Root Trait Variation and its Contribution to Drought Tolerance in Bambara Groundnut (*Vigna subterranea* (L.) Verdc.)

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Dedication

To my beloved mother Colleta Chamboko, thank you for being my guiding star.

"Since everyone requires food,

the agricultural field will always be appealing"

- Dr. W.T Chinembiri

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List of abbreviations

ANOVA - Analysis of variance

BD - Branching density

BI – Branching intensity

BN - Branching number

CFF-FRC - Crops For the Future-Field Research Center

C_i - Intracellular CO^2 concentration

E - Rate of transpiration

FSF - Future Smart Food

GGE - Genotype and genotype by environment

g_s - Leaf stomatal conductance

H^2 - Broad-sense heritability

HTC - Hard-to-cook

masl - Meters above sea level

NoL- Number of leaves

NUS - Neglected underutilised specie

PCA - Principal component analysis

P_n - Net photosynthetic rate

PPFD - Photon flux density

PVC - Polyvinyl chloride

R:S – Root to shoot ratio

RDia - Root diameter

RDW - Root dry weight

RH – Relative humidity

RL - Root length

RLD - Root length density

RSA - Root system architecture

RV - Root volume

SA - Surface area

SDW - Shoot dry weight

SH - Shoot height

TRL - Tap root length

VPD - Vapour pressure deficit

Publications Arising from This Work

Refereed Journal Articles Included as Part of This Thesis:

1. **Mateva, K.I**., Chai, H.H., Mayes, S., Massawe, F. (2020). Root Foraging Capacity in Bambara Groundnut (*Vigna Subterranea* (L.) Verdc.) Core Parental Lines Depends on the Root System Architecture During the Pre-Flowering Stage. Plants 2020, 9, 645. https://doi.org/10.3390/plants9050645

2. **Mateva, K. I**., Chai, H.H., Mayes, S., Massawe, F. (2022). Natural Genotypic Variation Underpins Root System Response to Drought Stress in Bambara Groundnut [Vigna subterranea (L.) Verdc.]. Frontiers in Plant Science, 13, Article 1664-462X. https://doi.org/10.3389/fpls.2022.760879

3. **Mateva, K.I**., Tan, X.L., Halimi, R.A., Chai, H.H., Makonya, G.M., Gao, X., Shayanoyako, A.I.T., Ho, W.K., Tanzi, A.S., Farrant, J., Mabhaudhi, T., King, G., Mayes, S., Massawe, F. Bambara Groundnut (*Vigna subterranea* (L.) Verdc). (Under review: Elsevier. In: Farooq M., Siddique KHM (eds) Neglected and Underutilized Crops: Future Smart Food).

4. **Mateva, K.I**., Gao, X., Chai, H.H., Mayes, S., Massawe, F. Screening Promising Drought Resistant Early-Generation Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) Lines Based on Shoot and Root System Traits Under Drought Stress. (Under review: Frontiers in Plant Science. Root Development - Proceedings from the joint Rooting 2021 and 11th Symposium of the International Society for Root Research).

Other Related Refereed Journal Articles Not Included as Part of This Thesis:

5. Gao, X., Chai, H. H., Ho, W. K., Kundy, A. C., **Mateva, K. I**., Mayes, S., Massawe, F. (2021). Genetic linkage map construction and identification of QTLs associated with agronomic traits in bambara groundnut (Vigna subterranea (L.) Verdc.) using DArTseq-based SNP markers. Food and Energy Security, 00, e353. https://doi.org/10.1002/fes3.353

6. Andlib Z., Makonya G.M., **Mateva K.I**. (2021). Understanding Gender Dimensions of Disaster Impacts on Agriculture in the Global South. In: Djalante R., Bisri M.B.F., Shaw R. (eds) Integrated Research on Disaster Risks. Disaster Risk Reduction (Methods, Approaches and Practices). Springer, Cham. https://doi.org/10.1007/978-3-030-55563-4_15

7. Gao, X., Bamba, A.S.A., Kundy, A.C., **Mateva, K.I**., Chai, H.H., Ho, W.K., Musa, M., Mayes, S., Massawe, F. (2020). Variation of Phenotypic Traits in Twelve Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) Genotypes and Two F_2 Bi-Parental Segregating Populations. Agronomy, 10, 1451. https://doi.org/10.3390/agronomy10101451

8. Mayes, S., Ho, W. K., Chai, H. H., Gao, X., Kundy, A. C., **Mateva, K.I**., …Azam-Ali, S. N. (2019). Bambara groundnut: an exemplar underutilised legume for resilience under climate change. Planta. https://doi.org/10.1007/s00425-019-03191-6

9. Mustafa, M., **Mateva, K.I**., Massawe F. (2019). Sustainable Crop Production for Environmental and Human Health – the future of agriculture. Annual Plant Reviews Online 2019 Volume 2, Issue 4**.** https://doi.org/10.1002/9781119312994.apr0700

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root length; RLD (60-90cm) – root length density in the 60-90cm soil depth; GY - grain yield column^{-1}; 100-SW – weight of 100 seeds. Trait names might overlap due to the statistical package used. .171

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ABSTRACT

Bambara groundnut (*Vigna subterranea* (L) Verdc), is an exemplar neglected African grain legume that thrives under strikingly contrasted environments relative to other grain legumes. Originating in West Africa, its distribution spans across aridity gradients from tropical dry climates in Senegal and Kenya, respectively, down to arid and semi-arid regions in sub-Saharan Africa. This is on soils more or less poor in nutrients and formed under variable pedoclimatic conditions. In these contrasting habitats, it is generally agreed that bambara groundnut has diversified due to domestication from its wild relative, *Vigna subterranea* var. spontanea (Harms) Hepper, as a result of steady changes through natural and artificial selection. Bambara groundnut is a close relative of cowpea (*Vigna unguiculata*) and morphologically fits into the same niche as groundnut (*Arachis hypogaea* L.). The wide distribution in natural environments and ability to tolerate both biotic and abiotic stresses better than cowpea and groundnut, make bambara groundnut an interesting model for examining diversification in response to ephemeral soil water resources. Although important, comprehensive variation assessment on below ground (root) traits in bambara groundnut have not been pursued. The hypothesis was that by focusing on naturally occurring genotypic variation in root system architecture and rooting distribution, bambara groundnut genotypes from dry agroecologies with periodic drought stress had developed root system traits that improved water foraging in deeper soil depths over time. This could be visualised and quantified using a low-cost polyvinyl chloride column (PVC) phenotyping system and image analysis.

To test this hypothesis, morphological variability in root system architecture was characterized in eight bambara groundnut parental lines of varying geographic origin (Gresik, LunT, IITA-686, DodR, S19-3, Tiga nicuru,

Ankpa-4, DipC1). The experiment was conducted over two seasons (2018 and 2019) under fixed rainout shelter at the Crops For the Future-Field Research Center (CFF-FRC) in Semenyih, Malaysia. Results revealed that in deeper (60-90cm) soil depths, genotypes S19-3 and DipC1 from drier regions of Sub-Saharan Africa had longer tap roots and greater root length distribution. Bambara groundnut genotypes from wetter regions in Southeast Asia and West Africa (i.e., Gresik, LunT, and IITA-686), on the other hand, had shallower and more branched root growth closer to the soil surface. Genotypes generally displayed two extremes in root foraging patterns and branching habits i.e., deep-cheap rooting in genotypes sourced from dry regions and shallow-costly rooting in genotypes adapted to higher rainfall areas with shallow soils.

Next, the natural genotypic diversity revealed in the eight genotypes was then investigated to detect adaptive changes in tap root length and root length density in response to periodic drought stress. Genotypes were grown in PVC columns in well-watered and 30-day drought stress (DS) treatments for two seasons (2018 and 2019). DS significantly ($P < 0.05$ - < 0.001) reduced average shoot height, number of leaves, and delayed flowering in 2018 and 2019. In 2018, the average root-to-shoot ratio was significantly higher ($P < 0.001$; 22%) under DS treatment. On average tap root length at 55 days after emergence (DAE) i.e., end of 30-d DS, was reduced by 14% and 22% in 2018 and 2019, respectively, and by 5% and 11% at 105 DAE (50-days of DS recovery) in 2018 and 2019, respectively, with some genotypes in 2018: DodR (55 DAE); LunT and Ankpa-4 (105 DAE) and in 2019: IITA-686 (105 DAE), increasing to measurements comparable to the well-watered (WW) treatment. In 2018 and 2019, root length density in the DS treatment was associated with significant grain yield advantage ($R^2 = 0.27$ and $R^2 = 0.49$) in 2018 and 2019, respectively. This indicates that the various agroecological conditions to which bambara groundnut has been exposed in its natural setting may have induced

phenotypic differentiation to adapt to ecotypic conditions, which may help offset the impact of adverse events like regular drought stress. When looking for superior genotypes, ecotypic distinction can be an interesting aspect to remember.

Finally, root traits such as tap root length and root length density in the 60-90cm soil layer were shown to be beneficial in screening and selecting superior lines from a bambara groundnut population. The population was derived from a cross between two parental lines i.e., S19-3 (maternal) × DodR (paternal). Across replicates, 100-seed weight had the lowest average repeatability (0.62), while high repeatability values were observed for root length density in the 60-90cm soil depth (0.99). Under DS environment (50-d of DS recovery), root length density in the 60-90cm soil depth was strongly correlated ($P < 0.05$ - $P < 0.001$) with shoot traits such as number of leaves (r = 0.69), shoot dry weight (r = 0.78), and shoot height (r = 0.67). This indicates that shoot traits are useful traits that can also be used as proxies to make estimations of root length density. According to a regression analysis, root length density in the 60-90cm soil depth was associated with grain yield (R^2 = 42%; $P < 0.001$). According to biplot analysis, the top three bambara groundnut lines in terms of yield under drought stress were 'Line12', 'Line35', and 'Line41'.

Overall, the work provides a novel and in-depth examination of bambara groundnut below-ground (root trait variation) and its role to drought tolerance. According to this research, bambara groundnut possess differential deep root foraging and density patterns with two extremes i.e., deep-cheap rooting in the genotypes mainly sourced from dry regions and a shallow-costly rooting system in genotypes suited to higher rainfall areas. Farmers have inadvertently selected for these variations over time due to their effect on yield in both dry and wet conditions. Drought tolerance breeding for bambara groundnut will more likely accelerate as a consequence of a better

understanding of root systems and foraging patterns. Selected high yielding lines from the S19-3 (maternal) × DodR (paternal) cross i.e., 'Line12', 'Line35' and 'Line41' — all exhibiting deep and extensive rooting in deeper soil depths, will be advanced as part of the current Future Food Beacon: Bambara Groundnut breeding (BamBREED) research project. Elite lines generated from this breeding programme could be registered as improved varieties and released to the general public for cultivation in drought-prone areas. This is projected to boost dietary diversity and significantly increase the nutritional value of people's diets.

Keywords: Bambara groundnut (*Vigna subterranea* (L) Verdc), root system architecture, tap root length, root length density, natural genotypic variation.

CHAPTER 1 : General Introduction and Background

1.1 Climate Change and Drought Stress

Climate change is posing a serious threat to global food sustainability (Lesk et al. 2016). Drought and heat stress have been the most significant limiting factors to crop production and, consequently, food production, as a result of climate change. Droughts are becoming more common all over the world as a result of decreased precipitation and altered rainfall patterns (Lobell et al. 2011). Droughts have a significant influence on crop yields due to detrimental effects on plant development, physiology, and reproduction (Barnabás et al. 2008). According to Mourtzinis et al. (2015), in the United States, soybean yields decreased by 2–4% during the growing season, resulting in a loss of US$11 billion. In addition, increased drought stress has been projected to reduce the areas suitable for bean (*Phaseolus vulgaris*) production (Beebe et al. 2011). In reality, drought stress has resulted in a 300% yield gap for legume crops grown by smallholder farmers in developing countries located in the tropics and subtropics where irrigation and high inputs are not available (Andrews and Hodge 2010).

A lack of crop diversity exacerbates harmful environmental impacts in existing cropping systems, with climate-related yield instabilities (Reckling et al. 2018). Stress adaptation processes have emerged in plants as a result of natural selection over long periods of time. Wild legume relatives often have stronger drought stress-resistance characteristics (Mickelbart et al. 2015). To increase production under stress conditions, these beneficial natural variations must be incorporated into current elite germplasm. This can be accomplished by determining beneficial natural variation, and then incorporating these natural variants into elite varieties. In the past, most drought phenotyping activities on field crops were concentrated on above ground shoot traits like yield, shoot vigour and disease resistance (Paez-Garcia et al. 2015), while below ground root phenotyping received little consideration. More recently, Saoirse

and colleagues (Saoirse et al. 2020) highlighted that the technical difficulties of accessing the soil while phenotyping root traits, particularly using non-destructive methods, is the main reason for this exclusion. Although the root system is critical for plant function, root phenotyping has only recently become more common. This crucial position is exemplified by the fact that plants can translocate 20–50% of total fixed carbon to their root system, and root traits are also highly significant from an agronomic perspective (Kuzyakov and Domanski 2000).

1.2 Ideotype Development

The optimal phenotype for a given environment is called an ideotype (Donald 1968). Matching phenology to the environment is a key component of drought stress adaptation, since it helps to prevent drought during crucial growth stages like flowering (Ullah et al. 2020). Shoot architecture and growth determinism have been ideotype targets in beans (Kelly 2001). For example, the main traits of an ideal bean have been described by Brothers and Kelley (1993), which includes an upright growth pattern, steep branch angles, a low number of seeds pod^{-1} and pods $plant^{-1}$. Donald (1968) proposed a wheat (*Triticum aestivum*) ideotype with small leaves that continues to influence wheat breeding today. These cases, as well as the majority of reported ideotypes, are based solely on aboveground (shoot) morphological characteristics.

In theory, an ideotype may also be defined based on below-ground (root) attributes. Lynch (2013) suggested the maize (*Zea mays*) ideotype as an example of an ideotype that contains a lot of root system architectural details. Considering that the intended environment for the maize ideotype is under dry rainfed conditions, a steep (sharp root angle), deep (long tap root), and cheap (few lateral branching) ideotype can enhance efficient soil resource foraging.

However, in cases where water is not limiting and soil nutrient resources such as phosphorus (P) is distributed in the topsoil, the same ideotype is unsuitable. A shallow and expensive (profuse lateral branching) ideotype can take full advantage of such an environment (Lynch 2013). These ideotype differences have been observed in barley (*Hordeum vulgare*) (Jia et al. 2019), wheat (Alahmad et al. 2019) and rice (*Oryza sativa*) (Uga et al. 2013). Ideotype breeding assumes that by combining favourable characteristics, breeders can engineer optimal plants for water and nutrient resource foraging.

1.3 Problem Statement

Climate change is already impacting agricultural development and food security, and unless immediate action is taken, millions of people will go hungry (Lesk et al. 2016). The global increase in drought stress is expected to reduce world production of particularly major crops such as maize, wheat, rice, and soybean (Zhao et al. 2017). This is the time to concentrate on diverse approaches to food safety and nutritional sustainability (Chivenge et al. 2015; Mustafa et al. 2019a). Currently, the world depends mainly on three plant species i.e., maize, wheat and rice (Ray et al. 2013), and these account for more than 60% of what we all eat (Zhao et al. 2017). In fact, a heavy reliance on a few major cereal crops can also be linked to not only monotonous diets, but diet-related diseases, mostly originating from micronutrient deficiencies (Hannah and Max 2017). For example, cereals contain relatively small amounts of proteins and micronutrients providing on average about 9g protein, 10-140mg calcium and 0.5mg iron $100g^{-1}$ serving (McKevith 2004). This is lower compared to grain legumes and knowledge of this alone should validate the need to not only diversify our food basket but complement current cereals-based food systems (Mustafa et al. 2019b).

Diversification — of both agricultural production systems and diets, is a practical and sustainable approach to address these challenges and to improve global food and nutritional security (Mustafa et al. 2019b). Some suggestions have been made to make use of indigenous and underutilised species (Massawe et al. 2015; Mabhaudhi et al. 2016b). Neglected and underutilized species (NUS) are staple food crops that have normally slight monetary significance and are not considered favourably by the plant breeding community (Foyer et al. 2016). NUS have been published as having resistance to numerous environmental shocks including drought (Mabhaudhi et al. 2016a). As opposed to cereal crops, many legume crops are known for their ability to resist water deficit stress and high temperatures, while fixing atmospheric nitrogen (Osakabe et al. 2014). Underutilized legumes combat incursions of new diseases and pests, survive in hostile or deficient soil environments, have lower greenhouse gas emissions and contribute to carbon sequestration in the soil (Peoples et al. 2009). In addition, these underutilized crops are considered as potential candidate crops against projected climate change and its consequences e.g., food insecurity (Mayes et al. 2019b).

Bambara groundnut is an interesting model underutilized grain legume that flourishes under strikingly contrasted environments relative to other grain legumes. Not only is bambara groundnut regarded as an ideal candidate for food security in the changing climate (Chivenge et al. 2015) but also exhibits different drought tolerance mechanisms, allowing it to tolerate a range of environmental conditions and durations of stress. This makes it an important crop for promotion in areas that are currently drought prone as well as an important future crop in areas, where climate change projections show an increased frequency and intensity in droughts. For example, Mabhaudhi et al. (2018) projected that yield and water productivity of bambara groundnut will increase by ~37.5% and 33%, respectively, in response to projected climate

change in South Africa. Furthermore, Mabhaudhi et al. (2016a) also demonstrated that under climate change, the areas suitable for bambara groundnut production would also expand in South Africa, confirming the resilience of the crop under climate change.

Studies to understand the underlying mechanisms of drought-resistance in bambara groundnut have been limited and largely focused and elucidated by above ground shoot morpho-physiological studies (Collinson et al. 1997; Collinson et al. 1999; Mabhaudhi and Modi 2013; Al Shareef et al. 2014; Chibarabada et al. 2015a). Furthermore, these studies have demonstrated that the degree of drought resistance varies between landraces and their place of origin; the severity and velocity of the drought and phenological stage effected (Collinson et al. 1997, 1999; Mabhaudhi and Modi 2013; Al Shareef et al. 2014; Chibarabada et al. 2015b). While bambara groundnut has demonstrated drought resistance, there are still substantial gaps that must be filled via crop improvement before this trait may be of greater value to farmers.

To completely comprehend and manage the plant's drought resistance, the spectrum of both above-and belowground variation found in bambara groundnut germplasm must be investigated (Mayes et al. 2019a). Unlike the former, belowground plant root research has been limited mostly by the inability to get access to the rhizosphere (Kuijken et al. 2015). Given that root characteristics influence water acquisition and, hence, yield (Kashiwagi et al. 2005), it is expected that natural variability in root traits of various bambara groundnut genotypes might be the missing link. This might be the first step toward completely understanding the superior drought tolerance of bambara groundnut compared to other crop legumes.

1.4 Research Aim

The overall aim was to investigate the response of diverse bambara groundnut genotypes root system architecture (RSA) to drought stress. The research project utilised a low-cost soil-filled polyvinyl chloride (PVC) column phenotyping system and image analysis software to quantitatively compare naturally occurring root trait variation.

1.4.1 The Overarching Hypothesis:

Bambara groundnut genotypes from dry agroecologies with periodic drought stress have throughout their years of cultivation in the same agroenvironment, developed root system traits that improve water foraging in deeper soil depths, which can be visualised and quantified using a low-cost PVC column phenotyping system and image analysis.

1.4.2 The following questions were addressed to achieve the overall aim:

1. Are there any differences in root system architecture (RSA) as plants approach a critical growth stage and is this RSA consistent with the environment the parental genotype was sourced from?

2. What are the effects of drought stress on deep rooting and root length density and does this determine different genotypes root foraging capacity?

3. Can shoot and root system traits be successfully used to identify promising lines for drought breeding?

1.5 Structure of the Thesis

This thesis is presented in paper format and is composed of a published and submitted papers. Each paper that is included as an experimental chapter has all the associated information relevant for that experiment. The thesis consists of seven chapters. **Chapter One**, provides a general introduction to the research study, its rationale, goals, hypothesis, and a summary of the thesis structure. **Chapter Two** reviews the literature on the origin, production, and drought tolerance potential of bambara groundnut. In addition, the chapter examines the literature on root system architecture under drought stress, root phenotyping methodologies, root image analysis, and data analysis. **Chapter Three** covers materials and methods that general apply to experiments conducted during the research work. Materials and methods that are more applicable to certain experiments are listed and described in more detail in specific sections in the chapters that present the experiment(s). **Chapter Four** presents results from a polyvinyl chloride (PVC) column system used to characterize the morphological variability in root system architecture (RSA) during the pre-flowering growth stage. This chapter was published in Plants (Mateva et al. 2020. 9, 645). **Chapter Five** visualised deep rooting profile and root length density in bambara groundnut in response to drought stress. Quantification of root system differences was possible. This chapter was published in Frontiers in Plant Science (Mateva et al. 2022. 13, 1664-462). **Chapter Six** reports the role of shoot and root system traits in identifying promising lines for future drought breeding work. This paper is currently under review (Frontiers in Plant Science) for publication. Lastly, **Chapter Seven** provides a general discussion and draws together the key conclusions and implications; a section on possible further work is included.

CHAPTER 2 : Literature Review

2.1 Summary

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) represents an untapped potential for developing robust food systems. This promising but underutilised African grain legume has high nutritional qualities comparable to popular and widely consumed legumes, as well as exceptional resistance to biotic and abiotic stresses. In addition, the crop can grow on a range of soils, fix atmospheric nitrogen, and enhance soil fertility, making its production truly climate-resilient. Third to peanut (*Arachis hypogaea* L) and cowpeas (*Vigna unguiculata* L. Walp.) in terms of production and consumption in sub-Saharan Africa, bambara groundnut is set to increase in importance as current food production systems become more diverse, and this is also evident in the steady increase in yield and area harvested across the west, east and southern Africa over the past 25 years. Despite these relevant characteristics, the potential of bambara groundnut in improving food systems is hindered by a number of challenges. Over the years, research efforts have led to a more optimistic outlook for bambara groundnut's ability to overcome these challenges. For example, substantial research has been conducted to uncover essential systems responsible for drought stress, with a special emphasis on shoot phenotyping. Root phenotyping on the other hand, is equally important as shoot phenotyping since the root system is responsible for the majority of the plant's success. To date, many phenotyping methods and tools that facilitate the acquisition, handling, and processing of phenotypic data have been created, redefining the landscape of not only shoot and root phenotyping but whole plant phenotyping in bambara groundnut. However, a concerted policy push by African governments, with technical and financial support from regional organisations, is still required to boost research uptake to realise the crop's full potential. The chapter provides comprehensive evidence of bambara groundnut as a "future smart food". It details the challenges that need to be addressed and production systems thinking solutions to harness the full potential of this less-mainstream crop.

Keywords: Bambara groundnut; Climate change; Food systems; Future smart food (FSF); Neglected underutilised species (NUS); Root phenotyping

2.2 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) — often called a "complete food" due to its high nutritional value — is an excellent example of a neglected and underutilised species (NUS) grown mainly in tropical dry and semi-arid climates of Africa. Originating in West Africa, its distribution spans across aridity gradients from dry tropical climates in Senegal and Kenya, respectively, down to arid and semi-arid regions in sub-Saharan Africa. Bambara groundnut is propagated from locally adapted landraces rather than true varieties (Massawe et al. 2005). The crop is mostly grown in a range of soils: from clay loams to deep sands — all of which are deficient in most essential nutrients and formed under varying pedoclimatic conditions. Farmers in these areas prize bambara groundnut for its resilience to drought (Hillocks et al. 2012; Adzawla et al. 2016) and ability to fix atmospheric nitrogen and increase soil fertility, especially in intercropping systems (Dakora 1998; Egbe et al. 2013).

As the climate crisis worsens and environmental stressors rise, there is a growing need for agricultural diversification by supporting crops that can be cultivated in the harshest of conditions (Chivenge et al. 2015; Massawe et al. 2016; Mustafa et al. 2019a). Acknowledging the role of legumes in responding to nutritional and climate challenges, the United Nations (UN) Sustainable Development Goal (SDG 2): Zero hunger — advocates for sustainable food production systems and resilient agricultural practices. Indeed, bambara groundnut is an important crop in the tropical dry and semi-arid climates of Africa (especially in Nigeria, Burkina Faso, Niger, Cameroon, Mali, Zimbabwe, Democratic Republic of Congo and Togo), with global production concentrated in the aforementioned African countries (Figure 2-1A; FAOSTAT 2019). However, although bambara groundnut output has increased by more than 57% from 98,198 tonnes harvested from 151,039 hectares (ha) of land in 1994 to 228,920 tonnes harvested from 370,953 ha of land in 2019 (FAOSTAT 2019),

productivity remains low (Figure 2-1B), due to insufficient financial investment in research and development.

