Evaluation Of Bambara Groundnut (*Vigna Subterranea* (L.) Verdc) Accessions for Drought Tolerance and Yield Performance Using Agro-Morphological and Physiological Traits

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Submitted in partial fulfilment of the academic requirements for the degree of Master of Science in Agriculture (Crop Science)

The Discipline of Crop Science

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Professor Alfred O. Odindo (*Supervisor*)

Date: 09/12/2021

Signed: Dr Abe Shegro Gerrano (*Co-Supervisor*)

Date: 09/12/2021

DECLARATION 1: PLAGIARISM

I, Sithembile Kunene, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

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

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the references sections.

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Signed: Sithembile Kunene (*Candidate*)

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PRESENTATIONS

Kunene, S.P., Gerrano, A.S., Matthew, I., Odindo, A.O., (12/03/2020). **A multivariate approach to assess the phenotypic variability among twenty-four bambara groundnut (vigna subterranea l. verdc) genotypes from two production sites (Brits and Ukulinga)** South African Plant Breeders Association (SAPBA) 2020.University of Pretoria, Future Africa conference centre, Poster presentation.

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ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc, is cultivated for improving food security and nutrition. The crop has high nutritional value and is productive even in poor soils. However, it is still neglected by researchers and research institutes. There are currently efforts underway in South Africa to promote and improve the nutritional status of Bambara groundnut. The objectives of this study were therefore: (1) to determine the extent of genetic variability and the traits relationship among Bambara groundnut accessions (Acc 25, Acc 55, Acc 61, Acc 78, Acc 82, Acc 87, Acc 95, Acc 96, Acc 97, Acc 100, Acc 105, Acc 117, Acc 121, Acc 131, Acc 150, Acc 151, Acc 175, Acc 177, Acc 179, Acc 184, Acc 190, Acc 197, Acc 199, Acc 200) (2) to screen Bambara groundnut genotypes for drought tolerance at the germination stage under simulated drought conditions; and (3) to assess the response of Bambara groundnut accessions to water stress conditions.

The first study characterized the agronomic performances of Bambara groundnut and evaluated the relationships among the traits. The performance of twenty-four (24) genotypes of Bambara groundnut grown in two different production environments (Ukulinga and Brits) in South Africa was investigated in order to assess the influence of environment on agronomic characteristics and to identify genotypes that may be more or less stable across locations. Differences in performance of Bambara genotypes and the influence of environment on agronomic trait performance were highly significant ($p < 0.001$). Ukulinga was a better site for growing Bambara groundnut compared to Brits. However, Acc 179, Acc 184, Acc 150, were associated with desirable grain yield traits at Brits site with yield ≤500kg/ha. Two (PCs), PC 1 and PC 2, accounted for 30.65% and 24.72% of the variation with a cumulative variation of 55.37% for Brits and 29.36% and 20.66% of the variation with a cumulative variation of 50.02% for Ukulinga. Grain yield (GY), hundred grain weight (HSW), petiole length (PL) and leaf width (LW) contributed more to PC 1 at Brits, five traits (GY, HSW, PL, LL and LW) contributed more to PC 1 at Ukulinga. Acc 117 had the highest grain yield (723 g/plot) at Ukulinga.

With the second study, we examined the genotypes and traits associated with drought resistance and evaluated the parameters for screening Bambara groundnuts at germination stage using polyethylene glycol (PEG 6000). The results showed that all genotypes differed significantly ($p<0.001$) under two germination conditions. For selecting drought tolerant genotypes under both stress conditions, correlation coefficients showed that indices such as mean productivity (MP), stress tolerance index (STI), and geometric mean productivity (GMP) were the most

appropriate measures. Five genotypes (Acc 25, Acc 87, Acc 97, Acc 100 and Acc 117) were more tolerant and have the ability to increase Bambara production in South Africa.

Plant growth and development, leaf gas exchange and chlorophyll fluorescence parameters were used in experiment three to determine how Bambara plants respond to water stress and to identify high yielding genotypes for breeding programmes in South Africa. The emergence of (> 80%) was observed in most genotypes. Drought stress caused significant (p<.001) effects on PL, TB, GY, gs, Ca/Ci, IWUE, F0', Fm', ETR, ETR/A, Fv'/Fm', and ΦPSII. Two principal components (PCs) were identified for leaf gas exchange and chlorophyll fluorescence parameters under water stress conditions and for growth and yield variables. Both Principal biplot and cluster analysis were used to identify drought tolerant genotypes such as Acc 177, Acc 199, Acc 197, Acc 151, Acc 75, Acc 184, Acc 64, Acc 200, Acc 97, Acc 175, Acc 25, Acc 100, Acc 121, Acc 87, Acc 61, Acc 105, Acc 121, Acc 82 and Acc 131 which can be recommended for growing Bambara groundnut.

The findings of the present study can thus be used to identify good performing genotypes for South African breeding programmes using environmental factors, germination stage, water stress, physiological and morphological parameters as well as leaf gas exchange parameters and chlorophyll fluorescence parameters. Many of the 24 genotypes (79%) were tolerant to drought. Despite extreme weather conditions, they can produce high yields.

Keywords: Bambara groundnut, genotypes, production environments, germination stage, water stress, underutilized crop, crop improvement.

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Most importantly I would like to thank **The Lord God Almighty** for giving me strength that saw me through this process. More than anything, I would like to give thanks and acknowledge all the grace and wisdom He has blessed me with. Thank you, Lord, for being my redeemer.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a member of the Fabaceae family of plants and one of the world's most important sources of food and nutrition. It was originally native to West Africa and has spread to the drier regions of Africa (Touré et al. 2012) including South Africa. Bambara groundnut has compound leaves that grow from the stem and form a crown above the soil surface. The plant can grow up to 0.30 -0.35 m in height (Katamssadan, 2016). Bambara groundnut is considered autogenous (Khan et al., 2021). After the plant is fertilized, the flowers turn yellow and the stem grows downwards, taking the growing seed with it (Tan XL et al., 2020). Bambara pods are 1.25 - 2.5 cm in diameter (Anhwange and Atoo, 2015). The pods usually produce one or two seeds (Atoyebi et al., 2018). Rubatzky and Yamaguchi, 1997 describe Bambara groundnuts as having a large variety of colors: black, brown, red, white, beige, cream, maroon, and mixtures thereof.

Bambara groundnut is grown throughout Africa mainly by smallholder and subsistence farmers (Thompson et al., 2010). Smallholder and subsistence farmers who grow these landraces conserve the genetic diversity and resources of the species on the farm by storing the seed on the farm and in traditional storage facilities for the next growing season. Most farmers use landraces to grow Bambara. It is possible to combine this crop with sorghum and maize (Sorghum bicolor) (Nyoki and Ndakidemi, 2016). Literature reports that Bambara groundnut is of great importance because it is adapted to different environmental conditions and can tolerate drought. Bambara groundnut is a legume that can improve soil because one of its functions is to fix atmospheric nitrogen (Mohale et al., 2014). Besides being a source of food for humans, seeds are also a source of food for animals. The seeds can be cooked and are used to make flour for cakes and biscuits (Nti, 2009). The nutrients contained in Bambara seeds are Carbohydrate (56.3%), Protein (20%), Fat (5.9%), Fiber (5.4%) and Minerals (2%) (Murevanhema and Jideani, 2013); (Bamshaiye et al., 2011).

Bambara groundnut is adapted to marginal areas characterized by hot and dry conditions (Mabhaudhi and Modi, 2013). Bambara plant can grow up to 2000 m above sea level on marginal soils. Climate conditions necessary to grow Bambara plants include temperature ranges between 20°C and 34°C, average rainfall ranging from 600 mm to 750 mm, and pH values between 5.0 and 6.5 (Durr et al., 2015; Taffouo et al., 2010). The species can also thrive under wetter conditions with annual rainfall of more than 2000 mm and in most soils with good

drainage (Laurette et al., 2015). Bambara groundnut is adapted to marginal areas characterized by hot and dry conditions (Mabhaudhi and Modi, 2013).

1.2 Bambara groundnuts production challenges

There are many constraints that limit the development and production of Bambara groundnut to fully realize its genetic potential (Gerrano et al., 2021). These constraints include lack of genetic improvement, growth and development, product quality, recurrent drought, and disease and pest resistance (Hillocks, 2012). Cultivation of unimproved crops such as landraces results in lower yields and less production (Mabhaudhi and Modi, 2013). Bambara groundnut yields are low in South Africa because farmers use unimproved landraces (Abejide et al., 2018) and poor production practices (Gerrano et al., 2021). Drought is a major challenge that affects the performance of landraces (Berchie et al., 2012). Furthermore, the problem of drought is further exacerbated by climate change (Thompson et al., 2010). Warmer temperatures can lead to higher rates of evaporation from the soil, resulting in drought and low rainfall (Koster et al., 2009).

Other challenges faced by smallholder farmers include lack of information on crop multiplication, taxonomy, genetics and agronomic practises, and disease and pest management (Ogwu et al., 2018; Majola et al., 2021). Bambara groundnut seeds take longer to emerge and require greater amounts of water to germinate (Chibarabada et al., 2014). This may be due to the poor quality of the seed, which may absorb water into the seed system due to prolonged storage under uncontrolled conditions of fluctuating temperature and relative humidity. These seeds often have low vigour, resulting in poor seedling germination, sprouting and establishment, which can lead to low plant population and affect yield (Temegne, 2018).

1.3 Problem statement

Much work remains to be done to identify agromorphological and nutritional traits for selection of Bambara groundnut accessions for increased production and yields. Zenabou et al. (2014) and Unigwe et al. (2016) have identified the most important agromorphological traits that could differentiate the potential of Bambara groundnut accessions and be used to develop selection strategies for Bambara groundnut improvement. At the International Institute of Tropical Agriculture (IITA), Ouedraogo et al. (2008) evaluated a mixture of Bambara groundnut accessions for phenotypic variability of agronomic and morphological traits; the accessions in this study were less variable. In Botswana, Karikari (2000) assessed variability between local and exotic Bambara groundnut landraces, while Unigwe et al. (2016) assessed morphological variation among groundnut accessions belonging to the Bambara tribe (*Vigna subterranea* L.

Verdc). The study by Jonah and colleagues (2014) also looked at the relationship between crop growth and yield of Bambara groundnuts. Not much work has been done to identify agromorphological traits that could be useful in selecting germplasm for use in Bambara groundnut improvement programs. To improve Bambara groundnut landraces in South Africa for breeding programs, further research and development work should be carried out (Ntundu et al., 2006). In Bambara groundnut, there are many phenotypic variations that can be utilized for crop improvement programs. Therefore, there is a large genetic variation in Bambara groundnut that makes it a valuable source of genetic material for crop improvement programs.

1.4 Justification

Smallholder farmers growing Bambara groundnuts with unimproved landraces cannot produce enough food, which may pose a risk to food and nutrition security. The risk is exacerbated by weather variability due to erratic rainfall and prolonged dry spells and climate change is causing these changes. The identification of drought tolerant landraces could provide useful germplasm for the improvement of Bambara groundnut crops, leading to improved livelihoods of smallholder farmers and food security.

1.5 Study aims

The study aims to understand the extent of agronomic and physio-locational variability in Bambara groundnut which may be useful in selecting and identifying potential Bambara groundnut lines for several traits for the improvement programme.

1.6 Specific objectives

The specific objectives of the study were

1.6.1 To determine the extent of genetic variability and the traits relationship among Bambara groundnut accessions by assessing the phenotypic variability among twenty-four Bambara groundnut genotypes.

1.6.2 To screen Bambara groundnut genotypes for drought tolerance at the germination stage under simulated drought conditions.

1.6.3 To assess the response of Bambara groundnut accessions to water stress conditions.

1.7 Thesis structure

The thesis comprises of six chapters as follows:

Chapter 1 – provides the background to the study. The chapter defines the problem statement, gives the justification, the aim and objectives.

Chapter 2 – this chapter reviews the literature and the current understanding on Bambara groundnut production, challenges and work done on the identification of agro-morphological and physiological traits that could be useful for crop improvement.

Chapter 3 – using phenotypic variability across twenty-four Bambara groundnut genotypes, the study examines genetic variation and traits relationships in Bambara groundnut accessions.

Chapter 4 – is experimental and reports on the screening of Bambara groundnut genotypes for drought tolerance at the germination stage under simulated drought conditions.

Chapter 5 – is experimental and reports on assessing the response of Bambara groundnut accessions to water stress conditions.

Chapter 6 – is a discussion which wraps up the entire thesis, concludes and gives recommendations for future research and development.

The experimental chapters 3, 4 and 5 are written as journal articles.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature review outlines the origin and distribution of Bambara groundnut as an underutilized crop in Africa. The botanical description and production of Bambara groundnut is also briefly discussed. In this chapter, we have also looked at the morphological, phenological and physiological characteristics to gain a better understanding of the growth and yield of Bambara as a legume. We have also discussed the uses and importance of Bambara groundnut for food security. This information is particularly important for smallholder farmers who grow this crop. Bambara groundnut is a drought-tolerant crop, so we also looked at drought adaptation and climate change impacts, as well as germination/emergence of Bambara groundnut. Since this crop can grow in different environments, we have also studied temperature, light and water stress as well as nutrient composition (proteins, lipids, minerals and phytochemicals) and their importance to Bambara groundnut. So, we intend to increase South Africa's production of Bambara groundnuts and increase its productivity by providing more information on the agronomy and physiology of this crop. It is hypothesized that drought stress can affect the growth and yield of Bambara groundnut. Future improvement opportunities are discussed as a useful tool for obtaining information to improve Bambara production in South Africa. An overview of Bambara groundnut (*Vigna subterranea*), one of the least understood and most underutilized crops in subsistence farming systems in Africa, is presented here.

2.2 Methodology

An analysis of the status of Bambara groundnut in South Africa is presented in this chapter based on both qualitative and quantitative research findings. Our literature search focused on African articles. Scopus and Google Scholar were primarily used for literature searches. We used 105 journal articles and 17 book chapters that have been peer-reviewed and reports as primary sources of information. The internet was searched using the terms "Bambara groundnut production", "neglected underutilized crops", "effects of drought" and "morphology and physiology". Abstract, title, keywords, and key terms were used in the literature search. Apart from grey literature, we used websites, dissertations, journal articles, reports and briefs. This was beneficial in that the search went beyond literature that is often inaccessible to audiences outside of academia.

2.3 Results

2.3.1 Origin and distribution of Bambara groundnut

The Bambara groundnut originated on the African continent and spread from the west to southern and central Africa; it was brought to South Africa by people in southern Africa (Feldman et al., 2019). It was originally grown by smallholder farmers who were so poor that they did not have access to agronomic production methods including irrigation and could not afford fertilizers (Hilson and Garforth, 2012; Gerrano et al., 2021). The area of origin of Bambara groundnut is probably in northeastern Nigeria and northern Cameroon. It is cultivated throughout tropical Africa as it occurs wild in central Nigeria and eastern Sudan (Parker, 2009). Originating in West Africa's high rainfall region, perhaps near the headwaters of the Niger River, Bambara groundnut is spread throughout the world. It then spread to Central Africa in ancient times and more recently to Madagascar, Asia and South America (Wamba et al., 2012). Bambara plant was first mentioned in the 17th century when it was still called Mandubi in Angola. In 1963, it was named *Glycine subterranean* by Limmaeus before Verdcourt suggested the present name *Vigna Subterranea* L verb (Akpalu, 2010). After being known as *Voandzeia subterranea* for over a century, it is now called *Vigna subterranea* (Adzalwa et al., 2016). Nigeria has spread this crop from the Jos Plateau and Yola. According to Dalziel, Bambara tribe was founded by him in northern Nigeria in 1901, thus giving it the name Bambara. Hepper then confirmed Dalziel's discovery in 1957. Africa has been cultivating Bambara groundnuts for many years and it contributes to food security (Hillocks et al., 2012).

The crop is grown mostly in the United States by women as most men consider it unprofitable. Therefore, there is not much marketing for Bambara in most countries (Mabhaudhi and Modi, 2013). It is adapted to the western and southern regions of Tanzania (Mubaiwa et al., 2018). Madagascar probably adopted it early from the Arabs. It was introduced by Brazilians and Surinamese in the nineteenth century, then by Indonesians and Filipinos (Omogbai and Aghahowa, 2017). In addition to its distribution throughout Africa, Bambara groundnut is now found in Madagascar, Mauritius, and Lowa, New Caledonia, northern Australia, tropical Central America, Suriname, and Brazil (Alake et al., 2015).

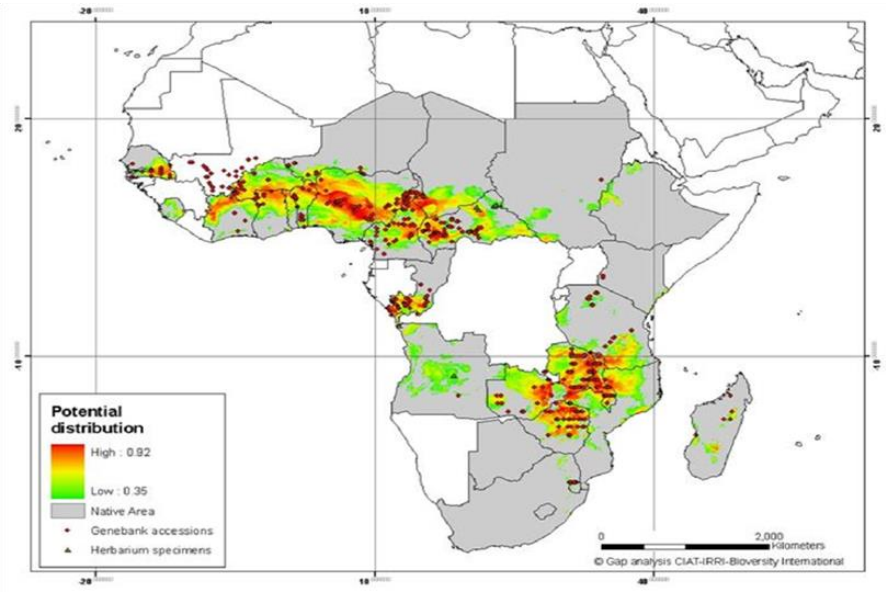

Figure 2.1: CIAT-IRRI-Biodiversity International distribution of Bambara groundnut (*Vigna subterranea* (L.) Verdc) in Africa, Temegne, 2018

2.3.2 Botanical description and production of Bambara groundnut

Bambara groundnut *(Vigna subterranea* L. Verdc*)* is a species of the genus *Vigna*, subtribe *Phaseolinae*, tribe *Phaseoleae*, and family *Papilionaceae* (Shanko et al., 2014). It was long known as *Voandzeia subterranea* before being renamed *Vigna subterranea* in 1980 (Mayes et al., 2019). In accordance with Linnaeus' system of nomenclature, Linnaeus named the plant *Glycine subterranea*. The plant was found in Madagascar by du Petit-Thouars in 1806 and was named *Voandzeia subterranea* (L) by researchers over a period of more than 100 years. Bambara groundnut is similar to plant species in the genus *Vigna* (Somta et al., 2011). In 1980, as soon as Verdcourt realized Marechal's work, he named it *Vigna subterranea* (L) Verdc. Since it is mostly grown by smallholder farmers, production figures for Bambara groundnut are difficult to obtain (Muzawazi et al., 2017). The average yield for Bambara groundnuts in Africa is only 650 kg/ha, far below the normal potential yield of 3000-4500 kg/ha. According to Hillocks et al (2012), under field conditions, seed yield is 4 t/ha. The highest combined seed yield was obtained by Effa et al. (2017) in their three-year study. The following year, a fungus caused viral leafroll disease, which greatly reduced yield. Many farmers have achieved high yields with fertilizer application; in Calabar, Shiyam et al. (2016) reported a yield of 1.95 t/ha with 2.5 t/ha of organic-mineral fertilizer. Toungos et al. (2009) reported yields of 432.5 kg/ha

after using the application of 60 kg/ha P_2O_5 in Yola. After application of 110 kg/ha P_2O_5 in Igbariam, southeastern Nigeria, Nweke and Emeh (2013) obtained the highest yield of 1.65 t/ha. It is mainly exported by Burkina Faso, Chad, Mali, Niger and Senegal, from where it supplies Bambara groundnut markets in Benin, Ghana, Nigeria and Togo (Sidibé et al., 2020). Crops such as these are not traded internationally as they are mainly grown for local consumption in the drier regions of tropical Africa. The largest exporter of Bambara groundnuts is Zimbabwe, which exports about 2 000-3 000 tons per year, most of which is shipped to South Africa (Van Heerden and Walker, 2016). The majority of production is produced in Zambia (Mubaiwa et al., 2017). Although Bambara groundnuts have high production, utilization and economic potential in Zambia, their yield is still very low (below 750 kg/ha) and there are several areas where research can be done to improve yield (Oyeyinka et al., 2018).

2.3.3 Morphological and phenological characteristics

Bambara groundnuts are annual herbaceous plants with spreading stems (Sidibé et al., 2020). The internodes vary in length, so that there are narrow, medium, and spreading varieties (Figure 2.2 a). The plants of this genus have taproots and lateral roots in their lower part (Chimwamurombe and Khulbe, 2011). Bambara taproots are dense, they have many short (up to 20 cm long) lateral stems through which the leaves grow (Redjeki et al., 2011). Ibny et al. (2019) describe that the roots produce nodules associated with Rhizobium (the bacteria that fix nitrogen). Bambara groundnut has easily recognisable morphological characteristics, including a distinctive colour. They are tripinnate, have erect petioles and are thick at the base. It has butterfly-shaped flowers on pedicels that grow from nodes on the stem and form a raceme (Tan et al., 2020). Ibny et al. 2019 reported that the pedicel and sepal dilate after fertilisation.

The seeds of Bambara are usually one or two and have a round or slightly oval shape (Figure 2.2 b). Early pods may be yellow or green, while mature pods are usually yellow or purple (Ibny et al., 2019). The fruit grows inside or outside of the soil surface (Ogwu et al., 2018). Bambara seeds are smooth and round (up to 1.5 inches in diameter). Their coloration varies from cream to brown to red and mottled (Gulu, 2019). The qualitative characteristics such as growth habit, pod shape and pod colour of Bambara groundnut accessions vary considerably. In Bambara groundnut, all the three growth forms namely racemose, semi racemose and spreading (open) have a low proportion of spreading form (Gbaguidi et al., 2018). After a plant is pulled out of the ground, most pods remain intact (Muhammad et al., 2020). Nevertheless,

spreading genotypes can be used in intercropping to suppress weed growth and form an effective ground cover (September, 2016).

Figure 2.2: Morphological features of Bambara groundnuts. Plants of Bambara groundnuts; (b) seeds of mature Bambara groundnuts, Mandizvo and Odindo, 2019.

2.3.4 Uses and importance of Bambara groundnut in relation to food security

Health and nutrition are the main uses of Bambara groundnut. Cattle, pigs and poultry can all be fed it. Pigs and poultry can eat the seeds while cows eat the stems (Aremu et al., 2016). Raw Bambara groundnuts are chewed and swallowed by pregnant women to treat nausea (Murevanhema and Jideani, 2013). The leaves of Bambara groundnut are rich in phosphorus and are also used as cattle feed (Murevanhema and Jideani, 2013). Since Bambara plant can fix atmospheric nitrogen and add it to the soil, it is an excellent rotation crop (Stagnari et al., 2017). The agronomic advantages of this crop include its nutritional value, drought tolerance and ability to grow in poor soils (Temegne et al., 2018). As a protein-rich source that contributes to improving nutritional status in rural areas, Bambara groundnut plays an important role in food security. Bambara groundnut provides a nearly balanced diet in terms of carbohydrates, fat, protein, and minerals (Murevanhema and Jideani, 2013). A diet high in carbohydrate (65%) and protein (18%) is said to be fully balanced (Unigwe et al., 2016). Moreover, Bambara contain 80% more protein than groundnuts (65%) and cowpea (64%) (Mubaiwa et al., 2018). As a source of protein and as a seasoning in soups, Bambara plants is important in the diets of communities in rural areas (Mayes et al., 2013). By providing a nutritious alternative to animal protein and a source of income for farmers, cultivation of the crop could improve the situation of smallholder farmers, according to William et al. (2016). Because of its drought tolerance and market potential, the crop can improve the welfare of smallholder farmers (William et al., 2016).

2.3.5 Drought adaptation and the effects of climate change

Bambara groundnut, which grows in an area with less than 500 mm of rainfall, is considered the most drought tolerant of all grain legumes (Mabhaudhi et al., 2018) and requires only 300 mm of rainfall to grow and reproduce. The optimum rainfall for the crop is between 750- and 1 400-mm. Rainfall above 3 000 mm can lead to yield loss (Temegne et al., 2018). Even in unfavourable environments, Bambara is productive; there are very few reports of its productivity associated with drought. According to the findings of Jorgensen et al. (2010), osmotic adjustment may be involved in maintaining turgor pressure in Bambara leaves, as well as reduced leaf area and functional stomatal regulation. According to the report by Mayes et al. (2019), Bambara groundnut can maintain a water potential of about - 2.0 megapascals (MPa). It can grow under dry weather conditions when rainfall is insufficient to complete its growth cycle during the rainy season. The plant uses the available water sparingly by developing the leaf area slowly to conserve water so that the plant can survive the reproductive period and produce some yield (Mabhaudhi et al., 2018).

Climate change affects processes such as germination, photosynthesis and respiration that determine the transport of gases in a Bambara (Khan et al., 2017). Extreme temperatures reduced water availability and changing soil conditions will make it difficult for the Bambara plant to survive. Therefore, it is important to understand these factors and their effects on the growth and yield of the Bambara plant (Mayes et al., 2019). Bambara groundnut depends on certain factors such as temperature, light, carbon dioxide (CO_2), rainfall and moisture to grow and reproduce. When CO_2 levels in the leaves increase, stomatal conductance decreases and the leaves are able to use water more efficiently (Mateva et al., 2020). The pace of climate change, weather patterns and drought stress are increasing in South Africa. Landraces for heat and drought tolerance have yet to be developed (Cleasby et al., 2016). Identification of these landraces would improve food security and nutrition by increasing production in drought-affected areas (Muhammad et al., 2020).

2.3.6 Current breeding and selection initiatives of Bambara groundnut

It has not been possible to develop major breeding programs for this crop so far. Only a few breeding populations have been described and used for genetic studies and cultivar development (Ahmad et al., 2016). Breeding programs depend on how much germplasm is available and the breeding strategies adopted by each plant species. Molecular markers can provide insight into these processes and indicate the best options for developing a breeding program to improve Bambara groundnut (Mayes et al., 2013). A number of markers of

molecular systems have been made and used to assess the genetic diversity and origin of Bambara groundnut landrace populations (Olukolu et al., 2012; Somta et al., 2011).

To identify and select genotypes that possess floral traits that contribute to pod yield, breeders need to determine variability of some floral traits of the plant (Oyinga and Uguru, 2011). Few reports have been published on the breeding, crop improvement, and genetics. Since the late 1950s, farmers have selected genotypes and growth traits that provide high yields and bushy growth (Khan et al., 2017). Some selections arise during cultivation, while others are developed from population patterns by sorting out seeds of the same color. Successful selection depends on information about genetic variability and the numerous relationships between pod yield and individual traits (Akpalu, 2010). A breeding objective has been established for Bambara groundnut (Aliyu et al., 2014) to develop improved genotypes. Muhammad et al (2020) reported that breeding programs for this crop have not been coordinated. Therefore, the available genotypes are selections from indigenous landraces. Thus, to meet the daily demand, high yielding genotypes need to be developed through breeding (Oyiga and Uguru 2011).

2.3.7 Bambara production in South Africa

Bambara groundnut production in South Africa declined significantly from 1946 (4000 tons) to 2016 (1,500 tons), which has been attributed to drought (Aliyu et al., 2014). South African farmers grow Bambara groundnut as landraces in the absence of newly released improved varieties (Cook, 2017). Landraces are a valuable resource that can be used by smallholder farmers (Abu and Buah, 2011). They originated in northern South Africa and spread to the lowlands of KwaZulu-Natal through indigenous migration. Currently, it is only found in the Northern Province, Swaziland, and KwaZulu-Natal (Akinola et al., 2020).

Most Bambara groundnut in South Africa is produced in five provinces (Limpopo, Mpumalanga, North-West, Johannesburg and KwaZulu-Natal) (Otto et al., 2020). Cook (2017) identifies Capricorn, Waterburg, Mopani and Vhembe districts as the main growing areas for Bambara groundnuts in Limpopo. South Africa produces most of its Bambara groundnuts for home consumption under traditional low-input cropping systems, and it is only recently that the varieties have been commercialized (Halimi et al., 2019). A significant percentage (<50%) of the supply for South Africa comes from Zimbabwe (Locks et al., 2012).

2.3.8 Production challenges that limit the production of Bambara groundnut

In addition to lack of genetic improvement, ignorance of taxonomy, reproductive biology and genetics of agronomic and qualitative traits and poor cultivation practises (Ogwu et al., 2018 and Majola et al., 2021), Bambara groundnut has several constraints to its development. Harnessing this knowledge will greatly improve the performance of the crop (Mayes et al., 2019). Cell wall assembly during plant growth is also affected by adverse environmental conditions (Mubaiwa et al., 2018).

Plant cell enlargement is slowed down during cold temperatures and drought, and the process is very sensitive to environmental conditions. Lack of favourable conditions may result in failure of seeds to germinate or flowers to open (Zondi, 2012). Due to the use of local lines, the productivity of Bambara groundnut is low. Fatimah and Ardiarini (2018) noted significant differences among the studied local lines of Bambara groundnut, both within and between lines, including plant growth habit, leaf shape and stem hairiness. They also emphasised the need to better Bambara local lines by cleaning the potential local lines as soon as possible. In addition, they noted that plant breeding could improve local lines by cleaning potential local lines. In addition, the study found that smallholder farmers cultivate Bambara groundnut similar to other crops in marginal agricultural areas. The marginal effect of household size states that farmers with larger households tend to grow more Bambara groundnut. Therefore, household members contribute significantly to agricultural production in a farming community (Sidibé et al., 2020).

