UNDERSTANDING DROUGHT TOLERANCE OF BAMBARA GROUNDNUT (*Vigna subterranea* (L.)Verdc.) IN DIFFERENT AGRO-ECOLOGIES OF NIGERIA

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ABSTRACT

Bambara groundnut (BG) (*Vigna subterranea* (L.) Verdc.) is a legume seed crop with high nutritional contents. It is a drought tolerant crop plant, and it is predominantly found in sub-Saharan Africa. Assessment of the available diversity using morphological and molecular approaches is an essential step to understand the genetics of complex traits including drought tolerance and to develop new high-yielding and climate-resilient varieties.

In the arid and semi-arid zones of Nigeria, drought is the major abiotic stress limiting crop performance. To study the response of BG to drought stress, multi-year field drought trials were conducted in research fields of Ahmadu Bello University (ABU), Mokwa, Niger state (lat. 9.345064, long. 5.031372), Ibadan, Oyo state (lat. 7.486354, long. 3.903728), and Zaria, Kaduna state (lat. 11.18656, long. 7.615368). A set of one hundred and thirty-five accessions of BG with diverse origins were established and characterized for drought under well-watered (WW) and water stressed (WS) regimes in two consecutive years 2019/2020 and 2020/2021. The experiment was laid out in an α-lattice design of 3 replicates per regime with 15 rows and 9 blocks per replicate. A 10m distance separated the regimes and WW and WS regimes were irrigated until flower emergence when water supply was withdrawn from WS plots. Twelve quantitative traits, namely: number of days to the first flower, days to 50% flowering, plant height (cm), plant spread (cm), number of petioles per plant, number of pods per plant, number of seeds per plant, seed weight per plant (g), 100 seeds weight (g), and shell weight per plant (g) were recorded using BG descriptors.

ANOVA revealed significant variations for most traits. Genotype, and treatment x genotype were highly significant ($p<0.05$). Drought stress imposed at flower emergence generally suppressed growth and yield. Consequently, significant yield reduction was observed in WS compared to WW conditions. The mean BLUPs values of seed weight accounted for 6.15g under WS and 8.49g under WW condition respectively. There was a highly significant correlation ($p<0.05$) between pod weight per plant and number of pods per plant.

A genome-wide association study (GWAS) was conducted on the same set of 135 BG accessions. The estimated best linear unbiased estimates (BLUEs) mean value was calculated for yield-traits and pod-shell trait in each location and across the locations. The BLUEs value of each accession and high-quality 5,395 genome-wide SNPs were used for marker-trait association analysis using both GLM and MLM models. A total of 15 SNPs QTL were significantly associated with five

phenotypic traits (numberof seeds per plant, number of pods per plant, seed weight per plant, shell weight per plant, and podweight per plant). The threshold of –log (p) > 5 was used to consider a significant association between SNP marker and trait. Out of 15 SNP QTL, 12 SNP QTL were common in both GLM and MLM statistical methods employed for the GWAS analysis. Six of these SNP markers were found in both WW and WS regimes. Eight unique SNP markers were found only in WS and wereabsent from WW regimes. The phenotypic variance explained (R square) by these QTLs were ranged 0.01-0.15 (GLM) and 0.10-0.14 (MLM) in GLM and MLM approaches respectively. By the MLM approach, six SNPs were associated with the number of pods per plant, five associated with number of seeds per plant, one associated with pod weight and seed weight while four were associated with shell weight.

A trimmed sequence (70-100 bp reads) of associated SNPs was blasted to Legume Information System database (https://www.legumeinfo.org) to identify candidate genes (1Mb region: 500Kb downstream and 500 Kb upstream) in reference genome of relative-legume crops cowpea (*Vigna unguiculata*) and mung bean (*Vigna radiata*). A total of 56 candidate genes were identified and they were located closely to the 15 SNPS QTLs associated with the number of pods per plant and number of seeds per plant. A candidate gene Vigun05g235600 (GTPase-activating protein 1-like) and four other candidate genes; Vigun04g138400, Vigun04g138600, Vigun04g139100, and Vigun04g139400 (receptor- like protein kinase 2) have been reported for their role under abiotic stresses including drought tolerance in field crops. A candidate gene search identified six significant candidate genes in proximity to the SNP markers associated with the number of pods per plant and number of seeds per plant; four SNPs were left for shell weight in the MLM. Seven SNPs QTL of total 15 QTLs showed pleiotropic effects and were associated with both pods per plant and seeds per plant traits. Two pairs of SNP markers were exhibiting pleiotropic effects on seeds per plant and shell weight. This is the first GWAS study reported for yield related traits under WS in BG. The associated SNPs could help to understand the genetic yield traits in BG for future genomic-assisted breeding research. The outstanding accessions under WS conditions could serve as parent lines for drought improvement while the significant positive correlations between pairs of the traits suggests the possibility of simultaneous improvement of the traits to enhance seed yield.

SNPs- Single nucleotide polymorphism, QTL- Quantitative trait loci, GWAS- genome wide association study

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And to the Lord, all knowing, most-high, my sustainer, my provider, inspiration, and source of strength, wisdom, knowledge and understanding and for always being right there for constant protection I received through thick and thin, my life owes you my praise.

DEDICATION

This work is dedicated to the Lord, my late parents, and my siblings.

CERTIFICATION

I certify that this work was carried out by Faloye Benjamin Busuyi under my supervision in the school of Biosciences, University of Nottingham, Malaysia.

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Primary Supervisor Festo Massawe (PhD.)

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Sean Mayes (PhD.)

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LIST OF ACCRONYMS

AfCFTA- African Continental Free Trade Area

BG- Bambara groundnut

BLUES- Best Linear Unbiased Estimation

BLUPS- Best Linear Unbiased Prediction

CE- Central Africa

CFFRC- Crops For the Future Research Center

CGIAR- Consultative Group for International Agricultural Research

DArTSeq- Diversity Array Technology Sequence

DNA- Di-ribonuclease Acids

EA- East Africa

EC- Emulsifiable Concentrate

GDP- Gross Domestic Product

GWAS- Genome Wide Association Studies

IBPGR- International Board for Plant Genetic Resources

IITA- International Institute of Tropical Agriculture

MAF- Minor Allele Frequency

NAERLS- National Agricultural Extension and Research Liaison Services.

NPK- Nitrogen Phosphorus Potassium

NPAFS- National Program for Agriculture and Food Security

PIC – Polymorphic Information Content

RH- Relative Humidity

RWC- Relative Water Content

SE- South-East

SNPs- Single Nucleotides Polymorphisms

SSA- sub-Sahara Africa

UK- United Kingdom

WA- West Africa

WS- Water stressed.

WW- Well watered.

CHAPTER ONE

1.0. INTRODUCTION

The world population is projected to reach 9.8 billion in 2050 (Dorling, 2021). In Africa, high population growth coupled with increased demand for food in the era of climate change will make it difficult for the continent to feed her people (Jat *et al*., 2016). Current global food supplies from major crops could not cater for all calorie and nutrient needs in sub-Saharan Africa as population increase and climate change challenge food security across the world (Ebi *et al.,* 2021). Population explosion on the other hand is a major threat to food security in sub-Sahara Africa. Most of the countries in SSA are characterized by rapid population growth of more than 2% annually (Eze *et al.,* 2012). Unfortunately, food production does not keep pace with this increase, resulting in nutrition challenges, diseases, famine, economic instability, migration, and social unrest (Buhaug *et al*., 2015). Nigeria population as a case study is growing rapidly, demanding for more imported food stuff for sustenance, which invariably affecting the country gross domestic product (GDP) (Apu *et al*, 2018; Ashaye *et al* 2017). Agricultural practices in most countries of Africa are not sustainable to provide food for her teeming population despite the years of persistent effort to improve the production and quality of food resources, food insecurity still exists,(Wudil *et al*., 2022). Gradual loss of crop diversity, neglect of underutilized but nutritious crops, reliance on few primary crops for food sustenance, degraded soil nutrient, erratic rainfall pattern and high temperature put risk to food production and nutrition.

The risk associated with our reliance on few major crops, especially in Africa where most countries depend on food imports and supplies and price shocks may result to diverse future food challenges. Diversifying our food sources beyond the major species, inclusion of the indigenous underutilized crop species and strengthening local food supplies, upscaling of modern technologies such as conservation and climate smart agriculture, developing multiple stress-tolerant crop cultivars and biotypes through biotechnological tools, restoration of degraded soils and waters and conservation of biodiversity are the available solutions that must be promoted at regional and country level to ensure continuous food and nutritional security.(Adelabu *et al.,* 2023).

Climate change is defined by the United Nations (https://www.un.org) as a long-term shift in temperatures and weather patterns. Human activities such as increase in gas emission, land overuse, urbanization have

been highlighted as the major driver of climate change and the activities has resulted to several negative implications such as alteration of rainfall pattern, drought, rapid changes in temperature, outbreak of new pests and diseases, altered yield of crops and reduction in quality and quantity of agricultural produce, all these has put serious challenges to crop yield and shortfalls in global food security. Drought stress alone has a detrimental influence on crop performance and has been described as the most damaging climate hazard impacting two-thirds of the global population threatening food security (Agyeman *et al*., 2023). On a global scale, Fuhrer (2003), Jones and Thornton (2003), and Araya *et al*., 2015) predicted that the future impact of climate change on global agricultural systems might bring about climate variability, shifting temperatures, and precipitation patterns. Declines in soil nutrients and soil moisture combined with a shift in pests and diseases, and loss of water from the soil due to a rise in atmospheric temperature are not exemptions. These may have a significant detrimental impact on crop survival, crop performance and yield and ultimately affect food systems and nutritional security. With all the above challenges, staple crops may be affected in many situations, which may result in increased hunger, malnutrition, and increase in the prevalence of diseases. Climate change will impact biotic and abiotic factors in crop systems threatening food sustainability (Adrian *et al*., 2011).

Recent research revealed that global food production and crop yields are already being impacted by climate change (Ray *et al*., 2019). Shifts in rainfall patterns and their frequency coupled with a temperature rise will accelerate more changes that are difficult to predict and affect crop performance and quality in the foreseeable future (Adrian *et al*., 2011). As arid and semi-arid zones in sub-Sahara Africa (SSA) experience extended drought, intermittent rainfall, salinity, and high temperature, all these could result in low yields and crop failures (Kazi *et al*., 2015; Ofori *et al*., 2021). Besides, drought stress occurs at different stages of plant growth and development resulting to a substantial loss of reproductive apparatus such as flower abortion and pollen sterility inhibits pollen viability, pollen germination on the stigma, and pollen tube growth leading to poor seed set and yield loss (Guo *et al*., 2023).

An urgent intervention for crop diversification is therefore imperative, to address over-dependence on a few major food crops and the need to tap into the abundant germplasm of various underutilized food legumes found in ex-situ conservation in both national and international genebanks for the provision of more nutritious foods (Adelabu *et al.,* 2023). All effort should be made to improve the locally grown food crops with proven record of resilience, adaptation, short maturity period, and high nutritional potential to

increase her food basket. Crop diversification and inclusion of new crops may be an appropriate response towards food security in many circumstances, particularly considering climate change, population growth and intermittent drought's effects on food production (Selena *et al*., 2016), as well as the extent of micronutrient deficiencies. Pellegrini *et al*., 2014; Akinola *et al.,* 2020 suggested that crop diversification could play a pivotal role by bridging the gaps of failure of major crops to produce yields under arid condition, it can also mitigate the worst effect of economic loss of significant crops, helping to satisfy the nutritional needs of rural dwellers and enhance small holders farming systems.

Many useful crops with high nutritional value are neglected, possibly due to a lack of information about their nutritional value or their low yield under varying climatic conditions. Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) is a distinct legume crop among other underutilized (leguminous) crops with nutritional potential which is fit to address the present and future food and nutrition challenges. It can be included in the major staples (Brought Azam Ali 1992). Bambara Groundnut is a hardy leguminous subterranean food crop cultivated for its nutritious seeds. It has different name tags which describe its potential as nutritious food crop; It is referred to as "complete food" due to the nutrients derived from the seed (Akpapunam *et al*., 1994). It is also referred to as a drought tolerance plant as it thrives and produces some yield in conditions too arid for other crops, (Mkandawire 2007, Enwere *et al*., 1996). It is referred to as a "future crop" due to its resilience and ability to produce some yields in a condition too arid for other crops. Bambara groundnut can contribute to the food and nutrition requirements of low-input farmers found across sub-Saharan African countries. Based on various importance of Bambara Groundnut nutritional value, it can be useful as a food security crop (Brough *et al*., 1992). Also, it has a production advantage, and can fill the gap in conservation agriculture (Hobbs et al 2008). Bambara Groundnut is one of the most economically important underutilized food legumes and is a rich source of proteins. It is cultivated in arid regions in sub-Saharan African countries and its cultivation has extended to Africa, Asia, and other parts of the world. Abiotic stress is a common and major limiting production factors. Heat stress, high temperature and low rainfall, low soil fertility and lack of minimum inputs etc., have led to a considerable yield loss and crop neglect.

Although, a report has shown that Nigeria is the highest producer of Bambara groundnut in Africa (Hillocks *et al*., 2012), report on grain yield per hectare of Bambara groundnut and most underutilized legume food crops are scanty. This is not far-fetched as most of these crops serves as companion crops to

the major crops, records of sole cropping in large hectarages of land and commensurate grain yield are very scarce. Most models are unreliable as they are based on farmer's mixed farming systems, this leads to ambiguity and bias in projecting grain yield of underutilized crops in such instances. Low yield of underutilized legumes including Bambara groundnut is a major challenge and in part, could be a possible reason for the neglect. In comparison to other major leguminous crops, Bambara groundnut yield per hectare is far from major crop yield. (Stagnari *et al.*, 2017). Collinson *et al.*, 2000 report that Bambara yield varied from 68.5 to 159.9 Kg ha^{-1}. Azam Ali *et al*., 2001 also report the yield range of Bambara to be between 0.5 and 3 tons per hectare in sub-Saharan Africa. The lack of improved Bambara varieties could also be an important factor for its low yield because, to date, no improved variety bred for abiotic stress conditions like other major crops, rather, various landraces are cultivated across all the growing regions of Nigeria and in sub-Sahara Africa. It is therefore imperative to do more studies on Bambara groundnut that will improve grain yield and tolerate drought effect rather than increasing cultivated land area to cater for present and foreseeing future food shortages in sub-Sahara Africa**.**

1.1 Abiotic stress factors on crop performance

Legume plant faces drought stress at different stages of growth and development resulting to substantial loss of reproductive apparatus such as flower abortion and pollen sterility inhibits pollen viability, pollen germination on the stigma, and pollen tube growth leading to poor seed set and yield loss (Guo *et al*., 2023). Although Bambara is a drought tolerance crop, drought stress at the vegetative stage and high-temperature stress during the reproductive and pod- filling stage are notable challenges which may cause considerable yield loss of Bambara groundnut. In Nigeria, terminal drought is very common in the areas of legume production which incapacitates legume production at its maximum. Drought tolerance and early varieties of Bambara groundnut that can circumvent the incident of drought and inconsistent rainfall are lacking but could help in drought circumstances. Despite being a hardy crop that produces some yields in an arid environment, extreme temperature and inconsistent rainfall are the basic cultivation challenges which incapacitate legume production at its maximum. These challenges are also expected to increase in near future owing to climate change thereby necessitating the development of stress-tolerant and climate-resilient varieties from its germplasm which can circumvent the incident of drought and inconsistent rainfall, heat, and low rainfall conditions. Moreover, this study seeks to understand the performance of various genotypes in Nigeria soils also determine the traits that confer drought tolerance in Bambara groundnut using both multi-location trialing and molecular approaches.

Genetic diversity, multi-locational trialing, and Genome Wide Association Study (GWAS) are the different approaches to be employed in this study for the determination of tolerance Bambara groundnut towards its yield improvement. A broad understanding of landraces' performance and responses under well-watered and water-stressed conditions could thereby open opportunities to develop stress tolerance Bambara groundnut. Therefore, this study is necessary for Bambara groundnut landraces improvement, their response under diverse environments especially in Nigerian soils where it is being cultivated is pertinent to its breeding.

1.2. Nigeria Population in the Africa Continent and future food security

The continent of Africa is categorized into North, West, Central, East, and South regions. Amongst the five categories, West Africa is the region with the highest number of countries and the most populous region with over 350 million people in 2015 (Ogunleye *et al*, 2018). Nigeria alone accounts for more than half of West Africa's population (over 200 million). The United Nations projects that the overall population of Nigeria will reach about 401.31 million by the end of the year 2050 (Ogunleye *et al*, 2018). Therefore, Nigeria is a country with the highest population out of the 54 countries in Africa and the 7th most populous country in the world in 2017. (Ibrahim *et al.,* 2023). This implies that more food is needed to cope with the growing population of African countries and most especially Nigeria.

The diets of resource poor people living mostly in the arid and semi-arid zones are based exclusively on cheap carbohydrates from rice (*Oryza sativa*), wheat (*Triticum aestivum*), or maize (*Zea mays*) resulting in stunted growth (Hefferon, 2015). Three major crop groups have supplied human foods in Nigeria and sub-Saharan Africa: legumes, roots and tubers, and cereals (Achike, 2004). From these groups, two or three crops have been selected with total reliance as sources of diet and income (NAERLS, 2010; NPAFS, 2010). Legume- (Cowpea (*Vigna unguiculata*), Groundnut (*Arachis hypogaea*) and Soybean (Glycine max.). Root and tuber (Cassava (*Manihot spp.*), Yam (*Dioscorea spp.*), and Cocoyam (*Colocasia spp*.) Cereals (Maize, Rice, and Sorghum). Meanwhile, many other useful crops with high nutritional value which could support sustainable agricultural productivity for climate change adaptation are neglected possibly due to low awareness of their potential or lack of information about them. The reliance on just a few staples could negatively affect food and nutritional security in Africa and the world at large. Countries have employed various means to ensure food security and attain food sufficiency for the ever-growing population. Karnataka state in India, for example, has employed crop diversification to maximize the use

of arable lands to grow diverse crops which can also address food requirements (Acharya *et al*., 2012.). The reliance on just a few staples with apparent negative effects on food and nutritional security in sub-Saharan Africa has generated a desire by various researchers to assess the potential of other crops to produce foods in a changing world. These steps can be a right step towards food security in Africa. (Mayes *et al*., 2012 ; Minde *et al*., 2021 ; Mustafa *et al*., 2019 ; Muhammad *et al*., 2020). Nonetheless, the cultivation of limited crop species with high-input and high-yielding characteristics embraced by modern agriculture has resulted in a decline in crop diversity and cultivation of traditional crop species. Traditional crop species and their peculiar mode of cultivation, information about means of preparation and their usage resides with the resource-limited farmers and rural communities. (Mubaiwa *et al*., 2018), therefore climate change and high population growth which significantly increasing the world's food demand can be improved by adaptation agriculture and inclusion of climate smart crops into the food basket.

1.3. Nature of Nigerian agroecology and legume production

Nigeria occupies a land mass of 923,768km^2 (NAERLS, 2010). Agriculture is the mainstay of her economy accounting for more than 45% of the gross domestic product (GDP) and employing more than half of the workforce. In Nigeria, agriculture is mainly in crop production, fishery, forestry, and livestock. Crop production is the largest segment of all, and it accounts for 87.6% of the total sector's output. (AfCFTA, 2020). There are six major agroecological zones in Nigeria-Mangrove Swamp, Rainforest, Derived savanna, Guinea savanna, Sudan savanna, and Sahel savanna zones. Differences in precipitation patterns make them unique in characteristics and that explains the farming and crops that survive in each agroecology. The main staple crops in Nigeria are cassava, yam, maize, sorghum, rice, and millet. These crops together cover 65% of the total cultivated area. Cassava, yam, cocoyam, and maize are the major staple crops in the humid area of the country, whereas maize, sorghum, millet, cowpea, and groundnut are the major staple crops in the sub- humid and semi-arid areas. Rainfall is bimodal in the Mangrove and Rainforest zone and parts of the derived savanna zones and unimodal in the Guinea, Sudan, and Sahel savannas. Annual rainfall varies from as low as 500 mm per year in the Sahel savanna zone to about 3000 mm in the mangrove swamp zone (NPAFS, 2010, NAERLS, 2010). The country has an agricultural land area of about 84 million hectares, of which 33 million hectares are currently under cultivation. About 3 million hectares of arable land are irrigable and only about 220,000 hectares are irrigated (NPAFS, 2010). Farming in Nigeria is primarily done in small scale with most farmers cultivating less than 1 hectares of land. Aworth.,2015 described a smallholder farmer as one with less than 10 hectares farmland. 80% of

Nigeria farmers are small scale farmers (Akinsuyi., 2011). There is limited access to modern improved technologies such as mechanization and inputs (Aworth.,2015).

1.4. Advantages of legume crops.

Grain legume crops represent an important component of agricultural food crops consumed globally. It ranks second to cereals in production and accounts for 27% of the world's food production and supplies 33% of protein needs (Metwally *et al*., 2019). The importance of legumes in Africa cannot be over emphasize, various traditional foods are made from legumes, they can be milled into flour, used to make various products such as chips, bread, doughnuts, bread spreads, Pop beans (Popenoe et al., 1989; Kindscher, 1992), and soybean candy (Genta et al., 2002). They are sources of proteins, essential micronutrients, energy, and vitamins for human.(Khatun *et al.*, 2021). It is grown majorly by small holders' farmers and its production requires low inputs. They complement cereals, roots and tuber crops which are the primary sources of carbohydrates. Grain legumes stand as a popular choice for farming in intensive cereal-based cropping systems due to their potential to survive in low fertile soils and their ability to fix atmospheric nitrogen. Effects of climate change including drought and high temperature are the major challenges to legume production and productivity. Increase in temperature alone can alter crop growing cycle, increase crop respiration rates, increase evapotranspiration, and affect the crop survival (Jat *et al*., 2016).

1.5. Roles of legumes in biological nitrogen fixation (BNF)

The biological nitrogen fixation process is one of the important ecosystem services that plays a crucial role in agricultural systems. It increases grain legume productivity and helps to improve the carbon and nitrogen content of soils (Houlton *et al.,* 2019). Biological nitrogen fixation is essential majorly in smallholder farming systems where access to nitrogen fertilizer is limited due to its high cost and inadequate supply. Because of their importance in soil health and soil deficiency improvement, they are considered as engines of sustainable farming as they intensify the productivity and interaction of the soil, crop, livestock, people, and other components. (Alliot *et al.,* 2015). Moreover, low inputs requirements of grain legumes for survival, capacity to withstand abiotic stress, and their characteristics deep root systems (Kumar *et al.,* 2018), are considered a vital crop for achieving food sustainability and nutritional security in developing countries. However, despite the diverse importance of grain legumes, their productivity is very low and far below the potential production of the legume species (Yirga *et al.,* 2019). Low

productivity is often associated with declining soil fertility of the farmland and reduced nitrogen-fixation. Their yield can be improved through inoculation of adaptable effective rhizobia (Desta *et al.,* 2015). Although, inoculating legumes with rhizobia has yielded many good results in terms of a substantial increases in nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels, but specificity exists between rhizobia strain and the legume variety, and compatibility between the two is essential for successful nodulation and nitrogen fixation (Emam and Rady, 2014).

1.6. Biological nitrogen fixation in agriculture

The process of turning atmospheric nitrogen into ammonia (NH3) via the enzyme nitrogenase is known as biological nitrogen fixation (BNF). It occurs in practically all ecosystems and contributes around 1.4 x 10^{11} kg nitrogen per annum to terrestrial systems, with natural ecosystems receiving about 76% of the nitrogen and agroecosystems receiving the remaining 22% (Galloway *et al.,* 2004). Biological nitrogen makes up around 80% of the earth's atmosphere (Abd-Alla *et al.,* 2014), however, most species cannot use it until it is converted, either through industrial processes or biologically by bacteria. -About 25% of the world's fixed nitrogen production comes from chemical fertilizer, while 60% comes from biological nitrogen fixation (Zahran, 1999), an important component of plants. Nitrogen is also one of the main factors in the production of food around the world. The production of the world's food, animal feed, and industrial products requires between 150 and 200 million tons of mineral nitrogen annually from plants in agricultural systems (Unkovich *et al.,* 2008). Nearly 100 million tons of nitrogen are fixed annually by the commercial Haber Bosch process to satisfy those needs. It is thought that biological nitrogen fixation is more environmentally friendly than industrial nitrogen because Nitrogenous fertilizers have high levels of pollution of water bodies. (Al-Sherif,1998).–Biological nitrogen fixation is an efficient source of nitrogen for low inputs and resource poor farmers. It plays a key role in sustainable grain legumes production. Approximately 2 tons of industrially fixed nitrogen is needed as fertilizer for crop production to equal the effects of 1 ton of nitrogen biologically fixed (Abd-Alla *et al.,* 2014). The developing countries are faced with high cost of fertilizer and the limited market infrastructure for farm inputs, efforts have been directed towards the integration of BNF of legumes in soil nutrient management, in which legumes play a crucial role (Chianu *et al.*, 2008; Giller *et al.,* 2019). More focus has been given to the symbiotic associations as they have the greatest quantitative impact on the nitrogen cycle.

Legumes are very important both ecologically and agriculturally because they are responsible for a

substantial part of the global flux of N2 to fixed forms. The interaction between legume and rhizobia leads to the development of nitrogen fixing symbiosis (Ohyama *et al.,* 2009). The legume-rhizobia symbiosis is the single most important source of biologically fixed nitrogen in agricultural systems (Cullis *et al.,*2017). A significant proportion of the total nitrogen added to agricultural land can be attributed to the legume-rhizobia symbiosis (Sprent, 2001). Generally, the relation between rhizobia and legume is a selective one, each rhizobia species has a distinct host range which allows nodule formation with a particular set of leguminous species, and each species form nodules only with a certain range of rhizobia. Nevertheless, a complex association between legume and rhizobia has been found and many leguminous species can form nodules with distinctive rhizobia species in different geographical regions (Han *et al.,*2005: Tong *et al.,* 2018). For a successful infection, a compatible pairing of legume cultivar and rhizobia strain is required. The symbioses between rhizobia and legume plants are mainly a mutualistic interaction (Lindström and Mousavi, 2010).

1.7. Factors affecting symbiotic nitrogen fixation.

Symbiotic nitrogen fixation efficiency depends on rhizobia strain, plant host, environmental factors, soil, and their interaction. Several biotic and abiotic factors affect symbiotic nitrogen fixation. The most important abiotic factors include drought, salinity, waterlogging, temperature, soil acidity, inadequate mineral nutrition, and mineral toxicities (Zahran, 1999; Abdel-Latef and Ahmed, 2015). These factors can prevent the survival of rhizobia in the soil, the infection process, nodule growth and nodule functioning and symbiotic nitrogen fixation (Serrage and Adu-Gyamfi, 2004). It is estimated that crops grown on 90 percent of arable lands experience one or more environmental stress (Abdel-Latef and Ahmed, 2015). All are interconnected in the control of N2 fixation, hence none of them could be considered in isolation. It is also difficult to isolate the effects of such factors on the success of inoculation from their effects on symbiosis functioning and nitrogen-fixation. Rhizobia populations vary in their tolerance ability to major environmental factors (Biswas *et al.,* 2008). The existence of genetic variability in tolerance to most stress factors has been shown in both legume host plants and their respective rhizobia strain (Hungria and Vargas, 2000). This suggests the possibility of overcoming the environmental constraints limiting symbiotic nitrogen fixation potential. Optimal performance of the nitrogen fixing symbiosis depends upon selection of both symbiotic partners for adaptation to the target environment (Sessitsch *et al.,* 2002). The symbiotic nitrogen fixation process also depends on the occurrence and survival of rhizobium in soils and their efficiency (Adamovich and Klasens, 2001).

1.8. The importance of primary nitrogen fixation in nodules in the productivity of legumes in Africa

One of the major drawbacks and limitations that continues to challenge food security is a decline in soil nutrient status. This has hindered food production in different capacities across African countries (Sanginga *et al.,* 2003). In East African countries especially Kenya, Ethiopia, Rwanda, Malawi, Zimbabwe, it is projected that yearly depletion of soil nutrient especially, soil organic carbon is at high rate, exceeds 30 kg/ha of N and 20 kg/ha of potassium for arable land, (Smaling 1993, Rubio *et al*., 2021). The consistent and striking rise in cereals yield in the developed part of the world is directly attributable to a triple fold increase in the use of Nitrogen fertilizer. The lesson learnt from the "Green Revolution" has been that the world staples were bred to respond favorably to high N2 fertilization rates. A typical instance of fertilizer use in the developed country has allowed cereal yield of 7 tons/ha^{-1} by the application of 200 to 300 kg N'ha^{-1} (Bockman *et at*., 1990; Waggoner, 1994). The side effects of such high N2 fertilizer application have resulted in volatilization of N2 oxides (greenhouse gases) into the atmosphere, depletion of non-renewable resources, an imbalance in the global N2 cycle, and leaching of NO-3 into groundwater (Kinzig and Socolow, 1994), soil acidity. Continuous use of N2 fertilizer has a negative impact leading to more than 1.5 billion ha of land around the world acidic. Hence, inorganic Nitrogen fertilizer use and its side effect to agricultural land is likely to increase in the developed countries if not checkmate as the result of acid rain, long-term N fertilization, and natural weathering (Graham and Vance, 2000).

By contrast, in developing countries the high cost of N2 fertilizer, the energy requirements for production, and the sub-optimal transportation capabilities limit its use, especially for small farms. The projected population explosion will require more foods to meet the caloric and nutritional needs of people. This will necessitate unprecedented increases in crop production. Nitrogen is the major limiting nutrient for most crop species. Acquisition and assimilation of N2 is second in importance only to photosynthesis for plant growth and development (Newbould, 1989). Production of high-quality, protein-rich food is extremely dependent upon the availability of necessary N2. Generally, nutrient depletion of soil is a peculiar problem for small holder farmers in developing countries, where much grain-legume production occurs, and many farmers cannot afford to use fertilizers.

Therefore, in meeting the food requirements of the present without compromising the needs of the future food security, agricultural sustainability will require the substitution of Nitrogen fertilizer with biologically fixed N2, this may enhance safe farming practices that are economically viable and a guaranteed healthy environment (Bohlool *et at.,*1992; Vance and Graham, 1995). Sustainable agriculture is broadly defined as agriculture that is managed toward greater resource efficiency and conservation while maintaining an environment favorable for evolution of all species (Bohlool *et at.,*1992; Golley *et at.,*1992). Therefore, the use of N2-fixing species in cropping systems reduces the need for N2 fertilizers and improves soil fertility. Additionally, biologically fixed N2 is bound in soil organic matter and thus is much less susceptible to soil chemical transformations and physical factors that lead to volatilization and leaching. Apart from the nutritional potential of legumes in food security, its advantages in the cropping system are numerous in terms of direct nitrogen transfer, residual fixed nitrogen, nutrient availability and uptake, effect on soil properties, breaking of pests' cycles, protein rich forage for livestock, inoculation with effective rhizobia, and the use of appropriate agronomic practices and cropping systems.

1.9. Effects of drought on nodule function

Drought is one of the most important constraints limiting crop production globally and prevents crop yield potential in cereals and legumes (Lesk *et al*. 2016). This problem will persist and will continue to increase in frequency and severity because of climate change (Schwalm *et al.* 2017). Under drought conditions, decreased root lengths and dry biomass accumulation have been reported in many soybean genotypes (Thu *et al.,* 2014). Drought not only changes root physiological features such as root depth, root branching density, and root angle but also partitioning of root to shoot biomass with an increase in root mass (Franco *et al.,* 2011; Fenta *et al.,* 2014). Several studies have also provided strong evidence that nodule numbers are only decreased when legume plants are subjected to severe drought conditions (Fernandez Luquen *et al.,* 2008; Márquez-García *et al.,* 2015). Nodule drought tolerance has been linked to the ability to sustain a supply of photosynthate to the nodules during drought and to greater nodule biomass (King and Purcell, 2001). The symbiotic interaction between legume and rhizobia facilitates atmospheric nitrogen fixation, a process that provides essential nitrogen to support plant growth and development. Symbiotic nitrogen fixation is important for sustainable agriculture, as it sustains plant growth on nitrogen-poor soils and limits fertilizer use.

1.9.1. Problem statement

Various constraints such as low soil fertility, intermittent drought, and insufficient agricultural inputs are some of the challenges faced by small scale farmers, especially in the semi-arid and arid regions of the world. Drought stress especially, water deficit amongst all various challenges is cited as a major issue causing significant yield loss to legume production especially in SSA because they are typically grown under rain fed conditions which make production vulnerable to drought effects (Tomer *et al*., 2020).
At any growth stage of legumes starting from germination to economic yield stage, drought stress causes varying levels of yield loss (Lu *et al*.,2021). Due to climate change, drought's severity has been predicted to increase in the foreseeable future leading to significant negative impacts on the livelihood of rural farmers affecting yield quality and quantity leading to food insecurity.

There is need for agricultural diversification and the inclusion of underutilized crops found in conservation in various genebanks across the world. A good example of such an important underutilized legume crop is Bambara groundnut (*Vigna subterranea*). It is a drought tolerance legume with high nutritional potential. It has been neglected by farmers due to low yield, sketchy information about its economic value and other inherent potential has not encouraged its commercial cultivation, low research attention and no breeding program like the primary legume crops such as soybean (*Glycine max*) and groundnut (*Arachis hypogaea*) has ever existed.

National, regional, and international genebanks across the countries of the world is playing major roles in not only conserving diverse seed crops for breeders, researchers, and farmers but also, their functions in the use of plant genetic resources (PGR) to address food security challenges under the context of climate change, biotic and abiotic stress mitigating agricultural systems cannot be over emphasized. Continuous exploration mission by the genebanks for germplasm collection and ex situ conservation and use in perpetuity of PGR with the financial supported by the Food and Agriculture Organization of the United Nations (FAO) and the Ford and Rockefeller Foundations has served as major intervention and has safeguarded diverse landraces and crop wild relatives' extinction due to war, industrialization, threatened habitat, and natural disasters. (Smale *et al.,* 2020). The international genebanks managed by the consultative groups on the international agricultural research (CGIAR) currently manage over 773,000 accessions PGR consists of cultivated and crop wild relatives that are treasure trove to achieving global food and nutrition security. (Crop Trust 2015). At IITA, over 2000 (Two thousand) accessions of Bambara

groundnut are conserved ex situ. Although the huge accessions of this crop can contain high diversity which could be useful for its improvement, the huge accessions could also remain under-exploited due to huge number of accession germplasm. Definition and characterization of each accession of the germplasm is very important to understand their inherent potential.

Advances in the molecular biology and the application of next generation genomic sequencing has revolutionized the science world by providing tools that help to cost effectively study genetic diversity, identify desirable genes and alleles as well as facilitating their transfer during crop improvement, thus reducing the time to deliver new varieties. Unfortunately, various underutilized legume crops(such as Bambara groundnut) conserved in various genebanks including IITA are lagging in the advances in molecular characterization especially in the use of genome wide association study (GWAS) compared with other primary and well-known food crops. Bambara groundnut yield varies from genotype to genotype. In-depth research is required on the genotypic response to drought and grain production performance of several Bambara groundnut genotypes in various agroecology. Different Bambara groundnut genotypes may have different tolerance levels to drought, and drought severity varies from one environment to the other. This study seeks to understand the drought tolerance accessions amongst the 135 accessions with the application of molecular techniques for the screening and to know the traits that confers drought. Identification of Bambara groundnut with drought tolerance characteristics with potential to produce yields under limited rainfall conditions will be an advantage for the breeders and researchers.

1.9.2. Aim of this study

This study seeks to investigate the genotypic differences in crop response to water stress at different locations, differences in drought tolerance, and some indications of the genetic basis of differences in drought response of 135 different Bambara groundnut genotypes. The investigation includes multi-location drought experiments carried out in two consecutive years in an open field at different agroecological zones in Nigeria. Additionally, the application of Genome-Wide Association Studies (GWAS) to identify associations between observed phenotypic differences in the 135 genotypes and the underlying genomic variations.

1.9.3. The objectives of this study are to:

a. Identify drought tolerance genotypes and the traits that confer drought tolerance using a diverse set of Bambara groundnut accessions collected from different countries.

b. Identify polymorphisms at the DNA level that may be associated with drought tolerance using Diversity Array technology sequencing (DArtSeq) and Genome-wide association studies (GWAS)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bambara groundnut (*Vigna subterranea* (L.) Verdc.).

The Bambara groundnut is a small annual herb with a trailing stem containing only one species which belongs to the Fabaceae family. Fabaceae contains over 20,000 flowering plants with fruits or pods and whose roots fix atmospheric nitrogen into the soil in association with soil-borne rhizobia. Bambara groundnut has two botanical forms:

i. Vigna *subterranea* var. *subterranean*

ii. Vigna *subterranea* var. *spontanea.*

The former is the cultivated variety while the latter is the crop's wild relative. (Basu *et al*., 2007). Wild relatives of cultivated Bambara groundnut have only been found in northeastern Nigeria and northern Cameroon and it is believed that the crop originated from this part of the continent (Olukolu *et al.,* 2012). The wild form *spontaneous* (Harms) Hepper has smaller seeds and slender stems as compared to the cultivated forms. They both have chromosome number 2n = 2x = 22. (Khan *et al.,* 2020). Electrophoretic studies carried out by Howell (1990), did not show a significant difference between the cultivated genotypes and the supposedly wild forms, and it was concluded that the wild plant might simply be an escape from the cultivated ecotype. The cultivated type *Vigna subterranea* var *subterranea* has pod lengths ranging between 1-2.2 cm long and 1.2-1.8 cm wide. Bambara seeds do not require any pre-treatment such as scarification before germination as some legume seeds do but sufficient soil moisture can enhance even germination during crop establishment (Naik *et al.,* 2021). Under normal seed and soil moisture conditions, it takes Bambara groundnut seeds 6 to 14 days to sprout, the flower emerges between 30 and 35 days after seedling emergence, and the pod ripens between 90 and 150 days depending on genotypes. Morphological features of the Bambara plant and pod are like peanuts (*Arachis hypogaea*). Bambara genotypes have geotropic pod development, most genotypes bear their pods underneath the soil and some genotypes bear their pods on the soil surface. One to two seeds per pod are common in Bambara groundnut but some genotypes also bear four seeds per pod.

2.2 Morphological description of Bambara groundnut plant and ripe pod

Bambara groundnut is an herbaceous, hardy, and leguminous annual crop cultivated for its nutritious subterranean pods. Morphologically, it has creeping stems at ground level (Smýkal *et al*., 2015). It is a

predominantly self-pollinated crop (Ahmad, 2013). Differences in the length of internodes result in bunched, intermediate (semi-bunched), and spreading types. The common appearance of the plant is bunched leaves arising from a branched stem (Massawe *et al*., 2016, Abu *et al*., 2011), which forms a crown on the soil surface. Stem branching begins very early, about one week after germination, and as many as 20 branches may be produced. Each branch is made up of internodes, and those near the base are shorter than the more distant ones. The roots form nodules for nitrogen fixation, in association with appropriate rhizobia (Majola *et al.,* 2021). Leaf and flower buds arise alternately at each node. Leaves are pinnately trifoliate, glabrous with erect petiole, thickened at the base.

The terminal leaflet of Bambara groundnut is slightly larger than the lateral leaflets, with an average length of 6 cm and an average width of 3 cm. The flowers are borne on hairy peduncles, which arise from the nodes of the stems. Usually, two flowers are attached to the peduncle by pedicels. Good knowledge of the flower structure is essential for breeding the crop and vital observations such as leaf size, days to flowering, and their response to drought conditions. Variation among landraces has been reported by Massawe *et al*., 2003; Mwale *et al*., 2000, and Smýkal *et al*., (2015). The interval between the openings of successive flowers in a raceme varies from 24 to 48 hours; that of flowers on the same peduncle does not exceed 24 hours, but rarely do they open at the same time (Kinet, 2018). When flowers open during the early hours of the morning, they are yellowish white, but yellowish brown in the evening and are light brown towards the end of the plant's life cycle.

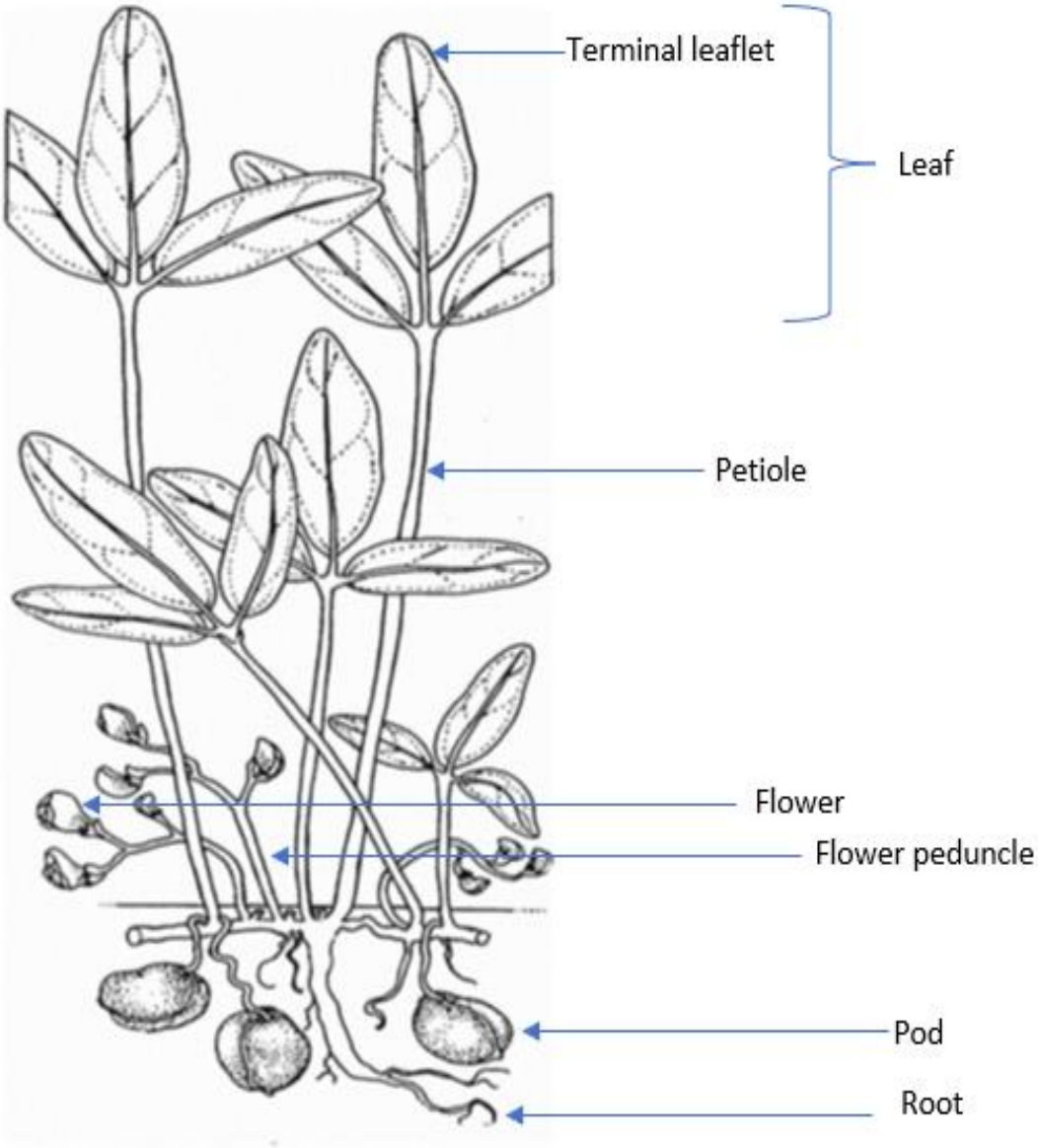


Figure 2. 1. The morphology of Bambara groundnut plant. (National Research Council, 2006).

2.3 Bambara groundnut botanical classification

Sub-kingdom- Tracheobionta

Super division-Spermatophyta

Division- Magnoliophyta

Class- Magnoliopsida

Sub-class- Rosidae

Order- Fabales

Family- Fabaceae

Sub-family- Papilionoidae

Tribe- Phaseplae

Sub-tribe- Phaseolinae

Genus- Vigna

Species- subterranea (L. Verdc.) Source Goli *et al*., (2015)



(Photo taken by Ben.)

Morphology of Bambara groundnut ripe pods.

2.4. Bambara groundnut nutritional value

Bambara groundnut beans are rich sources of protein (Tan *et al,* 2020) and can be used as a partial substitute for meat (Venter and Coertze 1997). Bambara importance as a potential nutritious food crop is growing especially in the rural areas of sub-Saharan Africa (Vurayai *et al*., 2012). Mature Bambara groundnut seeds contain 18-24% protein (Mayes *et al*., 2019, Paliwal *et al.,* 2020), 51-70% carbohydrate (Halimi *et al*., 2019) and 7.3 - 8.5 fat (Ominawo *et al*., 1999). The protein in Bambara groundnut has high lysine content (Adu-Dapaah and Sangwan, 2004) and has a beneficial complementary effect when consumed together with cereals that have low lysine content (Massawe *et al*., 2005). It is rich in eight of the nine essential amino acids, (Yao *et al*., 2015).

Bambara groundnut has many names tag due to its nutritional potential. It is referred to as a 'well-balanced food' because of its nutrient profile (Massawe *et al*., 2005, Paliwal *et al*., 2020). It is referred to as 'future crop', "complete food" perhaps due to the nutrients derived from the seed (Akpapunam *et al*., 1994). It is also referred to as a drought tolerance plant as it thrives and produces some yield in conditions too arid for other crops (Mkandawire 2007, Enwere *et al*., 1996). This crop can contribute to the food and nutrition requirements of low-input farmers found across sub-Sahara African countries. Based on its production advantage as a legume crop, and its nutritional value, and its potential to produce some yield where other crops fail (Collinson *et al*., 1997), it can therefore be recommended as food security crop (Brough *et al*., 1993. which can fill the gap in conservation agriculture (Hobbs et al 2008).