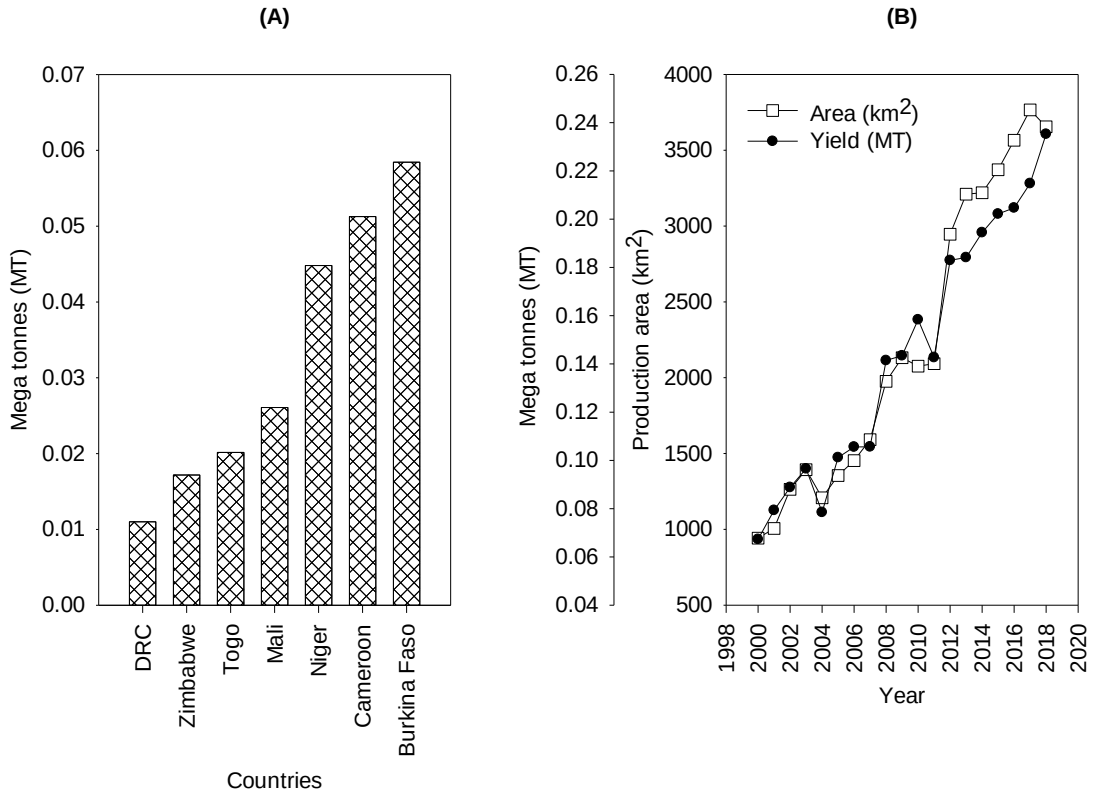

Figure 2-1: (A) Bambara groundnut yield and area harvested in Africa from the year 2000 – 2019 and (B) Bambara groundnut production of top ten countries in 2019. FAOSTAT (2019) data file is limited in information on Nigeria, which is a significant producer of bambara groundnut.
Source: FAOSTAT, (2019).

The Future Food Beacon: Bambara Groundnut breeding (BamBREED) research project at the University of Nottingham has been making progress in improving productivity. Using the decoded genome of the bambara groundnut (Chang et al. 2019; Salazar-Licea et al. under review: Springer Nature), multidisciplinary techniques, and strategic collaboration with universities, institutes, and farmers in South Africa and Ghana, high-yielding bambara groundnut varieties with increased adaptability and consumer demand-driven traits are now being developed.

Supporting bambara groundnut as a Future Smart Food (FSF) starts with quantifying the crop's value in terms of complementing and closing the gap on food and nutrition security to improve livelihoods (Feldman et al. 2019; Mayes et al. 2019a; Li and Siddique 2020; Khan et al. 2021a). This chapter demonstrates this and more, lending credence in bambara groundnut investment and production systems thinking by governments and food processors. Given bambara groundnut's versatility, now is the time to harness the full potential of this less-mainstream crop.

2.3 The Plant

Bambara groundnut, also known as roundnut, is an annual herbaceous plant (Bamshaiye et al. 2011) that gets its name from an area near Timbuktu, Mali. Vernacular names generally vary from one region to another and from one ethnic group to another. For example, bambara groundnut is named "Okpa" "Epiroro" and "Gurjiya" or "Kwaruru" in Ibo, Yoruba and Hausa, respectively in Nigeria, "Njugu mawe" in kiSwahili, "Nyimo" and "iNdlubu" in chiShona and isiNdebele, respectively in Zimbabwe, "iziNdlubu" in isiZulu and "Jugo beans", both in South Africa, "Mandubi d'Angola" in Portuguese in Brazil and "Thua rang" in Thailand, "Kacang manila" in Malaysia and "kacang bogor" in Indonesia.

The plant resembles peanut (groundnut, *Arachis hypogaea*) in growth habit since it forms a crown of leaves emerging from branches above the soil level (Figure 2-2A). Bambara groundnut has an extensive tap rooting system with numerous first, second and third-order lateral branching as early as 30 days after emergence (Mateva et al. 2020; see *CHAPTER 4*). On the rooting system, the development of nodules occurs as a consequence of a symbiotic relationship with *Bradyrhizobium* of the cowpea (*Vigna unguiculata* L. Walp.)

type and/or *Rhizobia* of the peanut type, which aid in nitrogen fixation (Doku 1968; Heller et al. 1995; Molosiwa 2012). On closer inspection, the stem located above ground is horizontal and grows from the main tap root. Differences in internode length (Figure 2-2B) results in a bunching phenotype (for example, genotypes: DipC1 and Uniswa red), semi-bunched (genotypes: S19-3 and Tiga nicuru), and spreading genotypes (DodR, Getso, Gresik, LunT and IITA-686), according to the International Plant Genetic Resources Institute (IPGRI 2000). The tip of each petiole is occupied by a node and reproductive parts, i.e., bright whitish-yellow coloured flowers (at the end closest to the soil level; Figure 2-2C), whilst trifoliate leaflets are at the top (Figure 2-2D). Leaflets are round to elliptic, ranging from 6-8cm and 3-4cm in length and width, respectively (IPGRI 2000).

The podding pattern of bambara groundnuts is similar to peanuts, with a positive gravitropic peduncle, or "peg" in peanuts, elongating and entering the soil (Figure 2-2E-F). The form of the pods is spherical or oval depending on the seed number contained in the pod (Figure 2-2G; Basu et al. 2007a). While most landraces have single-seeded pods, Amadou et al. (2001) reported some landraces from the Democratic Republic of Congo (DRC) contain pods with up to three seeds.

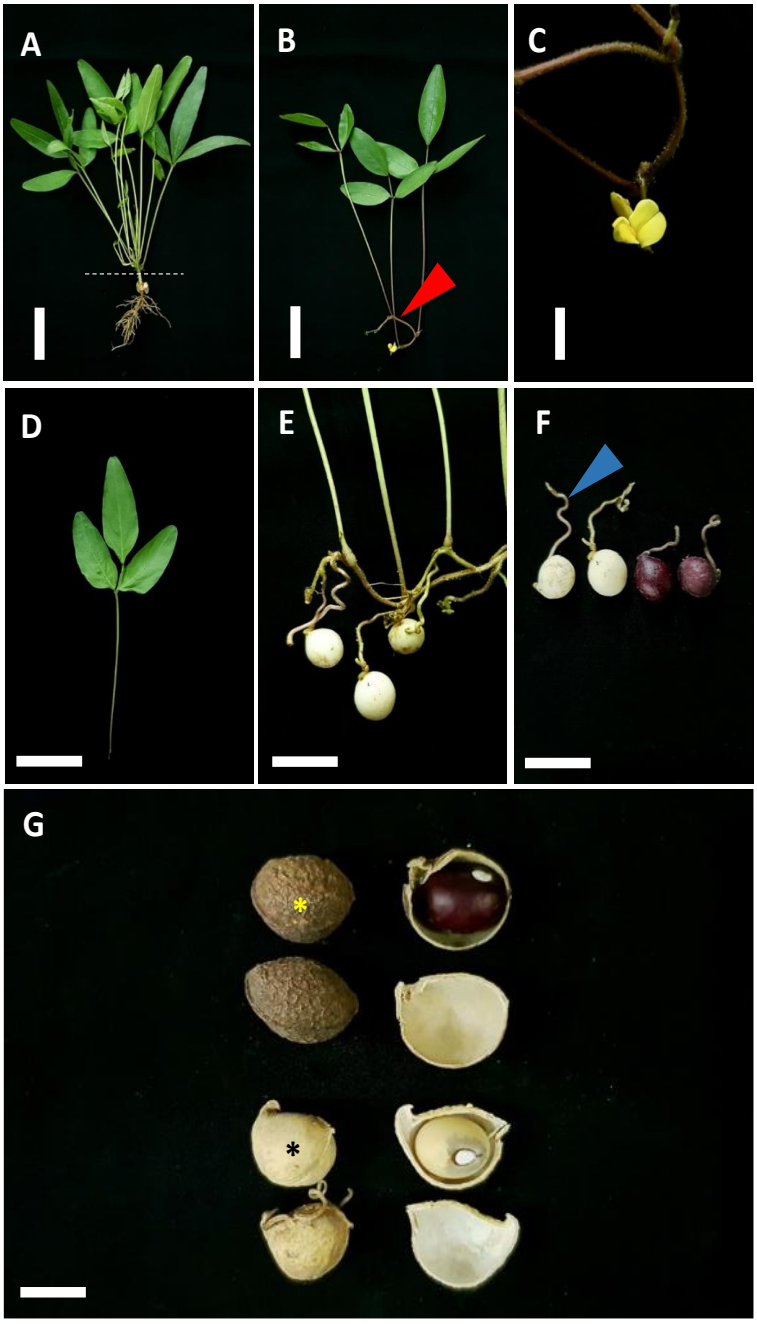

Figure 2-2: Bambara groundnut (*Vigna subterranea* (L) Verdc) plant: (**A**) bunched shoot-crown and root system at 20 days after sowing. White bar = 10cm; (**B**) three untangled leaflets. The red arrow (◄) indicates internode length. White bar = 10cm; (**C**) enlarged flower. White bar = 1mm; (**D**) enlarged single trifoliate leaflet on a 30 cm long petiole. White bar = 5cm; (**E**) elongated peduncles and immature seeds. White bar = 1mm; (**F**) elongated peduncles (◄) with two different coloured seeds. White bar = 1mm; (**G**) mature dry reddish-brown seeds: with little groove (brown) (*) pod texture and mature cream seeds: with smooth (yellowish-brown) (*) pod texture. White bar = 1cm. According to the International Plant Genetic Resources Institute (IPGRI 2000), all descriptions are for bambara groundnut.

2.3.1 Taxonomy

Bambara groundnut is indigenous to the Sahelian area of modern-day West Africa (Halimi et al. 2019). Legumes are part of the Fabaceae (Leguminosae) superfamily, which has over 20,000 different species. Bambara groundnut is classified in the genus *Vigna* within the Milletoid clade (warm-season legumes) of the Papilionoideae subfamily of Fabaceae. Papilionoideae is the economically dominant sub-family which includes legumes used for food (Wojciechowski et al. 2007) such as soybean (*Glycine max*), peanut, pea (*Pisum sativum*), and common bean (*Phaseolus vulgaris*). The *Vigna* genus contains more than 100 species distributed among six subgenera: *Vigna* (African *Vigna*), *Haydonia*, *Plectotropis*, *Ceratotropis* (Asian *Vigna*), *Lasiospron*, and *Sigmoidotropis* (Zuluaga et al. 2021), which grow in warm temperate and tropical regions (Sakai et al. 2016).

The divergence between the African and Asian *Vigna* subgenera was estimated to have occurred approximately 4.7 million years ago (MYA) (Kang et al. 2014). Phylogenetically, cowpea (*Vigna unguiculata*) is the closest relative of bambara groundnut (with both categorised in the *Vigna* subgenera), followed by mungbean (*Vigna radiata*), adzuki bean (*Vigna angularis*), moth bean (*Vigna aconitifolia*), which are classified in *Ceratotropis* or Asian *Vigna*. Analysis of legume phylogeny indicated that divergence between the *glycine-phaseolus* complex occurred approximately 19–22 MYA Kang et al. 2014), whilst divergence between *phaseolus-vigna* complex was determined to be ~5 MYA (McCrory et al. 2010) (Figure 2-3). Therefore, common bean *Phaseolus spp* member would be more closely related to bambara groundnut followed by soybean and chickpea (*Cicer ariteneum*). Bambara groundnut is also classified as a pulse (syn grain legume) which are a subset of legumes characterised by their edible seeds that are high in protein (20-40%) and low in lipid (<10% seed), used as vegetables (garden peas, green beans), for oil extraction (soybean,

peanut) or sowing/cover purpose: clover (*Trifolium angustifolium*) and alfalfa (*Medicago sativa*) (Duranti 2006; Xipsiti et al. 2017). Although technically inaccurate, the terms "pulse" and "legume" are frequently used interchangeably since all pulses are legumes but not all legumes are pulses (Singh 2017).

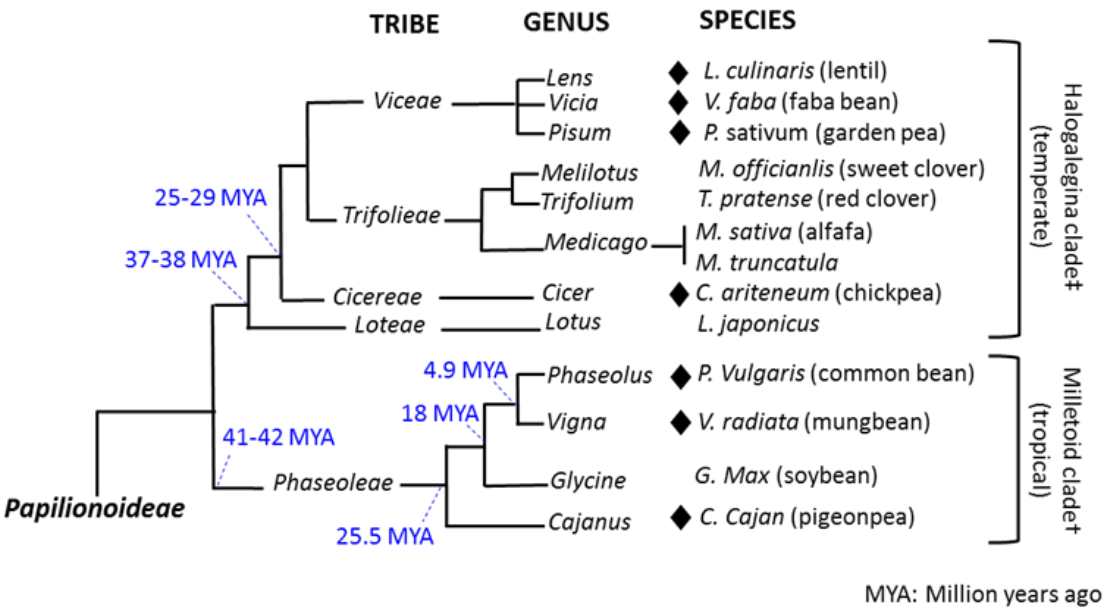

Figure 2-3: Expanded taxonomic relationships within the Halogalegina and Milletoid clades of sub-family Papilionoideae, with representative species indicated. Evolutionary divergence times are indicated at the tribe and genus level. Black diamond (◆) indicates species classified as pulses by the United Nations Food and Agriculture Organization (FAO) (Xipsiti et al. 2017).
Source: Halimi et al. (2019).

2.3.2 Origins and Distribution

In the 17th century, the Angolan word for bambara groundnut was referred to for the first time in literature as "Mandubi d'Angola" by Marcgrav de Liebstad in 1648 (National Research Council 2006). *Glycine subterranea* was later the name given to it in 1763, in line with Linnaeus' description of organisms in his book Species Plantarum (Heller et al. 1995). Bambara groundnut was given the name "Voandzou" in French by Du Petit-Thouars in

1806, based on the vernacular name "Voanjo" ("Voa" — means "seed' and "anjo" — means "filling"). On this basis, around a century ago, researchers coined and used the term *Voandzeia subterranea* (L.) Thouars (Heller et al. 1995). However, thorough botanical studies revealed a significant resemblance between bambara groundnut and other species of the genus *Vigna* (Maréchal et al. 1978). This then led Verdcourt — a botanist, to suggest the new name *Vigna subterranea* (L.) Verdc. in 1980 (Goli 1997). Bambara groundnut has subsequently expanded in popularity and are now cultivated by farmers in nations outside of Africa, including Brazil, India, Thailand, Malaysia, the Philippines, and Indonesia (Mayes et al. 2019a). According to Adhi and Wahyudi (2018), bambara groundnut was brought to Madagascar by the Arabians and subsequently spread to Brazil in the early 17th century before being introduced to the Philippines and Indonesia. However, no reliable statistics on yield and harvested area are available for these countries.

2.3.3 Growth and Development

Germination in bambara groundnut takes around 7-15 days and is hypogeal, which means the cotyledons remain below ground. Daytime temperatures of 20–28°C have been observed to be optimal for early growth (Linnemann and Azam-Ali 1993b; Linnemann 1993a; Karunaratne et al. 2013; Al Shareef et al. 2014), though there are often considerable differences in temperature (Dhanaraj 2018) and photoperiod (Kendabie et al. 2020) response between genotypes at later growth stages. Hepper (1963) first demonstrated that different bambara groundnut landraces could germinate from either fresh or dry seeds, with emergence of nearly 100%, at 5–21 days in both scenarios. Using a large set of different landraces, (Berchie et al. (2010) showed that the ideal temperatures for germination were generally 30–35°C, with slower germination reported below 15°C and above 40°C. The crop is cultivated in a

wide range of soils, from clay loams to deep sands, with the best growth and development in porous, well-drained soil with adequate aeration and soil pH of 5.0–6.5. Bambara groundnut can be grown effectively in regions with as little as 600–750 mm of rainfall distributed over a 4-5 month growing season in some countries. The root is one of the essential organs for transporting different materials from the soil (Lynch 1995). The root system is determined by the genotype and collection location (Mateva et al. 2020; see *CHAPTER 4*). Single genotypes of bambara groundnut originating from farmer landraces exhibit distinct natural genotypic variation for tap root length and root distribution or root length density. Known drought-resistant genotypes (S19-3, and DipC1) have long penetrating roots as early as 30 days after emergence (Mateva et al. 2020; see *CHAPTER 4*). The tap-root forms lateral secondary branching roots. The upper 30cm of soil usually contains 75-90% of the total dry weight of the root, with an even larger margin reported in Gresik, a genotype sourced from Indonesia (Mateva et al. 2020; see *CHAPTER 4*). Bambara groundnut benefits from bacterial symbiosis, which causes the roots to quickly develop nodules (Puozaa et al. 2017). This is important for the vegetative growth (up to first-pod), where the plant nitrogen partition is high (Ramolemana 1999).

2.3.3.1 Vegetative Growth

Temperatures between 20–28°C are optimum for plant vegetative growth (Linnemann 1993a; Karunaratne et al. 2013; Al Shareef et al. 2014). Reduced pod yield was observed under a high temperature of 33°C as compared to 23°C, from landraces Uniswa red and S19-3 collected from Swaziland and Namibia, respectively. However, low temperatures have a detrimental impact on bambara groundnut productivity. A study by Sesay et al. (2008) found reduced yields of bambara groundnuts associated with late sowing may result from cooling temperatures late in the season. Late in the

season, as temperature drops, leaf number and leaf size decrease, resulting in a reduction in yield: pods numbers and seed size. Working with unspecified seeds from Zimbabwe, Harris (1993) initially demonstrated that plants sown early developed much more vegetative dry matter than plants sown later. As a result, yields of certain bambara groundnut landraces can differ depending on the planting date. This was later corroborated by Mukakalisa et al. (2013), who working with a range of bambara groundnut landraces demonstrated that seeds sown in the field earlier in the growing season produced better yields ranging from about 204-337kg ha^{-1}, compared to landraces sown mid-season (58-134 kg ha^{-1}), with those sown during the winter doing extremely poorly (12-65kg ha^{-1}).

2.3.3.2 Root System Architecture (RSA)

The root system architecture (RSA) refers to a root system's shape and spatial organisation (Lynch 1995; Lynch 2013). The bambara groundnut root system is characterised by a single primary tap root that arises from the embryo, followed by several orders of lateral branching roots (Mateva et al. 2020; see *CHAPTER 4*). Also, bambara groundnut roots, like many legumes, develop nitrogen-fixing nodules, which allow for symbiotic relationships with soil microorganisms (Dakora 1998; Puozaa et al. 2017). Efforts have already begun to uncover intrinsic phenotypic variations in the RSA of bambara groundnut. The objective is to include genes for important root system traits into current elite lines to equip the crop and increase its resilience to climate change-related disasters such as drought. A deeper-cheaper RSA ideotype related to drought stress tolerance through increased and efficient water foraging in bambara groundnut genotypes has been established (Mateva et al. 2020; see *CHAPTER 4*). For example, an established drought-resistant genotype (S19-3, from Namibia) was confirmed to have a quick and longer reaching tap

root and more branching in deeper soil depths (Mateva et al. 2020; see *CHAPTER 4*). This corroborated Jørgensen et al. (2010) prior classification of S19-3 as a "water-spender" during drought, further validating this deeper-cheaper ideotype. In follow-up studies, Mateva et al. 2022; see *CHAPTER 5*), working with eight bambara groundnut genotypes, demonstrated that intrinsic phenotypic variations in RSA underpin drought resistance. In the same study, the authors demonstrated that root length density (RLD) in the deeper soil depth was an important trait, correlated to stomatal conductance and subsequently grain yield, especially in the genotype DodR (originating from dry regions in Tanzania).

2.3.3.3 Nodulation

Like other nodulating legumes, bambara groundnut establishes complex relationships with soil rhizobia by releasing chemical signals into the rhizosphere, resulting in the production of root nodules that contain *Bradyrhizobium* species (Dakora 1998; Puozaa et al. 2017; Dlamini et al. 2021). *Bradyrhizobium spp*. such as *B. pachyrhizi*, *B. yuanmingense*, and *B. daqingense* have been isolated from root nodules of bambara groundnut landraces growing in Ghanaian, Angolan, and Namibian soils (Ibny et al. 2019). The nitrogen can be used during bambara groundnut vegetative development while simultaneously replenishing the soil, making it useful in intercropping and rotational systems (Lengwati et al. 2020). Uguru and Ezeh (1997), showed that five different soil types in Nigeria did not impair the nodulating capacity of six bambara groundnut landraces except at very low pH values in *Entisol* and *Inceptisol*. Accordingly, Dakora (1998) reported that bambara groundnut could survive on a range of nitrogen-deficient soils and, hence, was able to revitalise overall soil health and boost production in integrated crop productions systems in Africa. Working on Nigerian soils, Yakubu et al. (2010) showed that bambara

groundnut nodules fixed around 28.4kg ha^{-1} of soil available nitrogen, with almost double (32–81kg ha^{-1}) reported by Musa et al. (2016) on acidic soils in Malaysia. A repeated intercropping study by Egbe et al. (2013) in Nigeria then found that including bambara groundnut into an intercropping system with maize (*Zea mays*) boosted maize productivity. Similarly, Lengwati et al. (2020), also using a repeated intercropping study, reported a 222% increase in marginal returns of maize after rotation with bambara groundnut, with no chemical nitrogen fertiliser applied. This demonstrates that the bambara groundnut always could enhance African soils while also assisting current cereal-based food production systems.

2.3.3.4 Flower Initiation, Pod and Seed Development

The reproductive biology of the bambara groundnut has been extensively studied (see PhD thesis by Dhanaraj (2018). According to Dhanaraj (2018), bambara groundnut has papilionaceous zygomorphic flowers that develop uniformly. The plant has brightly coloured flowers that are cleistogamous — a type of automatic self-pollination, where pollen is deposited before the flower opens. Although the whitish-yellow flowers attract insects, it is reasonable to infer that limited cross-pollination occurs in bambara groundnut. However, further research is needed to prove this. Flowering is temperature-dependent, with ideal temperatures ranging from 20–28°C (Karunaratne et al. 2013; Al Shareef et al. 2014). In contrast, Al-Shareef et al. (2013) found that at 33°C, pod numbers in Uniswa red (a Swaziland genotype) decreased, but S19-3 (a genotype sourced from Namibia) produced more pods. This might be due to the genotype's resistance to the sourced location's high daytime average temperatures. In addition to flower initiation, podding is affected by high and low temperatures (>30°C or <15°C, respectively), which reduce anther dehiscence, pollen germination, pod set, and pod filling

(Dhanaraj 2018). At optimum temperatures, landraces generally begin to flower at 30-35 days after planting, and this can extend to 60 days after planting, with peak flower numbers reported between 45-50 days after planting (Dhanaraj 2018). However, cold temperatures have been noted to decrease pollen activity (Dhanaraj 2018).

After successful fertilization in the closed flowers, a specialised hairy tube-like structure known as the peduncle emerges, sensing gravity and bending downward. The peduncle elongates and implants the embryonic pod into the soil — "geocarpic pod" development — with two separate embryonic pods from one peduncle common (Figure 2-4A). According to (Dalziel 1937), the peduncle elongates, transporting the embryonic pod into the soil. This view is shared by Cobley (1956), Rassel (1960), and Johnson (1968), who agreed that the elongation of the peduncle forces the embryonic pod into the soil for maximum pod development. In contrast, (Doku and Karikari 1970) later revealed that seeds can continue to develop even when peduncles have been obstructed from penetrating the soil — but "ageocarpic" pods formation can drastically reduce expected yield. Pods developing on or below the soil attain their maximum dry weight between 30-70 days after flowering (Mabhaudhi et al. 2013). From this point on, the seeds continue to mature, and the dense spongy parenchyma layer within the pod that protects the seed begins to dry out. Depending on the landrace, seeds are fully mature at 90-150 days after planting (Mabhaudhi et al. 2013). As a general rule, seed maturity can be verified by opening the pod and inspecting the formerly dense and spongy parenchyma layer, which should be thin and dehydrated at this point (Figure 2-4B, C). It's worth mentioning that bambara groundnut's fruiting habit means that flowering and podding can continue as long as growing conditions are ideal. However, this results in pods of varying maturity. Specifically, S19-3 (sourced from Namibia) is a landrace-derived genotype with a highly

determinant fruiting habit, but other landrace-derived genotypes, such as Ankpa-4 (sourced from Nigeria), might be indeterminate, especially during long photoperiods (Mayes et al. 2019b). As a result, harvesting bambara groundnut is dependent on the presence of 70-80% mature pods as a rule of thumb (Figure 2-4D). This range can be adjusted to account for breeding techniques that need rapid reproduction.