2.3.9 Germination and seed emergence in Bambara groundnut

Unlike germination, emergence involves the formation of an embryo from a seed that undergoes a series of metabolic processes, such as respiration (Singh, 2011). Bambara seeds germinate slowly compared to groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculate)* (Mabhaudhi et al., 2013). Development, phenology, and yield of a Bambara landrace under water stress showed poor emergence (below 30%) for many farmers (Mabhaudhi et al., 2013). Moreover, poor seed quality can lead to poor stand and yield in addition to poor field emergence (Sinefu et al., 2011). Seed coat colour and texture have been shown to affect germination rate (Mandizvo and Odindo, 2019). When seeds germinate, the film prevents them from accessing water (Mabhaudhi et al., 2013) and oxygen (Ijarotimi and Keshinro, 2020). Miya and Modi (2017) reported that scarification improves seed germination however, there was no emergence from the ground. For this reason, Bambara is not suitable for commercial cultivation and is not well accepted by farmers. The lack of germination and field emergence

hinders production success and productivity (Jost et al., 2016). Seedling emergence and lower plant population may be a result of poor germination of Bambara (Ilyas and Sopian, 2011).

The high germination rate of dark coloured varieties observed by Ijarotimi and Keshinro (2020) indicates that the seed was of high quality. Mandizvo and Odindo (2019) observed that seed lots with high germination have higher emergence rate than those with low germination. Strong, early germinating seed gives the emerging seedlings a competitive advantage against a variety of environmental stresses and diseases (Sinefu et al., 2011). Germination and emergence of Bambara groundnut are often unstable and variable (Touré et al., 2012). The seed does not begin to grow until it has absorbed sufficient water (Zondi, 2012). As a result of soil disturbance, the seed may also be exposed to environmental factors that lead to seedling colonisation by seed depth. Water level can affect seedling emergence and development (Lara-Viveros et al., 2020). Bambara becomes dry when excessively watered as the water fills all the pores in the soil and prevents oxygen from reaching the seeds (Umeugochukwu, 2016). The major factors affecting seed germination and emergence in semi-arid regions are dry soil and high temperature. Short-term flooding during sowing can also be caused by poorly drained soils or heavy rainfall (Tichavský et al., 2018). Seeds germinate only after they have absorbed enough water to reactivate their growth (Zondi, 2012).

2.3.10 Effects of environmental factors on germination

Muhammad et al. (2020) pointed out that it is critical to understand the environmental influences on growth and development of Bambara groundnut in different agroecological regions, as well as the possibility of transferring selections to other regions and cultivating the crop in general. Germination and emergence of Bambara groundnut are affected by environmental factors which include temperature, water stress and light (Mabhaudhi et al., 2013).

2.3.10.1 Temperature

Seed growth and development can be affected by temperature in Bambara (Figure 2.3). Bambara groundnut seeds can germinate at 30 °C to 35 °C and take 5 to 21 days to develop (Duerr et al., 2015). An average daily temperature ideal for Bambara development is between 20 °C and 28 °C (Mabhaudhi and Modi, 2013). Extreme temperatures (≤ 35°C) cause seed death, which affects plant survival and future plant species (Ambede et al., 2012). Temperature affects the movement of water and nutrients in the soil such as nitrogen (N), phosphorus (P) and potassium (K). According to Mueller et al. (2016), low temperature inhibits water uptake. Anjum et al (2011) found that drought is more severe than temperature stress in plants under

heat and water stress. At high temperatures, Kendabie et al. (2020) reported fewer cones and pods in Bambara groundnut. As a result of competition for assimilates with stem and leaf growth, plants become dwarfed. In hot weather, drought affects morphological and physiological processes more than in cold weather (Chai et al., 2016). Limited research has been conducted on the differences between landraces in response to drought and temperature. (Mayes et al., 2019). Temperature also affects how quickly roots take up water and nutrients. Plant growth is indirectly affected by soil temperature through soil physical processes such as evaporation rate at the soil surface (Latati et al., 2016). Temperature affects seed germination through enzymatic activity (the conversion of organic material into small molecules that can be taken up by the plant through its cell wall) and various other metabolic processes such as photosynthesis, respiration and nitrogen fixation (Hasan et al., 2018).

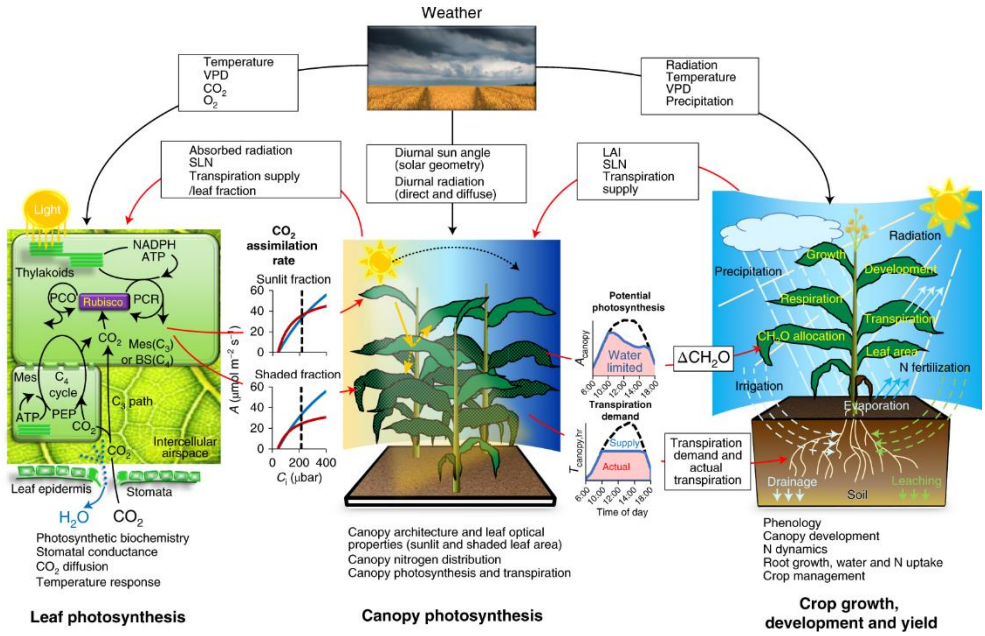

Figure 2.3: An integrated framework for modelling crop growth and development based on the diurnal canopy photosynthesis–stomatal conductance module, Wu et al., 2019

2.3.10.2 Light

Seed germination and seedling formation are affected by both light and temperature. Plant phytochromes are very important in determining the timing of germination, and thus they become an essential part of the evolutionary strategy to enforce conditional dormancy so that seedlings are protected from environmental extremes (Mabhaudhi and Modi, 2013). In high light, seeds germinate more quickly but too much light can damage them (Ambede et al., 2012). Photosynthesis will be very slow compared to respiration. As a result, more photosynthesis

products are being used than produced (AL Shareef et al., 2014). Kendabie et al., 2020) state that the dry matter allocation to Bambara pods will be reduced and more of an emphasis will be placed on yield and production. The response of germination to light probably varies from habitat to habitat. It has been demonstrated that strong light increases the probability of seedling establishment in shaded environments, such as tunnels (Mabhaudhi and Modi, 2013). Photosynthesis is the process by which Bambara groundnuts obtain energy from light. Without light, the Bambara would not be able to produce the energy it needs to grow and develop (Mayes et al., 2019).

Plants can be damaged by excessive light. Overexposure to direct light will prevent the germination of Bambara seeds. It is therefore necessary to protect Bambara seeds from too much direct sunlight during the summer months in order to ensure that they germinate properly (Klompong and Benjakul, 2015). The amount of light a plant receives determines how fast it grows and how long it remains active (Murevanhema and Jideani, 2013). In plants, photosynthesis is the most important metabolic process. Regardless of how much of any other variable Bambara receive, they will not reach their maximum growth rate or potential without enough light (12 to 16 hours of light per day) (Filli et al., 2013).

2.3.10.3 Water stress

According to a study (Mabhaudhi et al., 2011), seed germination and area of the leaf (LAI) of Bambara are reduced by water stress. When plants suffer from water stress, the stomata close, maximizing the plant's water consumption and reducing CO_2 emissions (Sinefu et al., 2011). Not only the seeds are affected by water stress, but also the leaves and roots, leading to a decrease in stomata conductance and photosynthetic rate (Chai et al., 2016). When stomata are closed, a small amount of CO_2 is sequestered while photosynthetic electron transport proceeds normally (Chai et al., 2016). A few papers have reported that water stress reduces Bambara production and total number of seeds. Mabhaudhi et al (2013) found that water stress reduces Bambara pod production. Water stress affects plant growth and productivity (Sinefu et al., 2011). Reduced leaf growth and stomata closure are two of the first signs of water stress in plants (Mabhaudhi et al., 2013). A number of researchers, including Mabhaudhi et al. (2013), indicate that Bambara groundnut grown in pots or in small soil volumes experiences less leaf loss due to water stress than Bambara plants planted in the field. The roots of plants grown in the field are mainly concentrated in the topsoil profile where most of the water is absorbed (Mubaiwa et al., 2017). Plants are more likely to maintain drought if their roots grow tremendously to draw water from deeper soil zones (Wang and Zhang, 2017).

Reduced photosynthetic rate and internal carbon dioxide concentration are caused by water stress (Sinefu et al., 2011). Plant growth and productivity decrease when stress increases (Ambede et al., 2012). By lowering the water potential of a cell in response to reduced soil moisture, Al-Yasi et al. (2020) found that osmotic adjustment is important for drought tolerance. Photosynthesis and chlorophyll content of Bambara plants suffering from water shortage decreases, resulting in slower growth and premature maturation of the plant (Ambede et al., 2012). Water scarcity affects Bambara groundnut physiologically, morphologically and molecularly (Chai et al., 2016). Under water stress, dry matter is more distributed among the roots. Through a diffusion process called osmosis, root hairs protrude into the soil to absorb water (Mohammed et al., 2016).

2.3.11 Macronutrient composition and importance in Bambara groundnuts

Groundnuts, such as Bambara groundnuts, are an important source of protein and help combat malnutrition. They are the cheapest and easiest legumes to buy (Keneni, 2011) and serve as a source of vegetable protein in developing countries. The composition of Bambara is 65% carbohydrate, 18% protein and 6.5% fat. The seeds contain an average of 54.5-69.3% carbohydrate, 17-24% protein and 5.3-7.8% fat and provide 367-414 calories per 100g (Okonkwo et al., 2018), making it a complete food. There is need to increase consumption in developing countries as it is very low (Murevanhema and Jideani, 2013).

2.3.11.1 Carbohydrates

Bambara groundnut contains starch and non-starch polysaccharides in its carbohydrate fraction (Mubaiwa et al., 2017). Bambara groundnut is predominantly composed of carbohydrates, which account for up to 69.3% of the dry weight of the seeds (Shiyam et al., 2016). It appears that raw Bambara groundnuts are less digestible than cooked groundnuts as they contain more carbohydrates. By improving the digestibility and availability of carbohydrates, cooking the seeds significantly increases the rapidly digestible starch. The carbohydrates in Bambara are sources of energy as they form solid walls around the plant cells. These solid cell walls form the scaffold that enables plants to stand and grow as they have no skeleton or weight bearing form (Hasanuzzaman et al., 2018).

2.3.11.2 Proteins

Bambara groundnut cells also contain proteins (Diedericks et al., 2020). Bambara groundnuts contain an average of 23.6% protein but can range from 9.6 to 40%. Vicilin was found to be the major storage protein in Bambara groundnuts followed by legumes (Tan et al., 2020). They are present in the seeds and serve as a source of amino acids for the developing seeds where

they account for about 40% of the seed weight (Yao et al., 2015). High protein content is an important characteristic of Bambara; however, both amino acid composition and protein digestibility are equally important characteristics (Chimonyo and Modi, 2013). In addition, protein's role is very important in the structural integrity of the cell wall (Jamet et al., 2006). Much of the growth of Bambara plant consists of proteins and amino acids (Lin Tan et al 2020). They provide a good source of lysine, which is frequently lacking in cereals but abundant in methionine. About 70-81% of proteins from raw and cooked Bambara peanuts are digestible in vitro (Lin Tan et al., 2020). Hellwig. (2019) reported that cooking increases protein digestibility in vitro by destroying factors like tannins amylase inhibitors, and protease inhibitors and breaking innate proteins to polypeptides that improve enzyme suitability and protein solubility.

2.3.11.3 Lipids

Bambara plants contain large amounts of lipids. They not only provide metabolic energy, but also protect against desiccation and pathogens, transport electrons, and absorb light (Yao et al., 2015). Lipids also influence membrane structure. Their structure serves as a barrier between the cells and the external environment (Mandizvo and Odindo, 2019). They have been reported to range from 1.4 to 9.7% in Bambara peanuts (Lin Tan et al., 2020). Mandizvo and Odindo (2019) describe lipids as structural components of cell membranes as well as permeable barriers that serve as barriers between cells and their external environment. Abiotically and biotically, we need to understand how plants survive in a changing climate and in times of food insecurity. Thus, farmers need to know how Bambara plants transmit lipophilic signals from roots to shoots while maintaining high levels of unsaturated fatty acids to grow Bambara plants that can survive adverse environments such as drought (Zhang and Zhu, 2018).

2.3.11.4 Minerals

In Bambara groundnuts, potassium is the most abundant mineral (1542-2205 mg/100g), followed by magnesium, calcium, then phosphorus (313-563 mg/100g) and sodium (16-25 mg/100g) (Yao et al, 2015). For rapid healthy growth, Bambara require constant access to these minerals. During seedling and leaf growth and flowering, the plant consumes every primary nutrient. According to Halimi et al (2019), these minerals are more abundant in lentils than in common legumes, but the levels vary depending on the variety and growing conditions. The concentration and bioavailability of nutrients in Bambara groundnut seeds may vary depending on factors such as storage time, processing method, and location of the seeds. These minerals

are found in Bambara groundnut (Gwala et al., 2020). Bambara groundnut can provide the required amount of each mineral (Ijarotimi and Keshinro, 2020).

2.3.11.5 Phytochemicals

Bambara contains flavonoids and tannins. The seeds with dark or red coat contain the highest concentrations of them (Murevanhema and Jideani, 2013). Mubaiwa et al (2018) found in raw and cooked red Bambara seeds and the number of flavonoids epicatechin and catechin increased with darkness of the seed coat. Bambara groundnut contains phytochemicals that protect cells from damage that can lead to cancer. Bambara plants are protected from fungi, bacteria, plant viruses, insects and other animals by these phytohormones (Shegro et al., 2013). Legumes contain a total of 478 phytochemicals. An estimated 19% of these are condensed tannins (n = 90), followed by flavanols (n = 79), isoflavones (n = 64) and phenolic acids (n = 63), which are associated with 17%, 13% and 13% of the total, respectively (Tor-Roca et al., 2020). Bambara seeds contain antioxidants in amounts comparable to those of legumes, but lower than ascorbic acid, the most potent antioxidant (Nyau et al., 2015). Although phytochemical compounds have beneficial effects on health, their nutritional effects should not be ignored (Lin Tan et al., 2020).

2.3.12 Future prospective of improvement

Bambara groundnut has been improved in various ways to meet the demand (Effa and Uko, 2017). In South Africa, there are various (more than 30) landrace varieties of Bambara groundnut (Cook, 2017). To improve the crop, a new Bambara groundnut accessions need high canopy cover and uniform establishment. Through the use of molecular markers and genetic resources, farmers will be able to better understand how traits are controlled genetically and use marker-assisted selection and genome-assisted breeding (Mayes et al., 2019). Mild, intermittent, and terminal droughts are not harmful to Bambara groundnuts. Adu-Dapaah and Sangwan (2004) suggested that gamma irradiation of Bambara groundnut could improve productivity and induce higher genetic diversity than an untreated control.

Genetic population structures are key to dissecting traits in underutilized crops. By partitioning traits into genetic and nongenetic components, effects of trait variation can be localized to specific loci (Mayes et al., 2019). Selection for higher number of pods or plants could increase seed yield of Bambara groundnut (Alake and Alake, 2016). Bambara groundnut can be improved by shortening the generation cycle to allow at least four cycles per year (Bationo et al., 2011). Before Bambara groundnut can offer major benefits to farmers, it needs to fill a number of significant gaps through crop improvement. According to Mayes et al. (2019), future

breeding programs for Bambara groundnut could aim to select individual lines with increased drought resistance and appropriate yield traits. Climate resilient agriculture has great potential for Bambara groundnut (Adu-Dapaah and Sangwan, 2004).

2.4 Conclusion

In South Africa, unimproved landraces of Bambara groundnut (*Vigna subterranea* L. Verdc) lead to low yields. Food security and nutrition with Bambara can be improved. Therefore, it is important to expand the distribution of new Bambara groundnut landraces, especially because conditions in the environment are changing. It was noted in the literature that few studies have been carried out on crop productivity. In crop production, this is another problem that needs to be addressed so that this crop can be better developed and produced more effectively. Therefore, further research and development efforts are needed in the area of Bambara groundnut production, especially in South Africa and beyond.

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CHAPTER 3: A MULTIVARIATE APPROACH TO ASSESS THE PHENOTYPIC VARIABILITY AMONG TWENTY-FOUR BAMBARA GROUNDNUT (*Vigna subterranea* L. Verdc) GENOTYPES FROM TWO PRODUCTION SITES (BRITS AND UKULINGA)

Abstract

Among the varieties of groundnuts grown on the African continent, the Bambara groundnut (*Vigna subterranea* L. Verdc) is the least utilized. Crops such as Bambara are essential for food security and nutrition but has been poorly characterized. The objective of this study was to characterize the agromorphological traits of Bambara groundnut landraces and to assess the relationships among the traits. Two different production environments (Ukulinga and Brits) were used to study twenty-four (24) genotypes of Bambara groundnut to evaluate the agronomic traits influenced by both environmental factors, and to identify stable genotypes in each location. Three replications were used in a randomized complete block design. The agromorphological traits measured were grain yield per plot (GYPlot), leaf petiole length (PL), 100 seed weight (HSW), leaf length (LL), plant canopy (PC), plant height (PH), leaf width (LW), and number of pods per plot (NPP). Data were analysed using the analysis of variance (ANOVA) for each location. The differences in performance between Bambara genotypes and environmental effects were significant ($p< 0.05$) for agronomic trait performance. Brits site had a cumulative variation of 55.37% after two principal components (PCs), PC 1 and PC 2, created by both PC 1 and PC 2, and Ukulinga had a cumulative variation of 50.02% after two principal components (PCs), PC 1 and PC 2. GYPlot, HSW, PL and LW contributed more to PC 1 at Brits site, five traits (GYPlot, HSW, PL, LL and LW) contributed more to PC 1 at Ukulinga. Acc 179, Acc 184, Acc 150 were associated with desirable grain yield traits at Brits site with yield ≤500 kg/ha. In contrast, at Ukulinga, all genotypes had a yield of ≤600 kg/ha. Ukulinga is a better production site for Bambara groundnut compared to Brits. Furthermore, this will give us a better idea of potential candidate genotypes for Bambara groundnut improvement programme.

Keywords: Bambara groundnut, multivariate approach, environmental effects, phenotypic variability, principal component, morphological variability

3.1 Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc) is considered a promising crop grown mainly for domestic consumption and nutritional security. It is mainly grown by women in Africa and Asia (Khan et al., 2020). As the most neglected crop species not recognized in breeding programs (Mayes et al., 2019), Bambara groundnut landraces remain the most important source of food security produced by smallholder farmers (Gao et al., 2020). Several researchers have obtained Bambara groundnut landraces from different parts of Africa and outside, but these helpful genetic resources have not yet been fully exploited (Uba et al., 2021). In South Africa, Bambara groundnut is grown under varying environmental conditions (temperature, rainfall and solar radiation) mainly in KwaZulu-Natal, Mpumalanga, Limpopo and North-West provinces (Mabhaudhi and Modi 2013). Despite the lack of intensive research efforts, it is one of the most important legumes in many parts of Africa (Akinola et al., 2020). Bambara groundnut fixes atmospheric nitrogen and thus can improve soil fertility. It is resilient to adverse temperature conditions and can yield on degraded and acidic soils compared to other crops (Tan et al., 2020). The challenge for farmers and researchers is to find ways to increase the efficiency of crop production and find suitable cultivars to meet the demands of a growing population (Mubaiwa et al., 2018).

Production environment can be one of the constraining factors for Bambara groundnut production (Unigwe et al., 2016). Bambara groundnut genotypes are landraces that have evolved under terrible conditions and are known for their yield consistency (Alake et al., 2015). To improve yield of Bambara groundnut and select suitable genotypes for different agroecological regions, breeders need to identify suitable genotypes that are adapted to growing conditions for high production and productivity (Aliyu et al., 2016). In recent years, Bambara groundnut landraces have been continuously grown in adverse and stressful environments because they are highly productive under these conditions. (Obidiebube et al., 2020). The ability of these landraces to survive in the poorest environments is clear evidence of their adaptation to stressful conditions when grown without agronomic inputs such as fertilizers, irrigation or pest and disease control (Khan et al., 2020).

In South Africa, Bambara groundnut landrace in South Africa are not fully characterised for agronomic and morphological traits (Rahmah and Setiawan, 2020). The main objective of this study was to characterize agronomic traits and evaluate trait relationships and identify high yielding and stable genotypes under two different production conditions (Figure 3.2) in South Africa.

3.2 Materials and methods

3.2.1 Plant material

The genotypes used in this study included twenty-four Bambara groundnut accessions; Acc 25, Acc 55, Acc 61, Acc 78, Acc 82, Acc 87, Acc 95, Acc 96, Acc 97, Acc 100, Acc 105, Acc 117, Acc 121, Acc 131, Acc 150, Acc 151, Acc 175, Acc 177, Acc 179, Acc 184, Acc 190, Acc 197, Acc 199, and Acc 200 (Figure 3.1) obtained from the Agricultural Research Council (ARC) genebank in Pretoria, South Africa. The 24 genotypes comprised of eight seed coat colour variations which are: cream, brown, spotted cream, black, cream, dark red, speckled brown, and cream brown (Figure 3.1).

Figure 3.1: Twenty-four Bambara groundnut accessions sorted into eight seed coat colours

3.2.2 Experimental sites

The genotypes were grown on the Brits (North-West Province) and Ukulinga (KwaZulu Natal Province) research farms of ARC and the College of KwaZulu Natal (UKZN), respectively, during the 2018/2019 summer cropping season. Figure 3.2 below shows the average daily rainfall at Brits and Ukulinga during the summer growing season (2018/2019). The Brits site is located at latitude 25.6276° South and longitude 27.7816° East, at an elevation of 1119.10 meters above sea level. The recorded maximum and minimum temperatures during the growing season ranged from 14.46 to 30.31ºC with an average temperature of 22.39ºC. The soil type is a loamy clay with a pH of 7.08 (ARC-VOPI, 2019). Ukulinga is located at 29°24'E latitude and

30°24'S longitude at an altitude of 840 meters above sea level. The locality receives an average annual rainfall of 750 mm over 113 rainy days, with 23% of the mean annual precipitation (MAP) falling in the winter months. The maximum and minimum temperatures recorded in Ukulinga during the growing season ranged from 14.7 to 26.8ºC, with an average temperature of 22.8ºC. The soil type is a loamy clay soil

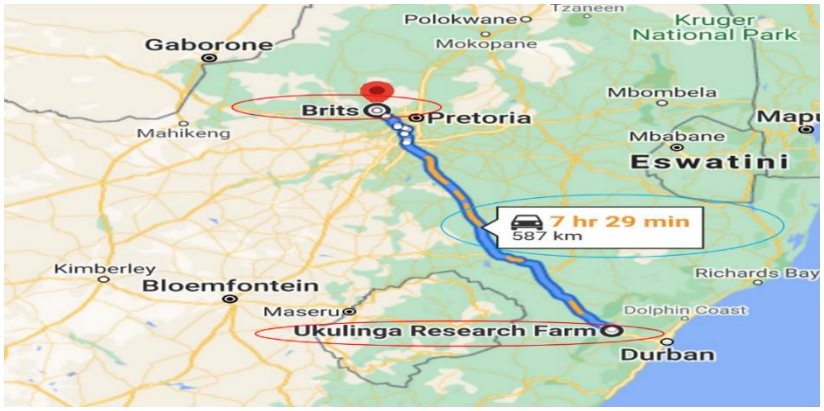

Figure 3.2: Locations for Ukulinga and Brits research farms

3.2.3 Experimental design, layout, and treatment structure

In each site, the experiment was set up as a one-factor analysis with the twenty-four genotypes as the main factor and replicated three times, giving a total of 72 experimental units (plots). Each plot was (2.5 × 2 m) with row and inter-row spacing of (0.3 × 0.45 cm). The experimental design used at the two sites was a randomized complete block. Three rows of 4 m length were planted at each site. Two seeds were hand-sown per hole and plants were thinned to one after seedling emergence. A central row of five randomly selected plants was used for data collection and analysis.

3.2.4 Data collection

Data collection began during the vegetative stage of the crop. Data collected included: leaf length, leaf width, leaf area, petiole length, plant height, number of pods per plant, plant canopy, days to maturity, number of seeds per plant, hundred seed weight, grain yield per plot using the descriptor for Bambara groundnut (IPGRI. 2000). Average daily rainfall data (Figure 3.3) was obtained from the Agricultural Research Council – Soil, climate, and water.

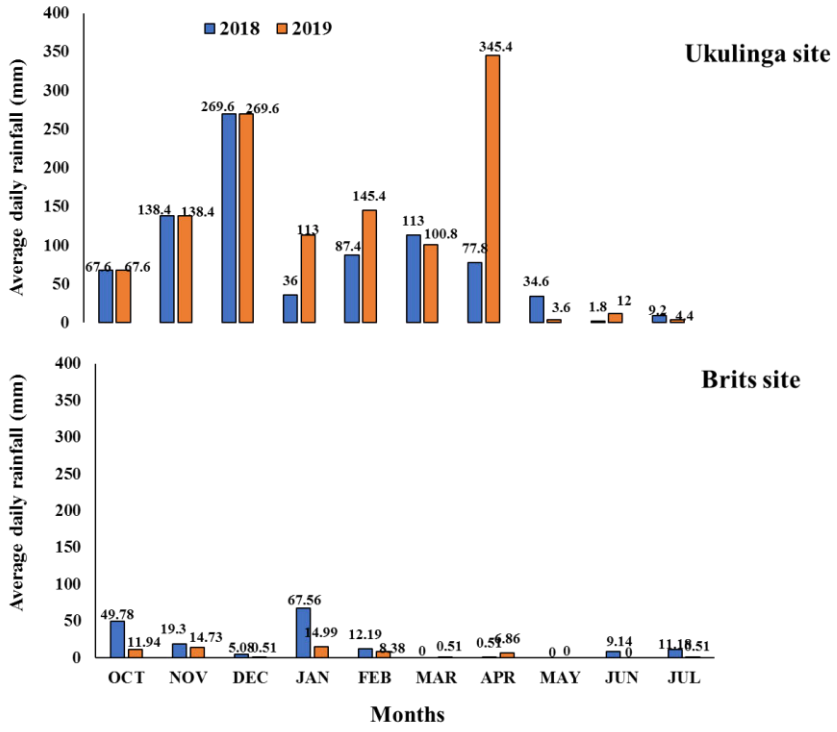

Figure 3.3: Average rainfall at Brits and Ukulinga during summer cropping season (2018/2019)

3.2.5 Parameters measured and data analysis

Twelve (12) quantitative morphological characters (Table 3.1) were measured among the 24 test Bambara groundnut accessions using the descriptor for Bambara groundnut.

Table 3.1: List of quantitative morphological characters recorded from 24 Bambara groundnut accessions

Quantitative characters	Code	Description	Measurement type
Leaf length (cm)	LL	Length of the leaf from the base to the tip	Measuring tape
Leaf width (cm)	LW	Width of the leaf from the widest part of the leaf	Tape measurement
Leaf area (mm^2)	LA	Area of the leaf	Leaf area meter
Petiole length (cm)	PL	Length of panicle from its base to the tip	Tape measurement
Plant Height (cm)	PH	Height of main stalk from the ground to the tip of the main panicle	Tape measurement
Plant canopy	PC	Measuring above ground portion of a plant	Tape measurement
Days to maturity (count)	DM	Number of days from emergence to maturity in each plot	Counting
Number of pods per plot (count)	NPP	Number of pods per each plant	Counting
Number of seeds per plot (count)	NSP	Total count of number of seeds per five plants	Counting
Yield per plant	YPP	Weight of seed per plant (average of five plants)	Weighing balance
Hundred seed weight	HSW	Weight of hundred seed counts at 12% moisture content	Weighing balance
Grain yield per plot	GYPlot	Total weight of seed per plot	Weighing balance

3.3 Data analysis

Data was analyzed using the Analysis of Variance (ANOVA) for each site. Means were compared using least significant difference (LSD) at the 0.05 significance level. Correlations were generated using GraphPad Software, La Jolla California USA. Phenotypic correlation coefficients were calculated to examine the degree of association between phenotypic traits. Data were also subjected to principal component analysis (PCA) using XLstat software France Addinsoft 40, rue Damrémont 75018 Paris, Japan. Only common data for both sites were

analyzed to compare the variability between these two environmental conditions. An F-test was performed to prove that the variances at the two sites (Ukulinga and Brits) were different and thus there was no interaction between Ukulinga and Brits sites (Appendix A Table 5).

