed to be resistant to drought through avoidance and the escape mechanism. Avoidance mechanisms in Bambara groundnut was observed through stomatal closure which is the plant's first line of defence to water stress. Closure of stomata helps plants to regulate water loss through transpiration. A decline of the stomatal conductance may limit the net photosynthetic rate and water transpiration with progressive water stress, leading to increased water use efficiency because the transpiration is inhibited more than photosynthesis(Xu, Zhou and Shimizu, 2010). In the glasshouse experiment, stomatal conductance was reduced significantly with water stress (Chapter 4). Similar observations were reported by Mabhaudhi *et al*. (2011), Mabhaudhi *et al*. (2013), Vurayai *et al*. (2011), Jørgensen *et al*. (2010), Jørgensen *et al*. (2011) and Collinson *et al*. (1997). Plants that were stressed in Chapter 4 completely recovered their stomatal conductance after rewatering.

The drought escape mechanism was observed both in field and the glasshouse experiments (Chapter 3 and Chapter 4) via reduced canopy size. Reduction in canopy size (plant height, leaf number, leaf area) is directly related to biomass accumulation and yield attainment, therefore there should be a balance between minimising water losses through transpiration and biomass production which is a function of transpiration (Mabhaudhi, 2012). Shorter crop canopy duration was also observed as a part of drought escape mechanism which was due to early senescence, indicating completion of the lifecycle. Early senescence provides the crop with shorter duration of time for build-up of harvest index resulting in lower yield. However, under conditions where terminal drought is present, the drought escape mechanism will ensure attainment of some yield as opposed to no yield at all (Mabhaudhi, 2012). This is particularly important for farmers during drought where Bambara groundnut can still survive where many other crops may fail.

Drought adaptation strategies at the canopy level were complemented by loss of chlorophyll – a heat dissipation strategy associated with drought avoidance (Mabhaudhi, 2012). In this study, the plants in the field experiment (Chapter 3) showed significant losses of Chlorophyll content (SPAD readings), however the plants in the glasshouse experiment maintained SPAD measurement readings in the drought treatment (Chapter 4). Reduction or no-change in

chlorophyll contents of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration (Kyparissis *et al*., 1995). Mabhaudhi *et al*. (2013) reported that there was no significant difference in chlorophyll content between different water regimes. This shows that Bambara groundnut plants can maintain high amounts of chlorophyll content despite the development of moisture deficit stress which can be considered to be a line of defence against drought which can result in drought resistance (Vurayai *et al*., 2011).

As discussed in Chapter 6, the QTLs identified in the present study and those identified in earlier studies can be used for breeding varieties which would be tolerant to water-stress conditions. A total of four significant QTLs and 17 putative QTLs in drought treatment and one significant QTL and 13 putative QTLs in irrigated treatment was identified. QTLs for *days to flowering*, *days to podding*, *pod width*, *pod length*, *seed width*, and *single seed weight* QTLs (1 significant and 4 putative) identified only in the drought conditions, and one QTL for *leaf width* (putative) identified only under irrigated conditions were unfortunately not consistent. Therefore, their utility may be somewhat limited. However, QTLs for *double seeded pods*, biomass, shelling percentage, stomatal conductance, SPAD readings and *seed length* were identified in both treatments which were located on LG2, LG 3, LG7, LG 9, LG 10 and unmapped markers,

suggesting that they are intrinsic differences between the parents. Combining the findings in the present study with previous findings, breeding programmes may be implemented for developing drought tolerant Bambara groundnut varieties. It is possible that the unmapped markers could be linked to a linkage group if additional markers are added to the current work in the future.

The ability of Bambara groundnuts to produce reasonable yield under limited water conditions makes them suitable to be cultivated in semi-arid and marginal areas. Therefore, the results produced in this study maybe a useful selection criterion for possible drought resistance in Bambara groundnut.