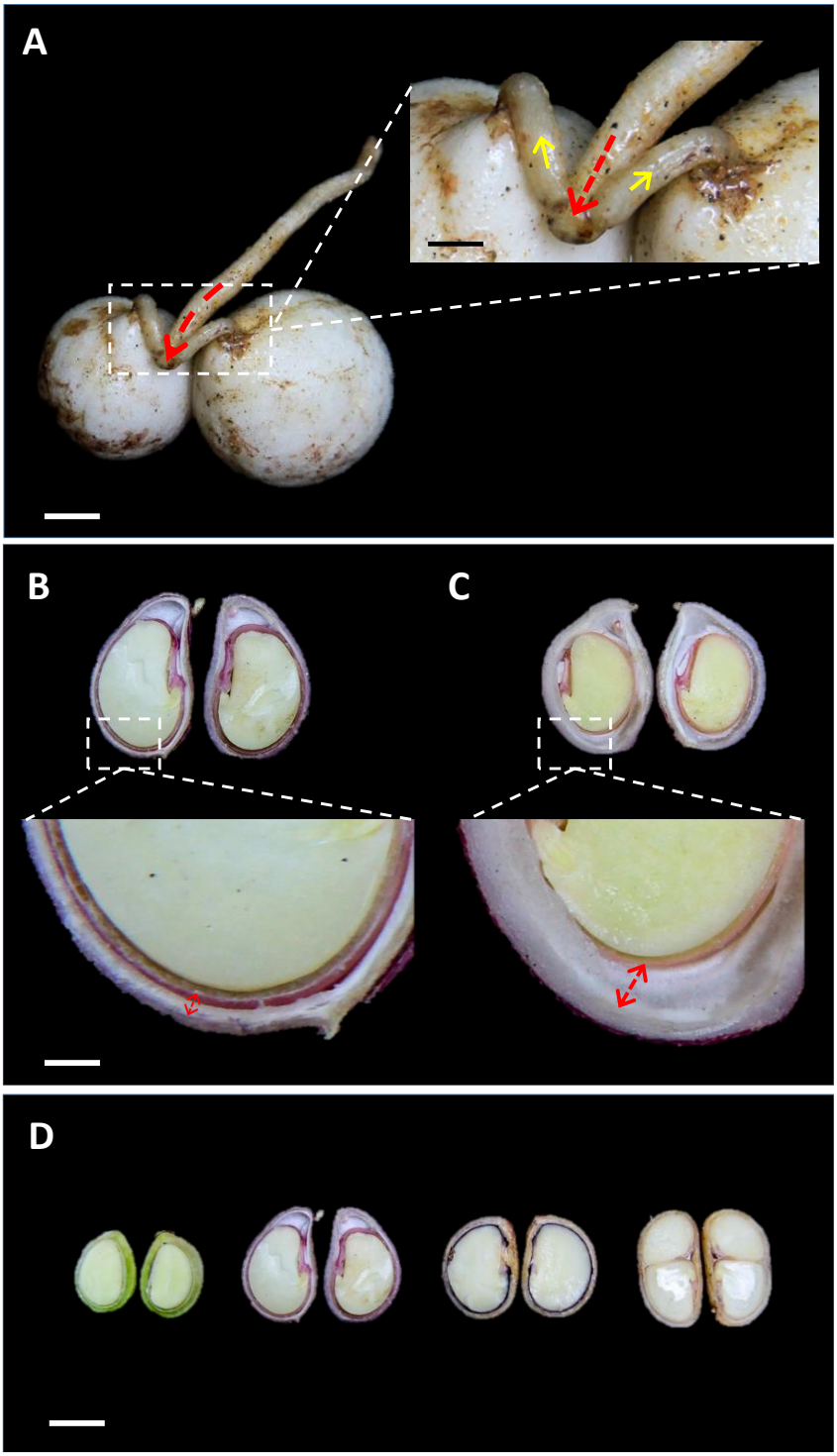

Figure 2-4: Bambara groundnut (*Vigna subterranea* (L) Verdc) pods: (**A**) elongated peduncle ( ) with enlarged section showing two separate embryonic pods ( ); (**B**) mature seed with thin, dehydrated parenchyma layer ( ); (**C**) immature seed with a dense and spongy parenchyma layer ( ); (**D**) four different single and double pod bambara landrace-derived genotypes harvested at 70-80% mature pods. White bar = 1cm.

2.4 Importance and Nutritional Density

Bambara groundnut can play an increasing role in the human diet (Mubaiwa et al. 2018). However, progress in increasing its use as a food crop is slow due to limited systematic efforts to collate and analyse compositional data for the crop and breeding efforts to improve the nutritional composition. In addition, data reflecting the range of nutritional variation within the species is scarce, with a limited comparison with major crops species. A literature review of the nutritional composition of bambara groundnut and four taxonomically related legumes (soybean, chickpea, mungbean and cowpea) (Halimi et al. 2019) provided a summary of the nutritional potential of bambara groundnut. The multi-species analysis indicated the following:

1) Bambara groundnut has higher mean seed lipid but lower mean seed protein in comparison to cowpea. In comparison to mungbean, bambara groundnut has higher mean seed lipid and protein (Figure 2-5).

2) Bambara groundnut is a good candidate for inclusion into diets for management of high cholesterol and diabetes due to the combination of relatively high resistant starch content (69.7-72.6% of total starch in comparison to 16.0-51.6% for chickpea), the amylose content of the starch (16-35% in comparison to 15-26% in cowpea starch), lower glycaemic index 40 (Oyeyinka et al. 2017). Pulse starches are classified as slowly digesting and resistant starches due to their high amylose content-typically 15-30%, with values up to 88% reported in wrinkled pea (Hoover et al. 2010). This leads to a lower glycaemic index (GI) in comparison to foods such as cereal.

3) Seed protein varies considerably (9.6-30.7% of seed) dependent on environment, cultivar or growing season, as shown from the compilation of 14 studies. It has already been well established that

variation in seed protein concentration in legumes are attributed to genetics (G), environment (E) and their interaction (G×E) (Agarwal 2017; Assefa et al. 2019). There is potential to increase bambara groundnut seed protein concentration through targeted breeding strategies. Bambara groundnut has seed proteins with higher proportions of sulphur-containing amino acids (methionine and cysteine) compared to cowpea and chickpea. The proportions of methionine and cysteine in bambara seed protein meet the recommended Food and Agriculture Organisation (FAO) guideline for amino acid intake (FAO 2003).

4) The predominant fatty acids in bambara seed are the same as those found in soybean (Medic et al. 2014), with oleic, linoleic, palmitic, stearic and linolenic representing >70% of total fatty acid. In some studies, oleic and linolenic acids have accounted for >40% of total fatty acid (Minka 2000; Adeleke et al. 2018). Based on clinical trial evidence, dietary intake of oleic acid has been associated with improved human immune response (Sales-Campos et al. 2013), reducing cardiovascular diseases and cancer (Piccinin et al. 2019). Polyunsaturated fatty acids such as linoleic (omega-6) and linolenic (omega-3) acids are of particular interest to dieticians due to their proposed roles in reducing hypercholesterolemia and improving cardiovascular function (Mensink et al. 2003; Salas-Salvadó et al. 2006; Wanders et al. 2010).

5) Concentrations of potassium, magnesium, iron and zinc have been reported to vary in each mungbean, chickpea and bambara groundnut. However, the relatively higher levels reported in the latter may reflect the specific availability in those trials (Anwar et al. 2007; Abiodun and Adepeju 2011; Dahiya et al. 2013; Nair et al. 2013; Alake 2016). Dependent on soil and uptake in the growing environment, a 100g serving of bambara groundnut has the potential to fulfil the child and

adult Recommended Daily Allowance for iron (7.0-15.1mg) and zinc (3-14mg) (Services and Agriculture 2015; National Health and Medical Research Council and Health 2017). Globally, two billion are affected by symptoms arising from iron and zinc deficiencies (Bailey et al. 2015).

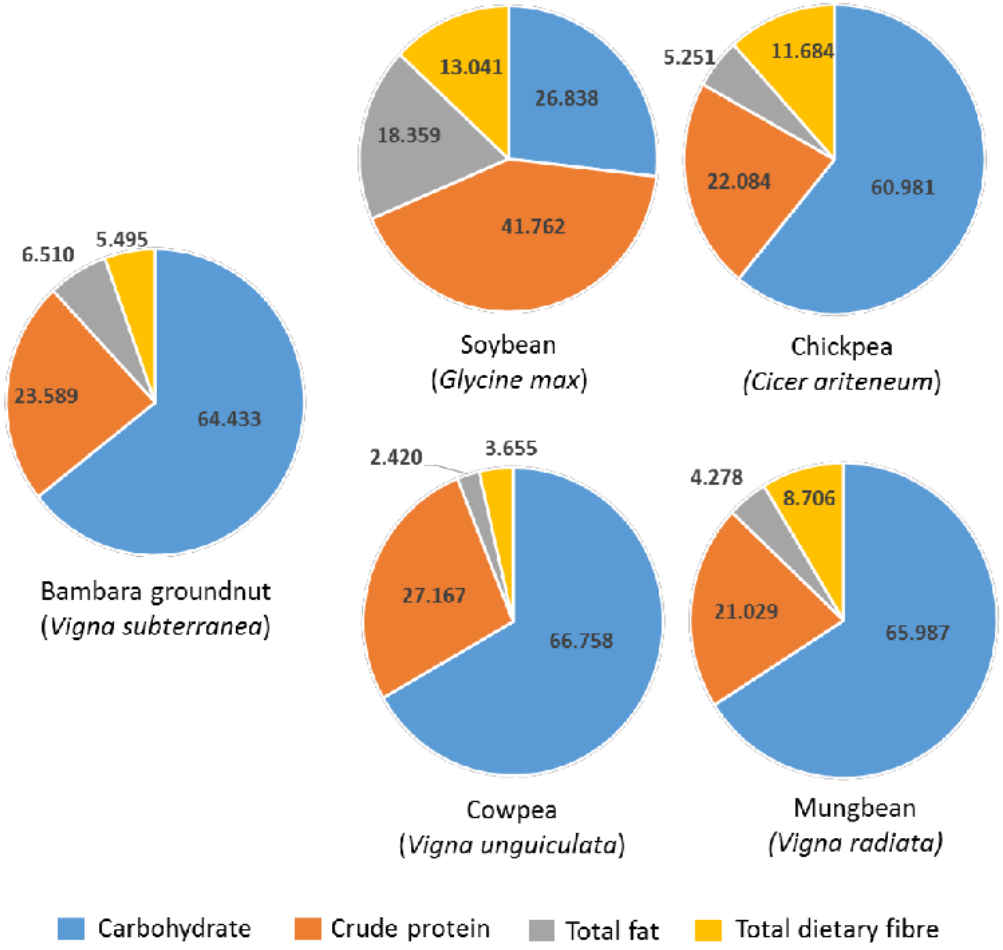

Figure 2-5: Compositional variation in the four proximate components for raw bambara groundnut (*Vigna subterranea* (L.) Verdc.) seeds and selected crop comparators: soybean, chickpea, cowpea and mungbean. Blue - carbohydrate, orange - protein, grey - total lipid, and yellow - total dietary fibre. Data are presented as calculated mean values expressed as % seed (g $100g^{-1}$).
Source: Halimi et al. (2019).

There are many information gaps concerning the nutritional composition of bambara groundnut that would allow direct comparison with

other legume crops. For example, there are gaps in data quantifying specific dietary compounds such as resistant starch and dietary fibre fractions (insoluble and soluble fractions). There also appears to be contradictory or incomplete evidence regarding reported relative concentrations of amino acids and specific seed storage proteins (Halimi et al. 2019). In addition, it is currently unknown whether the relative concentration of vitamins, anti-nutritional components and other secondary metabolites such as isoflavone differ significantly between bambara groundnut and other legumes. For example, a review of isoflavone variation in food and crop raw materials indicated relatively high levels in legumes such as soybean and chickpea (Bustamante-Rangel et al. 2018). Isoflavone has been linked to protection against osteoporosis, hormone-dependent cancers and loss of cognitive function (Gómez-Zorita et al. 2020).

2.4.1 Germplasm Collection and Conservation

Over 6,000 accessions of bambara groundnut are mainly collected from African countries, and international institutions hold these collections. The major germplasm collection is held by the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA) (*n = 2,031*) in Ibadan, Nigeria. This collection is gathered from over 25 African countries and has been characterized (Begemann 1997). Other institutions include the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) in France (*n = 1,416*), Department of Agricultural Research (DAR) in Botswana (*n =338*), Plant Genetic Resources Research Institute (PGRRI) in Ghana (*n = 296*), National Plant Genetic Resources Committee (NPGRC) in Tanzania and Zambia (*n = 515*), International Livestock Research Institute (ILRI) in Ethiopia (*n = 17*), and Genetic Resources Research Institute (GeRRI) in Kenya (*n = 12*). On a global scale, the Svalbard Global Seed Vault will surely aid future breeding

programmes by reducing climate change vulnerability and preserving bambara groundnut diversity.

2.4.2 Genetic Diversity, Genetics and Plant Breeding

Molecular plant breeding, such as marker-assisted breeding (MAB) using Deoxyribonucleic acid (DNA) markers, has been a worthwhile strategy for crop improvement (Moose 2008; Jiang 2018). Random amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphism (AFLP) markers, simple sequence repeat (SSR or microsatellite) markers, and more recently, Diversity Arrays Technology genotyping-by-sequencing (DArTseq) markers and single nucleotide polymorphism (SNP) markers have been developed and applied in several bambara groundnut studies (e.g., Massawe et al. 2003; Ntundu et al. 2004; Somta et al. 2011; Molosiwa et al. 2015; Ahmad et al. 2016; Chai et al. 2017; Ho et al. 2017; Redjeki et al. 2020). Massawe et al. (2003) reported high polymorphism levels among 12 landrace-derived genotypes of bambara groundnut using 16 RAPD primers, and Amadou et al. (2001) found high genetic variation among 25 African accessions of bambara groundnut using 15 RAPD primers. However, this technique is comparatively less reliable due to the low reproducibility of RAPD markers (Jones et al. 1997; Massawe et al. 2003). In a study to determine genetic variation among a diverse group of 100 bambara groundnut landrace-derived genotypes from Tanzania, two major clusters were identified using 11 AFLP primer pairs, which generated 49 polymorphic fragments across the bambara groundnut landrace-derived genotypes (Ntundu et al. 2004). Massawe et al. (2003) used seven AFLP primer pairs and generated 504 amplification products, ranging from 50-400 base pairs (bp) in 16 cultivated bambara groundnut landrace-derived genotypes. Aliyu (2013) used microsatellite markers alongside the characterisation of morphological features to analyse the level of genetic

diversity in a small collection of ten Ghanaian bambara groundnut landrace-derived genotypes. Eight individual landrace-derived genotypes out of the ten were clustered into seventeen units. Genetic distances both inter and intra between landrace-derived genotypes of bambara groundnut using SSR markers were in the range of 0.48–0.90, consistent with previous reports Massawe et al. (2003) obtained using RAPD markers. DArTSeq is a relatively new molecular marker technique, more comprehensive in terms of molecular variation underlying the polymorphisms with an affordable price, and has been reported in bambara groundnut (Olukolu et al. 2012; Ho et al. 2017; Redjeki et al. 2020). Olukolu et al. (2012) identified a relatively high genetic diversity using 554 DArT markers among 40 landrace-derived from genotypes of bambara groundnut collected from East Africa (Kenya, Madagascar, Tanzania and Zambia), West Africa (Nigeria, Ghana, Burkina Faso and Republic of Benin), and Central Africa (Cameroon). More recently, a total of 170 bambara groundnut accessions collected from Indonesia, East Africa, West Africa, Central Africa, and Southern Africa were used to evaluate the genetic diversity among landraces using 170 SSR markers and 168 DArTseq markers and indicate the likely original source of current Indonesian material (Redjeki et al. 2020).

Controlled crossing protocols have been established in bambara groundnut (Massawe et al. 2005; Suwanprasert et al. 2006; Kendabie et al. 2015), and these have been used successfully in artificial hybridisation efforts. For example, Basu et al. (2007b) reported on a second filial generation (F_2) population, derived from a domesticated landrace-derived from genotype from Botswana (DipC1; female parent) crossed with a wild accession collected in Cameroon (VSSP11; male parent). This was developed to investigate the inheritance of "domestication" traits in bambara groundnut. The results of this work suggested that traits including leaf area, specific leaf area (SLA), carbon

isotope discrimination (CID), and 100-seed weight are controlled by several genes. At the same time, internode length, stems per plant, days to emergence and seed eye pattern around the hilum are likely to be under largely monogenic control (Basu et al. 2007c; Chai et al. 2015) evaluated a fifth filial generation (F_5) breeding population derived from two bambara groundnut landrace-derived from genotypes (Tiga Nicuru × DipC1) to evaluate the effects of mild drought stress on the morpho-physiological characteristics. Strong genotypic variation was observed for many traits, including 100-seed weight, harvest index, stomatal density and leaf area (Chai et al. 2016). Five segregating populations have also been developed from crosses involving photoperiod-sensitive landrace-derived from genotypes (e.g., Ankpa-4 and LunT) and less-sensitive (S19-3, DipC1, DodR and IITA-686) to accelerate breeding for improved varieties in bambara groundnut (Kendabie et al. 2015; 2020). These populations include Ankpa-4 × IITA-686 (reciprocal), Ankpa-4 × DodR, Ankpa-4 × DipC1, S19-3 × Ankpa-4 and IITA-686 × LunT (Kendabie et al. 2015). Two F_2 bi-parental segregating populations derived from IITA-686 × Tiga Nicuru and S19-3 × DodR were developed and advanced to obtain structured populations and breeding lines for genetic analysis and trait dissection (Gao et al. 2020).

2.5 Climate Resilience

With modern agriculture, natural environment deterioration and climate change, biotic and abiotic stresses have become increasingly important factors severely affecting global crop production (Harris and Roach 2017). Drought, temperature fluctuations, and an increase in pest and disease incidence, all of which are mostly felt in Africa and other developing countries (Ali et al. 2017), are all significant pressures on the productivity of our present agriculture food systems, resulting in unpredictable and low yields. Africa needs diverse crops that fit its climate, soils and cropping systems and

researchers, governments, and investors must take the lead in promoting and supporting a new vision for African agriculture.

2.5.1 Resistance to Drought Stress

The mechanism of drought resistance in bambara groundnut has primarily been clarified by above-ground shoot phenotyping during the past 20 years (Collinson et al. 1997; Sesay et al. 2010; Chibarabada et al. 2015a, 2015b; Muhammad et al. 2016; Nautiyal et al. 2017; Fatimah et al. 2020). This has mainly been on gas exchange components, leaf area reduction, inorder to collectively maintain a reasonably high leaf water status. Bambara groundnut can maintain turgor through a combination of osmotic adjustment, reductions in leaf area index and effective stomatal regulation of water loss (Collinson et al. 1997).

Using three genotypes Botswana (DipC1), Tanzania (DodR) and Sierra Leone, Collinson et al. (1999) reported adaptive mechanisms to avoid drought in relation to maximizing seasonal radiation interception. The authors reported that water stress reduced seasonal light interception by 71% in LunT (sourced from Sierra Leone, wet habitat) and by 37–47% in DodR (from Tanzania) and DipC1 (from Botswana), both dry semi-arid regions. Later, Jørgensen et al. (2010) explored the diversity of drought adaptation strategies of the two contrasting landraces: S19-3 (from Namibia) and Uniswa red (from Swaziland), concluding that Uniswa Red could be defined as a "water-saver" and S19-3 as a "water-spender" with an early and late closure of stomata, respectively. These mechanisms were also corroborated by novel work (Muhammad et al. 2016; Nautiyal et al. 2017). With work by Chai et al. (2016), helping detect quantitative trait loci for several phenotypic traits on an initial genetic linkage map developed using a cross between DipC1 (from Botswana) and Tiga nicuru (from Mali).

A study by Kundy (2019) explored the effects of drought stress on bambara groundnut (landraces: Nalbam 2, Nalbam 4, DodR, S19-3) and a peanut (commercial check: Mnanje). Mnanje had the highest concentration of proline, an amino acid that protects and helps plants survive and recover from drought stress. However, minimal differences in yield decline were reported between the peanut variety and bambara groundnut landrace (i.e., S19-3; 55 and 59%, respectively). This shows that with equivalent effort in drought stress breeding, bambara groundnut lines could provide equivalent, if not higher, yields than that of the existing improved peanut. It's only a matter of establishing a level playing field.

In recent years, root phenomics in legumes and cereal crops has received more attention (Saoirse et al. 2020). Researchers working on bambara groundnut are interested in utilising this field to understand the root system's function in improving water acquisition. To this end, breeding efforts for bambara groundnut plant ideotypes with root traits suited for drought stress-prone regions is ongoing. Recent results revealed that bambara groundnut genotypes generally displayed two extremes in root foraging patterns and branching habits, i.e., deep-cheap rooting in landrace-derived genotypes sourced from dry regions and shallow-costly rooting patterns in genotypes adapted to higher rainfall areas with shallow soils (Mateva et al. 2020; see *CHAPTER 4*). When faced with periodic drought stress, variation in intrinsic root length density in deeper soil depths in the genotype DodR was found to influence water foraging ability (Mateva et al. 2022; see *CHAPTER 5*). This ability to penetrate and draw available water from deep within the soil profile backs up the idea that various agroecological conditions to which bambara groundnut has been exposed over thousands of years in its natural setting could have selected for ecotypic, phenotypic and possibly genetic differentiation in growth and root structure (Mateva et al. 2022; see *CHAPTER*

5). This could help offset the impact of drought stress. However, root gene expression and flow evidence will be required to fully explain genetic differentiation.

2.6 Root System Architecture in Legumes

The root system is considered critical for water absorption and, as a result, for enhancing legume seed production under drought stress (Gaur et al. 2008). Root traits are commonly defined as the root system architecture (RSA), referring to the form and spatial structure within the soil of a root system. In legumes, the number and size of lateral roots are determinants of RSA (Dubrovsky et al. 2006). The symbiotic relationships, as well as soil conditions, play a key role in RSA. The root system of legumes is characterised by a single main tap-root that emerges from the embryo, followed by consecutive orders of lateral branching roots (Rich and Watt 2013). This RSA reduces intra-plant root competition, and is efficient at acquiring resources. Figure 2-6 shows an example root system of 15-day old S19-3, a parental bambara groundnut genotype sourced from Namibia. Fitter et al. (1991) suggested that this RSA – which provides a deeper and more proliferative root system, is preferred in environments where soil-resource and water supply is limited, putting a premium on soil-resource acquisition, especially water. Legume roots can develop secondary root organs; nitrogen (N) fixing nodules. Nitrogen fixing nodules are present only on legume roots and allow for symbiotic interactions with soil bacteria (Oldroyd and Downie 2004). It is also important to note that legumes share a regulatory mechanism that regulates nodule formation and root growth (Bright et al. 2005).

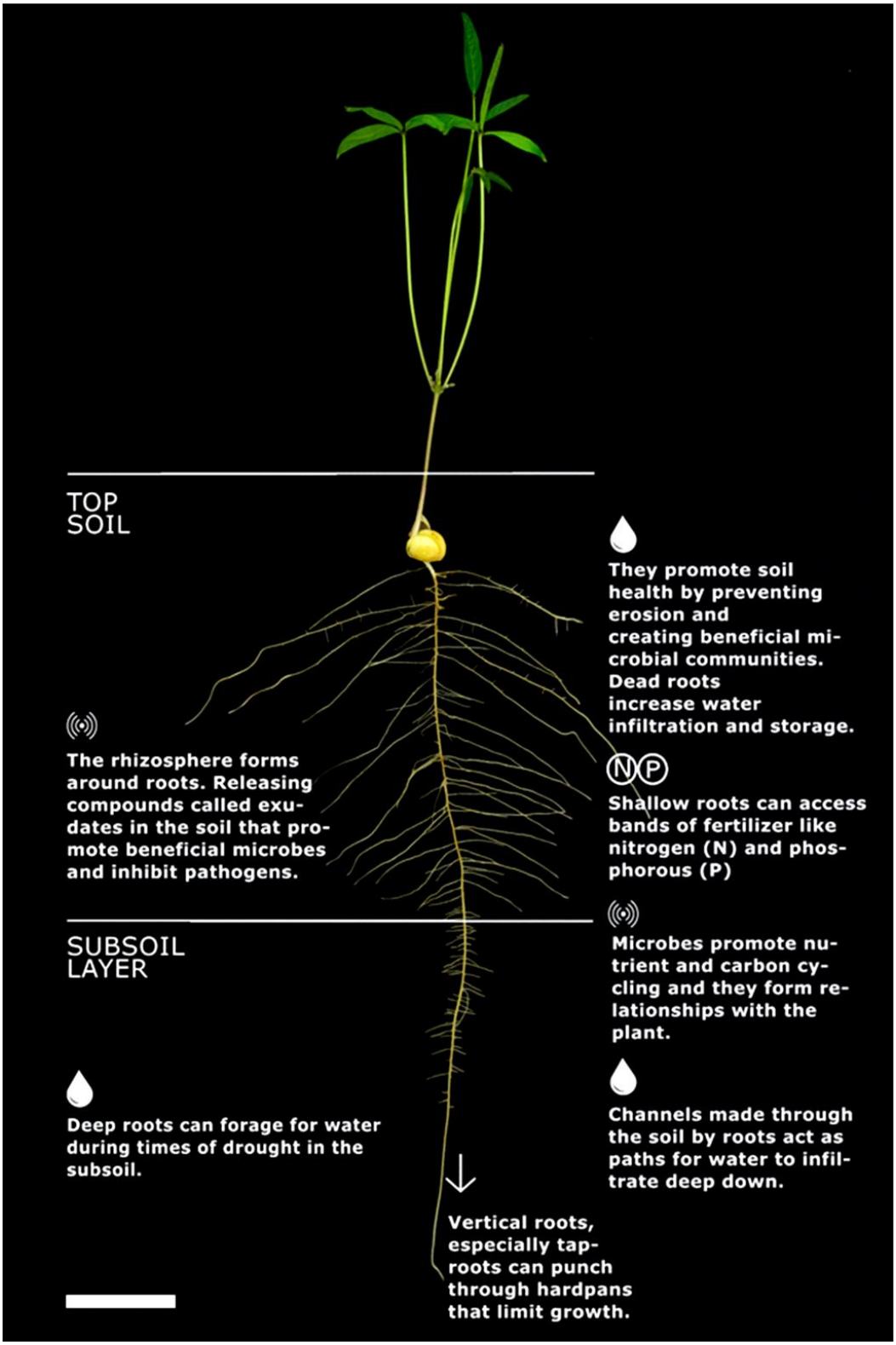

Figure 2-6 Example root system of 15-day post-emergence S19-3, a parental bambara groundnut (*Vigna subterranea* (L.) Verdc.) genotype sourced from Namibia. Bambara groundnut forms only one primary/tap root during its development which branches out through lateral roots. White scale bar = 5cm.