Bambara groundnut is ideal and fits into the African diet, but its cultivation is still sourced from landraces. The gross energy value of Bambara groundnut seed is greater than that of other common pulses such as cowpea (*Vigna unguiculata*), lentil (*Lens esculenta*), and pigeon pea (*Cajanus cajan)* (Adu-Dapaah, 2004). It is cultivated in arid regions in sub-Sahara African countries and its cultivation has extended to Africa, Asia, and other parts of the world. Abiotic stress is a common and major limiting production factors in these areas. Heat stress, high temperature and low rainfall, low soil fertility, lack of minimum inputs and lack of improved variety etc. can lead to a considerable low yield. Although, research has shown that Nigeria is the highest producer of Bambara groundnut in Africa (Hillocks *et al*., 2012), reports on grain yield per hectare of Bambara groundnut and most underutilized legume food crops are scanty. This is not far-fetched as most of these crops serve as companion crops to the major crops, records of sole

cropping in large hectarages of land and commensurate grain yield are very scarce. Most models are unreliable as they are based on farmer's mixed farming systems, this leads to ambiguity and bias in projecting grain yield of underutilized crops in such instances.

In comparison to other major leguminous crops, Bambara groundnut yield per hectare is far from major crop yield (Stagnari *et al.*, 2017). Collinson *et al.*, 2000 report that Bambara yield varied from 68.5 to 159.9 $kgha^{-1}$. Azam Ali *et al*., 2001 also reported the yield range of Bambara to be between 0.5- and 3-tons ha^{-1} in sub-Saharan Africa. The lack of improved Bambara groundnut varieties could also be an important factor for its low yield because, to date, no improved variety bred for abiotic stress conditions like other major crops, rather, various landraces are cultivated across all the growing regions of Nigeria and in sub-Sahara Africa. It is therefore imperative to do more studies on Bambara groundnut that will improve grain yield and enhance its tolerance to drought rather than increasing cultivated land area to cater for present and future food shortages in sub-Sahara Africa. Although, Bambara groundnut is a drought tolerance crop, this study will target the drought that occurred from flower emergence through pod filling until harvest. The notable changes in terms of yield loss caused by water deficit shall be recorded. A broad understanding of landraces' performance and responses under well-watered and water-stressed conditions could thereby an open opportunity to develop stress tolerance Bambara groundnut ecotype. Therefore, this is a pre-breeding study necessary for Bambara groundnut landraces improvement, their responses under two water regimes and diverse locations could help understand tolerance potential of different genotypes amongst the total accessions used in this study and this could serve as a pilot study for the whole Bambara groundnut germplasm conserved in the genebank of IITA Nigeria.

2.5 Bambara groundnut cultivation and yield potentials

Bambara groundnut plant cultivation and use is prominent in the traditions of rural communities in SSA where it has adaptation to diverse agro-ecologies. Deliberate selection by farmers over years is a common practice based on preferential traits of interest (Muhammed *et al*., 2020, Panzeri *et al*., 2022, Onwubiko *et al*., 2011). Globally, this crop is gaining more research interest, spreading through Africa, South America, Asia and Latin America (Baudoin *et al*., 2001) but the highest diversity is found in Nigeria and Cameroun that shares a border with Nigeria (Panzeri *et al*., 2022).

FAOSTAT 2017 reports a global estimated annual production of Bambara groundnut to be 160,378 tons, out of this; over a hundred thousand tons are produced in West Africa alone, for instance, Nigeria is the

highest producer of Bambara groundnut with an annual production estimate of 100,000 metric tons. (Muhammad *et al*., 2020).

The introduction of groundnut (*Arachis hypogaea*) led to a decline in the production of Bambara groundnut, but in recent years there has been a renewed interest to improve the crop (Ceasar, 2007). The reason for this interest is probably the high degree of drought resistance as well as the ability to produce reasonable pod numbers when grown on poor soils (Cheng *et al*., 2019). Further research into this crop could increase its acceptability and may sustain food security in Africa. According to Hillocks *et al.,* (2012), Bambara groundnut plays a key role in the traditional food and culture of people in the western and northern parts of Côte d'Ivoire. It is now widely distributed in the semi-arid zone of SSA, and most authors seem to support the view that it is the third most important food legume after cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*) (Mkandawire, 2007).

Various authors report Bambara groundnut as a crop with high tolerance to drought, (Azam Ali, 2001; Massawe *et al*., 2005; Jorgensen *et al*., 2010; Berchie *et al*., 2012; Muhammad *et al*., 2020) but drought affect its yield. Most published reports on Bambara groundnut yield are obtained from the crop's landraces, it has not received the ideal research attention like the other primary legumes by producing to its full capacity and genotypes bred to tolerate the changing weather condition and withstand arid environments are not common. Improved crop performance under the present and future climate change will require reducing crop losses due to environmental factors as well as well-structured plans to provide major gains in yields. (Bailey- Serres *et al*., 2019). Emerging strategies for enhancing sustainability, improved yield, and discovery of genotypes with resilience potential in a changing climate will require the concerted effort of various researchers across the countries of the world, international organizations, and the inclusion of farmers especially from countries where the crop is well cultivated. This can only be achieved by the integration of knowledge and more investment towards the deployment of molecular tools for the evaluation of genetic diversity of the available Bambara germplasm and exploitation of genes that confer tolerance to drought, improved yield, low anti-nutritional factors, less cooking time, and genotypes with improved performance under harsh and varying weather condition. In Nigeria, terminal drought, extreme temperature, and inconsistent rainfall are the basic cultivation challenges common in the areas where it is cultivated which generally incapacitates legume production at its maximum. Drought tolerance and early varieties of Bambara groundnut that can circumvent the incident of drought and inconsistent

rainfall are lacking in the region. These challenges are also expected to increase soon owing to climate change thereby necessitating the development of stress-tolerant and climate-resilient varieties from its germplasm to circumvent the incident of drought and inconsistent rainfall, heat, and low rainfall conditions. Moreover, this study seeks to understand the performance of various genotypes in Nigeria soils and to determine the traits that confer drought tolerance in Bambara groundnut using both multi-location trialing and molecular approaches.

Soil condition can also serve as a determinant factor to yield, understanding the soil texture, structure, soil moisture and soil nutrients for a subterranean crop plant like Bambara groundnut is essential for optimum performance. Nutrient deficiency can retard plant performance and ultimately lead to the death of the plant. Micronutrients are the soil nutrients required by plants in small quantities although their absence in the soil can cause a serious problem in crop production and in the health conditions of livestock that feeds on them (Deckers *et al*., 2004). The essential micronutrients for field crops are boron, copper, iron, manganese, molybdenum, and zinc. Other essential mineral nutrients needed at low concentrations are nickel and cobalt (Fageria *et al*., 2002). The availability of micronutrients (Zinc, Boron, Copper, Silicon), and macronutrients (Nitrogen, Phosphorus, Potassium, Calcium, Magnesium) are very essential to plant performance under drought stress. For instance, Phosphorus increases shoot biomass and grain yield in stressed plants (Jin *et al*., 2008; Faye *et al*., 2006) while Potassium plays an important role in plant's growth and development, it contributes significantly towards the plant's survival under drought condition by reducing the severity of drought stress on plant. (Hassan *et al*., 2017; Wilmer *et al*., 2022). Soil structure can increase the availability of soil nutrients. Limited or insufficient soil moisture at planting could result in delayed Bambara germination, low germination, and seed decay in the soil.

2.6 Center of Bambara groundnut cultivation in Nigeria

Bambara groundnut (Vigna *subterranea* (L.) verdc.) is predominantly cultivated in the Northern part of Nigeria where it receives the optimal temperature and minimum and maximum annual rainfall that best suited its production. (Abejide *et al.,* 2017). In Nigeria, landrace diversity is found in cultivation and the highest Bambara groundnut production comes from Nigeria (Uba *et al.,* 2021). Despite the production capacity and favorable weather requirement that supports its production in this zone, study has shown that drought has posed a major threat to the productivity of Bambara groundnut (Vuraiyai, 2011). The area of Bambara groundnut cultivation in Nigeria has declined (Mohammed 2014) and this has been attributed to

the effect of drought. Therefore, to have improved yield and drought tolerance of Bambara groundnut for arid zones and changing weather conditions in Nigeria, there is need to explore the available diversity of Bambara groundnut and determine suitable indices to be used for the selection of drought-tolerant genotypes from the available Bambara groundnut germplasm.

Farmlands under cultivation are best used when underutilized legumes are interplanted with other cash crops (Patil *et al.,* 2020). Bambara as an exemplary legume crop needs further research trials in Nigeria agro-ecologies to understand its performance and yield potential under water deficit and varying weather conditions. Depending on the genotype, three mechanisms for drought response can be exhibited by Bambara groundnut (avoidance, tolerance, and escape) (Muhammad *et al*., 2015; *Chai et al*. 2016). Moreover, differences found in grain yield (Kg\Ha) depend majorly on landraces, agricultural practices, and location of cultivation in Africa (Mayes *et al*., 2019).

The genetic resources of Bambara groundnut are widely conserved as landraces by indigenous farmers across SSA (Massawe *et al.,* 2005). Ex-situ conservation of Bambara germplasm is also found in Genetic Resources Centre, IITA, Nigeria (Mayes *et al.,* 2019). The crop lacks research attention especially in the context of breeding to develop varieties with better taste, reduced cooking time, improved yield, biotic, and abiotic stress tolerance that can fit into diverse agro-ecologies. Until now, the use and consumer preferences of Bambara groundnut are strongly linked to the variations found in its seed testa colour (Aviara *et al*., 2013). It is therefore imperative to improve Bambara groundnut with an application of scientific approaches such as molecular analysis to describe the potentials contained in its germplasm for further identification of useful traits and superior genotypes. In this study, the genetic diversity of Bambara groundnut landraces was explored as the first step towards the development of a breeding strategy for crop improvement. The rationale for this study is based on the crop's importance as a source of livelihood and its nutritional potential (Hillocks *et al*., 2012). Records of sole cropping in large hectarages of land and commensurate grain yield reports are very scarce. Most yield modelling is unreliable as most legumes' cultivation is based on farmer's mixed farming systems, they are grown as rotation crops with maize, millet, and sorghum as the root nodules fix nitrogen into the soil (Vanlauwe *et al.,* 2019), this leads to ambiguous and biased projections of grain yield of underutilized crops in such instances.

2.7 Effect of environmental factors on the crop performance and meeting food demand

Drought stress is the presence of water levels below normal that hamper crop growth and development or increase water loss resulting in negative impacts on crop production (Van Loon and Laaha 2014; Yaduvanshi *et al.,* 2015). It can also be described as short cessation of rainfall which results in plant distress and ultimate yield reduction. Drought has negatively impacted agriculture in SSA causing threats to food production. (Schlenker *et al.,* 2010, Haile *et al.*, 2005, Ahmed *et al.,* 2020).

According to Cole *et al.,* (2018), to meet global food demand, there must be a 60% increase in food production. Such a proposed astronomical increase in food production may not be realized with just a few staples in cultivation under changing climate and rainfed agricultural systems with unreliable rainfall patterns. Therefore, in meeting food security demand, there is a dire need to assess crop diversity, and diversify food baskets, especially the inclusion of underutilized but versatile crops for the various potential they exhibit especially their resilience to varying weather conditions and tolerance to drought. It is therefore essential to invest in underutilized legume crops to develop such crop varieties that can circumvent drought-aggravated hazards and to also diversify agriculture to improve food varieties and prevent hunger (Wiebe *et al.*, 2019). Crop performance is affected by drought coupled with the effect of climate change; therefore, staples are becoming insufficient to feed the growing population. For instance, the Nigerian population is one of the highest in SSA (Akinyemi *et al.,* 2014) and food availability is diminishing in supply as the yield capacity of staples dwindles due to the impact of climate change and limited resources for agricultural production such as irrigation and application of inputs for better crop performance. This need to be well addressed, hence, the chance of meeting food requirements may become difficult, especially with the few staples currently cultivated under the prevailing arid conditions and future food pattern in recent times is unreliable because of climate change (Idowu *et al.,* 2011), the drought-prone regions of northern Nigeria already face aridity and most water dams for agricultural productions are drying up (Medugu *et al.,* 2011, Sawa *et al.,* 2015).

Generally, crop plants need sufficient water for nutrient uptake and synthesis for optimum performance (Soares *et al.,* 2019). The rain-fed agricultural system is vulnerable to the impact of climate change (Olawale *et al.,* 2016). This is gradually enforcing a shift in the crop production cycles and thereby resulting in food security challenges. There is an urgent need to address the performance and inclusion of underutilized leguminous food crops and yield under drought conditions in Nigeria, the outcome of which could help in the choice of crop suitability that could help address food security and climate change in the

drier regions of Nigeria and other SSA countries facing related food security challenge.

2.8 Drought stress in plants

Drought stress, whether short mild, prolonged mild or severe types of stress, causes damage to crop plants throughout the growing cycle. The effect of drought on plants depends on the stage of the plant and the severity of the drought (Gray *et al.,* 2016) but in all cases, the drought effect results in some yield loss (Zhu *et al.,* 2002). Seeds planted need enough moisture as one of the external factors for germination. Drought can occur at the seedling, vegetative, or reproductive stage of the plant. The drought that occurs during the seedling stage causes a reduction in seed germination whereby the moisture in the soil is not sufficient for the seed for imbibition, swelling and softening of seed coat that ignites the germination process and enzyme activation. Seed germination and emergence are the vulnerable phase of crop establishment (Villalobos *et al.,* 2016). Lack of sufficient soil moisture affects seed germination, impedes nutrient uptake by the plant, alters photosynthesis, (Abobatta, 2019), causes economic loss through poor establishment, re-seeding costs and ultimately crop failure (Villalobos *et al.,* 2016)

The flowering and reproductive stage is the most vulnerable in a plant's life cycle (Hui *et al.,* 2015). At this stage, flower, fertilization, cell division, embryo and seed formation occur, these are highly sensitive to water deficiency. Flowering time and development are affected by drought stress-causing pollen grain sterility, flower abortion, and the seed set is hampered. Drought caused by water deficit during crop development poses major constraints to plant performance (Aslam *et al.,* 2012). The loss recorded by a prolonged and severe drought on crop plants exceeds losses from all other causes (Farooq *et al.,* 2009, Aslam *et al.,* 2012). Crops are more sensitive to drought that occurs at the reproductive stage of growth, and recovery may not be feasible or limited depending on the severity and rate of damage to the reproductive organ (Daryanto *et al.,* 2015).

2.9 Mechanisms of drought in legume crops

To have continuous crop production to meet food demand in SSA, drought research is one of the key areas that need more studies because of the present and future impact of climate change on crop production. Many drought studies and effects on crop plants especially on staples have been reported, however, how some crop plants survive and produce yield under limited soil water, semi-arid environmental conditions are unclear. Drought stress in leguminous crops is very complex (Waseem *et al.,* 2011) as it is controlled

by many genes. The study of tolerance and adaptation of Bambara groundnut genotypes to drought needs an in-depth and careful study.

Plants have evolved strategies to manage drought stress and water limitation conditions. Abobatta, (2019) elucidated strategies used by plants to manage temporary drought conditions to ensure survival and reproduction. These strategies are (broadly) drought escape, avoidance, and tolerance. These mechanisms are also used by Bambara groundnut (Mayes *et al.,* 2019), and genotype responses to drought in Bambara groundnut can be defined under these strategies.

Escape-escape strategies may rely on a higher rate of growth and more rapid development to produce seeds within a shorter life cycle. The plant completes the process of reproduction before the emergence of harsh environmental conditions. (Chaves *et al.,* 2003).

Avoidance- decreasing water loss from plants by control of these mechanisms involve stomatal closure, trichomes, reduced leaf area, senescence of older leaves, etc. or maximized water uptake by increased root growth.

Tolerance- When plant populations are subjected to drought conditions, the response to wilting can vary. While some are experiencing wilting, some are already dried, and some will maintain a measure of turgor and viability as the drought continues (Nguyen *et al.,* 1997). The tolerance mechanism varies from one species to the other and even from genotype to genotype; plants can reduce their resource utilization and adjust their growth to cope with adverse environmental conditions. Effects of water stress on plant performance result in different changes in the living plant, the changes include physiological, morphological, and biochemical changes. The physiological changes result in stomatal closure, photosynthesis is impaired, there can be a reduction in leaf water retention, reduction in relative water content, increase in internal temperature, end of translocation, high proline accumulation, internal carbon dioxide reduction, decrease in transpiration rate etc. (Guilin *et al.,* 2018). Morphological changes can include stunted growth, dwarf plant, early maturity, reduction in leaf size, leaf wilting, reduction in leaf area, leaf rolling, less leaf number, high root-to-shoot ratio, reduction in shoot length, changes in plant height leaf waxiness, presence of leaf hair, change in leaf colour, etc. Decrease in chlorophyll content, ABA accumulation, carbohydrate production, high proline accumulation, and decline in photochemical efficiency. All these have a direct impact on the plant performance and utmost yield.

In the past, major advances have been made in the improvement of staple crops in a bid to boost food production. The 'Green Revolution' employed modern approaches and technologies between I940 and 1970 and the outcome of this brought improvement to the yield and performance of primary food crops (Fita *et al.,* 2015). Recently, the yield of these staples cannot meet up with food demand due to population increase, the threat of climate change, genetic erosion of crop diversity, and most importantly, drought compounded by climate change. The staples are dependent on several inputs for optimum performance; hence, there is a necessity to focus on adaptive changes to manage the impact of drought on crop yield, especially in the arid part of the countries where most of the cereals and legumes crops are cultivated. To overcome over-dependence on just a few foodstuffs, (Jose *et al.,* 2020) and the continuous decline of crop yield due to the impact of drought, more work on drought study in leguminous crops is needed, the assessment and incorporation of various landraces of underutilized crops that have the potential to withstand the effect of drought and improve adaptation to climate change is essential.

Major constraints of Bambara groundnut concerning increased production are low yield compared to other well-researched leguminous crops, and the effect of limited water supply during the crop growing cycle as the plant is cultivated in the drier parts of the country with low and infrequent rainfall pattern (Majola *et al.,* 2021, Sesay *et al.,* 2004; Hampson *et al*., 2000). These constraints can be addressed by first understanding the performance of various genotypes in a diverse environment and by the application of molecular tools for in-depth assessment of its germplasm.

2.10 Physiological responses of Bambara groundnut to drought

At normal condition of plant growing cycle, seeds imbibe water from the soil moisture and that sparks germination process. Germination starts as water is trapped from the soil through the plant root, and it is translocated into the plants part giving the plant good condition to germinate, grow and develop when other conditions are satisfied. About 60–90% of water in the plant is located within plant cells, and the rest is mainly in cell walls (Gimez *et al.,* 2005) but when the plant water requirement cannot be fully satisfied, it leads to imbalance between water uptake and water loss. (Fahad *et al,* 2017). The imbalance between water uptake and water loss impacts the plant at all stages of growth and development from crop establishment (seedling) to crop maturity. Initial response of plants to drought starts with poor germination of the seeds due to insufficient moisture for imbibition, leading to early dieback or impaired seedling establishment. (Khaya *et al.,* 2006).

Morphological and physiological response of drought results in physical damages, physiological disruptions, and biochemical changes. (Fahad *et al,* 2017). The morphological response of Bambara groundnut to drought stress requires a critical review due to the impact of drought during different stages of crop growth and variation in rainfall across the growing environments. Investigation of the impact of drought stress at various physiological stages of Bambara groundnut with a view towards the development of strategies for improvement and to understand the genotypes that can survive water limited or drought prone environment where it is mostly cultivated is an important step towards its breeding for resilience. Bambara at germination - Germination of the seeds in water stressed environment is very slow (Gao *et al.*, 2020), while germination under non-stressed condition is vigorous when other condition for germination is supplied. Drought impaired seed imbibition leading to decreased seed germination. (Mayes *et al.,* 2019)

2.10.1 Above and below ground response of Bambara groundnut to water stress.

Bambara at vegetative stage- Impact of drought at vegetative and leaf water status of Bambara is measured by the leaf relative water content (RWC). RWC is an indicator of plant water status and has a value to determine its tolerance to drought (Ogbonnaya *et al.,* 1998, Galle *et al.,* 2007; Li *et al.,* 2011). Leaf relative water content significantly reduced in Bambara groundnut when drought is imposed. Vurayal *et al.,* 2011 report the physiological response of Bambara groundnut to short periods of drought imposition from two experiments and three regimes, one stressed at vegetative stage, (25 days after sowing (DAS), at flowering (46 DAS) and at pod filling stage (60 DAS) respectively and the second trial as control were well watered throughout. It was discovered that Water stressed during the three stages of growth of Bambara groundnut decreased, the relative water content reduction level ranged between 9% and 12.3%, showed Bambara groundnut plants can maintain relatively high RWC values despite the development of moisture stress (Vurayal *et al.,* 2011).

At reproductive stage- research has shown that Bambara groundnut plants established under drought conditions suffered a significant yield reduction in terms of low number of pods per plant, number of seeds per pod and biomass yield. Collinson *et al*., 1996) reported significant reductions in pod number per plant, harvest index and final seed yield due to terminal drought in a Zimbabwean Bambara groundnut landrace. Leaf dry mass ratio (LDMR) also decreased after two weeks of stress imposition and became severe at three weeks. (Muhammad *et al.,* 2016). Consequently, Nautiyal *et al*., (2017), Gao *et al*., (2023) elucidated

the responses and yield performance of Bambara groundnut under drought conditions are significantly influenced by genotype differences.

Several investigations of the morphological and molecular responses of Bambara groundnut to water stress has been reported and has included the impact of water stress to the vegetative and below the ground (subterranean pods and roots) performance of Bambara groundnut, the outcomes of which has defined genotype plasticity to drought and has helped in the classification of various genotypes based on their responses to drought at different stages of growth and that genotypes responds differently to water stress when subjected to similar condition of stress. Kundy *et al*., 2023 elucidated the effect of water stress on two Bambara groundnut genotypes and one groundnut variety (*Arachis hypogaea*) to understand tolerance superiority of the two-crop species as the two are similar in their morphological features bearing subterranean pods. In their study, it was discovered that the groundnut genotype exhibits restricted changes in RWC per unit reduction of water potential compared to the two Bambara groundnut used in the study. Genotypes with relatively low reduction in water content under stressed conditions are often considered to be relatively drought resistant. This implied that the groundnut has a higher drought potential in terms of the maintenance of RWC than the two Bambara genotypes. Agyeman *et al.,* 2023 report the impacts of water deficit on the above and below the ground performance of three Bambara groundnut genotypes imposed at three regimes. Their study showed differences in Bambara groundnut responses at different water regimes. Dry matter yield for above and below the ground was consistently reduced at water stressed regimes as compared to well-watered.

2.10.2 Molecular response of plants to drought

Drought stress is considered a major constraint to crop performance globally, it affects the physiological, morphological, molecular, and biological processes of crop plants leading to various challenges at different growth stages such as stunted growth, gradual changes in plants physiology, disrupts the defensive mechanisms as such plant becomes vulnerable or leads to substantial reduction in economic yield or even dies when it is prolonged. Various reports have projected that future drought will impair crop performances and reduce the yield of crops. Al-Kaisi and Broner, (2009) projected a yield reduction of 163 to 326kg/ha by 2050 because of drought impact. As such, understanding molecular response of crop plants to drought and devising a means to circumvent the negative impact could be a roadmap to food security. Plants could be stressed by cellular water deficit through drought, salt or extremely low or high temperatures. Moreover, many genes that are associated with drought are also coding for other traits, making drought tolerant genotypes difficult to develop. Understanding both the function of the gene

products and the mechanisms of gene expression is necessary to completely appreciate the molecular response to water deficiency. Numerous genes that are caused by water shortage are predicted to protect cellular function. These genes are also likely to be involved in metabolism, regulation, signaling, and stress recognition. The experimental conditions under which stress is applied, age of plant and severity of stress will also affect how specific processes are regulated (Kundy *et al*., 2023). The stressful situations that are administered in the lab could not fully reflect those that take place in the field. (Radin, 1993).

Molecular perspective of drought- Various processes are involved in the growth and development of crop plants. The processes such as photosynthesis, translocation of solutes, ion transport, nutrient metabolism, cellular development, all these are necessary for the proper functioning of plants and can only be carried out under a sufficient water condition but greatly impaired by the absence of water. When plants sense drought, they trigger certain resistance/defensive mechanisms to maintain the vital processes needed for their proper functioning (Yokota *et al*.,2006). Absence of moisture results to adaptation by changing certain biochemical pathway such as transcription and translational processes and altering physiological processes to adapt the new condition, however, this in turn affects the processes of photosynthesis, respiration, translocation of ions and nutrients in plants and activity of plant growth regulators (Farooq *et al*., 2009a, Farooq *et al*., 2009b).

It is hypothesized that several water-deficit-induced gene products could protect cellular structures from the impacts of water loss. The predictions are based on the inferred amino acid sequence and expression traits. These genes have also been found to be expressed throughout the maturation and desiccation stages of seed development in the early 1980s (Baker *et al*., 1988). ABRE, abscisic acid-response element; LEA, late embryogenesis abundant protein; lea, late embryogenesis abundant gene. Since then, it has been discovered that these genes are also expressed in vegetative tissues when water loss occurs because of osmotic, low-temperature, and water stress. There is a need for a better means to diagnose this challenge. Numerous genes that respond to drought, transcription factors, aquaporins, abundant proteins during late embryo, and dehydrins have all been discovered at the molecular level. Hadiarto *et al*., 2011. In recent years efforts have turned toward isolation of genes that are induced during water deficit to study the function of drought induced gene products and the pathways that lead to gene induction. Changes in gene expression are fundamental to the responses that occur during water deficit, and they control many of the short- and long-term responses. The expression of genes linked to water deficiency is regulated by the

tissue, organ, and developmental stage and may occur irrespective of the presence of stress. One set of genes, for instance, is expressed both throughout the maturity and desiccation stages of seed formation.

2.10.3 Importance of landraces in crop improvement

The term 'landrace' was first used by Von Ru¨nker in 1908 (cited by Zeven, 1998) but since then various definitions have evolved. For example, Harlan, (1975) defined landraces as populations that had evolved in subsistence agricultural societies because of 'millennia long', 'artificial' human selection pressures, with gene flow mediated through human migration, seed exchange as well as natural selection. Harlan (1975) believed that landraces have three basic characteristics:

(i) Variability of genotypes. (ii) Integrity and (iii) Local adaptation.

This conception of a landrace is the one still most widely applied. Hawkes (1983) extended the term by adding the association with marginal environments and a lack of direct competition with highly bred cultivars. There is a need for urgent conservation action for many crop landraces. Landraces remain a vital resource for contemporary plant breeding and are still widely grown in marginal environments (Villa *et al.,* 2005). They are threatened by replacement with highly bred cultivars which undoubtedly have led to widespread landrace extinction and genetic erosion of the underutilized crop gene pool. (Van de Wouw *et al.,* 2010). In local traditional farming systems where landraces are predominant, conservation of landraces has been made possible through common practices of seed exchange in collaborative farming, planting, harvesting, and saving some seeds for the next planting over many decades. All of these have enriched the genetic pool of crops by promoting intraspecific diversity (Frankel *et al.,* 1998).

Crop improvement often utilizes landrace diversity in the development of new cultivars (Frankel, 1977; Frankel *et al.*, 1998), particularly when developing cultivars for marginal environments. Landraces are still sources of specific traits for disease and pest resistance, nutritional quality, and tolerance of marginal environments (Frankel *et al.,* 1998). Genebanks conserve the crop landraces and some of their wild relatives for use in crop improvement, research, training etc. (Mohammad *et al.,* 2020). Both landraces and wild relatives conserved usually have distinct characteristics which may confer adaptive potential for a wide range of economically important traits (McCouch *et al.,* 2012).

Genebanks need to revamp all activities that will enhance the use of these plant genetic resources., this includes and not limited to the deployment of molecular tools for its characterization, diversity study and drought experiments that can reveal the potential of the conserved germplasm. IITA Genebank with the

highest Bambara germplasm in the world has characterized the germplasm using morphological Bambara descriptors (Olukolu *et al.,* 2012). Moreover, as an exemplary underutilized crop has received some breeding work as way back as 1978 at Malkerns research station in Swaziland. Doku and Karikari (1971) had an operational selection of wild Bambara groundnut towards its development. Karikari, 1972 had a correlation study between yield and some agronomic characteristics in Bambara groundnut. Major research has begun on underutilized legumes by BAMLINK, Crops for the Future Research Centre (CFFRC) and other international bodies to improve the yield, nutrition, and acceptability of underutilized legumes. With the advent of Molecular markers technology, it is important to increase effort in the analysis of diverse germplasm conserved in both national and international genebanks across the world for easy analysis of inherent potentials in the crop germplasm. Interest in Bambara groundnut as one of the underutilized legumes has increased across the globe possibly due to its nutrient potential and diversity available in its germplasm. The conserved germplasm is a great treasure for present and future breeding. Well-coordinated research and investment are required for cultivar development and the identification of outstanding genotypes that can produce a high number of pods under limited rainfall and the discovery of other hidden potential through molecular analysis and field evaluation. Other international genebanks with an appreciable number of accessions in conservation include Office De la Recherche Scientifique *et al.,* Technique Outre-mer (ORSTOM)

2.10.4 The importance of genetic diversity.

The variation found within genotypes of the same crop species with contrasting alleles of a gene in different individuals can be referred to as its genetic diversity. It is a basis for a crop's natural survival, and it is a valuable tool used by breeders in crop improvement (Bhandari *et al*., 2017). Crop genetic diversity may exist in the form of landraces, wild relatives, and mutant lines which may serve as a source of genetic variation in breeding climate-resilient crop varieties. The present study was undertaken with the broad objective of assessing different traits that confer drought tolerance in Bambara groundnut and to understand the yield performance of genotypes at different locations. The first step embarked for the realization of this objective was to understand the genetic variation present in the Bambara landrace. The availability of genetic diversity provides the possibility of crop adaptation to changing environments. Low genetic diversity results in uniformity and poor or reduced adaptation to current or changing conditions, leading to extinction (Frankham, 2005, Luan *et al*., 2006).

The genetic diversity analysis of plants is a critical component of plant genetics, crop improvement of plant genetic resources and crop evolution (Yildirim *et al*., 2013). Genetic diversity study in crop species has enhanced crop conservation; it reflects the presence of different alleles available in the crop gene pool and has revealed different genotypes within the crop's populations (Begna, (2021). The understanding of the available diversity informs the breeder of the selection criteria parallel with the breeding objectives and will also enhance the decision on the available diversity in a breeding program. Genetic diversity is important in breeding programs to accelerate gain from selection for yield improvement, for instance, breeders need to develop varieties resistant or tolerant to biotic and abiotic stresses. Moreover, a good understanding of the available diversity in crop germplasm is necessary for the development of appropriate conservation strategies as well as germplasm use.

2.10.5 Molecular markers as breeding tools for crop improvement

Molecular markers have provided a lot of the latest information on the genetic diversity of plants and their relationship between and within species. With the advent of genomic tools, molecular markers became the method of choice for genetic diversity assessment. It has been effective method of tracking loci and genome regions in crop plants (Kesawat *et al*., 2009, Younis *et al*., 2020) Various molecular markers have been used by plant breeders for the assessment of genetic diversity and selection, such as Amplified Fragment Length Polymorphisms (AFLP) (Dvorak *et al*., 1998; Cardoso *et al,*, 2018), Inter Simple Sequence Repeats (ISSR), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) (Deepashree *et al*., 2022) etc. Various markers have been used in Bambara genetic diversity studies. Aliyu *et al.,* (2015) used SSR markers to analyze the diversity in some landraces from Ghana.

Also, Igwe *et al*. (2017) used random amplified polymorphism of DNA (RAPD) for Bambara diversity study. This study employed Diversity Array Technology (DArT seq) for the genetic diversity analysis of one hundred and thirty-five Bambara groundnut genotypes sourced as landraces. (Table 3.2 and 3.3). An approach named genotyping-by-sequencing (GBS) has emerged to perform simultaneous discovery and genotyping (Pavan *et al*, 2013; Wang *et al*., 2020). It is advantageous as a high throughput and cost-effective tool for genome-wide analysis of genetic diversity, especially for non-model species and germplasm sets. Diversity Array Technology (DArTseq) (http://www.diversityarrays.com/dartapplication-dartseq) and Single Nucleotide Polymorphism (SNP) markers based on GBS technology have been successfully applied for linkage mapping, QTL

identification in bi-parental mapping populations, genome-wide association studies (GWAS), genetic diversity studies in many crops (Brinez *et al*., 2012; Grzebelus *et al*., 2014; Sharma *et al*., 2014; Yu *et al*., 2014; Vikram *et al*., 2016) as well as marker-assisted and genomic selection. Therefore, with DArTseq SNPs, it is possible to genotype thousands of markers in a single assay for many individual plants. DArTseq is valuable in the construction of high-resolution mapping and detailed genetic dissection of traits. DArTseq markers based on genotyping by sequencing allow SNP (Single Nucleotide Polymorphism) marker discovery. The quick progress in sequencing and genotyping technologies in the last decade has permitted the advancement of SNP and DArTseq arrays even for polyploid crops such as wheat (Peterson *et al*., 2014) and legume crops like Bambara groundnut. In summary, DArTseq has been increasingly used in genetic diversity analysis, linkage mapping, and QTL identification in many crop plants. (Ketema *et al.*, 2020, Hassani *et al*., 2020, Cui *et al*., 2014 ; Raman *et al*., 2014; Ziems *et al*., 2014).

Diversity Arrays Technology is a high-throughput genotyping method using the DArT-SeqTM. DArT technology. It relies on a complexity reduction method to enrich genomic representations with single-copy sequences and subsequently perform next-generation sequencing. It detects both SNPs and presence/absence sequence variants, collectively referred to as DArT-Seq markers (Raman *et al*., 2014). DArT-Seq targets genes and avoids sequencing repetitive sequences known as Junk. Parameters for the DArT marker assaying pipeline for quality control are the reproducibility, call rate, Polymorphic information content (PIC) and the Q-value which is the logarithm of the minimum false discovery rate at which the test may be called significant) (Kilian *et al*., 2012)

2.10.6 Genotype × Environment interaction

Bambara groundnut is grown mostly under rain-fed conditions in arid regions of SSA characterized by very low and erratic annual rainfall. Rainfall varies from one agro-geographical region to another, and this variation defines the amount of water available for crop use and performance. To determine the significance and pattern of interaction of genotypes across various environments, trial data of multiple locations with at least two seasons are used for such various G x E related studies. Such data is useful to determine whether any repeatable patterns of interaction exist between genotypes and environments and if there are any, it can guide the division of the target region into sub-regions or mega-environments and if not, the target location is treated as a single mega environment (Yan and Tinker, 2006). The response of genotypes to a given environment is not always similar (Canter *et al*., 2005). The discovery of genotypes

with superior performance in terms of grain yield from the test genotypes is a key step in breeding work. This study uses eight phenotypic traits to assess the performance of each genotype across three locations in two consecutive years. In the assessment of crop performance and their responses in diverse environments, stability analysis is essential. Grain yield stability along with the prevailing environmental factors is also affected by their response to the environment. High yield is one of the targets for any Bambara groundnut improvement program and as such lack of improved Bambara groundnut varieties could be an important factor for its low yield. Collinson *et al*., (2000) report that Bambara yields varied from 68.5Kg ha -1 to 159.9 Kg ha -1 in their evaluation. Azam Ali *et al*., (2001) also reported the yield range of Bambara groundnut to be between 0.5 and 3 tons per hectare in SSA. To date, no improved varieties bred for abiotic stress conditions, like other major crops, have been released rather; various landraces are in cultivation across all the growing regions of Nigeria and in SSA.

CHAPTER THREE

3.1. MATERIALS AND METHODS

3.1.1. Nature of experiment, year conducted, duration and field locations.

This experiment was conducted in Nigeria at three different agro-ecological zones and open field condition during the year 2019/2020 and 2020/2021 seasons. The field used for this study were located at Mokwa, Niger state (lat. 9.345064, long. 5.031372), Ibadan, Oyo state (lat. 7.486354, long. 3.903728), and Zaria, Kaduna state (lat. 11.18656, long. 7.615368) as presented in Figure 3.1 below.

3.1.2. Preliminary investigations of eleven years weather data of the selected locations and analysis of soil nutrients

A preliminary study of eleven-year data (2008-2018) comprising rainfall, minimum and maximum temperature of the three locations was undertaken. The data was obtained from IITA Geographic Information System Unit (GIS). The analysis helped to understand the history of the rainfall pattern of the selected locations and other weather indices which could indirectly impact the crop performance in each of the zones where these trials were established. It was observed in the preliminary analysis of the weather data that the three locations experiences drought at its peak every two months of the year (November, December and the first two months of the subsequent year i.e., January, and February) whereas the first two months of every year have the least and most times no rainfall. This result helped to determine the best time of the year to set up drought experiments at each location, since this research target is to identify drought tolerance genotypes that has potential to withstand stress at open field condition, such natural conditions with minimum rainfall is an important condition to address the objective of this study. Table 3.1 below showed the eleven years rainfall pattern of the studied locations ranging from (2008-2018) while figure 3.1 shows the map of Nigeria and the study locations. Materials needed for this work are Bambara groundnut seeds sourced from the genetic resources center of IITA, Nigeria, irrigable fields, Cutlass, measuring tape, Bamboo pegs for plot demarcation, planting labels, NPK 50:50:50 fertilizer, electronic field notebook for data recording, Ziplock bags for leaf sampling during leaf relative water content experiment, plastic bowl, oven for drying, 30 cm rule for measurement.

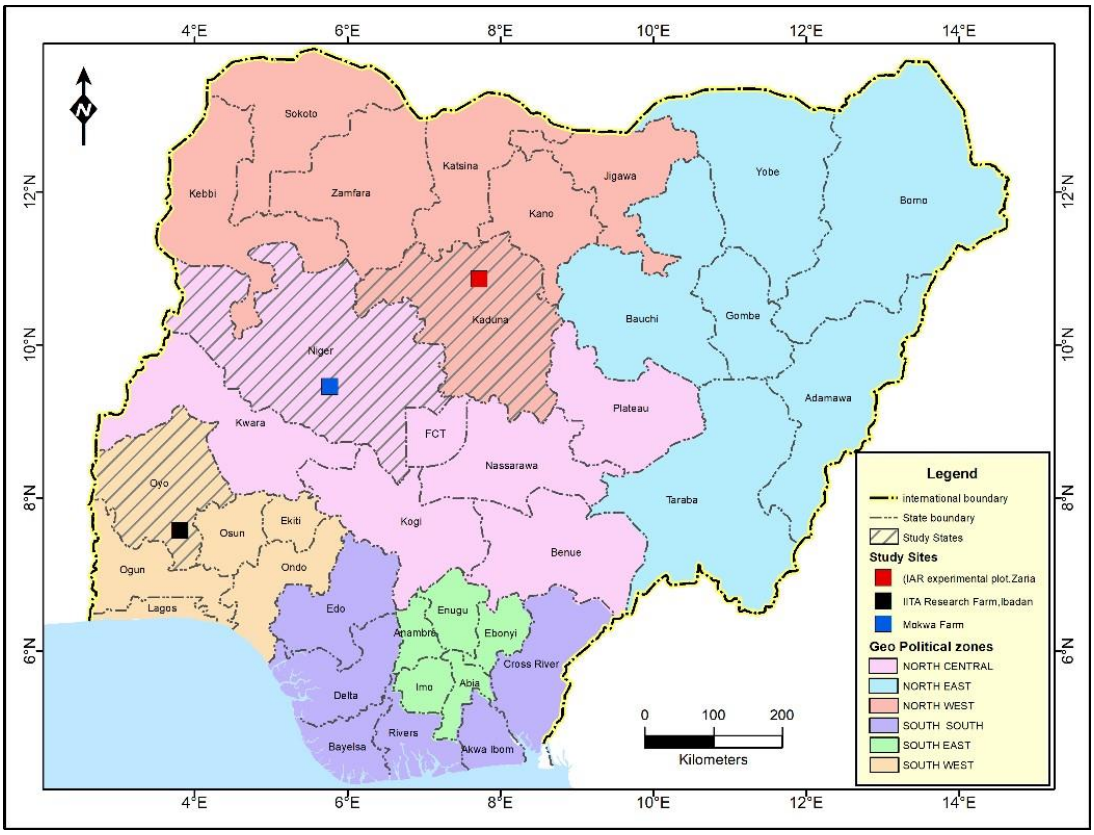


Figure 3.1. Map of Nigeria showing the study locations in the year 2019/2020 and 2020/2021

Methods

Land preparation.

Field layouts

Marking the fields into blocks.

Experimental design- (9*15 α- lattice)

Number of replicates 3

Number of seeds planted per hill-2 thinned to 1 after germination.

Number of seeds planted per replicate- 20 seeds.

Chemical application- pre-and post-emergent chemicals for weed control.

Drought imposition- at flower emergence.

Determination of leaf relative moisture content and Data collection.

Table 3.1. Eleven years total monthly rainfall history of the three locations.

Year		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Zaria	*Jan.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ibadan	*Jan.	0.0	0.3	0.1	0.0	0.0	1.1	0.0	0.0	0.4	0.0	0.0
Mokwa	*Jan.	0.0	0.3	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0	0.0
Zaria	*Feb.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ibadan	*Feb.	0.0	1.2	2.3	4.8	1.2	1.1	3.0	1.4	0.0	0.0	2.5
Mokwa	*Feb.	0.1	0.1	0.2	0.6	0.4	0.6	0.1	0.1	0.0	0.0	0.9
Zaria	Mar.	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.3	0.0	0.0
Ibadan	Mar.	3.2	0.8	1.6	2.3	3.4	3.3	5.0	2.0	6.0	2.1	4.8
Mokwa	Mar.	0.7	0.4	0.4	0.3	0.1	0.7	0.9	1.5	1.0	0.1	0.3
Zaria	Apr.	0.3	1.0	0.5	0.5	1.0	0.5	2.3	0.0	2.5	0.4	0.1
Ibadan	Apr.	4.4	5.8	4.2	3.4	2.8	8.1	5.5	3.9	6.4	3.4	3.0
Mokwa	Apr.	1.4	5.1	2.2	1.3	2.1	1.8	2.6	0.1	2.3	0.9	0.7
Zaria	May	2.2	1.6	1.8	1.9	5.1	0.9	2.2	0.6	2.5	5.2	2.0
Ibadan	May	5.3	6.0	5.6	4.7	5.9	3.3	5.7	5.1	8.2	6.1	6.2
Mokwa	May	1.6	4.3	4.4	3.7	5.1	2.7	3.1	1.3	4.0	5.9	2.8
Zaria	Jun.	3.3	2.9	5.9	2.1	6.6	2.1	3.1	1.9	4.0	6.4	3.5
Ibadan	Jun.	7.0	6.1	7.1	7.5	6.1	5.2	12.2	6.9	9.6	11.7	10.8
Mokwa	Jun.	5.2	4.9	4.2	4.4	4.5	4.4	2.9	2.4	2.7	8.1	6.3
Zaria	Jul.	7.5	5.1	7.2	4.1	8.2	5.5	4.9	8.7	8.4	7.8	6.8
Ibadan	Jul.	8.0	5.2	6.8	5.0	9.0	5.8	10.5	4.4	2.3	9.7	8.0
Mokwa	Jul.	7.3	6.2	6.3	5.3	6.6	4.0	3.0	4.5	5.7	3.8	9.9
Zaria	Aug.	9.3	7.6	6.4	7.4	10.5	5.8	8.0	12.3	6.2	8.2	6.8
Ibadan	Aug.	4.0	1.3	8.9	10.2	1.4	1.5	3.2	3.2	2.5	3.4	4.0
Mokwa	Aug.	9.6	7.5	7.6	6.3	8.0	3.2	6.6	3.9	4.6	5.8	6.1
Zaria	Sep.	7.1	3.4	7.6	6.5	8.3	2.5	7.0	7.4	8.4	3.4	5.3
Ibadan	Sep.	9.7	5.2	9.8	9.4	6.8	5.6	4.5	11.0	10.7	7.4	8.4
Mokwa	Sep.	7.9	5.4	7.9	5.7	7.6	3.6	6.5	5.3	7.9	4.3	7.6
Zaria	Oct.	2.1	1.0	2.6	2.3	2.9	1.1	1.2	1.5	1.0	0.3	1.4
Ibadan	Oct.	3.7	3.7	11.3	8.5	6.0	3.5	5.7	4.0	4.6	3.1	9.0
Mokwa	Oct.	3.4	4.3	4.8	3.5	5.4	1.8	3.3	1.7	2.6	2.3	3.1
Zaria	*Nov.	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ibadan	*Nov.	0.0	1.1	5.4	0.3	0.6	0.7	1.6	0.3	0.4	0.8	0.6
Mokwa	*Nov.	0.0	0.5	1.0	0.1	0.7	0.2	0.5	0.1	0.0	0.0	0.0
Zaria	*Dec.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ibadan	*Dec.	0.3	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.6	0.0
Mokwa	*Dec.	0.3	0.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.2	0.0

*- *Months with extremely low or no rainfall records are marked with an asterisk.*





Plate 3.1. Hydrosense equipment was used for measuring soil moisture and soil volumetric water content.

The analysis of soil types, moisture, soil structure, texture, and soil nutrient composition of all the locations is crucial in this study. Although, reports showed that Bambara groundnut can thrive on varying soil types and less fertile soils, however, the soil water retention ability, moisture availability, soil structure, and soil texture determine the ease of root penetration, water availability, and water holding capacity of the soil are a function of any crop performance (Chen *et al.,* 2022). The volumetric water content (VWC) and percentage moisture content %MC) of the soil were determined before crop establishment using an equipment known as Hydrosense (Plate 3.1). It measures soil moisture, relative water, and volumetric water content.

The Hydrosense system combines a compact, handheld display (CD620) and a sophisticated soil water sensing probe (CS620) in a portable package to provide quick and reliable soil water content measurements. Each measurement is obtained by inserting the 20cm long stainless probe rods into the soil and pressing a single button on the display unit. Hydrosense is a versatile tool for monitoring and managing soil water in a wide range of conditions. The microprocessor-controlled circuitry and two-line readout are contained in a splash-proof enclosure that includes two integral membrane buttons used to operate the system. A 5-ft coiled cable connects the display to the probe. The 5 mm diameter stainless steel rods are an essential part of the electronic circuitry encapsulated in the epoxy probe head. The parallel rods constitute a driven transmission line sensitive to dielectric permittivity and water content. Hydrosense has two modes of operation. The water content measurement mode uses standard laboratory calibrations to provide per cent volumetric water content from air drying to saturation. In the water deficit mode, Hydrosense measurements are taken at lower and upper water contents as specified by the user and stored in memory as reference values. The reference values are then applied to subsequent measurements to determine the water added to bring the soil to the upper water content.