7.3 Implications of study and future research

Bambara groundnut has high potential in the future due to its drought tolerance and high nutritional values in the seed. The current study that uses Bambara groundnut, a less researched crop is aimed to provide some information on drought tolerance of the given crop. A field experiment was carried out to evaluate the different genotypes under irrigated and rainfed treatments in field conditions. At the University of Nottingham, FutureCrop glasshouse were used to evaluate the effect of drought on Bambara groundnut F_3 segregating population. Thereafter, QTL analysis of agronomic and drought

related traits for the mapped populations was performed. The results obtained from this study provides a basis for further research and validation of the genotypes and lines used. The markers and QTLs identified could help in the research and development of drought tolerant cultivars of Bambara groundnut.

Although significant reduction in leaf area and leaf number was observed among all genotypes (DipC, Uniswa Red, S-19 and Gresik) that was used in the field study (Chapter 3) , the final yield produced in the rainfed treatment was comparable to the irrigated treatment in S19-3 and Gresik and higher in the case of DipC and Uniswa Red. In a study by (Jørgensen *et al.*, 2010), Uniswa Red was defined as a 'water-saver' as it maintains leaf water status to a great extent of soil water deficit which is important for survival during intermittent drought, while S19-3 was defined as a 'water-spender' with a late closure of stomata, hence a late declining of transpiration rate during soil drying allowed the landrace to maximize its water use despite giving up its leaf water relations. The study concluded that with such drought response together with a fast phenological development of S19-3, the landrace is capable of escaping from terminal drought while maximizing its water use and productivity when soil water is available. Repeating the experiment during drier months of the year can further validate the results from this experiment by leaving each genotype to finish their lifecycle completely as the crops were

harvested earlier due to rainy season. More physiology measurements are needed to be carried out to explain the variability in the yield of the genotypes studied under this field experiment.

A segregating population derived from a controlled cross hybridization between two genotypes, IITA-686 from Tanzania and LunT from Lungi, Sierra Leone was used to study the effect of drought stress in Bambara groundnut in the FutureCrop Glasshouse. A wide variation in trait values was observed between the parents (*peduncle length* and *shelling percentage*) and also within the F_3 population (*days to emergence*, *internode length*, *pod width*, *pod length*, *seed width*, *seed length*, *peduncle length*, *pod no per plant*, *pod weight per plant*, *single seed weight per plant*, biomass, harvest index and 100-seed weight). A number of lines such as L2, L34, L53, L77, L82, L121, L124, L131, L136 and L137 produced more than 20% more total seed weight under drought compared to irrigated treatment. These lines need to be further evaluated to confirm if the results obtained in this study is due to the drought tolerance mechanism in the selected lines or due to other environmental interactions. It is also suggested to use blocking in future experiments to test for differences in the glasshouse environments. Future experiments in the glasshouse should also be carried out in pots to have better

control of soil moisture levels for drought experiments, although this could effect plant strategies, such as deeper roots, to avoid drought

A complete and annotated genome sequence of the Bambara groundnut is still not available to date. Therefore, development of translational methodologies for the location of genetic components of traits and their underlying candidate genes is a priority for underutilised crop species (Ho *et al.*, 2017). In the present study, construction of a genetic linkage map using DArT markers is attempted with a F_2 population derived from a cross between IITA-686 and LunT. The number of polymorphic markers available for this study was lower when compared to Hui Hui, (2014) and Kendabie, (2014) due to the more limited genetic differences between the two parents. However, this map corresponds to the 11 chromosomes number reported by Hui Hui, (2014) and Kendabie, (2014) and were ordered from LG1 to LG11 based on previous intergrated genetic map produced by (Ho *et al.*, 2017) to facilitate the use of the map for future studies. The development and application of the DArT Seq technology provides a tool that allows comparison of results from genetic analysis in future crosses to the current work done in this study. Integrating genetic linkage maps across different segregating populations will provide a more thorough analysis of the genetic location of traits observed across multiple populations (Hui Hui, 2014).

This study is a small contribution to further evaluate and understand the drought responses in Bambara groundnut which is important for agricultural biodiversity and food security challenges. Future research should include more data on genetic molecular expression, drought prone biochemical metabolism and plant physiological processes to further understand the drought resistance mechanism in Bambara groundnut.

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