3.4 Results

3.4.1 Plant growth and development in response to production environment.

In this study, 12 morphological quantitative traits of Bambara groundnut were analysed. There were highly significant ($p< 0.05$) differences between the two production sites in plant growth and development. In terms of plant height, the genotypes at the Brits site differed significantly (Figure 3.4). The tallest plant at Brits site was Acc 100 (30. 74 cm) while the tallest plant from Ukulinga was Acc 61 with a height of 28.67 cm. Plant height varied from 19.33 cm (Ukulinga) to 30. 74 cm (Brits). Acc 100 from Brits had the longest leaf (8.44 cm) followed by Acc 55 (7.73 cm) from Ukulinga site. There was a significant difference ($p< 0.05$) in plant canopy between genotypes at Ukulinga site (Figure 3.5). At Ukulinga, plant canopy varied from 26.67 to 36.33 cm. At Brits, plant canopy varied from 33.6 to 82.1 cm. In addition, Acc 25 at Ukulinga had the largest canopy (36.33 cm) and Acc 175 at Brits had the largest canopy (82.1 cm).

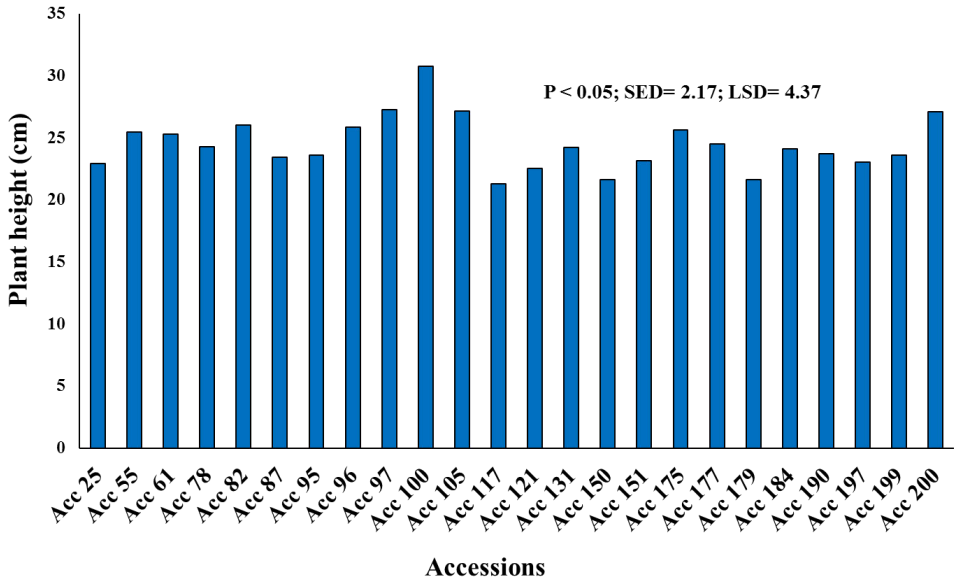

Figure 3.4: Variation of plant height among twenty-four (24) genotypes at Brits site

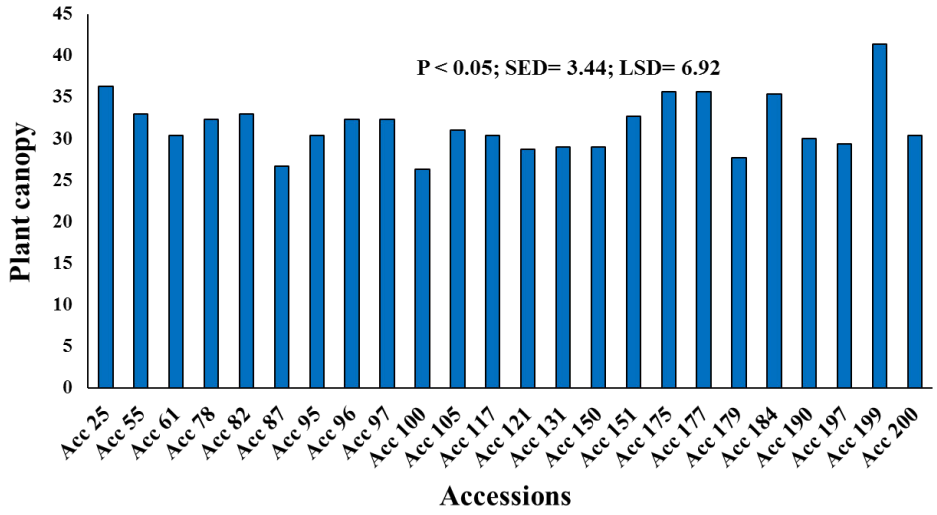

Figure 3.5: Variation of plant canopy among twenty-four (24) genotypes at Ukulinga site

3.4.2 Yield performance in response to production environment.

Yield performance was determined using grain yield per plot and converted to grain yield per ha (GY/ha) and hundred seed weight (HSW) (Figure 3.6). Highly significant differences (p<0.001) were observed among genotypes in Brits in terms of hundred seed weight and grain yield (Figure 3.6 a and b). Acc 179 obtained the highest grain yield (1083.04 kg/ha) in Brits followed by Acc 184 (727.76 kg/ha) and Acc 150 (705.15 kg/ha). Grain yield at Ukulinga varied from 652.00 to 1446.67 kg/ha with Acc 25 having the lowest yield. Grain yield of the 24 genotypes tested at both locations varied from 115.34 to 1446.67 kg/ha. All genotypes showed high yield (≤600 kg/ha) at Ukulinga compared to Brits, implying that environmental conditions were more favorable for Bambara groundnut production at Ukulinga. According to hundred seed weight, there were highly significant differences among genotypes in Brits (Figure 3.6 a). Acc 97 had the highest hundred seed weight in Brits followed by Acc 179 and Acc 150 (77.5, 72.2 and 71.1 g, respectively).

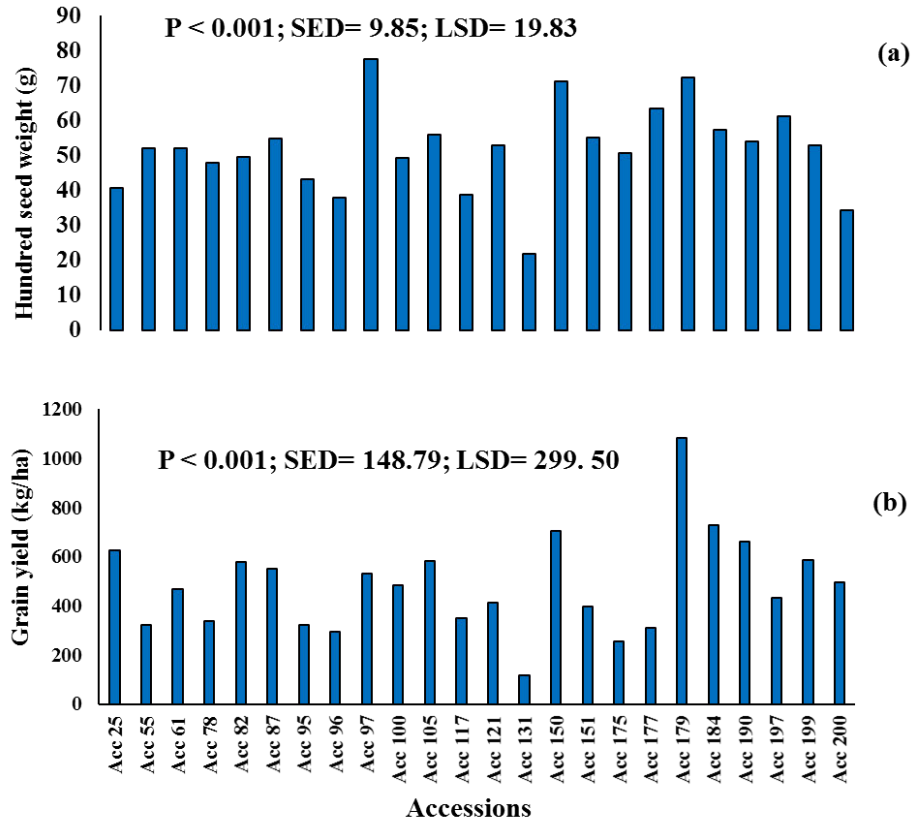

Figure 3.6: Variation of hundred seed weight (HSW) and grain yield (GY) at Brits.

Table 3.2: Mean of twenty-four genotypes in two locations for the vegetative growth parameters (leaf length (LL), leaf width (LW), plant canopy (PC), plant height (PH), and petiole length (PL))

Genotypes	LL		LW		PC		PH		PL	
	Brits	**Ukulinga**	**Brits**	**Ukulinga**	**Brits**	**Ukulinga**	**Brits**	**Ukulinga**	**Brits**	**Ukulinga**
Acc 25	6.51	6.37	2.90	2.90	41.30	36.30	22.92	25.67	1.88	1.87
Acc 55	6.73	7.73	2.50	3.50	39.50	33.00	25.49	23.67	1.63	1.83
Acc 61	6.99	6.67	2.40	3.20	37.90	30.30	25.27	28.67	1.79	1.70
Acc 78	6.39	6.60	2.00	2.80	37.30	32.30	24.29	25.67	1.93	1.93
Acc 82	6.51	7.33	2.40	3.20	39.50	33.00	26.00	25.67	2.32	1.47
Acc 87	6.20	6.83	2.60	2.80	38.20	26.70	23.46	25.33	2.28	1.60
Acc 95	6.79	6.80	2.40	2.50	45.10	30.30	23.58	24.33	1.83	1.70
Acc 96	6.48	7.50	2.50	2.60	36.40	32.30	25.87	22.67	1.90	1.87
Acc 97	6.93	7.50	3.20	3.20	52.70	32.30	27.28	23.00	2.33	1.97
Acc 100	8.45	6.33	2.50	3.00	44.00	26.30	30.74	24.00	2.42	1.83
Acc 105	6.66	5.50	2.70	2.50	40.60	31.00	27.17	24.33	2.51	2.00
Acc 117	6.71	7.47	2.80	3.00	34.60	30.30	21.29	26.67	2.26	1.93
Acc 121	6.49	6.17	2.90	3.30	33.60	28.70	22.52	19.33	2.32	1.43
Acc 131	6.17	6.50	2.60	3.50	42.00	29.00	24.24	22.33	1.86	1.57
Acc 150	6.78	6.83	2.80	3.00	44.10	29.00	21.63	23.67	2.33	1.83
Acc 151	7.13	6.43	2.50	2.60	43.80	32.70	23.16	25.67	2.03	1.90
Acc 175	6.30	6.60	2.40	2.70	82.10	35.70	25.63	24.00	2.21	1.87
Acc 177	6.27	7.47	2.40	2.40	45.70	35.70	24.52	21.33	2.04	2.13
Acc 179	6.92	6.83	2.90	3.00	40.50	27.70	21.64	24.33	2.01	2.03
Acc 184	6.58	6.00	2.50	2.50	46.10	35.30	24.12	26.33	2.17	2.07
Acc 190	6.28	6.00	2.60	2.80	47.10	30.00	23.71	26.67	1.85	2.23
Acc 197	6.60	5.83	3.00	2.30	38.10	29.30	23.04	24.67	2.10	1.87
Acc 199	6.13	7.00	2.70	3.90	36.20	41.30	23.62	25.00	2.20	1.80
Acc 200	6.83	6.67	2.80	3.60	45.70	30.30	27.09	19.67	2.43	2.07
LSD 5%	1.37		0.77		8.08		4.30		0.40	
% CV	12.54		16.83		13.22		10.64		12.37	
se	0.85		0.48		2.65		2.65		0.25	

LSD5% = least significance difference at 5 percent % CV = percentage coefficient of variation SE = standard error, LL = leaf length, LW = Leaf width, PC=plant canopy, PH= plant height, PL= petiole length

3.4.3 Principal component analysis

Principal components analysis (PCA) was used to determine whether the production environments influenced trait variation between the genotypes in Brits and Ukulinga where these genotypes were planted and grown, and for each composite trait they should be linearly independent and account for a maximum amount of variance in succession. Table 3.3 shows the results of the principal component analysis of the seven (7) physiological traits measured. The first five principal components accounted for 90.33% of the cumulative value at Brits and 90.86% at Ukulinga. Four traits (GYPlot, HSW, PL and LW) contributed more to PC 1 at Brits and five traits (GYPlot, HSW, PL, LL and LW) contributed more to PC 1 at Ukulinga. Moreover, three traits PH, PC and LW had high loadings (≥0.6) on PC 1, PC 2 and PC 5 respectively at Brits. At Ukulinga site, GYPlot, PH, PC and HSW had high loadings (≥0.6) on PC 1, PC 2, PC 3 and PC 4. The contribution of plant height was low on PC 1 but high on PC 2 at both sites. Grain yield was high at PC 1 and low at PC 2 at both sites. The first three traits with the highest loadings for PC 1 are GY, HSW and PL for the Brits. The first three traits with the highest loadings on PC 1 at the Ukulinga are: LL, GYPlot and LW.

Table 3.3: Principal component analysis showing eigenvectors, eigenvalues, and percent variability

Physiological traits	Brits					Ukulinga				
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 1	PC 2	PC 3	PC 4	PC 5
GYPlot	0.54	0.20	0.06	0.32	0.25	**0.81**	0.31	0.21	0.38	0.06
HSW	0.52	0.08	0.29	0.44	0.04	0.48	0.21	0.29	**0.65**	0.38
PH	0.02	**0.69**	0.09	0.02	0.05	0.03	**0.72**	0.43	0.18	0.38
PC	0.01	0.32	**0.84**	0.04	0.26	0.31	0.43	**0.66**	0.13	0.19
PL	0.41	0.31	0.05	0.57	0.56	0.41	0.54	0.50	0.15	0.44
LL	0.24	0.50	0.44	0.32	0.36	0.82	0.01	0.27	0.30	0.23
LW	0.47	0.17	0.06	0.53	**0.65**	0.49	0.57	0.22	0.50	0.20
Eigenvalue	2.15	1.73	1.06	0.85	0.54	2.06	1.45	1.12	0.97	0.76
Variability (%)	30.65	24.72	15.07	12.19	7.70	29.37	20.66	16.03	13.92	10.90
Cumulative (%)	30.65	55.38	70.45	82.64	90.33	29.37	50.02	66.05	79.96	90.86

Vector loadings ≥0.6 are boldfaced, grain yield per hectare (GYPH), hundred seed weight (HSW), plant height (PH), plant canopy (PC), petiole length (PL), leaf length (LL), Leaf width (LW); %=percentage.

3.4.4 Principal component biplot

At Brits, there were two principal components, PC 1 and PC 2, which accounted for 30.65% and 24.72% of the variation, respectively, with a cumulative variation of 55.37% (Figure 3.7). At Brits site, four genotypes (Acc 82, Acc 100, Acc 105 and Acc 97) were positively associated with PC 1 while Acc 61, Acc 96, Acc 151 and Acc 175 were positively associated with PC 2. In contrast, in Ukulinga, six genotypes (Acc 61, Acc 96, Acc 97, Acc117, Acc 177 and Acc 199) were positively associated with PC 1. Only three genotypes (Acc 100, Acc 150, and Acc 200) were negatively associated with PC 1. On the other hand, at Ukulinga, the first and second principal component analysis were 29.36% and 20.66%, respectively, with a cumulative variation of 50.02%. At the Brits site in the biplot diagram, only two parameters (LL and PL) were positively correlated with each other. Acc 200 and Acc 105 were more correlated in the first quadrant. At the Brits, Acc 131, Acc 78, Acc 177, Acc 117, Acc 25, Acc 150, Acc 55 and Acc 78 had no correlation with the traits in the third quadrant. In contrast, at the Ukulinga, only Acc 100, Acc 150, Acc 200, Acc 131, and Acc 121 had no association with any of the traits in the third quadrant. At Brits, Acc 200, Acc 82 and Acc 105 were jointly positively associated with leaf length and petiole length, and Acc 184 was jointly associated with hundred seed weight. At Ukulinga, Acc 61, Acc 96, Acc 97 and Acc 199 were jointly associated with plant canopy, hundred seed weight, grain yield and leaf length.

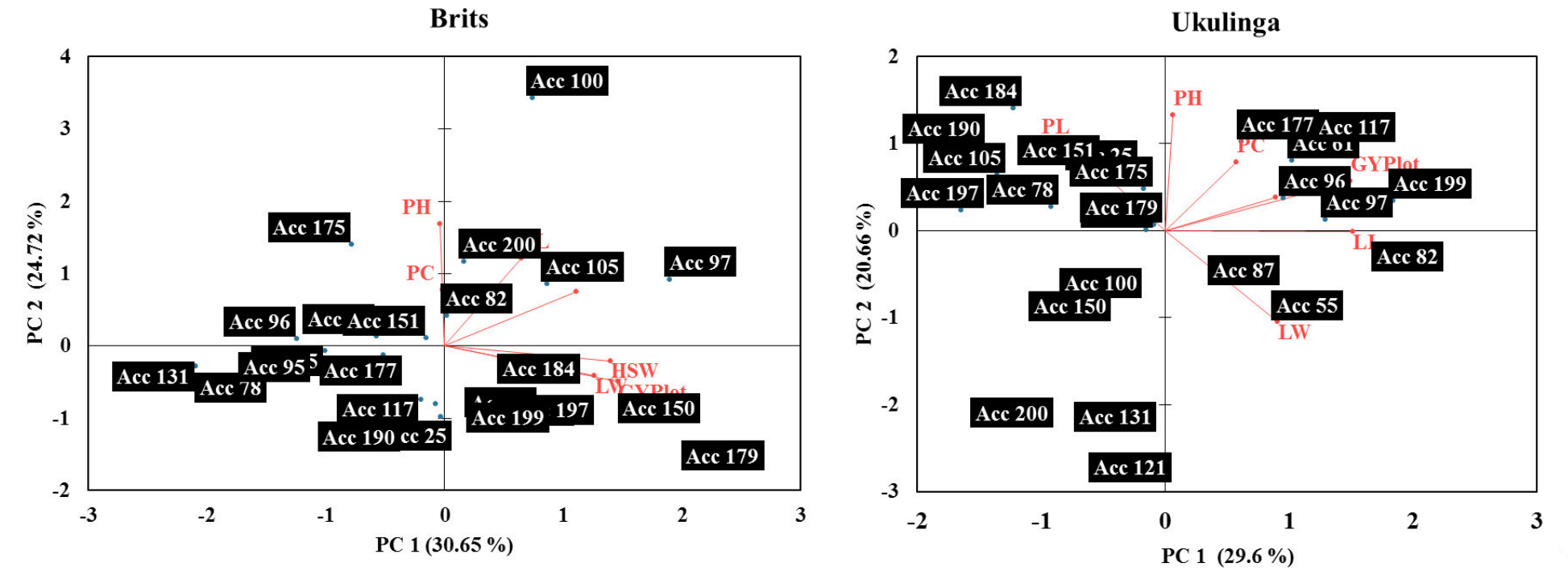

Figure 3.7: Rotated principal component scores and percentage explaining variance of PC 1 versus PC 2 and showing similarities among 24 Bambara groundnut genotypes. Descriptions of the sources of the landraces used are indicated in Table 3.1

3.4.5 Correlations among traits

Pearson correlation coefficients (r) between the traits assessed are shown in Figure 3.8. Following the Brits, only four phenotypic traits (HSW, GYPH, LL and PH) had high correlation values among themselves; hundred seed weight (HSW) had a highly significant (r=0.58) correlation with grain yield per plot (GYPlot). Plant height (PH) showed highly significant (r=0.51) correlation with leaf length (LL). At the Ukulinga, there is only one pair of highly correlated traits, grain yield had a highly significant (r=0.65) correlation with leaf length (LL). The following phenotypic traits are highly correlated with the Brits: grain yield and plant height as well as grain yield and leaf width. At the Ukulinga, a strong negative correlation was found between grain yield, petiole length and leaf width. There was a weak negative correlation between grain yield, plant canopy, hundred grain weight, plant height and leaf width at Brits and hundred seed weight, leaf length, plant height, and grain yield at Ukulinga.

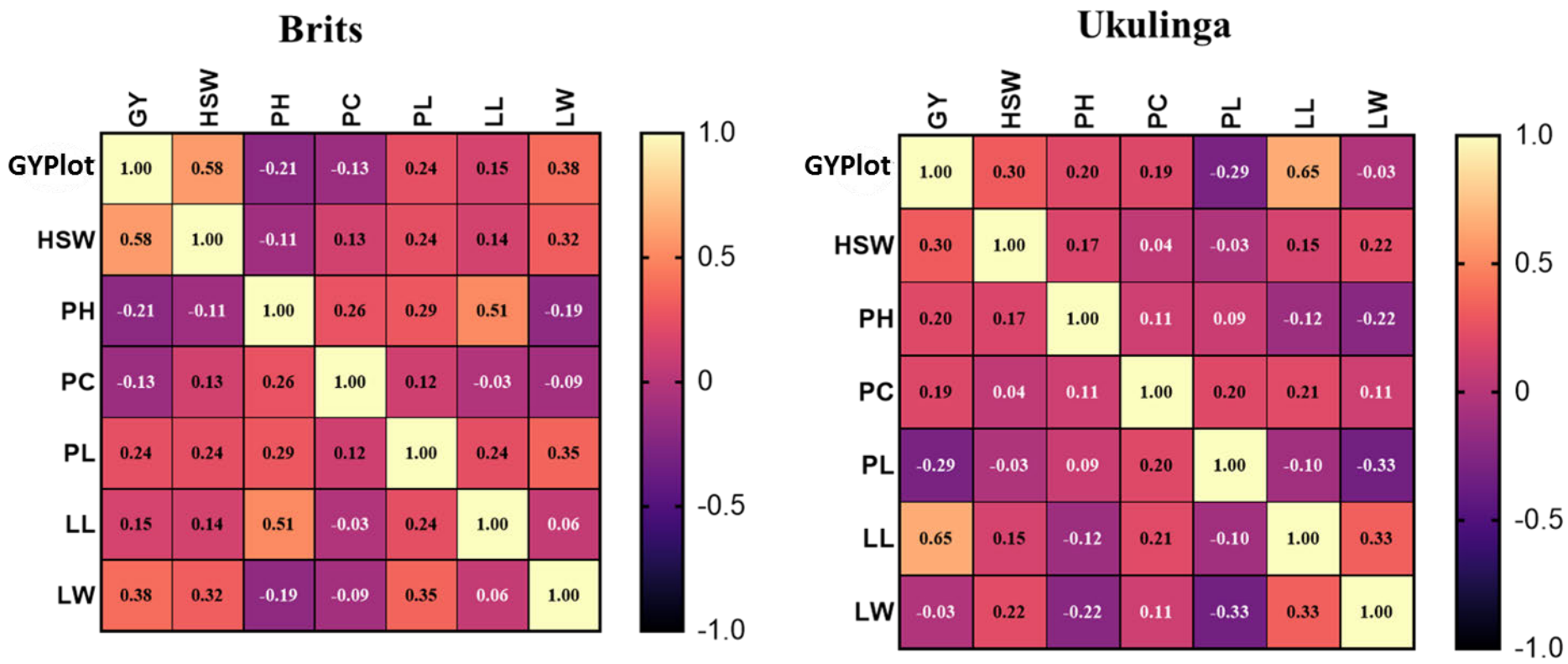

Figure 3.8: Pearson correlation coefficient among 7 phenotypic traits in Bambara groundnut accessions

3.5 Discussions

3.5.1 Morphological variability

The objective of this study was to evaluate and characterize agronomic traits and to identify high yielding and stable genotypes under two different production environments and to determine the relationships among the traits (plant height, grain yield, hundred grain weight, plant canopy, leaf width, leaf length and petiole length). In terms of plant growth, two traits (plant height and plant canopy) showed highly significant ($p \leq 0.01$) differences (Figure 3.4 and 3.5). This shows that the two production environments had different climatic conditions (soil texture, rainfall, temperature, humidity). Bambara groundnut grows best in climates with adequate sunshine, high temperature and adequate rainfall (Mabhaudhi and Modi, 2013). The genotypes evaluated showed high yields in Ukulinga compared to Brits, implying that the environmental conditions were more favorable for growing Bambara groundnut in Ukulinga compared to Brits. However, Acc 179, Acc 150 and Acc 25 from Brits showed high yields despite rainfall at this location. These genotypes can be used for high production of Bambara groundnut in breeding programs. Ukulinga environment was more suitable for Bambara growth and development in 2018/2019 cropping season. However, Unigwe et al (2016) reported that Bambara groundnut is very adaptable compared to other crops and grows well under harsh conditions (high wind, heavy rain, and cold temperature). At Brits, a highly significant ($p \leq 0.01$) difference was observed between grain yield and other yield related agronomic traits such as hundred seed weight, indicating that these traits have high genetic variation among themselves (Figure 3.6). It is reported that yield and other yield traits are highly influential parameters for crop improvement in most of the crops (Khan et al., 2020). Grain yields per hectare varied widely and ranged from 115.34 to 1446.67 kg/ha. In this study, the yield at Ukulinga (1069.01 kg/ha) was significantly higher than that reported by Unigwe et al. (2016) and Shegro et al. (2013). Their yields ranged from 9.90 to 126.03 kg/ha and 13.33 to 191.73 kg/ha respectively. This implies that the Bambara groundnut genotypes used in this study were able to adapt to the production environments in Brits and Ukulinga.

The environment had a significant effect ($p < 0.05$) on plant height which ranged from 21.29 to 30.74 cm for Brits and from 19.33 to 29.00 cm for Ukulinga (Figure 3.4). There are several factors that affect the growth of Bambara groundnut including temperature, altitude, rainfall, and soil type (Shegro et al., 2013). The use of pesticides to control Bambara plants is a common practice by small scale farmers from rural communities as environmental stress disturbs the plant and makes it more susceptible to disease and/or insect attack (Mohale et al. 2014).

Environmental factors affecting plant growth include light, temperature, water, moisture, rainfall, soil texture and nutrition (Mabhaudhi and Modi, 2013). On the other hand, production environment had significant effect ($p<0.05$) on grain yield at Brits (Fig. 3.6b). Yields of Bambara groundnut vary significantly among sites, seasons, and genotypes, with yields averaging 650-850 kg/ha as reported by Akpalu et al. (2012). Researchers have reported wide variation among landraces in grain yield and other traits (Alake et al., 2015). Berchie et al. (2010) also reported pod and seed yields of 4173.05 and 3084.43 kg/ha, respectively in Ghana. This indicates that Bambara groundnut grown in different environments can give high yields. Field observations indicate that Bambara groundnut cultivation by subsistence farmers is characterized by low and unpredictable yields and crop failure is common (Akpalu et al., 2012). In Brits, a significant difference in hundred seed weight was observed between genotypes and environment (Figure 3.6a). In this study, a range of 21.70 to 83.74 g was observed. These results demonstrate the importance of seed quality as it determines seed yield as reported by Akpalu et al. (2012). Seed size may be an indicator of seed vigor which is an aspect of seed quality (Mandizvo and Odindo, 2019). Hundred seed weight has been cited as an important yield enhancing trait in the evaluation of morphological traits (Unigwe et al. 2016). It is a valuable measure of yield and a suitable indicator to observe the tradition of quantitative traits influenced by genotype and environment (Rogé et al., 2016). Interestingly, the phenotypic variation among genotypes in the study was significant, suggesting that accessions had high genetic diversity for the traits of interest that could be used in breeding programs. Unigwe et al (2016) reported the same results when they studied the different morphologies of Bambara populations in South Africa.

For traits such as plant height, petiole length and hundred-seed weight, these phenotypic coefficients of variation were almost similar, suggesting that these traits vary due to genetic factors rather than environmental factors. Plant height was highly significant ($p\leq 0.05$) at Brits (Figure 3.4) and leaf petiole length was highly significant ($p\leq 0.05$) at Ukulinga among the accessions studied (Figure 3.5). Similar results were reported by Unigwe et al (2016). Agronomic, physiological, biochemical, and molecular characterization (Figure 2.3) of Bambara groundnut accessions should be carried out to determine the true extent of genetic diversity since cropping conditions may affect DNA sequence (Unigwe et al., 2016). Plant height and plant cover were also highly significant ($p\leq0.05$) among the studied accessions. Mabhaudhi et al. (2018) reported similar results and pointed out the lack of water use by plants resulting from reduced soil water accessibility in response to decreasing height and leaf length.

As a result of reduced leaf canopy size, water is used more efficiently through evaporation, although this is often at the expense of yield potential (Chimonyo et al., 2016).

3.5.2 Principal component analysis

Shegro et al (2013) demonstrated the usefulness of principal component analysis (PCA) for predicting trait relationships in Bambara groundnut accessions. Based on the seven (7) traits measured, the patterns of association and comparison among the 24 Bambara groundnut genotypes are examined in this study. In Figure 3.7, the biplots PC 1 and PC 2 show that the genotypes are distributed in four quadrants. Two different production environments show major differences in Bambara groundnut. The genotypes showed paring orientation, i.e., they shared most of the measured traits, indicating an association of Bambara groundnut landraces across environmental conditions. Comparison of PC analysis and PCA biplot showed that the landraces shared certain growth and yield traits. Similar observations were also made by Shegro et al (2013). To strengthen such relationships, they suggested the addition of molecular markers. The genotypes that were far apart on the axes were distantly related to genotypes within the same quadrant. According to the biplot, Acc 87, Acc 184, Acc 197, and Acc 199 were strongly associated with Brits. Acc 95 and Acc 179 showed strong association at Ukulinga. Ntundu et al (2006) also reported strong association between landraces. A plant with this strong relationship grows and produces high yield (Chijioke et al., 2010).