(Photo taken by Ben.)

Plate 3.2. Soil samples from the three locations, Mokwa, Ibadan and Zaria for analysis

This study also explored the soil types present in Mokwa, Ibadan and Zaria fields where this research was established (Plate 3.2). Soil was sampled prior to planting to assess the available soil nutrients. The samples were made at ten points per field using a soil auger with a depth of 0-30 cm. The soil sampled per location was thoroughly mixed and submitted to IITA soil analytical lab for routine analysis test. Soil pH, textural investigation, soil organic matter, cation exchange capacity (CEC), phosphorus, potassium, calcium, and Magnesium were analyzed. Soil types, soil nutrient composition and their effect on crop performance have been reported by various journals. Soil nutrient composition is an important factor in plants' performance. Soil nutrient deficient poses various threats to plant performance and ultimately reduce crop yield (Patil *et al*., 2020).

3.1.3. Sources of seeds for this work and the rationale

The Genetic Resources Centre (GRC) of the International Institute of Tropical Agriculture (IITA) is known for the conservation of plant genetic resources material. This center conserves various seed crops including underutilized legumes and clonally propagated crops in perpetuity for research purposes. IITA GRC holds in trust the largest germplasm of Bambara groundnut accessions *ex-situ* which are collected as landraces across countries in sub-Sahara Africa. These landraces house important traits which can be used in crop improvement. Their conservation is highly important to guard against genetic erosion and

extinction of valuable genes which might be used to address various biotic and abiotic challenges to food security. The 135 accessions were sorted from the total germplasm conserved in the genebank of the International Institute of Tropical Agriculture, IITA Ibadan Nigeria. This consisted of 112 accessions of landraces conserved ex-situ at the Genetic Resource Center and 13 genotypes from the Crops for the Future Research Center (CFFRC) University of Nottingham, UK and 10 newly explored landraces from the farmer's field generally grown in the middle belt zones of Nigeria. These three groups were combined to make a total of 135 accessions. The list and origin of the collection of each accession are represented in Tables 3.2 and 3.3. These accessions were planted in three locations for drought evaluation and GWAS studies.

Table 3.2. List of one hundred and twenty-six Bambara accessions collected from IITA.

S/Nb	Accession	Country	Region	S/Nb	Accession	Country	Region	S/Nb	Accession	Country	Region
1	TVSu-8	Nigeria	WA	43	TVSu-217	Ghana	WA	85	TVSu-378	Tanzania	EA
2	TVSu-9	Nigeria	WA	44	TVSu-218	Ghana	WA	86	TVSu-379	Tanzania	EA
3	TVSu-11	Nigeria	WA	45	TVSu-221	Ghana	WA	87	TVSu-386	Tanzania	EA
4	TVSu-14	Nigeria	WA	46	TVSu-222	Ghana	WA	88	TVSu-388	Sudan	EA
5	TVSu-25	Nigeria	WA	47	TVSu-224	Ghana	WA	89	TVSu-397	Cameroon	Cen
6	TVSu-29	Nigeria	WA	48	TVSu-225	Ghana	WA	90	TVSu-410	Cameroon	Cen
7	TVSu-46	Unknown	*	49	TVSu-227	Ghana	WA	91	TVSu-425	Cameroon	Cen
8	TVSu-47	Unknown	*	50	TVSu-229	Ghana	WA	92	TVSu-431	Cameroon	Cen
9	TVSu-50	Unknown	*	51	TVSu-231	Ghana	WA	93	TVSu-441	Cameroon	Cen
10	TVSu-66	Unknown	*	52	TVSu-232	Ghana	WA	94	TVSu-467	Cameroon	Cen
11	TVSu-75	Unknown	*	53	TVSu-233	Ghana	WA	95	TVSu-535	Cameroon	Cen
12	TVSu-82	Nigeria	WA	54	TVSu-234	Ghana	WA	96	TVSu-581	Nigeria	WA
13	TVSu-84	Unknown	*	55	TVSu-245	Gambia	WA	97	TVSu-593	Nigeria	WA
14	TVSu-85	B. Faso	WA	56	TVSu-253	Nigeria	WA	98	TVSu-600	Nigeria	WA
15	TVSu-87	Mali	WA	57	TVSu-262	Nigeria	WA	99	TVSu-645	Nigeria	WA
16	TVSu-89	Mali	WA	58	TVSu-266	Nigeria	WA	100	TVSu-671	Nigeria	WA
17	TVSu-94	Mali	WA	59	TVSu-267	Nigeria	WA	101	TVSu-2088	Nigeria	WA
18	TVSu-103	Mali	WA	60	TVSu-269	Nigeria	WA	102	TVSu-2089	Nigeria	WA
19	TVSu-110	Mali	WA	61	TVSu-275	Nigeria	WA	103	TVSu-2090	Nigeria	WA
20	TVSu-112	Mali	WA	62	TVSu-277	Nigeria	WA	104	TVSu-2091	Nigeria	WA
21	TVSu-115	Cote d'Or	WA	63	TVSu-280	Nigeria	WA	105	TVSu-2092	Nigeria	WA
22	TVSu-125	Nigeria	WA	64	TVSu-288	Benin	WA	106	TVSu-2093	Nigeria	WA
23	TVSu-137	Ghana	WA	65	TVSu-295	B. Faso	WA	107	TVSu-2094	Nigeria	WA
24	TVSu-143	Ghana	WA	66	TVSu-310	B. Faso	WA	108	TVSu-2095	Nigeria	WA
25	TVSu-144	Ghana	WA	67	TVSu-311	B. Faso	WA	109	TVSu-2096	Nigeria	WA
26	TVSu-145	Ghana	WA	68	TVSu-318	B. Faso	WA	110	TVSu-2097	Nigeria	WA
27	TVSu-146	Ghana	WA	69	TVSu-319	B. Faso	WA	111	TVSu-2098	Nigeria	WA
28	TVSu-151	Ghana	WA	70	TVSu-323	Nigeria	WA	112	TVSu-2099	Nigeria	WA
29	TVSu-154	Ghana	WA	71	TVSu-329	Nigeria	WA	113	TVSu-2100	Nigeria	Farmers
30	TVSu-156	Ghana	WA	72	TVSu-343	Nigeria	WA	114	TVSu-2101	Nigeria	Farmers
31	TVSu-158	Ghana	WA	73	TVSu-348	Nigeria	WA	115	TVSu-2102	Nigeria	Farmers
32	TVSu-160	Ghana	WA	74	TVSu-351	Nigeria	WA	116	TVSu-2103	Nigeria	Farmers
33	TVSu-161	Ghana	WA	75	TVSu-357	Nigeria	WA	117	TVSu-2104	Nigeria	Farmers
34	TVSu-164	Ghana	WA	76	TVSu-359	Nigeria	WA	118	TVSu-2105	Nigeria	Farmers

35	TVSu-167	Ghana	WA	77	TVSu-361	Nigeria	WA	119	TVSu-2106	Nigeria	Farmers
36	TVSu-170	Ghana	WA	78	TVSu-362	Nigeria	WA	120	TVSu-2107	Nigeria	Farmers
37	TVSu-183	Tanzania	EA	79	TVSu-364	Nigeria	WA	121	TVSu-2108	Nigeria	Farmers
38	TVSu-200	Benin	WA	80	TVSu-368	Nigeria	WA	122	TVSu-2109	Nigeria	Farmers
39	TVSu-211	Ghana	WA	81	TVSu-371	Tanzania	EA	123	TVSu-2110	Nigeria	Farmers
40	TVSu-212	Ghana	WA	82	TVSu-373	Tanzania	EA	124	TVSu-2112	Nigeria	Farmers
41	TVSu-214	Ghana	WA	83	TVSu-374	Tanzania	EA	125	TVSu-2113	Nigeria	Farmers
42	TVSu-216	Ghana	WA	84	TVSu-376	Tanzania	EA	126	TVSu-2114	Nigeria	Farmers

WA-West Africa, EA-East Africa, Cen-Central Africa, *- unknown region as found in IITA Bambara groundnut passport data.

Table 3.3. List of twelve Bambara groundnut accessions collected from the University of Nottingham, the United Kingdom, with their country of origin.

Sn	Genotype code	Country	Region	Longitude	Latitude
1	CFFR-1 (GresiK)	Indonesia	SE Asia	112.39^{0}E	7.09^{0}S
2	CFFR-2 (Lun T)	Sierra leone	WA	13.14^{0}W	8.29^{0}N
3	CFFR-3 (Tiga nicuru)	Mali	WA	7.59^{0}W	14.13^{0}N
4	CFFR-5 (Ankpa-4)	Nigeria	WA	7.38^{0}E	7.24^{0}N
5	CFFR-6 (IITA-686)	Tanzania	EA	35.46^{0}E	6.10^{0}S
6	CFFR-7 (DodR)	Tanzania	EA	35.57^{0}E	5.54^{0}S
7	CFFR-8 (Dip C)	Botswana	SA	25.55^{0}E	24.38^{0}S
8	CFFR-9 (S 19-3)	Namibia	SA	17.04^{0}E	22.33^{0}S
9	CFFR-10 (Uniswa red)	Swaziland	SA	31.22^{0}E	26.28^{0}S
10	CFFR-11 (Uniswa red-G)	Swaziland	SA	31.22^{0}E	26.28^{0}S
11	CFFR-12 (Kano-2)	Nigeria	WA	8.62^{0}E	12.19^{0}N
12	CFFR-13 (Getso)	Nigeria	WA	8.31^{0}E	12.0 ^{0}N

WA-West Africa, EA- East Africa, Cen-Central Africa, SE- South-east

3.1.4. Experimental design

A randomized Alpha lattice design of 9 X 15 blocks per replicate was used in this study. Two water regimes (Well-watered and Water stressed) were established and replicated three times. (Figure 3.3). Although, these trials were established at open field conditions, the time of establishment was planned towards the end of rainfall, this was also confirmed by the eleven years of rainfall history data analyzed before field establishment. Mokwa planting was done in October 20, Zaria was planted November 3rd and Ibadan was planted November 17th. The two regimes were given water until flower emergence when stress was imposed on water stress plot, at that time, there was no more rainfall, the only source of water was irrigation. Both well-watered until flower emergence at which irrigation was withdrawn from water stressed plot and was not restored until harvest (physiological maturity) and water stressed plots were planted 10-meter distance from each other (Figure 3.3). The two regimes were established at the same time at each location. The planting space was 25 cm within the row and 75 cm between rows. Two seeds were sown in a 5-6 cm depth hole for all accessions at all the reps and regimes, thinning was done two weeks after planting to one vigorous and healthy-looking plant. The two seeds sown initially were to ensure that one plant per stand was available (increase the chance of high plant population per accession) to prevent wide gaps in germination should there be any reason for replanting due to low germination. Irrigation was stopped at water stressed regime at flower emergence while water is given to the well water regime throughout the cropping period until physiological maturity.

All the fields were well managed throughout the experimental period, pre- and post-emergent agrochemical herbicide with a trade name called (Force-up) of active ingredients 480g/l glyphosate -isopropyl amine salt was sprayed a day after planting at the dilution ratio of 150 ml of chemical per 20 liters. of water, this was able to suppress the weed growth for three weeks. Subsequently, the weed was manually controlled at three weeks' intervals to ensure that the plots are always maintained clean. Cyperdiforce of active ingredients Cypermethrin 30 g/l + Dimethoate 250 g/l EC, 80 ml per 20 liters of water was applied three weeks after planting and was increased to 100 ml for the same water quantity at the flowering stage. Basal application of N: P: K (50:50:50) fertilizer was carried out at 20g per plant three weeks after planting. Earthen up was done at podding stage using hoes. The various chemicals used, and their dilution ratio is presented in Table

3.4.

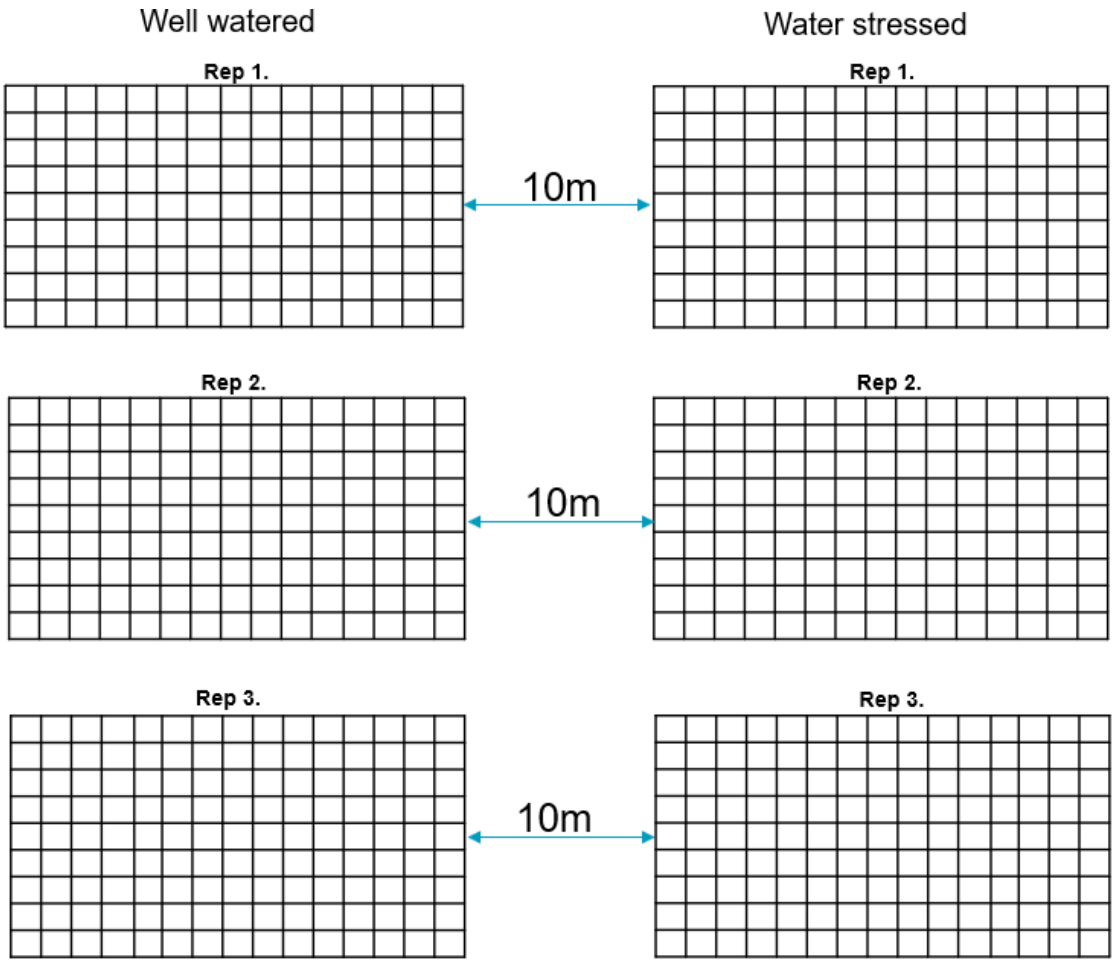


Figure 3.3. Pictorial view of the experimental plots used in this study.

Table 3.4. Various chemicals used and their dilution ratio.

Chemical trade name	Chemical type	Active ingredients	Concentration
Force-up	Contact herbicide	480g/l glyphosate-isopropylamine salt	150ml/20 litres water
Fertilizer	N:P: K 15:15:15	Nitrogen, Phosphorus and Potassium	20g/plant stand
Cyper-Diforce	Insecticide	Cypermethrin 30g/lt. +Dimethoate 250g/lEC	100ml/20 liters of water
Karate	Insecticide	50g/l lambda-cyhalothrin	100ml/20 liters of water

3.1.5 Determination of relative water content of Bambara groundnut leaves

Three terminal leaflets of BG were harvested into Ziplock bags. Ziplock bag weight were tared on a scale before the leaflets were put in the bag The fresh weight of the sample was measured with a sensitive scale, and the leaves were soaked in water overnight to become turgid under a light source. The leaf samples were removed, the surface water was gently cleaned, and the leaf was weighed after imbibition. The weighed leaves were dried in an oven at 80 degrees centigrade for two days. The dried weight was also recorded.

Relative water content was calculated as RWC = Fresh weight- Dry weight/Turgid weight – dry weight x100

3.1.6 Phenotypic measurements from well water and water stress plots

From each regime (well-watered and water stressed), five plants were tagged per replicate for each accession, making a total of fifteen plants per accession from the three replicates per regime that were measured and recorded for phenotypic traits. The International Board for Plant Genetic Resources (IBPGR) Bambara groundnut descriptors were used for the trait characterization during

data collection. The performance of 135 accessions of Bambara groundnut was measured based on the following eight morphological and quantitative parameters; plant height, number of petioles per plant, plant spread, number of days to 50% flower, number of pods per plant, the weight of pods per plant, number of seeds per plant and the weight of seeds per plant. Grain yield/hectare was estimated from the plot seed yield for each genotype across the three replications for each location. Table 3.5 below is a brief description of traits used for morphological characterization. Environmental indices that could interfere with crop performance were recorded before and during the crop establishment at the three locations and in the two years. Both weather and soil sampled from the experimental plots were analyzed. Table 3.6 is the number of environments used for this study.

Table 3.5. **Parameters measured using Bambara groundnut descriptor.**

Yield and yield component	Methods of Measurement
Plant height (cm)	Measured ten weeks after planting from the plant's base to the highest petiole of the plant.
Plant spread (cm)	The widest length between two opposite plants was measured using a cm ruler.
Number of petioles per plant	The number of petioles for each plant was counted 40 days after planting.
Number of days to the first flower	number of days taken for each genotype from planting to first flower emergence
Number of days to 50% flower	number of days taken for each genotype from planting to 50% flower
Number of flowers	number of flowers per plant was collected by counting the flower
Leaf length (cm)	measure the terminal leaf length from the leaf apex to the stalk
Leaf width (cm)	measured from the widest distance from the two sides of the leaf blade
Number of pods per plant	direct counting at harvest of the number of pods harvested per plant.
Weight of pods per plant (g)	number of seeds recovered after careful threshing and cleaning on a single-plant basis
Number of seeds per plant	number of seeds recovered after careful threshing and cleaning on a single-plant basis
Weight of seed per plant (g)	weight of the seed was measured after shelling, cleaning, and drying to 12% moisture content.
Growth habit	by observing the whole plant structure at flower emergence using BG descriptors
Terminal leaf shape	by observing leaf shape and recording based on BG descriptors

Table 3.6. Twelve environments where this drought research was conducted.

1. Ibadan-WW-2019	2. Ibadan-WS-2019	3. Ibadan-WW-2021	4. Ibadan-WS-2021
5. Mokwa-WW-2019	6. Mokwa-WS-2019	7. Mokwa-WW-2021	8. Mokwa-WS-2021
9. Zaria-WS-2019	10. Zaria-WW-2021	11. Zaria-WW-2019	12. Zaria-WS-2021

WS- water stressed. *WW*- well watered.



Plate 3.3. The well-watered regime at Ibadan location.



Plate 3.4. The Water stressed regime at Ibadan location.



Plate 3.5. The Well-Watered regime at Mokwa location



Plate 3.6. The Water stressed regime at Mokwa location.



Plate 3.7. The well-watered regime at Zaria location.



Plate 3.8. The water-stressed regime at Zaria location.



Plate 3.9. The 135 BG accessions screened for drought susceptibility and tolerance.

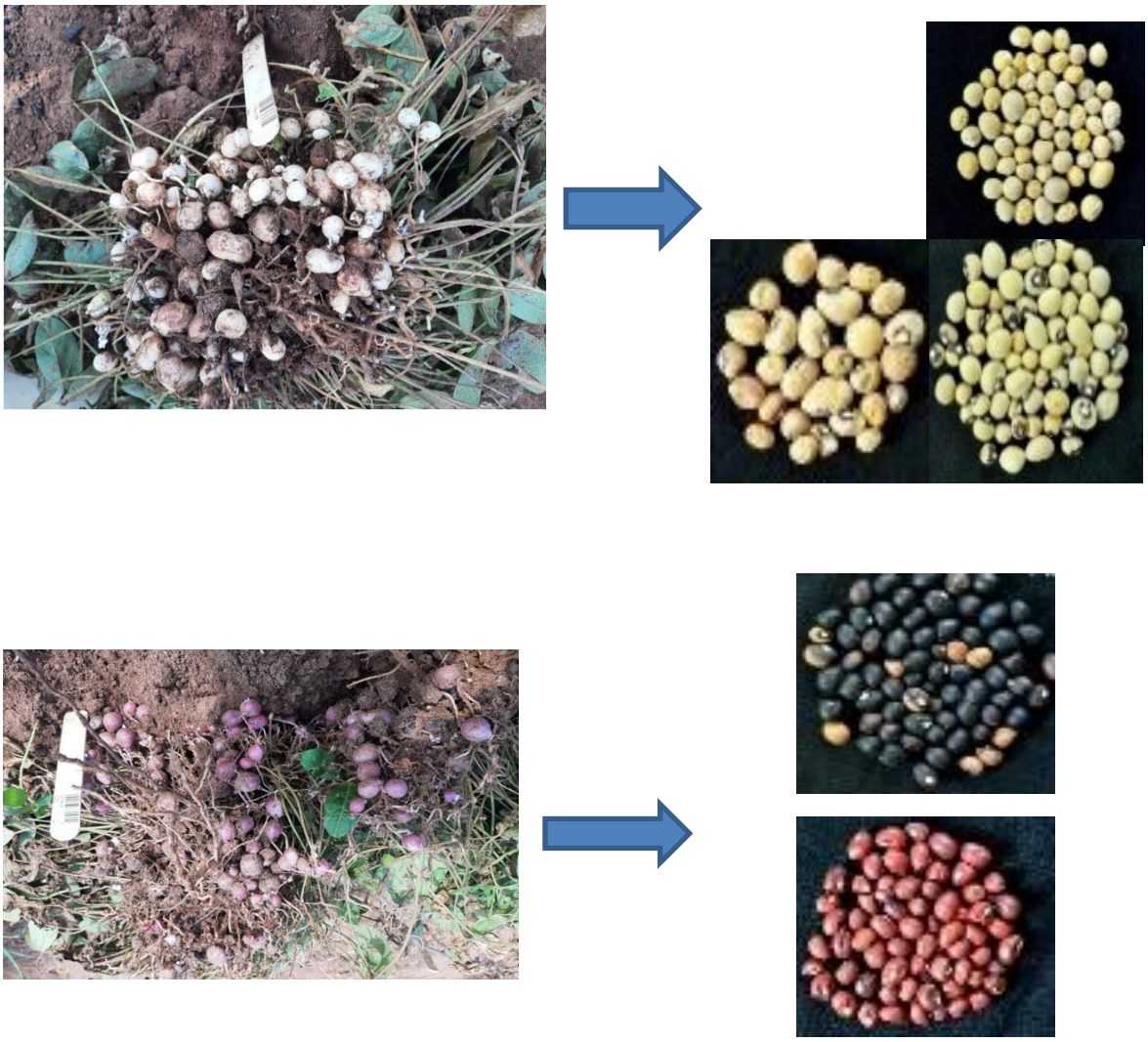


Plate 3.9.1. Examples of two common pod colors observed during harvest and the various seed color obtained after threshing.



Plate 3.9.2**.** Drying of Bambara groundnut at 7 ºC drying room to low moisture content before threshing.



(Photo taken by Ben.)

Plate 3.9.2b. Turning the net bags of Bambara groundnut for even drying of pods.

3.1.7. Genotyping and quality control

Leaf sampling, DNA extraction and quantification in preparation for Genome Wide Association Studies (GWAS).

Young Bambara groundnut leaves were harvested from each tagged plant three weeks after planting. The quality DNA was extracted from each accession at the Bioscience Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria using a modified CTAB protocol as described by Doyle, 1987. Quality and quantity DNA was extracted using 1% agarose gel and nanodrop200c spectrophotometer and was diluted to 50 nanogram per microliter for DArTseq analysis. 50mg samples of freshly collected young leaves were placed in a mortar and pestle and ground into a fine powder using liquid nitrogen. The powder was immediately placed in 2ml microtubes containing 1000µL of 2% CTAB extraction buffer (20mM EDTA, 0.1M Tris-hydrochloric pH8.0, 1.4M sodium chloride, 2% CTAB, plus 0.4% beta- mercaptol ethanol which was added just before use). The mixture was mixed properly ensuring that there were no lumps of the powdered leaves. It was then placed in a water bath to incubate at 65^{o}C for one hour, at 15min intervals, the mixture was gently mixed by inversion. 500 µL of chloroform- iso-amyl alcohol (24:1) was added to the mixtures in the tubes and was gently shaken for 10 minutes.

The tubes were placed in a centrifuge to spin down for 30 minutes at 13000rpm. The supernatant was then transferred to a fresh 2 mil tube, and 500 µL chloroform- isoamyl alcohol (24:1) was added to the supernatant and then mixed properly for another 10 minutes. The tube was then placed in a centrifuge to spin down for 10 minutes at 13000 rpm. The supernatant was then transferred to a fresh tube of 1.5 ml, 700 mL of ice-cold isopropanol stored in -20^{o}C for one hour was added to the solution and mixed gently by inversion for 30 minutes. It was placed in a -80^{o}C freezer for one hour. It was then centrifuged at 13000 rpm for 10 min, at this point it is possible to visualize the DNA pellet, usually white in colour, settling at the bottom of the tube. The liquid solution was carefully poured out of the tube and the DNA pellet was washed with 400 µL of 70% ethanol to eliminate salt residue that adhered to the DNA, and the DNA was set to dry until the next day, with the tubes inverted over a filter paper at room temperature. The pellet was then resuspended in 50 µL TE buffer consisting of 10 mM tris-hydrochloric pH 8.0, imM EDTA pH 8.0. 5 µL ribonuclease

(RNAse 10mgmL-1) was also added to each tube, this solution was incubated in an incubator at 37ºC for one hour, and after, stored at -20ºC.

3.1.8 Quantification of Bambara genomic DNA.

Agarose gel electrophoresis was used to quantify and test the quality of genomic DNA. Two 0.5% agarose tablets were placed in a beaker containing 100ml of TBE (trisborate EDTA) buffer, which was then placed in a microwave for about 15 minutes, the gel was left to cool and 5 µL of 10mg/ml safe view was added, the gel was swirled before pouring into the electrophoresis plate and the comb was gently removed. 2 µL of each sample was loaded onto a 1% agarose gel which was placed in 0.5XTBE (tris borate EDTA) buffer. In the first and the last well of the three lanes, 100ng/µL of DNA lambda marker was loaded. The DNA was then allowed to run in the gel for an hour, the gel was carefully placed in a trans illuminator, and the DNA picture was viewed on a computer screen attached to the illuminator. The nanodrop spectrophotometer was used to quantify the DNA.

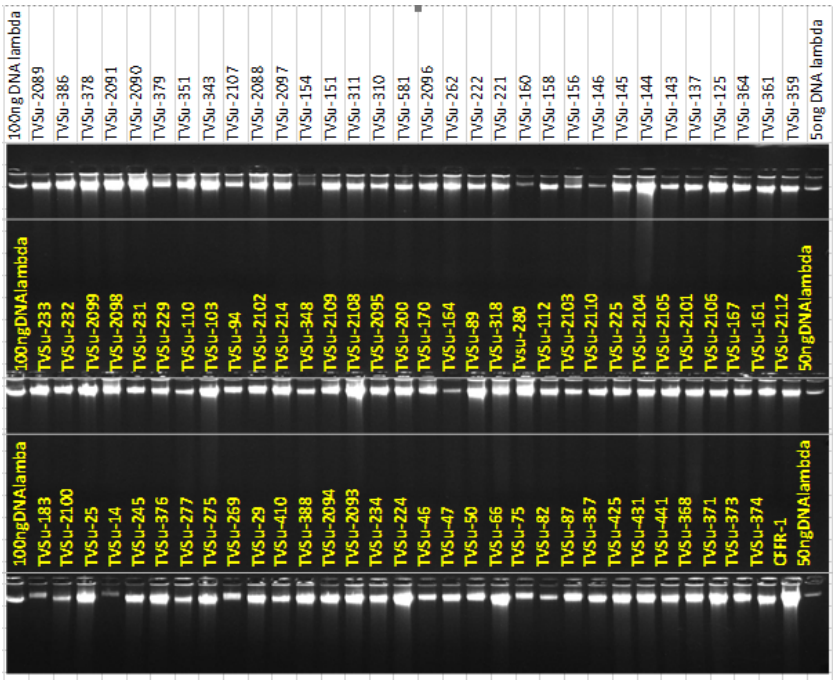


Plate 3.9.3a. Gel Picture of Bambara Groundnut

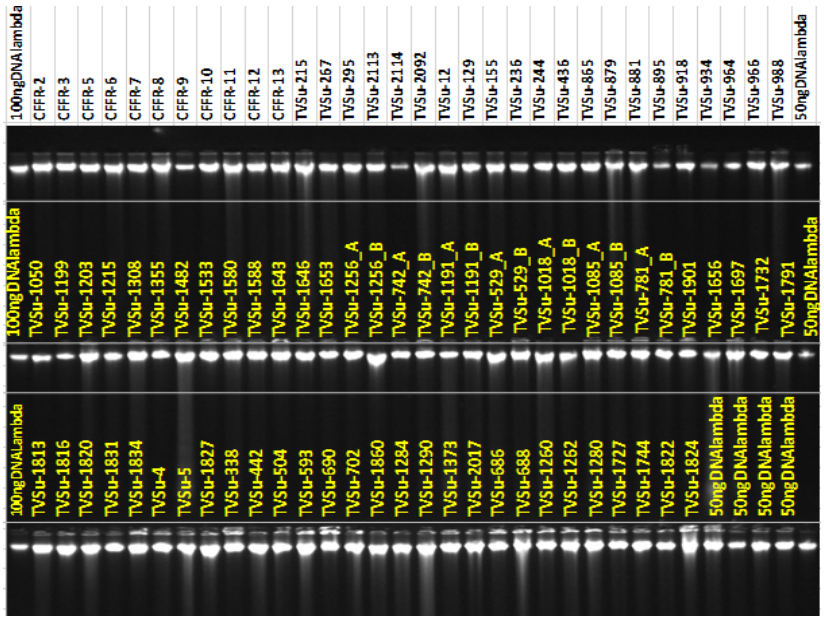
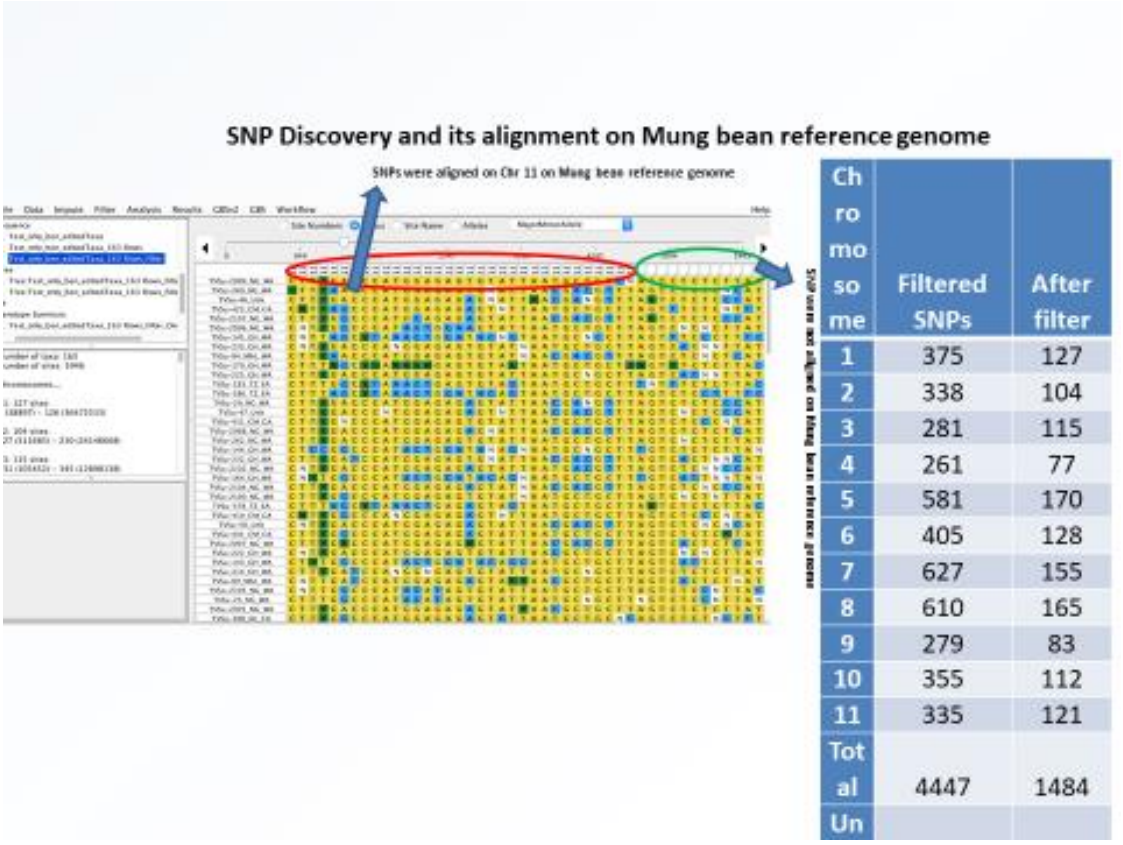


Plate 3.9.3b. Gel Picture of Bambara Groundnut

3.1.8. DArTseq SNP genotyping and SNP quality control

The high-quality DNA (100ng/µL) of 135 accessions was sent for genotyping-by- sequencing (GBS) at DArT Pvt Ltd, Canberra, Australia. SNP markers were generated using the next-generation high-throughput DArTseq approach. DArTseq is a genotyping-by-sequencing system which sequence genome-wide the most informative representation of DNA samples for SNP discovery using Next-Generation-Sequencing platforms Illumina HiSeq-2500 ((http://www.diversityarrays.com/dart-application-dartseq). This approach represents a combination of both complexity reduction and restriction enzymes method (Wenzel et al., 2004). The raw reads sequence generated in the FASTQ sequence files was further processed using proprietary DArT analytical pipeline known as DArT toolbox, which uses primary and secondary workflows (an automated genotypic data analysis program) to perform SNP calling and filtering and alignment of SNPs on reference genome of species or close relative species. The primary workflow analysis pipeline of DArT toolbox with DArTsoft14 algorithm utilize to remove poor quality sequence reads based on Q-scores and de-multiplexed individual genotypes by barcode, while secondary workflow analysis generates final SNP calling with several quality and information content including trimmed SNP read sequence, call rate, read depth, marker reproducibility, polymorphic information content (PIC) and observed heterozygosity (van Deventer *et al.,* 2020). The sequences were mapped to the reference genome of Mungbean_v6 (https://plants.ensembl.org/Vigna_radiata/Info/Index) (Kang *et al.,* 2014). A total of 24,386 SNPs was generated genome-wide in Bambara groundnut population. These 24.38K SNPs further filtered using call rate of ≥ 80%, and marker reproducibility > 95%, minor allele frequency (MAF) ≤ 0.01%, missing data ≤ 30% to remove bad SNPs. After SNP quality control, a total 5, 395 SNPs were finally retained for GWAS study.



Chromosome	Filtered SNPs	After filter
1	375	127
2	338	104
3	281	115
4	261	77
5	581	170
6	405	128
7	627	155
8	610	165
9	279	83
10	355	112
11	335	121
Total	4447	1484
Un		

Figure 3.4. Bambara groundnut SNPs Discovery and its alignment on mung bean reference genome.

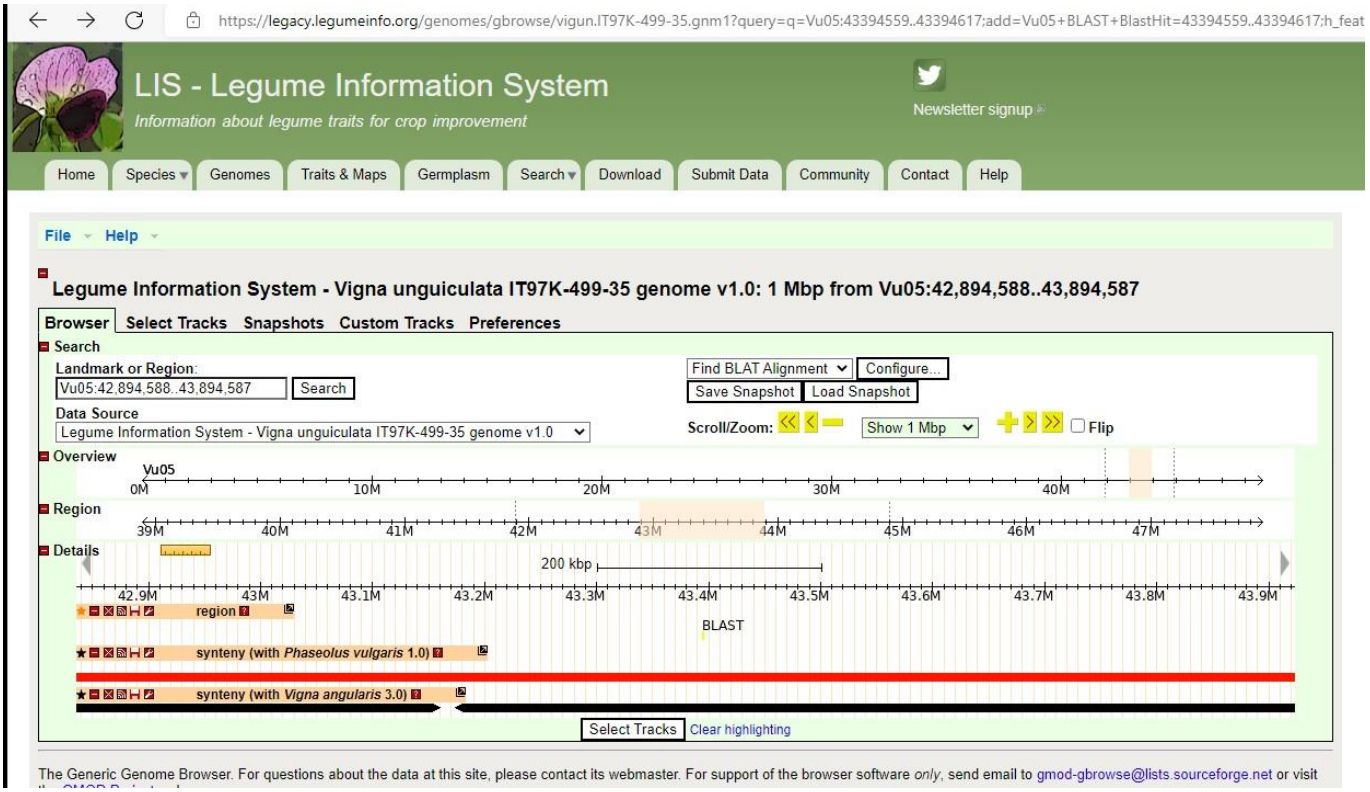


Plate 3.9.4. Blast search platform for the trimmed SNPs search.

CHAPTER FOUR

4.1. RESULTS AND DISCUSSION

Table 3.1 shows the rainfall records data for eleven years (2008-2018) obtained from Geospatial Information Systems Unit of the International Institute of Tropical Agriculture (IITA GIS) before the experiment was established. This record showed consistent monthly rainfall pattern but varied in volume over eleven years understudied. It is noteworthy that January, February, November, and December always possess very little or no rainfall for the eleven years' period. The highest rainfall that was recorded in those months was from November in the year 2010 at Ibadan with mean monthly rainfall of 5.4mm (Table 3.1). Others are either zero or very negligible (below 2.5mm). The analysis of the rainfall data showed a consistent trend in very low or no rainfall in November to February of every year across the three locations (Mokwa, Zaria and Ibadan). Therefore, having this fore knowledge of the rainfall pattern justifies the establishment of these trials in an open field condition. Figure 4.1 shows the history of rainfall pattern and volume (mm) in Mokwa, Zaria and Ibadan and the months of the years with the highest and the lowest rainfall for the period between 2008 and 2018. It can be inferred that every middle of October (last two weeks) can be the best period to establish drought experiment in these locations in Nigeria. Planting in mid- October of every year will give the plants some advantages of rainfall for crop establishment as the drought sets in before flower emergence. Figure 4 shows the trend of rainfall pattern for the eleven years' period at Ibadan, Mokwa and Zaria respectively.

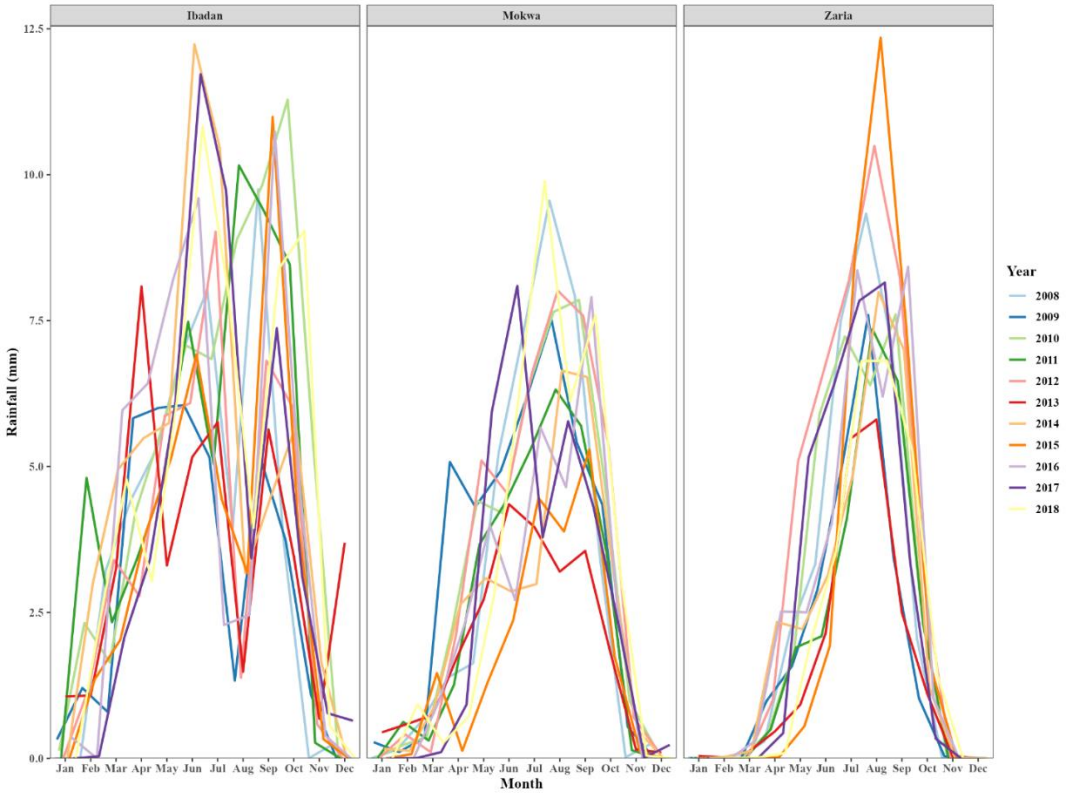


Figure 4. The figure above shows the history of eleven years rainfall trend in the three locations and the months with the least rainfall prior to the experiment establishment at respective locations. Each line represents the year and volume of rainfall in each month of the eleven years.

4.1.1. Meteorological data and soil analysis results

The rainfall (mm), temperature (°c), relative humidity (%), and soil nutrient compositions of the twelve sites were recorded from crop establishment until the experiments were terminated. The recorded data were analyzed and presented below.

Rainfall

Figure 4.1a shows the graphical representations of monthly total rainfall in Zaria, Mokwa and Ibadan in the year 2019/2020 and 2020/2021 respectively. From the figure presented below, Ibadan rainfall pattern in the two growing years is quite different from Zaria and Mokwa locations. In October 2019/2020 and 2020/2021 the volume of water available was higher in Mokwa and Zaria 2019/2020. (Table 4.1a). These two locations had at least (150 mm) volume of rainfall for two consecutive months of September and October whereas, it had a drastic decline in the subsequent months at both locations for those months. Mokwa 2019/2020 experienced more stable rainfall than other locations in that year followed by Zaria while Ibadan had the least. There were variations in Ibadan 2019/2020 and 2020/2021 as there was no rainfall at Ibadan 2020/2021 for a period of 4 months (Figure 4.1a).

Table 4. are the meteorological records of all locations taken during the crop establishment at Mokwa, Ibadan and Zaria. These records showed rainfall pattern and volume in the years 2019, 2020 and 2021, each from January to December. It showed that the month of October chosen for this experiment was the best month for the commencement of drought study in Bambara groundnut. Bambara groundnut planted from 20th of October allows the crop to enjoy the later rain for crop establishment. The rain ended before 30 days. Bambara groundnut flowers started to emerge 35 to 37 days in all locations before flower emergence. Then, drought was imposed at flowering stage at water stressed plots while water was supplied to the well-watered plots throughout the cropping cycle until physiological maturity. In all locations, September, October 2019, 2020, and 2021 had the highest amount of annual monthly rainfall while the month of November had the least rainfall in all locations throughout the year 2019/2020 and 2020/ 2021.(Table 4.1). It is important to note that the months of January and February are the months without any rainfall at all the three location in the two consecutive years. Table 4.

The mean monthly rainfall at Ibadan in the month of October 2019, 2020, and 2021 are 9.7 mm, 10.0 mm, and 6.8 mm respectively. (Table 4.1b. At the same location, mean monthly rainfall in November in the years 2019, 2020 and 2021 are 1.0 mm, 0.00 mm, and 0.00 mm respectively while in December, the volume of rainfall in mm are 0.3,0.00 and 0.00 mm respectively. Zaria had the mean monthly rainfall (mm) in October 2019, 2020, and 2021 as 4.99 mm, 3.10 mm and 0.55 mm. In November, it has 2.08 mm, 0.30 mm, and 0.00 mm respectively while it has 0.00 mm, 0.00 mm and 0.00 mm for the three years respectively in December. (Table 4.1c).