2.7 Root System Architecture Under Drought Stress

The growth and distribution of the root system can be considered important factors for more effective water absorption and, as a result, for controlling grain legume production under drought stress (Comas et al. 2013; Ghosh and Xu 2014). High-input irrigated conditions prefer a heavily branching root systems in the shallow topsoil layer, whilst according to (Kramer 1969), one important characteristic of drought resistance, is a deep root system. In order to access enhanced available soil moisture, plants adapt to greater rooting depth and root biomass (Blum 2011; Fenta et al. 2014) (Figure 2-6).

Drought resistance has been shown in a variety of crops to be partly based on having a deep and distributed root system, including rice (Nguyen et al. 1997; Uga et al. 2013), maize (Tuberosa et al. 2002; Hund et al. 2011), barley (Jia et al. 2019), wheat (Manschadi et al. 2006; Wasson et al. 2012), chickpea (Varshney et al. 2011; Chen et al. 2012), and soybean (Pantalone et al. 1996). Deep rooting is a dynamic characteristic that is influenced by root length and growth angle (Araki et al. 2002). The orientation of horizontal and vertical spread of roots in the soil is determined by root angle, which is recognised as an important trait for drought resistance in sorghum (*Sorghum bicolor*) (Mace et al. 2012), wheat (Christopher et al. 2013), and rice (Christopher et al. 2013; Uga et al. 2013). In rice (Kato et al. 2006), chickpea (Kashiwagi et al. 2015), and sorghum root angle was connected to rooting depth (Singh et al. 2011). During times of low rainfall, wide root angles may reduce energy inputs while accessing deeper horizons for water (Wasson et al. 2012). DEEPER ROOTING 1 (DRO1) was cloned from a natural genetic difference in root angle in rice (Uga et al. 2013). DRO1's ability to build a deep rooting system doubled yield under mild and extreme drought stress (Uga et al. 2013). Lateral root initiation and elongation, which typically corresponds to lateral root number, root length

density, and root surface area and thickness, are the key determinants of root distribution (Figure 2-7). Roots with a narrow diameter and a large specific root length increase the surface area of roots in contact with moisture, allowing more soil volume to be explored for water, as well as raising hydraulic conductance by lowering the apoplastic barrier to water entering the xylem (Comas et al. 2012; Hernández et al. 2010). Furthermore, a reduction in root diameter improves water access and enhances plant efficiency in water-stressed environments (Wasson et al. 2012).

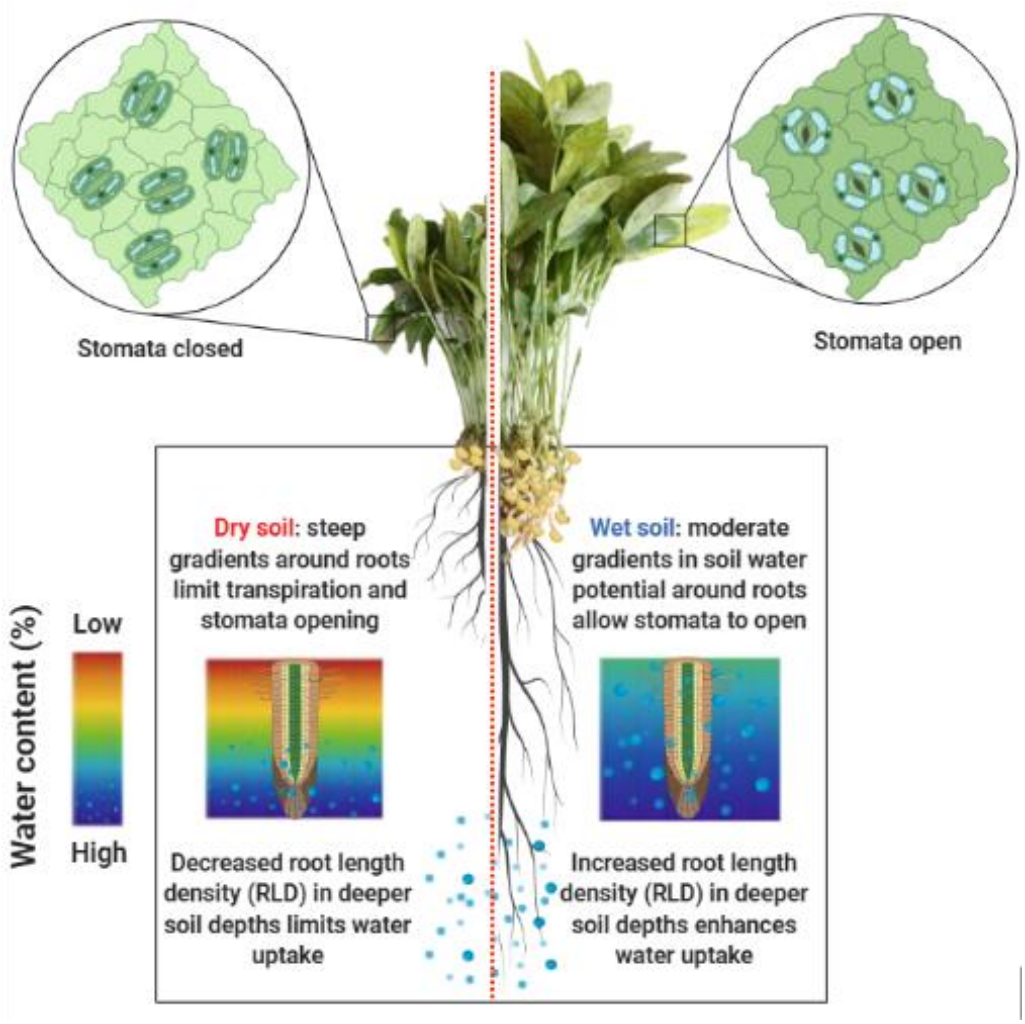

Figure 2-7 Bambara groundnut (*Vigna subterranea* (L.) Verdc.) genotypes with decreased root length distribution in deeper soil depths restrict water uptake thereby limiting stomatal conductance (g_s) and ultimate grain yield.

In water-stressed soils, well distributed roots have a reasonably high water uptake ability. Chickpea lines with a higher root length density have demonstrated increased yield and drought tolerant trait efficiency in drought stress conditions (Jaganathan et al. 2015). In addition to deep and well distributed rooting, drought stress triggers root system plasticity by increasing the number of fibrous roots, decreasing lateral root diameter, and modifying root biomass, (Nielsen et al. 1997), with some reports highlighting postponed root length density (RLD) (Bontpart et al. 2020). Under drought stress, improvements in root-to-shoot relationship metrics can compensate for moisture deficiency and sustain stomatal conductance (Maseda and Fernández 2006).

Stomatal conductance reduces under water-stressed conditions due to stomatal closing to preserve leaf water status (Liu et al. 2003). There is contradictory information about the mechanism that causes stomatal closure. Some findings support chemical signals e.g., abscisic acid; ABA (Assmann and Shimazaki 1999; Davies et al. 2002) as the cause of stomatal closing, although others support hydraulic signals (Sperry et al. 2002) as the cause. This activity lowers leaf moisture loss and decreases gaseous exchange between the plant and the environment, lowering the rate of photosynthesis and, as a result, lowering crop production, but allowing plants to survive drought stress in the short term (Goche et al. 2020). The mechanism governing stomatal conductance is unclear, but it may be caused by low root moisture status, which is transmitted to the leaf by ABA hormone signalling (Jia and Zhang 2008). According to the optimal partitioning principle, a plant distributes energy among its different organs in order to achieve optimal growth (Bloom et al. 1985). It also implies that, even though the plants are adapted to produce either a shallow or deep root, the shoot ratio and some degree of responsiveness can adjust the ratio to balance the resources that restrict plant growth (Shipley and

Meziane 2002). An increase in the number of fine roots and the rates of overall root development are two other root morphological characters that influence resource acquisition (Robinson et al. 1999). Root hairs help to extract soil water by increasing the contact area of roots with soil particles (Wasson et al. 2012). Many plants have root hairs, which are linked to increased water and nutrient absorption as well as stress resiliency (Jungk 2001; Carvalho and Foulkes 2018). Breeding programmes can benefit from a better understanding of these mechanisms in order to achieve optimum yield potential under suitable growth conditions.

2.8 Root Phenomics

Root phenotyping is an essential approach for identifying root development and foraging patterns. Equally, root phenotyping is as critical as shoot phenotyping since the root system is responsible for the majority of the plant's success (Lynch 1995). Various phenotyping methods and software that makes it easier to acquire, handle and process phenotypic data have been developed to date, transforming the landscape of root phenotyping. In the paragraphs to follow, commonly used phenotyping methods which range from field to laboratory and greenhouse-based methods (Tardieu et al. 2017) and provide an overview of advances in root image analysis and data analysis developed to date.

2.8.1 Field-Based Phenotyping

Field-based phenotyping enables quantitative analysis of root growth and distribution in the field. This technique can be used in conjunction with breeding field trials to test both aboveground and belowground traits simultaneously. This makes it possible to link adjustments in aboveground

traits to RSA. With the exception of minirhizotrons, which are designed to be inserted down into a transparent tube in the soil and used to image roots (Arnaud et al. 2019), in-field phenotyping (such as trenches, soil cores and shovelomics) cannot be used to view the whole root system. Trenching is used to manually trace and quantify only a section of vertical root distribution in the field and this is both laborious and time consuming. As a result, instead of tracing roots manually, it would be preferable to develop a tool that can extract and measure roots from a digital image (Yoshino et al. 2019).

The coring approach is utilised since the trench approach is time consuming and tedious, especially when phenotyping a larger plant population (Yoshino et al. 2019). Unlike trenches, cores rely on forcing a known diameter core-sampler into the ground using a manual or engine powered hammer. This method consists of three steps: core-sampling, root washing, and root scanning, with root traits such as volume and length quantified for each soil depth (Yoshino et al. 2019). Unfortunately, the cores only provide a two-dimensional representation of the roots whilst missing root segments or inclusion of extraneous segments could result in the root system being underestimated or overestimated.

Shovelomics is a less time-consuming phenotyping method than coring and trenching. Shovelomics was developed in maize (Trachsel et al. 2011) and is basically the digging up, washing and visual scoring of plant root crown traits. This method has also been used widely in wheat (York et al. 2018), common bean (*Phaseolus vulgaris*) and cowpea (Burridge et al. 2016) due to the ease of excavating only the basal section of the roots. When combined with scanning and imaging software such as RootReader2D (Clark et al. 2013), Rhizo Vision Explorer (Seethepalli et al. 2021) and DIRT (Das et al. 2015), shovelomics greatly simplifies the measurement of root traits such as root angle and lateral root numbers down to a depth of about 25cm. Measurements, on the other

hand, are destructive and highly variable, necessitating extensive sample replicates.

2.8.2 Lab and Greenhouse-Based Phenotyping

To decrease variability caused by climate and the varied composition of the soil in the field, lab and greenhouse-based phenotyping methods have been developed. Depending on the objective, lab-based phenotyping approaches often utilise aeroponics (Selvaraj et al. 2019), full or semi-hydroponics (Clark et al. 2011; Chen et al. 2017; Qiao et al. 2019), hydrogel media (Ma et al. 2019), instead of soil substrate. These soil-free methods allow for in-situ characterisation of roots without the need for destructive sampling to remove the substrate. Without affecting the plant's growth, a series of root development images can be captured across a full growing season. Also, plants growing in a semi-hydroponic (growth pouch), with a standardised Hoagland nutrient solution, can have the solution refreshed or changed as and when needed. However, one clear disadvantage of a hydroponic phenotyping method is that roots do not have a spatial arrangement that is true to the form in actual soil, unless additional support systems such as glass beads (Sandhu et al. 2016) and mesh netting (Uga et al. 2018) are used — especially in the case of full hydroponics. Nevertheless, the hydroponic system over the years has proved useful for basic root system characterisation, although gel and soil (in greenhouse) phenotyping methods would give more spatial-temporal data (Yoshino et al. 2019).

The agar-based phenotyping method uses clear cylinders filled with agar (Xu et al. 2013; Nagel et al. 2020). These agar-filled cylinders rotate on a turntable allowing the full 360° capture of images enabling both two and three-dimensional in-situ plant roots to be visualised at various time points during plant growth and development (Iyer-Pascuzzi et al. 2010; Uga et al.

2018). However, creating three-dimensional images from big volume and high quality two-dimensional images takes time — easily 4 to 10 minutes, which is one of the key limitations of this phenotyping approach (Yoshino et al. 2019).

Since soil is opaque, using it as a substrate is more challenging than using a hydroponic solution or a gel medium phenotyping method in the lab. In this case rhizotrons or rhizoboxes, which have one transparent face are filled with soil allowing plant root distribution to be captured using either spectral imaging (Bodner et al. 2017) or tracing (Nagel et al. 2020) of root distribution in two-dimensions. Also, soil-filled polyvinyl chloride (PVC) columns/pipes/pots have over the years been used to grow plants and extract complete roots by washing off the soil especially in greenhouses. Although this screening method is time-consuming, it has enabled the replication of natural soil and physical qualities such as bulk density (Mateva et al. 2020; see *CHAPTER 4*). This has allowed plant scientists and breeders to capture key root traits, such as: deep rooting and root distribution (Serraj et al. 2004; Kashiwagi et al. 2006; Lalitha et al. 2015; Figueroa-Bustos et al. 2019; Mateva et al. 2020; see *CHAPTER 4*) in soils more or less true to form. The soil-filled PVC phenotyping system, though highly effective, is not high-throughput (Mateva et al. 2020; see *CHAPTER 4*). Magnetic resonance imaging (MRI) and X-ray computed tomography (CT) can help with this problem. Both imaging methods have gained popularity over the years with regard to lab-based root phenotyping in 3D without the need for destructive sampling (van Dusschoten et al. 2016). However, MRI and X-ray CT both have the disadvantage of having a narrow scanning area and taking a long time to scan: 25–50 minutes with a processing time of 110–186 minutes. (Flavel et al. 2017).

2.8.3 Image Analysis

There is an increased demand for quick and accurate software solutions to accurately quantify root traits as high-throughput phenotyping and image capture of root systems expands. Over the years, two-dimensional output from imaging software has been a standard, primarily explored using manual [e.g., DART (Le Bot et al. 2010)), semi-automatic (e.g., MyROOT (Betegón-Putze et al. 2019), Semi-automated Root Image Analysis: saRIA (Narisetti et al. 2019), SmartRoot (Lobet et al. 2011) and completely automated (e.g., RhizoVision (Seethepalli et al. 2021), EZ-Rhizo (Armengaud 2009), GiA Roots (Galkovskyi et al. 2012), DIRT (Bucksch et al. 2014)] softwares. Traditionally, software in this field has depended heavily on the assumption that the root system stands out against the scan-background. In such circumstances, image thresholding using GiA Roots (Galkovskyi et al. 2012), has been explored and shown to be successful in identifying root systems as consistently brighter or darker than the scan-background. However, performing thresholding increases image noise which occurs when image pixels are misidentified as either root material or the scan-background, and corrective filtering before analysis is required to reduce this (Atkinson et al. 2019). When the root material is correctly recognised from an image, the root width is accurately estimated. The software WinRhizo (Arsenault et al. 1995), specifically designed for washed roots, can easily perform this task by estimating the width of each root using pixel-distance transformations. WinRhizo automates this procedure, however it becomes less reliable in root systems with a lot of root bunching and crosses, which is typically prevalent among older plants. In such cases, sectioning the root system according to depth, for example (Mateva et al. 2020; see *CHAPTER 4*), may be effective in decreasing underestimations caused by bunched roots and crossings. X-ray CT imaging is employed if destructive root sampling is not desired. With the distinct advantage of tracing roots inside a soil PVC

column, the software RooTrak (Mairhofer et al. 2012) employs a tracking algorithm that has been tweaked to accommodate bunched roots and crossings. However, software for this kind of root analysis is less developed, and there are fewer tools available (Atkinson et al. 2019).

2.9 Conclusion

This chapter gives a comprehensive review of the literature on bambara groundnut. It also highlighted the potential of bambara groundnut as a future smart food, as it is an underutilized crop with outstanding agronomic features, such as drought resilience under minimal input systems. Understanding the belowground root system mechanisms responsible for drought resilience in bambara groundnut is limited. The next chapters concentrated on filling these gaps.

CHAPTER 3 : General Materials and Methods

3.1 Introduction

This chapter presents general materials and methods common to chapters 4, 5 and 6. As stated in Chapter 2 (see *1.5 Structure of the Thesis*), this thesis is made up of a published paper in "paper format" and submitted papers. Materials and methods that are more applicable and unique to specific experiments are listed in more detail in the respective materials and methods sections of the individual chapters where the experiments are presented.

3.2 Materials and Methods

3.2.1 Plant Material

Eight bambara groundnut single genotypes derived from landraces of contrasting geographic origin were used in Chapters 4 and 5 for the root trait variability studies (Table 3-1; Figure 3-1). In detail, the seeds were collected from seven countries and four geographical regions, i.e., West Africa ($n = 3$), East Africa ($n = 2$), Southern Africa ($n = 2$), and Southeast Asia ($n = 1$; Table 3-1), with most of the landrace names based on the place the seeds were collected (Massawe et al. 2005). These genotypes are representatives of the bambara groundnut core parental line collection currently being screened for drought resistance as part of a project by Crops for the Future (CFF) (Gregory et al. 2019). Chapter 6 was based on data from a bi-parental segregating population of bambara groundnut. The F_4 segregating population was derived from a controlled cross between two genotypes to produce Population SD: S19-3 (maternal) x DodR (paternal). A more detailed discussion of the plant materials is provided in the specific Chapter 6.

Table 3-1 List of genotypes, respective seed color, and country collected, used for the soil-filled PVC column experiment in Chapter 4 and 5.

Geographical region	Designation	Genotypes [1]	Seed color	Country collected	Climate	Rainfall mean (mm year^{-1})
Southeast Asia	1	Gresik	Dark	Indonesia	Tropical wet	>2,000
West Africa	2	LunT	Cream	Sierra Leone	Tropical wet	>2,000
	3	Ankpa-4	Brown	Nigeria	Tropical dry	>2,000
	4	Tiga nicuru	Cream	Mali	Subtropical	450
East Africa	5	IITA-686	Dark	Tanzania	Tropical dry	>750
	6	DodR	Red	Tanzania	Tropical dry	>570
Southern Africa	7	S19-3	Dark	Namibia	Subtropical desert	350
	8	DipC1	Cream with black eye	Botswana	Semi-arid	500

[1] Names mostly based on the place the seeds were collected, e.g., Gresik, city found in East Java, Indonesia; LunT, Lungi the northern province of Sierra Leone; Ankpa, an area in Kogi State Nigeria; IITA-686, International Institute of Tropical Agriculture (IITA) Nigeria; Dodoma Red (DodR), the national capital of Tanzania; and Diphiri Cream (DipC1), the region of Kweneng, Botswana.

Figure 3-1 Bambara groundnut single genotypes seeds used during the course of the experiments. Black bar = 1cm

3.2.2 Study Site and PVC Column Screening System

Experiments (Chapter 4, 5 and 6) were conducted under a rainout shelter at the Crops For the Future-Field Research Center (CFF-FRC) located at 2°55′52.2″N 101°52′45.7″E, altitude 42m above sea level in Semenyih, Malaysia. The bambara groundnut were grown in light weight PVC pipes. The pipes originally 20 × 580cm (inside diameter and length, respectively; Figure 3-2A), were cut into 20 × 116cm (inside diameter and length, respectively; Figure 3-2B). Although the column diameter restricts vertical development, this was not an issue since the thesis was primarily concerned with horizontal tap rooting and root branching from the taproot. Four holes were made one half of the PVC pipe for moisture measurements in Chapter 4 and 5 (Figure 3-2C). The pipes were placed on top of a detachable perforated plate which allowed free drainage of excess water. In addition, a wooden frame was constructed to support the columns and keep them upright (Figure 3-2D).

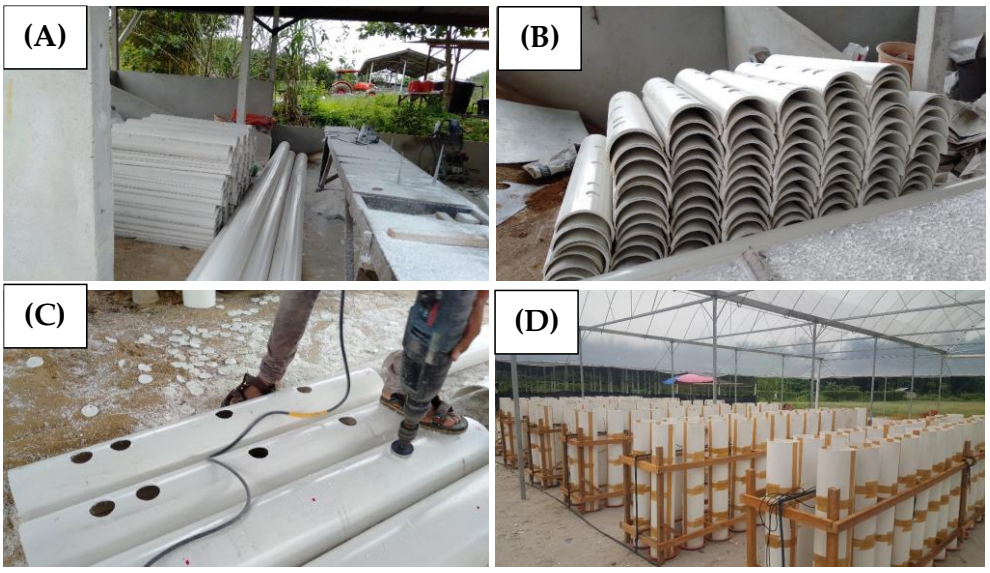

Figure 3-2 (**A**) PVC pipes originally 20 × 580cm (inside diameter and length, respectively, (**B**) PVC column of 20 × 110cm (inside diameter and length, respectively), placed on a perforated plate, (**C**) Four holes made on one half of the PVC pipe for moisture measurements (**D**) PVC column setup under rainout shelter including a wooden frame for structural support.

To facilitate root harvesting, each column was cut longitudinally along both sides and the two halves taped together with 4.8cm (by width) packing brown tape before filling with soil. CFF-FRC native soil have been identified as Oxisols (according to USDA classification) high in the element iron (Fe) oxides and a source of infection for a number of plant bacterial and fungal foliar infections. According to unpublished preliminary findings, bambara groundnut genotypes (particularly, S19-3) adapted to and sourced from hot-dry environments was susceptible. As a result, the soil used in the experiments came from an open field 2 km away from the CFF-FRC. Since previous bambara groundnut genotypes grown on the same soil had no visible infection signs and symptoms, the soil was preferred and chosen. The soil was composed of a mixture of air-dried sand and clay (2:1 w:w).

The textural class of the mixed soil was sandy clay. Detailed physical and chemical properties of the soil are: silt (10%), sand (48%), clay (42%) and 4.2% organic matter, 0.13% N, 41.0 (mg L^{-1}) P, 0.16 (meq 10 g^{-1}) K, 0.11 (meq 100 g^{-1}) Mg, 1.09 (meq 10 g^{-1}) Ca, 151.3 (mg L^{-1}) Fe, and had a pH of 5.7 according to a soil test performed by Applied Agricultural Resources Sdn Bhd, Malaysia. Malaysia generally has acidic soils that retain a lot of moisture, increasing iron (Fe) availability. However, this was not a concern since it had no effect on plant development and the soil was homogeneous throughout the experiments (removing any bias). In terms of texture, the soil mixture matched the native soil at CFF-FRC.The soil was sieved through a 3 mm mesh to eliminate >3 mm diameter soil particles. The soil was poured into the PVC pipe and manually packed using an ~1.5kg concrete base tamper, with base plate diameter equal to the internal diameter of the pipe. The amount of soil packed into the PVC columns depended on: bulk density of the sandy clay soil and the volume of the PVC columns. The soil surface was lightly scraped after every interval pack to provide hydraulic connectivity between the soil portions, preventing a

layering effect (Lewis and Sjöstrom 2010). Each pipe was packed to allow for a homogeneous continuum, rather than stratified layers. The downward movement of water and growth of roots is limited by horizontally stratified layers. As such, soil packing allowed all soil fractions to be exposed equally to water, promoting uniform water distribution rather than preferential flow pathways (Hardie et al. 2013). Pipes were filled with 55.3kg of soil up to 110cm high achieving a constant bulk density of 1.6g cm^{-3} and hereafter referred to as PVC columns. Despite high bulk density, the soil was adequate for plant growth and root assessment (Kundy, 2019). Basal fertilizer (10kg ha^{-1} N as urea (46%), 50kg ha^{-1} P as Christmas Island rock phosphate (CIRP) (30%), and 50kg ha^{-1} K as muriate of potash (MOP) (60%) was surface applied and incorporated into the topsoil layer (0 to 10cm) of the column (Musa et al. 2016). Based on unpublished preliminary results from a separate experiment, 90g of granular Agromate ABC micronutrients (Agromate International, Ltd) were dissolved in 10L of water and added to the soil columns at two and three weeks after emergence (WAE). The solution consisted of Mn EDTA (3.8%), Fe EDTA (4.0%), Cu EDTA (1.5%), B (0.5%), Zn EDTA (1.5%), Co (0.03%), Mo (0.10%), and Mg (5.10%).

After removing broken and damaged seeds, uniform sized seeds were selected and surface sterilized in a 10% (v/v) Clorox solution (sodium hypochlorite 0.5%) for 2 min on a rotary shaker at 150 rpm. Following this, seeds were rinsed thrice using distilled water. Sterilized seeds were placed in 9cm diameter petri dishes and allowed to imbibe water for 15h at a temperature of 28 ± 1°C, in the dark. For each genotype, two seeds were sown in individual columns. One healthy representative plant per column was maintained and the plants were grown under field conditions and protected from rainfall using a fixed-location transparent acrylic rainout shelter. The columns were irrigated until seedlings emergence (an average of 6 days to emergence across the

studied genotypes) then irrigated with 250mL of water four times on alternate days until harvest, i.e., 35-days after emergence (DAE) for Chapter 3. Based on recommendations by Kundy, (2019), for all experiments two insecticides, i.e., Agus 24SC at a rate of 16 mL 10 L^{-1} of water and Akosu 9.5SC at a rate of 7.5mL 10 L^{-1} of water (active ingredient, diafenthiuron 24.0% and chlorfenapyr 9.5% both suspension concentrates) were prepared as a tank mix and sprayed every seven days to protect the plants from white flies (*Aleyrodidae* spp) and red spider mites (*Tetranychus* spp), respectively. Fungicide with active ingredient: didenoconazole 20.0% emulsifiable concentrate was sprayed once at three WAE at a rate of 10mL 10 L^{-1} of water (Kundy, (2019).