3.5.3 Correlation among the traits

According to Silva et al (2016), the specific coefficient is a correlation estimate for the purpose of selection for direct and indirect breeding, as it indicates how closely two or more traits are genetically and non-genetically related. They also mentioned the importance of correlations and explained how these relationships are used to determine the probability of indirect selection for yield improvement in correlated traits. In both production environments, genotypic correlation coefficients were generally greater than phenotypic correlation coefficients (Figure 3.8). These results suggest that genetic factors dominate the putative relationship between traits with lower environmental influence (Alake et al., 2015). The following traits were negatively correlated at the Brits based on phenotypic traits. Plant height, leaf width, and grain yield per plot. Grain yield, petiole length and leaf width were negatively correlated in Ukulinga. Oyiga and Uguru in 2011 suggested that these traits should be allowed in the early cropping stage of Bambara groundnut production. In Brits, grain yield, plant canopy, hundred seed weight and plant height were negatively correlated with grain yield and at Ukulinga,

hundred seed weight, petiole length, plant height and leaf length were negatively correlated with grain yield. Changing rainfall conditions in the production environment may contribute to weak correlations. To breed Bambara groundnut for future production, it may be useful to select for these traits (Alake and Ayo-Vaughan, 2017). Plants need to compete for photosynthetic resources to grow (Oyiga and Uguru 2011). The following phenotypic traits show strong positive linear correlation in both environments: Grain yield, leaf length, and plant height. Unequal accessions may differ in seed size and color as explained by Unigwe et al (2016). Generally, farmers believe that large and apartment seeds germinate faster and produce larger plants than seeds of other shapes and sizes (Laris et al., 2015). Development of vegetative growth and yield collections is important for breeding programs (Gao et al., 2020). The positive correlations among and between the various traits signify that selecting for any of these traits will have a positive influence on selecting for related traits in a Bambara groundnut improvement program (Unigwe et al., 2016).

3.6 Conclusion

This study has shown that Bambara groundnut production can vary between different production environments. The wide variation in performance of Bambara genotypes that was observed, gives an opportunity for selection of potential parental lines for breeding and is indicative of the genotypes with high genetic diversity which can be exploited for use in breeding programs. Production environments was significant on Bambara groundnut genotypes and Ukulinga was more suitable for Bambara groundnut production. The genotypes in this study demonstrated significant variation in phenotypic characteristics. The Acc 179, Acc 184, Acc 150, were associated with desirable grain yield characteristics at Brits site. These genotypes may be appropriate for development of population as well as recommended for Bambara groundnut production in the country. At Ukulinga site all genotypes had the yield of ≤600 kg/ha. The genetic potential of the genotypes in this study can assist in selecting desirable parental lines and increase the effectiveness of Bambara groundnut breeding programs. In this study, we have characterized agronomic traits and evaluated the relationships among the traits in two different production environments. Different genotypes and production environments can be used to establish breeding programmes on Bambara groundnut. It will help in creating more genetic diversity mainly for the most valuable agronomic traits in Bambara groundnut.

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CHAPTER 4: SCREENING BAMBARA GROUNDNUT (*Vigna subterranea* L.) GENOTYPES FOR DROUGHT TOLERANCE AT THE GERMINATION STAGE UNDER SIMULATED DROUGHT CONDITIONS

Abstract

Smallholder and subsistence farmers traditionally grow Bambara groundnut (*Vigna subterranean* L. Verdc) in marginal and drought-prone areas. Crop growth and yield can be severely affected by drought. The early stages of seedling germination and establishment can be affected by drought, affecting yield. Therefore, it is important to test Bambara groundnut genotypes for drought tolerant traits at germination. Bambara crop improvement programmes can identify drought tolerant traits at early growth and developmental stages of Bambara groundnut genotypes by testing them for drought tolerant traits during early germination. Using polyethylene glycol (PEG 6000) to simulate drought conditions, the germination stages of Bambara groundnut genotypes were screened for drought tolerance. The study was carried out using 24 genotypes. The experiment was conducted using a 2 x 24 factorial experimental design which included simulated drought conditions - 2 levels (no stress - no PEG (distilled water) and stress conditions (5% PEG solution) and replicated three times giving a total of 144 experimental units. Data were collected on seed germination percentage, germination velocity index, mean germination time and seven drought tolerance indices: mean productivity (MP), tolerance index (TOL), geometric mean productivity (GMP), stress susceptibility index (SSI), yield index (YI), harmonic mean yield stability index (YSI) and stress tolerance index (STI). The data were applied to the mean observation of a genotype under drought stress conditions (Ys) and the mean observation of a genotype under non-stress conditions (Yp). The results showed that all genotypes were significantly different under the two germination conditions (p<0.001). There was also a highly significant (p<0.001) difference between the stress conditions. Correlations were generated using GraphPad Prism software. The correlation coefficients showed that indices such as MP, STI and GMP were the best measures of the collection of drought tolerant genotypes under both stress conditions. Acc 25, Acc 87, Acc 97, Acc 100, Acc 117, Acc 82, Acc 184, Acc 51, Acc 131, Acc 175, Acc 177, Acc 179 and Acc 199 were identified as drought tolerant genotypes showing considerable ability to improve Bambara production in South Africa.

Keywords: Bambara performance, drought indices, drought tolerance, genotypes, germination stage, screening polyethylene glycol (PEG)

4.1 Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc) is not only an important legume but also contributes to yield enhancement initiatives that can improve food and nutrition security (Lin Tan et al., 2020). This crop is rich in protein and carbohydrates (Murevanhema and Jideani, 2013). In Africa, Bambara groundnut is one of the most important legumes (Mayes et al., 2019). However, production is affected by abiotic factors such as drought. With the critical problem of water scarcity and climate change in most countries, farmers and researchers are looking for methods to improve Bambara productivity under water stress conditions (Sinefu et al., 2011). According to Khan et al (2021), South African research programs do not prioritize this crop. Research on Bambara groundnut improvement is limited (Shegro et al., 2013; Mubaiwa et al., 2018; Gerrano et al., 2021). However, only scattered research has been conducted in the country (Shegro et al., 2013). Muhammad et al., 2020) reported that there is lack of sufficient information on drought tolerance, physiology and agronomy of Bambara groundnut in South Africa. Bambara takes time to germinate compared to legumes such as groundnut (Arachis hypogaea) and cowpea (*Vigna unguiculate*). Poor seed quality may result in low germination (Mabhaudhi and Modi, 2013). According to Kapoor et al. (2020), plants respond to drought stress through a number of biochemical and molecular processes (Figure 4.1). Good quality seeds sprout quickly from the soil and have a high germination rate. Poor quality seeds germinate slowly and do not emerge as seedlings (Matthews et al., 2006). Poor plant stand may be the result of low germination or poor seed quality (Sinefu, 2011). Seed scarring and seed coat color may affect seed quality of Bambara groundnuts (Mandizvo and Odindo, 2019). Seed coat color and texture may not only affect germination but also create barriers that inhibit oxygen diffusion and water uptake during the first stage (imbibition) of seed germination (Chimonyo and Modi, 2013). Bambara is popular in smallholder farming systems and subsistence agriculture, but poor seed quality in terms of germination and vigour can delay and affect seedling emergence and establishment, resulting in reduced plant population and unproductive seedlings (Jost et al., 2016), thus reducing yield (Ilyas and Sopian, 2011). Studies on Bambara groundnut have shown that drought tolerance traits are identifiable during germination (Chibarabada et al., 2015). The ability to identify such traits is important because drought can affect yield performance at the stage of germination and seedling establishment (Richards et al., 2010). Therefore, osmolytes such as polyethylene glycol (PEG) should be used to test Bambara groundnut genotypes for drought tolerance at germination.

Polyethylene glycol (PEG) has been reported to reduce the ability of seeds to grow in water (Draweel et al., 2021). Water potential is lowered by PEG which is an osmolyte. The water potential of the seed drops from high to low during the germination phase (Miranda et al., 2014). Addition of PEG to water increases its concentration and decreases its water potential so that water cannot be absorbed by the seeds due to decreased water potential in the surrounding solution. PEG has generally been used in plants to simulate drought stress conditions during germination (Farshadfar et al., 2012).

If drought tolerant traits are observed at germination stage, it can provide valuable information for plant breeders to identify drought tolerant traits at early stages of growth and development (Mabhaudhi and Modi, 2013) and provide useful information for drought tolerant crops for high productivity (Mabhaudhi and Modi, 2013). The objective of this study was to test Bambara groundnut genotypes for drought tolerance under simulated drought conditions using polyethylene glycol (PEG 6000). Bambara groundnut genotypes from Agricultural Research Centre (ARC) Pretoria were also tested for drought tolerance.

4.2 Materials and methods

4.2.1 Plant material

The twenty-four genotypes of Bambara groundnut shown in Figure 4.1 were obtained from the Agricultural Research Council (ARC) genebank. The genotypes were sorted and categorised into eight seed coat colours (cream, brown, cream mottled, black, dark red, brown mottled and cream) (Figure 4.2). Seeds were visually scored at the Seed Science Laboratory, School of Agricultural Earth and Environmental (SAEES), University of KwaZulu-Natal, School of Agriculture, Engineering and Science (CAES), Pietermaritzburg, South Africa.

Figure 4.1: Twenty-four Bambara groundnut genotypes received from the ARC and grouped into eight categories based on seed coat colour variation.

4.2.2 Standard germination test (SG)

The different categories based on seed coat colour were subjected to the standard germination test (SG). A batch of seeds was subjected to germination under non-stress conditions (distilled water only without PEG solution, 0%) and simulated drought stress conditions (with 5% PEG solution). The determination of PEG concentration for the simulated drought stress conditions was justified based on the report of Rahmah et al (2020).

4.2.3 Procedures

Bambara groundnut seeds were placed on brown germination filter paper measuring 55.8 cm long × 30.3 cm wide (Figure 4.2). Ten seeds per genotype were placed on germination paper which was moistened with distilled water for control treatment and 5% PEG solution for treatment under simulated drought conditions. Seeds were cleaned three times with distilled water (according to Rahmah et al. (2020)). From day one after the experiment, new germinated seeds protruding at least 2 mm from the radicle were counted daily for up to 10 days. Observation under non-stressed and under drought stressed conditions (Ys and Yp) was determined by the average observation of a genotype under drought stress conditions (Yp) and the average observation of a genotype under non-stressed conditions (Ys) by weighing the fresh mass of germinated seedlings for each genotype. Fresh mass for both stress and non-stress conditions was measured on day 10 using micro cw30 digital bench scale. The percentage of germinated seeds was calculated by counting the number of germinated seeds and dividing by

10 (the total number of seeds in each filter paper), all multiplied by 100. The germination velocity, defined by the germination velocity index (GVI), was calculated according to the formula of Zondi (2012); GVI = G1/N1 + G2/N2 +… + Gn/Nn

where:

GVI = germination velocity index,

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

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

The mean germination time was also calculated as described by Koné *et al*., 2015

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

where:

MGT= mean germination time,

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

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

Seven selection indices; mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), tolerance index (TOL), stress susceptibility index (SSI), yield index (YI) and yield stability index (YSI) were estimated for each genotype based on yield under non-stress (Ys) and drought-stress (Yp) conditions. Quantitative drought resistance indices were calculated using the following formulas:

1) Stress susceptibility index = SSI= $\frac{[1-Ys/Yp]}{[1-(\bar{Y}s)/(\bar{Y}p)]}$ Ayed et al. (2021)
2) Tolerance = TOL = YP – YS Belay et al. (2021)
3) Mean productivity= MP= $\frac{Ys+Yp}{2}$ Pour-Aboughadareh *et al*. (2019)
4) Stress tolerance index= STI= $\frac{Ys \times Yp}{\bar{Y}p^2}$ Ekbic et al. (2017)
5) Geometric mean productivity=GMP= $\sqrt{[(Yp)(Ys)]}$ Grzesiak et al. (2019)
6) Yield index = YI= $\frac{Ys}{\bar{Y}s}$ El-Hashash et al. (2018)
7) Yield stability index= YSI= $\frac{Ys}{Yp}$ Sánchez-Virosta *et al*. (2021)

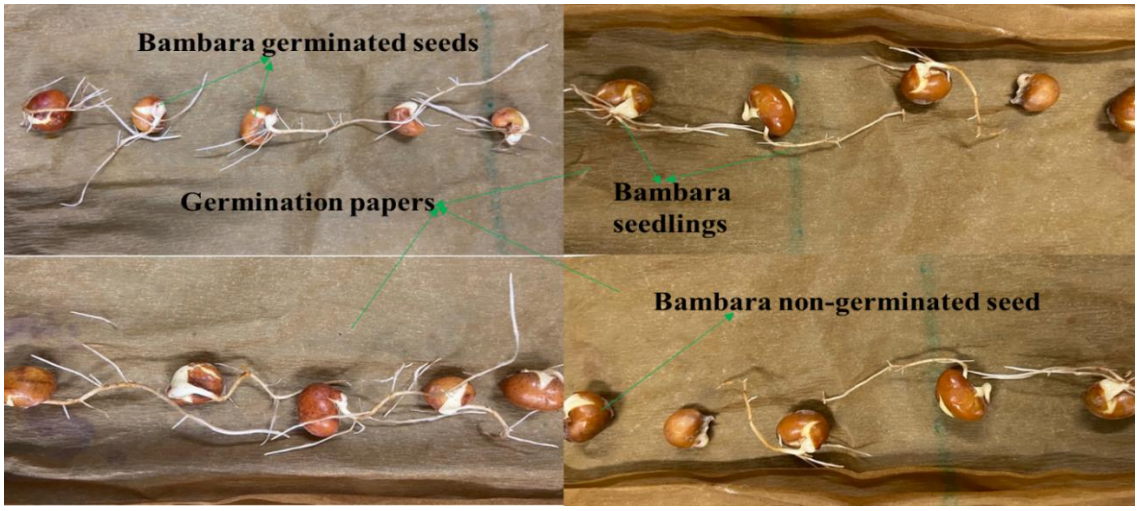

Figure 4.2: Bambara groundnut seeds germinated in brown filter papers.

4.2.4 Data analysis

Data were analysed using analysis of variance (ANOVA) by GenStat® (VSN International, UK). Means were separated using the Tukey test in GenStat® at 5% significance level. Cluster analysis and principal component analysis were performed using XLstat software. Correlations were performed using GraphPad Prism (version 9) computer software. Principal component analysis and correlations were carried out in this study to identify relationships and associations among 24 Bambara groundnut genotypes and among the 7 drought indices used. Cluster analysis was carried out to identify more and less tolerant genotypes.

4.3 Results

4.3.1 Seed germination under simulated drought conditions compared to the control.

The results obtained showed highly significant differences (p<0.001) between simulated drought conditions and the control treatment among genotypes with respect to germination percentage (Figure 4.3). There were highly significant differences (p<0.001) with respect to number of seeds germinated (NSG) between the two treatments over the 10 days (Figure 4.4). There was also highly significant difference (p<0.001) with respect to seedlings fresh mass (SFM) among two stress conditions (Figure 4.5). Seeds germinated under the 5% PEG concentration to simulate drought showed reduced germination and seedlings fresh mass of Bambara groundnut seeds. The seedlings fresh mass under non-stressed was between 0.22 and 0.40g/seedling while under drought-stressed, it was between 0.02 and 0.16g/seedling. Germination was between 40.33 and 65% for non-stressed and between 18 and 61.33% under drought-stressed. Under drought-stress Bambara seeds were slow to germinate in the first 5 days. Without PEG concentration most seeds were able to attain 100% germination by the 5th

day. The highest seedling fresh mass under drought stress was observed from Acc 184 (0.16g/seedling). Drought stress reduced germination percentage (Figure 4.4).

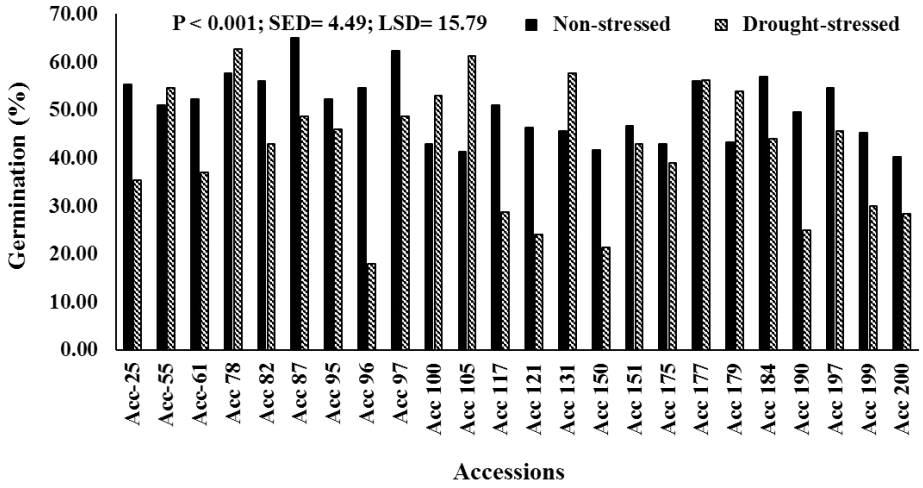

Figure 4.3: The germination percentage of seeds subjected to simulated drought conditions using 5% PEG compared to a control treatment using distilled water.

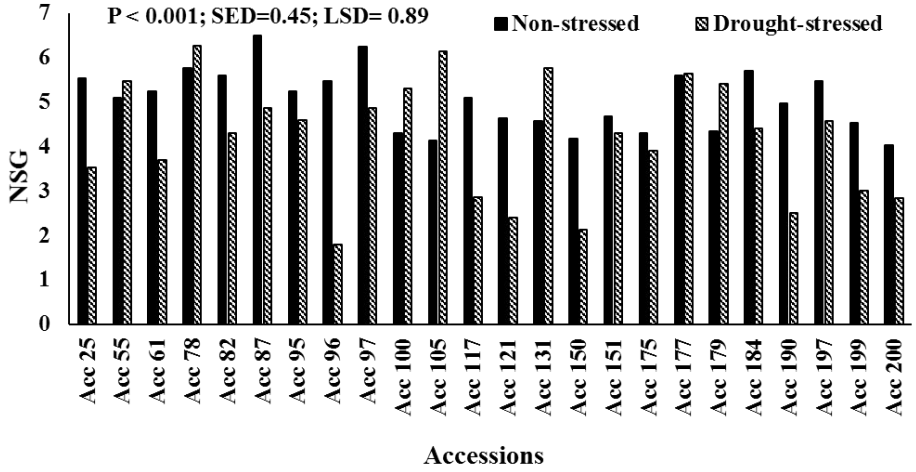

Figure 4.4: Number of seeds germinated (NSG) among twenty-four (24) genotypes subjected to simulated drought conditions using 5% PEG compared to a control treatment using distilled water

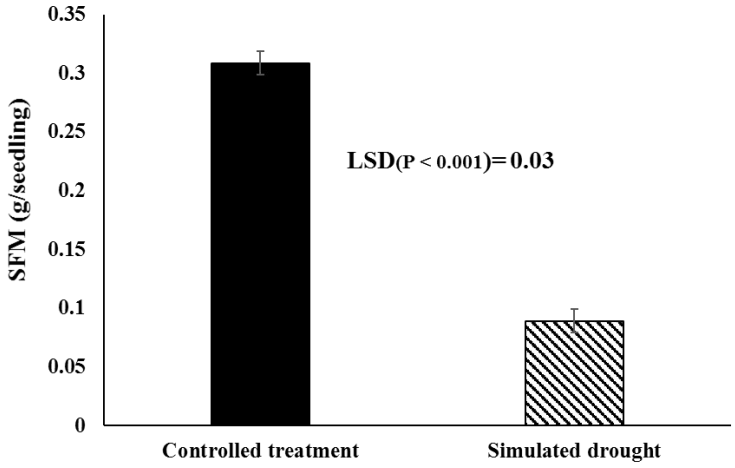

Figure 4.5: Seedling fresh mass (SFM) of seeds subjected to simulated drought conditions using 5% PEG compared to a control treatment using distilled water.

4.3.2 The effect of simulated drought conditions on seed vigour

The results obtained showed that there were highly significant differences ($p< 0.01$) among the tested genotypes with respect to GVI and MGT (Figure 4.6 and Figure 4.7). Germination velocity index (GVI) was between 0.693 and 1.327 for non-stressed, and between 0.245 and 1.019 for drought stress (Figure 4.6). Following mean germination time (MGT) there was highly ($p< 0.001$) significant different among accessions and between two stress conditions and with the interaction of genotypes and stress conditions. Drought stress reduced the germination velocity index in many genotypes except Acc 55, Acc 131, Acc 100, and Acc 179 these genotypes can be regarded as more drought tolerant. Mean germination time ranged from 0.40 to 0.65 under non-stressed, and from 0.21 to 0.63 under drought stressed (Figure 4.6).

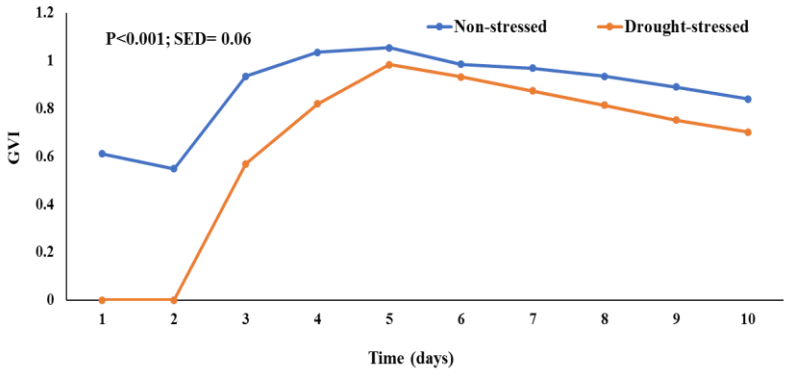

Figure 4.6: Germination velocity index (GVI) in each day subjected to simulated drought conditions using 5% PEG compared to a control treatment using distilled water.

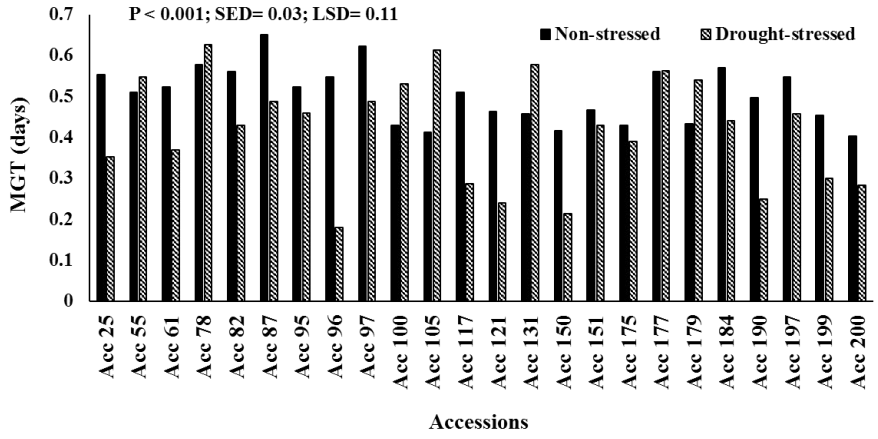

Figure 4.7: Mean germination time (MGT) among twenty-four (24) genotypes subjected to simulated drought conditions using 5% PEG compared to a control treatment using distilled water.

Table 4. 1: Table of mean squares of germination percentage (G %), germination velocity index (GVI), mean germination time (MGT), seedling fresh mass (SFM), and Number of seeds germinated (NSG) of 24 Bambara groundnut genotypes tested under nonstress and drought-stress conditions

	MS					
Source of variation	**DF**	**G (%)**	**GVI**	**MGT**	**SFM**	**NSG**
Rep	2	10662.7	10.1981	1.06627	0.118	106.627
Genotype	23	3801.9**	1.3405**	0.38019**	0.00583	38.019**
No. of days	9	110804.4**	9.8958**	11.08044**		1108.044**
Treatment	1	26608.4**	19.9866**	2.66084**	1.73356	266.084**
No. of days. Genotypes	207	209.2	0.1446	0.02092		1.368
Genotypes. Treatment	23	2832.2**	1.2271**	0.28322**	0.00838	28.322**
Genotypes. No of days. Treatment	207	1	1	1		1
Residual	958	300.8	0.2506	0.03008	0.00908	3.008
Total	1439					

G (%); germination percentage, GVI; germination velocity index, MGT; mean germination time, SFM; seedling fresh mass, NSG; number of seeds germinated, DF; degree of freedom, Rep; replication, ** Significant at the 0.01 probability level, MS; mean square.

4.3.3 Drought indices

4.3.3.1 Principal component analysis for drought indices

Principal component analysis showed that the first two PCs accounted for about 98.88% of total variation. The first PC, accounted for 64.24% of variation among all variables. The second PC accounted for 34.64% of all variation (Figure 4.8). In the first quadrant it is shown that Acc 179 and Acc 131 are highly correlated with each other on the other hand Acc 78, Acc 61, Acc 197, and Acc 95 are highly correlated with each other as well. These genotypes have high stress tolerance index (STI), high geometric mean productivity (GMP), and high yield index (YI). The first quadrant can be named as the yield potential and drought tolerance. Considering the high and positive value of this component, genotypes that have high values of these indices will be high yielding under stress and non-stress conditions. Looking at the second quadrant; Acc 87 and Acc 25 are highly correlated with each other and they are more associated with tolerance (TOL). Acc 117 and Acc 199 are more associated with stress susceptibility index (SSI). In the third quadrant four genotypes (Acc 121, Acc 190, Acc 150, and Acc 200). These genotypes are not associated with any of the drought indices. Acc 177 and Acc 175 are also not associated with any of the drought indices. On the forth quadrant three genotypes (Acc 105, Acc 97, and Acc 100) are highly correlated with each other, these genotypes have low PC 1 and PC 2. Acc 55 and Acc 184 with high PC 1 were more appropriate for stress and non-stress conditions. PC 2 can be regarded as a stress-tolerant dimension and capable of separating stress-tolerant from non-stress tolerant genotypes.

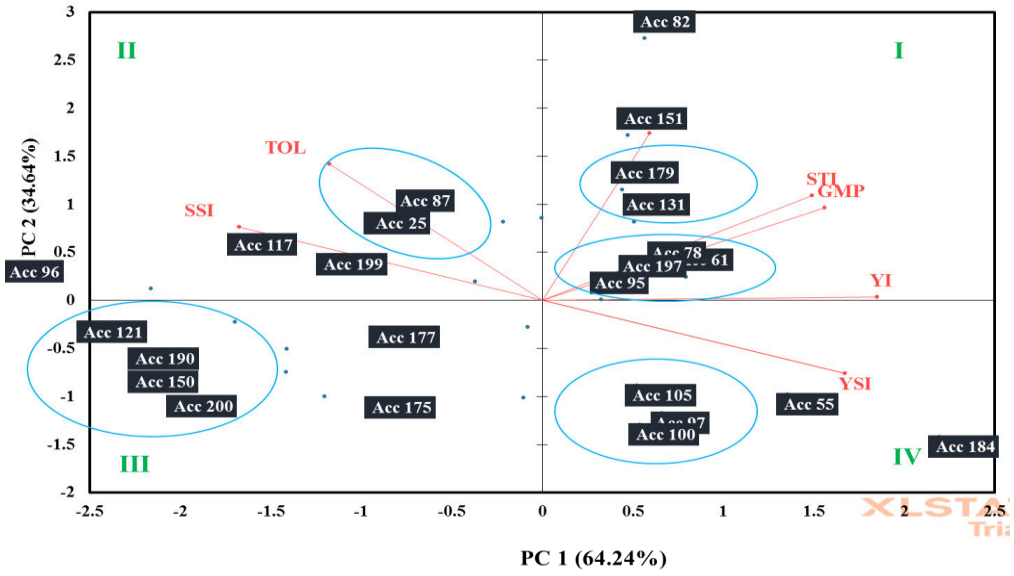

Figure 4.8: Biplot of drought tolerance indices based on the first two principal components axes (PC 1 and PC 2) for 24 Bambara genotypes in non-stress and drought stress conditions.

SSI: stress susceptibility index, TOL: stress tolerance, MP: mean productivity, STI: stress tolerance index, GMP: geometric mean productivity, YI: yield index, YSI: yield stability index.

4.3.3.2 Correlations among traits

The Pearson correlation coefficients (r) among the following evaluated drought indices; stress susceptibility index (SSI), stress tolerance (TOL), mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), yield index (YI), and yield stability index (YSI) are given in diagram below (Figure 4.9). SSI has highly significant correlation with (TOL). On the other hand, drought tolerance is negatively correlated with yield stability (r=-0.88), yield index (r=-0.62) and geometric mean productivity (r=-0.15).

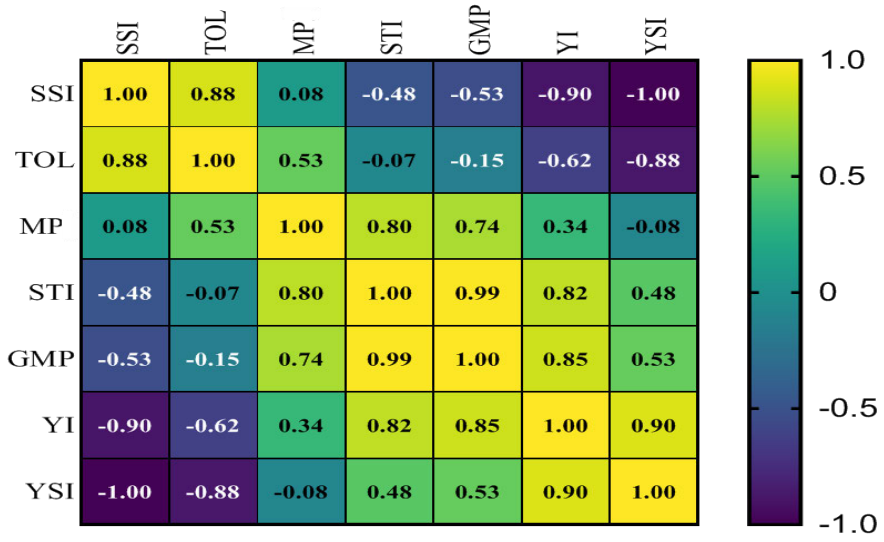

Figure 4.9: Pearson correlation among drought tolerance indices.