4.1.2. Relative humidity (%Rh)

The figures below showed the percentage relative humidity of all locations where the experiment was established in the years 2019/2020 and 2020/2021. Mokwa 2019/2020 and Mokwa 2020/2021 had the same pattern of relative humidity (Figure 4.1b). The values in percentage (%) decline as the rainfall declines in Mokwa and Zaria 2019/2020 and 2020/2021 off seasons. Ibadan 2019/2020 and Ibadan 2020/2021 did not follow this pattern. % Relative humidity in Ibadan was very high in the two years and the highest overall relative humidity was found in Ibadan 2020/2021 season {Figure 4.1b)}, From Ibadan 2020/2021, other environment cannot match up the least % relative humidity (90% found in Ibadan 2020/2021. (Figure 4.1b).

4.1.3. Temperature (◦C.)

Figure 4.1c below is the temperature readings during crop establishment at the three locations in the year 2019/2020 and 2020/2021. The temperature of all the environments was not stable. It had gradual increase especially in Ibadan and Mokwa 2019/2020 and 2020/2021. The peak of temperature was found in all environments to occur in February (figure. 4.1c). The highest temperature range was found in Ibadan between 29.2◦C and 37.5◦C followed by Mokwa 29.5◦C and 36.3◦C.

Table 4. Meteorological records of all locations taken during the experiment.

Location	Month	Rain(mm)	Temp (°c)	Rel Hum (%)	Location	Month	Rain(mm)	Temp (°c)	Rel Hum (%)
Zaria	Sept.2019	207.96	28.5	83.3	Mokwa	Sept.2020	225.42	29.6	83.8
Mokwa	Sep.2019	279.75	29.6	85.6	Ibadan	Sept.2020	76.5	34.5	98.7
Zaria	Oct.2019	154.6	28.3	83.4	Zaria	Oct.2020	95.96	28.6	76.3
Mokwa	Oct.2019	256.97	29.5	85.5	Mokwa	Oct.2020	101.85	31.4	77.1
Ibadan	Oct.2019	299.95	29.2	93.5	Ibadan	Oct.2020	312.75	30.5	94.8
Zaria	Nov.2019	62.49	30.6	61.8	Zaria	Nov.2020	9.04	27.4	69.8
Mokwa	Nov.2019	27.59	32.8	68.9	Mokwa	Nov.2020	0.89	34.0	61.1
Ibadan	Nov.2019	32.4	32.3	93.1	Ibadan	*Nov.2020	0	33.0	94.7
Zaria	*Dec.2019	0	28.8	43.5	Zaria	*Dec.2020	0	28.5	59.8
Mokwa	Dec.2019	1.95	32.9	53.8	Mokwa	*Dec.2020	0.02	34.9	52.8
Ibadan	Dec.2019	9	33.8	89.6	Ibadan	*Dec.2020	0	33.6	94.9
Zaria	*Jan.2020	0	29.3	35.9	Zaria	*Jan.2021	0	29.0	47.7
Mokwa	*Jan.2020	0	33.3	48.0	Mokwa	*Jan.2021	0.13	35.1	45.9
Ibadan	*Jan.2020	0	34.7	69.3	Ibadan	*Jan.2021	0	33.6	90.5
Zaria	*Feb.2020	0	32.6	28.5	Zaria	*Feb.2021	0	30.3	37.0
Mokwa	*Feb.2020	0.01	36.3	41.8	Mokwa	*Feb.2021	0.02	36.2	36.0
Ibadan	*Feb.2020	0	37.5	87.1	Ibadan	*Feb.2021	0	35.2	90.3
Zaria	Sept.2020	274.53	27.3	86.5	Ibadan	Mar.2021	54.3	33.8	96.5

*- *the months with little or no rainfall are denoted by asterisk.*

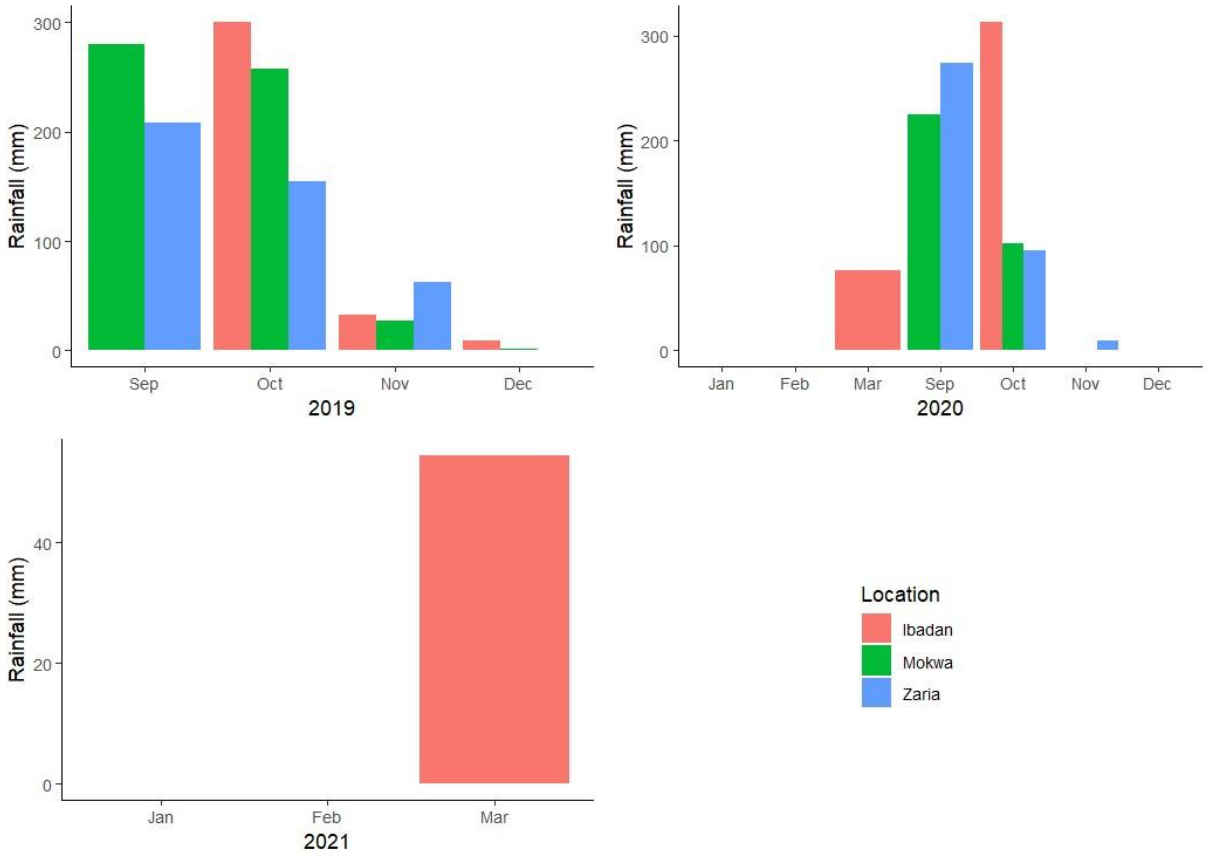


Figure 4.1a- Graphical representation of the volumes of rainfall and patterns in three locations in the year 2019/2020 and 2020/2021.

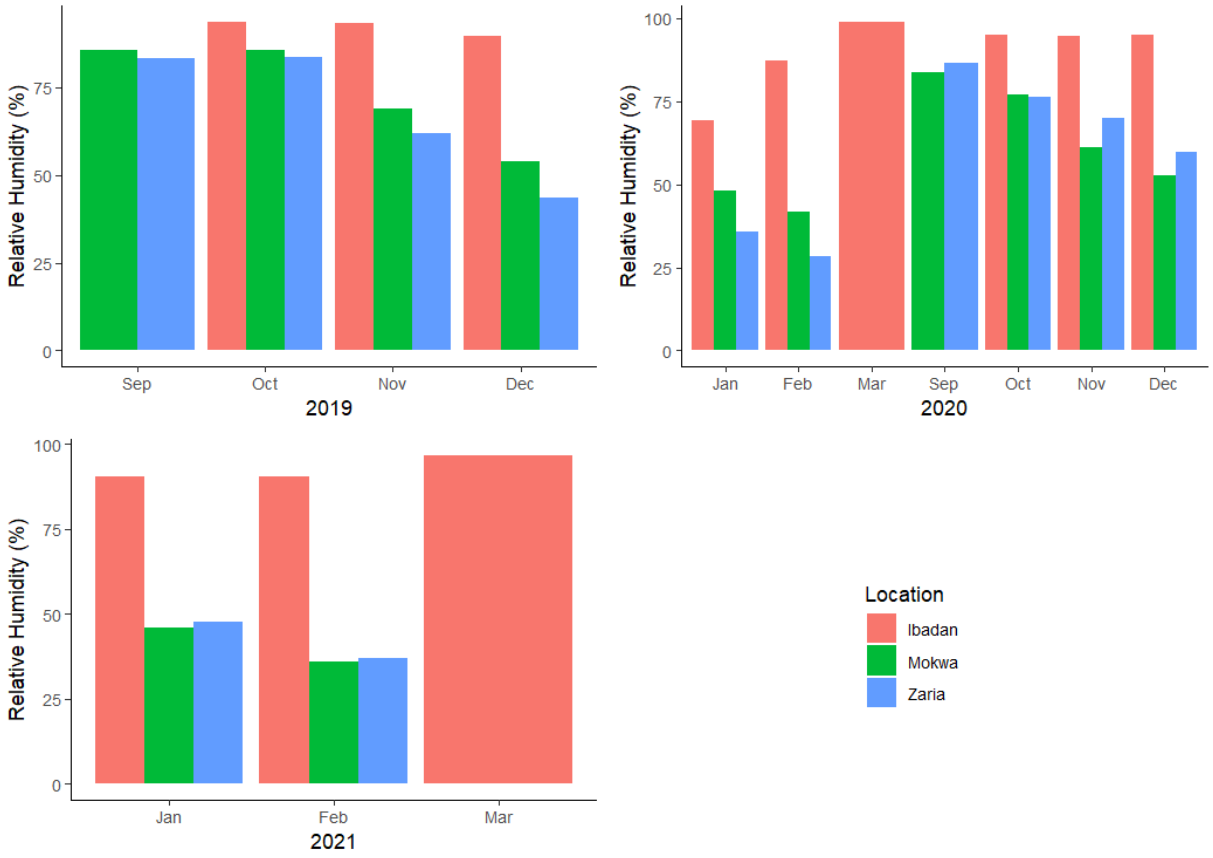


Figure 4.1b- Graphical representation of the % relative humidity in three locations in the year 2019/2020 and 2020/2021.

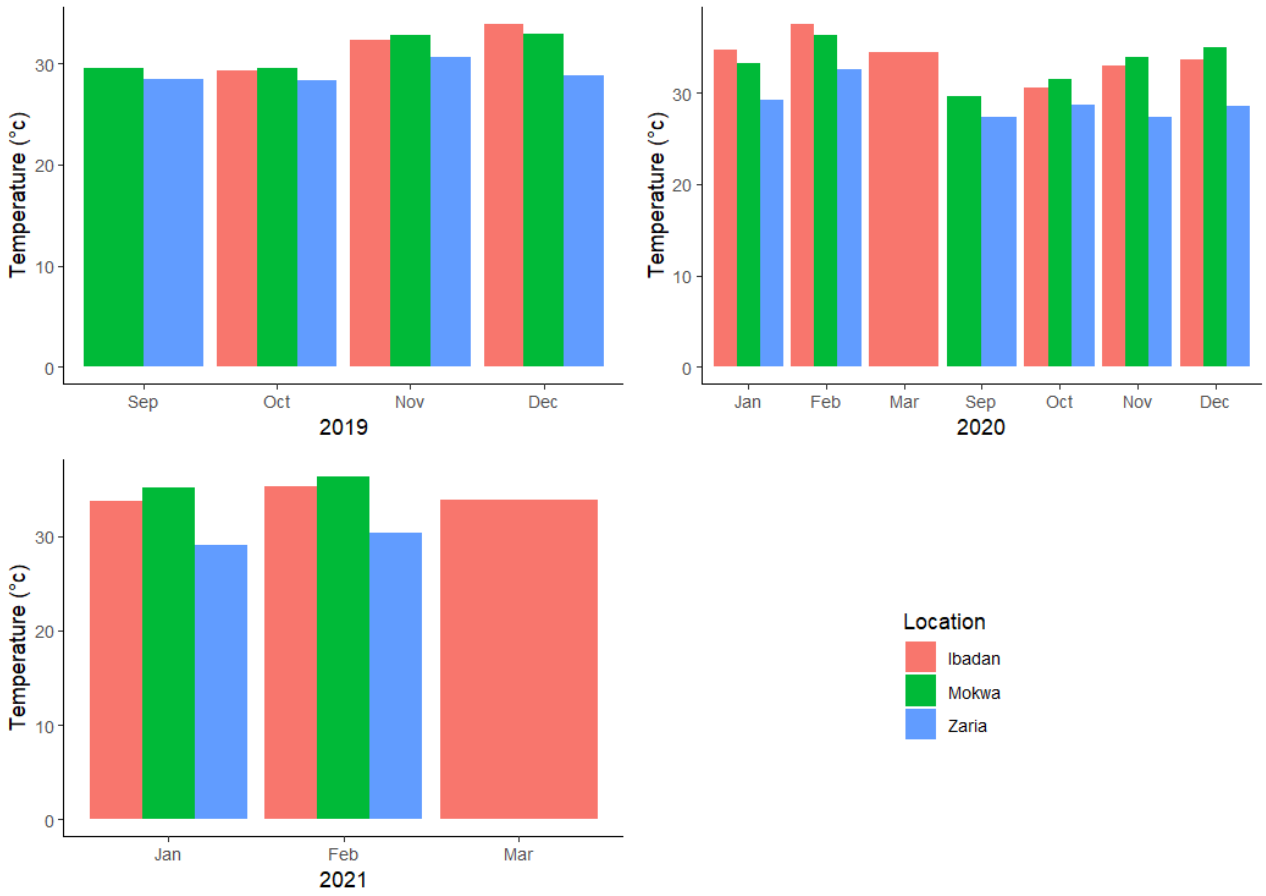


Figure 4.1c - Graphical representation of the temperature range in three locations in the year 2019/2020 and 2020/2021.

4.1.4. Soil analysis

Table 4.2a is the soil analysis results carried out by IITA soil analytical lab. Soil pH, textural investigation, soil organic matter (%), Cation Exchange Capacity (CEC) (Cmol/kg), %Nitrogen, extractable Phosphorus, Potassium (cm/kg), Calcium (cm/kg), and Magnesium (cm/kg), are analyzed.

Table 4.2a Soil Analysis of Experimental Sites

Location samples	***pH(H_2O) 1:1***	***%OC***	***%N***	***CEC (cmol/kg)***	***%Sand***	***%Silt***	***%Clay***
Ibadan	4.696	0.371	0.089	6.312	72.000	18.000	10.000
Mokwa	4.890	0.253	0.020	1.690	39.000	49.000	12.000
Zaria	6.460	0.051	0.076	5.493	16	24	49.000

Year	Location	P (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	Ca (cm/kg)	Mg (cm/kg)	K (cm/kg)	Na (cm/kg)
2019	Ibadan	34.82	188.12	8.69	81.03	208.89	2.90	1.09	0.24	0.14
2019	Mokwa	13.15	10.67	4.08	151.22	103.34	1.53	0.85	0.18	0.14
2019	Zaria	1.10	12.01	1.77	54.71	216.29	1.93	0.83	0.26	0.14
2021	Zaria	1.66	13.34	1.77	37.97	175.56	1.32	0.55	0.24	0.11
2021	Ibadan	23.80	108.07	5.23	291.59	157.04	2.51	0.93	0.26	0.13
2021	Mokwa	13.57	9.34	3.50	76.25	71.86	1.25	0.84	0.14	0.13

P- Phosphorus, ppm- parks per mole, cm/kg- centimeter per kilogram, Cu- Copper, Mn- Manganese, Fe- Iron, Ca- Calcium, Mg- Magnesium, Na- Sodium. %N- percentage Nitrogen.

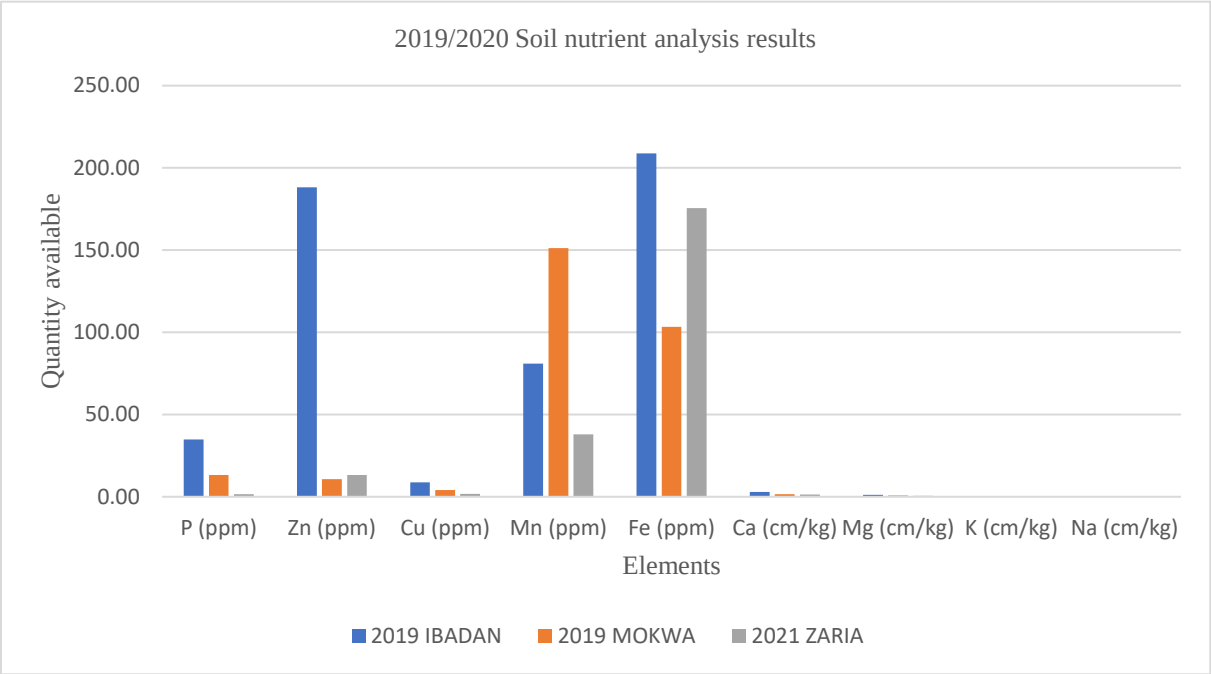


Figure. 4.9.3. Summary of the soil nutrient results at the three locations in the year 2019/ 2020.

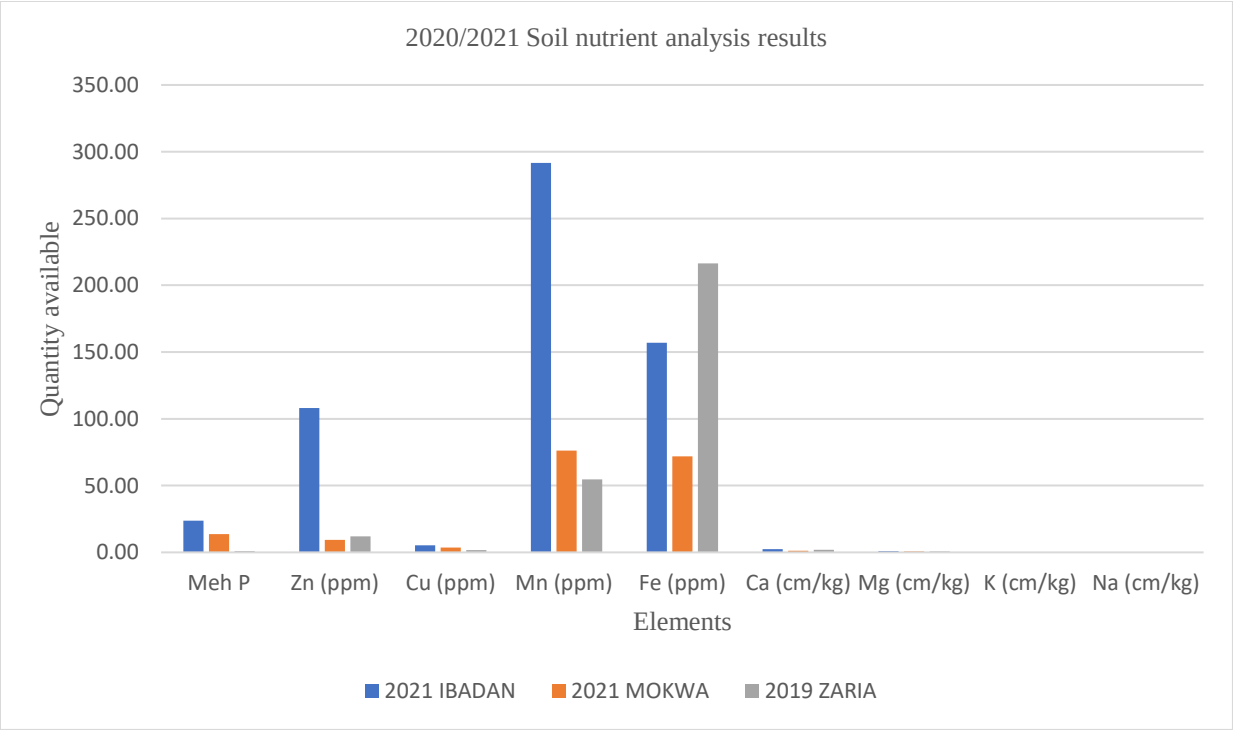


Figure. 4.9.4. Summary of the soil nutrient results at the three locations in the year 2020/2021

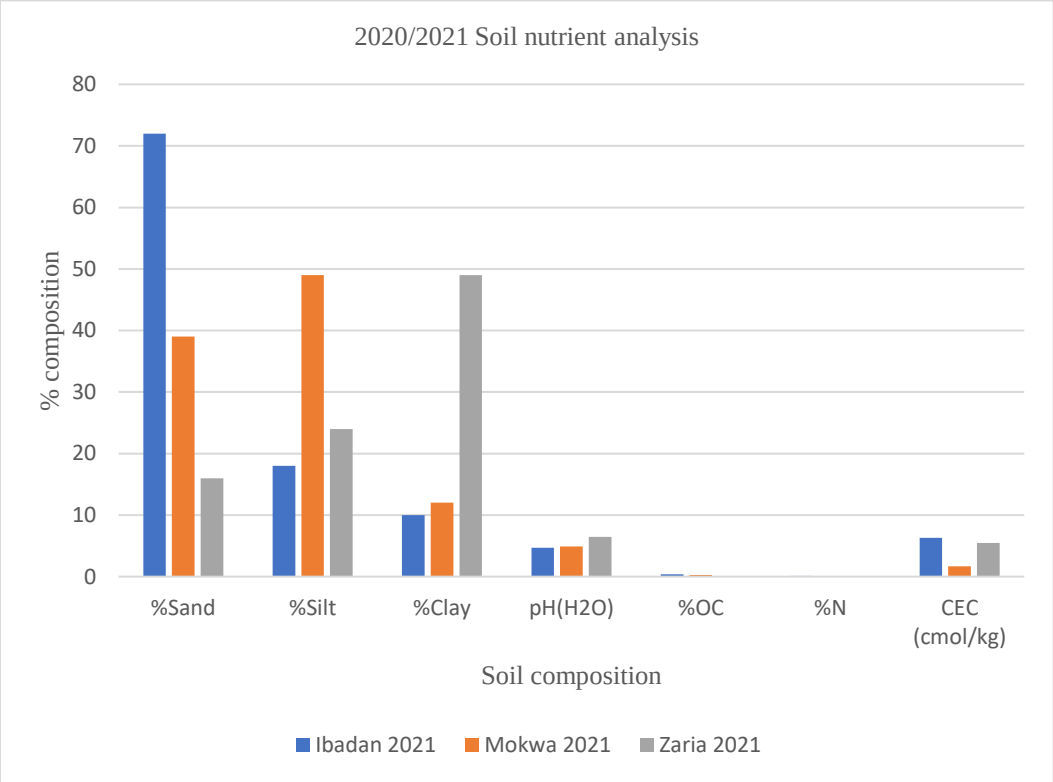


Figure.4.9.5. Summary of the soil nutrient results at the three locations in the year 2020/2021.

General comparison of the three sets of Bambara groundnut based on the total mean seed yield.

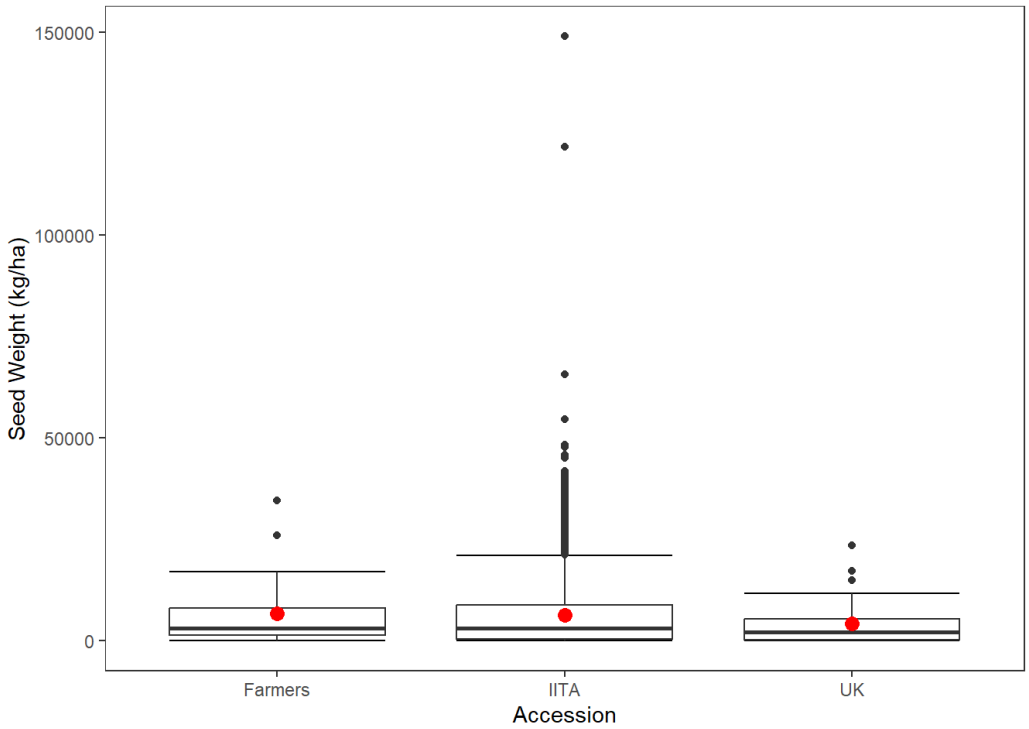


Figure 4.9.6. Comparison of the three sets of Bambara groundnut accessions used in this experiment.

The above is a graphical comparison of the three sets of Bambara groundnut accessions used in this experiment. Twelve accessions from Crops for the future, fourteen landraces newly collected from farmer's fields from the middle belt in Nigeria and one hundred and ten accessions from IITA gene bank. (Table 3.2 and 3.3)
The combined mean seed weight of all accessions was used for the ANOVA to understand the significant difference of yield performance of each set.

4.2b. ANOVA results and significant level across the three sets of Bambara groundnut accessions.

Set of Bambara G. nut	Number	Yield (Kg)	(P≤0.05)
Farmers	15 accessions	6834.023	a
IITA	109 accessions	6380.843	a
UK	12 accessions	4213.448	a

Mean yield (Kg) with the same letter is not significantly different.

4.2. Phenotypic data analysis

The data generated from this study arose from multi environmental drought trials. Data were scored from twelve environments: Table 3.6. (3 locations * 2 regimes * 2 years). Among traits scored in this study, five yield and seed related traits were used for both phenotypic and genotypic analysis. These are number of pods per plant, number of seeds per plant, pod weight, seed weight, and shell weight. The data from each site were first analyzed separately for each character using a linear mixed model which includes row and column effects. The resulting BLUPS (Best linear unbiased prediction) and BLUEs (Best linear unbiased estimator) values for each genotype were used to fit all the environment in which environment were treated as random effects. The significance of both random and fixed effects was assessed using Wald's test (Wald 1943). The phenotypic data were subjected to both BLUEs and BLUPs. While BLUPs are used for the field evaluation report on drought, the BLUEs was used for the GWAS study (appendix IX). The broad sense heritability was estimated using formula-
H2 = σˆ 2g/(σˆ 2g+σˆ 2ge/t+σˆ 2e/rt)h^2 =σ^g 2/(σ^g 2+σ^ge 2/t+σ^e 2/rt) where σˆ 2gσ^g 2 is genotypic variance, σˆ 2geσ^ge 2 denotes the interaction variance between genotypes and environment and σˆ 2eσ^e 2 experimental error variance. t and r are the numbers of environments and replications within an environment, respectively (Smith *et al*., 2005). All phenotypic analysis was done using R statistical software (R Core Team, 2022).

4.3. Response of 135 accessions of Bambara groundnut to drought.

There were significant differences in the performances of the 135 accessions of Bambara groundnut with reference to the significant mean square values for the 12 traits studied over a period of two years under water stress and well water condition (Table 4.3). Highly significant differences ($p<0.001$) were obtained for Days to 50% flower, plant spread, plant height, leaf length, leaf width, pod per plant, number of seeds per plant seed weight per plant and one hundred seed weight. Significant differences ($p<0.001$) were also observed among the 135 accessions for some reproductive traits (Table 4.3). Across environments, the response of the traits depends on either of the two levels or regimes (WW and WS).

Table 4.4 presents the mean, minimum, maximum, heritability, standard error, genotypic variance, and least significant difference values of the performances of 135 Bambara groundnut genotypes. The stress was imposed at flower emergence and both regimes were understudied throughout the growth and developmental cycle. Significant difference was observed in regimes ($P \leq 0.05$). Genotypes also respond differently under the two

regimes (P ≤0.05).

Table 4.3 Mean squares of the analysis of variance (ANOVA) for yield component of 135 accessions of Bambara groundnut measured across three sites under water stressed and well - watered conditions in 2019/2020 planting seasons.

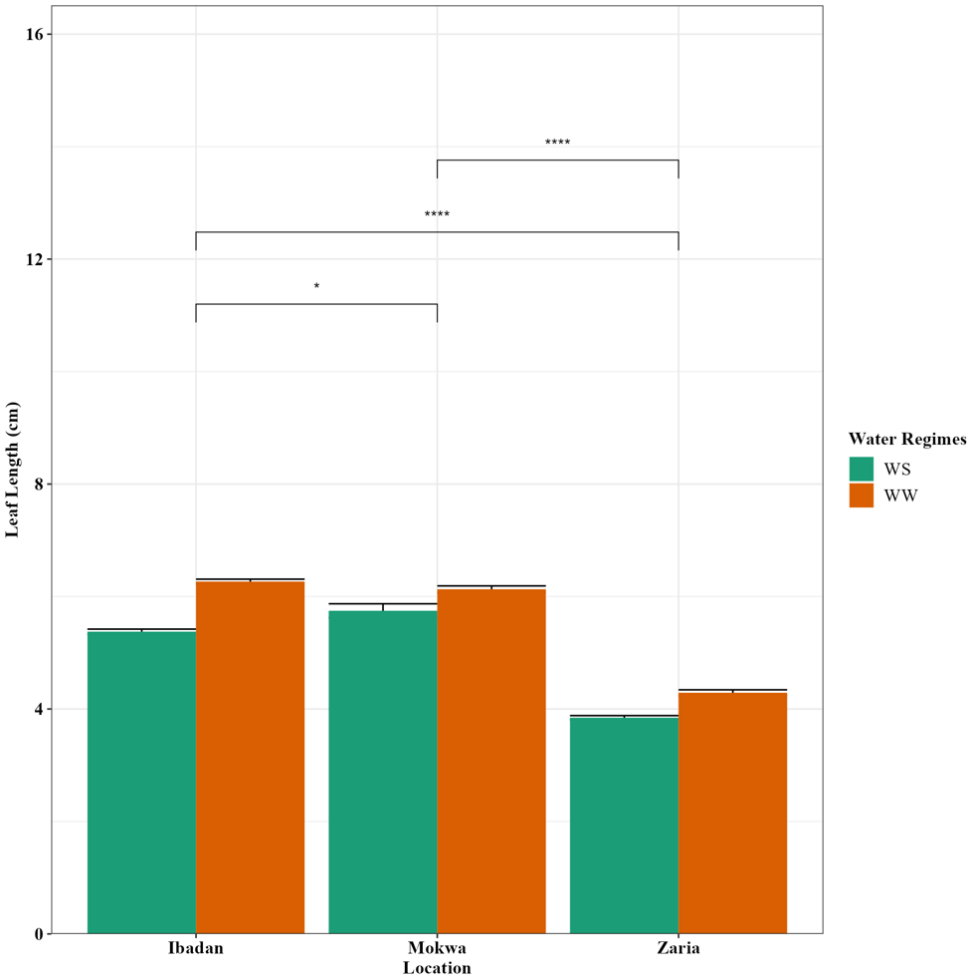
Source	DF	Days to flower	Days to 50% flower	Plant spread(cm)	Plant height(cm)	Leaf length (cm)	Leaf width(cm)
Replicate	2	0.3	$76.6^{0.001}$	$1030.7^{0.001}$	$495.57^{0.001}$	$6^{0.001}$	$2.42^{0.001}$
Accn	134	$19.8^{0.001}$	$33.4^{0.001}$	$226.9^{0.001}$	$61.4^{0.001}$	$6.9^{0.001}$	$1.85^{0.001}$
Locations	2	$8246^{0.001}$	$14673.2^{0.001}$	$14416.2^{0.001}$	$551.64^{0.001}$	$7039.6^{0.001}$	$1624.89^{0.001}$
Water regimes	1	0.8	4.3	$3677.8^{0.001}$	$899.75^{0.001}$	$74.6^{0.001}$	$8.33^{0.001}$
Accession: Location	247	$11.2^{0.001}$	$14.5^{0.001}$	$90.5^{0.001}$	$19.99^{0.001}$	$1.1^{0.001}$	$0.57^{0.001}$
Accession:Regimes	133	3.4	5.9	38.4	12.77	0.5	0.17
Location: Regimes	2	$69.6^{0.001}$	$114.3^{0.001}$	$328.9^{0.01}$	$192.91^{0.001}$	$17.3^{0.001}$	$2.42^{0.001}$
Accn:Location:Regime	242	3.6	6.3	49.9	9.75	0.5	0.19
CV	1318	4.71	4.99	22.24	19.89	18.63	28.65

Significant difference (0.1, 0.01and 0.001 levels of probability). Ns = not significant. Accn- Accessions

ANOVA contd.

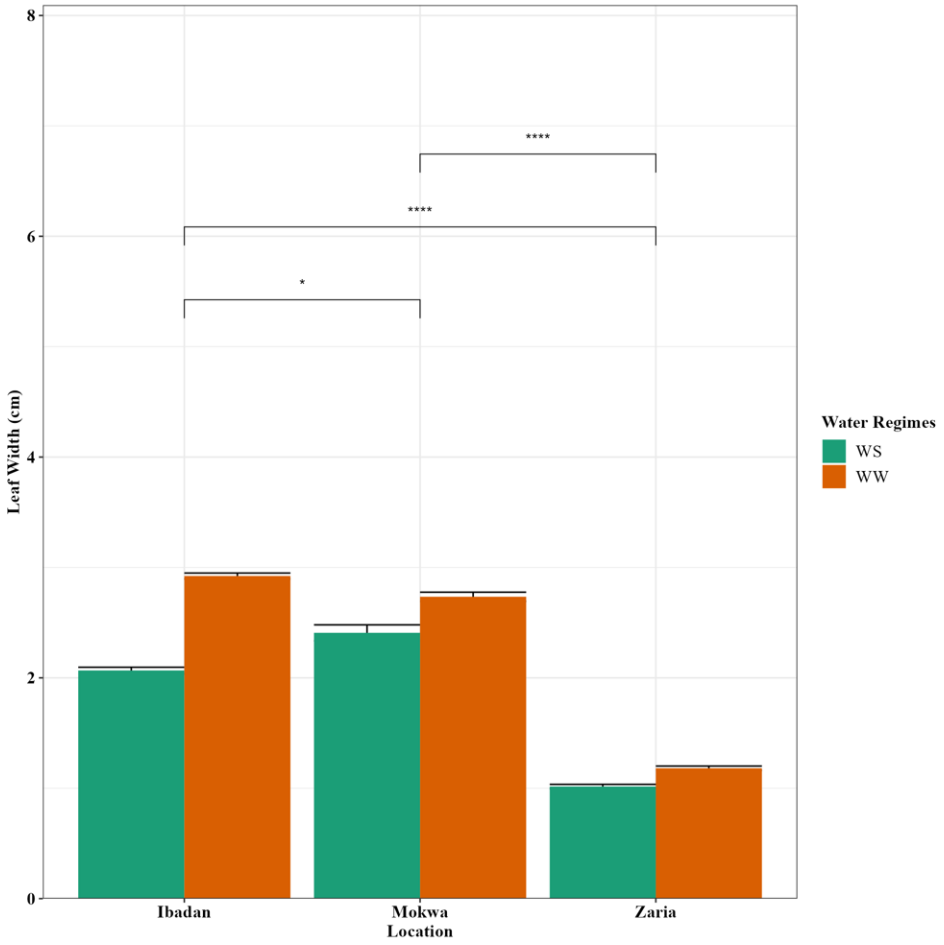
Source	DF	Pod/plant	Pod weight (g)	Nb of seeds	Seed weight (g)	Shell weight (g)	SW100
Replicate	2	$1972.6^{0.001}$	25320	$1905.8^{0.001}$	$462.45^{0.001}$	$57.54^{0.1}$	$2680.7^{0.001}$
Accession	134	$273.2^{0.001}$	8789	$225^{0.001}$	$98.95^{0.001}$	$39.43^{0.001}$	$482.9^{0.001}$
Locations	2	$18715.5^{0.001}$	$180659^{0.001}$	$4638.2^{0.001}$	$1825.27^{0.001}$	$1252.4^{0.001}$	$3320.2^{0.001}$
Water regimes	1	$2477.9^{0.001}$	11208	$882.8^{0.01}$	$395.54^{0.01}$	34.45	$3058.7^{0.001}$
Accn: Location	219	$265.9^{0.001}$	$12037^{0.001}$	$212^{0.001}$	$95.97^{0.001}$	$36.45^{0.001}$	$290.8^{0.001}$
Accn:Regimes	130	$249.6^{0.001}$	4105	$168.8^{0.001}$	$63^{0.001}$	15.33	194.1
Location Regimes	2	$3618.4^{0.001}$	6625	$1695.9^{0.001}$	$239.51^{0.01}$	$65.73^{0.01}$	$2138.7^{0.001}$
Accn: Location: Regime	92	$227.4^{0.001}$	10888	$169.3^{0.001}$	$109.94^{0.001}$	$26.31^{0.001}$	178.9
CV		86.2	456.4	92.25	132.65	165.42	38.87

Significant difference (0.05, 0.01and 0.001 levels of probability). Ns = not significant, Accn- Accessions



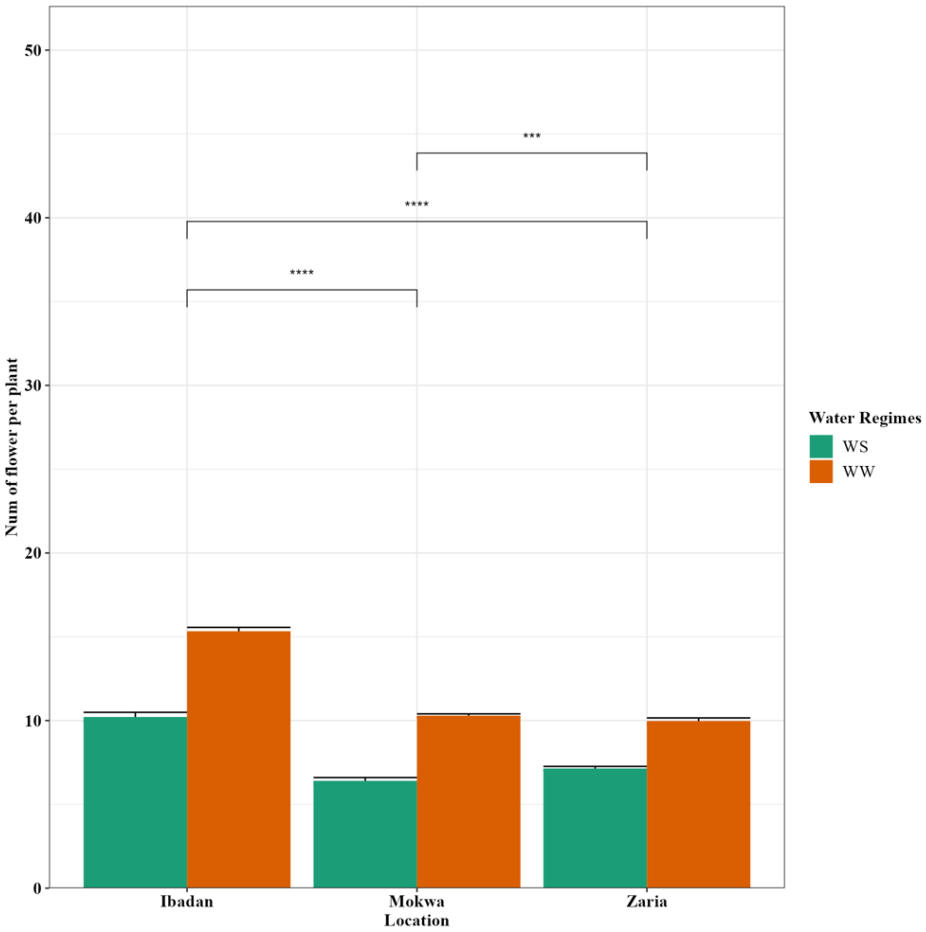
*, ***, Significant difference at 0.05, 0.0001 levels of probability respectively.

Figure 4.9.7. Multiple comparisons of leaf length across the three environments under two water regimes.



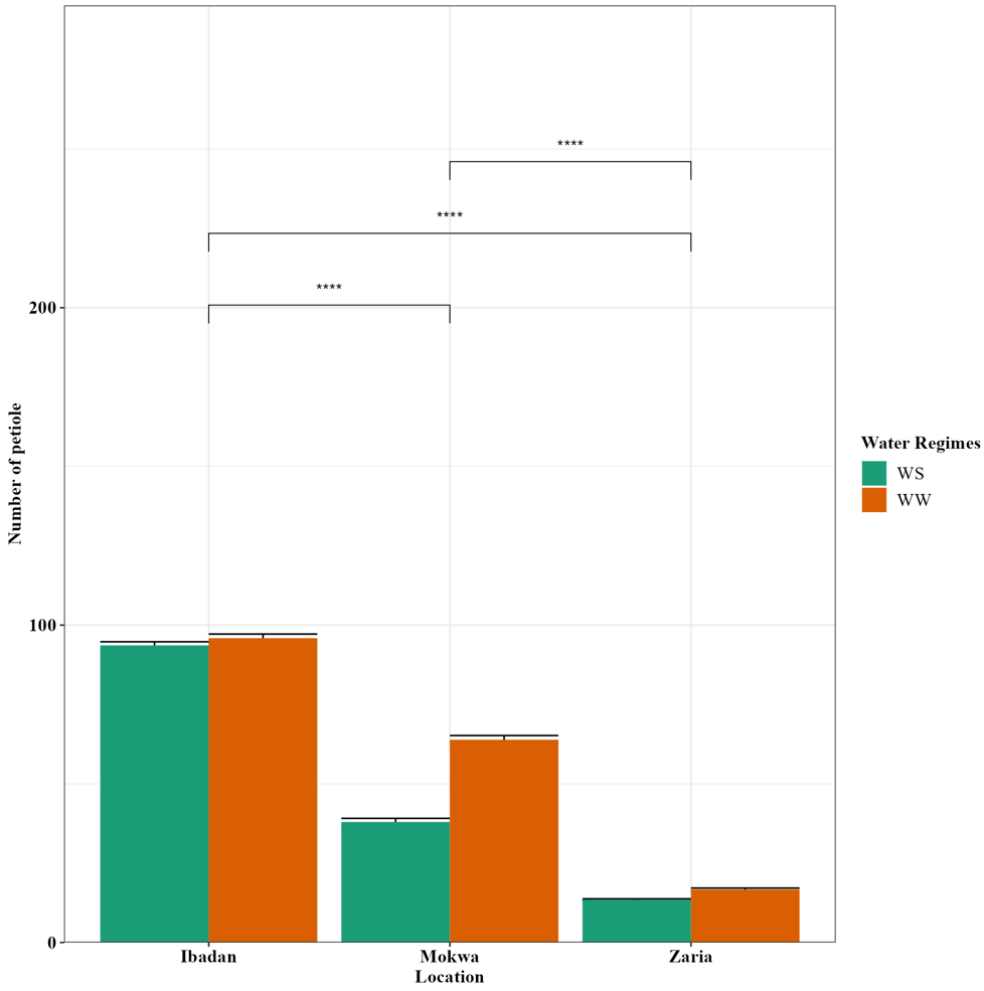
*, ****, Significant difference at 0.05, and 0.0001 levels of probability respectively

Figure 4.9.8. Multiple comparisons of leaf width across the three environments under two water regimes.