3.2.3 Experimental Design and Layout

In the first experiment (Chapter 4), the experimental design used was a completely randomized (CRD) design in six replications for each season. Eight bambara groundnut single genotypes derived from landraces of contrasting geographic origin were used. A CRD design was used considering the experimental units were homogeneous. In the successive experiments (Chapter 5 and 6), the experiments consisted of a factorial treatment combination of genotypes/lines and two water managements i.e., well-watered (WW) and drought stress (DS) with three replicates. Chapter 6 was based on data from Population SD: S19-3 (maternal) × DodR (paternal) which has contrasting traits for plasticity under drought as elucidated in Chapter 5. A detailed description of the plant materials is given in Chapter 6.

3.2.4 Water Treatments

For the two water treatments i.e., well-watered (WW) and drought stress (DS), the PVC columns were slowly irrigated once every three days at 17.00h

to field capacity until 25 DAE. Before sowing, soil moisture at field capacity (FC) was determined (13%) as a basis for controlling the amount of irrigation - water. For determination of soil moisture at FC, the columns with soil were saturated with water then left overnight. This was followed by determination of available soil moisture by volume. Soil moisture content in the column was monitored using a PR2 theta probe (Delta T Devices, UK) for compensation of moisture loss while maintaining the amount of soil moisture in respective treatments. The irrigation was continued in the WW treatment right up to the end of the experiment, while the DS treatment received no further irrigation from 25 DAE. Plants were subjected to DS for a period of 30-days. The DS treatment was terminated at 55 DAE. This was the period when no significant changes in gaseous exchange could be observed. Irrigation was resumed bringing the soil moisture in the columns back to field capacity. All the plants i.e., WW and DS (recovery) were then slowly irrigated once on alternate days until final harvest (105 DAE).

3.2.5 Plant Sampling and Common Measurements

To extract the roots, the PVC column was laid down and tilted at a 20° angle to the root washing station (Figure 3-3A). The column was split in half longitudinally. The soil was gradually removed to expose the roots in a bottom-up manner using soft a spray watering head (Figure 3-3B). After complete removal of the soil, the shoots (i.e., leaves and stems) were submerged in water-filled zip lock bags of 22 × 30cm (width and length, respectively) and transported to the laboratory for further assessment (Figure 3-3C).

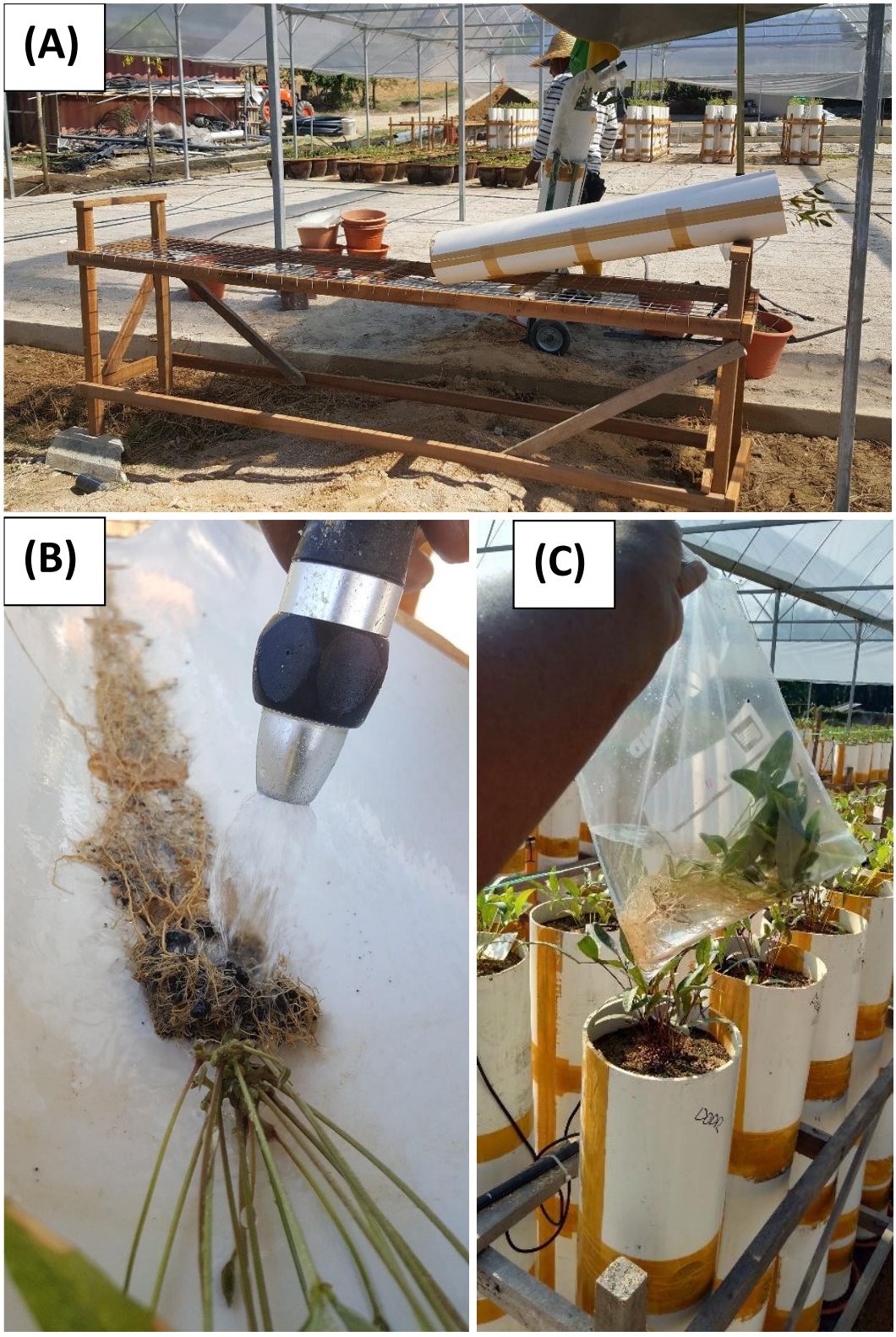

Figure 3-3 (A) Root washing station (with two PVC pipe washing capacity) (**B**) Gradually removal of soil to expose the roots using soft spray watering head. (**C**) Root systems submerged in water-filled zip lock bags of 22 × 30cm (width and length, respectively).

To identify and measure the tap root length (TRL), entire roots were laid flat and stretched against a two-meter ruler, giving an estimate of the deepest extent of the root system. Entire root systems (i.e., totals) were analyzed first. Following the entire analysis, root systems were cut into different segments with respect to varying 30cm soil depth (i.e., 0-30, 30-60, 60-90, and 90-110cm) and analyzed as such (Figure 3-4). In both cases, roots were spread in a shallow A3 size, 297 × 420mm (height × width, respectively) clear acrylic tray filled with water and disentangled using plastic forceps to reduce overlapping. Root traits were all computed from the scanned images in greyscale at 400 dots per inch (DPI) using a flatbed Epson Scanner (Epson Perfection V700, CA, USA) with WinRhizo Pro software v2009 (Regent Instruments, Montreal, QC, Canada).

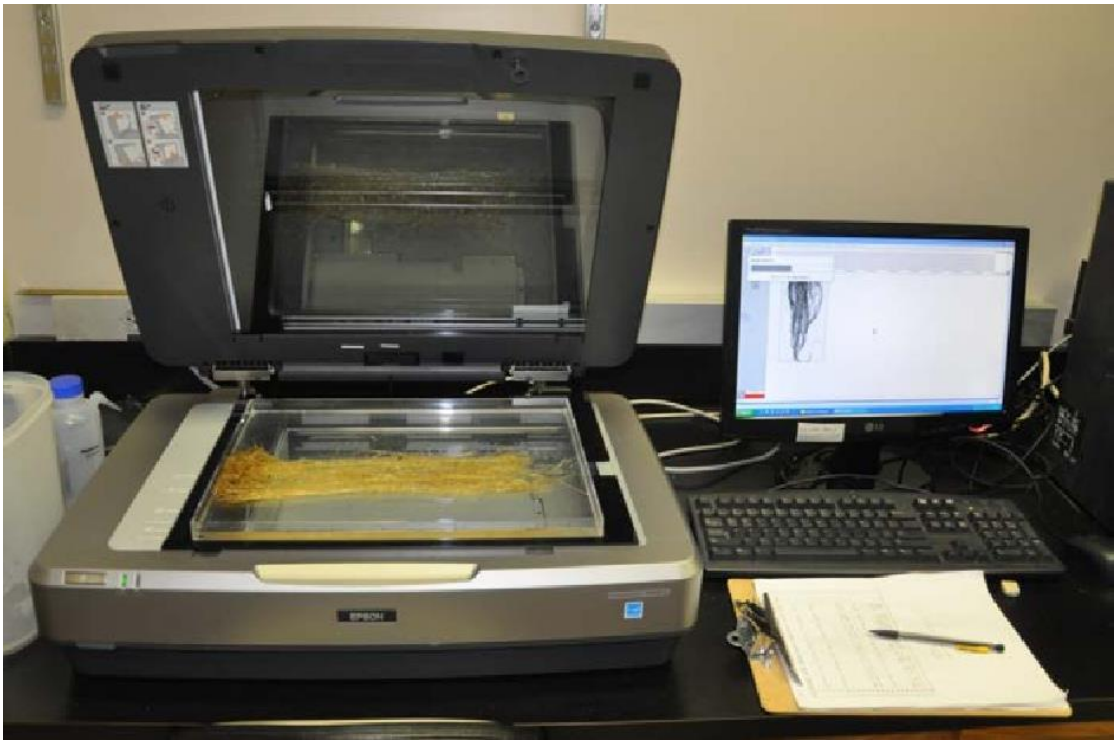

Figure 3-4 Bambara groundnut roots suspending on a clear acrylic tray on a flatbed Epson Scanner (Epson Perfection V700, CA, USA) with WinRhizo Pro software v2009.

The analyzed roots traits include root length (RL cm), representing root lengths in the network. Branching number (BN), the number of first-order lateral roots emerged from the tap root. Root surface area (SA cm^2), root volume (RV cm^3) and root diameter (RDia mm) were assessed as proportionate estimations of RL and expected to exhibit the same pattern and trend of variation (Lalitha et al. 2015). These traits subsequently allowed for the calculation of root length density (RLD cm cm^3), branching density (BD), and branching intensity (BI) using the following formulae:

$$\text{Root length density (RLD)} = \text{root length (cm)} / \text{soil volume } (cm^{-3}) \quad (1)$$

The soil volume was calculated by following the mathematical equation (2), where $\pi = 3.14$; r = soil column inner radius; h = segment-column height:

$$\text{Soil volume} = \pi \times r^2 \times h \quad (2)$$

$$\text{Branch density (BD)} = \text{number of branches/tap root length,} \quad (3)$$

$$\text{Branch intensity (BI)} = \text{number of branches/root length depth segment}^{-1}. \quad (4)$$

Shoot height (SH) was recorded on a fresh plant basis from the root crown to the apex of the longest plant stem using a ruler. Number of leaves (NoL) were measured on a fresh plant basis and recorded as the number of fully expanded trifoliate leaves. To measure biomass accumulation, shoot dry weight (SDW) and root dry weight (RDW) were recorded after drying in an oven at 80°C for 72 h and expressed as g $plant^{-1}$. Root to shoot (R:S) ratios were calculated on a dry mass basis by dividing RDW by SDW.

3.2.6 Developmental Traits

Days to 50% emergence (D50% Em) was recorded as the number of days after planting when 50% of the plants per genotype had emerged from the soil. Days to 50% flowering was recorded as the number of days after emergence (DAE) when 50% of the plants had flowered.

3.3 Statistical Analysis

The data obtained from all PVC column experiments were analysed using Statistica Version 13.3 software (TIBCO Inc, USA). In cases where data was non-normally distributed (tap root length (TRL), root length (RL), surface area (SA), root diameter (RDia), root volume (RV) (all 90-110cm depth), branching density (BD) 60-90cm, branching intensity (BI) 30-60cm, and 60-90cm depths, in Chapter 4), data were transformed before analysis of variance (ANOVA) using the square root transformation. The square root transformation was used to normalize skewed distribution. Mean comparisons were performed on the transformed scales and for the presentation of the results, the means were back-transformed. For all experiments means were compared using post-hoc Tukey's honest significant difference (HSD) at significance level of 95%.

CHAPTER 4 : Root Foraging Capacity in Bambara Groundnut (*Vigna Subterranea* (L.) Verdc.) Core Parental Lines Depends on the Root System Architecture during the Pre-Flowering Stage

4.1 Summary

Characterizing the morphological variability in root system architecture (RSA) during the sensitive pre-flowering growth stage is important for crop performance. To assess this variation, eight bambara groundnut single genotypes derived from landraces of contrasting geographic origin were selected for root system architecture and rooting distribution studies. Plants were grown in a polyvinyl chloride (PVC) column system under controlled water and nutrient availability in a rainout shelter. Days to 50% plant emergence was characterized during the first two weeks after sowing, while tap root length (TRL), root length (RL), root length density (RLD), branching number (BN), branching density (BD) and intensity (BI), surface area (SA), root volume (RV), root diameter (RDia), root dry weight (RDW), shoot dry weight (SDW), and shoot height (SH) were determined at the end of the experiment, i.e., 35 days after emergence. Genotypes S19-3 and DipC1 sourced from drier regions of sub-Saharan Africa generally had longer tap roots and greater root length distribution in deeper (60-90cm) soil depths. In contrast, bambara groundnut genotypes from wetter regions (i.e., Gresik, LunT, and IITA-686) in Southeast Asia and West Africa exhibited relatively shallow and highly branched root growth closer to the soil surface. Genotypes at the pre-flowering growth stage showed differential root foraging patterns and branching habits with two extremes, i.e., deep-cheap rooting in the genotypes sourced from dry regions and a shallow-costly rooting system in genotypes adapted to higher rainfall areas with shallow soils. I propose specific bambara groundnut genotypes as donors in root trait driven breeding programs to improve water capture and use efficiency.

Keywords: bambara groundnut (*Vigna subterranea* (L.) Verdc.); branching; deep rooting; drought adaptation; root traits

4.2 Introduction

Root system architecture (RSA) describes the form and spatial structure within the soil of a root system (Rogers and Benfey 2015). This has significant implications for plant development and enables plants species to adapt to environmental cues in order to flourish in various ecological habitats (Lynch et al. 2005). Variations in RSA are related to differences in soil nutrient and water acquisition among landraces of a similar developmental form but originating from contrasted ecological niches (Paula and Pausas 2011). Bambara groundnut (*Vigna subterranea* (L) Verdc), is an exemplar neglected African grain legume that thrives under strikingly contrasted environments relative to other grain legumes. Originating in West Africa, its distribution spans across climatic gradients from Senegal to Kenya and from the Sahara to South Africa with recent introductions in Southeast Asia (Feldman et al. 2019). In these contrasting habitats, bambara groundnut has diversified due to domestication from its wild relative, *Vigna subterranea* var. spontanea (Harms) Hepper, as a result of steady changes through natural and artificial selection (Doku and Karikari 1971).

Looking at the different patterns of root distribution, theoretical and experimental research propose that a root system comprising of a deep-cheap rooting system associated with a long tap root system and few primary first-order laterals, would favor deep water foraging and mobile nutrients acquisition in low-resource habitats (Taub and Goldberg 1996). Conversely, a shallow-costly rooting system associated with a shorter tap root system and greater primary first-order laterals, would favour water and nutrient acquisition in the shallow soil depths of high and low-resource habitats (Taub and Goldberg 1996). Generally, leguminous crop species cultivated in hot-dry environments exhibit a particular root topology, i.e., long tap root system with few primary first-order laterals (Chen et al. 2017). However, detailed

descriptions are currently missing for root trait differences among bambara groundnut genotypes of contrasted habitats.

Considering the increasing shortage of agricultural water and that no single shoot trait has yet been identified for its unique and dominant contribution to drought resistance (Collinson et al. 1997, 1999; Jørgensen et al. 2010; Sesay et al. 2010; Vurayai et al. 2011; Mabhaudhi and Modi 2013; Chibarabada et al. 2014; Chai et al. 2016a, 2016b; Muhammad et al. 2016), current bambara groundnut breeding efforts could investigate root system function and its manipulation in order to improve water and nutrient capture (de Dorlodot et al. 2007; Lynch and Brown 2012; Lynch et al. 2014). Bambara groundnut genotypes, such as S19-3 (from Namibia) are promising candidates for investigating and expanding plant ideotypes suited for dry environments (Jørgensen et al. 2010; Kundy 2019). In reality, for many centuries, southern African farmers have selected local bambara groundnut genotypes for their drought resistance (Abu and Buah 2011). As it occurred in other crop species (Palta and Fillery 1993; Hammer et al. 2009; Wasson et al. 2012; York et al. 2015), indirect selection by farmers for improved rooting capacity is likely to have occurred in bambara groundnut through its influence on yield over the years. Investigating the morphological variability in RSA and primarily the contrast between a diverse collection of bambara groundnut genotypes sourced from various agroecologies, would help test the hypothesis of indirect selection, while also defining root system ideotypes that could improve soil resource uptake.

Since grain legumes suffer significant yield reduction due to water deficit stress that occurs earlier on during the reproductive growth stages (Farooq et al. 2017), characterizing the root system at final harvest cannot reveal the range of maximum variation for drought resistance breeding. Significant variation in root growth is observable just before flowering, i.e., 35 to 45 days

after emergence (DAE) (Gregory et al. 1978; Krishnamurthy et al. 1996; Kashiwagi et al. 2005; Chen et al. 2011). As such, initial characteristics in plant root development and branching manner are essential to the ultimate establishment of the plants and their subsequent exploration of the soil volume for water. Therefore, root trait variation observed at this stage would aid in determining the most informative root traits that significantly reduce grain yield penalties for subsequent breeding (León et al. 2011).

From this, an initial exploration of genetic variability in root characteristics in bambara groundnut would be an important step to developing varieties for target environments. The underlying speculation is that bambara groundnut plants from low resource agroecologies have throughout the years developed root traits that improve resource foraging in deep soil depths. All the more explicitly, it is expected that bambara groundnut plants from low resource habitats would have more extensive root extension in the deep soil depths during the early growth stage. By means of a polyvinyl chloride (PVC) column study, the present experiment's objective is to explore the developmental variation in tap root and branching patterns at the pre-flowering stage using a collection of single genotypes derived from landraces of contrasting agroecological backgrounds.

4.3 Materials and Methods

4.3.1 Plant Material

The description for the experiment is presented in Chapter 3. Eight bambara groundnut single genotypes derived from landraces of contrasting geographic origin were used. For more details, see *3.2.1 Plant Material*.

4.3.2 Study Site and PVC Column Screening System

The experiments used modified a light weight PVC column screening system (Figure 4-1A-C). Experiments were conducted during two consecutive seasons (2017–2018 and 2018–2019) at the Crops For the Future-Field Research Center (CFF-FRC), Malaysia. The description for the study site and PVC column screening system is presented in Chapter 3. For more details, see *3.2.2 Study Site and PVC Column Screening System*. The experimental design and layout are described in *3.2.3 Experimental Design and Layout*.

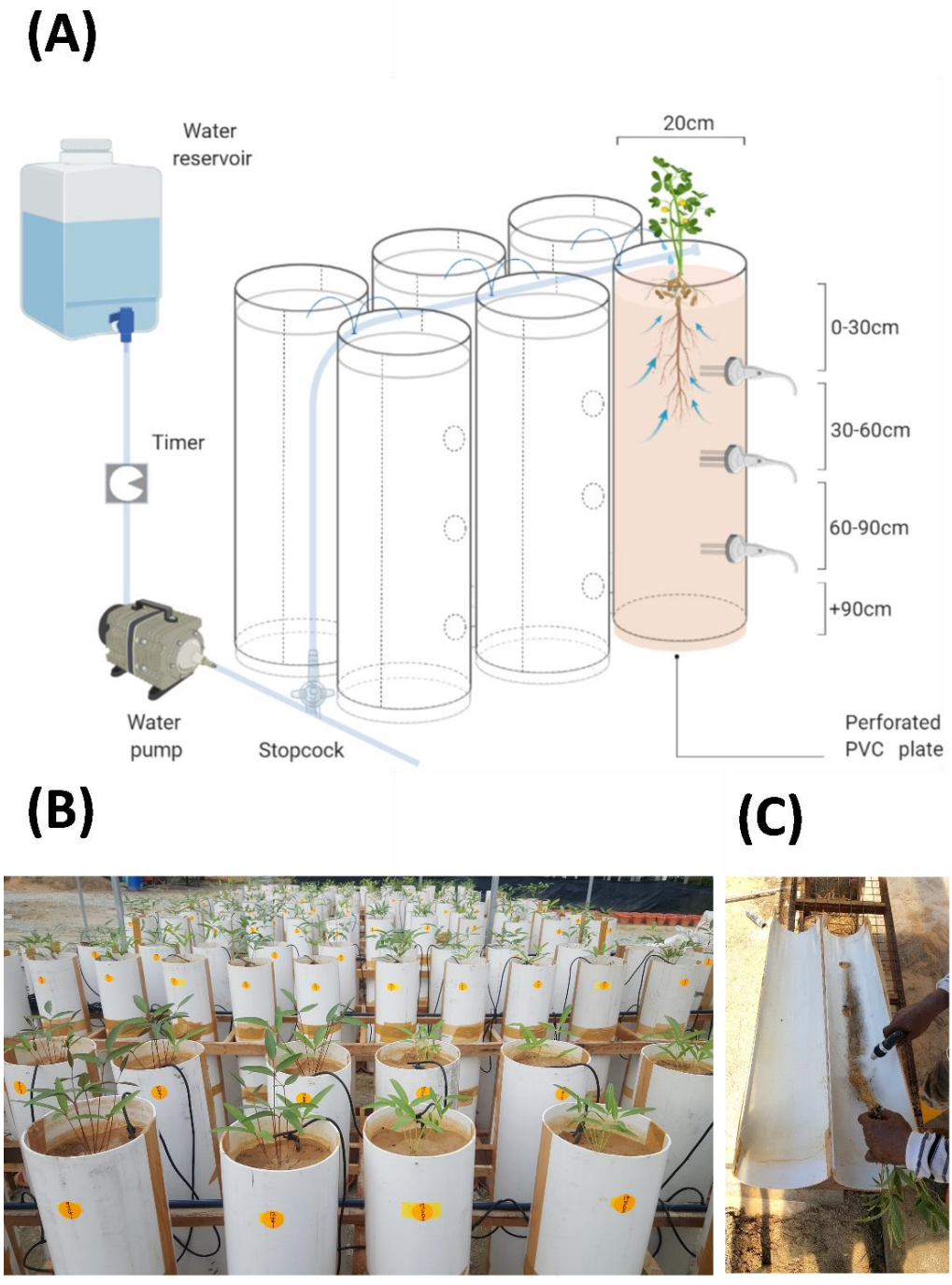

Figure 4-1 (**A**) Schematic representation of soil-filled PVC column of 20 × 110cm (inside diameter and length, respectively), placed on a perforated plate (**B**) PVC column setup under rainout shelter including a wooden frame for structural support and (**C**) column split and root washing.

4.3.3 Plant Sampling and Measurements

The procedure in this experiment was common to the other experiments and therefore is described under Chapter 3 (see *3.2.5 Plant Sampling and Common Measurements*). This included root length (RL cm), branching number (BN), root surface area (SA cm^2), root volume (RV cm^3), root diameter (RDia mm), root length density (RLD cm cm^3), branching density (BD), branching intensity (BI), Shoot height (SH), shoot dry weight (SDW) and root dry weight (RDW); number of leaves (NoL). Twelve biological replicates were used per root and shoot traits measurements per bambara groundnut genotype, except for NoL (n = 6). Developmental traits, particularly days to 50% emergence (D50% Em), are also described in Chapter 3 (see *3.2.6 Developmental Traits*).

4.3.4 Data Analysis

General linear model (GLM) multivariate analysis was performed for genotypes as main effects using Statistica Version 13.3 software (TIBCO Inc, USA). The significance of the main effect of the season was assessed using the (Wald 1943) statistic that asymptotically follows a χ^2 distribution. Wald statistics revealed that the error components across the years (G × Y) for the traits, were homogenous, and therefore it was necessary to draw inferences combined across years for the measured traits. For a detailed description of traits transformed, and mean comparisons refer to Chapter 3 (see 3.3 *Statistical Analysis*). Correlations between traits were performed using the cor ( ) and corrplot ( ) functions from the corrplot package in R (R Core Team, 2017). Since the traits have different units, they were scaled to have a variance of one and a mean of zero, using the Standardize function in Statistica Version 13.3. The eight bambara groundnut genotypes exhibited distinctly variable morphologies, and therefore reduced the complexity of the data, K-means clustering was used to generate homogeneous clusters of genotypes. K-means

clustering was run with different numbers of clusters (Clusters 3-4). Four clusters provided the most interpretable output, in terms of genotype clustering by traits.