4.3.4 Cluster analysis

Based on the results, the test genotypes which had the highest PC 1 and PC 2, was located in the first cluster (Figure 4.10). According to cluster diagram Acc 25, Acc 87, Acc 97, Acc 100, and Acc 117 are more drought tolerant. Followed by Acc 82 and Acc 184. Acc 51, Acc 131, Acc 175, Acc 177, Acc 179, and Acc 199 are less drought tolerant. However, Acc 55, Acc 96, Acc 121, and Acc 190 are more sensitive to drought. Genotypes which had high PCA 1 and low PCA 2, were placed in the second cluster. Therefore, cluster analysis supported the results of principal component analysis.

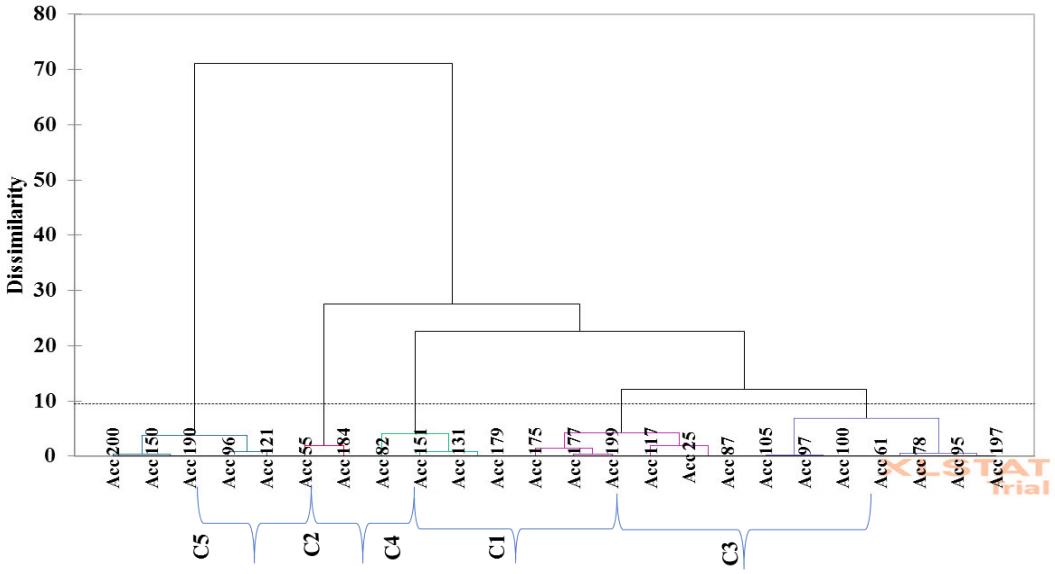

Figure 4.10: Dendrogram of cluster analysis of 24 Bambara genotypes using Ward's method based on drought tolerance indices.

4.4 Discussions

The aim of this study was to screen Bambara groundnut genotypes for drought tolerance at the germination stage under simulated drought conditions using polyethylene glycol (PEG 6000). The study also sought to identify traits that can be associated with drought-tolerance among the Bambara groundnut. The germination percentage and seed vigour of seeds subjected to simulated drought conditions was significantly lower than the control using distilled water. The highly (p<0.001) significant different between simulated drought conditions and the control treatment with respect to seedling fresh mass could possibly be attributed to reduced water potential as a result of the addition of PEG (Pei et al., 2010) which affected dry matter accumulation under simulated drought conditions. The two treatments (simulated drought and control with distilled water) were also highly significant different (p<0.001) with respect to germination percentage over the 10 days duration (Figure 4.3). Osmotic compounds like polyethylene glycol (PEG) have been used to simulate drought stress and decrease germination percentage through changes in the biochemical and physiological processes during the first and second phases of the germination process (Naderi et al., 2020). Acc 78 and Acc 131 had the highest germination (62.67%, 57.67%, respectively) under drought stress. Zondi (2012) reported that high germination does not necessarily result in rapid and uniform germination or vigorous stand under existent germination conditions. Germination proceeded slowly during the first three days for seeds subjected to the simulated drought conditions. These results are similar to those by Rahmah et al. (2020) who reported that the differences could be due to the fact that seeds under non-stressed conditions (control) were more vigorous than seeds under simulated drought stress. The reduced water potential in the PEG solution hinders seed imbibition, reduces cell division activities and water uptake hence germination also decrease (Mayes et al., 2019). The ability of seeds to germinate under drought stress (lower water potential) differs among genotypes (Shahriari et al., 2014).

The highly significant differences (p<0.01) between tested genotypes with respect to germination velocity index and mean germination time, suggests that there is high genetic variability among the genotypes (Rahmah, 2020). Slow and prolonged germination in seed has often been related with low final germination which can lead to poor seedling establishment, lower plant populations and consequently reduced yield (Tian et al., 2014). Drought stress did not lower mean germination time for Acc 78, Acc 105, Acc 131, Acc 55, Acc 179, and Acc 100. Chibarabada et al. (2014) reported that seed colour is more associated with seed quality.

They confirm that seed viability may not necessarily imply good seed vigour. Too much water can also affect speed of germination in seeds (Maraghni et al., 2010). A germinating seed is in the heterotrophic state and needs oxygen for metabolism until the transition to the autotrophic phase where process of photosynthesis takes over (Allahverdiyeva et al., 2015).

Based on drought indices under non-stress and simulated drought conditions, the most tolerant genotypes were Acc 25, Acc 87, Acc 97, Acc 100, and Acc 117. These genotypes were able to imbibe water under lower water potentials and this is a good indicator for drought tolerance. The most sensitive genotypes based on these indices were Acc 55, Acc 96, Acc 121, and Acc 190. The tolerance of different genotypes is because of their genetic and physiological ability to influence water absorbed in the plant system throughout stress conditions (Khakwani et al., 2011). Generally, the best indices to select Bambara groundnut genotypes are MP, STI and GMP (Abejide et al., 2017). These results agree with those of Zare (2012) on Iranian barley and Rahmah (2020) on Bambara groundnut that MP, STI, and GMP were the most appropriate indices to screen genotypes for adaptation to drought conditions.

Based on the results of principal component analysis, biplot (Figure 4.8) and cluster analysis diagram (Figure 4.10), Acc 25, Acc 87, Acc 97, Acc 100, and Acc 117 were identified as the most tolerant genotype and showed considerable genetic potential to improve in South Africa. Acc 87 with high PC 2 is more suitable for stress than for non-stress condition. Acc 87 and Acc 117 cultivars were identified as high drought tolerant. While Acc 96, Acc 121, and Acc 190 were identified as high drought susceptible and low yield stability. Cluster analysis has been widely used to report genetic diversity and grouping based on comparable features (Pervaiz et al., 2010). Zare (2012) reported that selection of genotypes that have high PC 1 and PC 2 are suitable for both stress and non-stress conditions. Therefore, Acc 82, Acc 151, and Acc 131 with higher PC 1 and PC 2 are excellent genotypes under both stress conditions. Acc 87 with high PC 2 was appropriate for stress than for non-stress condition (Figure 4.8). Acc 105, Acc 97, and Acc 100 had the lowest PC 1 and PC 2. A similar result was reported by Ahmadizadeh et al. (2012). When he was investigating behaviour of durum wheat genotypes under normal irrigation and drought stress conditions in the greenhouse in 2012. PCA has been used to confirm main parameters and to estimate the drought tolerance levels of various genotypes at the germination stage (Rahmah et al., 2020). Seven drought indices mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), tolerance index (TOL),

stress susceptibility index (SSI), yield index (YI) based on PCA were used to estimate the drought tolerance levels of Bambara genotypes, and 13 genotypes (Acc 25, Acc 87, Acc 97, Acc 100, Acc 117, Acc 82, Acc 184, Acc 51, Acc 131, Acc 175, Acc 177, Acc 179, and Acc 199) out of 24 genotypes were considered as tolerant to drought. These genotypes had also high seed vigour, high germination, and high seedling fresh mass which is an indication of drought tolerance. Liu et al. (2017) specified that the three best indicators for classifying the drought tolerant lettuce genotypes under PEG 6000 treatment are final germination percentage, relative germination rate, and relative sprout potential. These indicators can be used to classify the genotypes as drought tolerant, moderately tolerant, and susceptible to drought (Ibny et al., 2019). Stress susceptibility index (SSI) has highly significant correlation with tolerance (TOL). This means that an increased in susceptibility index caused an increase of tolerance. Stress tolerance index (STI) and geometric mean productivity (GMP) had highly significant correlation with mean productivity (MP). On the other hand, yield index (YI) and mean productivity (MP) have positively highly significant correlation with stress tolerance index (STI).

The correlations valuation indicated a relationship of drought indices with the characters of seedlings mass. Correlated parameters can be used in the main component analysis (Rahmah et al., 2020; Shen et al., 2020). The parameters that are significantly correlated with each other can be used in the assessment and determination of the drought tolerance levels of the different genotypes (Nouri et al., 2011). The correlation is an important tool for the researchers to make the traits to be combined into the genotype selection program (Mohammadi and Amri, 2011). Hao et al., 2014 emphasised that screening for drought tolerance must involves observing parameters and indices that assess alterations, these parameters can be easily identified using correlations.

4.5 Conclusion

In Bambara groundnut, germination may be affected by drought stress when the seeds are at the germination stage. Although the concentration of PEG reduced seedling growth, it did not affect seedling vigour too much. Bambara groundnut genotypes can be evaluated during germination using seedling papers containing 5% PEG 6000 solution. For the production of Bambara, it is very important to analyse the seeds at the germination stage. Principal component analysis, biplot analysis and cluster analysis identified the following genotypes as drought tolerant: Acc 25, Acc 87, Acc 97, Acc 100, Acc 117, Acc 82, Acc 184, Acc 51, Acc 131, Acc 175, Acc 177, Acc 179 and Acc 199. Bambara groundnut is an underutilised indigenous crop in South Africa and this study confirmed that drought stress can affect seedling growth. Thus, this study was able to identify landraces capable of developing and improving the crop. Drought tolerant genotypes can be identified at germination stage based on seed vigour, seedling fresh mass, germination and drought indices. In addition, studies should be conducted to identify important agronomic traits such as yield and drought indices under stress conditions or in controlled environments. The next experiment (chapter 5) will test genotypes under real water stress conditions in a tunnel.

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CHAPTER 5: ASSESSING THE RESPONSE OF BAMBARA GROUNDNUT (*Vigna subterranea L*) ACCESSIONS TO WATER STRESS CONDITIONS

Abstract

Bambara groundnut (*Vigna subterranea* L. Verdc) is adapted to marginal lands as it is considered a drought tolerant legume. In this study, drought tolerance of 24 genotypes of Bambara groundnut was investigated by evaluating their morphophysiological traits during plant growth and development. The objective of this study was to identify high yielding genotypes with traits that could be useful for breeding in South Africa. The trial was conducted in a tunnel at the University of KwaZulu-Natal Agriculture Campus in Pietermaritzburg. The trial was laid out in a Randomised Complete Block Design (RCBD) and replicated in triplicate. Data were collected on the following variables: Grain yield per plot, leaf length, leaf width, petiole length, plant height, total biomass, number of seeds per plant, seed emergence, leaf gas exchange and chlorophyll fluorescence parameters. Most of the genotypes showed high emergence rate (> 80%). Water stress had a significant effect (p<0.001) on PL, TB, GY, gs, Ca/Ci, IWUE, F0', Fm', ETR, ETR/A, Fv'/Fm' and ΦPSII. Using principal component analysis, 33.40% of the variance observed among genotypes in response to water stress was found to be explained by PC 1 and attributed to variations in leaf gas exchange and chlorophyll fluorescence measurements. This PC was attributed to growth and yield parameters and explained 63.76% of the observed variation. Using the main plots and cluster analysis, drought tolerant genotypes such as Acc 177, Acc 199, Acc 197, Acc 151, Acc 75, Acc 184, Acc 64, Acc 200, Acc 97, Acc 175, Acc 25, Acc 100, Acc 121, Acc 87, Acc 61, Acc 105, Acc 121, Acc 82 and Acc 131 were identified. The physiological and penal traits identified in this study may also help in crop improvement programmes and selection of drought tolerant genotypes of Bambara groundnut.

Keywords: Water stress, Bambara groundnut, genotypes, drought tolerance, growth and yield parameters, leaf gas exchange parameters, chlorophyll fluorescence parameters.

5.1 Introduction

Bambara groundnut (*Vigna subterranea* (L) Verdc.) is an African legume crop which is drought tolerant (Chai et al., 2016). The crop is able to endure long periods of dryness without failing (Filli et al., 2013). With its drought tolerance, Bambara groundnut has the ability to be developed in the dry areas of Africa in order to improve food security (Berchie et al., 2012). It has been cultivated using unimproved landraces (Mabhaudhi and Modi, 2013). Landraces of Bambara groundnut are the key source of planting materials for small holder farmers (Wamba et al., 2012). There are many landraces that are planted in South Africa hence, it is very essential to evaluate the variation in this crop for use in the breeding programs (Unigwe et al., 2016). Bambara is one of the most underutilised and neglected crop species which lack recognition in breeding programmes (Muhammad et al., 2020). Because of its reported drought tolerance and high-water use efficiency there are now renewed efforts to study Bambara groundnut with a view to promoting it to marginal production areas (Chibarabada et al., 2014). Bambara groundnut has been referred to as a drought tolerant plant because of higher yield they produce compared to other legumes under the same condition (Mayes et al., 2019). Muhammad et al. 2016 observed a reduction in yield among Bambara groundnut landraces after drought imposition, even though the results pointed at the resilience potential of the species to drought. Mabhaudhi and Modi (2013) also reported that water stress can have negative impact on yield responses and emergence of Bambara groundnut.

Studying the effects of water stress on growth and yield performance of Bambara groundnut is very important to investigate the possible production and yield of Bambara groundnut landraces in various regions and to improve food security for small holder farmers (Abu and Buah, 2011). Water stress is a limiting factor in most legume crops, uncoupling the effects of drought in the field is difficult (Al Shareef et al., 2014). Therefore, experiments in controlled environments that complement drought studies can be used to identify the effects of water stress on growth and development of Bambara groundnut.

In this study, it was hypothesised that water stress may have influence on growth and yield performance of Bambara groundnut. It was further hypothesised that drought tolerance may be linked to seed different genotypes. Mechanisms associated with drought tolerance in Bambara groundnut would be determined in the context of different seeds genotypes (Mabhaudhi and Modi, 2013). Hence, the specific objective of this study was to determine the effects of water stress on growth and yield performance of twenty-four Bambara genotypes.

5.2 Materials and methods

5.2.1 Plant Material

Twenty-four genotypes of Bambara groundnut were obtained from the Agricultural Research Council genebank (ARC). The 24 genotypes comprised eight seed coat colour variants: cream, brown, black, dark red, and speckled brown (Table 5.1).

Table 5.1: The list of Bambara groundnut accessions used in this study with their seed colours

No	Accessions names	Seed colour
1	Acc 25	Brown
2	Acc 55	Black
3	Acc 61	Cream
4	Acc 78	Dark red
5	Acc 82	Dark red
6	Acc 87	Brown
7	Acc 95	Speckled brown
8	Acc 96	Brown
9	Acc 97	Dark red
10	Acc 100	spotted cream
11	Acc 105	Brown
12	Acc 117	Speckled brown
13	Acc 121	Black
14	Acc 131	Brown
15	Acc 150	Speckled brown
16	Acc 151	Brown
17	Acc 175	Speckled brown
18	Acc 177	Brown
19	Acc 179	Black
20	Acc 184	Dark red
21	Acc 190	Dark red
22	Acc 197	Black
23	Acc 199	Dark red
24	Acc 200	Dark red

5.2.2 Tunnel environment

The study was conducted between October 2020 and February 2021 in the tunnel at the University of KwaZulu Natal, Pietermaritzburg campus. Pot experiments were conducted under tunnel conditions 27/15°C day and 65% relative humidity and natural day length) at the Controlled Environment Research Unit (CERU), University of KwaZulu-Natal, Pietermaritzburg, South Africa.

5.2.3 Experimental design and trial management

There were 144 experimental units (ten-litre pots) set up in a randomised complete block design with a 2 x 24 factorial treatment design and 3 replications, resulting in a total of 144 experimental units (ten-litre pots). The treatments were as follows: Water stress – 2 levels (Well-watered and stressed); Genotypes – 24 levels (accessions from the ARC).

The non-stressed (well-watered) plants were well watered and maintained at field capacity throughout all the stages of growth (vegetative stage, flowering stage, and pod filling stage).

For the stressed plants, watering was withheld and done at two-week intervals (14 days). Three rows of ten litre pots were planted for each treatment. The pots spacing was 0.4m between pots and 1 m between rows pot. Three seeds were hand sown per pot; the plants were then thinned to one after seedlings emergence. Large seeds were selected for sowing because they germinate faster, and develop into more vigorous seedlings (Shankar et al., 2006).

5.2.4 Seed characterisation

The seeds were sorted into twenty-four genotypes (Figure 5.1). The seeds were not treated, the criterion used to select them was according to seed colour based on previous studies that suggested seed coat colour may influence the growth and performance of Bambara groundnut (Mabhaudhi and Modi, 2013).

Figure 5.1: Twenty-four Bambara groundnut genotypes used in this study

5.2.5 Data collection

Two weeks after planting, data collection began. The following data were collected: Seedling emergence up to the 15th day after planting, leaf length, leaf width, plant height, petiole length, number of petioles per plant, total biomass, seed weight and number of seeds per plant. From the emergence of 90% of the seeds, the rate of emergence was monitored daily. The quantitative morphological parameters are listed in Table 5.2 along with their code, description and mode of measurement. Plant height was measured from the soil surface to the base of the tallest leaves. Leaves with at least 50% green area were counted. The seeds, leaves and roots of each plant were harvested in succession and dried at -10°C for three days at the University of KwaZulu Natal Seed Laboratory for Horticulture and Plant Sciences. Once the leaves and roots of the plants were dried in the laboratory, the total seed biomass, seed weight and number of seeds per plant were measured (Figure 5.2B). During harvest, the number of seeds produced per plant (NS) was determined by removing the pods, drying them in the sun, and counting the number of seeds determined by their size and firmness. Grain yields under non-stress and drought stress (Ys and Yp) were used to calculate drought indices for cluster analysis (Table 5.3).

Table 5.2: List of quantitative morphological characters recorded from 24 Bambara groundnut accessions

Quantitative characters	Code	Description	Measurement type
Leaf length (cm)	LL	Length of the leaf from the base to the tip	Measuring tape
Leaf width (cm)	LW	Width of the leaf from the widest part of the leaf	Measuring tape
Seeds weight (g)	SW	Weight of the seeds per plant	Weighing balance
Petiole length (cm)	PL	Length of panicle from its base to the tip	Measuring tape
Plant Height (cm)	PH	Height of main stalk from the ground to the tip of the main panicle	Measuring tape
Total biomass (G)	TB	Weight of the whole plant	Weighing balance
Number of seeds per plant	NSP	Number of seeds for each plant	Counting
Seed emergence	SE	Number of seeds emerged for each day up to 15 days	Counting

Table 5.3: Seven drought tolerance indices used to evaluate Bambara groundnut to drought conditions for cluster analysis

Drought tolerance indices	Equation	Reference
Stress Susceptibility Index (SSI)	$\mathbf{SSI} = \frac{\left[1 - \frac{Ys}{\bar{Y}p}\right]}{\left[1 - \frac{\bar{Y}s}{\bar{Y}p}\right]}$	(Ayed *et al*., 2021) [Equation 4]
Tolerance (TOL)	$\mathbf{TOL} = Y_p - Y_s$	(Belay *et al*., 2021) [Equation 5]
Mean Productivity Index (MPI)	$\mathbf{MPI} = \frac{Y_p + Y_s}{2}$	(Pour-Aboughadareh *et al*., 2019*)* [Equation 6]
Stress Tolerance Index (STI)	$\mathbf{STI} = \frac{Y_p \times Y_s}{(\bar{Y}_p)^2}$	(Ekbic *et al*., 2017) [Equation 7]
Geometric Mean Productivity (GMP)	$\mathbf{GMP} = \sqrt{(Y_p)(Y_s)}$	(Grzesiak *et al*., 2018) [Equation 8]
Yield Index (YI)	$\mathbf{YI} = \frac{Ys}{\bar{Y}s}$	(El-Hashash *et al*., 2018) [Equation 9]
Yield Stability Index	$\mathbf{YSI} = \frac{Y_s}{Y_p}$	(Sánchez-Virosta *et al*., 2021) [Equation 10]

Ys; yield of each genotype under non-stress, Yp; yield of each genotype under stress, $\bar{Y}s$; mean yield of all genotypes under non-stress conditions, $\bar{Y}p$; mean yield of all genotypes under drought stressed conditions

5.2.6 Emergence data collection

From the seventh day after sowing, the seedlings were observed daily for emergence. The cotyledons were considered fully developed when the seedling emerged. Measurements were taken for 15 days. Thereafter, irrigation was discontinued for 14 days to allow water stress treatment. The percentage of seedlings emerged was calculated by dividing the number of seeds emerged by the number of seeds planted and then multiplying all numbers by 100. Using the formula of Bewley and Black (1994), the mean emergence time was calculated as follows:

$$MET = \frac{f(x)}{\Sigma f}$$

where: *MET* = mean emergence time, *f* = number of newly germinate seeds at a given time (day), and *x* = number of days from date of sowing

Gas exchange and chlorophyll fluorescence parameters

The LI -6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) was used in the tunnel to measure leaf gas exchange and chlorophyll fluorescence simultaneously. The system is attached to a leaf chamber with a Licor Bioscience, Inc. LCF (6400-40B, 2 cm leaf area). Leaf temperature was maintained at 25^0C, while artificially saturated photosynthetic active radiation (PAR) was fixed at 400 mmol mol1 and 1000 mmol m^2 s^1, respectively, and leaf external CO_2 concentration (Ca) was fixed. A water volume of 500 mm and a relative humidity of 43% were maintained. To prevent stomatal closure, the vapor pressure deficit between leaf and air was maintained at 1.70 kPa due to the low humidity effect. During the day between 11:00 and 14:00, fully developed leaves were measured at the top of the plant by clamping the leaf into the sensor head. Each genotype was measured in and on one leaf under non-stressed and water-stressed conditions. After measuring stomatal conductance and stomatal CO_2 concentration, intercellular CO_2 concentration (Ci) and the ratio of intercellular to intercellular were determined. In agreement with Mashilo et al., 2017, A/Ci was calculated to measure the net CO_2 assimilation rate. Water use efficiency can be divided into two categories, intrinsic water uses efficiency and instantaneous water use efficiency (Mashilo, 2017). Intrinsic water use efficiency is calculated as the ratio of A to gs, while instantaneous water use efficiency is calculated as the ratio between A and T. Fluorescence values were measured at the lowest (Fo') and highest (Fm') intensities. A steady state of photosynthesis was characterized by uniform fluorescence (Fs). For the fluorescence changes, equation (2) was used to calculate the first equation (1).

$$F_v' = F_m' - F_o' \quad (1)$$

$$DF = F_m' - F_s \quad (2)$$

Using the chlorophyll fluorescence parameters, several photochemical variables, including Fv'/Fm', photochemical quenching (qP), non-photochemical quenching (qN), and electron transport rate (ETR), were calculated according to Snider and Omary (2014) and Zhang and Lin (2014). The ratio of ETR to A was used to calculate electron transport to oxygen molecules (Flexas et al., 2002). Dos Santos et al. (2013) observed that the alternative electron sink was determined by dividing the effective quantum efficiency of the photosystem II by the effective quantum efficiency of CO_2 assimilation (A).

5.2.7 Harvesting

During harvesting, the soil was completely removed from the roots and the roots were soaked, after which the biomass was weighed using a Sartorius BSA224S-CW 230 X 310 X 305mm micro-CW balance. The seeds were then removed from the pods by hand threshing. As shown in Figure 5.2, the biomass and seeds were weighed for each genotype and the results were used for data analysis.

5.2.8 Data Analyses

ANOVA was used to analyse all data using GenStat® version 18 (VSN International, UK). To compare the means of the treatments, Duncan's test in GenStat® was used with a probability level of 5%. The significance of statistical differences was also determined using the standard errors, which were accepted as $P \leq 0.05$. These correlations were performed using Prism 9 software installed on the GraphPad Prism computer. To identify relationships between 24 Bambara groundnut genotypes and morphological and physiological parameters, principal component analysis and correlations were performed in this study. Cluster analysis was carried out using XLstat to identify more and less tolerant genotypes.

5.3 Results

5.3.1 Emergence

The results showed highly significant differences ($p<0.001$) between days with respect to seedling emergence (%) (Table 5.4). Highly ($p<0.001$) significant differences were also observed between the genotypes. There were also highly ($p<0.001$) significant interactions between number of days and genotypes. Number of seeds emerging increased in each and every day as shown in table 5.4. The genotypes differed in the seedling emergence most genotypes showed high emergence, however, a few showed low emergence. Acc 25, Acc 55, Acc 95, Acc 96, Acc 151, Acc 177, and Acc 200 had the highest emergence (100%). Followed by Acc 61, Acc 78, and Acc 87 (89%) then followed by Acc 82, Acc 97, Acc 131, Acc 117, Acc 179, and

Acc 184 (78%). Acc 100, and Acc 199 were at 67%. Followed by Acc 105, Acc 150, Acc 175, and 197 with 55-56% emergence rate. Acc 121 and Acc 190 had the lowest emergence throughout with 11% and 33% respectively. Furthermore, in terms of mean emergence time (MET) the analysis of variance indicated that there were highly significant ($p<.001$) differences between the number of days after planting genotypes (Table 5.5). The same results appeared between number of days and between genotypes.

Table 5.4: Emergence percentage for twenty-four Bambara groundnut genotypes in 15 days

	Emergence %														
Accessions	**day1**	**day2**	**day3**	**day4**	**day5**	**day6**	**day7**	**day8**	**day9**	**day10**	**day11**	**day12**	**day13**	**day14**	**day15**
Acc 25	0	0	0	0	0	0	0	22	22	78	89	89	89	100	100
Acc 55	0	0	0	0	0	0	0	0	33	56	78	78	89	100	100
Acc 61	0	0	0	0	0	0	0	0	0	44	67	78	78	78	89
Acc 78	0	0	0	0	0	0	0	0	0	22	56	78	78	78	89
Acc 82	0	0	0	0	0	0	0	0	0	78	78	78	78	78	78
Acc 87	0	0	0	0	0	0	0	0	0	56	56	67	89	89	89
Acc 95	0	0	0	0	0	0	0	0	0	67	89	89	89	100	100
Acc 96	0	0	0	0	0	0	0	0	0	56	89	89	89	89	100
Acc 97	0	0	0	0	0	0	0	0	0	44	67	67	78	78	78
Acc 100	0	0	0	0	0	0	0	0	0	44	44	56	67	67	67
Acc 105	0	0	0	0	0	0	0	0	0	11	22	22	22	22	55
Acc 117	0	0	0	0	0	0	0	0	0	56	78	78	78	78	78
Acc 121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
Acc 131	0	0	0	0	0	0	0	0	0	56	67	67	67	78	78
Acc 150	0	0	0	0	0	0	0	11	22	33	33	44	44	44	56
Acc 151	0	0	0	0	0	0	0	22	33	78	100	100	100	100	100
Acc 175	0	0	0	0	0	0	0	0	0	33	44	44	56	56	56
Acc 177	0	0	0	0	0	0	0	22	33	100	100	100	100	100	100
Acc 179	0	0	0	0	0	0	0	0	0	22	33	56	56	67	78
Acc 184	0	0	0	0	0	0	0	0	0	44	56	67	67	78	78
Acc 190	0	0	0	0	0	0	0	0	0	33	33	33	33	33	33
Acc 197	0	0	0	0	0	0	0	0	0	22	22	33	56	56	56
Acc 199	0	0	0	0	0	0	0	0	0	22	44	56	67	67	67
Acc 200	0	0	0	0	0	0	0	0	0	78	89	89	89	89	100

Table 5.5: Mean emergence time (MET) for twenty-four Bambara groundnut genotypes in 15 days

	Mean emergence time (MET)														
	day1	**day2**	**day3**	**day4**	**day5**	**day6**	**day7**	**day8**	**day9**	**day10**	**day11**	**day12**	**day13**	**day14**	**day15**
Acc 25	0	0	0	0	0	0	0	0	0	2	2	2	2	3	3
Acc 55	0	0	0	0	0	0	0	0	1	1	2	2	2	3	3
Acc 61	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2
Acc 78	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2
Acc 82	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2
Acc 87	0	0	0	0	0	0	0	0	0	1	1	2	2	2	3
Acc 95	0	0	0	0	0	0	0	0	0	1	2	2	2	3	3
Acc 96	0	0	0	0	0	0	0	0	0	1	2	2	2	2	3
Acc 97	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2
Acc 100	0	0	0	0	0	0	0	0	0	1	1	1	2	2	2
Acc 105	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2
Acc 117	0	0	0	0	0	0	0	0	0	1	2	2	2	2	2
Acc 121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acc 131	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2
Acc 150	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
Acc 151	0	0	0	0	0	0	0	0	1	2	2	2	3	3	3
Acc 175	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2
Acc 177	0	0	0	0	0	0	0	0	1	2	2	2	3	3	3
Acc 179	0	0	0	0	0	0	0	0	0	0	1	1	1	2	2
Acc 184	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2
Acc 190	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Acc 197	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2
Acc 199	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2
Acc 200	0	0	0	0	0	0	0	0	0	2	2	2	2	2	3

5.3.2 Phenotypic response to water stress

5.3.2.1 Plant growth and development in response to water stress

Plant growth and development was determined in terms of plant height, leaf length, petiole length, and leaf width. Results showed that over the growing period, there was no significant differences (p>0.05) between water stress with respect to plant height. There was also no significance difference observed among genotypes with respect to plant height (p>0.05). However, Acc 175 had the highest height (33.38 cm) followed by Acc 199 (33.09 cm). Acc 96 was the lowest with the height of 28.66 cm followed by Acc 78 with 29.32 cm. The interaction between genotypes and water stress was not significant (p>0.05) with respect to plant height. Water stress had no significant difference (p>0.05) with respect to petiole length. However, there was high significant difference (p<0.05) among genotypes (Figure 5.2) with respect to petiole length. The interaction of water stress and genotypes had no significant difference (p>0.05). In terms of leaf length there was no significant difference (p>0.05) with respect to water stress. Interaction between water stress and genotypes had no significance (p>0.05) impact as well with respect to plant height. With respect to leaf width no significant different (p>0.05) observed in terms of water stress and there was also no significance (p>0.05) impact between genotypes. However, leaves of Acc 100 had the length (8.77 cm) followed by Acc 82 with 8.73cm. In terms of leaf width, no significant different in terms of water stress and among genotypes. The interaction between water stress and genotypes had also no significance impact (p>0.05) on leaf width as well. Acc 100 had width (3.40 cm) followed by Acc 82 (3.30 cm).