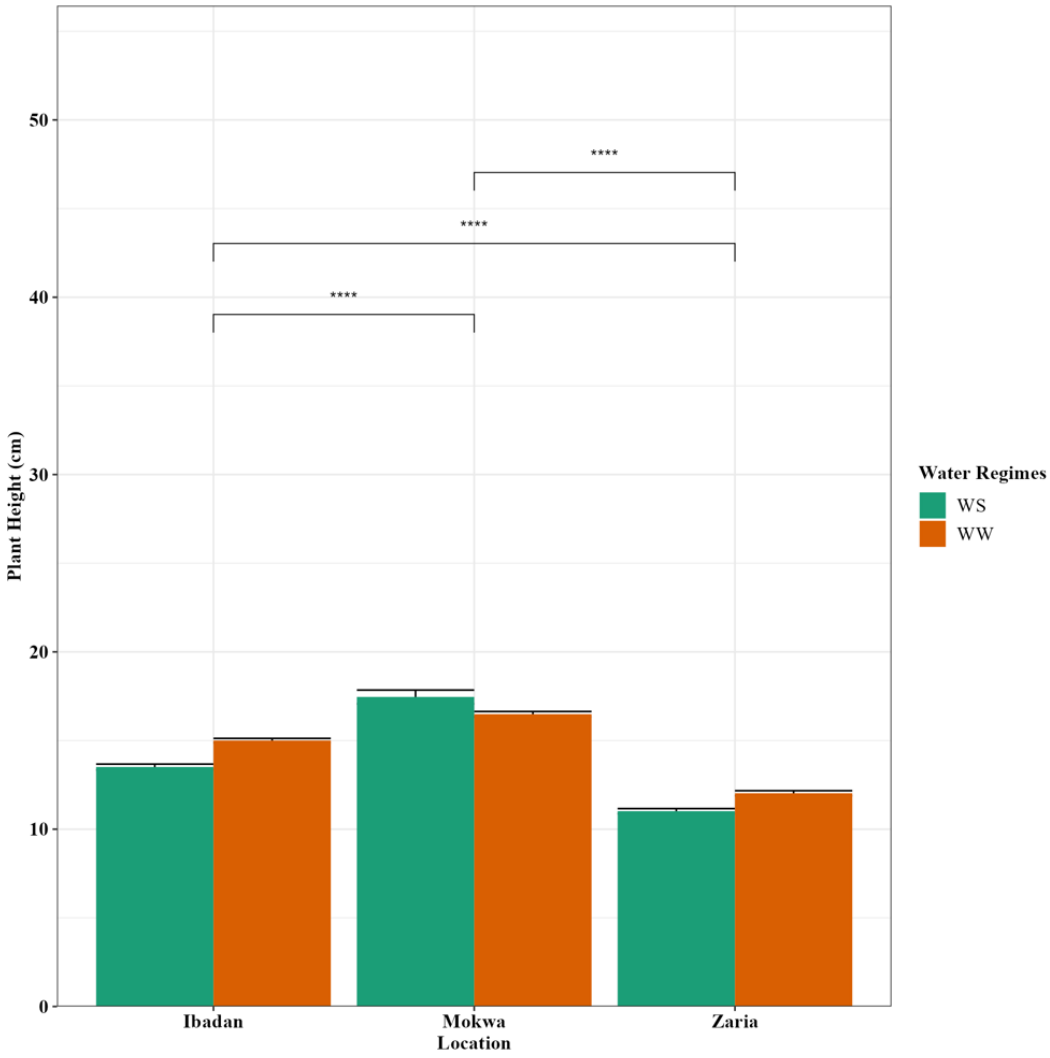
, *, Significant difference at 0.001, and 0.0001 levels of probability respectively

Figure 4.9.9. Multiple comparisons of the number of flowers across the three environments under two water regimes.



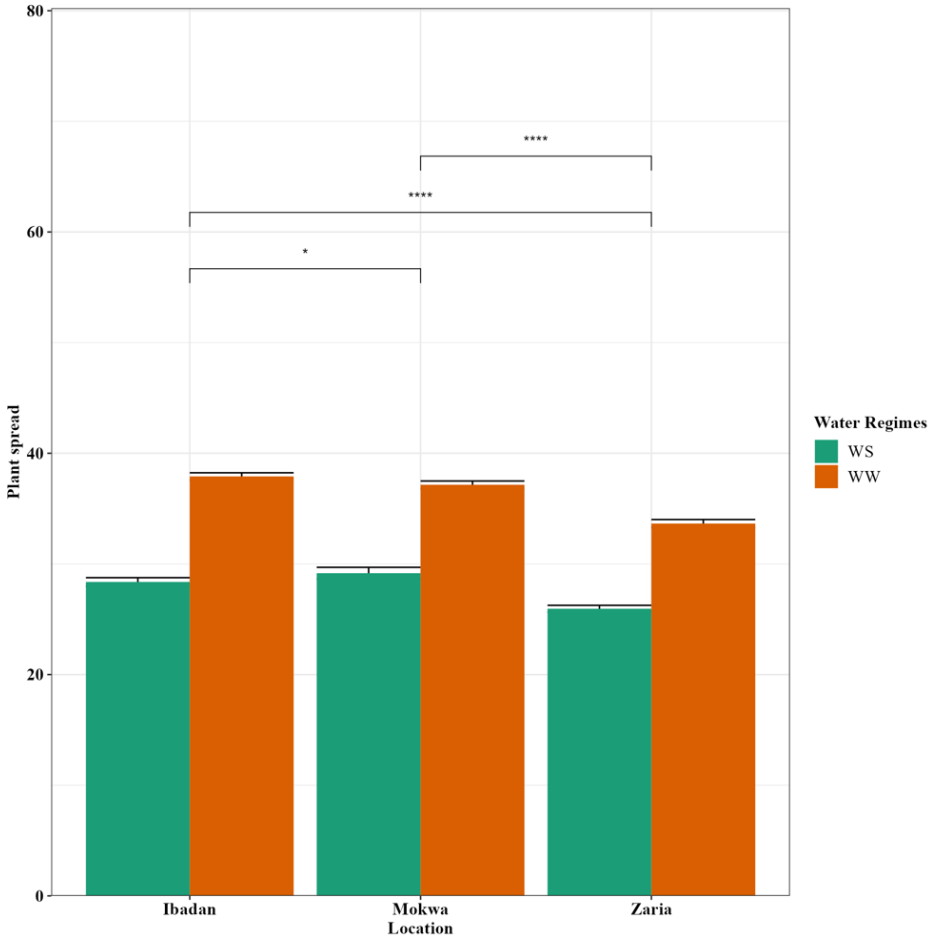
**** Significant difference at 0.0001 levels of probability.

Figure 5.0. Multiple comparisons of the number of petioles across the three environments under two water regimes.



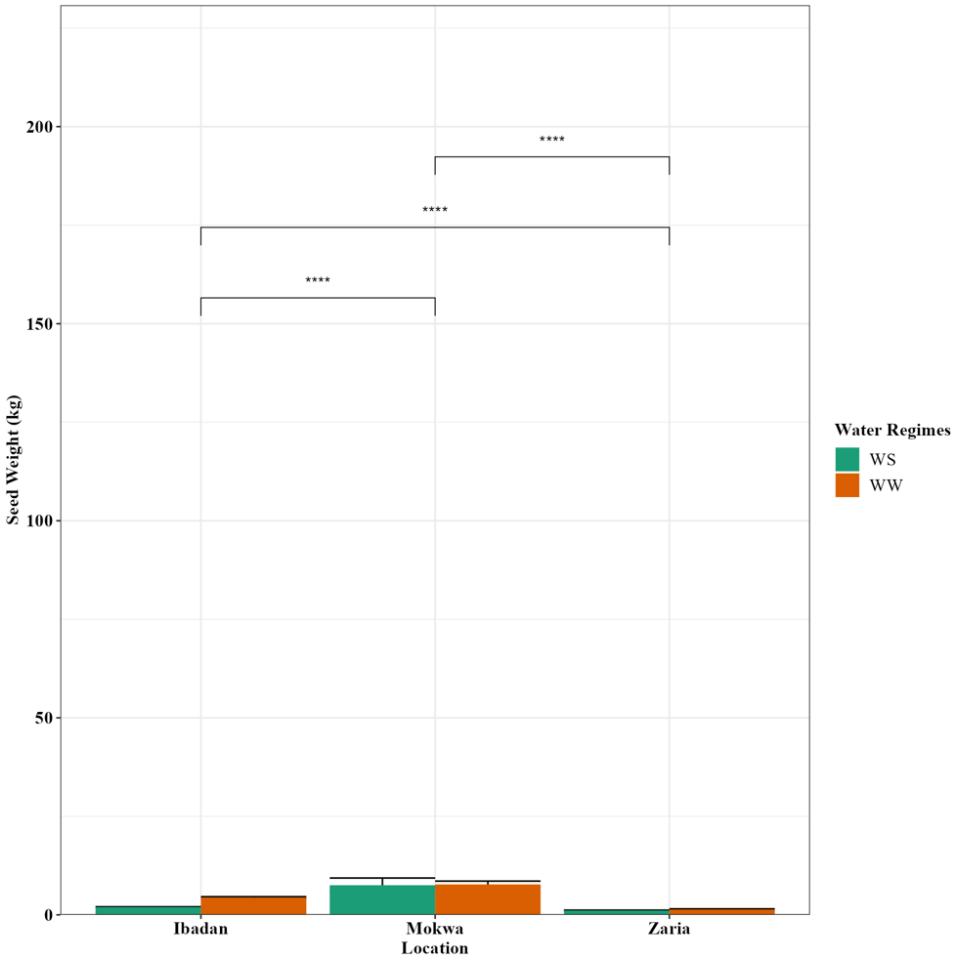
**** Significant difference at 0.0001 levels of probability.

Figure 5.1. Multiple comparisons of plant height of Bambara across the three environments under two water regimes.



*, **** Significant difference at 0.05 and 0.0001 levels of probability.

Figure 5.2. Multiple comparisons of plants spread across the three environments under two water regimes.



****, 0.0001. Seed weight with level of significant across locations

Figure 5.3. Multiple comparisons of seed weight across the three environments under two water regimes.

Table 4.4 Genotype mean, minimum, maximum, heritability, standard error, genotypic variance,and least significant difference values for both regimes in the twelve test environments.

Environments	Traits	Mean	Min.	Max.	H2	SE	Gen.Variance.	LSD
Iba-WW-19	First flower	40.65	40.65	40.65	0.00*	0.08	0.00	0.00
Iba-WS-19	First flower	40.06	39.86	40.63	0.18	0.39	0.18	1.08
Iba-WW-19	50% flower	42.91	42.54	43.24	0.23	0.28	0.10	0.78
Iba-WS-19	50% flower	43.17	41.88	45.59	0.29	1.31	2.37	3.67
Iba-WW-19	Leaf length	6.15	5.12	7.91	0.76	0.28	0.33	0.79
Iba-WS-19	Leaf length	5.41	4.99	6.00	0.24	0.30	0.11	0.83
Iba-WW-19	Leaf width	2.93	2.32	4.57	0.77	0.26	0.18	0.58
Iba-WS-19	Leaf width	2.63	2.07	3.65	0.49	0.20	0.14	0.74
Iba-WW-19	Plant height	18.17	16.54	20.25	0.35	1.08	1.79	3.04
Iba-WS-19	Plant height	17.71	16.74	19.99	0.20	0.93	1.04	2.58
Iba-WW-19	Plant spread	34.84	31.71	38.68	0.36	1.91	5.59	5.36
Iba-WS-19	Plant spread	32.91	32.91	32.91	0.00*	0.52	0.00	0.01
Iba-WW-19	Pod plant	7.14	6.26	9.16	0.15	1.19	1.57	3.28
Iba-WS-19	Pod/plant	6.34	5.28	8.69	0.17	1.83	2.30	3.90
Iba-WW-19	Pod weight	5.42	4.22	8.82	0.24	1.31	2.15	3.63
Iba-WS-19	Pod weight	3.08	2.44	4.13	0.19	0.71	0.61	1.98
Iba-WW-19	Seed/plant	8.12	6.26	12.67	0.29	1.86	4.79	5.22
Iba-WS-19	Seed/plant	7.06	6.40	8.46	0.09*	1.17	1.38	3.18
Iba-WW-19	Seed weight	3.65	2.81	5.68	0.25	0.87	0.96	2.41
Iba-WS-19	Seed weight	2.22	1.8	2.90	0.17	0.48	0.27	1.33
Iba-WW-19	Shell Weight	1.56	1.12	3.10	0.29	0.22	0.29	1.29
Iba-WS-19	Shell weight	0.83	0.67	1.24	0.17	0.46	0.06	0.62
Iba-ww-21	First flower	44.24	44.06	51.85	0.75	0.45	0.82	1.27
Iba-ws-21	First flower	43.8	43.22	48.27	0.71	0.45	0.70	1.26
Iba-ww-21	50% flower	44.72	44.14	56.93	0.80	0.72	2.56	2.03
Iba-ws-21	50% flower	44.51	43.26	57.37	0.82	0.74	3.07	2.08
Iba-ww-21	Leaf length	6.07	5.15	7.05	0.67	0.27	0.22	0.77
Iba-ws-21	Leaf length	6.38	5.17	7.64	0.78	0.27	0.33	0.76
Iba-ww-21	Leaf width	2.73	2.05	4.17	0.82	0.17	0.15	0.47
Iba-ws-21	Leaf width	2.74	1.94	4.38	0.89	0.14	0.19	0.40
Iba-ww-21	Plant height	14.96	11.16	19.43	0.71	0.82	2.33	2.31
Iba-ws-21	Plant height	14.95	11.56	21.16	0.69	0.90	2.55	2.53
Iba-ww-21	Plant spread	38.00	32.44	43.25	0.57	2.31	12.36	6.51
Iba-ws-21	Plant spread	30.92	24.04	36.32	0.58	2.37	13.34	6.67
Iba-ww-21	Pod/plant	12.89	7.22	27.74	0.55	3.20	22.34	8.94
Iba-ws-21	Pod/plant	6.16	3.12	11.76	0.48	1.83	6.39	5.17
Iba-ww-21	Pod weight	17.39	10.28	40.89	0.47	5.55	57.06	15.52
Iba-ws-21	Pod weight	4.22	3.35	5.49	0.19	0.93	1.00	2.54
Iba-ww-21	Seed/plant	11.09	5.57	19.44	0.52	2.81	15.96	7.79
Iba-ws-21	Seed/plant	5.66	4.55	8.26	0.25	1.29	2.15	3.60
Iba-ww-21	Seed weight	4.28	2.01	7.96	0.44	1.23	2.60	3.40
Iba-ws-21	Shell weight	1.70	1.40	2.18	0.17	0.42	0.19	1.11
Iba-ww-21	Shell weight	0.59	0.46	0.95	0.23	0.18	0.04	0.49
Iba-ws-21	Seed weight	1.86	1.52	2.49	0.17	0.38	0.16	1.03
Iba-ww-21	Petiole/plant	95.5	62.96	144.3	0.65	9.04	228.52	25.38
Iba-ws-21	Petiole/plant	98.5	59.32	136.38	0.69	8.22	212.5	23.04

Table 4.4 contd.

Environments	Traits	Mean	Min.	Max.	H2	SE	Genotypic variance	LSD
Mok-19-ww	First flower	43.74	40.60	44.95	0.20	2.02	1.68	3.27
Mok-19-ws	First flower	44.52	37.10	47.70	0.43	2.26	3.89	4.23
Mok-19-ww	50% flower	47.81	45.30	48.82	0.15	2.12	1.42	3.11
Mok-19-ws	50% flower	48.95	43.0	51.09	0.33	2.34	3.01	4.02
Mok-19-ww	Plant spread	40.60	30.50	51.74	0.55	3.40	20.88	8.67
Mok-19-ws	Plant spread	36.84	28.60	48.86	0.56	4.10	33.16	10.81
Mok-19-ww	Pod/plant	30.38	22.60	46.60	0.40	4.17	26.34	11.24
Mok-19-ws	Pod/ plant	26.42	19.30	48.19	0.45	5.07	44.35	14.0
Mok-19-ww	Pod weight	26.48	19.30	35.33	0.36	3.62	18.55	9.77
Mok-19-ws	Pod weight	18.74	15.90	22.24	0.17	2.65	7.27	6.97
Mok-19-ww	Seed/plant	34.19	26.10	42.95	0.37	4.45	27.96	11.88
Mok-19-ws	Seed/plant	28.97	20.10	51.11	0.44	5.42	49.46	14.92
Mok-19-ww	Seed weight	19.25	14.80	24.16	0.33	2.49	8.24	6.66
Mok-19-ws	Seed weight	16.57	13.70	19.91	0.19	2.43	6.58	6.53
Mok-19-ww	Shell weight	6.36	4.20	9.490	0.41	1.14	2.11	3.15
Mok-19-ws	Shell weight	5.05	3.34	8.17	0.33	1.02	1.49	2.82
Mok-21-ww	First flower	40.20	37.0	42.84	0.63	1.11	3.29	3.11
Mok-21-ws	First flower	44.12	40.50	46.58	0.48	1.45	3.90	4.04
Mok-21-ww	50% flower	44.05	40.30	47.12	0.61	1.39	4.88	3.90
Mok-21-ws	50% flower	47.87	46.70	52.42	0.14	1.51	2.47	4.12
Mok-21-ww	Leaf length	6.05	5.09	7.05	0.52	0.32	0.22	0.91
Mok-21-ws	Leaf length	6.35	4.83	8.03	0.69	0.34	0.36	0.95
Mok-21-ww	Leaf width	2.72	2.26	4.82	0.54	0.28	0.17	0.80
Mok-21-ws	Leaf width	2.79	2.38	4.67	0.46	0.29	0.15	0.81
Mok-21-ww	Plant height	16.61	16.61	16.61	0*	0.15	0.00	0.00
Mok-21-ws	Plant height	20.38	16.40	25.20	0.65	1.28	4.63	3.59
Mok-21-ww	Flower/plant	6.29	6.09	6.54	0.10	0.29	0.08	0.78
Mok-21-ws	Flower/plant	6.19	5.54	7.03	0.28	0.5	0.33	1.38

Table 4.4 contd.

Environments	Traits	Mean	Min.	Max.	H2	SE	Genotypic variance	LSD
Zar-19-ww	Leaf length	5.84	5.63	6.08	0.16	0.21	0.05	0.59
Zar-19-ws	Leaf length	5.39	5.21	5.93	0.15	0.20	0.05	0.57
Zar-19-ww	Plant height	19.18	15.80	23.17	0.68	1.00	3.13	2.83
Zar-19-ws	Plant height	17.9	15.10	22.54	0.53	1.39	4.15	3.93
Zar-19-ww	Plant spread	34.77	34.77	34.77	0*	0.32	0.00	0.00
Zar-19-ws	Plant spread	32.11	30.60	33.65	0.12	1.28	1.75	3.52
Zar-19-ww	Pod/plant	15.43	9.62	29.40	0.57	3.07	21.64	8.66
Zar-19-ws	Pod/plant	18.25	11.4	28.0	0.56	2.67	16.3	7.54
Zar-19-ww	Pod weight	11.72	5.85	27.84	0.6	2.77	19.15	7.82
Zar-19-ws	Pod weight	13.12	9.36	23.33	0.48	2.12	8.52	5.96
Zar-19-ww	Seed/plant	17.32	10.50	31.33	0.58	3.38	27.35	9.56
Zar-19-ws	Seed/plant	20.28	11.90	31.56	0.56	3.06	21.31	8.63
Zar-19-ww	Seed weight	6.83	3.62	15.25	0.57	1.56	5.56	4.40
Zar-19-ws	Seed weight	8.60	6.70	12.42	0.38	1.31	2.71	3.67
Zar-19-ww	Shell weight	3.27	1.95	5.98	0.54	0.73	1.14	2.05
Zar-19-ws	Shell weight	3.88	2.70	5.99	0.47	0.72	0.97	2.03
Zar-21-ww	First flower	44.36	50.1	57.76	0.71	2.16	2.02	1.27
Zar-21-ws	First flower	44.01	43.3	57.41	0.83	0.74	3.11	2.07
Zar-21-ww	50% flower	44.69	55.1	0.63	0.48	0.61	1.34	2.03
Zar-21-ws	50% flower	44.8	43.2	48.27	0.71	0.45	0.70	1.26
Zar-21-ww	Flower/plant	6.79	9.04	0.26	0.98	1.25	2.72	0.80
Zar-21-ws	Flower/plant	7.13	5.59	9.88	0.43	0.84	1.20	2.34
Zar-21-ww	Leaf length	4.29	4.72	0.21	0.26	0.08	0.73	0.77
Zar-21-ws	Leaf length	3.84	3.57	4.08	0.23	0.20	0.05	0.54
Zar-21-ww	Leaf width	1.17	1.41	0.37	0.13	0.03	0.36	0.47
Zar-21-ws	Leaf width	1.01	0.78	1.42	0.52	0.14	0.04	0.38
Zar-21-ww	Num of petiole	16.55	24.3	0.34	2.72	11.0	7.61	25.38
Zar-21-ws	Num of petiole	13.50	11.0	19.52	0.47	1.61	4.76	4.51
Zar-21-ww	Plant height	11.98	14.4	0.41	0.95	1.51	2.67	2.31
Zar-21-ws	Plant height	10.98	8.85	14.97	0.60	1.03	2.58	2.88
Zar-21-ww	Pod weight	2.48	13.5	0.43	2.0	6.87	5.59	15.52
Zar-21-ws	Pod weight	2.30	1.44	8.31	0.23	1.84	4.21	5.10
Zar-21-ww	Plant spread	33.43	34.70	0.12	1.29	1.57	3.33	6.51
Zar-21-ws	Plant spread	26.09	26.09	26.09	0*	0.37	0.00	0.00
Zar-21-ww	Seed/plant	1.76	1.40	4.58	0.09*	1.23	1.44	3.24
Zar-21-ws	Seed/plant	3.51	2.29	7.54	0.20	1.94	4.38	5.31
Zar-21-ww	Pod per plant	3.61	19.0	0.44	2.87	14.3	8.00	8.94
Zar-21-ws	Pod per plant	2.12	2.12	2.12	0*	0.35	0.00	0.01
Zar-21-ww	Seed weight	0.59	0.56	0.75	0.02*	0.25	0.04	0.53
Zar-21-ws	Seed weight	1.47	1.08	3.01	0.16	0.77	0.65	2.09
Zar-21-ww	Shell weight	0.55	0.45	0.75	0.1*	0.19	0.03	0.50
Zar-21-ws	Shell weight	0.20	0.20	0.23	0.01*	0.07	0.00	0.12

4.4. Yield Components

In this study, there are highly significant (P<0.01) correlation in the yield related traits taken. Number of pods per plant, pod weight, seed weight and number of seeds has revealed the existence of adequate genetic variability among 135 Bambara groundnut accessions evaluated for drought tolerance at two water regimes (Water stressed (WS) and Well-watered WW), three contrasting locations in two years. Genotypes performed differently to the number of pods per plant, pod weight, seed number and seed weights under the two regimes. The mean pod per plant from Ibadan WW plots outstands its counterpart's WS regime (Table 4.4) for instance, mean number of pods per plant in Ibadan WS (6.34) whereas it was 7.14 at WW condition. Mean pod per plant in Ibadan 2021 WS (5.66) differed from mean pod per plant at WW regime (11.09). Moreover, Mokwa 2019 had the same trend of values for this trait at WW plot (34.19) and WS (28.97). Contrariwise, Zaria 2019, 2021 mean seed pod per plant at WS are (20.28), (3.51) slightly higher than Zaria 2019, 2021 WW (17.32), (1.76) respectively. Yield in Zaria 2019, 2020 is lower than other locations.

Pod weight per plant: The mean pod weight per plant from all locations also varied within and across regimes. The mean pod per plant at Ibadan WS and WW in 2019 are (3.08g) and (5.42g) respectively. While same trend was also recorded in Ibadan 2021 WS regime (4.7g) and (17.39g). Mokwa 2019 pod weight per plant value of (18.74g) at WS regime and (26.48g) at WW regime. Zaria mean pod weight for 2019 WS was also slightly higher than WW (11.72g) condition. (Table 4.4).

Days to first flower: At Ibadan 2019, the number of days to first flower emergence was not significantly different at both regimes (WW and WS). At this location, the minimum number of days was 39.86, the mean 40.65 at WW and 40.06 at WS respectively. At this location, number of days to first flower emergence ranged from 39.86 and 40.63 at WS regime whereas, it was 40.65 at WW condition (Table 4.4)
At Ibadan 2021, there was no significant difference between the two regimes for the number of days to the first flower (Table 4.4). At the WW regime, the mean, minimum and maximum number of days to first flower are 44.24, 44.06 and 51.85 respectively whereas it was 43.8, 43.22 and 48.26 at the WS regime. In general, the number of days to first flower was earlier in WS regimes than WW regimes.

Pods per plant: The main effect of regimes was highly significant, and the main effect of location was significant as well as year based on numbers of pods per plant, while the interaction effect was also significant.

Mokwa WS 2019 had the highest records of pods per plant (48.19) followed by Mokwa WW 2019 with maximum number of pods per plant (46.60). The lowest number of pods per plant (2.12 and 1.07) were from Zaria 2019 at WS and WW respectively. (Table 4.4), this might be due to the hereditary disparity of genotypes to respond differently in well-watered and water stressed condition or differences in soil nutrient profiles from each location. This result agrees with Murut *et al*, 2014 who found substantial differences in the number of pods per plant among haricot bean varieties in his multi location trials at rain fed conditions. This can be possible despite the regime. Bambara groundnut accessions were impacted differently at WS regimes across location. Some genotypes could not continue to survive the stress condition when drought was imposed at flowering stage, such genotypes aborted good number of their flowers starting from one to two weeks after water withdrawal from WS plots. Some genotypes exhibited escape mechanisms whereby they utilized the available moisture, to complete their life cycle under water stressed condition. Therefore, regimes had a significant effect on the number of pods per plant possibly due to genotypic differences and response of genotypes to water deficit.

Pod weight: Impact of water regime on pod weight showed significant difference across all locations. The highest pod weight was found in Ibadan well-watered regime (40.89 g) followed by Mokwa well-watered (35.33 g), while the least pod weight was found in Zaria (0.90 g), the corresponding highest pod weight under water stressed regime was found in Zaria 2019 with pod weight (23.33 g) followed by Mokwa 2019 with pod weight (22.24 g). The deviation in accession's pod weight across location could be associated to the genetic makeup, nutrients uptake and utilization, soil structure, moisture etc. as it varies across locations. Like other parameters, the well-watered regimes gave the highest pod weight (40.89g) whereas water stressed regimes had (23.33g).

Number of seed per plant: Table 4.4 presents mean seeds per plant across the twelve environments in the year 2019 and 2021. The mean number of seeds per plant in Ibadan 2019 under WW and WS are (8.12) and (7.06) respectively. Also, Ibadan 2021 had mean seed per plant of (11.09) at the WW regime and (5.66) at WS regime. Mokwa mean seed number per plant at the WW (19.25) and WS (16.57). Zaria mean number of seeds per plant varied from other locations.

4.5. Plant growth habits.

Plant growth habits were scored using Bambara groundnut descriptors. Regimes do not influence plant growth habits. The growth habits as recorded in the descriptors are -semi-bunch, bunchy and spreading. Tables 4.5.1, 4.5.2, and 4.5.3 show the growth habit of the total accessions in this study 3 of 135 accessions (2.2%) of the total accessions had spreading growth habit, 21 out of 135 accessions (15.5%) had semi-bunch growth habit while 111 accessions (82.2%) out of 135 accessions had bunchy growth habit.

Leaf length responded positively under the well-watered condition but was significantly low under water stressed regime. Genotypes with the highest leaf length were found in Ibadan at well-watered regime followed by Mokwa well-watered plot while the least leaf length was in Zaria water stressed regime.

Table 4.5. The mean, minimum, maximum, standard error value, heritability, and least significant difference for five pod traits scored in all the twelve environments.

Traits	Locations	Regime	Year	Mean	Min	Max	SE	H2	LSD
Pod/Plant	Ibadan	WS	2019	6.34	5.28	8.69	1.40	0.17	3.90
	Ibadan	WW	2019	7.14	6.26	9.16	1.19	0.15	3.28
	Ibadan	WS	2021	6.16	3.12	11.76	1.83	0.48	5.17
	Ibadan	WW	2021	12.89	7.22	27.74	3.20	0.55	8.94
	Mokwa	WS	2019	26.42	19.31	48.19	5.07	0.45	14.00
	Mokwa	WW	2019	30.38	22.56	46.60	4.17	0.40	11.24
	Zaria	WS	2019	18.25	11.36	28.00	2.67	0.56	7.54
	Zaria	WW	2019	15.43	9.62	29.40	3.07	0.57	8.66
	Zaria	WS	2021	2.12	2.12	2.12	0.35	0.00	0.01
	Zaria	WW	2021	3.61	1.07	18.96	2.87	0.44	8.00
Pod weight									
	Ibadan	WS	2019	3.08	2.44	4.13	0.71	0.19	1.98
	Ibadan	WW	2019	5.42	4.22	8.82	1.31	0.24	3.63
	Ibadan	WS	2021	4.22	3.35	5.49	0.93	0.19	2.54
	Ibadan	WW	2021	17.39	10.28	40.89	5.55	0.47	15.52
	Mokwa	WS	2019	18.74	15.87	22.24	2.65	0.17	6.97
	Mokwa	WW	2019	26.48	19.26	35.33	3.62	0.36	9.77
	Zaria	WS	2019	13.12	9.36	23.33	2.12	0.48	5.96
	Zaria	WW	2019	11.72	5.85	27.84	2.77	0.60	7.82
	Zaria	WS	2021	2.30	1.44	8.31	1.84	0.23	5.10
	Zaria	WW	2021	2.48	0.90	13.47	2.00	0.43	5.59
Seeds per Plant									
	Ibadan	WS	2019	7.06	6.40	8.46	1.17	0.09	3.18
	Ibadan	WW	2019	8.12	6.26	12.67	1.86	0.29	5.22
	Ibadan	WS	2021	5.66	4.55	8.26	1.29	0.25	3.60
	Ibadan	WW	2021	11.09	5.57	19.44	2.81	0.52	7.79
	Mokwa	WS	2019	28.97	20.10	51.11	5.42	0.44	14.92
	Mokwa	WW	2019	34.19	26.05	42.95	4.45	0.37	11.88
	Zaria	WS	2019	20.28	11.85	31.56	3.06	0.56	8.63
	Zaria	WW	2019	17.32	10.46	31.33	3.38	0.58	9.56
	Zaria	WS	2021	3.51	2.29	7.54	1.94	0.20	5.31

	Zaria	WW	2021	1.76	1.40	4.58	1.23	0.09	3.24
Seed weight									
	Ibadan	WS	2019	2.22	1.80	2.90	0.48	0.17	1.33
	Ibadan	WW	2019	3.65	2.81	5.68	0.87	0.25	2.41
	Ibadan	WS	2021	1.86	1.52	2.49	0.38	0.17	1.03
	Ibadan	WW	2021	4.28	2.01	7.96	1.23	0.44	3.40
	Mokwa	WS	2019	16.57	13.66	19.91	2.43	0.19	6.53
	Mokwa	WW	2019	19.25	14.81	24.16	2.49	0.33	6.66
	Zaria	WS	2019	8.60	6.70	12.42	1.31	0.38	3.67
	Zaria	WW	2019	6.83	3.62	15.25	1.56	0.57	4.40
	Zaria	WS	2021	1.47	1.08	3.01	0.77	0.16	2.09
	Zaria	WW	2021	0.59	0.56	0.75	0.25	0.02	0.53
Shell weight									
	Ibadan	WS	2019	0.83	0.67	1.24	0.22	0.17	0.62
	Ibadan	WW	2019	1.56	1.12	3.10	0.46	0.29	1.29
	Ibadan	WS	2021	0.59	0.46	0.95	0.18	0.23	0.49
	Ibadan	WW	2021	1.70	1.40	2.18	0.42	0.17	1.11
	Mokwa	WS	2019	5.05	3.34	8.17	1.02	0.33	2.82
	Mokwa	WW	2019	6.36	4.20	9.49	1.14	0.41	3.15
	Zaria	WS	2019	3.88	2.70	5.99	0.72	0.47	2.03
	Zaria	WW	2019	3.27	1.95	5.98	0.73	0.54	2.05
	Zaria	WS	2021	0.55	0.45	0.75	0.19	0.10	0.50
	Zaria	WW	2021	0.20	0.20	0.23	0.07	0.01	0.12

Bambara Groundnut Growth Habit

Table 4.5. 1. Accessions with a Semi Bunchy Growth Habit

Serial	Accession	Serial	Accession
1	TVSu-144	12	TVSu-318
2	TVSu-160	13	TVSu-357
3	TVSu-431	14	TVSu-379
4	CFFR12	15	TVSu-47
5	TVSu-154	16	TVSu-8
6	TVSu-2088	17	TVSu-82
7	TVSu-2098	18	TVSu-85
8	TVSu-267	19	TVSu-87
9	TVSu-269	20	TVSu-9
10	TVSu-277	21	TVSu-94
11	TVSu-280	22	TVSu-467

Table 4.5.2 Accessions with a Spreading Growth Habit

Serial	Accession
1	TVSu-112
2	TVSu-143
3	TVSu-295

Table 4.5.3. Accessions with a Bunchy Growth Habit

Serial	Accession	Serial	Accession	Serial	Accession	Serial	Accession	Serial	Accession	Serial	Accession
1	CFFR9	22	TVSu-2113	43	TVSu-167	64	TVSu-218	85	TVSu-329	106	TVSu-645
2	CFFR1	23	TVSu-217	44	TVSu-170	65	TVSu-221	86	TVSu-343	107	TVSu-671
3	CFFR10	24	TVSu-266	45	TVSu-183	66	TVSu-222	87	TVSu-348	108	TVSu-75
4	CFFR11	25	TVSu-361	46	TVSu-200	67	TVSu-224	88	TVSu-364	109	TVSu-89
5	CFFR13	26	TVSu-373	47	TVSu-2089	68	TVSu-225	89	TVSu-368	110	TVSu-2109
6	CFFR2	27	TVSu-535	48	TVSu-2091	69	TVSu-227	90	TVSu-371		
7	CFFR3	28	TVSu-84	49	TVSu-2092	70	TVSu-229	91	TVSu-374		
8	CFFR5	29	TVSu-110	50	TVSu-2093	71	TVSu-231	92	TVSu-376		
9	CFFR6	30	TVSu-103	51	TVSu-2094	72	TVSu-232	93	TVSu-378		
10	CFFR7	31	TVSu-11	52	TVSu-2097	73	TVSu-233	94	TVSu-386		
11	CFFR8	32	TVSu-212	53	TVSu-2099	74	TVSu-234	95	TVSu-388		
12	TVSu-137	33	TVSu-115	54	TVSu-2101	75	TVSu-245	96	TVSu-397		
13	TVSu-156	34	TVSu-125	55	TVSu-2102	76	TVSu-25	97	TVSu-410		
14	TVSu-2090	35	TVSu-14	56	TVSu-2103	77	TVSu-262	98	TVSu-425		
15	TVSu-2095	36	TVSu-253	57	TVSu-2106	78	TVSu-275	99	TVSu-441		
16	TVSu-2096	37	TVSu-145	58	TVSu-2108	79	TVSu-288	100	TVSu-46		
17	TVSu-2100	38	TVSu-146	59	TVSu-211	80	TVSu-29	101	TVSu-66		
18	TVSu-2104	39	TVSu-151	60	TVSu-2112	81	TVSu-310	102	TVSu-50		
19	TVSu-2105	40	TVSu-158	61	TVSu-2114	82	TVSu-311	103	TVSu-581		
20	TVSu-2107	41	TVSu-161	62	TVSu-214	83	TVSu-319	104	TVSu-593		
21	TVSu-2110	42	TVSu-164	63	TVSu-216	84	TVSu-323	105	TVSu-600		

Table 4.6. Accessions with an Elliptic Leaf Shape

Serial	Accession	Serial	Accession
1	CFFR9	12	TVSu-2107
2	TVSu-137	13	TVSu-2110
3	TVSu-144	14	TVSu-2113
4	TVSu-156	15	TVSu-217
5	TVSu-160	16	TVSu-266
6	TVSu-2090	17	TVSu-361
7	TVSu-2095	18	TVSu-373
8	TVSu-2096	19	TVSu-431
9	TVSu-2100	20	TVSu-535
10	TVSu-2104	21	TVSu-84
11	TVSu-2105		

Table 4.6.2. Accessions with Lanceolate Leaf Shape

Serial	Accession	Serial	Accession	Serial	Accession	Serial	Accession	Serial	Accession	Serial	Accession
1	CFFR1	22	TVSu-151	43	TVSu-2106	64	TVSu-267	85	TVSu-371	106	TVSu-75
2	CFFR10	23	TVSu-154	44	TVSu-2108	65	TVSu-269	86	TVSu-374	107	TVSu-8
3	CFFR11	24	TVSu-158	45	TVSu-211	66	TVSu-275	87	TVSu-376	108	TVSu-82
4	CFFR12	25	TVSu-161	46	TVSu-2112	67	TVSu-277	88	TVSu-378	109	TVSu-85
5	CFFR13	26	TVSu-164	47	TVSu-2114	68	TVSu-280	89	TVSu-379	110	TVSu-87
6	CFFR2	27	TVSu-167	48	TVSu-214	69	TVSu-288	90	TVSu-386	111	TVSu-89
7	CFFR3	28	TVSu-170	49	TVSu-216	70	TVSu-295	91	TVSu-388	112	TVSu-9
8	CFFR5	29	TVSu-183	50	TVSu-218	71	TVSu-310	92	TVSu-397	113	TVSu-94
9	CFFR6	30	TVSu-200	51	TVSu-221	72	TVSu-311	93	TVSu-410		
10	CFFR7	31	TVSu-2088	52	TVSu-222	73	TVSu-318	94	TVSu-425		
11	CFFR8	32	TVSu-2089	53	TVSu-224	74	TVSu-319	95	TVSu-441		
12	TVSu-110	33	TVSu-2091	54	TVSu-225	75	TVSu-323	96	TVSu-46		
13	TVSu-103	34	TVSu-2092	55	TVSu-227	76	TVSu-329	97	TVSu-467		
14	TVSu-11	35	TVSu-2093	56	TVSu-229	77	TVSu-343	98	TVSu-47		
15	TVSu-112	36	TVSu-2094	57	TVSu-231	78	TVSu-348	99	TVSu-50		
16	TVSu-115	37	TVSu-2097	58	TVSu-232	79	TVSu-351	100	TVSu-581		
17	TVSu-125	38	TVSu-2098	59	TVSu-233	80	TVSu-357	101	TVSu-593		
18	TVSu-14	39	TVSu-2099	60	TVSu-234	81	TVSu-359	102	TVSu-600		
19	TVSu-143	40	TVSu-2101	61	TVSu-245	82	TVSu-362	103	TVSu-645		
20	TVSu-145	41	TVSu-2102	62	TVSu-25	83	TVSu-364	104	TVSu-66		
21	TVSu-146	42	TVSu-2103	63	TVSu-262	84	TVSu-368	105	TVSu-671		

Table 4.6.3. Accessions with Oval Leaf Shape

Serial	Accession
1	TVSu-2109
2	TVSu-212
3	TVSu-253
4	TVSu-29

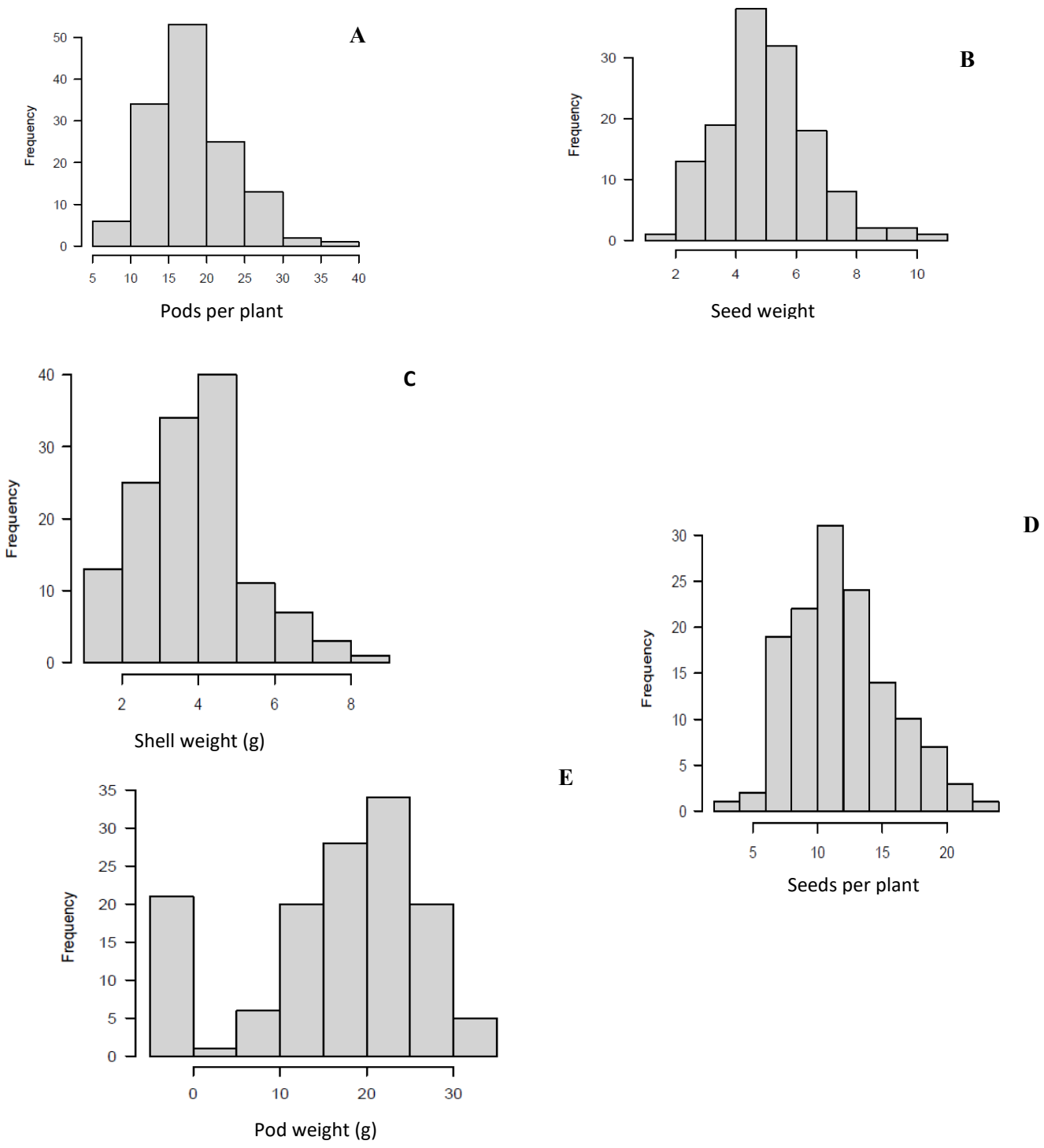


Figure 5.4 (a, b, c, d, and e). Phenotypic distribution of the five yield related traits; (A)-Pods per plant, (B) Shell weight (gram), |(C) Seeds per plant (gram); (D) Pod weight per plant (gram), (E)-Seed weight per plant

(gram) in Bambara groundnut population.

Table 4.7. Presents the correlation coefficient of drought related traits employed for the 135 accessions of Bambara groundnut for the genotype response to drought. There was a positive and highly significant ($P \leq 0.001$) correlation amongst the traits. Days to first flower had significant correlation ($P \leq 0.001$) with days to 50% flower and number of flowers per plant. The number of petioles and number of flowers per plant had a highly significant correlation ($P \leq 0.001$). Most of the vegetative traits used are highly correlated with each other except for days to 50% flower where there was no significant correlation (Table 4.7).

Table 4.7. Correlation coefficients between common drought indices among accessions of Bambara groundnut in the year 2019/2020 and 2020/2021

	DFF	50%F	NFLWPP	PetPP	PLTSPRD	PLT-HGT	LF-LGHT	LF-WID	POD-PLT	POD-WGT	SD-PLT	SD-WGT	Sh-WGT	%RWC	SW-100
DFF	0.74***														
D50%F	0.74***	0.01 ns													
NFLWPP	0.17***	-0.01 ns													
PetPP	0.04	-0.1 ns	0.23***												
PLTSPRD	-0.13**	-0.08 ns	-0.01 ns	0.29***											
PLT-GHT	-0.21***	1 ns	-0.007	0.18***	0.1 ns										
LF-LGHT	-0.14***	-0.06 ns	0.07 ns	0.48***	0.22***	0.59***									
LF-WID	-0.18***	-0.1 ns	0.05 ns	0.55***	0.26***	0.59***	0.82***								
POD-PLT	-0.16***	0.02 ns	-0.13**	0.04 ns	0.12**	0.30***	0.21***	0.26***							
PODWGT	-0.14***	-0.04 ns	-0.07 ns	-0.01 ns	0.08 ns	0.1 ns	0.05 ns	0.09 ns	0.34***						
SD-PLT	-0.13***	0.001 ns	-0.09 ns	-0.03 ns	0.05 ns	0.21***	0.09 ns	0.15***	0.85***	0.33***					
SD-WGT	-0.16***	-0.05 ns	-0.08 ns	-0.04 ns	0.06 ns	0.16***	0.07 ns	0.13**	0.72***	0.33***	0.85***				
Sh-WGT	-0.22***	-0.06 ns	-0.12*	-0.06 ns	0.05 ns	0.22***	0.12**	0.17***	0.71***	0.35***	0.76***	0.91***			
%RWC	-0.16***	0.09 ns	0.02 ns	0.32***	0.29***	0.32***	0.36***	0.43***	0.19***	0.06 ns	0.09 ns	0.09 ns	0.13**		
SW-100	-0.15***	-0.19***	-0.02 ns	-0.07 ns	0.03 ns	-0.08 ns	-0.08 ns	-0.06 ns	0.14***	0.11*	0.19***	0.36***	0.31***	-0.1	

DFF-Days to first flower, 50%F- days to 50% flower, SW-100- 100seed weight, NFLWPP-Number of flower per plant, PetPP- Number of petioles per plant, PLTSPRD- Plant spread, PLT-HGT- Plant height, LF-LGHT- Leaf length, LF-WID-Leaf width, POD-PLT- Pod per plant, POD-WGT- Pod weight, SD-PLT- Seed per plant, SD-WGT- Seed weight,Sh-WGT-Shell weight, %RWC- relative water content. Levels of significance *- P ≤ 0.05, **- P ≤ 0.01 ***-P ≤0.001, ns- not significant)

Table 4.8. Percent yield reduction and drought susceptibility index of the five accessions with lowest yield reduction due to drought, best DSI ranking, and best performing under well-watered and water-stressed conditions.