4.4 Results

4.4.1 Plant Emergence, Size, Biomass Production, and Root to Shoot Ratio

According to results of analysis of variance, seed emergence, shoot height, and root to shoot ratio were significantly affected by genotype (Table 4-1). Seed emergence started three days after sowing (Gresik) and continued up to 10 days (Ankpa-4) with a mean of six days. The genotype Gresik showed the fastest emergence, resulting in higher shoot height and biomass production at 35 DAE, as well as higher number of leaves, although this was not statistically different from the other genotypes. Days to 50% emergence was negatively and highly correlated to the root dry weight ($r = -0.39$, $P < 0.006$) and, subsequently, the root to shoot ratio ($r = -0.47$, $P < 0.001$; Figure 4-2), demonstrating that root biomass decline as a result of slow seedling emergence. At that time, Tiga nicuru, the least vigorous of the studied genotypes, was only 74% the size of IITA-686 in terms of shoot height and only 43% the size of Gresik in terms of root dry weight. LunT, IITA-686, DodR, S19-3, Tiga nicuru, and DipC1 showed intermediate and statistically similar values for emergence, with Tiga nicuru less productive (for root dry weight, 0.28g plant^{-1}) than Gresik (0.64g plant^{-1}; Figure 4-3), essentially due to lower shoot dry weight (1.19g plant^{-1}). Despite contrasted growth capacities, the genotypes Gresik and S19-3 showed similar biomass allocation patterns with root to shoot ratios of 0.35 and 0.28, respectively, significantly higher than those observed in Ankpa-4 (0.16; Table 4-1).

Table 4-1 Effect of genotypes on days to 50% emergence (D50% Em) and shoot height (SH), number of leaves (NoL), and root to shoot (R:S) ratio at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively).

Treatment [1]	D50% Em (number of days)	SH (cm plant $^{-1}$)	NoL[2] (number plant $^{-1}$)	R:S ratio (RDW/SDW)
G				
Gresik	4.67 ± 1.03 ^{c}	21.50 ± 1.10 ^{a}	17 ± 1.91 ^{a}	0.35 ± 0.03 ^{a}
LunT	5.67 ± 1.03 ^{bc}	24.51 ± 0.91 ^{ab}	15 ± 0.95 ^{a}	0.19 ± 0.02 ^{bc}
IITA-686	6.50 ± 1.05 ^{abc}	26.18 ± 1.04 ^{ab}	13 ± 0.80 ^{a}	0.21 ± 0.02 ^{bc}
DodR	5.33 ± 1.03 ^{bc}	25.20 ± 0.96 ^{ab}	14 ± 0.48 ^{a}	0.23 ± 0.02 ^{bc}
S19-3	6.17 ± 1.47 ^{abc}	23.75 ± 0.66 ^{ab}	14 ± 0.54 ^{a}	0.28 ± 0.02 ^{ab}
Tiga nicuru	5.50 ± 1.05 ^{bc}	19.26 ± 2.15 ^{b}	14 ± 1.09 ^{a}	0.23 ± 0.04 ^{bc}
Ankpa-4	7.83 ± 1.47 ^{a}	20.03 ± 1.52 ^{b}	14 ± 0.71 ^{a}	0.16 ± 0.02 ^{c}
DipC1	7.33 ± 1.03 ^{ab}	24.04 ± 0.88 ^{ab}	15 ± 1.42 ^{a}	0.21 ± 0.02 ^{bc}
Mean	6.13	23.06	14.60	0.23
F probability				
G	0.000 ***	0.009 **	0.27 ^{ns}	0.000 ***

[1] Treatments G – genotype.

[2] NoL values rounded to the nearest integer because NoL represents discrete data.

The data is mean ± se values (n = 12), except for NoL (n = 6), with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns = not significant.

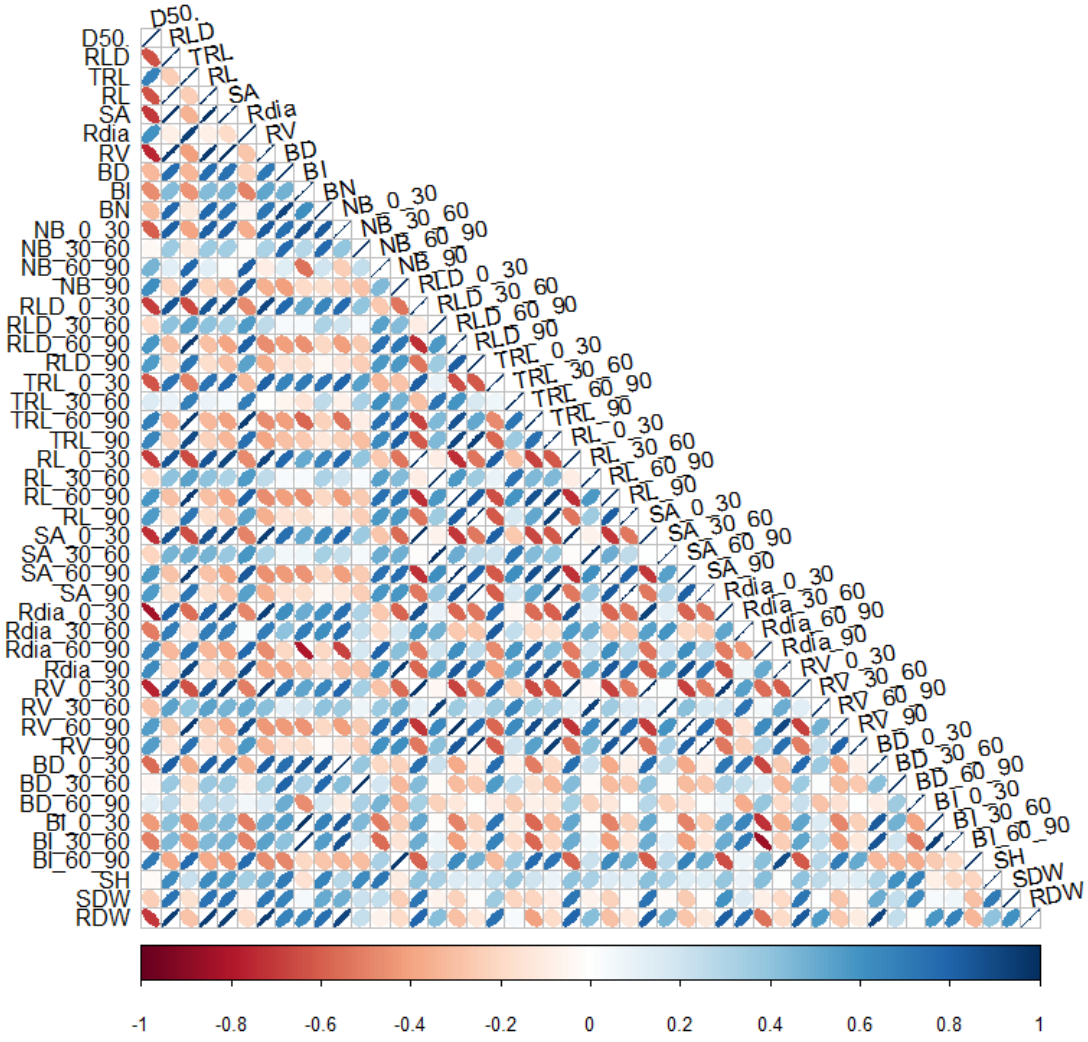

Figure 4-2 Pearson correlation coefficients between various root traits of bambara groundnut genotypes at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). Eccentricity and color of the ellipses represents the correlation value. The scale is indicated in the bar below the matrix.

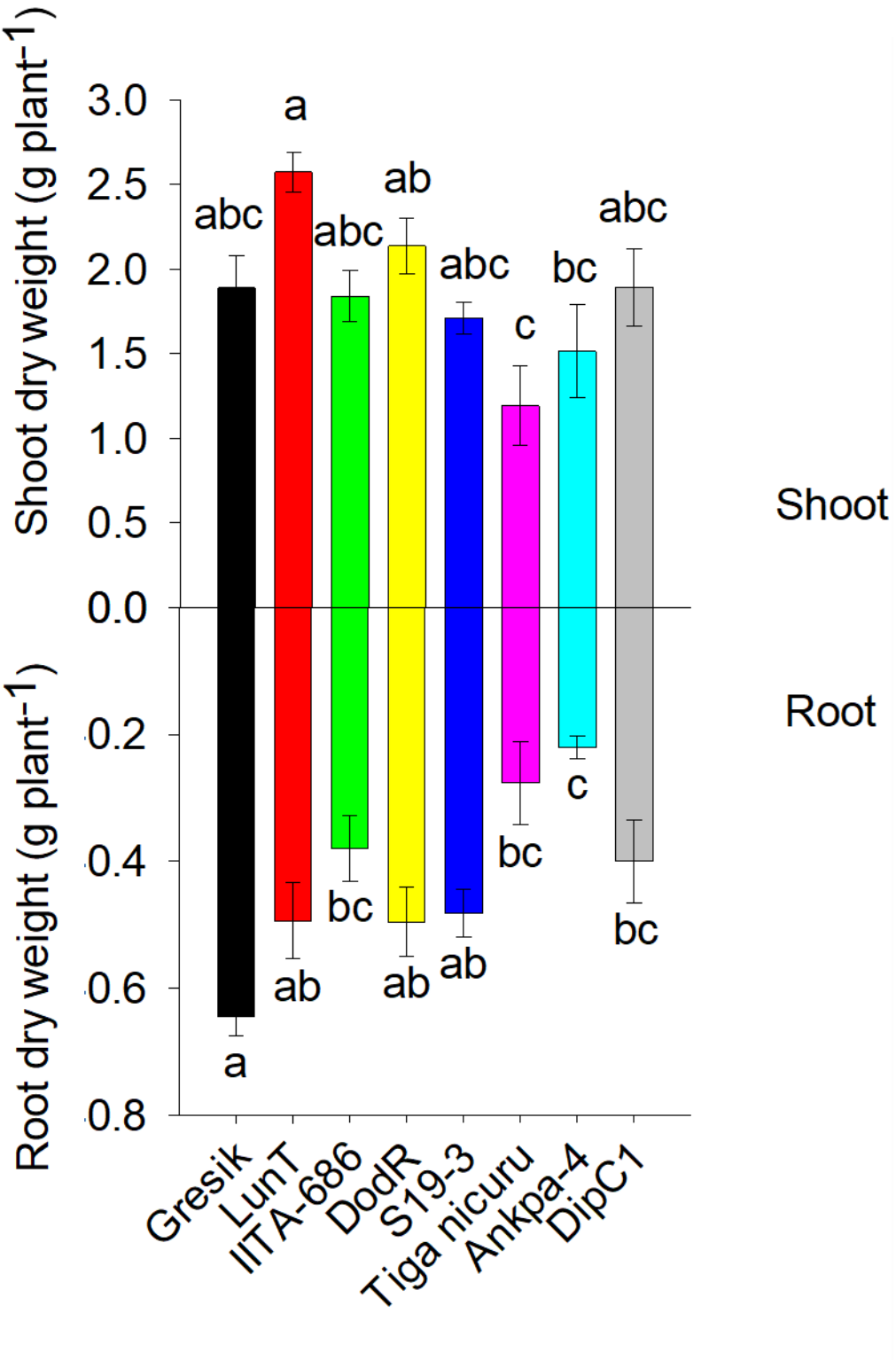

Figure 4-3 Shoot dry weight (SDW) and root dry weight (RDW) for bambara groundnut genotypes at 35 d after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). Mean ± se values (n = 12) are shown. Different letters indicate significant differences (HSD, $P < 0.01$).

4.4.2 Deep Rooting Profile

After 35 DAE, the vertical growth of the root system showed significant differences among genotypes (Figure 4-4A,B). The genotypes varied significantly for the rooting depth, i.e., tap root length. This ranged from 58.9cm (Gresik) to 100.6cm (DipC1) with average tap root length of 78.6cm (Figure 4-4B). Compared to DipC1, the genotypes Tiga nicuru and Gresik showed significantly less deep rooting, although the former still penetrated deep soil, i.e., >60cm while the latter was almost exclusively limited to the 30-60cm layer (Figure 4-4B). The genotypes S19-3 and Ankpa-4 showed significantly higher root depth, recording the second largest tap root length (95.1cm and 89cm, respectively), although S19-3 was not statistically different ($P > 0.05$) from the deepest rooting genotype DipC1 (Figure 4-4B). Plants of DipC1 had substantially longer tap roots, with up to 19-fold more tap root length in the 60-90cm depth as compared to the shallowest genotype (Gresik, 1.36cm; Figure 4-4C).

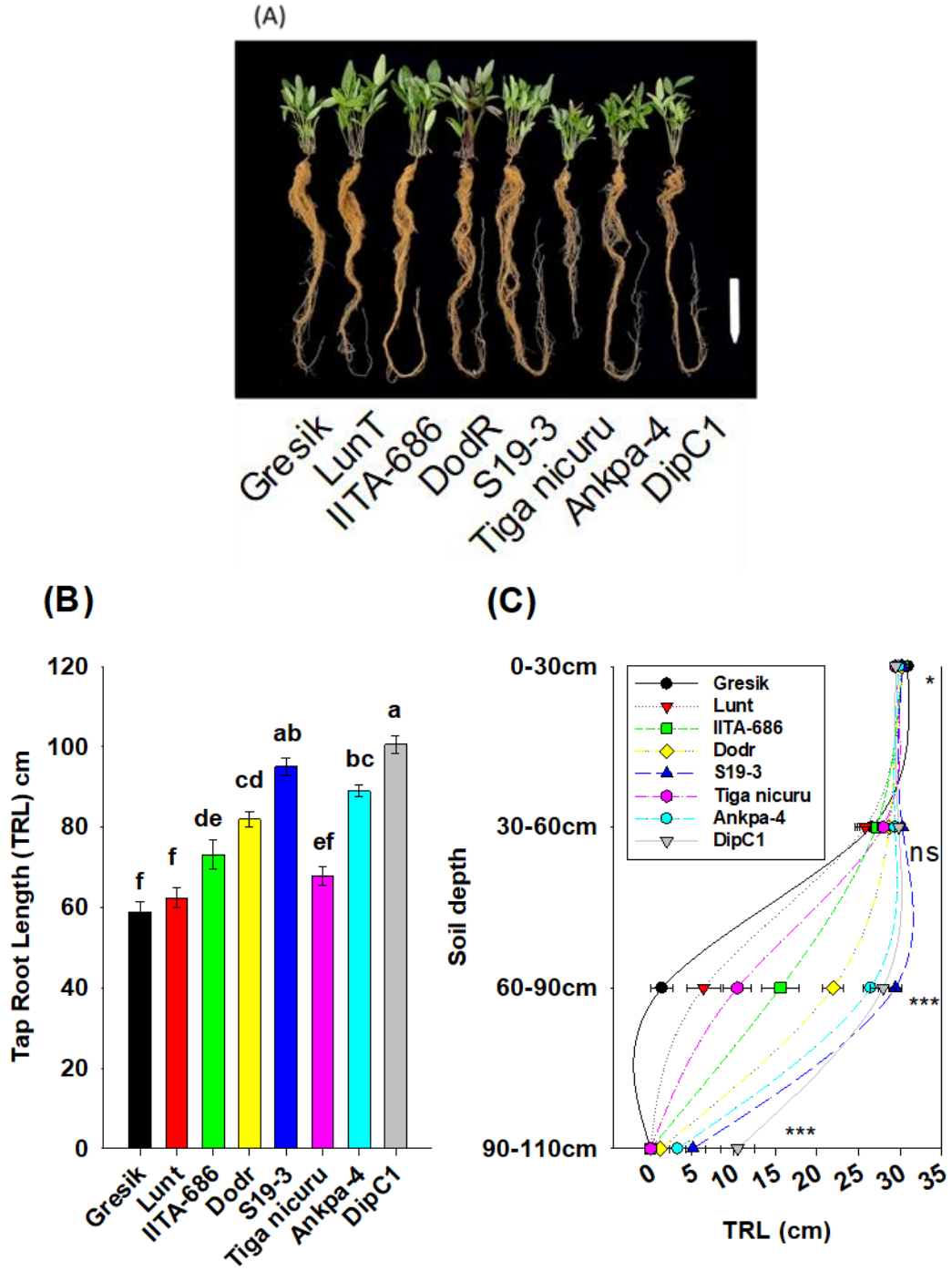

Figure 4-4 (**A**) Images of the entire root system for bambara groundnut genotypes at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). White bar = 15cm; (**B**) Total tap root length (TRL) in bambara groundnut genotypes. Mean ± se values (n = 12) are shown. Different letters indicate significant differences (HSD, $P < 0.01$); (**C**) TRL's per soil depth segments. Mean ± se values (n = 12) are shown. Significant differences as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and ns = not significant, among individual genotypes.

4.4.3 Root System Branching and Density Dynamics

The bambara groundnut root systems at 35 DAE were limited to the tap root, first-order, and second-order lateral branching (Figure 4-6A). The analysis of branching numbers and branching density, i.e., first-order lateral roots, from the tap root, revealed contrasted dynamics among the studied genotypes. Total branching numbers ranged from 120 (Tiga nicuru) to 278 (Gresik) with an average of 209 (Figure 4-6B), with the largest variance in the shallow soil depth (0-30cm; (Figure 4-6C) . Branching density followed a somewhat similar trend, ranging from 1.7cm^{-1} (Ankpa-4) to 4.5cm^{-1} (Gresik; Figure 4-7A,B). The genotypes, Gresik had the most BD (Figure 4-7B), although not statistically different from IITA-686, (3.6cm^{-1} tap root length) and in the case of Gresik, this was largely distributed in the shallow soil depth (0-30cm), as shown by data from the branching intensity (Figure 4-7C). As a result, root length and root length density, direct components of first- and second-order lateral branching were highest in the genotype LunT (4603.5cm and 0.13cm root cm^3 soil, respectively) followed by the second highest genotype Gresik (4545.5cm and 0.13cm root cm^3 soil, respectively; Figure 4-6A and Figure 4-7A).

A more detailed look into the different soil depth segments revealed that branching numbers in the 0-30cm depth ranged from 75 (Tiga nicuru) to 207 (Gresik; Figure 4-6C). The least branching genotype, Tiga nicuru, was statistically similar to S19-3 and DipC1 (117 and 116, respectively; Figure 4-6C). It appeared that changes in branching numbers reflected changes in root length in the 0-30cm, 30-60cm soil depths (r = 0.64, $P < 0.001$ and r = 0.31, $P < 0.03$, respectively; Figure 4-2) and not in the 60-90cm soil depth (r = -0.19, $P > 0.19$), a direct result of low mean BI values (0.000276cm^{-1} root length) realized in that soil depth (Figure 4-7C). The genotypes LunT and Gresik ranked highest for root length (3700.2 and 3430.6cm, respectively; Figure 4-8A) and root length density (0.39 and 0.36cm root cm^3 soil, respectively; Figure 4-9A), whilst Tiga

nicuru and Ankpa-4 allocated the least. However, S19-3 and DipC1 (genotypes originating from drier regions), branching number and subsequently branching intensity was highest at 60-90cm as soon as 35 DAS, when most of the other genotypes had yet reached that depth. In the 60-90cm soil depth, S19-3 and DipC1 had up to 55- and 34-fold, respectively, longer root length density (0.07cm root cm^3 soil) as compared to Gresik (0.001cm root cm^3 soil; Figure 4-9C).

In the shallow 0-30cm soil depth, shoot dry weight was closely and positively correlated ($P < 0.05$) with a wide range of traits, including root length density (r = 0.73), root length (r = 0.73), root surface area (r = 0.74), and root volume (r = 0.73; Figure 4-2). Additionally, shoot height was closely and positively correlated ($P < 0.05$) with branching number in the deep 60-90cm of the soil ($R^2 = 0.53$), and this was largely among genotypes originating from drier versus wetter environments (Figure 4-5).

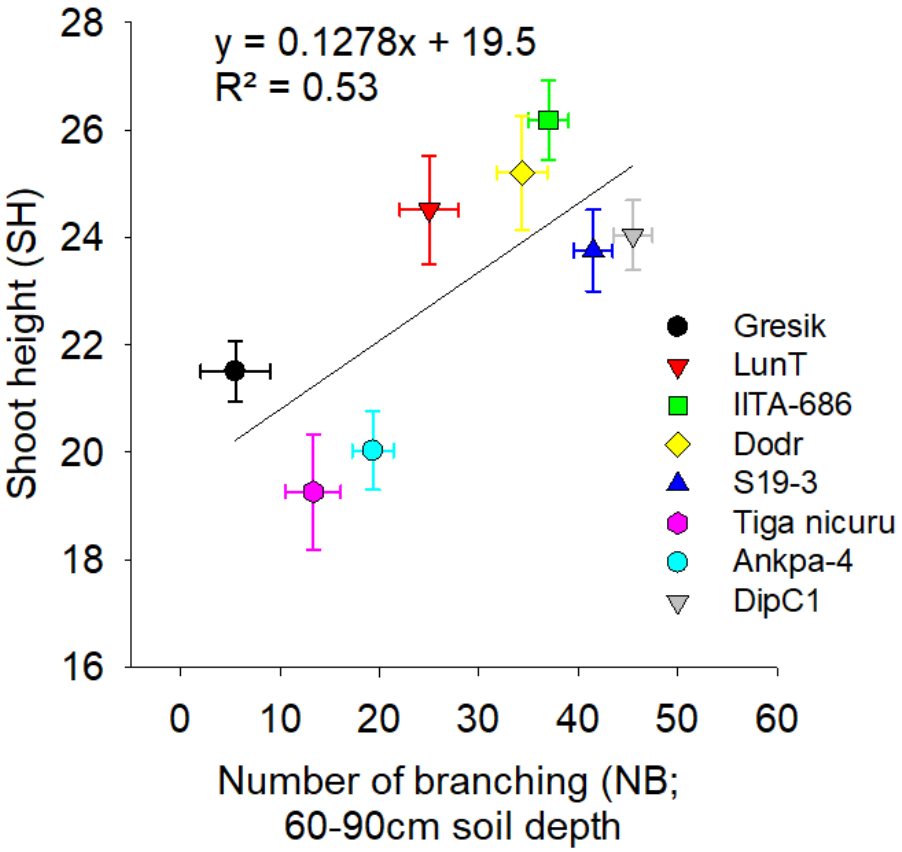

Figure 4-5 Correlation of bambara groundnut genotypes shoot height (SH) and number of branching in the 60-90cm soil depth segment at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). The data represents mean ± se values (*n = 12*).

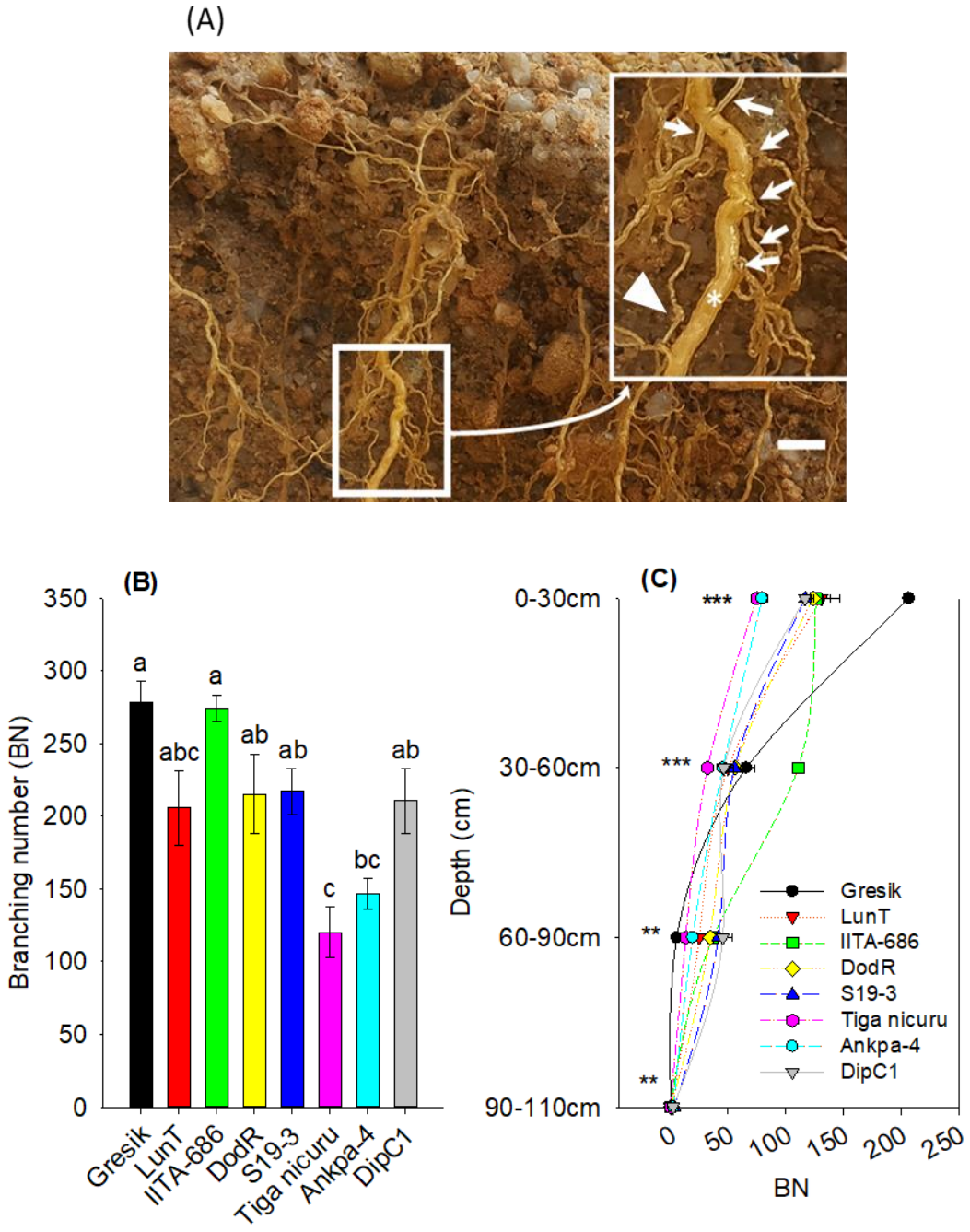

Figure 4-6 (**A**) An enlargement of the bambara groundnut tap root (asterisk), first-order laterals (arrows) and second-order lateral roots (arrowhead) for the genotype Gresik at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). White bar = 0.5mm; (**B**) Total branching number (BN) of first-order lateral roots. Mean ± se values (n = 6) are shown. Different letters indicate significant differences (HSD, P < 0.01); (**C**) BN's per soil depth segments. Mean ± se values (n = 6) are shown. Significant differences as follows: * P < 0.05; ** P < 0.01; *** P < 0.001; and ns = not significant, among individual genotypes.