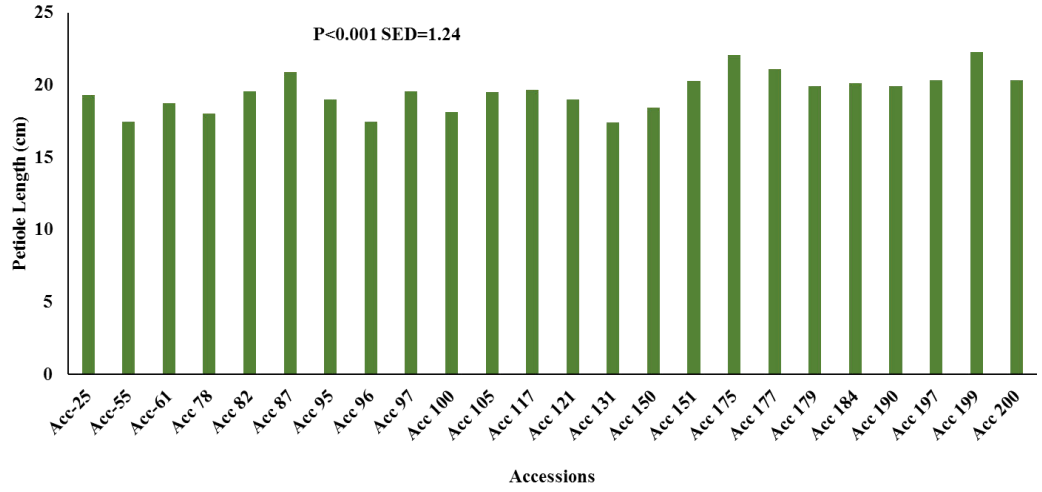

Figure 5.2: Petiole length (PL) variation among Bambara groundnut genotypes

5.3.2.2 Yield performance in response to water stress

Highly significant differences (p<0.01) were observed between those genotypes where irrigation was withheld (water stress) and well-watered plants with respect to biomass production (Figure 5.3). However, there were no significant difference (p>0.05) among genotypes. The interaction effect between water stress and genotype was also not significant (p>0.05) with respect to the total biomass production. The results showed that non-stressed plants produced more total biomass than those subjected to water stress. Acc 190 had the highest total biomass (27.33 g) followed by Acc 131 (24.67g) and Acc 121 (23.37g). Acc 117 had the lowest total biomass (12.17 g). The results obtained on grain yield showed that there were highly (p<.001) significant differences between water stress conditions (Figure 5.3b), but genotypes showed no (p< 0.05) significant variation among each other nor the interaction between water stress and genotypes. Water stress reduced the seed yield in all genotypes. The observed yield in non-stressed genotypes ranged between 11.33 and 26.71g while stressed genotypes ranged between 1.71 and 8.71 g. There were highly significant differences (p>0.001) between the no stress and stressed treatments with respect to the number of seeds produced (Figure 5.3c). However, the interaction among genotypes and water stress didn't show any (p>0.05) significant difference. Acc 200, Acc 25, and Acc 55 had the highest number of seeds (21). Acc 175 produced lowest number of seeds (9).

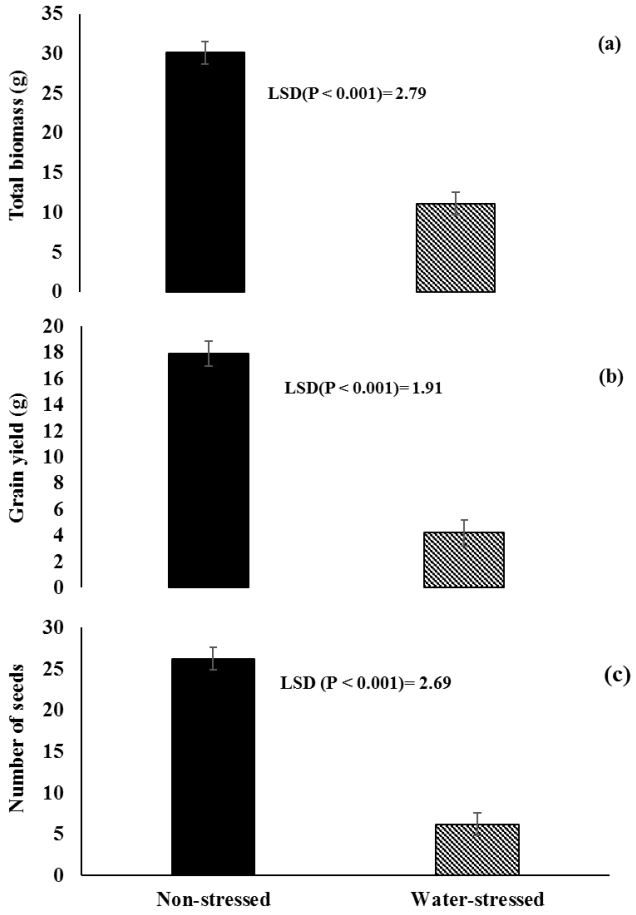

Figure 5.3: Effects of water stress on Bambara groundut genotypes: total biomass (a), grain yield (b), and number of seeds (c).

5.3.3 Agglomerative Hierarchical Clustering (AHC) cluster analysis

Agglomerative Hierarchical Clustering (AHC) is a clustering method which works from the dissimilarities between the substances to be classified together, it is one of the most general clustering methods (Das et al., 2021). The cluster analysis of the 24 Bambara groundnut genptypes are presented in Figure 5.4. The dendrogram clustered the accessions into five clusters. Cluster I consisted of four genotypes which are; Acc 105, Acc 61, Acc 121, and Acc 82. The second cluster comprised of four genotpes which are; Acc 55, Acc 87, Acc 200, and Acc 25. The third cluster included seven genotypes namely; Acc 179, Acc 96, Acc197, Acc 131, Acc 151, Acc 78, and Acc 184. The fourth cluster three genotypes namely; Acc 199, Acc 175, and Acc 190. The last cluster contained five genotypes, namely Acc 95, Acc 117, Acc 100, Acc 177, and Acc 150. The Pie chart (Figure 5.5) is showing that among twenty-four (24) genotypes used; the results observed suggest that 40% are more drought tolerant, 35% less drought tolerant, and only 25% of the genotypes could be sensitive to drought.

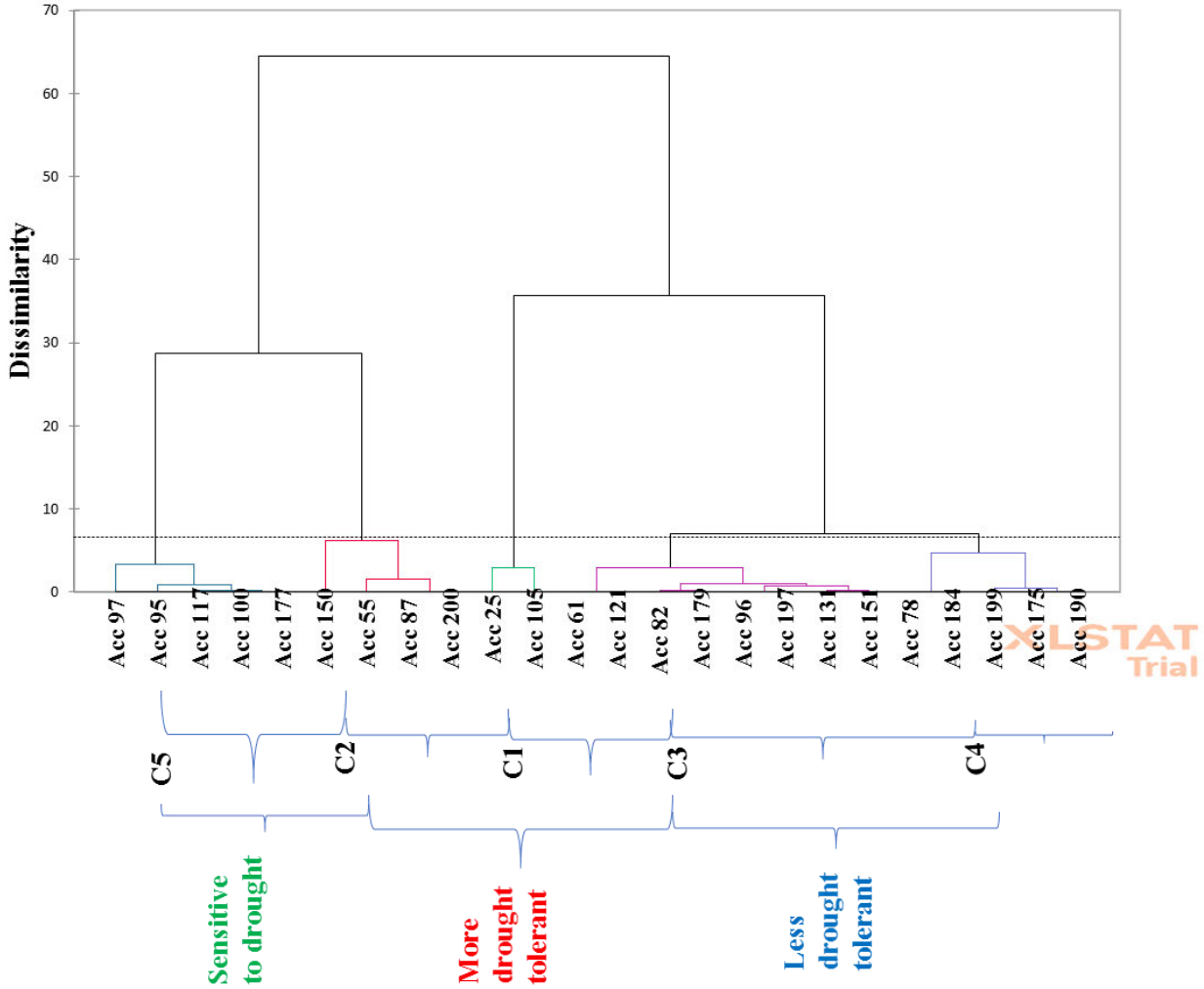

Figure 5.4: Dendrogram of cluster analysis of 24 Bambara genotypes using Ward's method based on drought tolerance indices.

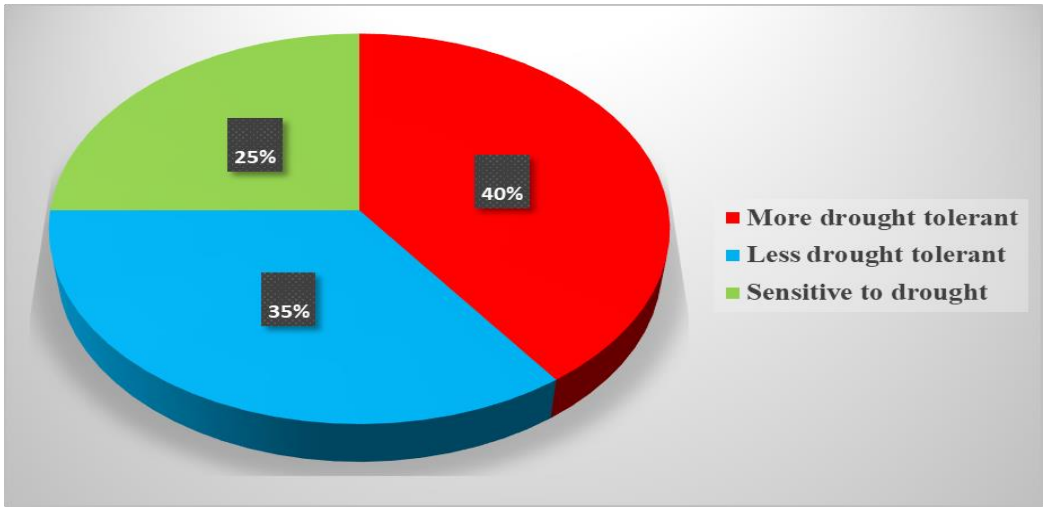

Figure 5.5: Pie chart showing drought tolerance among 24 Bambara groundnut genotypes based on the cluster diagram.

5.3.4 Principal component analysis for growth and yield traits.

Principal Component Analysis (PCA) is extensively used in plant research for trait arrangement and genotype organization. The existing phenotypic variation among the studied Bambara groundnut genotypes was explained by the PCA biplot between PC 1 and PC 2 (Figure 5.6), which shows the performance of yield and growth parameters of Bambara groundnut genotypes. The biplot explains the relationships and similarities among Bambara groundnut genotypes with respect to the seven (7) measured traits for growth and yield. In terms of their genetic variability, the genotypes exhibited mating orientation within the axes irrespective of geographical location, indicating that they share most of the traits for the seven(7) measured traits. A principal component analysis (Table 5.6) was performed to investigate whether the trait variation observed between genotypes was influenced by the water stress conditions under which these genotypes were grown. The two principal components PC 1 and PC 2, accounted for 42.57% and 29.57% of the variation, respectively, with a cumulative variation of 72.14% for non-stressed, and accounted for 38.53% and 25.23% of the variation, respectively, with a cumulative variation of 63.76% for water-stressed (Figure 5.6). Plant height (PH) and total biomass (TB) showed strong association with the other phenotypic traits. Water stress reduced the association between genotypes, with Acc 117 and Acc 151 strongly associated under water stress. It is likely that these genotypes share the same traits. Other strongly associated genotypes under water stress were: Acc 190 and Acc 184.

Table 5.6: Principal component analysis showing eigenvectors, eigenvalues, and percent variability of of plant growth and yield parameretrs of twenty four bambara groundnut genotypes.

	Non-stressed					Water-stressed				
Physiological traits	**PC 1**	**PC 2**	**PC3**	**PC4**	**PC5**	**PC 1**	**PC 2**	**PC3**	**PC4**	**PC5**
LL	0.39	0.29	0.43	0.35	**0.66**	0.18	0.26	**0.73**	0.26	0.40
LW	0.37	0.13	**0.65**	0.12	0.64	0.30	0.23	0.43	0.71	0.39
PL	0.27	0.59	0.04	0.18	0.19	0.13	**0.68**	0.13	0.01	0.52
PH	0.24	**0.61**	0.02	0.10	0.26	0.19	0.57	0.20	0.53	0.47
TB(g)	0.42	0.40	0.11	0.48	0.04	0.51	0.17	0.33	0.01	0.28
GY(g)	0.51	0.15	0.28	0.33	0.15	0.56	0.05	0.29	0.17	0.17
NS	0.38	0.02	0.55	**0.69**	0.18	0.50	0.24	0.19	0.34	0.31
Eigenvalue	2.84	1.94	1.01	0.56	0.34	2.67	1.71	1.27	0.75	0.33
Variability (%)	40.52	27.71	14.42	7.93	4.91	38.08	24.44	18.18	10.69	4.66
Cumulative %	40.52	68.23	82.65	90.58	95.49	38.08	62.52	80.70	91.39	96.06

Vector loadings ≧0.6 are boldfaced, leaf length (LL), Leaf width (LW), petiole length (PL), plant height (PH), total biomass (TB), grain yield (GY), and number of seeds (NS)

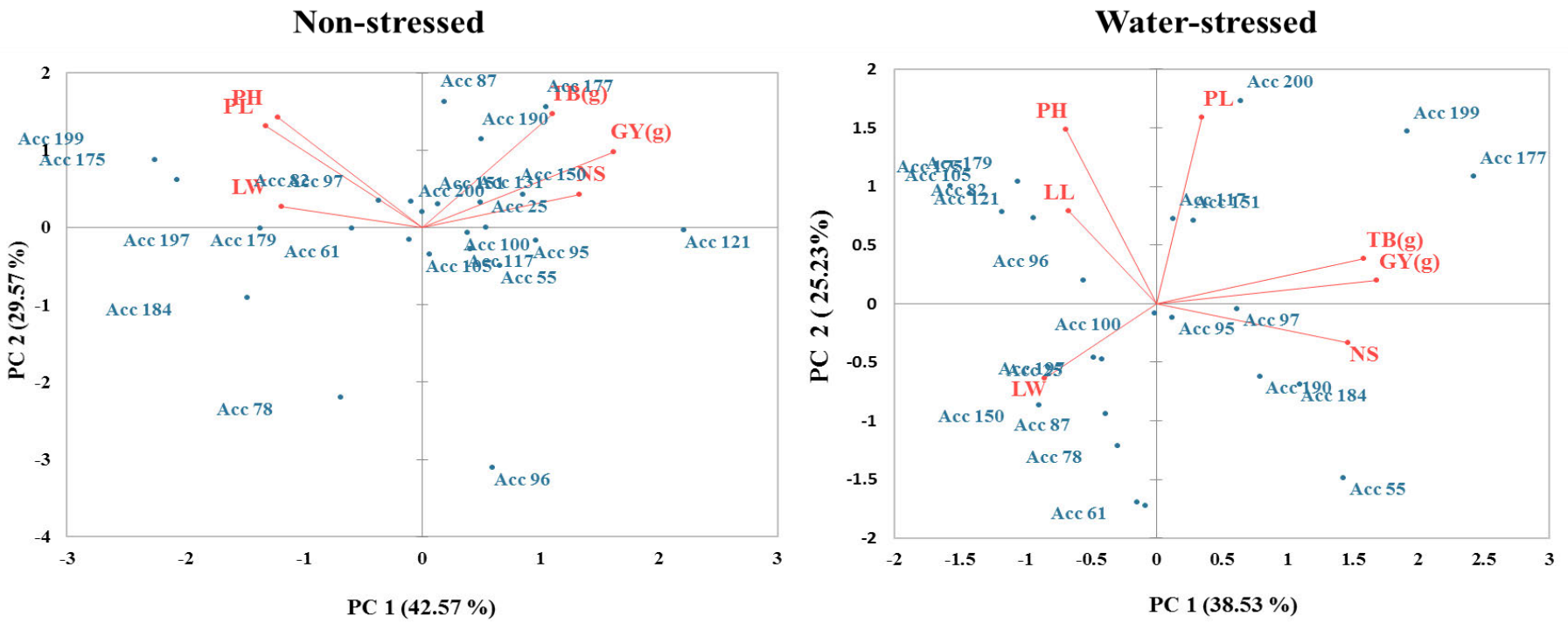

Figure 5.6: Principal component score plot of PC 1 and PC 2 describing the overall variation among Bambara groundnut genotypes under non-stressed and water stressed conditions.

5.3.5 Correlations among traits

Figure 5.7 below shows the correlation between growth and yield parameters. Number of seeds was found to be positively correlated with grain yield and total biomass, and leaf width and leaf petiole length with plant height, and leaf length with plant height under both water conditions. Weak negative correlations were observed between leaf length and total biomass and leaf length and number of seeds under both water conditions.

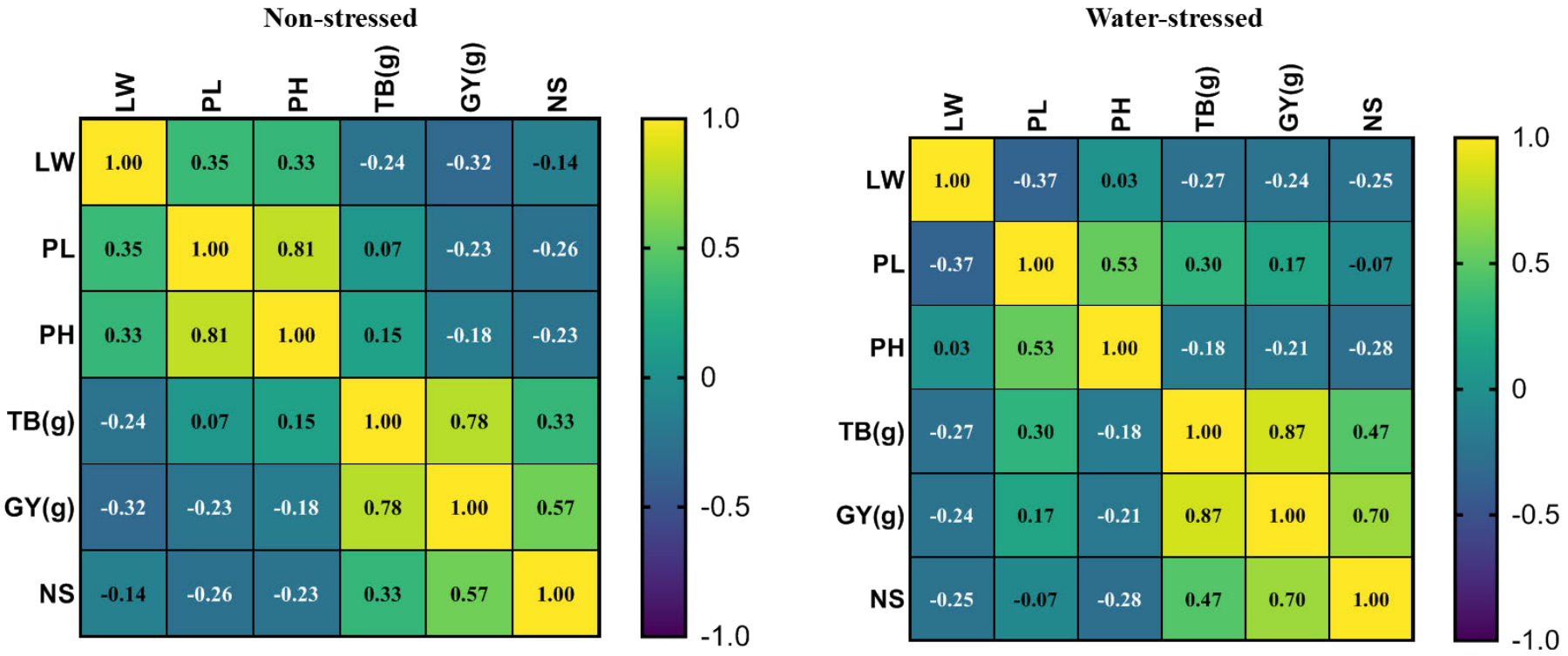

Figure 5.7: Correlation variation among growth and yield traits under non-stressed and drought stressed.

5.3.6 Changes in leaf gas exchange in response to water stress

The changes in leaf gas exchange parameters in response to water stress in the tested Bambara groundnut genotypes are shown in Figure 5.8 and 5.9. Highly significant (p<0.001) difference was observed in several leaf gas exchange parameters, indicating the presence of genotypic variability under non-stressed and water stressed conditions. Non-significant (p>0.05) differences were observed between genotypes under non-stress and water stress conditions for intercellular CO_2 (Ci). The interaction of water stress and genotypes was not significant. Significant differences (p<0.05) were observed with respect to the ratio of intercellular and atmospheric CO_2 (Ci/Ca) under both conditions (Figure 5.8b), but there was no significant difference between genotypes and in the interaction of water stress and genotypes. Regarding transpiration rates (T), differences were observed among the genotypes.

No (p>0.05) significant difference was found with respect to T (transpiration rates). Acc 55, Acc 78, Acc 87, Acc 150 and Acc 184 had low T values (< 10 mmol H_2O m^{-2} s^{-1}), while Acc 25, Acc 61, Acc 95, Acc 96, Acc 97, Acc 100, Acc 105, Acc 117, Acc 121, Acc 131, Acc 151, Acc 175, Acc 177, Acc 179, Acc 190, Acc 197, Acc 199 and Acc 200 had T values > 10 mmol H_2O m^{-2} s^{-1} under non-stressed conditions. Water stress did not affect transpiration rate too much, but the degree of reduction was higher in Acc 25, Acc 55, Acc 61, Acc 82, Acc 95, Acc 100, Acc 105, Acc 117, Acc 121, Acc 131, Acc 151, Acc 175, Acc 197 and Acc 199. Acc 10, Acc 117, Acc 121, Acc 190 and Acc 199 showed the greatest reduction with a decrease in values under non-stress (12.09 mmol H_2O m^{-2} s^{-1} - 8.92 mmol H_2O m^{-2} s^{-1}), (12.51 mmol H_2O m^{-2} s^{-1} - 6.83 mmol H_2O m^{-2} s^{-1}), (13.26 mmol H_2O m-^{2} s^{-1} - 7.72 mmol H_2O m^{-2} s^{-1}), (10.44 mmol H_2O m^{-2} s^{-1} - 7/.10 mmol H_2O m^{-2} s^{-1}), and (13.26 mmol H_2O m^{-2} s^{-1} - 10.99 mmol H_2O m^{-2} s^{-1}).

In terms of CO_2 assimilation rate (A), no (p>0.05) significant interaction was observed, and Acc 78, Acc 87, Acc 95, Acc 100, Acc 117, Acc 121, Acc 150, Acc 175, Acc 177, Acc 184, Acc 199, and Acc 200 had slightly lower values (< 50 mmol CO_2 m^{-2} s^{-1}), while Acc 25, Acc 5, Acc 61, Acc 96, Acc 97, Acc 105, Acc 131, Acc 151, Acc 179, Acc 190 and Acc 197 had slightly higher values under non-stressed conditions (> 50 mmol CO_2 m^{-2} s^{-1}). Water stress did not have a major effect on CO_2 assimilation rate (A), as many genotypes recored high values under both non-stress and water stress. In terms of stomatal conductance (gs), a highly significant difference (p<0.001) was observed under water stress conditions (Figure 5.9a).

There was no significant difference between genotypes. According to the results of assimilation rate/intercellular CO_2 concentration (A /ci), there was no significant difference in relation to water stress. Significant (p<0.05) differences were also observed with respect to intrinsic water use efficiency (IWUE) under both conditions (Figure 5.10). Drought stress increased intrinsic water use efficiency in all genotypes except Acc 87 [from 881 (CO_2) m_2 (H_2O) to -596 (CO_2) m^{-2} (H_2O)] and Acc 199 [from 976 (CO_2) m^{-2} (H_2O) to 457 (CO_2) m^{-2} (H_2O)]. In terms of instantaneous water use efficiency (WUEins), there were no significant differences in response to water-stress, but highly significant differences were observed among genotypes (Figure 5.10). Water stress increased instantaneous water use efficiency by about 31.8% in Acc 25, Acc 55, Acc 61, Acc 82, Acc 95, Acc 100, Acc 105, Acc 117, Acc 121, Acc 151, Acc 175, Acc 179, cc 190 and Acc 197.

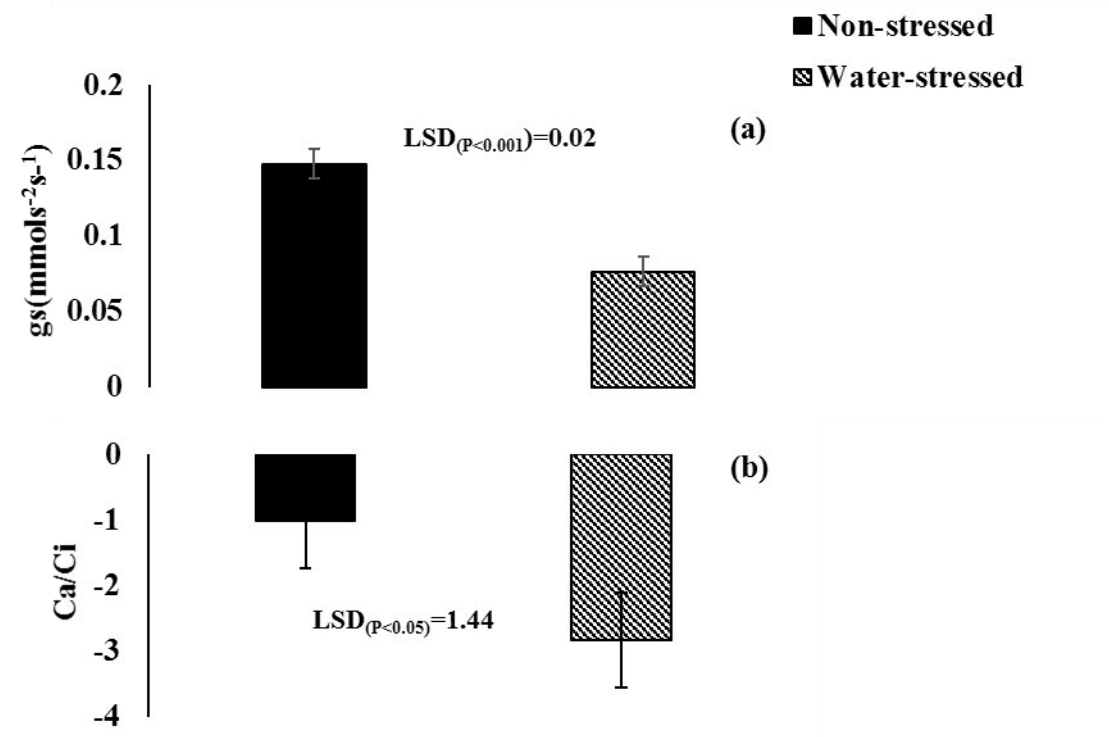

Figure 5.8: Effects of water stress on Bambara groundnut : stomatal conductance (gs) ratio of intercellular and atmospheric CO_2 (Ci/Ca).