Accession	%SW reduction	Rank
Accessions with lowest yield reduction		
TVSu170	0.23	23
TVSu221	1.09	24
TVSu225	1.16	25
TVSu310	1.19	26
TVSu227	1.87	27
Accessions with the best DSI ranking		
TVSu144	-27.78	2
TVSu374	-23.58	5
TVSu288	-13.54	11
TVSu397	-3.03	20
TVSu277	6.33	37
Best performing accessions under well-watered condition		
CFFR10	60.19	117
TVSu2094	79.12	133
TVSu2088	47.18	107
TVSu29	53.04	109
TVSu2093	83.62	135
TVSu2104	68.62	112
TVSu357	55.99	114
Best performing accessions under water-stressed condition		
TVSu288	-13.54	11
TVSu200	2.21	29
TVSu14	7.68	40
TVSu216	-15.88	8
CFFR9	4.39	36
TVSu2108	5.62	23
TVSu410	-18.70	7

%SW reduction = Percent seed weight reduction; DSI= Drought Susceptibility Index

4.6. Association Analysis

A genome-wide association study (GWAS) was conducted on the same set of 135 BG accessions.
The estimated BLUEs mean value was calculated for yield-traits and pod-shell trait in each location and across the locations. The BLUE value of each accession and high-quality 5,395 genome-wide SNPs were used for marker-trait association analysis using both GLM and MLM models. A total of 15 SNPs QTL were significantly associated with five phenotypic traits (number of seeds per plant, number of pods per plant, seed weight per plant, shell weight per plant, and pod weight per plant). The threshold of –log (p) > 5 was used to consider a significant association between SNP marker and trait. Out of 15 SNP QTL, 12 SNP QTL were common in both GLM and MLM statistical methods employed for the GWAS analysis. Six of these SNP markers were found in both WW and WS regimes. Eight unique SNP markers were found only in WS and were absent in WW regime. The phenotypic variance explained (R square) by these QTLs were ranged.
0.01-0.15 (GLM) and 0.10-0.14 (MLM) in GLM and MLM approaches respectively. By the MLM approach, six SNPs were associated with the number of pods per plant, five associated with number of seeds per plant, one associated with pod weight and seed weight while four were associated with shell weight. Two pairs of pleiotropic makers (24346038|F|0-5:A>G-5:A>G and 24346837|F|0-52:G>A-52:G>A) and (4183888|F|0-39:A>G-39:A>G and 4183987|F|0-65:G>A-65:G>A) were found. The first pair were associated with seed per plant and shell weight respectively while the second pair associated with seed per plant and seed weight respectively.

Manhattan and Q-Q plots of the SNP-based associations mapping for the four traits based on GLM and MLM are presented in Figures 4.21, 4.22, 4.23 4.24. The observed p-values for all traits aligned with expected p-values as shown by the Q-Q plots. Significant markers at the set threshold are those above the blue lines in the Manhattan plots. A candidate gene Vigun05g235600 (GTPase-activating protein 1-like) and four other candidate genes; Vigun04g138400, Vigun04g138600, Vigun04g139100, and Vigun04g139400 (receptor-like protein kinase 2) have been reported for their role under abiotic stresses including drought tolerance in field crops. A candidate gene search identified six significant candidate genes in proximity to the SNP markers associated with the number of pods per plant and number of seeds per plant; four SNPs were left for shell weight in the MLM. Seven SNPs QTL of total 15 QTLs showed pleiotropic effects and were associated with both number of pods per plant and number of seeds per plant traits.

Table 4.9. DArTseq SNPs markers having significant association with pod per plant, seeds per plant, shell weight, seed weight and number of seeds per plant of 135 accessions of Bambara groundnut evaluated during the 2019/2020 and 202/2021 cropping seasons at three locations in Nigeria.

			GLM		MLM	
Marker	**Trait**	**Chromosome**	**P- Value**	**R Square**	**P-Value**	**R Square**
24346038|F|0-5:A>G-5:A>G	PP	0	6.75E-06	0.15398	0.00	0.12
24346038|F|0-5:A>G-5:A>G	SP		2.11E-06	0.1299	0.00	0.13
24346837|F|0-52:G>A-52:G>A	ShW	0	2.85E-05	0.09653	0.00	0.12
27641616|F|0-8:T>A-8:T>A	ShW	0	6.42E-05	0.09809	0.00	0.11
27641765|F|0-13:C>T-13:C>T	ShW	0	0.0006	0.08688	0.00	0.10
37316456|F|0-39:C>T-39:C>T	PP	0	5.25E-06	0.15392	0.00	0.13
37316456|F|0-39:C>T-39:C>T	SP	0	0.00	0.14255	0.00	0.13
4178140|F|0-38:A>G-38:A>G	ShW	0	2.95E-05	0.11701	0.00	0.12
4181073|F|0-9:G>C-9:G>C	PP	0	2.03E-05	0.13825	0.00	0.14
4181073|F|0-9:G>C-9:G>C	SP	0	3.28E-05	0.1286	0.00	0.14
4181321|F|0-36:C>T-36:C>T	PP	0	7.88E-06	0.13552	0.00	0.12
4181321|F|0-36:C>T-36:C>T	SP	0	0.00	0.12687	0.00	0.11
4182521|F|0-47:G>A-47:G>A	PP	0	7.55E-05	0.12268	0.01214	
4182521|F|0-47:G>A-47:G>A	SP	0	4.45E-05	0.12512	0.00704	
4182848|F|0-68:T>C-68:T>C	PP	0	2.55E-05	0.1247	0.00194	
4182848|F|0-68:T>C-68:T>C	SP	0	5.85E-05	0.11161	0.00	0.12
4183175|F|0-44:T>C-44:T>C	PW	0	0.00026	0.11262	0.00	0.12
4183652|F|0-30:C>T-30:C>T	PP	0	5.66E-06	0.15604	0.00	0.13
4183652|F|0-30:C>T-30:C>T	SP	0	0.00	0.13094	0.00	0.14
4183856|F|0-6:T>G-6:T>G	PP	0	2.47E-05	0.1386	0.00	0.12
4183888|F|0-39:A>G-39:A>G	SP	0	4.27E-05	0.10237	0.00386	
4183987|F|0-65:G>A-65:G>A	SW	0	7.86E-05	0.12473	0.0019	
27641616|F|0-8:T>A-8:T>A	ShW-ww	0	5.00E-06	0.13506	0.00	
27641616|F|0-8:T>A-8:T>A	PW-ww	0	0.00074	0.0823	0.00	
27641765|F|0-13:C>T-13:C>T	ShW-ww	0	1.24E-05	0.14355	0.00	
4178140|F|0-38:A>G-38:A>G	SW-ww	0	0.00069	0.09013	0.00	
4178140|F|0-38:A>G-38:A>G	PW-ww	0	0.00054	0.09124		
4178140|F|0-38:A>G-38:A>G	ShW-ww	0	2.15E-06	0.15243		
4181321|F|0-36:C>T-36:C>T	PW-ww	0	0.000263	0.08582		
4182848|F|0-68:T>C-68:T>C	PP-ww	0	1.66E-06	0.15841		
4182848|F|0-68:T>C-68:T>C	SP-ww	0	7.31E-05	0.11825		
4183987|F|0-65:G>A-65:G>A	SP-ww	0	9.89e-05	0.11646		
4183987|F|0-65:G>A-65:G>A	PP-ww	0	0.000148	0.11087		
4183987|F|0-65:G>A-65:G>A	SW-ww	0	0.00042	0.0942		

PP- number of pods per plant, SP- number of seeds per plant, ShW- shell weight, PW- pod weight. Sw- seed weight, ww- alsofound in well water.

Evaluations in the years 2019 and 2021 at Ibadan as two of the environments did not reveal any pleiotropic marker at water stress regime for both years. Pleotropic marker was mostly found in Mokwa and Zaria in the two consecutive years. For instance, 24346038|F|0-5:A>G-5:A>G was found associated with two traits -pod per plant and number of seeds per plant in Zaria 2019, and Zaria 2021. Meanwhile, 37316456|F|0-39:C>T-39:C>T related to pod per plant and seed per plantin Mokwa 2019. Moreover, combined analysis of all the environment revealed two sets of marker overlaps; one set is associated to yield related traits found in both regimes and the second set only detected in water stressed regime. (Table 4.9).

Seven markers (1). 37316456|F|0-39:C>T-39:C>T (2). 4178140|F|0-38:A>G-38:A>G,
(3) 4181321|F|0-36:C>T-36:C>T. (4) 4183652|F|0-30:C>T-30:C>T; (5) 4183856|F|0-6:T>G- 6:T>G, (6) 24346038|F|0-5:A>G-5:A>G, and (7) 4182521|F|0-47:G>A-47:G>A associated with the five Bambara groundnut yield related traits in the combined location analysis were found on *Vigna unguiculata* and *Vigna radiata* genome. These significant Bambara groundnut markers were found on the cowpea and mung bean chromosomes. The blast search revealed that several genes whose encoding protein products are known to regulate drought tolerance are located close to these markers at less than 500 kbp upstream and downstream. These genes had been reported in *Vigna unguiculata* (Table 4.9).

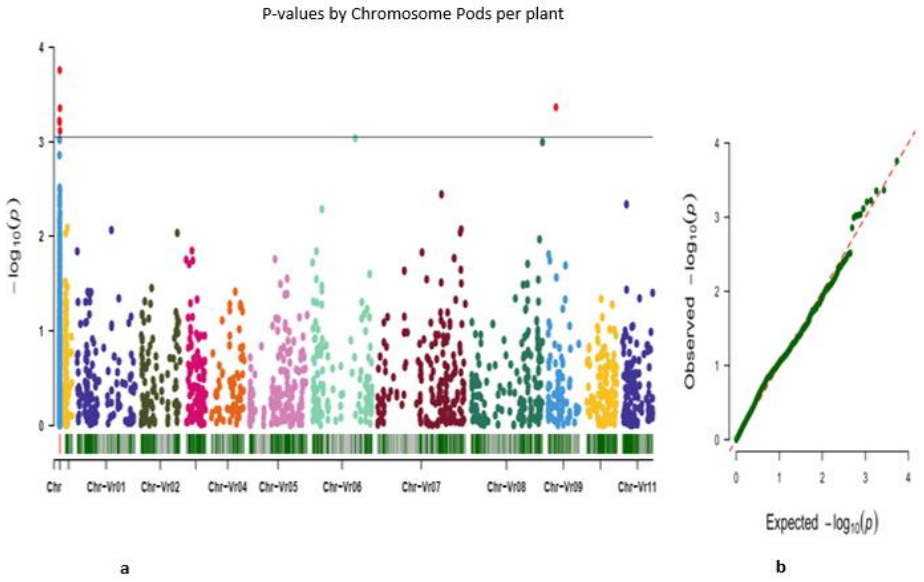


Figure 5.5. Genome -wide association analysis of pods per plant in Bambara groundnut population. (**a**) Manhattan plot for mixed linear model (MLM) approach for pods per plant. Negative log10 P-values are plotted on Y- axis against SNP position. on X-axis for each of the 11 chromosomes. Genome wide significant threshold is depicted as harsh color. horizontal line, each dot in different colors represents the SNPs on the 11 chromosomes. (**b**) Quantile-quantile (Q-Q) plot of the MLM for the number of pods per plant.

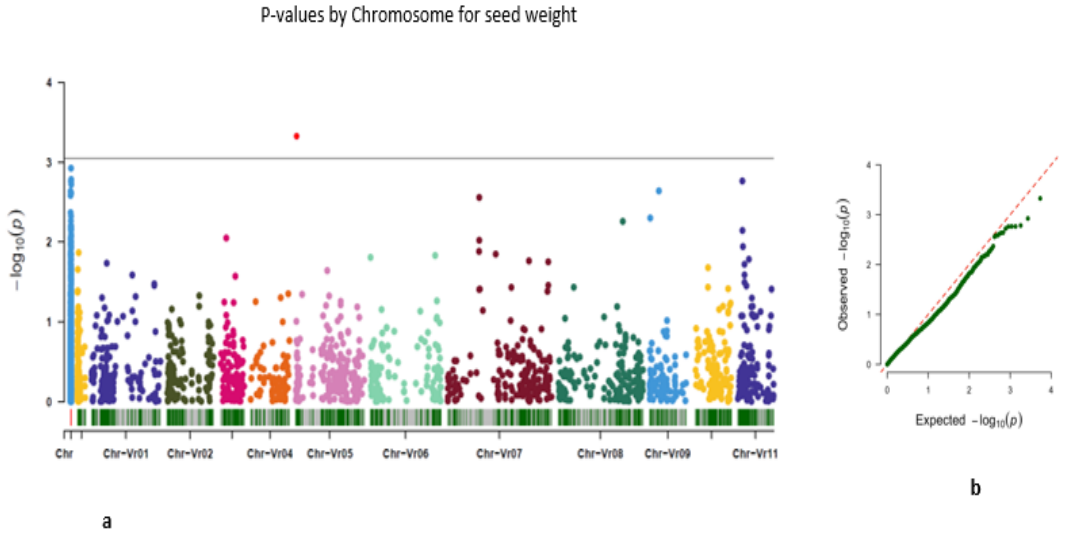


Figure 5.6. Genome -wide association analysis seed weight in Bambara groundnut population. (**a**) Manhattan plot for mixed linear model (MLM) approach for seed weight. Negative log10 P-values are plotted on Y- axis against SNP position on X-axis for each of the 11 chromosomes. Genome wide significant threshold is depicted as harsh color horizontal line, each dot in different colors represents the SNPs on the 11 chromosomes. (**b**) Quantile-quantile (Q-Q) plot of the MLM for seed weight.

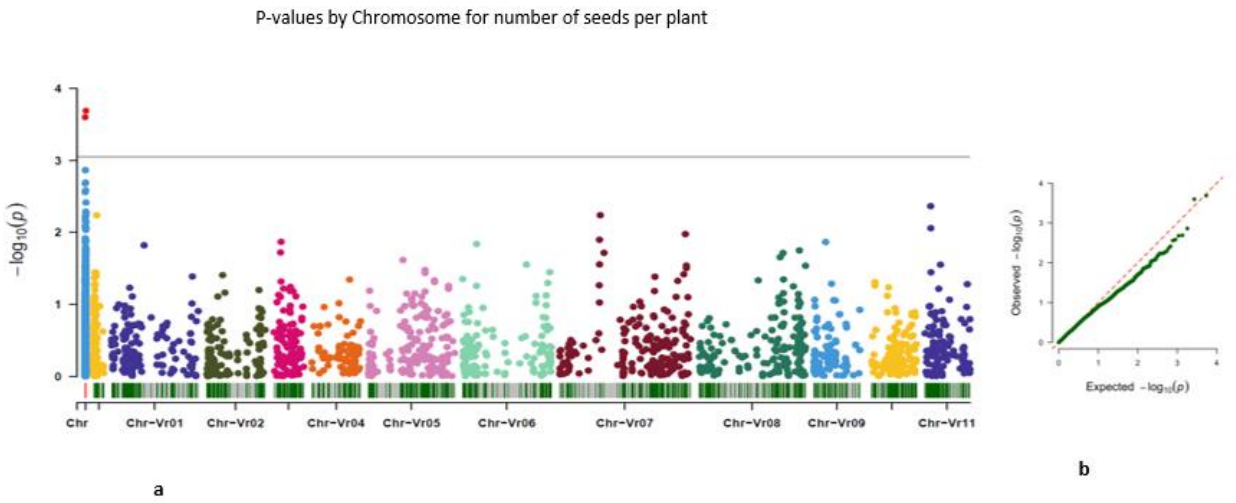


Figure 5.7. Genome -wide association analysis of seeds per plant in Bambara groundnut population. (**a**) Manhattan plot for mixed linear model (MLM) approach for the number of seeds per plant. Negative log10 P-values are plotted on Y- axis against SNP position on X-axis for each of the 11 chromosomes. Genome wide significant threshold is depicted as harsh color horizontal line, each dot in different colors represents the SNPs on the 11 chromosomes. (**b**) Quantile-quantile (Q-Q) plot of the MLM for seed per plant.

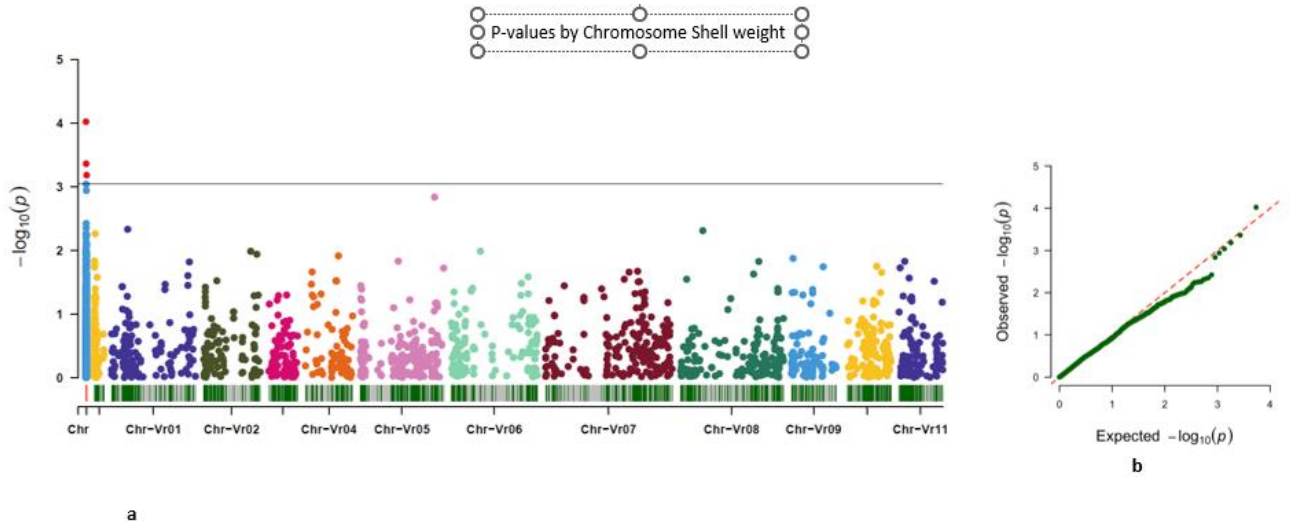


Figure 5.8: Genome -wide association analysis of shell weight in Bambara groundnut population. (**a**) Manhattan plot for mixed linear model (MLM) approach for shell weight. Negative log10 P-values are plotted on Y- axis against SNP position on X-axis for each of the 11 chromosomes. Genome wide significant threshold is depicted as harsh color horizontal line, each dot in different colors represents the SNPs on the 11 chromosomes. (**b**) Quantile-quantile (Q-Q) plot of the MLM for shell weight.

Table 4.9.1. Bambara groundnut nucleotide sequence with close identity and local alignment with Cowpea and mung bean genomes.

Clone ID	Gene ID	Location	annotation end product	References
37316456	Vigun05g237400	Vu05:43046869..43053981	protein HIRA-like isoform X2 [Glycine max]; IPR011494 (TUP1-like enhancer of split), IPR015943 (WD40/YVTN repeat-like-containing domain); GO:0005515 (protein binding), GO:0005634 (nucleus)	
	Vigun05g235200	Vu05:42866784..42867398	germin-like protein 9-3-like [Glycine max]; IPR001929 (Germin); GO:0030145 (manganese ion binding), GO:0045735 (nutrient reservoir activity)	Li et al., 2016
	Vigun05g235600	Vu05:42882671..42886280	rho GTPase-activating protein 1-like	Su et al., 2013. Flower development under drought stress
	Vigun05g235700	Vu05:42888937..42891109	Calcium-binding EF-hand family protein; IPR011992 (EF-hand domain pair); GO:0005509 (calcium ion binding)	
	Vigun05g236600	Vu05:42967140..42968998	ABSCISIC ACID-INSENSITIVE 5-like protein 1-like [Glycine max]; IPR004827 (Basic-leucine zipper domain); GO:0003700 (sequence-specific DNA binding transcription factor activity), GO:0043565 (sequence-specific DNA binding)	Daszkowska-Golec, A. (2016)
	Vigun05g237900	Vu05:43120779..43124414	fatty acyl-CoA reductase 3-like [Glycine max]; IPR016040 (NAD(P)-binding domain), IPR026055 (Fatty acyl-CoA reductase); GO:0080019 (fatty-acyl-CoA reductase (alcohol-forming) activity	

	Vigun05g239400	Vu05:43249595..43251295	Ribosomal protein L24e family protein; IPR000988 (Ribosomal protein L24e-related), IPR023441 (Ribosomal protein L24e domain), IPR023442 (Ribosomal protein L24e, conserved site)	
4178140	Vigun04g138400	Vu04:34453250..34456659	receptor-like protein kinase 2; IPR001611 (Leucine-rich repeat), IPR003591 (Leucine-rich repeat, typical subtype), IPR013210 (Leucine-rich repeat-containing N-terminal, type 2); GO:0005515 (protein binding)	Ouyang *et al*,.2010; Mizuno et al 2007
	Vigun04g138500	Vu04:34472480..34487953	general transcription factor group E6; IPR001487 (Bromodomain), IPR027353 (NET domain); GO:0005515 (protein binding)	Receptor-like kinase OsSIK1 improves drought and salt stress tolerance in rice (Oryza sativa) plants.
	Vigun04g138600	Vu04:34489311..34490922	receptor-like protein kinase 2; IPR001611 (Leucine-rich repeat); GO:0005515 (protein binding)	Ouyang, S. Q., Liu, Y. F., Liu, P., Lei, G., He, S. J., Ma, B., ... & Chen, S. Y. (2010). Receptor-like kinase OsSIK1 improves drought and salt stress tolerance in rice (Oryza sativa) plants. The Plant Journal, 62(2), 316-329.
	Vigun04g139100	Vu04:34537199..34540469	receptor-like protein kinase 2; IPR001611 (Leucine-rich repeat), IPR003591 (Leucine-rich repeat, typical subtype), IPR013210 (Leucine-rich repeat-containing N-terminal, type 2), IPR025875 (Leucine rich repeat 4); GO:0005515 (protein binding)	The role of receptor-like protein kinases (RLKs) in abiotic stress response in plants. Ye et al., 2017

	Vigun04g139400	Vu04:34588849..34592675	receptor-like protein kinase 2; IPR001611 (Leucine-rich repeat), IPR003591 (Leucine-rich repeat, typical subtype); GO:0005515 (protein binding)
4181321	Vigun01g121700	Vu01:29848622..29849563	geranylgeranyl pyrophosphate synthase 1; IPR017446 (Polyprenyl synthetase-related); GO:0008299 (isoprenoid biosynthetic process)
	Vigun01g121800	Vu01:29853957..29857872	alpha/beta hydrolase family protein; IPR019149 (Protein of unknown function DUF2048)
	Vigun01g122100	Vu01:29869351..29876221	TGACG-sequence-specific DNA-binding protein TGA-2.1-like isoform X1 [Glycine max]; IPR004827 (Basic-leucine zipper domain), IPR025422 (Transcription factor TGA like domain); GO:0003700 (sequence-specific DNA binding transcription factor activity), GO:0043565 (sequence-specific DNA binding)
	Vigun01g122900	Vu01:29964497..29969354	squamosa promoter binding protein-like 9; IPR004333 (Transcription factor, SBP-box); GO:0003677 (DNA binding), GO:0005634 (nucleus)
4181458	Vigun05g102300	Vu05:10225018..10232336	alcohol dehydrogenase 1; IPR002085 (Alcohol dehydrogenase superfamily, zinc-type), IPR011032 (GroES (chaperonin 10)-like), IPR013149 (Alcohol dehydrogenase, C-terminal), IPR016040 (NAD(P)-binding domain); GO:0008270 (zinc ion binding), GO:0016491 (oxidoreductase activity), GO:0055114 (oxidation-reduction process)
	Vigun05g103100	Vu05:10291207..10295562	protein serine/threonine kinase activity

	Vigun05g103300	Vu05:10317562..10319287	phosphoglucan, water dikinase, chloroplastic-like isoform X1 [Glycine max]; IPR013783 (Immunoglobulin-like fold), IPR013784 (Carbohydrate-binding-like fold), IPR015902 (Glycoside hydrolase, family 13); GO:0003824 (catalytic activity), GO:0030246 (carbohydrate binding), GO:2001070 (starch binding)
	Vigun05g104100	Vu05:10366814..10372931	auxin transporter-like protein 2-like isoform X1 [Glycine max]; IPR013057 (Amino acid transporter, transmembrane)
	Vigun05g104200	Vu05:10404080..10405997	plasma membrane intrinsic protein 1; 4; IPR000425 (Major intrinsic protein), IPR023271 (Aquaporin-like); GO:0005215 (transporter activity), GO:0006810 (transport), GO:0016020
4183652	Vigun09g121200	Vu09:26926663..26929305	cation/H+ exchanger 23; IPR006153 (Cation/H+ exchanger); GO:0006812 (cation transport), GO:0015299 (solute:hydrogen antiporter activity), GO:0016021 (integral component of membrane), GO:0055085 (transmembrane transport)
	Vigun09g120500	Vu09:26693025..26693723	Plant invertase/pectin methylesterase inhibitor superfamily protein; IPR006501 (Pectinesterase inhibitor domain); GO:0004857 (enzyme inhibitor activity), GO:0030599 (pectinesterase activity)
	Vigun09g121500	Vu09:27111895..27127791	Regulator of chromosome condensation (RCC1) family protein; IPR009091 (Regulator of chromosome condensation 1/beta-lactamase-inhibitor protein II)

4183856	Vradi07g12260	Vr07:30419497..30428351	RING/FYVE/PHD zinc finger superfamily protein; IPR013083 (Zinc finger, RING/FYVE/PHD-type), IPR016181 (Acyl-CoA N-acyltransferase); GO:0005515 (protein binding), GO:0008080 (N-acetyltransferase activity), GO:0008270 (zinc ion binding);*-**; AT5G36670.1
	Vradi07g11670	Vr07:29529212..29530069	putative DNA-binding protein ESCAROLA-like [Glycine max]; IPR014476 (Predicted AT-hook DNA-binding) ; ***-; gi|356512004|ref|XP_003524711.1|
	Vradi07g11760	Vr07:29631073..29633831	NAC domain protein; IPR003441 (NAC domain); GO:0003677 (DNA binding); ***-; UniRef90_UPI00042B9626
	Vradi07g11780	Vr07:29667949..29672503	Protein phosphatase 2C family protein; IPR001932 (Protein phosphatase 2C (PP2C)-like domain), IPR015655 (Protein phosphatase 2C); GO:0003824 (catalytic activity);*-*-; AT2G46920.1
24346038	Vigun09g120900	Vu09:26830351..26836023	Cyclin associated with protein kinase Kin28p n=2 Tax=Komagataella pastoris RepID=C4R4Y7_PICPG; IPR015429 (Cyclin C/H/T/L); GO:0000079 (regulation of cyclin-dependent protein serine/threonine kinase activity), GO:0016538 (cyclin-dependent protein serine/threonine kinase regulator activity), GO:0019901 (protein kinase binding), GO:0070985 (TFIIK complex)

	Vigun09g121200	Vu09:26926663..26929305	cation/H+ exchanger 23; IPR006153 (Cation/H+ exchanger); GO:0006812 (cation transport), GO:0015299 (solute:hydrogen antiporter activity), GO:0016021 (integral component of membrane), GO:0055085 (transmembrane transport)
	Vigun09g121600	Vu09:27152901..27153224	SAUR-like auxin-responsive protein family; IPR003676 (Auxin-induced protein, ARG7)
24383534	Vigun09g196800	Vu09:37166203..37171118	dehydration-responsive protein RD22; IPR004873 (BURP domain)
	Vigun09g196900	Vu09:37187071..37203918	bromo-adjacent homology (BAH) domain-containing protein; IPR001025 (Bromo adjacent homology (BAH) domain), IPR012677 (Nucleotide-binding, alpha-beta plait); GO:0000166 (nucleotide binding), GO:0003682 (chromatin binding)
	Vigun09g197100	Vu09:37216330..37217378	uncharacterized protein LOC100798503 [Glycine max]
	Vigun09g197500	Vu09:37244552..37254525	Pentatricopeptide repeat (PPR) superfamily protein; IPR002885 (Pentatricopeptide repeat), IPR011990 (Tetratricopeptide-like helical); GO:0005515 (protein binding)
27640410	Vigun01g235400	Vu01:40693587..40696720	myo-inositol-1-phosphate synthase 2; IPR002587 (Myo-inositol-1-phosphate synthase); GO:0004512 (inositol-3-phosphate synthase activity), GO:0006021 (inositol biosynthetic process), GO:0008654 (phospholipid biosynthetic
	Vigun01g236500	Vu01:40800303..40801136	hypothetical protein; IPR000008 (C2 domain); GO:0005515 (protein binding)

	Vigun01g237300	Vu01:40858856..40865484	ATP-binding/protein serine/threonine kinase [Glycine max]; IPR001611 (Leucine-rich repeat), IPR011009 (Protein kinase-like domain), IPR013210 (Leucine-rich repeat-containing N-terminal, type 2), IPR013320 (Concanavalin A-like lectin/glucanase, subgroup), IPR025875 (Leucine rich repeat 4); GO:0004672 (protein kinase activity), GO:0004674 (protein serine/threonine kinase activity), GO:0005515 (protein binding), GO:0005524 (ATP binding), GO:0006468 (protein phosphorylation)
27640415	Vigun03g167200	Vu03:19381795..19383203	glucuronoxylan 4-O-methyltransferase 3-like [Glycine max]; IPR021148 (Putative polysaccharide biosynthesis protein)
	Vigun03g167500	Vu03:19442656..19444901	HCP-like superfamily protein with MYND-type zinc finger; IPR001810 (F-box domain), IPR002893 (Zinc finger, MYND-type), IPR011990 (Tetratricopeptide-like helical); GO:0005515 (protein binding)
	Vigun03g168200	Vu03:19701513..19707086	caffeoyl-CoA 3-O-methyltransferase; IPR002935 (O-methyltransferase, family 3); GO:0008171 (O-methyltransferase activity)
4176853	Vigun01g018800	Vu01:2013315..2013798	Nodule Cysteine-Rich (NCR) secreted peptide related
	Vigun01g01990	Vu01:2107168..2114107	bifunctional purine biosynthesis protein purH-like [Glycine max]; IPR002695 (AICARFT/IMPCHase bienzyme), IPR016193 (Cytidine deaminase-like), IPR024051 (AICAR transformylase domain); GO:0003824 (catalytic activity), GO:0003937 (IMP cyclohydrolase activity), GO:0004643

			(phosphoribosylaminoimidazolecarboxamide formyltransferase activity), GO:0006164 (purine nucleotide biosynthetic process)	
	Vigun01g020100	Vu01:2136204..2153281	THO complex subunit 1 isoform X2 [Glycine max]; IPR021861 (THO complex, subunit THOC1)	
4176853	Vigun01g018800	Vu01:2013315..2013798	Nodule Cysteine-Rich (NCR) secreted peptide related	Zhou *et al*., 2021
	Vigun01g019700	Vu01:2098924..2102035	PENTATRICOPEPTIDE REPEAT 596; IPR002885 (Pentatricopeptide repeat), IPR011990 (Tetratricopeptide-like helical); GO:0005515 (protein binding)	Guo *et al*., 2021
4182227	Vigun02g133100	Vu02:28381022..28382703	Protein of unknown function (DUF620); IPR006873 (Protein of unknown function DUF620)	
	Vigun02g133800	Vu02:28420643..28425098	zinc finger protein MAGPIE-like [Glycine max]; IPR013087 (Zinc finger C2H2-type/integrase DNA-binding domain); GO:0003676 (nucleic acid binding), GO:0046872 (metal ion binding)	
4182227	Vigun02g133100	Vu02:28381022..28382703	Protein of unknown function (DUF620); IPR006873 (Protein of unknown function DUF620)	

	Vigun02g133500	Vu02:28400724..28402416	Plasma membrane mannitol transporter n=1 Tax=Arachis hypogaea RepID=B2Z3Y4_ARAHY; IPR005828 (General substrate transporter), IPR016196 (Major facilitator superfamily domain, general substrate transporter); GO:0005215 (transporter activity), GO:0006810 (transport), GO:0016020 (membrane), GO:0016021 (integral component of membrane), GO:0022857 (transmembrane transporter activity), GO:0022891 (substrate-specific transmembrane transporter activity), GO:0055085 (transmembrane transport)
	Vigun02g135200	Vu02:28514386..28518885	BES1/BZR1 homolog 1; IPR008540 (BZR1, transcriptional repressor)
	Vigun02g135500	Vu02:28536905..28540816	Ubiquitin-conjugating enzyme family protein; IPR016135 (Ubiquitin-conjugating enzyme/RWD-like), IPR023313 (Ubiquitin-conjugating enzyme, active site); GO:0016881 (acid-amino acid ligase activity)
	Vigun02g137000	Vu02:28660119..28670226	Protein DA1-related 1-like isoform X2 [Glycine max]; IPR001781 (Zinc finger, LIM-type), IPR003903 (Ubiquitin interacting motif), IPR022087 (Protein DA1 like); GO:0008270 (zinc ion binding)
4182521	Vigun11g003900	Vu11:371421..377900	FACT complex subunit SSRP1; IPR000969 (Structure-specific recognition protein), IPR009071 (High mobility group box domain), IPR011993 (Pleckstrin homology-like domain), IPR013719 (Domain of unknown function DUF1747), IPR024954

		(SSRP1 domain); GO:0003677 (DNA binding), GO:0005634
Vigun11g005700	Vu11:609412..611378	Ankyrin repeat family protein; IPR020683 (Ankyrin repeat-containing domain), IPR026961 (PGG domain); GO:0005515 (protein binding)
Vigun11g006000	Vu11:634616..642145	WD repeat-containing protein 13-like [Glycine max]; IPR015943 (WD40/YVTN repeat-like-containing domain); GO:0005515 (protein binding)

4.7. Description of the functions of the genes that confers drought stress.

The current study used a reference cowpea and mung bean reference genome. The Bambara groundnut nucleotide sequence with close identity and local alignment with Cowpea and mung bean genomes revealed some genes of importance and their function in drought stress study (Su *et al.,* 2013) Drought and extreme temperatures are some of the most challenging threats to crop plants which negatively affect plant performance leading to cellular water deficit, cell membrane injury, loss of enzyme activities, and other defects which may result in severe reductions of crop yields and sometimes death. Identification of the key genes responsible for drought stress has been a major interest in plant breeding research. (Su *et al.,* 2013: Ye *et al.,* 2017). Genes that are responsible for acclimation to water deficit and help to minimize loss of plant productivity during drought have been identified and analyzed in many studies, especially during vegetative development. One of the plant responses to drought is to increase the expression levels of genes involved in osmolyte synthesis and metabolism, thereby enhancing drought tolerance. Genes that adapt to low water availability reduce plant output losses during dry spells. Research has identified and examined these genes, particularly during vegetative development. (Kawasaki *et al*., 2001; Zhu, 2001; Chaves *et al*., 2009).

In this study, some genes for protein kinases have been identified. They are known to play important roles in abiotic stress response in plants. Gene ID Vigun05g237400 discovered in this study is a Receptor like protein kinases (RLKs). RLKs and their descriptions and function in relation to the study objectives are as follows; - RLKs are the largest gene family in plants, they play critical roles in plant life cycles particularly in the regulation of plant developmental processes as well as in hormone and stress responses. (Dievart and Clark, 2004).

The RLK gene family overall represents 2.5% of Arabidopsis genes (Gene2, Shiu and Bleecker, 2001). Leucine- rich repeat receptor- like linases (LRR-RLKs) are the largest group of RLKs, with over 200 members represented in Arabidopsis. While most of their functions remain unknown, quite a number of well characterized LRR-RLKs play important roles in exogenous signal perception in plant's environment. (Li *et al,.* 2002). Many RLKs have been shown to be involved in abiotic stress responses, including the abscisic acid response, calcium signaling and antioxidant defense (Ye *et al*.,2017) Vigun05g235600 is a-rho GTPase-activating protein 1-like. It helps flower development under drought stress. (Su *et al,.* 2013)

Vigun05g236600 abscisic acid-insensitive 5-like protein 1-like reveals the roles of root hair as an

environmental sensor to maintain plant functions under water-deficiency conditions. Daszkowska-Golec, A.(2016). Vigun04g138400 receptor-like protein kinase 2 has been found to improve drought and salt stress tolerance in rice (Oryza sativa) (Ouyang *et al*., 2010). Nodule Cysteine-Rich (NCR) secreted peptide related- reveals key genes currently known to be involved in the regulation of nodule in legumes (Zhou *et al*., 2021)

CHAPTER FIVE

5.1. DISCUSSION

Bambara groundnut is an important underutilized crop cultivated in many parts of Nigeria. It contributes to food and nutrition of the people living in Nigeria especially in the eastern and northern part of the country. Three key factors leading to low Bambara groundnut production as identified in the studied environments are- (a). Unpredictable periodic rains followed by a long dry season and (b) Poor soil with low fertility. (c) Genotypic differences among landraces of Bambara groundnut. These are the identified major constraints accounting for low production and productivity. The overall objectives of this study are to understand the response of 135 genotypes of Bambara groundnut to water stress in three different locations.

It was discovered that there were locational differences in climatic conditions of the test environments which impacted crop performances. Rainfall patterns, especially in quantity, frequency, and months of falls are different (Figure 4.2). The 11 years rainfall records taken prior to this study showed that Ibadan has bi- modal rainfall pattern which could allow cultivation in two seasons, Mokwa and Zaria are unimodal. Moreover, rainfall data during the experimentation period (2019 –2021) clearly confirmed the rainfall pattern of all the locations used for this study (Figure 4.3).

The long rainy season (May – October) was interrupted by a 5-month drought (December – April) before the onset of rain in the subsequent month of May. Mokwa and Zaria locations have unimodal rainfall pattern, although, duration of months of rainfall differs with Mokwa having 5 months of rainfall (June-October) and Zaria 4 months of rainfall (July- October) followed by dry season. At Ibadan, the long rain duration is received between March and July followed by August break, the second rainy period is between August and October. This rainfall trend is also supported by Ogungbenro *et al*., 2014, Willoughby *et al*., 2020: Dairo *et al*., 2020. Such rainfall conditions require irrigation support for consistent cultivation. However, low or no input constraints usually limit the farmers to be crop specific, risk-prone, and limited planting time in every year leading to low productivity. The highest (312.75 mm) and the least (207 mm) total monthly rainfall in the months that preceded cultivation in these locations were recorded in 2019/2020 and 2021/2022 in Ibadan and Zaria respectively. Whereas during drought trialing period of months November to February (2019/2020 and 2020/2021), total rainfall in these locations were- 62.49 in Zaria 2019/2020 planting season, 105mm in Zaria 2020/2021, - 29.55 in Mokwa

2019/2020mm and - 102.91mm in Mokwa 2020/2021. - 117.9 mm in Ibadan 2019/2020, -62.4 in Ibadan 2020/2021 planting season.

It showed clearly that rain cessation and period of drought from these locations are different ranging from 4-5 months. This can have a significant effect on both the staples and other companion crops causing low crop performance and sometimes crop failure especially under the prevailing rainfed agricultural systems, meeting food quantity as required by consumers may be difficult. Farmers in Mokwa and Zaria especially need Bambara groundnut genotype with early maturity and high adaptation characteristics which can flower and set its pod within short period of rainfall, farming in those environments could be very risky if the rain cease abruptly during flower initiation or when the crop is just setting their pods. Bambara groundnut with early maturity characteristics as described above will be suitable for cultivation in these areas with short months of rainfall. Short duration genotypes that can utilize the moisture and produce reasonable pods within short period of rainfall are related to escape mechanism genotypes which mature within short growing cycle.

The most sensitive stage in Bambara cultivation is the reproductive stage starting from flower emergence to pod initiation. We observed that the drought imposed to WS regime at flower emergence affected both vegetative and reproductive organs of Bambara groundnut. Plate 3.6, 3.8, and 3.9 (WS regime) showed the leaf gradual wilting, hastened senescence because of water deficit from flower emergence until maturity as compared to the plants under WW regimes which continue to generate new flushes of leaves, more branches, and new flowers until physiological maturity (Plate 3.3, 3.5, and 3.7). At WS regime, declined soil moisture due to drought imposition resulted to obvious changes in plant's morphology at one week after imposition. This is in support of studies conducted to understand Bambara groundnut response to a short period of water stress, drought was applied at different developmental stages of Bambara groundnut. (Vuraya *et al.,* 2011; Hui *et al.,* 2015). From their results, the stress imposition at different stages caused reduction in relative water content, but the effect of drought was severe at the reproductive stage imposition. Moreover, when water was supplied after the mild drought imposition, plants stressed during the pod filling failed to fully recover. Such genotypes that can harness resources for pod production during low moisture availability will be the best candidate genotype under limited water condition like Mokwa and Zaria location.

Nigerian rainfall pattern in terms of intensity and frequency has received some attention from various researchers. A good understanding of the past and current trend of hydrological changes is relevant to future food production. Going by research history on Nigerian climate, various experts research report on Nigerian rainfall history, Adefolalu (1986) analyzed 70-year rainfall data between the year 1911 and 1980 from 28 meteorological stations, Bello (1998) also compared the seasonality of rainfall distribution in Nigeria for two climate periods of 1930–1961 and 1962–1993. Ati *et al.,* (2009) reported rainfall over nine stations in northern Nigeria between 1953 and 2002. Their results elucidated historical changes in the climatic system of Nigeria as it reported a general decline of dry season's contribution to annual rainfall, they all concluded that the dry period is getting drier. Their results complied with Oguntunde *et al.,* (2011) who analyzed Nigerian rainfall data from 1901–2002 from Global Gridded Climatology of Climate Research Unit Time series (CRU TS.2.1). The result suggested a significant reduction in annual rainfall in more than 20% of the country landscape and a high reduction in volume of rainfall 50- 350 mm which equals 64% portion of Nigeria. (Ogungbenro *et al*., 2014). Conversely, projections of the effect of climate change at a global scale revealed increase in aridity, heat, and crop's vulnerabilities to effect of climate change, African countries with pronounced aridity and rainfed agricultural system will share the higher impact. It may therefore be important to increase research effort into underutilized crops like Bambara groundnut especially in the research towards gene discovery and traits that confers drought tolerance.

5.2. Soil Characteristics

Although, several literatures confirmed Bambara groundnut as a hardy crop with potential to thrive well in drought prone environments. Feldman *et al.,* 2019 ; Gunjal *et al*., 2009 ; Mayes *et al*., 2019; Mubaiwa *et al*., 2018). Nevertheless, soil condition also determines crop performance, one of the other causes of low yield apart from landraces cultivated at farmer's field is the conditions of the soil for farming; soil texture, structure and available soil nutrients including moisture which transports the nutrients are all important factors to crop performances. The low micro and macro nutrient elements below limit are an important limiting factor for Bambara groundnut production. The soil analysis results showed the soil types, the levels of micro and macro nutrient element of the fields where this research was carried out in Nigeria. The dominant soil texture class was sandy clay in Mokwa, sandy in Ibadan and silty clay in Zaria (Plate 3.2). According to FAO (2001) report, Nigeria is one of the countries with high declining soil fertility, this is a serious challenge in agricultural sector especially arid and semi- arid poor

subsistence farmers with low or no inputs availability to boost yield, more attention is needed for diversifying agriculture for yield continuum. Despite the declining soil fertility, Nigeria is still ranked the highest producer of Bambara groundnut. This requires an in-dept study if nutrient availability in the soil has any relationship with crop yield or Nigeria is utilizing more crop land area and not increase in yield per square meter space. Crop yield per hectare should be improved, increased genetic gain of crop plants and crop diversification may reduce pressure on crop land, improve the soil status, reduce desertification, protect soil erosion, and improve crop yield.

Soil nutrient analysis results for all locations are also presented (Table 4.2a).
Major challenges to crop yield can be attributed to soil condition during crop growth and development. Various soil factors mitigating crop production and productivity are soil nutrient availability, soils with micro and macro nutrient deficient will have negative impact on crop performance. Soil moisture and nutrient retention capacity, accessibility of the available soil nutrient and uptake by plant root depends on the soil condition. Soil types, soil nutrient composition and their effect on crop performance has been reported by various journals, soil nutrient composition is an important factor to plants performance. Soil nutrient deficiency poses various threats to plant performance and ultimately reduces crop yield (Patil *et al*., 2020). Nigeria especially in the semi- arid tropics, declining soil fertility has been identified as a big challenge and fundamental cause of poor crop performance. Soils in most of Nigeria farmlands have inherently low fertility and do not receive adequate nutrient replenishment. Most subsistence farmers who depend solely on rainfed agriculture are mostly affected.

Low soil fertility is adversely altering yield stability leading to vulnerability of crop plants, fundamental cause of crop's poor performance in Nigeria. It is very important to understand the soil, weather and other environmental factors that contribute to crop performance in each environment where the crop is cultivated. Various methods have been employed for the identification of drought tolerance genotypes for legume crops. This study included the analysis of other environmental factors apart from genetic factors to gain a better understanding of genotypic adaptation in the two conditions (well-watered and water stressed). Various studies confirmed the effect of environmental factors as mitigating factors to crop performance especially in drought conditions. (Masson-Delmotte *et al*., 2018)

High temperature has been considered a major cause of crop's poor performance as it alters the morphological or physiological and molecular characteristics of crop plants. The present study

considered the temperature, rainfall, relative humidity, wind speed of the three locations in the two consecutive years as peculiar challenges that could enhance poor yield of crops. The record showed that the weather conditions and edaphic factors of the twelve environment differs; each has their level of contributions to crop performance. The range in temperature, relative humidity, rainfall, during the growing cycle as seen in figures 4.1a, 4.1b, and 4.4c. Figure 4.1a showed the monthly records of rainfall and volume in Zaria 2019/2020 and 2020/2021 season. In Zaria 2019/2020, the rainfall decreased in frequency and intensity from September 2019 (207.96 mm), October (154.6 mm) and 62.49 mm in November, no rainfall in December and January of the subsequent year.