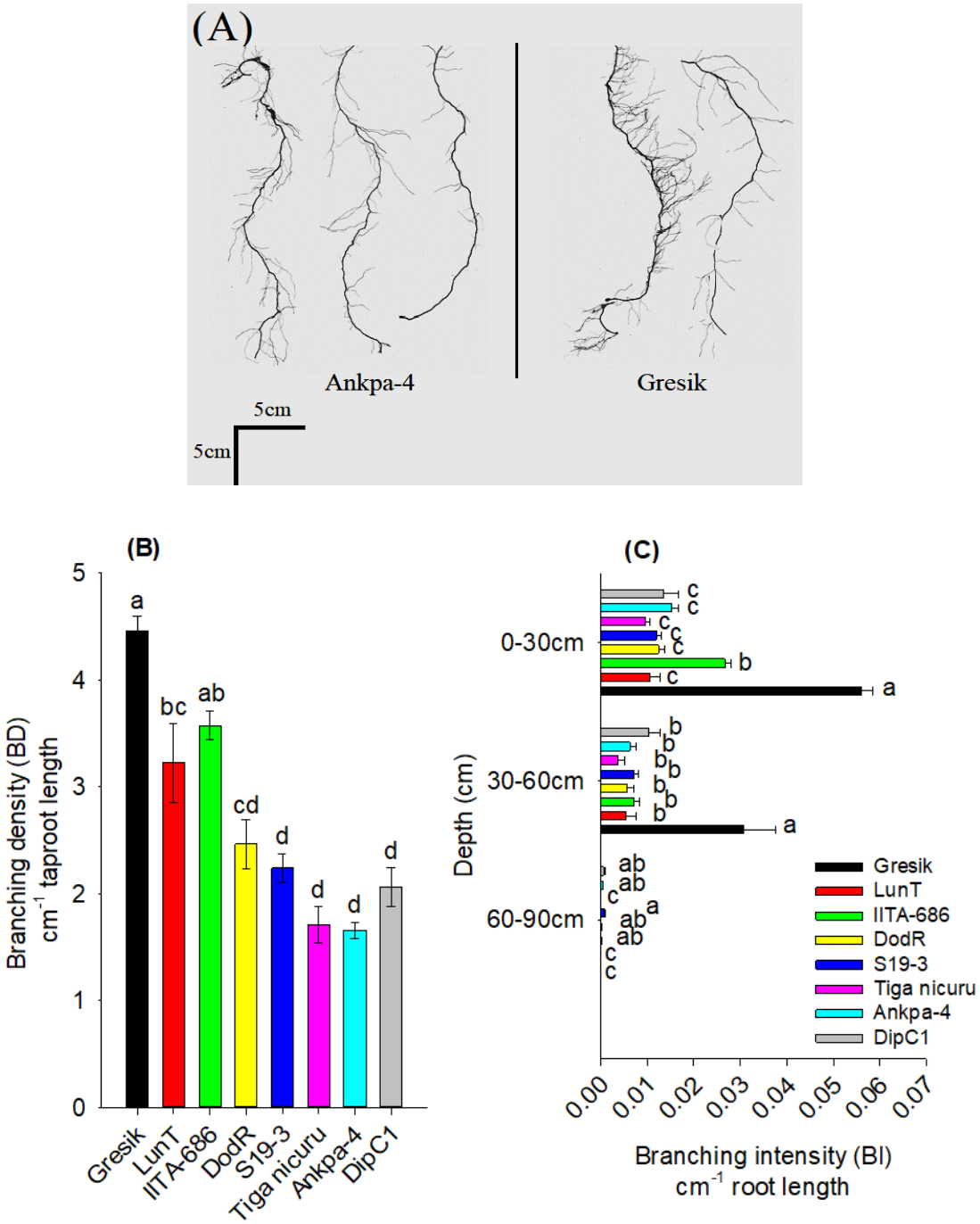

Figure 4-7 (**A**) Example bambara groundnut roots from different soil depths Ankpa-4 (from left to right 0-30, 30-60, and 60-90cm) and Gresik (from left to right 0-30 and 30-60cm) at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively); (**B**) Total branching density (BD) among different bambara groundnut genotypes; (**C**) Branching intensity (BI) among different bambara groundnut genotypes in different soil depth segments, i.e., 0-30, 30-60, and 60-90cm. Mean ± se values (n = 6) are shown. Different letters indicate significant differences (HSD, P < 0.01).

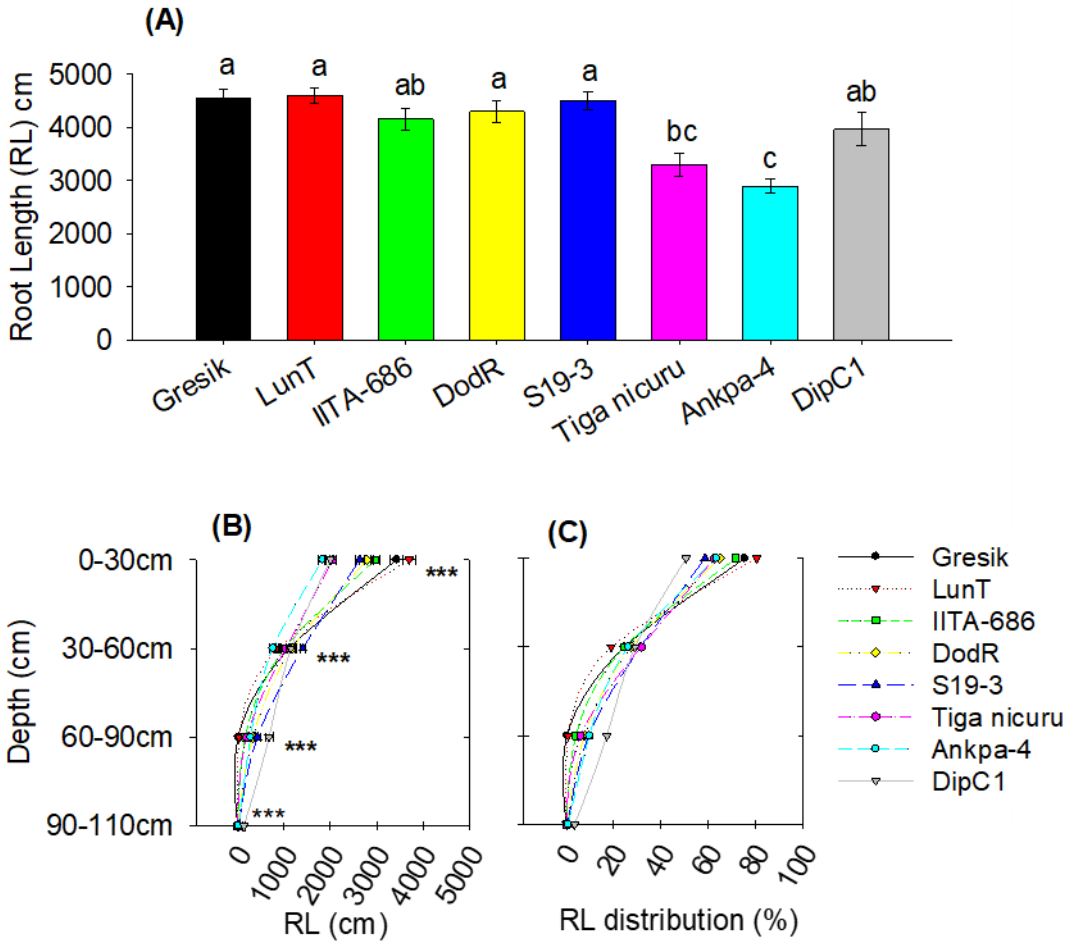

Figure 4-8 (**A**) Total root length (RL) (first- and second-order lateral roots). Mean ± se values (n = 12) are shown. Different letters indicate significant differences (HSD, $P < 0.01$); (**B**) RL's per soil depth segments; (**C**) Average percentages of RL distribution per soil depth segment. Mean ± se values (n = 12) are shown. Significant differences as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and ns = not significant, among individual genotypes.

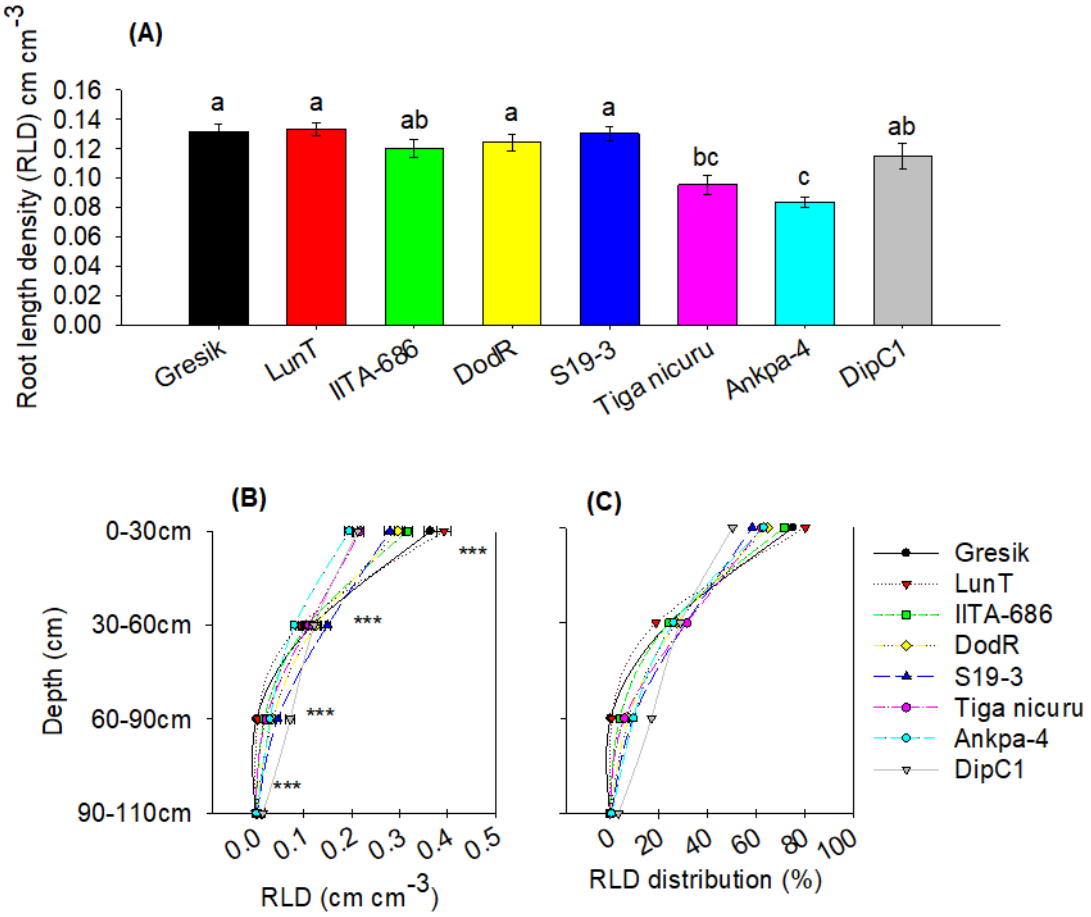

Figure 4-9 (**A**) Total root length density (RLD) (first- and second-order lateral roots). Mean ± se values (n = 12) are shown. Different letters indicate significant differences (HSD, $P < 0.01$); (**B**) RLD's per soil depth segments; (**C**) Average percentages of RLD distribution per soil depth segment. Mean ± se values (n = 12) are shown. Significant differences as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and ns = not significant, among individual genotypes.

4.4.4 Root Surface Area, Volume, and Diameter

Total root surface area ranged from 422.7 to 793.9cm² (for genotypes Tiga nicuru and Gresik, respectively; Figure 4-10A). Surface area in the topsoil (0-30cm) depth ranged from 275.1cm² (Tiga nicuru) to 645.1cm² (LunT) with average surface area of 434.9cm² (Figure 4-10B). The genotypes, Gresik had the second largest surface area (630.3cm²) in the 0-30 cm soil depth segment, although LunT was not statistically different ($P > 0.05$) from Gresik. However,

in deeper soil depths (60-90cm) the genotype DipC1 had substantially more surface area (74.7cm^2), with up to 35-fold more surface area as compared to the least (Gresik, 2.1cm^2; Figure 4-10C).

Total root volume ranged from 4.31 to 9.78cm^3 (for genotypes Ankpa-4 and Gresik, respectively; Figure 4-11A). Root volume in the 0-30cm topsoil depth varied among genotypes (Figure 4-11B). Ranging from 3cm^3 (Ankpa-4) to 8.3cm^3 (Gresik) with an average of 5.2cm^3. Although genotype Gresik had the largest root volume (8.3cm^3) it was not statistically different ($P > 0.05$) from LunT (Figure 4-11B). The genotypes LunT and Gresik ranked highest for root volume in the 0-30cm soil depth segment (86%, and 85%, respectively, Figure 10F). While DipC1 and S19-3 allocated the least root volume in the same topsoil segment (53% and 58%, respectively).

Total root diameter ranged from 1.07 to 1.83mm (Gresik and S19-3, respectively; Figure 4-12A). Root diameter in the 60-90cm subsoil depth varied among genotypes and ranged from 0.05mm (Gresik) to 0.52mm (S19-3) with an average of 0.38mm (Figure 4-12B). The genotypes Gresik and LunT ranked highest for root diameter in the 0-30cm soil depth segment (49% and 41%, respectively; Figure 4-12C). While S19-3, DipC1 and Ankpa-4 allocated the least root volume in the topsoil segment (25%, 28%, and 29%, respectively) and more in the deeper soil (20, 18, and 12%).

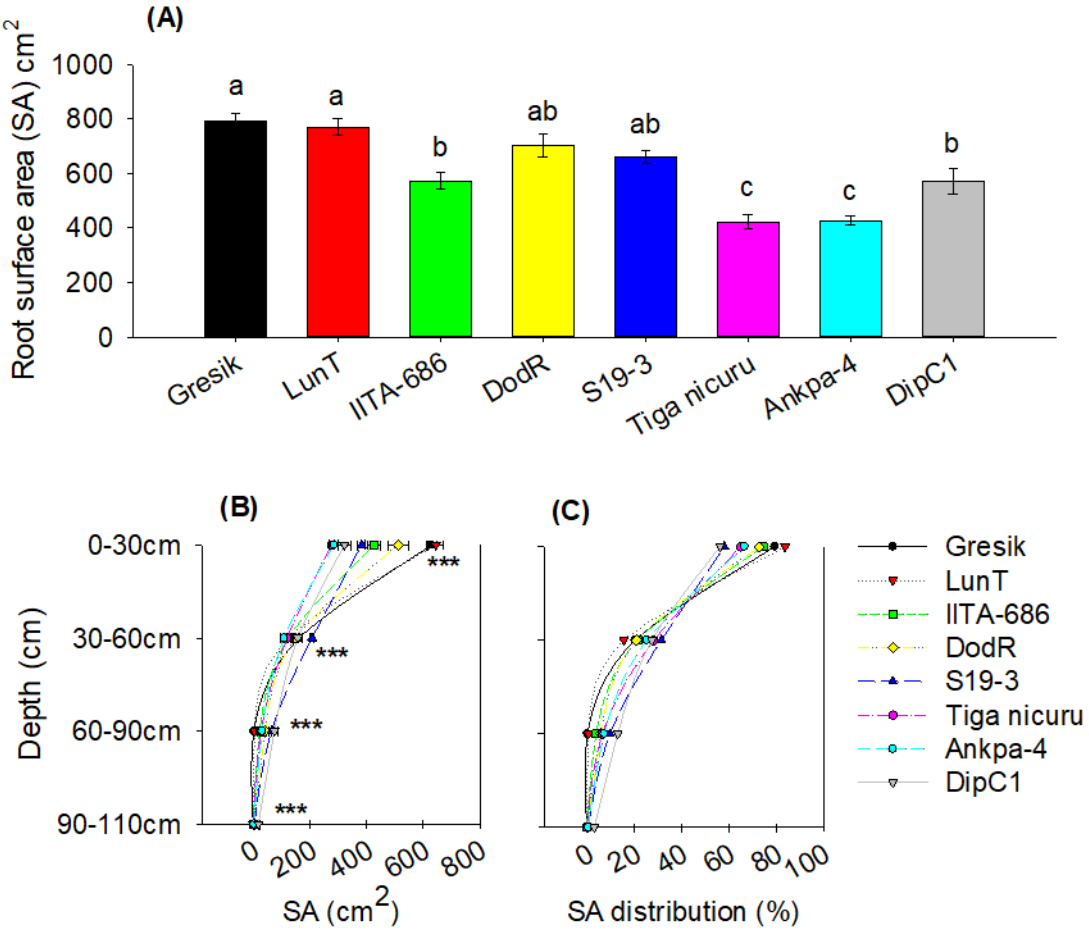

Figure 4-10. (**A**) Total root surface area (SA) in bambara groundnut genotypes. Mean ± se values (*n = 12*) are shown. Different letters indicate significant differences (HSD, *P < 0.01*); (**B**) SA per soil depth segments. Mean ± se values (*n = 12*) are shown. Significant differences as follows: * P < 0.05; ** P < 0.01; *** P < 0.001; and ns = not significant, among individual genotypes; and (**C**) Average percentages of SA distribution per soil depth segment.

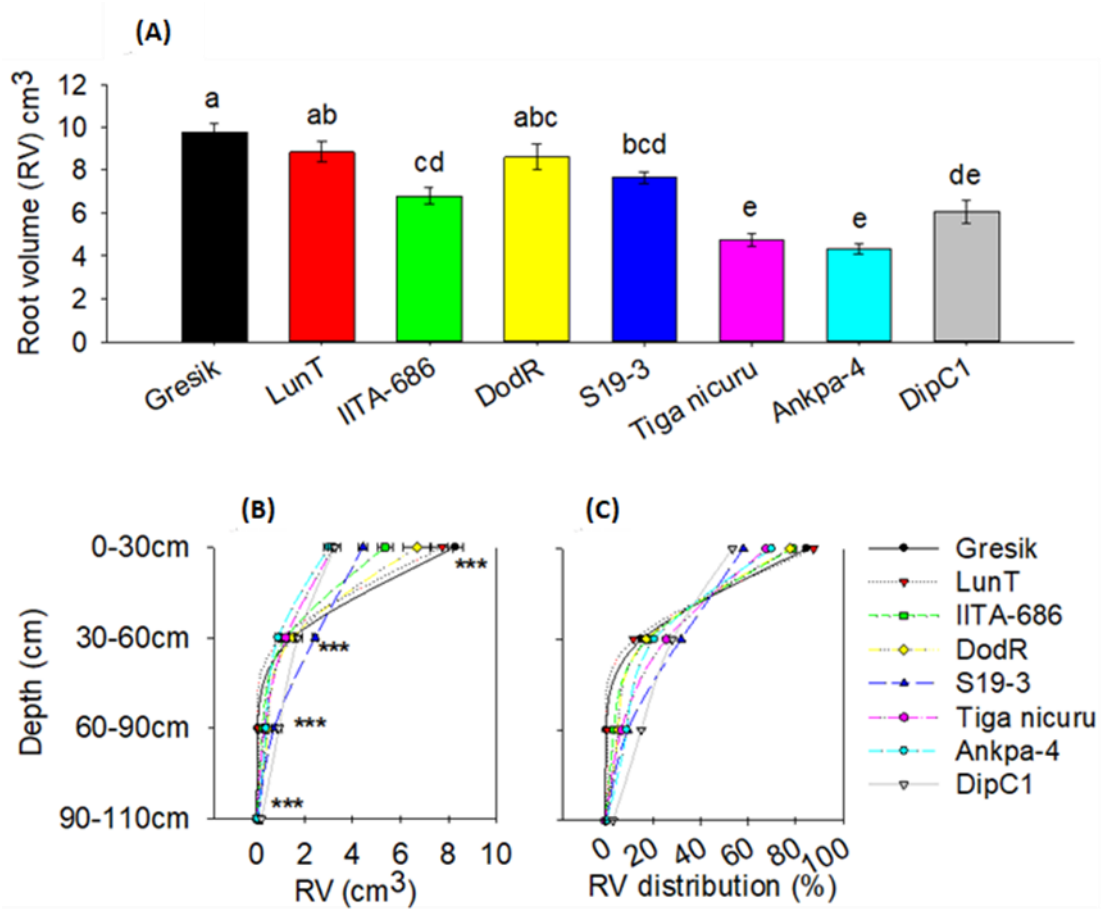

Figure 4-11 (**A**) Total root volume (RV) in bambara groundnut genotypes. Mean ± se values (n = 12) are shown. Different letters indicate significant differences (HSD, $P < 0.01$); (**B**) RV's per soil depth segments. Mean ± se values (n = 12) are shown. Significant differences as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and ns = not significant, among individual genotypes; (**C**) Average percentages of RV distribution per soil depth segment.

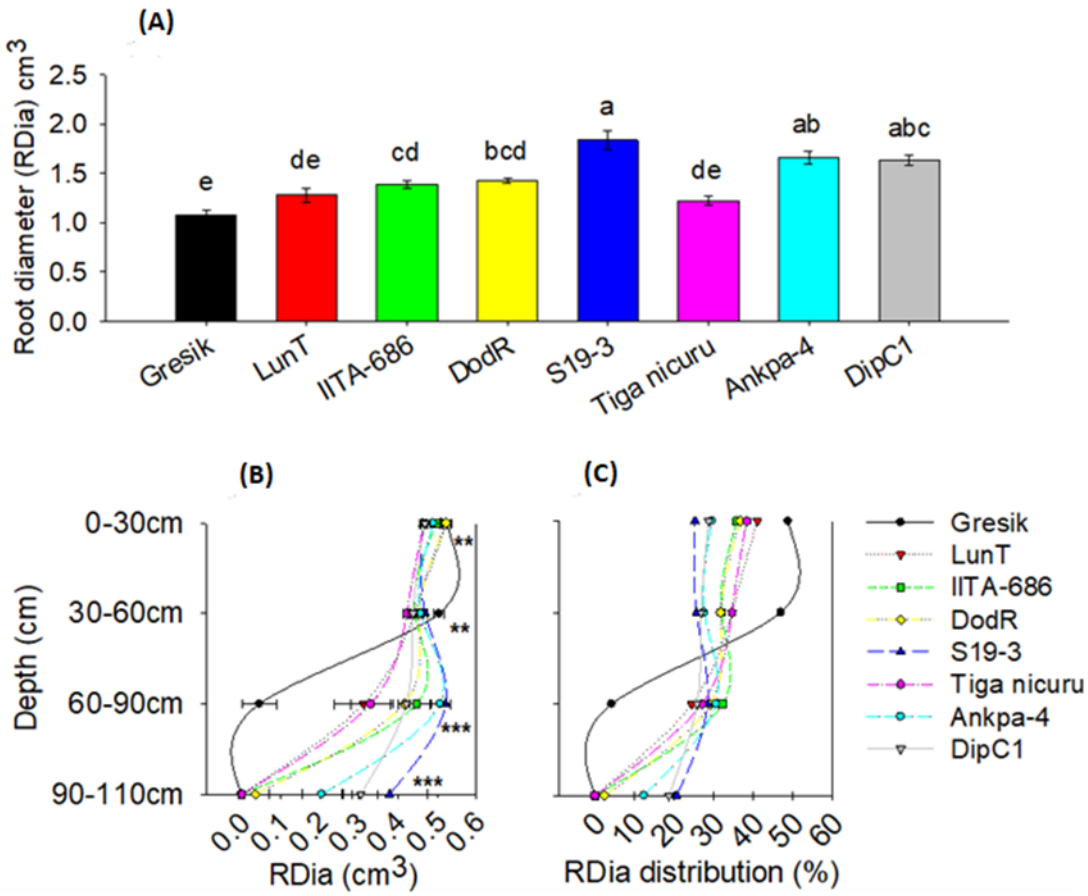

Figure 4-12 (**A**) Total root diameter (RDia) in bambara groundnut genotypes. Mean ± se values (n = 12) are shown. Different letters indicate significant differences (HSD, $P < 0.01$); (**B**) RDia per soil depth segments. Mean ± se values (n = 12) are shown. Significant differences as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and ns = not significant, among individual genotypes; and (**C**) Average percentages of RDia distribution per soil depth segment.

4.4.5 Identification of Grouping of Genotypes with Relatively Homogeneous Root Traits

In the bambara groundnut parental line collection, the number of genotypes included from Southeast Asia was (n = *1*), West Africa (n = *3*), East Africa (n = *2*), and from Southern Africa (n = *2*). Four relatively homogeneous genotype groups were determined based on a K-means clustering analysis (Figure 4-13). This indicated that high contrasting genotypes for the root traits

studied could be distinguished and confirmed from the soil-filled PVC column system.

The outlier genotype, Gresik (Cluster 1), from Southeast Asia, was separated from the others and recorded a significantly shorter tap root (Figure 4-14A) with the highest ranked RLD in the 0-30cm topsoil depth (Figure 4-14B). Cluster 2 contained three genotypes, i.e., LunT (west Africa), IITA-686, and DodR (both East Africa) representing an intermediate tap root and RLD in the topsoil 0-30cm depth. Similarly, Cluster 3 had two genotypes (Tiga nicuru and Ankpa-4 both from West Africa) also representing an intermediate tap root system and RLD in the same soil depth. In contrast, genotypes that originated from Southern Africa, i.e., DipC1 and S19-3 (both in Cluster 4), had significantly deeper tap root systems than the other three regions (Figure 4-14A) with also significantly less RLD in the shallow soil depth (Figure 4-14B).