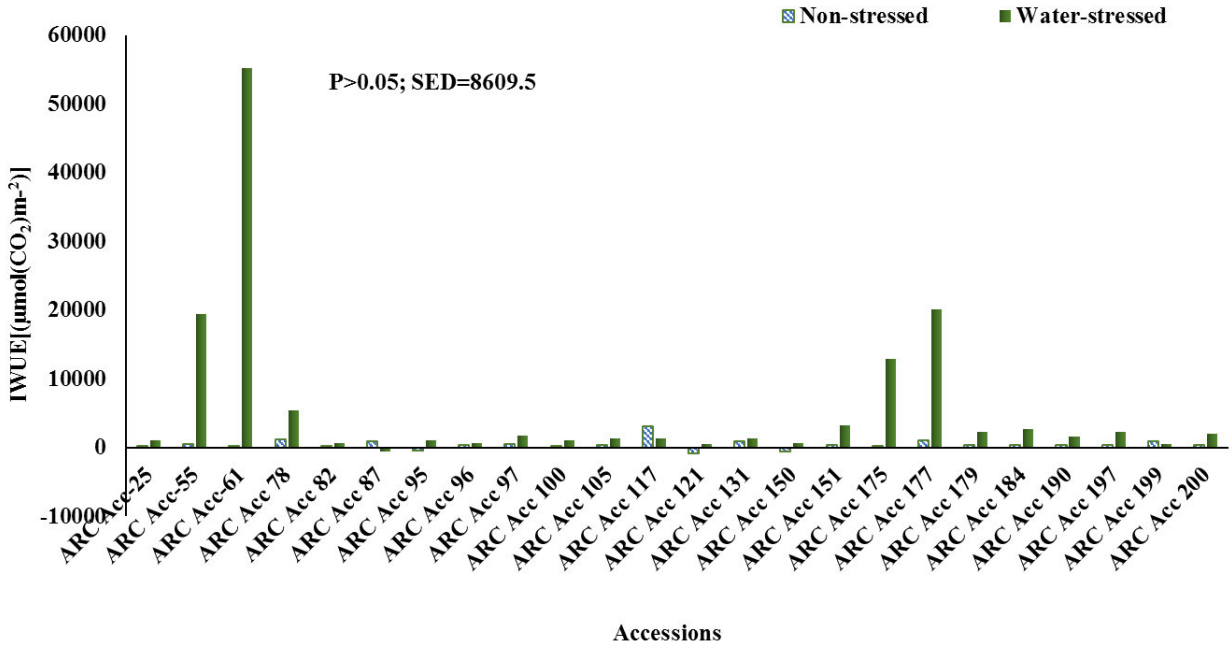

Figure 5.9: Effect of water stress on intrinsic water use efficiency (IWUE) among 24 Bambara groundnut genotypes.

5.3.7 Changes in chlorophyll fluorescence parameters in response to water stress

The effects of water stress on Bambara groundnut genotypes on chlorophyll fluorescence parameters are summarized in Figure 5.10 and 5.11. Significant interaction between genotype and water stress was observed for several chlorophyll fluorescence parameters, indicating different responses of the twenty-four Bambara genotypes under both non-stressed and water-stressed conditions. The genotypes showed highly significant (p<001) differences in terms of minimum fluorescence F_0' under non-stress and drought stress conditions (Figure 5.10a). A significant (p<0.05) difference was observed with respect to Fm' under stress conditions (Figure 5.10b). However, there was no significance between genotypes and the interaction between genotypes and water stress was not significant. Acc 179 showed increased levels of Fm' under water stress compared to other genotypes under the same water conditions. In relation to water stress, maximum quantum efficiency of photosystem II photochemistry (Fv'/Fm') was also highly significant (p<0.001) (Figure 5.11). Genotypes were also significant (p>0.05), but the interaction of water stress and genotypes was not significant. In terms of qP, there were also no (p>0.05) significant differences between genotypes in relation to water stress and even in the interaction of water stress and genotypes. Photochemical quenching (qP) was increased by water-stress in Acc 61, Acc 78, Acc 82, Acc 87, Acc 95, Acc 100, Acc 105, Acc 121, Acc 150, Acc 175, Acc 177, Acc 184, and Acc 200 (>0.4), but reduced in Acc 25, Acc 55, Acc 96, Acc 97, Acc 117, Acc 131, Acc 151, Acc 179, Acc 190, Acc 197, and Acc 199. In

terms of non-photochemical quenching (qN), there were also no significant differences (p>0.05) among genotypes in relation to water stress conditions and even in the interaction of genotypes and water stress. The responses of genotypes with respect to non-photochemical quenching (qN) were observed under non-stress conditions. There was a very high significant (p<0.001) difference with respect to water conditions for electron transport rate (ETR) (Figure 5.10c). However, there was no significant difference among genotypes and interaction of water stress and genotypes had no significant difference (p>0.05). ETR increased in all genotypes under stress conditions except Acc 197, and the effective quantum efficiency of photosystem II photochemistry (ΦPSII) was not significant in the twenty-four genotypes tested under both non-stress and water stress conditions (p>0.05) (Figure 5.10f). Water-stress increased ΦPSII in Acc 100, Acc 177, Acc 179, Acc 190, Acc 199 and Acc 200. There was a highly significant difference (p<0.001) in the relative measure of electron transport to oxygen molecules (ETR/A) compared to treatment without water stress (Figure 5.10d). However, no significant difference was observed between genotypes. For alternative electron sink (AES), there was no significant difference between stress conditions and between genotypes (p>0.05) (Figure 5.10e).

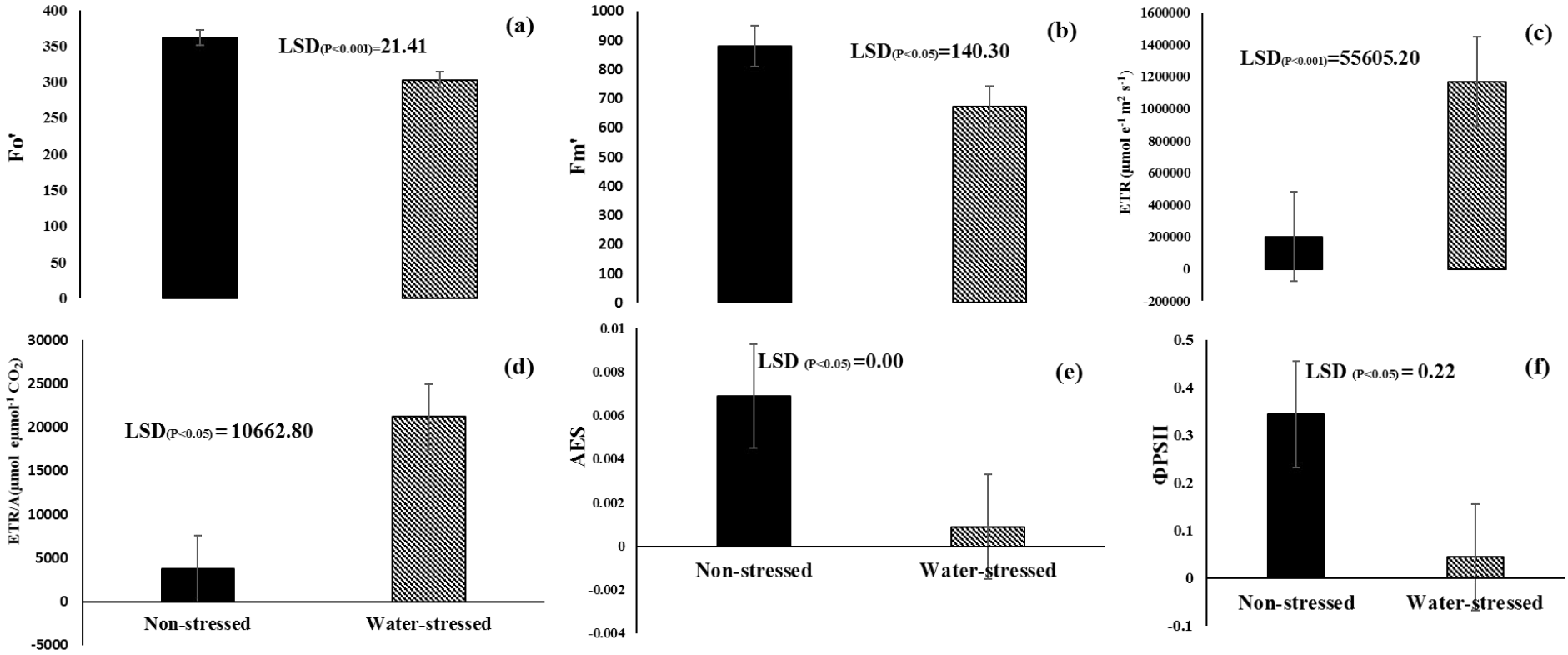

Figure 5.10: Effects of water stress in of Bambara groundnuts genotypes: Minimum (a) and maximum (b) fluorescence, electron transport rate (c), electron transport to O_2 molecules (d), alternative electron sink (e), and the effective quantum efficiency of photosystem II photochemistry (f).

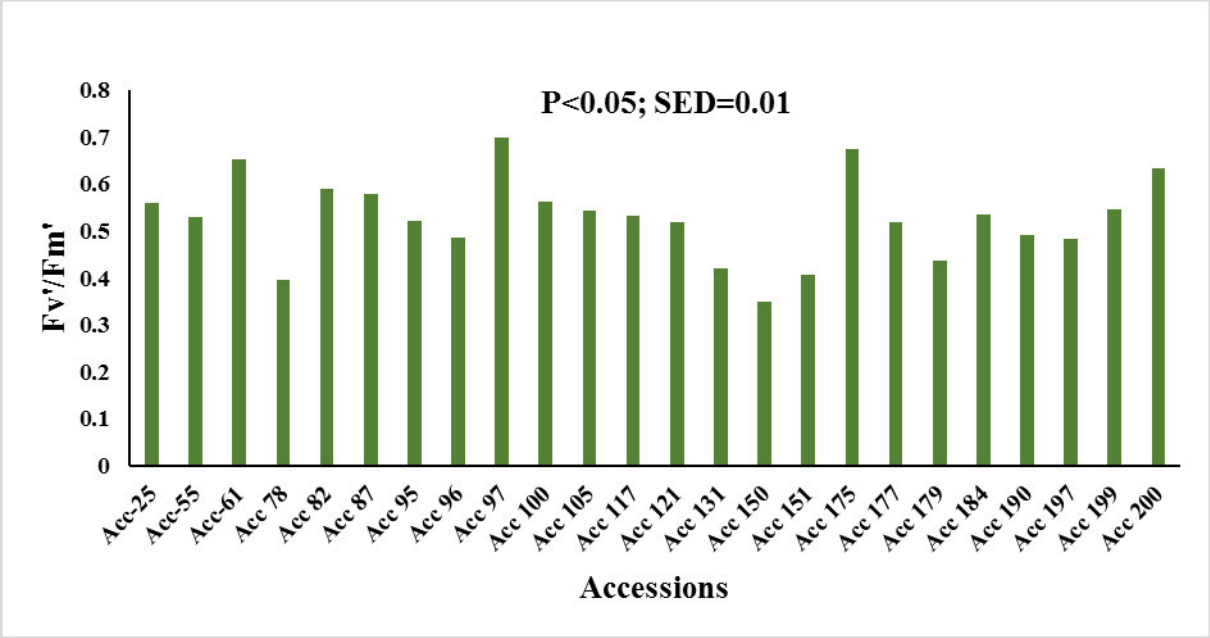

Figure 5.11: Variation of maximum quantum efficiency of photosystem II photochemistry (Fv'/Fm').

Table 5. 7: Analysis of variance showing mean squares and significance tests of leaf gas exchange and chlorophyll fluorescence parameters of 24 Bambara groundnut genotypes tested under non-stress and drought-stress conditions.

Source of variance	df	Leaf gas exchange parameters							
		gs	Ci	Ci/Ca	T	A	A/Ci	IWUE	WUEins
Water stress	1	<0.001**	0.30	0.014*	0.24	0.26	0.19	0.004**	0.18
Genotypes	23	0.294	0.43	0.76	0.46	0.71	0.60	0.016*	0.66
Genotypes. Water stress	23	0.573	0.89	0.72	0.24	0.87	0.53	0.016*	0.67
	df	Chlorophyll fluorescence parameters							
		F0'	Fm'	Fv'/Fm'	ΦPSII	qP	qN	ETR	ETR/A	AES
Water stress	1	<.001**	0.004**	0.001**	0.009**	0.72	0.30	<.001**	0.002**	0.015*
Genotypes	23	0.026	0.67	0.048*	0.75	0.65	0.27	0.60	0.80	0.81
Genotypes. Water stress	23	0.549	0.38	0.09	0.86	0.99	0.30	0.68	0.77	0.83

gs; stomatal conductance; Ci, intercellular CO_2 concentration; Ci/Ca; ratio of intercellular and atmospheric CO_2; T transpiration rate, A; net CO_2 assimilation rate; A/Ci, CO_2 assimilation rate/intercellular CO_2 concentration;; IWUE, intrinsic water use efficiency; WUEins, instantaneous water-use efficiency; F0' ; Minimum fluorescence, Fm'; maximum fluorescence, Fv'/Fm', maximum quantum efficiency of photosystem II photochemistry; ΦPSII, the effective quantum efficiency of PSII photochemistry; qP, photochemical quenching; qN, non-photochemical quenching; ETR, electron transport rate; ETR/A, relative measure of electron transport to oxygen molecules; AES, alternative electron sink.* Significant at the 0.05 probability level, ** Significant at the 0.01 probability level, non-significant difference. df, degrees of freedom

5.3.8 Principal component analysis for gas exchange and chlorophyll fluorescence parameters

Principal component analyses of leaf gas exchange and chlorophyll fluorescence parameters under nonstress and water stress conditions are shown in Table 5.7. The PCA-based principal component biplot showing the percentage explained variance of PC 1 versus PC 2 was used to determine the relationships among Bambara groundnut genotypes for leaf gas exchange and chlorophyll fluorescence parameters under non-stress and drought stress conditions (Figure 5.12) to identify drought tolerant genotypes. Smaller angles between dimension vectors in the same direction indicated high correlation of variables in terms of distinguishing genotypes. The two principal components PC 1 and PC 2, accounted for 40.84% and 24.87% of the variation, respectively, with a cumulative variation of 65.71% for non-stressed, and 17.81% and 15.59% of the variation, respectively, with a cumulative variation of 33.4% for water-stressed. IWUE, ETR, A/Ci, Fm', qP, ETR, ETR/A, qP, Fv'/Fm', gs, T, A,Ci, Ci/Ca, qN, ΦPSII, and AES showed positive correlation with PC 1; whereas WUEins, A/Ci, and Fo' were negatively correlated with PC 1.There were no positively correlated variables for PC 2. However, only Fo', A/Ci and WUEins were negatively correlated with PC 2, which accounted for 24.87% of the total variation. Under drought stress conditions, PCA analysis revealed PC's total variability to be 33.4%. AES , Ci, T, gs, qP , IWUE, Fo', qN, and A/Ci were positively correlated with PC 1, which accounted for 17.81% of the total variation. A, Ci/Ca and WUEins were negatively correlated with PC 2, while ETR, ETR/A, Fm' and , ΦPSII were positively correlated with PC 2, accounting for 15.59% of the total variation.

Table 5. 8: Principal component analysis showing eigenvectors, eigenvalues, and percent variability.

Physiological traits	Non-stressed					Water-stressed				
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 1	PC 2	PC 3	PC 4	PC 5
Ci	0.02	0.46	0.06	0.25	0.17	0.13	0.32	0.32	0.04	0.03
gs	0.21	0.36	0.06	0.05	0.04	0.39	0.12	0.18	0.02	0.26
Ci/Ca	0.04	0.44	0.10	0.31	0.18	0.09	0.04	0.38	0.15	0.36
T	0.30	0.16	0.05	0.40	0.19	0.29	0.21	0.20	0.04	0.35
A	0.02	0.21	0.58	0.35	0.07	0.39	0.06	0.28	0.26	0.01
A/C	0.01	0.15	0.28	0.28	0.81	0.20	0.01	0.10	0.19	0.32
IWUE	0.11	0.41	0.16	0.03	0.18	0.21	0.04	0.35	0.43	0.11
WUEins	0.19	0.29	0.20	0.03	0.27	0.25	0.24	0.26	0.09	0.21
F0′	0.26	0.03	0.35	0.18	0.08	0.01	0.06	0.41	0.15	0.18
Fm′	0.30	0.05	0.29	0.25	0.00	0.12	0.40	0.12	0.07	0.05
Fv′/Fm′	0.35	0.01	0.05	0.02	0.03	0.40	0.02	0.02	0.06	0.23
qP	0.28	0.08	0.28	0.09	0.16	0.31	0.09	0.20	0.09	0.37
qN	0.26	0.02	0.44	0.14	0.25	0.23	0.09	0.04	0.04	0.53
ETR	0.22	0.29	0.05	0.33	0.05	0.25	0.40	0.04	0.39	0.07
ETR/A	0.34	0.10	0.04	0.30	0.14	0.25	0.41	0.03	0.36	0.07
AES	0.34	0.11	0.04	0.31	0.13	0.01	0.34	0.29	0.43	0.06
ΦPSII	0.33	0.00	0.05	0.24	0.05	0.00	0.38	0.29	0.41	0.04
Eigenvalue	6.94	4.23	1.52	1.06	0.93	3.03	2.65	2.58	2.15	1.85
Variability (%)	40.84	24.87	8.94	6.23	5.46	17.81	15.59	15.18	12.65	10.86
Cumulative (%)	40.84	65.71	74.65	80.88	86.34	17.81	33.41	48.58	61.23	72.09

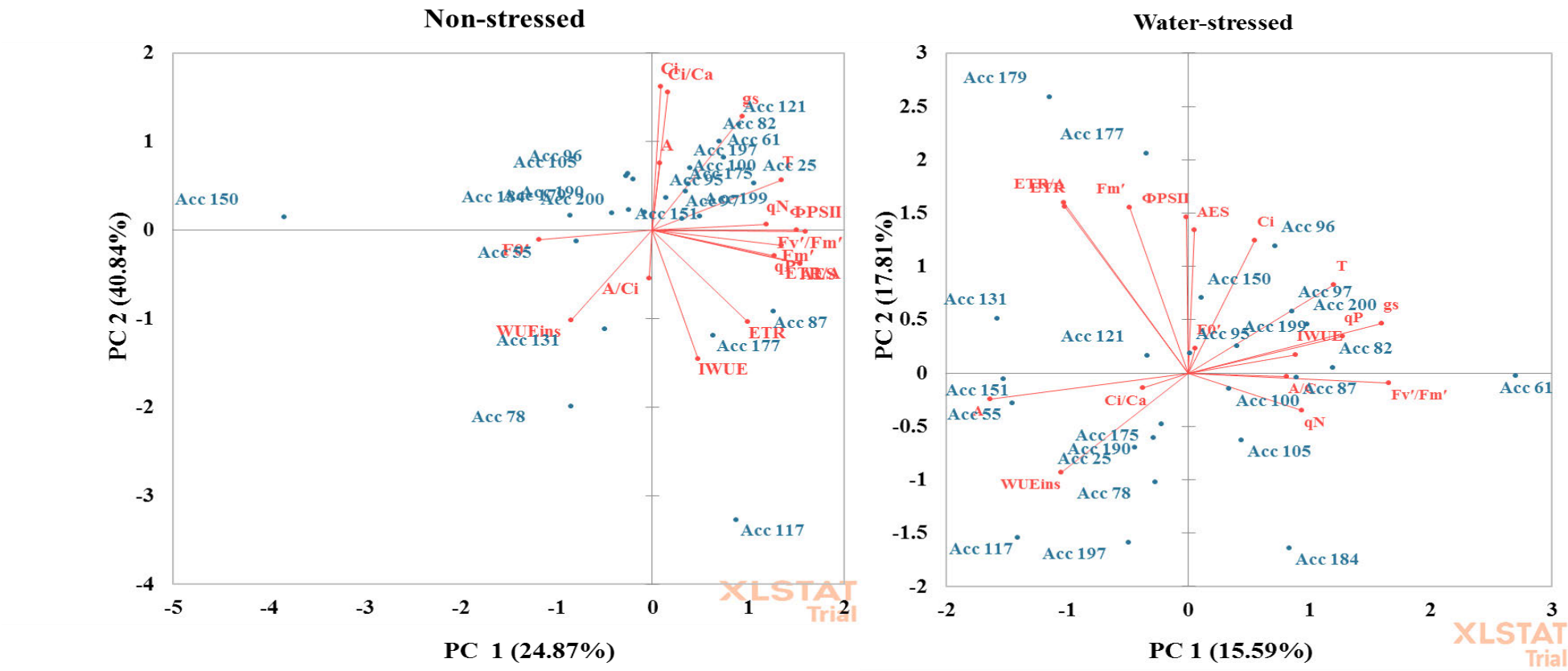

Figure 5.12: Biplot of gas exchange and chlorophyll fluorescence parameters based on the first two principal components axes (PC1 and PC2) for 24 Bambara genotypes in non-stress and water stress conditions.

5.3.9 Correlations among gas exchange and chlorophyll fluorescence parameters

Table 5.9 and Figure 5.13 below show the Pearson correlation coefficients (r) for the traits assessed. The correlation between chlorophyll fluorescence parameters and gas exchange parameters was reduced by water stress. Under non-stressed conditions, there were high positive correlations between IWUE and ETR, Fm' with Fv/'Fm', qN, ΦPSII, ATR and ETR/A. There was also a high correlation of gs with Ci and Ci/Ca. Fv'/Fm' had a high correlation with T, Fm', qN, ETR/A, AES, ΦPSII and ETR. On the other hand, gs had high positive correlation with Ci and Ci/Ca. Similarly, qP had high positive correlation with ETR/A, ARS and ΦPSII, while qN had high positive correlation with Fm' and Fv'/Fm'. ETR had positive correlation with IWUE, Fm', Fv'/Fm', ETR/A and AES. ETR/A had high positive correlation with T, Fm', Fv'/Fm', qP, ETR, AES and ΦPSII. AES had a high positive correlation with Fv'/Fm', qP, ETR, ETR/A, and ΦPSII. In addition, ΦPSII had a high positive correlation with T, Fm', Fv'/Fm', qP, ETR/A, AES, and ΦPSII. Under water stress, there were only two pairs with high positive correlation (ΦPSII with AES) and ETR and ETR/A

Table 5.9: Correlation coefficient among gas exchange and chlorophyll fluorescence parameters of 24 Bambara groundnut genotypes grown under non-stressed and water -stressed.

Non-stressed																
Variables	**Ci**	**Ci/Ca**	**T**	**A**	**A/Ci**	**IWUE**	**WUEins**	**F0′**	**Fm′**	**Fv′/Fm′**	**qP**	**qN**	**ETR**	**ETR/A**	**AES**	**ΦPSII**
Ci	1	0.984	0.216	0.285	-0.214	-0.798	-0.566	-0.120	-0.092	0.026	-0.137	0.045	-0.452	-0.060	-0.063	-0.028
Ci/Ca	0.984	1	0.196	0.241	-0.208	-0.780	-0.567	-0.103	-0.016	0.068	-0.136	0.066	-0.339	0.006	0.002	0.006
T	0.216	0.196	1	0.320	-0.113	0.014	-0.700	-0.485	0.600	0.695	0.615	0.580	0.142	0.476	0.473	0.757
A	0.285	0.241	0.320	1	-0.214	-0.195	-0.053	-0.267	-0.062	0.138	0.113	-0.287	-0.282	-0.121	-0.118	0.003
A/Ci	-0.214	-0.208	-0.113	-0.214	1	0.140	0.195	0.108	0.167	0.001	0.018	0.021	0.102	0.024	0.018	0.025
IWUE	-0.798	-0.780	0.014	-0.195	0 140	1	0.263	-0.223	0.212	0.271	0.336	0.087	0.735	0.406	0.418	0.086
WUEins	-0.566	-0.567	-0.700	-0.053	0 195	0.263	1	0.273	-0.296	-0.381	-0.173	-0.475	-0.019	-0.251	-0.250	-0.370
F0′	-0.120	-0.103	-0.485	-0.267	0 108	-0.223	0.273	1	-0.258	-0.793	-0.511	-0.296	-0.292	-0.633	-0.638	-0.484
Fm′	-0.092	-0.016	0.600	-0.062	0 167	0.212	-0.296	-0.258	1	0.787	0.378	0.746	0.529	0.626	0.626	0.731
Fv′/Fm′	0.026	0.068	0.695	0.138	0.001	0.271	-0.381	-0.793	0.787	1	0.560	0.643	0.533	0.801	0.804	0.768
qP	-0.137	-0.136	0.615	0.113	0.018	0.336	-0.173	-0.511	0.378	0.560	1	0.243	0.406	0.753	0.744	0.785
qN	0.045	0.066	0.580	-0.287	0.021	0.087	-0.475	-0.296	0.746	0.643	0.243	1	0.305	0.510	0.512	0.624
ETR	-0.452	-0.339	0.142	-0.282	0 102	0.735	-0.019	-0.292	0.529	0.533	0.406	0.305	1	0.731	0.738	0.367
ETR/A	-0.060	0.006	0.476	-0.121	0.024	0.406	-0.251	-0.633	0.626	0.801	0.753	0.510	0.731	1	1.000	0.736
AES	-0.063	0.002	0.473	-0.118	0.018	0.418	-0.250	-0.638	0.626	0.804	0.744	0.512	0.738	1.000	1	0.725
ΦPSII	-0.028	0.006	0.757	0.003	0.025	0.086	-0.370	-0.484	0.731	0.768	0.785	0.624	0.367	0.736	0.725	1
water-stressed																
Variables	**Ci**	**Ci/Ca**	**T**	**A**	**A/C**	**IWUE**	**WUEins**	**F0′**	**Fm′**	**Fv′/Fm′**	**qP**	**qN**	**ETR**	**ETR/A**	**AES**	**ΦPSII**
Ci	1	0.285	0.347	-0.009	0.067	-0.219	-0.438	0.221	0.349	0.060	0.048	0.059	0.111	0.134	0.102	0.095
Ci/Ca	0.285	1	-0.193	0.163	0 102	-0.272	0.067	0.228	0.248	0.114	-0.147	-0.192	0.103	0.102	-0.422	-0.393
T	0.347	-0.193	1	-0.175	-0.026	0.040	-0.516	0.344	0.082	0.246	0.027	0.412	0.013	0.034	0.030	0.031
A	-0.009	0.163	-0.175	1	-0.003	-0.779	0.141	0.258	0.191	-0.371	-0.507	-0.273	0.011	0.042	-0.071	-0.066
A/C	0.067	0.102	-0.026	-0.003	1	-0.131	-0.127	0.082	0.015	0.300	0.306	-0.043	-0.181	-0.180	0.044	0.037
IWUE	-0.219	-0.272	0.040	-0.779	-0.131	1	-0.081	-0.401	-0.158	0.229	0.161	0.290	0.252	0.205	-0.036	-0.053
WUEins	-0.438	0.067	-0.516	0.141	-0.127	-0.081	1	-0.330	-0.150	-0.169	-0.008	-0.229	-0.080	-0.083	0.060	0.033
F0′	0.221	0.228	0.344	0.258	0.082	-0.401	-0.330	1	0.075	-0.063	-0.186	0.109	-0.080	-0.058	-0.048	-0.074
Fm′	0.349	0.248	0.082	0.191	0.015	-0.158	-0.150	0.075	1	-0.096	0.027	0.014	0.368	0.361	0.290	0.322
Fv′/Fm′	0.060	0.114	0.246	-0.371	0 300	0.229	-0.169	-0.063	-0.096	1	0.377	0.101	-0.222	-0.203	-0.064	-0.064
qP	0.048	-0.147	0.027	-0.507	0 306	0.161	-0.008	-0.186	0.027	0.377	1	-0.234	-0.140	-0.142	0.190	0.203
qN	0.059	-0.192	0.412	-0.273	-0.043	0.290	-0.229	0.109	0.014	0.101	-0.234	1	-0.317	-0.323	-0.074	-0.106
ETR	0.111	0.103	0.013	0.011	-0.181	0.252	-0.080	-0.080	0.368	-0.222	-0.140	-0.317	1	0.995	0.015	0.095
ETR/A	0.134	0.102	0.034	0.042	-0.180	0.205	-0.083	-0.058	0.361	-0.203	-0.142	-0.323	0.995	1	0.038	0.120
AES	0.102	-0.422	0.030	-0.071	0.044	-0.036	0.060	-0.048	0.290	-0.064	0.190	-0.074	0.015	0.038	1	0.985
ΦPSII	0.095	-0.393	0.031	-0.066	0.037	-0.053	0.033	-0.074	0.322	-0.064	0.203	-0.106	0.095	0.120	0.985	1

Ci; intercellular CO_2 concentration, gs; stomatal conductance, Ci/Ca; ratio of intercellular and atmospheric CO_2, T; transpiration rate, A; net CO_2 assimilation rate; A/Ci, CO_2 assimilation rate/intercellular CO_2 concentration, IWUE; intrinsic water use efficiency, WUEins; instantaneous water-use efficiency, F_0' Minimum fluorescence, F_m'mamixum fluorescence; Fv′/Fm′ maximum quantum efficiency of photosystem II photochemistry, qP, photochemical quenching; qN, non-photochemical quenching, ETR, electron transport rate; ETR/A, relative measure of electron transport to oxygen molecules; AES, alternative electron sink, ΦPSII; the effective quantum efficiency of PSII photochemistry

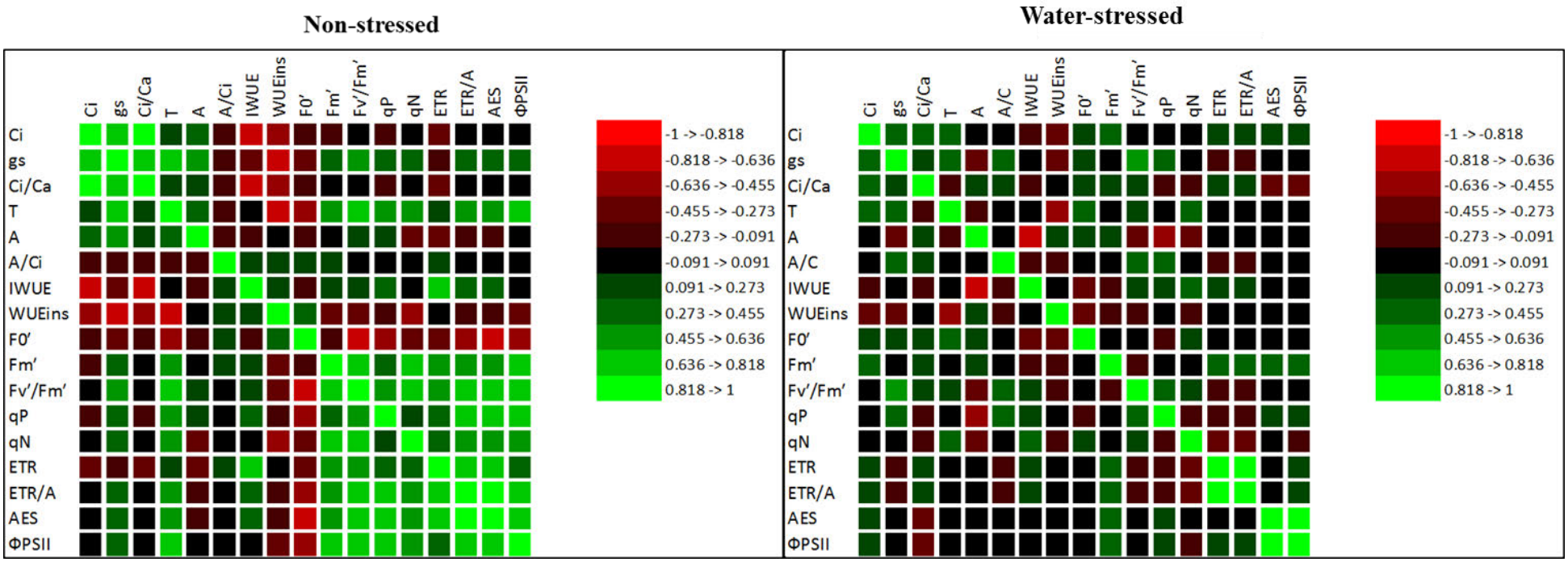

Figure 5.13: Correlation variation among gas exchange and chlorophyll fluorescence parameters under non-stressed and water -stressed.