5.3. Relative Humidity (RH)

Mean monthly percent RH for the three locations in the cropping years (2019/2020) and 2020/2021) looks similar (Figure 4.1b) with relatively lower humidity (ℚ8.5%) recorded at Zaria in February (2019/2020) season (Figure 4.1b). During trials (2019/2020) and (2020/2021), the highest RH was recorded in Ibadan 2019/2020 followed by Ibadan (2020/2021) locations i.e., 98.7% and 96.5% respectively (Figure 4.1b).

Temperature (^{o}C.)

The mean monthly maximum temperature had similar trend across locations (2019/2020) and (2020/2021) (Figure 4.1c). The least (27.3^{o}C) and the highest (37.53^{o}C)mean maximum temperature was recorded in Zaria (2020/2021) and Ibadan in February (2019/2020) respectively. Nigeria practices rainfed agriculture, understanding of rainfall pattern of Nigeria will improve our knowledge in the performances of crop as its contribution to genotype performance and way forward. Investigation and assessment of nutrient availability prior to crop establishment is essential to meet plant nutrient requirements. From the soil analysis, figures 4.11, 4.12 and 4.13 showed that the key mineral element Phosphorus (P) are found with limited availability in Ibadan and deficient in Mokwa and Zaria, this may result in poor performance and low yield of Bambara groundnut. To ensure improved pod yield of Bambara groundnut is achieved in the three locations under such weather conditions as recorded for (Mokwa, Zaria and Ibadan), there is also need for soil nutrient replenishment. Phosphorus is considered one of the primary macronutrients, along with nitrogen and potassium. (Masobirova *et al*., 2022; Patil *et al*., 2020) Phosphorus is a crucial component for converting solar energy into food, fiber, and other plant products. Phosphorus also plays a key role in the metabolism of sugars and energy storage. Decline in

soil moisture can lead to decrease in Phosphorus availability to plant for Phosphorus uptake (He *et al*., 2011; Sardans & Peñuelas, 2012)

This showed that most arid and semi-arid regions of Nigeria use for agriculture with low Phosphorus nutrients could lead to crop failure or yield loss and death of other crops that cannot withstand such harsh environment in Nigeria. This could also be the reason for low pod yield of some of the Bambara groundnut genotypes used in this study. Haruna *et al*., 2011 had similar results in his study on the effect of phosphorus fertilizer on the growth and yield of cowpea. He made a recommendation and the selection of cowpea lines that produce good yield under low soil phosphorus or lines with high phosphorus use efficiency. Genotypes with high water use efficiency or drought tolerance may be useful in such soil low in phosphorus and may be able to survive low soil phosphorus environment. This study also explored the soil types present in Mokwa, Ibadan and Zaria fields where this research was established. Table 3 presents the soil analysis results of the micro and macro nutrients. The following deductions were made from the soil analysis results. Soil texture (Particle size) location Ibadan sandy soil with 72%, Mokwa soil is sandy clay while Zaria is a mixture of clay and silt (Figure 3.2).

Although Ibadan had the highest (0.37%) organic carbon but generally, organic carbon content of the three locations is very low. 1- Ibadan (0.37%), 2- Mokwa (0.25%) 3- Zaria with (0.05%).

The Nitrogen content of the 3 locations (Zaria, Ibadan and Mokwa) are generally low particularly Mokwa. Nitrogen is an important element which helps foliage and plant leaf development. Table 4.2a presents Ibadan as the only location with the highest Phosphorus values (34.82 ppm and 23.80 ppm) in the two years. Zaria 2019 and 2021 had the least phosphorus values (1.10 and 1.66 ppm respectively. Phosphorus is an important element which enhances root growth and development, responsible for flower production, helps plants survive harsh climates and environmental stressors, no crop can give good yield when this element is lacking unless supplemented with phosphorus fertilizer. Deficiency of phosphorus leads to stunted growth with short internodes, results in few pods formation and low pods per plant.

Table 4.3 shows the mean values for both vegetative and reproductive traits performances. At the vegetative stage of plant development, both regimes performed relatively well across locations. This can be attributed to the availability of soil moisture at this stage before water was withdrawn from the water stressed plot at flower emergence. After water withdrawal, the water stress regime impacted the flowering and crop performances at this stage. The days to first flower was 40 days in Ibadan well-watered but had

39 days at water stressed condition from the same location. Likewise, it had 40 days at Mokwa under well-watered condition whereas, plant attained first flower at 37 days after planting at water stressed regime from the same location. Zaria had its days to first flower in 44 days after planting under well water condition and 43 days after planting under water stress regime (Table 4.3). it showed that water stressed regimes speed up the day to flower of genotypes than genotypes under regular water conditions.

The ability of any crop species to produce a reasonable yield under stressed environmental conditions is a guarantee of such species for stability of production. The relative yield performance of genotypes in drought-stressed and non-stressed environments can be used as an indicator to identify drought resistant varieties in breeding for drought-prone environments. Several drought indices have been suggested for the analysis of yield performances under well water and water- stressed conditions. These indices are based on either drought resistance or drought susceptibility of genotypes. (Raman *et al*., 2012)

The growth stage of plant at the drought incidence also determines the plant's response and severity of the impact. The outlook of a breeding program is to select from the pool of genotypes, the outstanding genotypes that its yield performance surpasses the currently grown varieties in a target environment. Environment varied greatly in climatic factors and soil factors, hence there is need for precision in selection of genotypes for a targeted environment. Performances of genotype must not be blown out of proportion to have stability of production even in unpredictable weather conditions. Several indices have been used by the breeders to calculate genotype performances under varying drought conditions. The yield of a particular genotype in stressed and non-stressed environments varied and the ability of a genotype to produce reasonable yield in drought-stressed environments is an indication of production stability. From this study, analysis of variance showed highly significant ($P<0.01$) differences for genotypes yield in ww and ws regimes. The coefficient of variation (CV values) varied between 4.71% (days to first flower) 28.65% (leaf length and pod related traits 38% (100 seed weight) to 456.4% pod weight. The results displayed low, moderate, and higher influence of environment for each trait and implies that the genotypes had exploitable genetic variability for the studied metrics.

A CGIAR led project on breeding pipelines for respective legume crops with enhanced genetic gains has made a progressive step in finding solution to the yield of tropical legumes especially in the context of characterization and utilization of diverse crop germplasm found in local, national, region and

international genebanks including farmer's preferred landraces (Ojiewo *et al*.,2019). The objective is to enhance crop utilization, trait discovery, pre-breeding and forward breeding with a focus on priority traits to enhance genetic gains on legume (Ojiewo *et al*.,2019). This study examined twelve morphological traits from various traits found in Bambara groundnut descriptors. At the morphological level, most of the traits are ordinal (they lack a unit of measurement) for instance, leaf color in Bambara is mostly green or sometimes pink, also is leaf shape, petiole color etc. In a study conducted by Mesay *et al*., 2022 on Bambara groundnut characterization for yield and yield related traits, they also confirmed that leaf color, leaf shape and other vegetative metrics' coefficient of variation are mostly not significant whereas highly significant differences were observed in the measurement for number of pods per plant, number of seeds per plant, and a hundred seed weight. Their results showed that genotypic variance ranged from 0.01 to 36.64 with relatively higher genotypic variance for the number of pods per plant and grain yield observed among the accessions characterized, these traits can be sought out for as trait of importance in legume breeding.

Seed weight and pod weight of Bambara groundnut differed from genotype to genotype also, do the number of pods and seeds per plant differed. These traits are known to contain vast genetic variability. Although, till date, seed testa color is mostly used for selection both by farmers and researchers; most populations are developed by sorting out seeds of the same colour from the mixture, this is mostly common in underutilized legumes, selection based on seed weight (100 seed weight) has not been reported in Bambara groundnut breeding. Seed testa colour are the commonly used criteria (Mayes *et al*,.2019). (Adewale *et al*., 2010 ; Adewale *et al*., 2012a. Adewale *et al*..2010 had earlier suggested the use of six seed characters (seed length, width and thickness and their ratios) as unique indices for discriminating among African Yam Bean (S*phenostylis stenocarpa*) (AYB) accessions. The significant accession × location × year effects for all traits indicated the distinctiveness of the environments in discriminating among the accessions. For instance, the performance of number of flowers was high across all locations under a well-watered and water stressed conditions (Fig 4.9.9), although there was a high significant differences number of flower based on regime WW and WS, the highest number of flowers was found at Ibadan location but that does not suggest the seed weight performance as Mokwa location had the highest seed weight in this experiment(Fig. 5.3). Moderate to high heritability estimates observed for all traits implied increased power of SNP detection in the accession, hence, identification of true associations between a marker and putative gene (Adewale *et al*., 2020). The significant positive

correlations between pairs of traits e.g 100 seed weight had highly significant and positive correlation with days to 50% flower, pods per plant had a highly significant and positive correlation with pod weight and seed weight highly correlated with the plant height suggests the possibility of simultaneous improvement of the traits to enhance seed yield.

The genetics of traits that confer drought tolerance have been studied using GWAS in this report. Significant variations were discovered in the population of Bambara groundnut used and the traits used significantly differed from each other. Association analyses between specific phenotypes and genotypes within a genome is an important step towards the discovery of genes controlling the traits. (Mwadzingeni *et al*., 2017; Adewale *et al*., 2020). Twelve out of 135 accessions used in this study which were collected from UK has been previously utilized for genome-wide association studies for various traits such as nutritional traits and several significant SNPs were found to be associated with the studied traits (Mayes *et al* 2019). In this study, model fitness for the GWAS was confirmed by the Q-Q plots. The alignment of observed and expected p-values in the Q-Q plots for all the measured traits indicated spurious associations because of population structure and familial relatedness were largely corrected. The contribution of all the significant markers to the phenotypic variation which ranged from 5.0 to 7.4% suggested that the markers could be useful for marker-assisted selection in Bambara groundnut improvement. Although Chan *et al* 2019 report the release of the first genome of Bambara, this is a great breakthrough in the breeding of this crop but till date, it has not been made available for gene annotation and other molecular analysis on Bambara groundnut. Therefore, the exact locations of the markers on the chromosomes remain unknown. The identification of more than one significant marker for specific traits further confirms the polygenic and complex nature of the selected traits in this study. Such pleiotropic markers could be useful in the simultaneous improvement of correlated traits. The six significant markers that were consistent in one location and combine location analysis for the same trait in this study could be considered as putative makers. The six significant markers found in *Vigna unguiculata* genome at a location close to genes whose encoding proteins had been reported in other crops to regulate same traits as those to which they are associated with in Bambara groundnut, can also be considered as candidate makers. Efforts should be directed towards the validation of the identified significant makers using several mapping populations before they can be targeted for use in maker-assisted selection for seed-size traits in Bambara groundnut.

Drought stress has long been a major challenge to crop performance and yield stability, its intense is

getting higher in African countries all day long. It causes significant yield losses in minor and major seed crops. In this study, five BG accessions have been identified as drought tolerance through a two-year three location drought experiment with drought imposition at flower emergence until harvest; however, the response of accession to water deficit was different, especially to the seed yield, the morphological characteristics of seeds harvested from WW and WS regimes and their corresponding 100 seed weight. Morphological assessment of the harvested seeds showed the impact of water deficit on seed and the corresponding seed weight. It was observed that genotype performance of seed weight from WW regimes were superior to same genotypes under WS regimes. The drought studies have identified genotypes with minimal yield reduction under drought (Table 4.8). Among the 135 accessions understudied, Table 4.8 showed the percent yield reduction and drought susceptibility index (DSI) of the five accessions with lowest yield reduction due to drought, best DSI ranking, and best performing genotypes under well-watered and water-stressed conditions.

Meanwhile, the identified genotypes with stable yield characteristics across diverse environments under regular water supply is a treasure to the farmers who seek to cultivate BG as a companion crop with their major crop under reasonable and evenly distributed rainfall conditions. The following accessions can be recommended for such an environment- TVsu-29, TVsu-397, TVsu-2088, TVsu-2091, TVsu-2093, TVsu-2095, TVsu-2108 and CFFR-10 (Uniswa-red) (Table 4.8). Moreover, the recommendation of the best accessions under water stressed condition are critical features that must be carefully selected. Various constraints mitigate crop performance in dry environments, and such should be considered when recommending genotypes for such instances. For instance, in an environment with short duration of rainfall accompanied with high temperature, a short duration genotype that completes its cycle (early maturing with escape potential) is needed in such an environment. A location with problem soils (low soil nutrient and soil structure) is another challenge, Bambara groundnut performance in term of seed production were very low in location with low soil nutrients (micro and macro nutrients), high percentage of clay composition, low organic carbon, low phosphorus, and low nitrogen.

Despite these deficiencies, some genotypes can be recommended with appreciable yield in terms of number of pods they produced. The accessions with this potential from the total 135 accessions are TVsu-2104 (bunchy growth habit with elliptic terminal leaf shape), CFFR-2 (bunchy growth habit and lanceolate terminal leaf shape), TVsu-2112 (bunchy growth habit and lanceolate terminal leaf shape), and 2105 (bunchy growth habit and elliptic leaf shape). It is noteworthy to observe that the accessions

under this category are all bunchy genotypes, moreover, all these genotypes except CFFR-2 (Lun-T) belong to one of the three groups of Bambara groundnut used for this study. They are the newly explored landraces from the farmer's fields in the middle belt of Nigeria. Further study is needed to explore the genetic basis of this potential inherent in the set of accessions above. Assessment of their nutrient profile will be useful to understand the presence of protein fibers and vitamins present in these accessions.

Another sought after trait is high yielding genotypes with short maturity period. This allows the optimal use of farmland by rotating other crops with Bambara groundnut genotypes of early maturity characteristics. TVsu-2110, TVsu-2108 are the best genotypes of consideration for such instance. Nevertheless, accession to recommend for an environment with inconsistent rainfalls and locations with unimodal annual rainfall should have reasonable yield, seeds that are not wrinkled due to drought, are-TVsu-14, TVsu-200, TVsu-216, TVsu-288, TVsu-410, CFFR-9 (S19-3). The accessions with late maturity characteristics from the total 135 accessions in this study are TVsu-467 (semi bunchy genotype with lanceolate leaf shape) and TVsu-295 (spreading growth habit and lanceolate terminal leaf shape). Nigeria holds huge potentials of biodiversity which if well managed and utilized, can sustain the food security, and improve the well-being of Nigerian. The crop diversity and their genetic diversity alone can turn the agricultural sector into a wealth creation sector. The challenge to bring this to reality is to adopt crop diversification to increase the number of crops in cultivation by small scale farmers, especially those crops that require low inputs. All adaptation strategies and measures to reduce the effects of climate change on crop yield leading to food security should be integrated to Nigerian agriculture to guarantee continuous provision of food and economic stability. The national genebanks have a major role to play in ending food and nutrition supply deficit that costs the country millions of dollars in food import annually.

The focus of national genebanks in food security has been extended, they have led the key role in developing small scale farmers and establishing food security through the intervention of the agricultural finance and donor community such as the crop diversity trust (Crop Trust, Bill, and Melinda gates foundation, Rockefeller foundation etc. National genebanks are also collaborating with international genebanks for a joint analysis of accessions, capacity building and identification of useful traits that are resilient to drought and can yield under a water limited conditions. A large investment is spent on the use of molecular markers such as genetic diversity study, genome wide association studies (GWAS) and the use of phenotyping tools to accelerate the identification of tolerance, high yielding and disease

resistance genotypes among the diverse crop germplasm conserved in genebanks. The characterization of diverse crop accessions and species into core and mini core by genebank has been a breakthrough to both research and farming communities. Evaluation of the conserved accessions of major crops in various genebanks has resulted in the identification of the genes that confers drought tolerance and disease resistance , these have been used for further breeding while the drought tolerance landraces are used as parent stock and treasure trove by the crop breeders.

The national genebank can also play important role by working directly with small scale and low-income farmers, sharing of diverse species of crop landraces including the underutilized legumes with outstanding potentials as a diversification, adaptation and risk management strategies against crop and yield loss of major crops, especially the core and mini core with unique features such as drought tolerance, disease resistance, early maturing etc. for the development of on- farm diversity. With the support of extension agents, they can build farmers' capacity especially in the awareness of climate change and its impacts on crop performance and biodiversity. Farmers can be encouraged to cultivate diverse crops which would significantly improve biodiversity, fix nitrogen into the soil, protect soil from erosion and mitigate the effect of climate change. Increase in the cultivation of underutilized legume can be facilitated by genebank assistance through the fabrication of mini- underutilized crop processors, this could make the cultivation of diverse underutilized crops attractive. Enhancing and maintaining on-farm diversity could improve the farming systems, resilience, and sustainability of food security. Renard *et al*., 2019; Sinami *et al.,* 2019 attests to the fact that diversification strategies have shown some promise to increase biodiversity and ecosystem services and reduce total dependence of external inputs and has improved yield and yield stability. The identified genotypes from this study with unique characteristics such as drought tolerance and outstanding yield can be given to the farmers or established as a demonstration plot beside farmer's field along with farmer's varieties where he can make selection of the outstanding genotypes among the genebank or "farmer's variety" . These identified Bambara groundnut genotypes can really improve farmer's income and improve family wellbeing.

SUMMARY, CONCLUSION AND RECOMMENDATION.

A lack of concerted effort to improve underutilized legumes in sub- Sahara Africa may lead to what is referred to as “hunger in a world of plenty”. This study revealed the potential of Bambara groundnut, as important crop tolerance to drought, resilient and opportunity food crop. It can be cultivated with low input, and by women for its characteristics as a crop that needs little agronomic inputs in its production. Unfortunately, climate crises such as intense drought and aridity disproportionately affect crop production in vulnerable countries posing serious challenges on the livelihood. Drought tolerance genotypes of Bambara groundnut can ameliorate yield loss due to water stress crises and the suffering encounter by women farmers in arid and semi-arid zones of cultivation can be improved by the inclusion of Bambara groundnut drought tolerance genotypes. An extensive investigation was made in this study using both molecular analysis and field evaluation to elucidate tolerance ability of Bambara groundnut under water deficit and diverse environmental conditions. This study has informed us about the potentials inherent in diverse Bambara groundnut accessions used in this study. High yielding genotypes under two extremes of water conditions and traits that conferred drought tolerance have been discovered. It has shown the possibilities and the gap the underutilized crops like Bambara groundnut can bridge in the context of food security and climate change.

In this report, the identified drought tolerance genotypes and genotypes with high yielding characteristics could enhance continuous production of Bambara groundnut in water limited zones and guarantee food security in Nigeria and other parts of sub-Saharan Africa with similar challenges. Genebank is known for conservation and distribution of plant genetic resources material to meet the present and future food challenges. Several accessions of Bambara groundnut are conserved in various genebanks and are well preserved against genetic erosion and future food security. A large investment is being used for their ex-situ conservation, regeneration, and characterization. Unfortunately, lack of sufficient information on genetic variation phenotypic traits and nutritional potential limits their use in crop breeding. This experiment has employed both molecular and a two-year drought study to identified drought tolerance, early maturing, best performing genotypes under water stressed and best performing genotypes under well-watered condition, and late maturing genotypes from 135 accessions out of thousands of accessions conserved in IITA genebank in Nigeria. The identified genotypes with high yielding and drought tolerance abilities can be retrieved from genebank back to the farmers from where it was originally collected but have been eroded and lost by farmers. Such genotypes can also be recommended to

researchers as parent lines for further research. This in a way can increase patronage of the crop germplasm in the genebank where various accessions are conserved, the outstanding genotypes can be grown in diverse environment of cultivation. Although, Bambara groundnut is an underutilized legume food crop, this research output has shown its drought tolerance potential to address the emerging abiotic challenges that are mitigating legume production in diverse environments especially in the arid and semi-arid with low soil fertility and unimodal rainfall zones in Nigeria.

Drought stress is a global problem challenging food security in many parts of the world, it is projected that no one will be exempted by its impact in the foreseeable future. Therefore, the amelioration of degraded soils for crop production, and food security for global population is imperative. Various ways such as irrigation, fertilization, cover cropping etc. used to deal with drought impact may not be feasible and practicable by resource poor farmers in arid areas and are not economically viable by many farmers cultivating a large farmland. The recommended option based on this study is crop diversification and the use of drought tolerance crop species. For instance, Bambara groundnut genotype with drought tolerance potential in this study is the most promising alternative in the environment with low and unreliable rainfall conditions. While drought tolerance genotypes are recommended to areas with limited rainfall, the susceptible but high yielding genotypes can be recommended to areas with sufficient rainfall.

The identified outstanding genotypes with unique and important traits such as early maturity, high yield and tolerance to drought features or the combined features is a gateway to the improvement of this crop and could reduce hunger and malnutrition of Nigerian farmers. This research has addressed a major bottleneck in the selection of the best genotypes which could be effectively introduced across different cropping seasons and environments. A set of Bambara groundnut genotypes that can recover water stress and deficit soil nutrient condition amongst the total genotypes used in this study could be a major asset to the farmers that cultivate this crop. Because of the growing need for more protein rich food crops and future projected population increase, coupled with climate change, the outcome of this research could play a major role in its future breeding. This study therefore identified and recommends that, to improve the yield of Bambara groundnut, the number of seeds per pod, number of pods per plant, weight of seeds per plant are the important determinant traits. TVsu 2108 and S19-3 also known as CFFR-9 in this study are the outstanding genotypes suitable for global cultivation or recommended for cultivation in all environments. This research outcome is expected to benefit consumers and scientists in Nigeria research

institutes and other countries, especially those in the Sahelian countries that requires drought tolerance Bambara groundnut germplasm.

Table 5.1.Twenty identified accessions with high rank seed weight adapted to different locations under a WW condition.

Num	Genotype	Loc-1	Loc-2	Loc-3	Reg	Remarks
1	TVsu164	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
2	TVSu2094	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
3	TVSu234	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
4	TVSu280	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
5	TVSu288	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
6	TVSu29	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
7	TVSu329	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
8	TVSu50	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
9	TVSu2094	Mokwa	Zaria	Ibadan	WW	Stable in all locations at WW condition only
10	CFFR10	Mokwa	Zaria	-	WW	Stable in two locations at WW condition only
11	CFFR11	Mokwa	Zaria	-	WW	Stable in two locations at WW condition only
12	TVSu143	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
13	TVSu2089	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
14	TVSu2101	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
15	TVSu2105	-	Zaria	Ibadan	WW	Stable in two locations at WW condition only
16	TVSu2106	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
17	TVSu2109	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
18	TVSu2110	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
19	TVSu2114	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only
20	TVSu216	Mokwa	-	Ibadan	WW	Stable in two locations at WW condition only

Table 5.2 Twenty identified accessions with high rank seed weight adapted to different locations under a WS condition.

Num..	Genotype	Loc-1	Loc-2	Loc-3	Reg.	Remarks
1	CFFR13	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
2	TVSu14	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
3	TVSu2100	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
4	TVSu25	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
5	TVSu357	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
6	TVSu431	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
7	TVSu441	Mokwa	Zaria	Ibadan	WS	Stable in all locations at WS condition only
8	CFFR3	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only

9	TVSu145	Mokwa	Zaria	-	WS	Stable in two locations at WS condition only
10	TVSu200	Mokwa	Zaria	-	WS	Stable in two locations at WS condition only
11	TVSu2094	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only
12	TVSu2097	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only
13	TVSu2098	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only
14	TVSu2099	Mokwa	Zaria	-	WS	Stable in two locations at WS condition only
15	TVSu2105	Mokwa	Zaria	-	WS	Stable in two locations at WS condition only
16	TVSu2108	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only
17	TVSu2112	Mokwa	Zaria	-	WS	Stable in two locations at WS condition only
18	TVSu2114	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only
19	TVSu216	Mokwa	Zaria	-	WS	Stable in two locations at WS condition only
20	TVSu225	Mokwa	-	Ibadan	WS	Stable in two locations at WS condition only





(Photo taken by IITA communication unit.)

3.9.5. The outstanding samples of Bambara groundnut accessions were identified from this study.

FUTURE WORK

This study has been able to identify genotypes with tolerance abilities and traits that confer drought in Bambara groundnut based on the multi-environment two-year trials and application of GWAS studies to address the research objectives. There are many reports on the effect of drought on vegetative development in crop plants especially the primary crops but very few on Bambara groundnut, most especially of drought on reproductive growth and below ground traits in Bambara groundnut are not common. Future study on Bambara groundnut especially the accessions used in this study should include the effect of drought stress on nodulation, plant root characteristics, correlation of above and below ground characteristics to drought tolerance, and nitrogen fixation. This could give a better understanding of genotype response to nodule production under water stressed regimes. Moreover, future studies should compare the nutrient composition of seeds harvested under well-watered condition and water stressed condition perhaps drought has any significant impact on the nutritional composition of genotypes harvested under water stressed regime.

Various receptor like protein kinases (RLKs) have been isolated from various plants but RLKs research has been focused on some staple crops like rice (Oryza sativa) soybean (Glycine max) and Common bean (Pisum sativum) and some high tolerance plants such as alfalfa have also been investigated (Zhang *et al*. 2014; Guo *et al*. 2016). None of such studies have been reported in underutilized legumes like Bambara groundnut. Research needs to focus on investigating the functions of RLKs and their associated signaling pathways and gene networks under abiotic stress. The relationship between abiotic stress and RLK-mediated signal transduction is a compelling area for future research in underutilized legume crops.

Lastly, the drought tolerance genotypes identified in this study can be sorted from genebank for distribution to the farmers especially those living in arid and semi- arid zones with limited resources and inputs and can be used by the researchers and breeders as parent stock for crop improvement.

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APPENDIXES

Table 4.10.1 Combined analysis of pod weight for combined BLUPS for all environment and stability rank.

Accession	Ibad-WS-19	Ibad-WS-21	Ibad-WW-19	Ibad-WW-21	Mok-WS-19	Mok-WW-19	Zar-WS-19	Zar-WS-21	Zar-WW-19	Zar-WW-21	Combined	Stability.Rank
TVSu112	2.89	4.50	5.50	16.26	19.41	26.56	13.68	2.52	11.44	1.69	10.57	1
TVSu89	2.97	4.25	4.73	15.10	17.95	24.86	11.86	1.84	11.27	1.14	9.78	2
TVSu600	3.03	4.02	6.64	16.25	19.25	26.75	12.36	2.22	10.09	2.01	10.52	3
TVSu2104	2.94	4.41	5.34	17.85	18.36	27.26	12.77	1.46	13.51	1.45	10.32	4
TVSu46	2.77	3.90	5.11	16.57	17.89	24.45	12.97	2.63	9.33	1.45	9.73	5
TVSu8	2.94	4.67	5.14	15.87	18.63	25.05	13.83	1.71	9.64	2.19	10.35	6
TVSu2114	3.61	5.09	5.27	19.93	18.38	27.25	13.78	1.94	12.70	1.74	11.19	7
TVSu2110	3.03	4.70	5.81	17.90	18.36	26.17	14.11	2.15	13.84	1.01	10.79	8
TVSu319	2.87	4.03	4.81	17.44	19.38	24.28	13.06	1.88	9.28	1.98	9.88	9
TVSu2093	2.81	4.04	5.53	16.53	18.38	28.15	12.14	1.79	9.48	1.75	9.96	10
TVSu2095	3.22	4.46	6.47	18.89	18.39	27.49	14.53	2.62	10.02	1.82	10.99	11
TVSu2105	3.15	4.40	7.13	18.03	18.60	26.24	12.42	2.90	13.51	4.88	11.40	12
TVSu2108	2.96	4.62	5.38	18.47	18.36	26.20	12.75	1.90	8.77	2.23	10.33	13
TVSu318	3.03	5.23	5.97	18.07	17.12	25.26	12.48	1.86	9.67	1.24	10.57	14
TVSu2096	2.96	3.99	4.99	17.66	18.38	25.67	10.89	1.91	13.63	1.45	9.89	15
TVSu2098	4.13	3.92	4.97	17.19	18.36	25.67	11.47	1.84	8.77	2.49	10.59	16
TVSu277	3.30	4.25	6.04	17.11	19.53	28.34	13.75	2.14	9.41	1.95	10.82	17
TVSu431	3.64	4.00	5.16	20.24	20.47	28.78	14.64	4.31	13.39	2.68	11.42	18
TVSu262	2.87	3.53	4.44	15.08	17.92	24.14	14.10	1.99	9.35	2.15	9.57	19
TVSu671	3.14	4.36	5.14	17.52	19.17	24.25	15.29	1.67	12.57	1.59	10.55	20
TVSu160	3.27	4.02	5.90	15.80	17.09	28.27	13.33	1.93	9.83	1.48	10.36	21
TVSu170	3.04	4.22	4.81	15.24	20.49	26.89	13.03	3.55	13.13	3.54	10.79	22
TVSu359	3.57	4.27	5.38	14.97	20.60	27.16	12.98	1.93	10.60	1.08	10.70	23
TVSu110	2.84	4.22	5.81	19.89	18.36	24.08	13.12	2.27	11.72	1.76	10.24	24
TVSu2099	3.44	4.39	4.89	15.85	18.38	27.81	12.72	1.91	14.40	1.43	10.64	25
TVSu275	4.13	4.37	4.82	15.90	19.45	28.63	15.29	1.89	12.01	1.74	11.21	26
TVSu25	3.97	5.04	4.91	17.21	18.48	29.46	15.76	2.07	12.13	2.65	11.58	27
TVSu323	3.60	3.93	5.04	17.77	17.92	24.50	10.01	1.85	9.06	2.12	10.00	28
TVSu214	3.14	4.04	5.90	17.81	18.56	24.19	14.63	2.13	9.26	3.24	10.53	29
TVSu2113	3.13	5.18	5.55	18.39	16.84	25.09	12.97	2.53	8.66	1.62	10.60	30
TVSu200	2.78	4.36	5.60	17.74	20.32	28.65	16.41	2.05	13.58	1.80	10.88	31
TVSu245	2.88	3.48	5.24	13.55	17.27	26.29	11.75	1.84	8.38	1.83	9.42	32
TVSu66	2.67	3.92	4.62	12.88	17.38	23.19	11.20	1.76	9.94	1.06	9.22	33
TVSu2091	3.07	5.02	5.25	14.61	18.39	27.07	12.10	1.87	8.36	1.57	10.42	34
TVSu221	2.75	4.29	6.06	16.80	20.03	27.31	16.96	1.82	11.67	1.85	10.80	35
TVSu227	3.79	4.56	6.08	18.19	20.01	29.19	15.71	2.20	10.14	1.57	11.48	36
TVSu310	3.42	4.12	4.88	13.46	17.98	23.89	10.64	1.92	9.27	1.12	9.82	37

TVSu410	3.15	3.92	5.68	15.77	22.24	27.84	12.55	1.85	12.31	1.98	10.64	38
TVSu295	2.87	4.15	5.78	20.18	19.36	26.74	12.32	1.67	14.74	1.55	10.43	39
TVSu2097	3.36	4.68	5.37	17.99	18.39	26.67	9.36	1.71	9.43	1.03	10.19	40
TVSu368	3.38	5.22	5.48	16.64	19.37	25.58	16.38	2.58	14.51	1.93	11.61	41
CFFR3	3.12	5.33	4.22	16.20	18.39	27.12	16.64	2.26	12.45	1.41	10.93	42
TVSu361	2.83	3.62	4.40	12.94	18.48	27.48	13.43	1.83	11.08	1.54	9.67	43
TVSu397	3.10	3.76	4.35	15.12	19.41	23.71	14.25	3.21	13.33	2.33	10.25	44
TVSu225	3.61	4.18	4.75	17.10	19.50	23.55	14.08	2.17	14.35	1.43	10.67	45
TVSu467	3.85	4.88	4.82	17.61	19.71	29.93	11.92	1.88	9.85	2.16	11.10	46
TVSu231	3.15	4.26	4.58	20.72	20.27	28.16	14.60	2.16	14.34	1.44	10.69	47
TVSu386	2.78	4.58	5.40	14.26	18.42	27.59	13.42	2.01	14.21	4.02	10.65	48
TVSu645	2.62	4.93	5.27	15.61	19.69	26.63	14.64	1.87	7.99	1.71	10.39	49
TVSu229	3.49	4.50	5.65	18.63	20.17	27.14	10.65	3.20	8.62	2.02	10.87	50
TVSu144	3.41	3.85	5.35	16.22	19.78	26.39	13.32	2.35	7.15	1.95	10.37	51
TVSu11	2.90	4.23	5.36	14.83	18.77	28.59	14.72	2.93	8.89	2.10	10.49	52
TVSu388	3.13	4.32	4.91	12.39	17.54	26.82	13.36	1.95	10.22	1.79	10.14	53
TVSu288	2.99	4.33	6.43	14.71	20.62	25.65	16.19	1.76	12.46	3.83	11.17	54
TVSu115	2.94	4.02	5.48	21.12	18.68	26.11	10.70	2.19	9.91	3.08	10.04	55
TVSu348	2.96	3.80	4.75	13.05	17.44	25.88	13.39	1.51	13.33	3.71	9.96	56
TVSu2103	3.17	3.77	5.49	15.61	18.36	27.66	10.25	1.76	7.26	1.61	9.76	57
TVSu2112	3.62	4.08	6.18	15.19	18.39	28.63	14.51	2.45	8.31	1.83	10.87	58
TVSu2089	3.64	4.37	5.66	22.16	18.39	27.26	13.29	1.88	10.80	1.44	10.85	59
TVSu161	2.96	4.07	5.11	13.77	18.72	29.19	14.40	1.91	10.05	2.23	10.28	60
TVSu84	3.53	4.52	6.01	13.74	18.64	23.59	10.06	1.89	9.18	2.06	10.42	61
TVSu351	3.15	3.71	5.09	14.37	20.63	29.71	12.39	1.92	11.70	1.76	10.27	62
TVSu125	2.81	4.22	5.26	14.04	17.45	28.33	14.92	4.37	12.04	1.58	10.58	63
TVSu329	3.21	3.79	8.82	19.32	17.57	24.45	15.13	2.08	13.43	3.53	11.40	64
TVSu311	3.23	3.45	4.56	14.91	15.87	21.08	10.34	2.23	9.86	1.74	9.16	65
TVSu371	2.70	4.04	5.21	15.38	17.16	28.07	11.85	2.42	9.69	5.81	10.19	66
TVSu373	2.93	3.80	5.18	12.91	18.32	24.36	10.49	2.05	9.99	4.07	9.80	67
TVSu103	2.97	4.15	5.07	13.31	17.14	22.11	10.98	1.90	8.13	1.56	9.42	68
TVSu2092	3.21	4.37	4.83	12.61	17.81	28.12	10.96	1.88	11.41	1.92	10.12	69
TVSu156	2.53	4.19	5.31	13.24	19.68	26.20	15.09	1.72	10.80	4.23	10.36	70
TVSu2094	3.04	4.34	6.03	17.65	18.38	28.29	14.74	2.04	17.01	2.32	11.03	71
TVSu425	2.54	4.99	4.89	12.61	19.36	25.59	11.58	1.93	13.30	2.73	10.24	72
TVSu2107	2.81	4.51	5.58	13.01	21.54	26.46	13.16	1.94	12.23	1.81	10.54	73
TVSu75	2.80	3.99	4.81	11.89	18.08	23.65	9.86	1.88	8.98	1.46	9.30	74
TVSu82	3.24	4.07	6.03	13.50	18.52	26.00	9.55	2.00	13.68	1.47	10.24	75
TVSu362	2.50	3.86	4.96	12.90	18.79	26.78	13.20	2.40	14.69	2.07	9.96	76
TVSu266	3.08	4.19	5.92	20.78	18.37	23.05	12.62	2.10	13.85	1.54	10.43	77
TVSu137	3.01	4.11	5.08	11.61	17.75	24.88	13.28	1.60	8.79	1.59	9.77	78
TVSu85	2.85	4.28	4.91	11.36	17.77	26.43	11.36	1.58	9.10	0.95	9.60	79
TVSu47	3.07	4.08	5.96	18.88	18.40	31.30	14.78	1.84	14.96	1.50	10.87	80

TVSu143	2.66	3.95	6.65	21.59	18.46	25.85	12.10	1.94	8.57	2.29	10.17	81
TVSu535	2.84	4.29	4.75	13.40	17.85	24.39	15.97	1.83	12.95	3.34	10.36	82
TVSu212	2.87	3.62	5.70	14.53	16.95	20.76	12.98	1.44	8.38	1.47	9.41	83
TVSu9	3.03	4.25	5.22	11.34	17.40	25.54	13.67	1.96	9.67	1.87	10.07	84
TVSu378	2.70	3.86	5.96	11.92	17.75	26.79	11.64	1.92	12.29	3.88	10.08	85
CFFR1	3.33	4.22	5.83	14.52	20.06	27.31	10.38	4.83	10.33	5.12	11.23	86
TVSu441	3.76	4.25	4.72	22.23	19.11	28.13	16.87	4.29	10.78	2.00	11.46	87
TVSu158	2.97	4.07	5.55	11.96	18.97	26.09	13.43	3.82	9.34	0.90	10.31	88
TVSu216	3.12	4.52	6.12	22.34	20.37	28.70	15.25	2.32	9.23	2.66	11.17	89
TVSu376	2.69	4.20	4.37	12.99	20.43	28.59	10.65	1.72	13.26	0.91	9.72	90
TVSu232	3.17	4.41	5.98	13.93	20.45	30.56	13.76	2.07	14.46	1.66	11.11	91
CFFR2	2.87	4.17	5.15	11.04	17.64	25.29	10.34	1.91	7.75	1.65	9.51	92
TVSu2102	3.11	4.01	4.87	11.93	19.85	24.30	14.22	1.87	8.15	1.58	10.02	93
TVSu50	2.79	3.69	6.52	23.19	18.12	26.45	12.94	1.50	14.52	2.51	10.36	94
TVSu14	2.95	5.02	4.86	14.62	20.16	30.29	17.21	2.34	13.60	2.10	11.25	95
TVSu2090	3.04	3.83	5.73	11.34	18.38	25.94	10.16	1.91	7.89	1.76	9.70	96
TVSu2106	3.39	4.58	7.27	23.71	19.15	25.41	15.09	4.00	14.55	1.96	11.81	97
TVSu94	2.60	4.45	5.19	10.72	16.40	26.16	13.92	1.72	9.99	1.65	9.78	98
TVSu183	2.89	3.57	5.23	14.45	19.70	30.62	10.83	2.24	8.34	1.38	9.76	99
TVSu218	2.89	4.22	5.42	14.63	16.79	20.01	13.98	2.88	11.05	1.65	9.92	100
TVSu379	2.51	3.52	4.68	15.74	19.24	27.53	12.74	8.31	10.23	3.42	10.50	101
TVSu269	3.40	3.79	6.31	13.30	17.91	23.12	13.70	1.92	15.62	1.56	10.57	102
CFFR13	2.81	5.23	4.61	24.06	18.39	25.34	15.18	3.11	11.39	3.12	10.96	103
TVSu233	2.44	4.74	5.65	23.82	20.10	28.82	13.39	1.80	10.15	1.89	10.51	104
TVSu374	3.24	3.35	5.50	11.27	17.99	23.22	15.52	1.99	9.31	1.59	9.90	105
TVSu145	3.50	4.22	5.41	12.08	17.90	24.06	13.39	1.98	14.51	5.50	10.99	106
TVSu2100	3.54	4.66	5.78	21.72	18.86	24.72	11.76	3.36	10.77	8.16	11.64	107
CFFR6	2.87	3.62	4.99	11.13	17.38	25.69	11.28	2.00	15.43	3.46	9.80	108
TVSu2101	3.42	4.25	5.99	23.41	19.54	32.85	12.82	3.15	11.08	2.42	11.31	109
TVSu146	2.67	3.81	4.79	10.28	17.40	22.78	14.72	4.16	12.21	2.73	9.94	110
TVSu267	3.02	4.62	5.55	23.33	19.07	24.56	13.18	7.90	10.23	2.12	11.37	111
CFFR11	2.99	4.09	4.32	18.69	17.33	26.07	13.82	1.88	19.73	2.79	10.42	112
TVSu222	2.93	3.91	5.15	25.64	20.49	29.75	13.45	1.73	11.57	3.38	10.60	113
CFFR5	2.97	3.92	5.24	18.04	19.84	24.64	17.21	2.19	19.28	2.28	10.94	114
TVSu2088	3.06	4.56	4.55	22.53	18.83	33.84	13.81	1.65	12.89	1.39	10.68	115
TVSu593	2.48	3.81	5.64	25.65	19.05	24.75	12.51	1.69	10.59	2.91	9.96	116
TVSu164	3.04	3.82	5.54	24.48	18.96	24.90	11.41	2.37	15.80	6.14	10.79	117
TVSu29	2.97	4.07	8.46	23.22	18.15	30.72	10.18	2.19	17.08	3.93	11.42	118
CFFR8	2.68	3.42	5.36	15.36	18.06	24.11	9.63	2.61	5.85	8.08	9.70	119
CFFR12	2.99	4.70	4.80	18.10	19.68	19.91	11.92	1.93	19.09	4.00	10.68	120
TVSu357	2.77	5.08	5.40	17.10	18.03	28.66	23.33	1.91	14.40	1.05	11.49	121
TVSu167	2.83	3.55	5.48	17.83	19.88	35.33	10.98	3.35	7.09	1.64	10.17	122
CFFR9	2.82	4.05	4.71	11.10	18.36	25.47	10.51	3.28	9.18	10.67	10.52	123

TVSu234	3.19	3.39	6.60	20.89	17.71	24.75	12.86	1.88	23.17	1.57	10.76	124
CFFR7	2.55	4.28	5.04	15.72	18.54	26.22	11.41	1.87	8.26	13.47	10.78	125
TVSu364	3.21	4.50	5.28	29.77	19.02	25.55	13.44	1.58	11.85	3.57	10.88	126
TVSu581	2.71	5.49	6.17	28.06	19.32	28.92	9.96	2.29	7.44	2.49	10.81	127
TVSu217	3.55	4.52	4.75	30.55	18.05	31.14	12.67	1.84	12.48	3.48	11.07	128
TVSu224	3.39	4.65	5.95	31.47	20.81	28.04	12.81	1.94	13.53	2.68	11.46	129
TVSu2109	3.04	3.85	6.85	31.45	18.49	24.26	15.42	1.93	13.35	1.87	10.90	130
CFFR10	2.93	3.97	5.78	14.55	18.68	29.54	14.38	2.40	25.72	2.47	11.23	131
TVSu154	3.09	3.74	4.75	32.45	20.25	28.66	13.10	2.60	14.98	3.76	10.86	132
TVSu280	3.36	3.97	5.08	32.66	20.25	27.62	12.46	1.85	15.55	3.11	10.97	133
TVSu343	3.92	4.02	4.95	14.77	17.79	19.26	13.04	1.57	27.84	1.51	11.02	134
TVSu253	3.20	4.09	5.20	40.89	19.48	29.50	11.91	4.81	9.50	3.87	11.19	135
MIN	2.44	3.35	4.22	10.28	15.87	19.26	9.36	1.44	5.85	0.90	9.16	
MEAN	3.08	4.22	5.42	17.39	18.74	26.48	13.12	2.30	11.72	2.48	10.50	
SE	0.71	0.93	1.31	5.55	2.65	3.62	2.12	1.84	2.77	2.00	0.69	
MAX	4.13	5.49	8.82	40.89	22.24	35.33	23.33	8.31	27.84	13.47	11.81	
H2	0.19	0.19	0.24	0.47	0.17	0.36	0.48	0.23	0.60	0.43	0.34	
LSD	1.98	2.54	3.63	15.52	6.97	9.77	5.96	5.10	7.82	5.59	1.87	

Table 4.10.2. The combined analysis of pod per plant for combined BLUPS for allenvironment and stability rank.