5.4 Discussions

5.4.1 Emergence

The objective of this study was to determine the effects of water stress on growth and yield performance of 24 Bambara groundnut genotypes and whether variation among the genotypes can be associated with seed coat colour. During the experiments, seeds of the Bambara groundnut genotypes emerged on average after 8-15 days. It was considerably faster than the 28–35 days reported by Mabhaudhi and Modi (2013). According to these results, 16 genotypes exhibited high emergence (>80%) when it came to seedling final emergence. According to Sinefu (2011) and Zondi (2012), most Bambara seeds they used exhibited emergence rates of >80%. The emergence of Acc 121 was the lowest (Table 5.4). Lacer and Nortjé (2019) reported that soils with high mechanical strengths can result in poor emergence of crops like legumes because the hypocotyl is prevented from breaking through the soil surface. In most sandy soils, Karunaratne et al. (2011) found that crusting prevents emergence. When the crust was not reduced, 64.8% of his seeds emerged, while 87.9% of the seeds emerged when the crust was reduced. Several factors such as soil type, water conditions, temperature, and seed coat color can affect seedling performance, according to Travlos et al. (2020). This indicates that tunnel conditions were optimum given the high emergence observed in the study. Seed colour can affect seed quality according to a study conducted by Mandizvo and Odindo (2019). In other words, seed coat color can affect emergence. Furthermore, they found that Bambara groundnut production can be improved by controlling seed coat colour.

5.4.2 Plant growth and yield

Plant growth and yield variables including plant height, leaf length, leaf width, petiole length, total biomass, grain yield, and number of seeds per pot were measured to determine yield performance. Water stress affected the growth of Bambara groundnuts. Despite this, water stress did not affect plant height, leaf length, or leaf width. Despite the fact that the roots of Bambara groundnut can store water continuously, the plant grows in adverse conditions as well (Mayes et al., 2019). Plant petiole growth can be affected by rainfall and solar radiation (Harner et al., 2019). Since plant growth is a turgor driven process, limiting water naturally affects growth in plants (Fatichi et al., 2014). Al Shareef et al. (2014) also reported similar results when they looked at the effects of drought and temperature on Bambara groundnut development and growth. According to their research, landraces behave similarly to some temperatures and water stresses, but they responded differently to other stresses due to specific genetic differences. There was a significant decline in grain yield, number of seeds, and biomass due to water stress. In a study of Bambara groundnut landraces, Zondi et al. (2012)

observed a significant decrease in seeds number. According to Mahabhaudhi and Modi (2013), under stressed conditions total biomass is lower than under irrigated conditions. The stomatal closure increases under water-limited conditions, resulting in decrease water loss via transpiration (Sadok and Sinclair, 2011). In response to water stress, stomatal closure is widely considered the first line of defense (Rodriguez-Dominguez and Brodribb, 2020). Using Bambara groundnut landrace selections, we examined how crop yield was affected by water stress in the current study. A lack of water caused stomatal closure, which results from water stress. An analysis of the PCA biplot between PC 1 and PC 2 was used to explain the phenotypic variation among the genotypes of Bambara groundnuts studied (Figure 5.7) which shows growth parameters and yield performance of Bambara groundnut genotypes. Based on their phenotypic variability, genotypes appeared to behave coupling-wise, regardless of where they were on the axes, which indicates that they contribute significantly to most of the measured traits. For non-stressed and water-stressed conditions, the first four principal components with eigenvalue greater than one explained 72.14% of the cumulative variation and 63.76% for the non-stressed conditions, respectively (Table 5.6). Plant height, petiole length, and total biomass were some of the factors that contributed most to the overall variation. PCA can be used to compare genotypes based on differences in numerical values. This implies traits in the genotypes that can be used to compare them (Adebisi et al., 2013). Under water stress, fewer leaves and reduced petiole length could lead to high grain yield (GY) according to the present study. There is a negative correlation between leaf width (LW) and petiole length (PL), plant height (PH) and total biomass (TB), which suggests that fewer leaves and reduced PL may contribute to high grain yield. (Figure 5.8). A study by Gao et al (2020) reported that the increased number of generative shoots and fruiting nodes negatively affected the harvest index. Furthermore, plant height was negatively correlated with seed number, biomass, and grain yield. In Bambara groundnut, Mabhaudhi and Modi (2013) reported that premature flowering caused early maturation and decreased seed yields when water stress was present. In determining genetic divergence and its nature, clustering gives a very convincing and significant indicator (Barreiro and Quintana-Murci, 2010). The cluster analysis presented as a dendrogram for 24 Bambara groundnut genotypes and the drought indexes used to calculate them (Figure 5.5) identified different clusters indicating associations between these genotypes: Acc 55, Acc 87, Acc 200, Acc 25, Acc 105, Acc 61, Acc 121, and Acc 82 which showed a higher level of drought tolerance, as indicated by dendrograms in Figure 5.5. Few genotypes (Acc 95, Acc 117, Acc 100, Acc 177, and Acc 150) were sensitive to water stress. In order to

categorize accessions based on their relationships, we grouped them into five major clusters (Figure 5.5).

5.4.3 Gas exchange and chlorophyll fluorescence parameters

In order to identify drought tolerant cultivars for crop production, it is essential to understand the mechanisms of alterations to water limited conditions (Fang and Xiong, 2015). Based on leaf gas exchange and chlorophyll fluorescence parameters, we investigated drought tolerance traits of diverse Bambara groundnut genotypes to classify the best performing genotypes for drought tolerance improvement programmes. According to the current study, water stress significantly decreased stomatal conductance (gs), intercellular and atmospheric CO_2 concentrations (Ci/Ca), minimum and maximum fluorescence (F_0') and (Fm′), alternative electron sinks (AES), and the effective quantum efficiency of PSII photochemistry (ΦPSII). A study by Mashilo et al. (2017) which investigated drought tolerance in selected bottle gourd reported similar results. During drought, the photosynthetic processes are impaired mainly through non-stomatal means (Mashilo et al. 2017). Based on the results of these experiments, it appears that there is a certain threshold level at which stomatal conductance should decrease so that WUE won't have an effect on Bambara leaves among genotypes, as reported by Singh and Reddy (2011). According to their study, the decline in gs to 0.04 $molm^{-2}S^{-1}$ resulted in a rise in IWUE in cowpea. Water stress can also enlarge the oxygenase activity of the RuBP carboxylase (Rubisco), decreasing carboxylation effectiveness (Parry et al., 2010). The level of transpiration rate reduction was greater for some genotypes, but not all genotypes were significantly affected by water stress. Plant transpiration rates are influenced by environmental conditions and water stress (Pereira et al., 2016). The moisture loss rate increases when conditions are hot, the air is dry, and too much light is present (Ouldboukhitine et al., 2014). Intercellular CO_2 was non-significant among all genotypes, and it was not affected by water stress. This means Bambara groundnut genotypes have fewer stomatal closures. Acc 179 showed increased values of Fm′ under water stress conditions compared to other genotypes in the same treatment. In a study of high light intensity and water stress, Hazrati et al. (2016) reported that decreases in Fm′ can be attributed to the neutralization of proteins in the chloroplast structure. Fv′/Fm′ measurements showed that most genotypes were above 0.5 when no stress was applied. In the present study, water stress significantly affected photosystem II photochemistry. In this study, water stress increased IWUE except for Acc 87 genotype. The genotype x water stress interaction on F_0' was non-significant in this study, which indicates none of the 24 Bambara genotypes varied in minimum fluorescence under both non-stress and

water stress conditions. The effects of interaction of drought and water stress on ΦPSII have been investigated widely, showing that drought affect photoinbition which influence ΦPSII (Wang et al., 2014). In addition, several genotypes have been demonstrated to reduce ΦPSII as a mechanism for maintaining photosynthetic under water stress conditions (Ghanbari et al., 2019). The responses of plants to multiple stresses have been reported to be unpredictably complex by a single-factor analysis, and a combination of factors can result in an increase and coinciding effect (Creusot et al., 2020). The genotypes differ significantly in Fv′/Fm′. Preserving Fv′/Fm′ may be a protective mechanism to avoid photoinhibitory injuries, which may allow photosynthesis to resume after water stress has been relieved (Singh and Reddy, 2011). There was a relative decrease in photorespiration in the current study as measured by ETR and ETR/A (Pilon et al., 2018). The ETR is used for comparing plant species treatments in established trials by measuring the volume of photosynthetic movement (George et al., 2018). Under water stress conditions, most genotypes showed a decrease in alternative electron sink (AES). Further, Acc 97 and Acc 61 had significantly higher AES values than other genotypes. Mashilo et al. (2017) found that AES activity was related to the capacity of defensive mechanisms that defend against excessive light damage, such as antioxidants and photorespiration. Under water stress, a positive correlation (Table 5.9) was observed between stomatal conductance and transpiration, suggesting stomatal control significantly affected transpiration rate in Bambara groundnut genotypes (Chai et al., 2016). Due to the drawbacks in RuBP synthesis caused by ATP deficiency, the reduction in net CO_2 assimilation (A) in most genotypes is mostly due to dysfunctions in the biochemical reactions that are involved in CO_2 fixation (Silva et al., 2016; Mashilo et al., 2017; Kapoor et al., 2020). There is a strong correlation between net CO_2 assimilation rate (A) and stomatal conductance (gs) indicating that A is reduced by stomatal closure and weak association indicates that it is decreased by non-stomatal factors (Mathobo et al., 2017; Olsovska et al., 2016). Moreover, the relationship between the intrinsic water use efficiency and the intrinsic CO_2 concentration demonstrates that the intrinsic CO_2 concentration does not modify photosynthesis in Bambara groundnut (Beena et al., 2012). According to Singh and Reddy (2011) and Mashilo et al. (2017), decreased CO_2 assimilation under water stress conditions may cause electron transport to oxygen molecules to intensify. Intercellular CO_2 concentration is negatively correlated with electron transport to oxygen molecules (ETR/A). Under non-stress and water stress conditions, intrinsic water use efficiency (IWUE) and instantaneous water use efficiency (WUEins) were negatively correlated with the ratio of intercellular and atmospheric CO_2 (Ci/Ca). Further, intercellular

CO_2 concentration (Ci) was also negatively correlated with IWUE and WUEins. Water-use efficiency is described as a negative function of Ci/Ca ratio (Condon, 2002). A number of factors can influence the IWUE, including evaporation, irrigation, soil depth, and texture. Maximum fluorescence (Fm′) is negatively correlated with non-photochemical quenching (qN) under water stress condition indicating an increase in Fm′ results in decreased heat (Shin et al., 2021).

Principal component analysis under water stress conditions showed that IWUE, ETR, A/Ci, Fm', qP, ETR, ETR/A, qP, Fv'/Fm', gs, T, A/Ci, Ci/Ca, qN, ΦPSII, and AES were positively correlated with PC 1 under non-stressed condition (Figure 5.14). According to Fatimah et al., 2020, this suggests that Bambara genotypes showed variations in physiological responses under water stress conditions. Principal component analysis identified drought tolerant Bambara groundnut genotypes such as Acc 177, Acc 199, Acc 197, Acc 151, Acc 75, Acc 184, and Acc 64, which were grouped together because of their high AES, gs, A and ETR/A values and Acc 200, Acc 97, Acc 175, Acc 25, Acc 100, Acc 121, Acc 87, Acc 61, and Acc 131, which grouped based on high values of Fv'/Fm', qN, Fm′ and F_0'. These results confirm those of the following authors who have reported effectiveness of the physiological parameters to estimate drought tolerance of landraces (Sánchez et al., 2018; Mashilo et al., 2017; Liu et al., 2018; Pereira et al., 2016). We have identified 16 genotypes in the present study which can be recommended for smallholder production in drought prone areas in South Africa. These genotypes also have the potential to supply plant genetic resources for Bambara groundnut crop improvement programmes.

5.5 Conclusion

Using 24 Bambara groundnut genotypes, this study evaluated emergence, growth, yield, leaf gas exchange, and chlorophyll fluorescence during water stress. Most of Bambara genotypes used in this study showed good emergence. This has shown that Bambara groundnut genotypes can avoid drought/water stress by reducing water losses through stomatal closure, decreasing petiole length, total biomass, grain yield, and number of seeds in response to reduced water accessibility under water stress. Water stress also reduced some of the gas exchange and chlorophyll parameters which include stomatal conductance (gs), ratio of intercellular and atmospheric CO_2 (Ci/Ca), minimum and maximum fluorescence (F_0' and F_m'), electron transport rate (ETR), electron transport to O_2 molecules (ETR/A), alternative electron sink (AES), and the effective quantum efficiency of photosystem II photochemistry (ΦPSII). In Bambara groundnut genotypes, phenotypic variation in drought tolerance was noted. Principal component analysis and cluster analysis were performed to identify drought tolerant genotypes (Acc 177, Acc 199, Acc 197, Acc 151, Acc 75, Acc 184, Acc 64, Acc 200, Acc 97, Acc 175, Acc 25, Acc 100, Acc 121, Acc 87, Acc 61, Acc 105, Acc 121, Acc 82, and Acc 131) that can be recommended for production in drought areas. Breeders may find it useful to use physiologic parameters, including gs, A, Fm′, Fv'/Fm', qN, and ETR/A to increase drought tolerance in Bambara groundnuts. The Bambara groundnut is also a valuable genetic resource that can be used to study genetic mechanisms related to drought tolerance. Additional studies should be done among those concentrating on the performance of selected genotypes under drought to determine important agronomic, morphological and physiological characters that are useful in crop improvement programmes and improved food security.

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CHAPTER 6: GENERAL OVERVIEW, SUMMARY AND CONCLUSIONS

6.1 General discussion

This study investigated how environmental conditions, germination stage and water stress affect the production of Bambara groundnut (*Vigna subterranea* (L.) Verdc) in South Africa. In this study, we described and analysed Bambara groundnut, especially its importance to smallholder farmers in Africa. A report by Mohammed et al. (2016) highlighted that about 80% of farmers rely on cultivation of unimproved landraces. As a result, Bambara groundnut is considered an underutilised crop in South Africa due to its low yields. To improve Bambara groundnut, farmers need good varieties with high germination, high field emergence, drought tolerance and vigorous growth in different production environments. These aspects lead to good plant stand and yield. Each chapter in this study served to fulfil the above factors.

There is evidence in the literature that the use of unimproved landraces leads to low yields especially in South Africa. Previous reports on different landraces suggested that low yields in Bambara may be caused by water stress, environmental factors such as rainfall, humidity and weather conditions (Unigwe et al., 2016; Sinefu et al., 2011; Chai et al., 2016; Mabhaudhi and Modi, 2013). Mandizvo and Odindo (2019) emphasised that seed quality can be a valuable initial criterion for crop performance selection, therefore, it is very important to evaluate Bambara at germination stage. The objective of this study was to estimate the extent of agronomic and physio-locational variation in Bambara groundnut that could be helpful in selection and identification of potential Bambara groundnut lines for improvement programmes in South Africa. To achieve this, the study took an approach that involved the following: (i) by assessing the phenotypic variability of 24 Bambara groundnut genotypes, we were able to determine the extent of genetic variability and relationship among the traits of Bambara groundnut accessions, (ii) screening Bambara groundnut genotypes for drought tolerance at germination stage under simulated drought conditions, and (iii) evaluating the effects of water stress on 24 Bambara genotypes in terms of yield and growth.

The first objective (Chapter 3) provided an answer to the question: “why Bambara groundnut production varies in different production environments?”. In this chapter, it was shown that variations in environmental conditions such as rainfall and temperature affect the growth and yield of Bambara groundnut. The genotypes in this study showed significant differences in phenotypic traits. Ukulinga site proved to be a better location for growing Bambara groundnut. However, genotypes Acc 179, Acc 184, Acc 150, Acc 190, Acc 25, Acc 199, Acc 105, Acc 82,

Acc 87 and Acc 97 were associated with high grain yield traits at Brits location with yield ≤500 kg/ha. Bambara groundnut grows best in climates that have adequate sunshine, high temperature and sufficient rainfall (Mabhaudhi and Modi, 2013).

The second objective (Chapter 4) has shown the importance of screening Bambara groundnut for drought stress at the germination stage. Sinefu (2011) emphasised that a combination of low germination and poor seed quality can lead to poor plant stand. In the current study, thirteen genotypes (Acc 25, Acc 87, Acc 97, Acc 100, Acc 117, Acc 82, Acc 184, Acc 51, Acc 131, Acc 175, Acc 177, Acc 179 and Acc 199) were identified as drought tolerant at germination stage. These genotypes showed significant potential to improve drought tolerance through Bambara breeding programmes.

The third objective (Chapter 5) proved that water stress can affect the physiological and phenological traits of Bambara groundnut. Water stress caused significant (p<.001) effects on PL, TB, GY, gs, Ca/Ci, IWUE, F_0', Fm', ETR, ETR/A, Fv'/Fm', and ΦPSII. Water stress is a limiting factor in most legumes. Al Shareef et al., 2014 reported that it is difficult to decouple the effects of drought in the field.

6.1 Conclusion

More research is needed to improve the cultivation of Bambara groundnut in South Africa. To achieve this, researchers need to gain a better understanding of the physiology, morphology, environmental factors and drought tolerance. Currently, more research is needed to understand the effects of drought tolerance and different environmental conditions, especially to identify high yielding genotypes under these conditions and desirable agronomic traits for screening Bambara groundnut. Based on our findings in this study, we concluded that drought stress has an impact on the growth, development and yield of Bambara groundnut.

6.2 Recommendations

To improve the production of Bambara groundnut, it is necessary to study the factors affecting its growth and productivity. The analysis of these factors will also be helpful in improving food and nutrition security in South Africa, especially in these difficult times that our country is experiencing. It is recommended that the experiments be repeated with more locations and seasons using accessions of Bambara groundnut landraces from the ARC genebank that are of high quality for high production.

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Appendix A: Analysis of variance (ANOVA) for chapter 3

Table 1: ANOVA table for plant height (PH) at Brits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	324.303	14.1	1.99	0.023
Residual	46	325.12	7.068		
Total	71	652.142			

Table 2: ANOVA table for plant canopy (PC) at Ukulinga

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	824.88	35.86	2.02	0.02
Residual	48	854	17.79		
Total	71	1678.88			

Table 3: ANOVA table for hundred seed weight (HSW) at Brits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	10621.6	461.8	3.17	<.001
Residual	46	6694	145.5		
Total	69	15446.5			

Table 4: ANOVA table for grain yield per plot (GYPlot) at Brits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	2759483	119978	3.61	<.001
Residual	46	1527535	33207		
Total	71	4341861			

Table 5: F-Test Two-Sample for Variances

	Brits	Ukulinga
Mean	483.683333	1069.012
Variance	39992.5987	58501.2
Observations	24	24
df	23	23
F	0.68362014	
P(F<=f) one-tail	0.18418524	
F Critical one-tail	0.49641961	

Appendix B: Analysis of variance (ANOVA) for chapter 4

Table 6: ANOVA table for germination percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	87444.4	3801.9	12.64	<.001
No_of_days	9	997240	110804	368.4	<.001
Treatment	1	26608.4	26608.4	88.47	<.001
Genotypes. No_of_days	207	43302.2	209.2	0.7	0.999
Genotypes. Treatment	23	65139.9	2832.2	9.42	<.001
No_of_days. Treatment	9	4350.6	483.4	1.61	0.109
Genotypes. No_of_days. Treatment	207	33417.7	161.4	0.54	1
Residual	958	288141	300.8		
Total	1439	1566969			

Table 7: ANOVA table for number of seeds germinated (NSG)

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Genotypes	23	874.444	38.019	12.64	<.001
No of days	9	9972.4	1108.04	368.4	<.001
Treatment	1	266.084	266.084	88.47	<.001
Genotypes. No of days	207	433.022	2.092	0.7	0.999
Genotypes. Treatment	23	651.399	28.322	9.42	<.001
No of days. Treatment	9	43.506	4.834	1.61	0.109
Genotypes. No of days. Treatment	207	334.177	1.614	0.54	1
Residual	958	2881.41	3.008		
Total	1439	15669.7			

Table 8: ANOVA table for seedling fresh mass (SFM)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	0.13399	0.00583	0.64	0.888
Treatment	1	1.73356	1.73356	190.83	<.001
Genotypes. Treatment	23	0.19275	0.00838	0.92	0.57
Residual	94	0.85393	0.00908		
Total	143	3.15022			

Table 9: ANOVA table for germination velocity index (GVI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	30.8315	1.3405	5.35	<.001
No_of_days	9	89.0623	9.8958	39.49	<.001
Treatment	1	19.9866	19.9866	79.76	<.001
Genotypes. No_of_days	207	29.9325	0.1446	0.58	1
Genotypes. Treatment	23	28.2238	1.2271	4.9	<.001
No_of_days. Treatment	9	13.2846	1.4761	5.89	<.001
Genotypes. No_of_days. Treatment	207	23.0813	0.1115	0.44	1
Residual	958	240.064	0.2506		
Total	1439	494.863			

Table 10: ANOVA table for mean germination time (MGT)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	8.74444	0.38019	12.64	<.001
No_of_days	9	99.724	11.0804	368.4	<.001
Treatment	1	2.66084	2.66084	88.47	<.001
Genotypes. No_of_days	207	4.33022	0.02092	0.7	0.999
Genotypes. Treatment	23	6.51399	0.28322	9.42	<.001
No_of_days. Treatment	9	0.43506	0.04834	1.61	0.109
Genotypes. No_of_days. Treatment	207	3.34177	0.01614	0.54	1
Residual	958	28.8141	0.03008		
Total	1439	156.697			

Appendix C: Analysis of variance (ANOVA) for chapter 5

Table 11: ANOVA table for emergence percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	110877	4820.8	18.66	<.001
No of days	15	1099796	73319.7	283.82	<.001
Genotypes. No of days	322	167903	521.4	2.02	<.001
Residual	718	185485	258.3		
Total	1080	1566811			

Table 12: ANOVA table for mean emergence time (MET)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	64.8238	2.8184	19.60	<.001
No of days	14	771.1476	55.0820	383.10	<.001
No of days. Genotypes	321	102.6243	0.3197	2.22	<.001
Residual	719	103.3784	0.1438		
Total	1079	1043.3076			

Table 12: ANOVA table for petiole length (PL)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	240.803	10.470	2.26	0.004
water stress	1	6.903	6.903	1.49	0.225
Genotypes. Water stress	23	128.008	5.566	1.20	0.265
Residual	83	383.660	4.622		
Total	132	724.088			

Table 13: ANOVA table for total biomass (TB)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	2178.85	94.73	1.34	0.168
water stress	1	12951.76	12951.8	183.36	<.001
Genotypes. Water stress	23	1209.63	52.59	0.74	0.786
Residual	83	5862.62	70.63		
Total	132	20527.55			

Table 13: ANOVA table for grain yield (GY)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	696.83	30.3	0.91	0.587
water stress	1	6772.55	6772.55	203.13	<.001
Genotypes. Water stress	23	736.94	32.04	0.96	0.522
Residual	83	2767.32	33.34		
Total	132	9956.95			

Table 14: ANOVA table for number of seeds (NS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	1632.52	70.98	1.08	0.382
water stress	1	14459.69	14459.7	220.45	<.001
Genotypes. Water stress	23	2051.92	89.21	1.36	0.157
Residual	83	5444.09	65.59		
Total	132	22564.81			

Table 15: ANOVA table for stomatal conductance (gs)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	0.09349	0.00407	1.17	0.294
water stress	1	0.182706	0.18271	52.65	<.001
Genotypes. Water stress	23	0.073428	0.00319	0.92	0.573
Residual	83	0.291505	0.00347		
Total	132	0.658597			

Table 16: ANOVA table for the ratio of intercellular and atmospheric CO_2 (Ci/Ca)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	335.84	14.6	0.77	0.757
water stress	1	118.79	118.79	6.26	0.014
Genotypes. Water stress	23	349.01	15.17	0.8	0.722
Residual	83	1574.05	18.96		
Total	132	2447.99			

Table 17: ANOVA table for the intrinsic water use efficiency (IWUE)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	4.95E+09	2.15E+08	1.94	0.016
water stress	1	1.00E+09	1.00E+09	9.01	0.004
Genotypes. Water stress	23	4.93E+09	2.14E+08	1.93	0.016
Residual	83	9.34E+09	1.11E+08		
Total	132	1.75E+10			

Table 18: ANOVA table for the minimum fluorescence (F_0')

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	174704	7596	1.82	0.026
water stress	1	123172	123172	29.52	<.001
Genotypes. Water stress	23	90092	3917	0.94	0.549
Residual	83	350461	4172		
Total	132	678320			

Table 19: ANOVA table for the maximum fluorescence (F_m')

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	3464708	150639	0.84	0.672
water stress	1	1575484	1575484	8.79	0.004
Genotypes. Water stress	23	4453829	193645	1.08	0.383
Residual	83	15048372	179147		
Total	132	24208120			

Table 20: ANOVA table for the electron transport rate (ETR)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	5.86E+13	2.55E+12	0.9	0.598
water stress	1	3.37E+13	3.37E+13	11.9	<.001
Genotypes. Water stress	23	5.44E+13	2.37E+12	0.84	0.679
Residual	83	2.38E+14	2.83E+12		
Total	132	4.15E+14			

Table 21: ANOVA table for the electron transport to O_2 molecules (ETR/A)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	1.75E+10	7.63E+08	0.74	0.795
water stress	1	1.09E+10	1.09E+10	10.55	0.002
Genotypes. Water stress	23	1.81E+10	7.87E+08	0.76	0.769
Residual	83	8.91E+10	1.04E+09		
Total	132	1.30E+11			

Table 22: ANOVA table for the alternative electron sink (AES)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	0.003446	0.00015	0.73	0.805
water stress	1	0.001281	0.001281	6.22	0.015
Genotypes. Water stress	23	0.00334	0.000145	0.71	0.828
Residual	83	0.017707	0.000206		
Total	132	0.025591			

Table 23: ANOVA table for the effective quantum efficiency of photosystem II photochemistry (ΦPSII)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	23	8.0421	0.3497	0.78	0.748
water stress	1	3.2477	3.2477	7.22	0.009
Genotypes. Water stress	23	6.9673	0.3029	0.67	0.858
Residual	83	37.7688	0.4496		
Total	132	56.8493			