GENOTYPE	Ibad-WS-19	Ibad-WS-21	Ibad-WW-19	Ibad-WW-21	Mok-WS-19	Mok-WW-19	Zar-WS-19	Zar-WW-19	Zar-WW-21	Combined	Stability.Rank
TVSu2095	6.58	5.92	7.24	11.83	24.91	29.97	17.90	13.38	2.66	13.58	1
TVSu368	6.49	6.41	6.77	10.33	24.55	28.67	16.47	13.96	2.77	12.87	2
TVSu262	5.98	3.84	6.57	13.49	24.09	30.13	18.20	13.81	2.69	12.57	3
TVSu2098	6.84	6.76	6.91	12.18	24.63	29.25	16.68	12.95	3.26	13.63	4
TVSu2101	7.00	6.02	7.36	13.12	27.51	31.35	15.81	14.49	3.36	14.30	5
TVSu2089	6.90	6.45	7.13	15.00	24.91	30.33	18.94	14.65	2.15	14.47	6
TVSu89	6.47	8.39	6.77	12.80	26.51	30.76	18.17	17.10	1.75	14.34	7
TVSu112	5.91	5.81	6.88	10.25	24.70	26.43	16.09	14.10	2.50	12.26	8
TVSu600	6.20	4.69	7.29	9.55	23.70	30.40	15.90	14.19	3.07	12.73	9
TVSu295	6.22	4.49	6.80	9.27	23.11	28.74	14.70	13.46	2.07	11.72	10
TVSu103	6.07	7.94	7.29	12.06	23.57	29.70	17.51	13.35	2.31	13.56	11
TVSu2110	6.24	6.97	7.05	12.48	24.63	32.31	16.28	15.22	1.64	13.62	12
TVSu2094	6.09	5.19	7.23	9.79	24.76	31.01	14.93	15.46	2.80	12.79	13
TVSu310	6.59	7.26	6.79	10.55	27.29	27.83	17.26	16.37	1.60	13.52	14
TVSu351	6.13	4.48	6.64	10.04	28.41	30.30	15.40	13.95	2.71	12.25	15
TVSu161	6.35	5.32	6.70	9.79	24.45	31.61	16.34	11.86	3.12	12.48	16
TVSu85	5.46	6.22	6.51	7.75	25.67	28.99	15.54	12.96	1.57	11.51	17
TVSu2088	5.85	6.12	6.26	13.72	25.62	34.08	18.21	15.81	1.78	13.34	18
TVSu2104	6.07	8.10	6.96	11.22	24.63	28.70	14.43	14.81	2.08	13.06	19
TVSu2108	6.16	7.08	6.72	13.05	24.63	30.77	15.90	11.36	3.09	13.01	20
TVSu671	8.69	6.85	7.03	13.73	27.83	27.98	20.22	17.06	2.22	15.63	21
TVSu75	6.11	5.76	6.68	9.76	27.07	28.53	13.87	13.00	2.22	11.91	22
TVSu319	5.77	6.76	6.62	11.13	26.97	25.67	18.19	14.76	2.94	12.81	23
TVSu277	6.33	5.34	7.47	10.40	27.61	32.97	19.18	13.27	2.97	13.86	24
TVSu425	5.33	8.82	6.54	12.28	26.67	28.24	15.24	16.14	3.87	13.21	25
TVSu222	5.37	4.76	6.78	16.26	29.37	31.97	18.69	15.34	4.92	13.92	26
TVSu361	5.89	4.02	6.39	9.01	25.39	32.67	18.24	15.49	2.48	12.44	27
TVSu125	6.31	6.16	7.65	12.29	22.70	31.51	19.94	16.94	2.28	14.57	28
TVSu359	6.99	4.49	6.95	9.33	28.34	29.67	16.39	13.00	1.61	12.73	29
TVSu158	6.54	5.13	7.14	9.47	27.27	30.45	18.70	12.05	1.07	12.99	30
TVSu137	6.30	4.67	6.72	8.13	23.31	26.16	17.73	13.61	2.27	11.86	31
TVSu66	5.68	7.19	6.69	11.82	22.16	26.51	16.80	15.03	1.39	12.43	32
TVSu25	6.73	7.35	6.58	10.42	22.80	32.34	17.81	14.19	3.80	13.72	33
TVSu9	7.15	8.52	7.38	10.74	25.20	33.85	18.34	14.74	2.88	15.12	34
TVSu441	8.28	7.29	6.72	16.78	28.68	32.15	20.99	13.73	3.22	15.99	35
TVSu645	5.60	8.96	7.16	13.66	26.98	30.43	18.47	11.51	2.43	13.92	36
TVSu2102	6.60	6.19	6.65	13.42	27.24	27.45	16.40	10.82	2.26	12.80	37
TVSu156	5.30	4.51	7.36	10.45	27.76	27.93	20.03	15.41	6.09	13.43	38
TVSu2106	7.03	4.15	7.79	14.75	26.14	26.63	19.94	14.03	2.88	14.17	39

TVSu2107	5.59	5.25	6.84	8.70	29.12	28.31	16.52	13.44	2.56	12.06	40
TVSu245	5.82	3.46	6.79	9.99	20.36	27.75	17.03	12.03	3.07	11.33	41
TVSu2090	6.25	6.60	7.59	8.53	24.76	30.43	14.15	13.38	2.51	12.92	42
TVSu200	5.86	6.74	7.59	13.77	31.61	31.81	21.46	17.94	3.12	15.42	43
TVSu467	7.71	9.50	7.34	13.65	30.20	33.00	16.64	14.68	3.51	16.00	44
TVSu288	6.30	6.10	7.76	13.33	31.33	28.15	19.47	15.76	4.56	15.00	45
TVSu217	6.86	5.48	6.85	17.71	25.74	33.51	18.18	16.48	3.91	14.86	46
TVSu2092	6.63	6.04	6.75	8.92	23.27	29.63	13.27	12.71	2.81	12.01	47
TVSu115	6.07	5.25	7.25	14.64	22.11	28.91	15.15	15.13	4.62	13.22	48
TVSu2097	6.22	8.21	6.90	11.23	24.91	30.65	13.81	12.54	1.43	12.77	49
TVSu8	6.45	6.20	6.92	10.58	21.61	25.85	16.75	12.31	3.15	12.40	50
TVSu167	5.82	4.59	7.32	12.51	26.19	32.55	15.48	10.70	2.40	12.58	51
TVSu144	6.90	3.93	7.12	13.83	28.04	29.78	17.84	10.53	3.25	13.29	52
TVSu227	7.89	5.84	7.63	11.47	30.05	31.62	20.57	13.31	1.95	15.11	53
TVSu2096	5.98	6.58	6.77	13.30	24.76	29.25	15.04	19.39	2.15	13.34	54
TVSu397	6.90	6.09	6.42	13.35	29.48	27.93	21.90	18.28	3.58	14.70	55
TVSu2112	6.50	5.96	7.97	11.80	24.91	33.70	21.09	12.43	2.98	14.68	56
TVSu2099	7.38	5.50	6.74	9.34	24.76	31.55	13.04	14.26	1.89	12.68	57
CFFR2	6.30	8.58	6.97	9.80	23.47	28.53	14.90	11.47	2.44	12.71	58
TVSu2103	6.40	5.36	7.00	12.23	24.63	31.24	13.61	10.36	2.62	12.33	59
TVSu386	6.15	7.02	7.42	12.63	30.09	36.08	21.74	18.17	6.55	16.23	60
TVSu593	5.56	5.00	7.03	18.32	27.97	28.76	18.08	16.83	4.11	14.06	61
TVSu214	6.61	7.26	7.48	18.27	29.85	32.37	23.26	14.72	5.51	16.46	62
TVSu323	7.96	5.70	6.90	12.80	23.42	27.94	13.45	11.51	3.63	13.22	63
TVSu2105	6.16	4.05	7.61	13.95	21.76	26.57	15.55	15.80	4.53	13.09	64
TVSu110	6.07	6.16	7.07	13.52	24.63	23.81	18.25	15.43	2.71	12.89	65
TVSu2114	7.44	10.13	7.13	15.14	24.76	29.88	18.67	20.08	2.48	16.23	66
TVSu2100	7.24	5.61	7.52	12.64	23.62	28.13	15.80	13.60	7.83	14.31	67
TVSu364	6.65	7.86	6.83	20.00	27.80	30.59	18.65	16.15	5.02	15.66	68
TVSu329	6.58	4.23	9.16	12.85	20.75	28.19	18.53	15.21	4.44	14.73	69
TVSu348	6.07	5.61	6.72	9.24	22.03	28.25	17.00	18.40	4.63	12.83	70
TVSu2113	6.94	5.47	6.93	13.97	21.15	26.19	17.63	11.55	2.38	12.89	71
TVSu212	6.15	4.77	7.15	9.71	20.65	23.49	16.19	11.40	2.05	11.41	72
TVSu388	6.16	7.69	6.93	9.06	23.63	32.00	22.37	14.59	2.54	14.06	73
TVSu84	7.14	6.63	7.31	9.44	27.38	27.40	12.47	12.09	3.00	12.86	74
TVSu311	6.66	3.61	6.65	12.32	19.36	26.15	17.58	15.37	2.53	12.17	75
TVSu29	6.27	4.94	8.39	14.73	21.64	29.85	13.71	14.46	5.04	13.91	76
TVSu145	6.61	6.16	6.95	11.55	22.64	26.05	20.07	16.23	6.69	14.17	77
TVSu216	6.85	5.94	7.51	15.11	32.18	29.45	20.91	12.65	3.57	15.03	78
CFFR5	6.38	4.66	7.13	11.99	28.40	30.36	22.29	21.12	2.67	14.85	79
TVSu160	6.35	5.63	7.25	13.02	20.54	33.54	17.42	12.50	2.04	13.37	80
TVSu47	6.19	5.55	7.09	8.87	20.87	33.54	17.16	15.14	2.30	12.95	81
TVSu275	8.48	6.44	6.77	10.78	29.22	36.51	18.91	18.24	2.65	15.69	82

TVSu143	6.12	4.66	8.31	17.67	23.21	29.55	15.71	12.64	4.19	14.05	83
TVSu431	7.09	6.27	7.03	16.55	33.35	30.67	22.67	18.91	3.97	16.31	84
TVSu371	5.85	4.42	7.44	17.02	24.04	33.70	19.72	15.75	8.19	15.09	85
TVSu11	5.87	8.91	7.24	12.49	29.59	36.39	20.33	13.27	3.28	15.27	86
TVSu82	6.72	5.43	7.04	9.42	21.40	26.19	11.36	14.75	2.07	11.81	87
TVSu266	6.03	5.73	7.81	14.76	23.56	28.77	21.50	20.73	2.25	15.03	88
TVSu362	5.65	5.69	7.18	9.05	25.17	29.93	18.57	21.42	3.04	13.78	89
TVSu2093	5.78	8.46	7.07	10.69	24.76	32.03	12.65	10.86	2.47	12.55	90
TVSu229	7.15	8.43	7.58	16.50	31.37	32.03	16.31	12.21	2.94	15.58	91
CFFR12	5.98	5.67	6.86	14.10	28.76	24.32	17.60	18.53	4.62	13.68	92
TVSu94	5.54	7.81	7.11	9.30	22.38	31.42	22.78	13.69	2.36	13.74	93
TVSu232	6.94	7.00	7.31	14.34	31.76	30.65	23.18	21.57	2.44	16.55	94
TVSu14	5.94	10.17	6.80	14.31	32.14	33.42	24.83	18.09	2.94	16.43	95
TVSu2109	5.96	3.91	7.87	15.95	21.93	29.12	22.53	13.91	2.69	14.08	96
TVSu46	5.84	3.79	6.93	9.24	19.31	22.56	15.76	11.01	2.07	10.47	97
TVSu2091	6.28	11.20	7.20	11.12	24.91	30.24	18.30	9.94	2.40	14.28	98
TVSu233	5.28	10.89	7.24	16.02	33.75	33.10	19.21	16.75	2.62	15.83	99
TVSu535	5.43	5.37	7.05	8.44	20.48	28.34	20.64	16.98	5.01	13.09	100
TVSu357	6.05	8.46	7.07	11.97	22.65	32.62	23.99	16.84	1.51	15.00	101
CFFR1	6.82	6.16	8.14	11.48	31.98	33.99	15.63	15.45	8.47	15.99	102
TVSu221	5.59	7.59	7.47	14.90	32.68	30.35	25.04	17.06	2.90	15.80	103
TVSu224	5.62	5.20	8.17	20.65	29.58	30.53	17.05	18.70	3.98	15.44	104
TVSu269	6.73	4.71	7.98	10.63	23.67	27.62	21.33	21.15	2.20	14.80	105
TVSu379	5.47	6.03	6.66	14.26	34.87	35.03	21.43	17.21	8.01	15.53	106
TVSu378	6.07	4.35	7.67	9.34	30.78	34.94	22.12	19.30	7.11	15.61	107
TVSu343	6.91	6.54	6.96	11.06	22.93	24.06	18.93	20.01	1.91	13.78	108
TVSu267	6.04	7.27	7.58	20.95	30.82	28.09	18.47	15.43	3.31	15.35	109
TVSu218	5.80	6.16	7.14	11.00	21.34	25.01	23.54	14.84	2.31	13.16	110
CFFR10	5.91	5.12	7.21	11.33	25.34	30.91	18.72	24.22	3.91	14.61	111
TVSu318	6.60	11.76	7.43	15.10	23.19	33.92	17.91	11.08	1.71	15.26	112
TVSu154	6.34	3.25	7.22	20.62	29.38	34.35	16.38	16.36	5.17	14.90	113
TVSu164	6.22	4.41	7.36	17.04	25.67	32.00	16.01	22.67	9.25	15.63	114
CFFR13	5.93	6.10	6.78	22.18	24.91	28.09	20.04	15.99	4.11	14.80	115
CFFR8	5.79	3.86	7.13	15.40	25.33	30.27	16.08	9.62	10.06	13.24	116
TVSu146	5.62	6.80	7.06	7.29	19.75	27.27	21.81	17.17	4.46	13.36	117
TVSu225	7.43	7.06	7.18	13.83	32.88	30.07	24.74	23.22	2.48	17.04	118
CFFR11	5.86	4.97	6.43	15.05	20.73	33.90	18.09	22.34	4.59	14.21	119
TVSu581	5.87	7.66	7.63	20.46	27.55	33.03	14.37	10.94	3.70	14.52	120
TVSu231	6.22	8.50	6.59	18.42	34.66	35.17	21.74	23.07	2.07	16.78	121
TVSu183	6.11	4.00	7.02	10.99	34.31	35.68	19.31	12.22	1.82	13.54	122
CFFR3	6.64	11.59	6.39	12.80	24.91	32.21	28.00	18.93	2.25	16.68	123
TVSu410	6.21	3.29	7.13	10.19	35.74	33.29	17.91	12.43	3.40	13.30	124
TVSu374	6.87	3.12	6.99	7.22	29.96	28.42	26.07	14.67	2.34	13.71	125

CFFR6	6.23	3.87	6.84	11.06	23.43	33.16	19.13	25.56	6.51	14.89	126
TVSu50	5.97	4.07	7.64	14.20	37.53	28.24	17.60	19.12	3.59	14.67	127
TVSu170	6.13	6.16	6.65	12.49	38.43	32.60	24.25	17.67	5.20	15.94	128
TVSu373	6.04	6.70	7.84	10.57	37.56	39.81	18.22	15.63	8.54	16.54	129
TVSu253	6.71	3.77	7.02	24.89	22.68	28.84	15.85	11.85	4.76	13.98	130
TVSu234	6.74	5.57	9.05	18.72	25.29	30.78	20.73	29.40	2.20	17.84	131
TVSu280	5.92	6.39	7.90	27.74	28.25	30.94	17.78	20.69	4.44	16.85	132
CFFR9	6.09	5.83	6.78	13.86	24.63	30.48	17.09	15.44	18.05	15.62	133
CFFR7	5.69	7.16	7.16	11.40	25.41	33.04	17.80	11.45	18.96	15.70	134
TVSu376	5.69	6.18	6.52	14.05	48.19	46.60	19.69	22.51	2.00	16.93	135
MIN	5.28	3.12	6.26	7.22	19.31	22.56	11.36	9.62	1.07	10.47	
MEAN	6.34	6.16	7.14	12.89	26.42	30.38	18.25	15.43	3.61	14.08	
SE	1.40	1.83	1.19	3.20	5.07	4.17	2.67	3.07	2.87	1.10	
MAX	8.69	11.76	9.16	27.74	48.19	46.60	28.00	29.40	18.96	17.84	
H2	0.17	0.48	0.15	0.55	0.45	0.40	0.56	0.57	0.44	0.48	
LSD	3.90	5.17	3.28	8.94	14.00	11.24	7.54	8.66	8.00	3.05	

Table 4.10.3. The combined analysis of the number of seeds per plant for combined BLUPS for all environment and stability rank.

GENOTYPE	Ibad-WS-21	Ibad-WW-19	Ibad-WW-21	Mok-WS-19	Mok-WW-19	Zar-WS-19	Zar-WS-21	Zar-WW-19	Combined	Stability. Rank
CFFR9	5.39	7.43	9.79	27.22	34.58	19.10	4.05	18.01	15.43	1
TVSu2098	5.34	7.42	11.92	27.22	33.13	19.25	3.26	14.79	15.18	2
TVSu262	5.30	6.88	11.25	26.51	34.25	19.25	3.15	14.90	14.73	3
TVSu600	5.11	8.25	8.90	26.33	33.44	17.54	3.29	15.44	14.57	4
TVSu2095	5.77	8.21	10.38	27.48	32.58	19.80	3.10	13.86	15.22	5
TVSu2089	5.45	7.88	13.51	27.48	35.31	21.55	2.94	16.86	16.36	6
TVSu310	5.32	7.27	9.03	29.33	30.84	19.07	2.50	18.08	14.35	7
TVSu85	5.73	6.72	7.27	26.38	34.12	18.56	2.61	14.88	13.88	8
TVSu222	4.69	7.61	13.11	31.90	36.02	21.07	3.40	17.61	16.27	9
TVSu368	5.74	7.18	9.03	24.76	32.35	18.38	3.12	14.45	14.29	10
TVSu2101	6.39	8.43	11.52	29.38	35.88	17.43	3.80	15.87	16.59	11
TVSu2106	5.63	11.02	10.82	29.71	31.98	20.55	3.16	16.90	16.81	12
TVSu361	5.28	6.51	7.68	28.50	35.47	19.93	3.17	18.16	14.78	13
TVSu89	7.27	7.42	11.81	30.21	33.82	21.07	2.63	21.04	17.11	14
TVSu359	5.69	7.57	8.94	30.38	32.93	17.26	2.65	14.49	14.42	15
TVSu66	5.81	6.94	10.66	24.98	30.88	18.94	3.68	17.03	14.96	16
TVSu425	5.05	6.67	10.27	29.75	31.14	17.02	4.17	17.72	14.67	17
TVSu2110	6.19	8.01	11.97	27.22	35.85	16.89	2.61	16.19	15.76	18
TVSu112	5.34	7.38	9.35	26.13	28.96	17.92	3.09	15.17	13.85	19
TVSu2091	7.52	8.10	8.82	27.48	34.23	19.36	3.02	13.79	16.20	20
TVSu156	5.49	8.35	9.00	30.37	31.56	22.32	4.29	18.16	16.26	21
TVSu2104	6.36	7.89	9.01	27.22	31.58	15.55	2.88	16.25	14.77	22
TVSu115	5.25	8.15	9.32	25.25	30.96	16.82	3.85	16.84	14.48	23
TVSu75	5.62	6.98	9.16	31.48	33.22	16.93	2.89	15.95	14.39	24
TVSu2090	5.70	8.92	7.71	27.36	33.53	16.30	2.93	14.76	14.44	25
TVSu103	6.01	8.76	12.39	26.65	33.50	19.77	2.98	13.30	15.91	26
TVSu295	4.97	7.61	7.19	24.27	31.47	16.95	3.51	14.25	13.32	27
TVSu441	5.46	7.18	14.99	29.99	35.88	23.08	3.39	16.62	16.87	28
TVSu351	4.79	7.10	8.62	29.74	34.21	15.49	3.01	14.84	13.54	29
TVSu154	5.23	7.71	13.14	31.62	36.83	18.12	4.13	16.45	16.32	30
TVSu277	5.32	8.81	9.49	30.65	36.51	22.18	3.05	14.57	15.95	31
TVSu200	6.06	9.27	11.36	33.71	35.99	24.36	3.28	19.66	18.15	32
TVSu8	5.77	7.36	9.47	23.89	30.88	18.98	3.35	13.58	14.29	33
TVSu158	5.16	7.61	7.90	30.28	33.63	21.41	2.52	13.33	14.43	34
TVSu137	5.05	7.53	6.33	26.04	30.30	20.69	2.99	14.45	13.63	35
TVSu214	6.01	9.34	15.73	34.10	35.68	24.33	4.72	18.32	19.27	36
TVSu25	6.36	6.49	9.35	24.94	36.46	19.86	3.54	15.72	15.38	37
CFFR2	6.19	7.67	8.53	26.88	32.74	16.15	3.01	12.24	14.07	38
TVSu2097	6.22	7.62	11.82	27.48	33.87	15.53	4.05	14.49	15.44	39

TVSu2088	5.48	6.26	11.27	26.43	38.11	20.41	2.56	18.02	15.27	40
TVSu217	4.89	7.17	15.19	26.68	35.45	18.99	4.03	17.41	16.12	41
TVSu9	6.73	8.55	8.30	26.74	37.07	22.47	3.30	17.18	16.87	42

TVSu319	5.84	6.89	9.98	30.40	29.26	21.42	3.16	16.70	15.15	43
TVSu2100	5.49	8.71	10.58	25.61	30.38	16.86	4.82	15.81	15.48	44
TVSu323	5.11	7.42	11.51	26.33	32.12	14.98	3.58	13.60	13.93	45
TVSu160	5.06	7.94	11.89	24.93	36.47	20.54	2.86	14.37	15.21	46
TVSu388	6.50	7.41	8.16	27.76	37.82	22.30	3.03	17.88	16.26	47
TVSu227	5.30	9.51	10.62	32.00	34.48	23.50	2.78	14.49	16.45	48
TVSu245	4.77	7.81	8.70	22.51	32.22	18.25	3.10	13.61	13.40	49
TVSu2107	5.25	7.60	7.77	31.60	31.52	16.89	3.09	14.88	13.93	50
TVSu645	6.75	7.79	11.99	30.27	32.94	20.54	2.98	12.22	16.02	51
TVSu2092	6.03	7.37	8.22	24.77	33.46	14.88	3.23	13.94	13.74	52
TVSu2096	5.55	7.60	12.36	27.36	33.13	16.21	2.86	20.45	15.38	53
TVSu378	4.92	10.37	9.45	32.08	37.62	24.58	6.42	20.25	19.10	54
TVSu161	5.27	6.98	12.61	26.09	34.81	17.16	3.34	12.56	14.48	55
TVSu2102	5.76	6.78	11.35	29.25	30.68	18.02	2.89	11.65	14.17	56
TVSu371	5.19	8.81	13.28	25.42	37.33	23.01	4.10	17.51	17.26	57
TVSu318	8.26	8.75	11.69	25.04	37.01	20.16	2.62	16.17	17.60	58
TVSu2105	5.28	9.59	11.62	26.36	31.55	19.45	7.54	17.33	17.76	59
CFFR1	5.13	10.08	9.71	33.15	35.23	17.17	4.44	16.93	16.55	60
TVSu671	6.02	8.36	13.54	34.43	34.28	23.80	2.96	21.51	18.08	61
TVSu216	5.33	9.08	13.57	33.11	33.77	22.47	3.40	14.12	16.79	62
TVSu125	6.10	8.42	11.42	23.29	35.46	20.70	2.94	18.63	16.50	63
TVSu2114	7.59	8.58	14.47	27.36	34.82	23.27	3.04	22.74	19.12	64
TVSu47	5.19	7.83	8.73	23.01	36.00	17.40	2.94	15.55	14.14	65
TVSu2108	6.31	7.04	11.95	27.22	35.34	17.39	3.29	11.57	15.16	66
TVSu431	5.65	8.48	14.14	34.32	33.83	24.90	3.73	21.21	18.36	67
TVSu467	6.50	6.78	12.05	33.10	38.12	18.64	3.54	15.60	16.41	68
TVSu110	5.05	7.97	9.80	27.22	27.19	20.28	3.34	17.32	14.09	69
TVSu2094	6.19	8.59	10.44	27.36	39.33	17.12	3.00	17.90	16.32	70
TVSu348	5.59	7.03	6.58	24.84	32.36	19.63	3.85	20.25	14.78	71
TVSu357	6.38	7.63	10.05	24.11	33.71	24.07	4.16	18.08	16.76	72
TVSu143	5.01	10.53	14.87	26.30	33.29	18.41	3.77	14.14	16.64	73
TVSu221	6.23	9.34	11.77	35.45	34.91	25.40	3.15	19.34	18.40	74
TVSu144	4.57	8.17	10.06	32.62	34.00	19.74	3.37	11.84	14.73	75
TVSu2103	5.32	8.09	10.51	27.22	34.93	15.45	3.24	11.05	14.17	76
TVSu2113	5.91	7.76	11.42	22.64	30.82	19.86	2.92	12.73	14.65	77
TVSu84	5.90	8.28	8.51	32.32	32.07	15.95	3.30	13.47	14.75	78
TVSu364	6.82	7.43	17.98	30.55	32.94	19.51	4.29	17.48	18.01	79
TVSu593	5.14	7.92	17.81	30.20	33.13	19.10	2.72	17.53	16.30	80
TVSu145	5.07	7.74	10.02	24.66	28.18	21.37	5.00	17.34	15.27	81
TVSu50	4.59	9.82	13.24	25.72	32.98	21.15	3.48	22.75	17.05	82
TVSu11	7.12	8.14	11.64	32.13	40.61	25.49	3.54	16.62	18.83	83
CFFR7	5.36	7.92	9.06	28.22	38.71	20.26	6.49	13.40	16.73	84
CFFR5	4.96	7.96	7.61	31.30	34.24	22.85	3.16	22.80	15.94	85

TVSu253	4.79	8.25	16.62	28.25	35.12	17.81	4.48	13.94	16.34	86
TVSu2093	6.62	8.00	9.61	27.36	35.14	13.96	3.03	12.03	14.64	87
TVSu146	6.00	7.76	5.57	24.16	33.53	22.47	3.96	18.04	15.49	88
TVSu275	5.39	7.15	10.41	33.28	40.91	21.41	3.25	19.74	16.68	89
CFFR8	4.94	8.13	10.16	27.99	33.52	16.96	6.24	10.46	15.14	90
TVSu288	5.72	10.15	12.43	36.81	33.07	24.60	4.23	19.82	18.89	91
TVSu2099	5.13	7.05	8.00	27.36	34.54	14.28	7.02	14.01	15.04	92
TVSu2109	4.64	9.86	16.03	25.27	34.68	24.46	3.15	16.35	17.25	93
TVSu267	5.96	8.95	17.38	34.99	32.84	23.80	3.74	17.83	18.71	94
TVSu82	5.41	7.50	8.46	25.11	28.43	11.85	2.79	15.51	12.86	95
TVSu329	4.99	12.67	10.72	22.85	32.84	19.25	3.56	16.55	16.70	96
TVSu229	6.29	9.07	14.59	36.38	37.60	18.64	3.27	17.36	18.10	97
TVSu311	4.55	6.98	9.96	20.10	31.27	18.34	2.98	17.80	13.33	98
TVSu167	5.06	8.32	10.67	29.15	38.12	16.40	3.02	11.57	14.79	99
TVSu224	5.63	10.31	18.76	33.20	34.36	19.40	3.47	20.92	18.87	100
TVSu535	5.15	7.71	9.17	22.42	30.98	22.63	2.61	19.90	14.69	101
TVSu46	4.60	7.09	7.52	20.52	27.02	16.04	2.81	11.67	11.59	102
CFFR13	5.92	7.08	17.60	27.48	32.06	24.64	3.70	18.55	17.85	103
TVSu362	5.33	8.05	7.19	27.73	33.22	19.74	3.31	24.04	15.65	104
TVSu232	6.08	8.43	13.29	36.14	35.19	25.74	3.02	23.53	18.87	105
TVSu266	5.51	9.93	14.21	29.09	31.91	23.66	2.97	24.96	18.25	106
TVSu14	7.52	7.06	12.74	36.16	38.21	27.45	3.29	21.75	19.45	107
TVSu29	5.42	11.97	13.56	26.00	36.93	14.77	2.82	17.49	16.96	108
TVSu233	7.97	8.65	13.81	38.54	39.52	24.90	3.57	18.24	20.35	109
TVSu386	6.45	8.46	8.57	31.82	41.11	26.35	5.05	22.30	19.34	110
TVSu2112	5.32	9.45	11.50	27.48	40.65	25.04	3.25	13.82	17.18	111
TVSu280	6.42	9.68	19.44	32.96	33.83	19.88	4.05	23.57	19.84	112
TVSu94	5.20	7.90	6.85	24.61	34.84	26.42	2.91	15.67	15.08	113
TVSu397	5.47	6.66	11.84	31.58	31.12	26.02	3.56	23.61	17.03	114
TVSu218	5.66	8.12	9.36	23.38	28.65	24.99	2.95	17.01	15.03	115
CFFR12	5.15	7.32	10.44	30.43	27.21	18.60	4.15	22.30	15.41	116
TVSu212	5.11	8.68	7.70	20.75	26.05	16.64	2.92	12.59	12.83	117
TVSu410	5.15	8.06	10.43	37.06	35.78	18.53	3.81	14.25	15.72	118
TVSu231	6.91	6.65	17.54	36.74	39.50	24.03	2.85	23.49	19.41	119
CFFR3	7.60	6.31	12.80	27.48	35.96	29.72	2.92	19.50	18.50	120
CFFR6	5.16	7.36	9.13	24.95	35.92	21.12	5.88	25.30	17.31	121
TVSu373	5.97	9.00	9.93	38.28	39.45	20.29	5.20	16.42	18.47	122
TVSu164	5.06	8.59	15.68	29.00	35.57	18.26	6.18	26.80	18.95	123
TVSu269	4.96	11.28	8.32	27.20	30.55	23.01	2.95	24.99	16.96	124
TVSu581	6.26	9.28	17.12	30.75	36.50	14.85	3.61	11.79	16.77	125
CFFR11	5.08	6.62	11.49	22.88	36.53	22.17	3.39	25.18	16.36	126
TVSu225	6.27	8.25	12.95	35.86	33.26	26.80	2.60	26.53	19.02	127
TVSu170	5.83	7.17	8.90	40.17	35.30	24.98	4.35	17.79	17.58	128

CFFR10	4.77	8.07	9.69	28.22	33.63	21.34	3.42	28.93	16.79	129
TVSu343	5.66	7.91	9.18	22.47	28.04	19.01	2.29	24.87	14.68	130
TVSu379	5.42	7.25	10.47	39.46	42.63	25.02	5.17	17.78	18.34	131
TVSu183	4.77	7.99	9.50	38.36	42.20	21.70	2.77	14.74	16.15	132
TVSu374	5.00	7.81	6.48	34.55	32.92	31.56	2.96	14.33	15.90	133
TVSu234	5.28	12.00	18.09	26.85	34.34	21.11	2.86	31.33	19.93	134
TVSu376	5.61	6.49	11.81	51.11	42.95	20.58	2.70	24.88	18.53	135
MIN	4.55	6.26	5.57	20.10	26.05	11.85	2.29	10.46	11.59	
MEAN	5.66	8.12	11.09	28.97	34.19	20.28	3.51	17.32	16.13	
SE	1.29	1.86	2.81	5.42	4.45	3.06	1.94	3.38	1.26	
MAX	8.26	12.67	19.44	51.11	42.95	31.56	7.54	31.33	20.35	
H2	0.25	0.29	0.52	0.44	0.37	0.56	0.20	0.58	0.56	
LSD	3.60	5.22	7.79	14.92	11.88	8.63	5.31	9.56	3.49	

Table 4.10.4 showed the combined analysis of seed weight for combined BLUPS for allenvironment and stability rank.

GENOTYPE	Ibad-WS-19	Ibad-WS-21	Ibad-WW-19	Ibad-WW-21	Mok-WS-19	Mok-WW-19	Zar-WS-19	Zar-WS-21	Zar-WW-19	Combined	Stability.Rank
TVSu2104	2.11	2.04	3.53	4.30	16.15	19.33	8.48	1.26	7.61	7.23	1
TVSu112	2.10	1.88	3.77	3.81	17.26	19.21	9.00	1.28	6.30	7.11	2
TVSu295	2.14	1.76	3.89	3.79	17.00	19.49	8.33	1.61	7.70	7.15	3
TVSu600	2.17	1.85	4.44	3.65	16.81	19.15	8.16	1.37	6.96	7.21	4
CFFR9	2.18	1.77	3.18	3.53	16.15	18.99	7.39	1.61	5.83	6.82	5
TVSu8	2.11	1.98	3.42	4.30	16.15	18.34	9.14	1.46	5.78	7.17	6
TVSu89	2.09	2.06	3.25	4.51	15.95	18.08	7.87	1.15	7.23	7.06	7
CFFR3	2.36	2.13	2.81	4.80	16.18	19.26	9.57	1.26	7.21	7.40	8
TVSu158	2.17	1.77	3.51	3.16	16.76	19.44	8.88	1.17	5.69	6.92	9
TVSu2095	2.25	1.95	4.20	4.06	16.18	19.36	9.17	1.36	5.61	7.31	10
TVSu2110	2.29	1.99	3.96	5.10	16.15	19.34	9.01	1.16	8.19	7.48	11
TVSu378	2.03	1.70	3.96	3.63	15.46	19.26	8.11	2.16	7.14	7.11	12
TVSu671	2.53	2.00	3.47	5.03	16.83	18.59	9.81	1.28	7.57	7.56	13
TVSu2100	2.40	1.99	3.88	4.12	16.78	18.12	7.89	2.23	6.30	7.53	14
TVSu361	2.11	1.82	2.99	2.92	16.93	19.89	8.59	1.34	6.89	6.91	15
CFFR1	2.37	1.76	4.08	3.57	17.04	19.30	7.30	1.79	6.49	7.19	16
TVSu2114	2.62	2.21	3.74	5.97	16.18	19.45	8.78	1.31	7.31	7.81	17
TVSu85	2.06	1.85	3.27	2.72	16.25	19.51	7.50	1.17	5.81	6.74	18
TVSu373	2.14	1.73	3.64	4.19	16.07	19.75	7.37	1.72	5.65	6.97	19
TVSu2105	2.26	1.80	4.40	4.38	16.44	18.28	8.10	2.44	6.52	7.46	20
TVSu2098	2.87	1.93	3.41	4.56	16.15	18.68	7.97	1.35	5.42	7.44	21
TVSu359	2.47	1.84	3.56	3.71	17.93	19.12	8.21	1.16	6.12	7.18	22
TVSu2106	2.46	1.88	4.66	4.72	16.64	18.03	9.15	1.33	6.91	7.51	23
TVSu2092	2.27	1.93	3.20	3.30	16.13	19.76	7.05	1.38	6.50	6.99	24
TVSu245	2.02	1.65	3.49	3.35	15.01	19.13	7.82	1.32	5.22	6.62	25
TVSu214	2.24	1.89	4.08	5.66	16.55	18.74	8.27	1.71	6.05	7.37	26
TVSu2097	2.36	2.06	3.55	4.48	16.18	19.11	6.89	1.56	6.02	7.27	27
TVSu431	2.56	1.85	3.51	5.16	17.88	18.82	9.61	1.53	7.94	7.57	28
TVSu115	2.12	1.81	4.03	3.74	16.69	18.78	7.07	1.66	5.74	6.98	29
TVSu160	2.40	1.72	3.83	4.60	15.11	19.96	8.78	1.25	6.20	7.16	30
TVSu310	2.32	1.74	3.25	3.18	16.19	17.36	7.36	1.12	5.77	6.75	31
TVSu2096	2.18	1.89	3.45	4.70	16.18	18.68	7.53	1.23	8.04	7.10	32
TVSu2108	2.16	2.48	3.62	4.92	16.15	19.23	8.25	1.45	5.26	7.57	33
TVSu156	1.94	1.82	3.62	3.05	16.92	18.68	9.76	1.82	6.65	7.07	34
TVSu125	2.09	1.73	3.58	4.60	14.89	19.96	8.77	1.27	6.13	6.92	35
TVSu137	2.33	1.81	3.18	2.60	15.52	18.01	8.78	1.26	5.57	6.86	36
TVSu75	2.05	1.80	3.68	3.30	16.13	17.77	7.24	1.24	5.04	6.74	37
TVSu368	2.40	2.07	3.68	4.32	16.65	18.54	9.79	1.35	8.40	7.57	38
TVSu227	2.63	1.84	4.17	4.02	17.97	20.66	9.66	1.25	6.55	7.55	39

TVSu319	2.07	1.92	3.21	3.96	17.43	17.71	8.38	1.36	5.61	6.96	40
TVSu66	1.95	1.79	3.14	4.21	15.25	17.28	8.71	1.56	6.47	6.86	41
TVSu50	2.03	1.64	4.77	4.90	16.25	19.28	8.64	1.38	8.56	7.22	42
TVSu425	1.88	1.87	3.31	3.84	17.27	18.11	7.77	1.81	7.90	7.00	43
TVSu441	2.59	1.86	3.19	5.44	17.16	20.63	10.20	1.40	6.96	7.56	44
CFFR2	2.10	1.93	3.42	3.12	15.64	18.89	7.23	1.28	4.76	6.81	45
TVSu2091	2.21	2.03	3.48	3.56	16.18	20.14	8.09	1.28	5.06	7.11	46
TVSu371	1.98	1.80	3.58	4.34	14.50	19.62	8.04	1.56	6.13	6.92	47
TVSu46	2.12	1.66	3.45	3.76	15.36	17.21	7.89	1.23	5.09	6.63	48
TVSu277	2.36	1.82	4.07	3.91	17.18	20.63	9.09	1.32	5.52	7.26	49
TVSu362	1.85	1.75	3.35	2.80	16.26	19.40	8.10	1.38	8.23	6.77	50
TVSu388	2.28	2.04	3.27	3.29	15.91	20.95	8.49	1.30	6.69	7.20	51
TVSu2090	2.21	1.69	4.10	2.78	16.18	18.59	7.14	1.27	5.17	6.78	52
TVSu323	2.58	1.80	3.19	4.75	15.67	17.51	7.67	1.42	5.44	7.10	53
TVSu262	2.07	1.75	2.95	4.06	15.67	17.68	9.55	1.38	5.42	6.82	54
TVSu318	2.22	2.49	3.91	5.03	15.27	20.28	8.45	1.22	5.85	7.63	55
TVSu535	2.15	1.77	3.19	3.84	15.44	18.21	9.91	1.14	7.59	6.96	56
TVSu2089	2.38	1.81	3.81	6.40	16.18	19.56	8.12	1.27	6.50	7.35	57
TVSu2099	2.47	1.74	3.24	3.08	16.18	19.93	8.07	3.01	6.50	7.41	58
TVSu364	2.28	1.96	3.25	6.47	16.83	18.62	8.58	1.77	7.11	7.48	59
TVSu9	2.24	1.92	3.59	2.77	15.21	19.49	9.68	1.35	6.26	7.13	60
TVSu94	1.91	1.79	3.49	2.74	14.42	18.74	8.92	1.27	5.64	6.67	61
TVSu2113	2.19	1.96	3.76	3.93	14.73	17.96	8.71	1.25	5.15	7.05	62
TVSu379	1.87	1.78	3.12	3.55	18.17	20.15	8.58	1.87	5.93	6.91	63
TVSu154	2.20	1.78	3.11	5.28	17.50	21.39	8.55	1.64	7.59	7.26	64
TVSu2107	2.04	1.83	3.74	3.36	18.44	18.66	7.74	1.32	6.98	6.96	65
TVSu170	2.17	1.73	3.28	3.13	18.29	20.27	9.08	1.60	7.42	7.07	66
TVSu2102	2.19	1.85	3.23	3.99	17.57	18.21	9.06	1.26	4.94	6.99	67
TVSu233	1.80	2.13	3.81	4.34	18.15	21.07	8.73	1.52	6.19	7.30	68
TVSu84	2.52	1.92	3.91	3.48	16.53	17.39	7.03	1.41	5.48	7.16	69
CFFR7	1.98	1.85	3.43	3.35	15.62	19.93	7.68	2.62	5.34	7.09	70
TVSu221	1.90	1.94	4.10	4.00	17.81	19.57	10.93	1.38	6.86	7.33	71
TVSu222	1.97	1.66	3.45	5.67	18.16	21.03	8.68	1.40	6.74	7.08	72
TVSu2093	2.04	2.17	3.73	4.48	16.18	21.37	8.02	1.31	5.93	7.28	73
TVSu266	2.20	1.87	3.56	5.49	16.46	17.58	8.33	1.28	8.11	7.23	74
TVSu82	2.34	1.86	3.93	3.44	16.42	17.75	6.74	1.23	7.67	7.06	75
TVSu348	2.15	1.66	3.21	2.83	15.96	17.69	9.19	1.69	7.93	6.91	76
TVSu275	2.90	1.83	3.27	4.24	17.33	21.79	9.06	1.33	7.71	7.56	77
TVSu25	2.79	2.17	3.13	4.36	16.40	21.29	10.49	1.52	7.63	7.86	78
TVSu232	2.27	1.87	3.83	4.26	18.71	18.71	9.08	1.29	8.51	7.38	79
TVSu2109	2.11	1.72	4.50	6.57	15.91	18.85	9.20	1.34	6.78	7.36	80
TVSu143	1.97	1.68	4.64	5.60	16.21	19.20	8.40	1.50	4.97	7.14	81
TVSu161	2.22	1.81	3.42	5.51	17.50	20.69	8.62	1.43	5.31	7.18	82

TVSu288	2.17	1.97	4.45	4.88	18.61	19.17	11.01	1.97	8.17	7.83	83
TVSu329	2.21	1.55	5.68	4.55	15.59	18.25	9.21	1.61	7.12	7.37	84
TVSu374	2.33	1.73	3.71	2.95	15.90	17.07	8.92	1.28	4.92	6.86	85
TVSu2094	2.23	2.06	4.18	4.94	16.18	20.80	9.71	1.29	9.36	7.64	86
TVSu386	2.07	1.94	3.72	3.07	16.44	20.85	9.09	1.86	8.74	7.33	87
TVSu645	1.89	2.18	3.46	4.42	17.94	18.85	9.39	1.28	4.75	7.20	88
TVSu267	2.18	1.97	3.59	5.81	16.90	17.23	8.10	1.49	6.02	7.28	89
CFFR13	2.03	2.18	3.13	6.36	16.18	18.79	10.21	1.55	6.53	7.55	90
TVSu351	2.24	1.63	3.41	3.15	18.79	20.24	8.55	1.30	6.52	6.94	91
TVSu224	2.45	1.73	4.05	6.48	18.90	20.01	8.40	1.42	7.75	7.56	92
TVSu397	2.34	1.70	2.90	5.08	16.98	17.50	9.45	1.43	8.32	7.16	93
TVSu146	1.96	1.99	3.17	2.01	15.00	17.56	8.57	1.52	7.26	6.86	94
TVSu593	2.11	1.71	3.77	6.69	16.68	18.17	8.58	1.23	6.24	7.11	95
TVSu216	2.40	1.85	4.01	5.55	18.68	20.39	9.63	1.46	5.38	7.51	96
TVSu144	2.51	1.52	3.79	3.55	18.08	19.32	8.82	1.41	4.45	7.01	97
TVSu2112	2.46	1.82	4.20	4.47	16.18	21.00	10.18	1.35	5.07	7.43	98
TVSu229	2.40	1.94	3.89	4.66	18.00	20.75	7.22	1.37	5.27	7.32	99
TVSu467	2.73	2.04	3.02	4.30	16.93	21.74	7.76	1.41	5.74	7.44	100
TVSu183	2.07	1.59	3.56	2.80	17.09	21.14	7.92	1.23	5.14	6.67	101
TVSu11	2.13	1.93	3.65	3.43	17.21	21.57	9.80	1.51	5.59	7.26	102
CFFR6	2.07	1.67	3.36	3.14	15.34	19.35	8.19	2.19	9.20	7.04	103
TVSu2103	2.28	1.79	3.76	4.42	14.95	20.22	7.55	1.38	4.27	6.99	104
TVSu225	2.56	1.82	3.24	5.08	17.64	17.50	9.48	1.15	8.92	7.41	105
TVSu200	2.01	1.98	3.96	4.33	18.67	21.11	11.22	1.39	8.38	7.57	106
TVSu311	2.34	1.57	3.02	4.08	13.66	16.63	7.21	1.29	5.94	6.58	107
TVSu145	2.37	1.81	3.65	3.77	15.49	16.53	8.95	2.26	7.77	7.39	108
TVSu103	2.16	1.88	3.49	4.59	14.76	16.44	7.65	1.26	4.62	6.85	109
TVSu231	2.24	2.01	3.06	6.09	18.47	21.15	9.59	1.26	9.38	7.57	110
TVSu269	2.36	1.72	4.39	3.01	15.70	16.94	8.60	1.29	8.49	7.14	111
TVSu280	2.35	1.99	3.30	6.74	17.80	20.12	7.93	1.64	9.44	7.67	112
TVSu47	2.20	1.83	4.05	4.52	15.61	22.50	9.25	1.28	8.00	7.35	113
TVSu212	2.09	1.58	3.95	3.14	14.94	15.76	8.32	1.29	5.00	6.62	114
TVSu376	1.95	1.82	2.88	3.09	18.55	20.76	7.57	1.14	8.45	6.78	115
TVSu253	2.26	1.70	3.52	6.60	17.16	21.36	8.33	1.71	5.22	7.28	116
CFFR8	1.96	1.68	3.69	3.76	16.23	18.27	6.70	2.50	3.62	6.81	117
TVSu357	2.03	2.18	4.59	4.53	16.17	19.98	12.42	1.74	7.62	7.88	118
TVSu167	2.01	1.55	3.85	3.97	17.64	21.69	7.98	1.29	4.55	6.78	119
TVSu218	2.12	1.86	3.65	3.47	14.78	15.80	9.35	1.29	5.39	6.85	120
TVSu217	2.54	1.81	3.17	6.09	15.81	22.50	8.19	1.75	7.41	7.50	121
TVSu2101	2.14	2.02	3.96	4.77	17.52	23.03	8.26	1.62	6.42	7.49	122
TVSu410	2.24	1.74	3.80	3.56	19.91	20.44	7.93	1.49	5.80	7.10	123
TVSu29	2.11	1.83	5.53	5.21	16.32	22.03	6.84	1.24	7.45	7.43	124
TVSu110	2.08	1.73	3.85	3.84	16.15	15.06	8.60	1.34	6.83	6.83	125

TVSu14	2.09	2.15	3.09	4.09	18.36	22.77	10.83	1.42	8.31	7.57	126
TVSu164	2.17	1.78	3.78	5.90	16.57	18.23	7.91	2.31	10.47	7.56	127
CFFR5	2.12	1.73	3.50	3.09	17.44	18.08	11.31	1.36	9.72	7.21	128
TVSu2088	2.05	1.96	3.02	4.84	16.47	24.16	8.62	1.17	7.86	7.19	129
TVSu581	2.00	2.16	4.11	7.96	16.95	20.29	7.00	1.51	4.37	7.48	130
TVSu234	2.26	1.71	4.65	5.65	15.24	18.49	8.53	1.25	12.09	7.49	131
CFFR11	2.17	1.67	2.93	4.19	15.41	18.98	9.35	1.45	12.43	7.15	132
CFFR12	2.14	1.89	3.18	4.03	18.02	15.27	8.15	1.93	11.36	7.29	133
TVSu343	2.74	1.86	3.28	3.40	15.25	14.81	8.28	1.08	12.50	7.36	134
CFFR10	2.00	1.65	3.93	4.02	16.76	21.70	8.91	1.40	15.25	7.38	135
MIN	1.80	1.52	2.81	2.01	13.66	14.81	6.70	1.08	3.62	6.58	
MEAN	2.22	1.86	3.65	4.28	16.57	19.25	8.60	1.47	6.83	7.19	
SE	0.48	0.38	0.87	1.23	2.43	2.49	1.31	0.77	1.56	0.38	
MAX	2.90	2.49	5.68	7.96	19.91	24.16	12.42	3.01	15.25	7.88	
H2	0.17	0.17	0.25	0.44	0.19	0.33	0.38	0.16	0.57	0.32	
LSD	1.33	1.03	2.41	3.40	6.53	6.66	3.67	2.09	4.40	1.02	