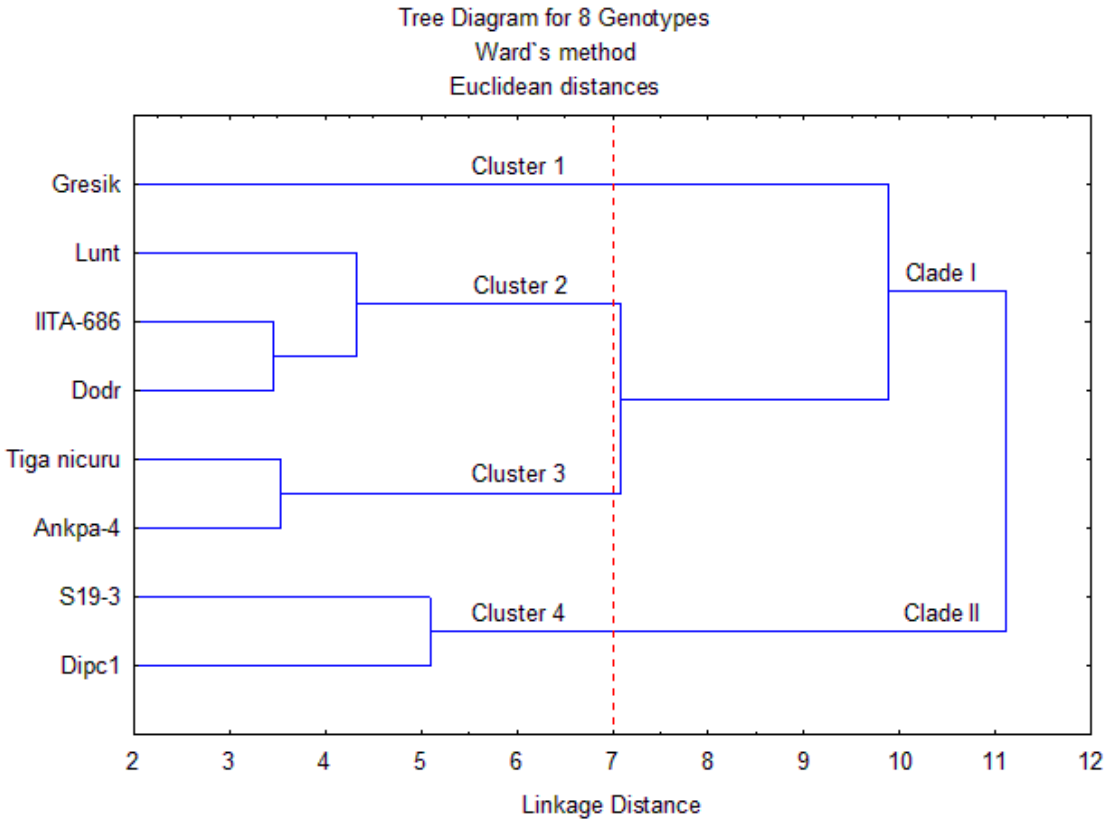

Figure 4-13 Dendrogram of agglomerative hierarchical clustering (AHC) using the Euclidean distances of bambara groundnut genotypes from the core parental line set originating from different geographical regions at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). The eight bambara groundnut genotypes were assigned to one of two general clades (Clade I and II) and further into one of four general clusters (Cluster 1, 2, 3, and 4). The horizontal red line indicates the cut-off used to form the four clusters.

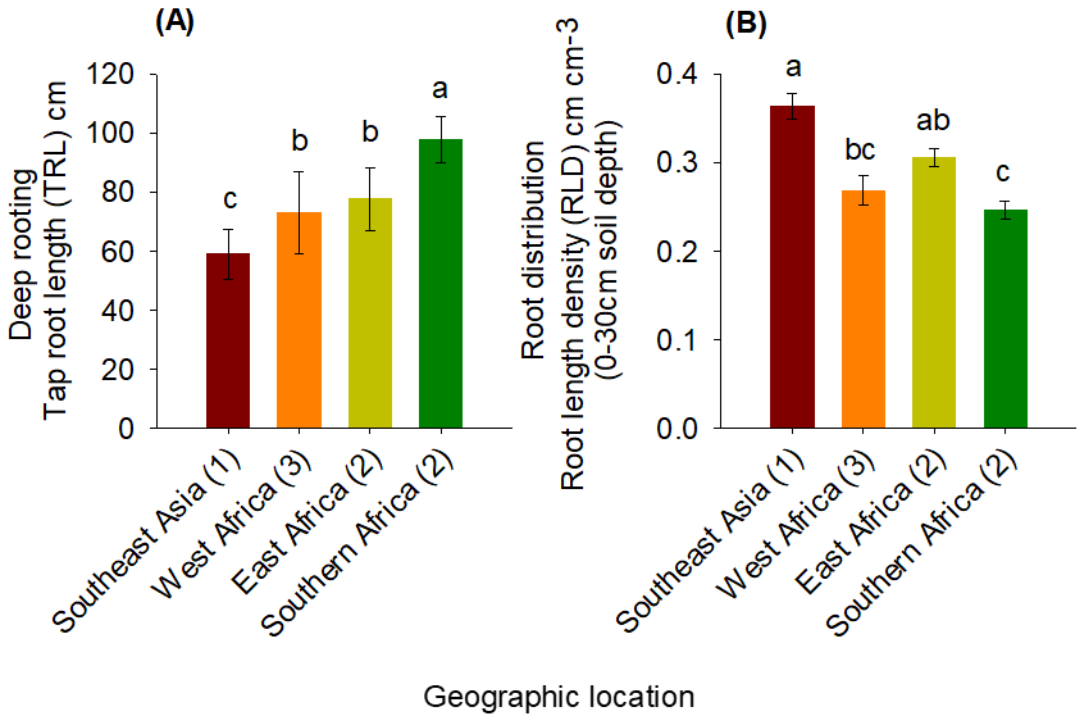

Figure 4-14 Graphical depiction of (**A**) mean deep rooting (tap root length in the 0-110cm soil depth; TRL) and (**B**) root distribution (root length density in the 0-30cm soil depth) bambara groundnut genotypes from the core parental line set originating from different geographical regions at 35 days after emergence (DAE) grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively).

4.5 Discussion

In the last 30 years, drought breeding work on bambara groundnut has been limited and largely focused and elucidated above ground shoot morpho-physiological studies (Mwale et al. 2007; Jørgensen et al. 2010, 2012; Berchie et al. 2012; Mabhaudhi and Modi 2013; Mayes et al. 2013; Al Shareef et al. 2014; Chibarabada et al. 2015a, 2015b; Chai et al. 2016b; Nautiyal et al. 2017). In order to fully understand and better manipulate the ability to tolerate water-limited conditions, the range of both above- and belowground variation present in bambara groundnut germplasm needs to be explored (Mayes et al. 2019a). Unlike the former, belowground plant root research has been mainly hampered

by the difficulty to access the rhizosphere (Kuijken et al. 2015). Considering that root traits influence water acquisition and, subsequently, yield (Kashiwagi et al. 2005), it is anticipated that the variability in the root traits of different bambara groundnut genotypes reported in this paper could be the missing link and initial step toward finally understanding bambara groundnut's superior drought adaptation.

4.5.1 Significance of PVC Columns Phenotyping System and Root Sampling at 35 Days After Emergence

Various platforms have been proposed to study the root system, most of which do not allow plant research and root analysis in a soil substrate. By modifying an efficient low-cost soil-filled PVC column phenotyping system (Lalitha et al. 2015; Serraj et al. 2004), quantitative comparisons for root traits among different bambara groundnut genotypes were made possible. Although not high throughput, the screening system allowed for natural soil and physical properties such as bulk density to be mimicked. Using this system, previously unknown root variation in a contrasting collection of bambara groundnut genotypes at 35 days after emergence (DAE) was determined. 35 DAE represents the mean pre-flowering stage of bambara groundnut, a growth stage sensitive to drought stress with strong subsequent effects on yield and yield parameters (Collinson et al. 1997; Kundy 2019). Studies by (Jongrungklang et al. 2013; Figueroa-Bustos et al. 2019), found that genotypes with larger early and high pre-flowering root length densities penetrate deeper soil depths which improves drought resistance with significantly higher grain yield under early water deficit conditions. In addition, wide genotypic variation has been reported at the pre-flowering growth stage in a number of cereal and legume root-related studies and our current work on bambara groundnut is no exception. Studies on chickpea, lupin (*Lupinus angustifolius*), common bean (*Phaseolus vulgaris*), and wheat indicate that significant variation in root growth

is observable from 35 to 45 DAE (Gregory et al. 1978; Krishnamurthy et al. 1996; Kashiwagi et al. 2005; Polanía et al. 2009; Chen et al. 2011). Therefore, root trait variation observed at this stage could aid in determining the most informative root traits that confer grain yield advantage during terminal drought stress.

4.5.2 Natural Genotypic Variation in Root System Architecture and Rooting Distribution

Bambara groundnut root systems, as with many dicotyledons, are characterized by a well-defined tap root system, with numerous first-order lateral branches. These lateral roots further branch into second- and third-order laterals. While the topology, i.e., tap root and primary laterals, remained virtually similar among the eight bambara groundnut genotypes, differences in rooting depth and branching were observed in different soil depth segments at 35 DAE. The genotype Gresik had the earliest emergence, i.e., 4.8 days after sowing (DAS), however DipC1 plants emerged 2.4 days later (Table 4-1) still managing to penetrate deep soil depths faster than the wet region genotypes. Even so, days to 50% emergence was found to be correlated to the total tap root length ($r = 0.44$, $P < 0.001$; Figure 4-5) and this particular case confirms DipC1's elongation dynamics associated with a quick and deep rooting system, an adaptation mechanism to dry environmental conditions.

The genotype LunT produced higher total root length in the first 0-30cm of the soil than Gresik (the outlier genotype; see Figure 4-13) and all the other studied genotypes, though the difference between the LunT and Gresik appeared marginal. In addition, the genotype DodR, LunT and IITA-686 were found to have similar extensive lateral branching (consisting of large root surface area, volume and diameter). A more detailed analysis of root length revealed that root branching density and intensity were not only higher in the topsoil layer: they were also more abundant in genotypes originating from the

wetter regions that experience sporadic rainfall throughout the growing season, than in the dry region genotypes. Conversely, Gresik had statistically shorter tap root length but consistently high root branching density and intensity values, bringing about higher total root length and root length densities in the shallow soil layer 0-30cm at the end of the 35 DAE. Such compensation between tap root length and branching, bares a general ecological significance as revealed by (Nicotra et al. 2002) who found that by studying independent contrasts among Australian perennial plants, species originating from rainy habitats typically have high root proliferation in the shallow soil depth as compared to species from dry habitats. In humid climates such as Indonesia from where Gresik was collected, rainfall wets the soils frequently. With frequent topsoil wetting the genotype Gresik's roots do not need to forage for deep water reserves. From a functional perspective, a costly highly branching system in the shallow topsoil layer improves the root absorption of phosphorus, however, in the case of a drought, this would enhance water depletion in that layer because of acute root competition (Lynch 2013).

In the most arid parts of Southern African, bambara groundnut is often grown after or intercropped with major cereal crops such as sorghum and maize (Graham and Vance 2003), and this is towards the end of the main wet season. The crop is forced to survive on residual soil moisture exposing the crop to terminal drought stress. In such cases, the crop has to quickly establish and develop a deep rooting system with an optimal lateral root length investment in energy to maximize capture of stored soil moisture at depth more efficiently. This attribute was indeed observed in genotypes from dry regions (DipC1, S19-3 and DodR) which produced limited lateral roots in the shallow soil depths but had long deep tap roots >90cm depth at 35 DAE, when none of the other genotypes had reached that depth yet (Figure 4-4B). At the pre-

flowering growth stage, plant roots that are able to reach deeper soil depths would support flowering with improved yield formation under drought (Comas et al. 2013). Differences in root architecture among genotypes from hot-dry and humid-wet regions suggest an adaptive response of bambara groundnut for soil resource capture by means of an improved foraging capacity of the root system in the hot-dry region sourced genotypes (Reynolds et al. 2004; Alvarez-Flores et al. 2014). Interestingly, Gresik was derived from an introduction into Indonesia from Africa not so long ago and most likely from Southern and East Africa (Redjeki et al. 2020). Practically, it would make sense that Gresik would need to have adapted quickly into a costlier, highly branching root system in the shallow topsoil layer, given the conditions in Indonesia. In such a case, environmentally responsive genes could have played a role, leading to root plasticity in order to enhance its growth in wetter environments. A study by Jørgensen et al. (2010), defined S19-3 as a "water-spender" exhibiting late closure of stomata and, consequently, a slow decline in transpiration rate during drought. Accordingly, this mechanism is now best supported by our root findings and classification of S19-3 as a genotype with an extensive root length density in the deeper soil depths as compared to the topsoil layer. The importance of increasing root and soil contact through greater root length density is that it allows plants to access greater quantities of soil (Lynch 2007; Blum 2011) in water-limited environments. Such positive correlations have been observed between deep root systems and drought resistance of chickpea, common bean, sugar beet (*Beta vulgaris*), and maize (Sponchiado et al. 1989; Kashiwagi et al. 2006; Varshney et al. 2013). Similarly, Kirkegaard et al. (2007) demonstrated that an increase of 30cm rooting depth allows for the capture of an extra 10 mm of water in the deeper soil depths, resulting in an additional (0.5t ha^{-1}) of wheat grain. Allowing the crop access to deep water reserves long after a drought event has started. Therefore, early selection (i.e., 35 DAE) for greater root length density at depth can be expected

to help enhance the genetic gains and yield improvement in bambara groundnut breeding efforts. Such a root system reduces the metabolic costs that come with having to maintain an elaborate root architecture, thus, allocating more resources towards deep soil foraging in order to access deep water and mobile nitrogen (Zhan and Lynch 2015). Consequently, because S19-3, much like DipC1, originated from drier regions (Namibia and Botswana, respectively), where increased vapor pressure deficit (VPD) increases atmospheric demand for transpired water. It is tempting to speculate that DipC1, with the genetic predisposition for long tap root system, could also be coined a "water spender" and could adopt similar physiological mechanisms such as the ones of its documented counterpart. Further work is needed to test this hypothesis.

The differences in root systems observed between the four geographic locations, reflect contrasting strategies for adaptation to environments with different rainfall patterns. This result, along with previous bambara groundnut shoot phenotyping, presents proof that root trait variability in bambara groundnut is as important as shoot trait phenotyping and contributes to plant survival and yield under water limited conditions. Given that the root system is a hidden and complex organ, the prospects of indirect selection by utilizing aboveground plant parts becomes highly desirable. Shoot dry weight was positively associated with root length density, root length, root surface area, and root volume all in the shallow soil depth. In addition, shoot height was positively correlated with the number of branches in the deep 60-90cm of the soil. This indicates that shoot dry weight and shoot height are good traits that can be used as proxies to make estimations of several shallow and deep root traits, respectively, in bambara groundnut and could both be prioritized for large-scale breeding phenotyping. As such, I conclude that farmers over the years have indirectly selected for differences in deep rooting and root length

density through their influence on yield under dry environments (Alvarez-Flores et al. 2014; Lalitha et al. 2015; York et al. 2015). Furthermore, bambara groundnut genotypes that evolved in drier areas could have adapted by increasing tap root length and reducing their branching distribution to capture deep water more efficiently. However, these traits cannot be of any advantage in humid environments with high annual average rainfall (Serraj et al. 2004) where a short rooting and highly branched system in the superficial soil layers would seem less adapted to drought.

4.6 Conclusion

The present study is an initiative to better understand an ignored African grain legume with superior drought resistance relative to other cultivated grain legumes in Africa. To the best of our knowledge, I provide the first itemized report of RSA in core bambara groundnut parental lines. In general, the deep tap root systems and fewer first-order lateral root branching conferring an efficient soil exploration, make a suite of root traits that have over the years fundamentally improved the water foraging capacity of S19-3, DipC1 and DodR as compared to Gresik, LunT, IITA-686, and DodR. This is especially valid for hot-dry-habitat S19-3 and DipC1, which flourish in an area of deep sandy soils under very dry and hot climate and, in the case of our study, demonstrated the most noteworthy rooting traits. With respect to the outlier genotype Gresik, it showed a particular root growth pattern best suited to shallow soils that receive frequent wetting. In the two circumstances, specific sets of RSA are expressed from the pre-flowering growth stage to support initial plant establishment. The distinctly differentiated root morphologies concur with two differential foraging strategies in dry environments, namely shallow-costly root systems exploring topsoil layers to benefit from occasional rainfall, versus deep-cheap root systems foraging water stored in deeper soil

depths (Paula and Pausas 2011). Bambara groundnut root trait study could be exploited in breeding for enhanced drought adaptation or low-input farming, though this would require some correlative investigations to confirm whether pre-flowering root traits translate into improved performance of mature plants in the field (Manschadi et al. 2008; Singh et al. 2010). More so, the genotypes originating from southern and eastern African regions possessed the best deep rooting, indicating potential for further selection from these regions. From an evolutionary point of view, it is important to note that crop domestication and natural selection depend on phenotypic selection (Lynch and Brown 2012). Considering that the larger shoot height is related to more branching in the deep soil depths of dry region genotypes and often results in higher yield than in its wet region sourced bambara groundnut genotypes counterpart. I hypothesize that farmers, over the years, have indirectly selected for differences in deep rooting and root length density, in particular through their influence on yield under dry environments. Moving forward, instead of adopting a strict "back to the roots" framework, bambara groundnut root phenotyping could prioritize these specific root traits.

In the next experimental chapter, the natural genotypic diversity revealed in the eight genotypes was then investigated to detect adaptive changes in tap root length and root length density in response to periodic drought stress.

CHAPTER 5 : Natural Genotypic Variation Underpins Root System Response to Drought Stress in Bambara Groundnut (*Vigna Subterranea* (L.) Verdc.)

5.1 Summary

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is mostly grown in rainfed production systems and suffers from periodic drought stress at critical growth stages, leading to yield reductions. Natural genotypic variation for root traits is essential for adaptation to water deficit conditions. However, root traits — especially rooting depth and distribution, have not been fully utilised as selection criteria to improve drought stress in bambara groundnut. The present study explored the natural genotypic variation found in single genotypes of bambara groundnut derived from landraces to identify adaptive differences in tap root length (TRL) and root length density (RLD) in response to periodic drought stress. A diverse core collection of eight bambara groundnut genotypes from various locations (namely, Gresik, LunT, IITA-686, DodR, S19-3, Tiga nicuru, Ankpa-4, DipC1), were grown for two seasons (2018 and 2019) in polyvinyl chloride (PVC) columns in well-watered (WW) and 30-day drought stress (DS) treatments. Plant samples were collected at 55 days after emergence (DAE) (30-d of DS) and at 105 DAE (30-d of DS plus 50-d of recovery). The results show that DS significantly ($P < 0.05$ - < 0.001) reduced shoot height (SH), number of leaves (NoL) and delayed flowering in 2018 and 2019 except for one genotype (LunT) in 2018. Root to shoot (R:S) ratio was significantly higher ($P < 0.001$; 22%) under DS in 2018. Under DS, average tap root length (TRL) at 55 DAE was significantly decreased by 14% and 22% in 2018 and 2019 and by 5% and 11% at 105 DAE (50-d of DS recovery) in 2018 and 2019, respectively. Average root length density (RLD) under DS treatment was associated with substantial grain yield advantage ($R^2 = 0.27$ and $R^2 = 0.49$) in 2018 and 2019, respectively. Variation in intrinsic RLD in deeper soil depths in the studied genotypes determines root foraging capacity when facing periodic drought stress. This suggests that different agroecological environments to which bambara groundnut is subjected in its natural habitat have promoted a phenotypic differentiation — in growth and root system, to adapt to ecotypic conditions, which may help offset the impact of adverse events like regular drought stress. This differentiation may be considered an important feature when selecting for superior genotypes. The natural genotypic variation exhibited especially by DodR could be exploited to identify potential quantitative trait loci (QTL) controlling deep rooting and root length density.

Keywords: Bambara groundnut (*Vigna subterranea* (L.) Verdc.); Drought stress; Root length density (RLD); Stomatal conductance (g_s)

5.2 Introduction

Drought is a major abiotic stress that lowers the yield of grain legume crops, including bambara groundnut (*Vigna subterranea* (L.) Verdc). Bambara groundnut is well-known for its ability to endure dry environments in comparison to other grain legumes, although adaptation mechanisms are unclear. In semi-arid Africa, it is third to groundnut and cowpea in terms of production and consumption (Sellschop 1962), bambara groundnut is set to increase in importance in marginal areas as production systems become more diverse to adapt to climate change (Mayes et al. 2012; Massawe et al. 2015; Feldman et al. 2019; Mustafa et al. 2019a; Mustafa et al. 2019b). According to various climate models, many drought-stricken areas in eastern and southern Africa are expected to become drier in the coming decades (Rippke et al. 2016). To mitigate this and satisfy the growing demand for food amid population growth, efforts are underway to develop climate resilient and nutritious crop varieties (Halimi et al. 2019; Mustafa et al. 2019a; Tan et al. 2020). In addition to genetics and genomics approaches, a detailed above-ground to below-ground phenotyping strategy in bambara groundnut breeding needs to be the focus.

Progress in drought phenotyping in bambara groundnut for the past 30 years, has been elucidated by above ground shoot traits (Collinson et al. 1997, 1999; Jørgensen et al. 2010; Sesay et al. 2010; Vurayai et al. 2011; Mabhaudhi and Modi 2013; Chibarabada et al. 2015a, 2015b; Chai et al. 2016a, 2016b; Muhammad et al. 2016). This has proved fruitful, revealing the potential in selecting individual lines with improved drought resistance. However, less explored is the below ground root system architecture (RSA). RSA is an important developmental trait which plays a vital role in plant adaptation and productivity especially under drought stress (Lynch 2013). Indeed, bambara groundnut have evolved drought stress adaptation mechanisms under natural selection (Mateva et al. 2020; see *CHAPTER 4*). As a result, natural genotypic

variation in RSA in bambara groundnut native to various agroecological conditions ranging from tropical wet to semiarid would be worthwhile to investigate.

However, the various interactions between RSA and different agroecological environments, make it difficult to establish a root system ideotype that enhances both the capture of mobile water and nutrients (Lynch and Wojciechowski 2015). For example, under drying soil conditions there is generally an increase in root elongation (Raja and Bishnoi 1990; Schmidhalter et al. 1998), but there is also evidence of reduced (King and Bush 1985; Ogawa et al. 2005) and postponed (Bontpart et al. 2020) root length density (RLD). Moreover, when the soil surface slowly dries-down, a reduction in RLD in deeper soil depths may aggravate the effects of water stress, triggering the reduction of essential processes such as stomatal conductance. This discrepancy might result from natural genotypic differences. For example, in consistently dry environments, bambara groundnut genotype such as S19-3 (a classic 'drought escape-type' sourced from Namibia) possesses a quick-deep-cheap rooting system, as opposed to a shallow-costly rooting system, which is considered to be more beneficial in drought-prone regions (Mateva et al. 2020; see *CHAPTER 4*). As a result, uncovering and integrating such beneficial variants into new elite bambara groundnut varieties may be crucial in optimising efficiency and establishing plant ideotypes for drought environments.

In consistent agroecological environments, genotypic variation for root system traits has been shown to distinguish functional plant types (Leva et al. 2009). This has been included in crop breeding programmes that are oriented towards low-input systems in which the availability of soil resources are spatio-temporally dynamic (Lynch and Brown 2012). However, Schneider and Lynch (2020) argue that an architectural model rigidly formed by the plant genome

would make the root system poorly reactive, irrespective of the benefits of particular root traits, whereas root system developmental plasticity would be desirable in highly variable agroecological environments. Considering that plasticity is difficult to achieve due to the inability to reliably generate the best phenotype, evolving environmental signals, and/or the fact that phenotypic plasticity is expensive (Via and Lande 1985). Therefore, natural genotypic variation could be explored further in bambara groundnut, in order to assign genotypes: rigidly under genome control, to specific agroecological environments and production systems.

Bambara groundnut is an interesting drought-tolerant crop for exploring such a line of inquiry in research. This grain legume flourishes under contrasted environments. Originating in West Africa, its distribution spans across aridity gradients from tropical dry climates in Senegal and Kenya, respectively, down to arid and semi-arid regions in sub-Saharan Africa. This is on soils more or less poor in nutrients and formed under variable pedoclimatic conditions. Indeed, farmers have grown bambara groundnut as genetically variable landraces for many centuries in the same agroecological climates. Comparing eight core parental lines of bambara groundnut — single genotypes derived from landraces of contrasting geographic origin, our previous studies found great variation in several shoot (Gao et al. 2020) and root system architecture (RSA) traits (Mateva et al. 2020; see *CHAPTER 4*). As a provisional explanation, I hypothesize that farmers, over the years may have indirectly selected for differences in the root system, particularly deep rooting and RLD in landraces from dry environments. This adaptation mechanism would be critical for plants exposed to drought stress especially at the flowering stage when plants are most vulnerable to drought stress. Therefore, the objective if the experiment was to compare rooting dynamics at two key stages, i.e., 55 days after emergence (DAE): (30-d of DS) and at 105 DAE: 50-d of drought stress

recovery in eight bambara groundnut genotypes sourced from different location.

5.3 Materials and Methods

5.3.1 Plant Material and the Polyvinyl Chloride (PVC) Column System

For a detailed description of the plant material see Chapter 3; *3.2.1 Plant Material* and soil-filled polyvinyl chloride (PVC) column setup see section *3.2.2 Study Site and PVC Column Screening System*. Eight single genotypes, representing a geographical gradient of aridity (see Figure 5-1A) below for country of origin) were used i.e., Gresik sourced from southeast Asia (humid and high rainfall habitat) while genotypes LunT, Ankpa-4, Tiga nicuru (West Africa), IITA-686, DodR (East Africa) from dry environments with rainfall >570 per annum. Lastly, genotypes S19-3 and DipC1 both from southern Africa (hot-dry habitat). The genotypes were compared under two water management regimes in a factorial treatment combination with three replicate plants per Genotype (G) × Water Management (WM), giving a total of 96 individual plants. Air temperature and relative humidity (RH) were monitored at 150cm height level from the ground using Tip-Temp EL-USB-2-LCD thermo-hygrometers (Tip-Temperature, Burlington, NJ). Daily air temperature values and RH were used to estimate the daily values of vapor pressure deficit (VPD).

$$\text{VPD (kPa)} = (100 - \text{RH} / 100) \times \text{SVP} \quad (1)$$

where RH is relative humidity and SVP is the saturation vapour pressure calculated as (Murray 1967):

$$\text{SVP} = [610.7 \times 10^{\,7.5(\text{T}) / 237.3 + (\text{T})}] \quad (2)$$

where T is the air temperature.

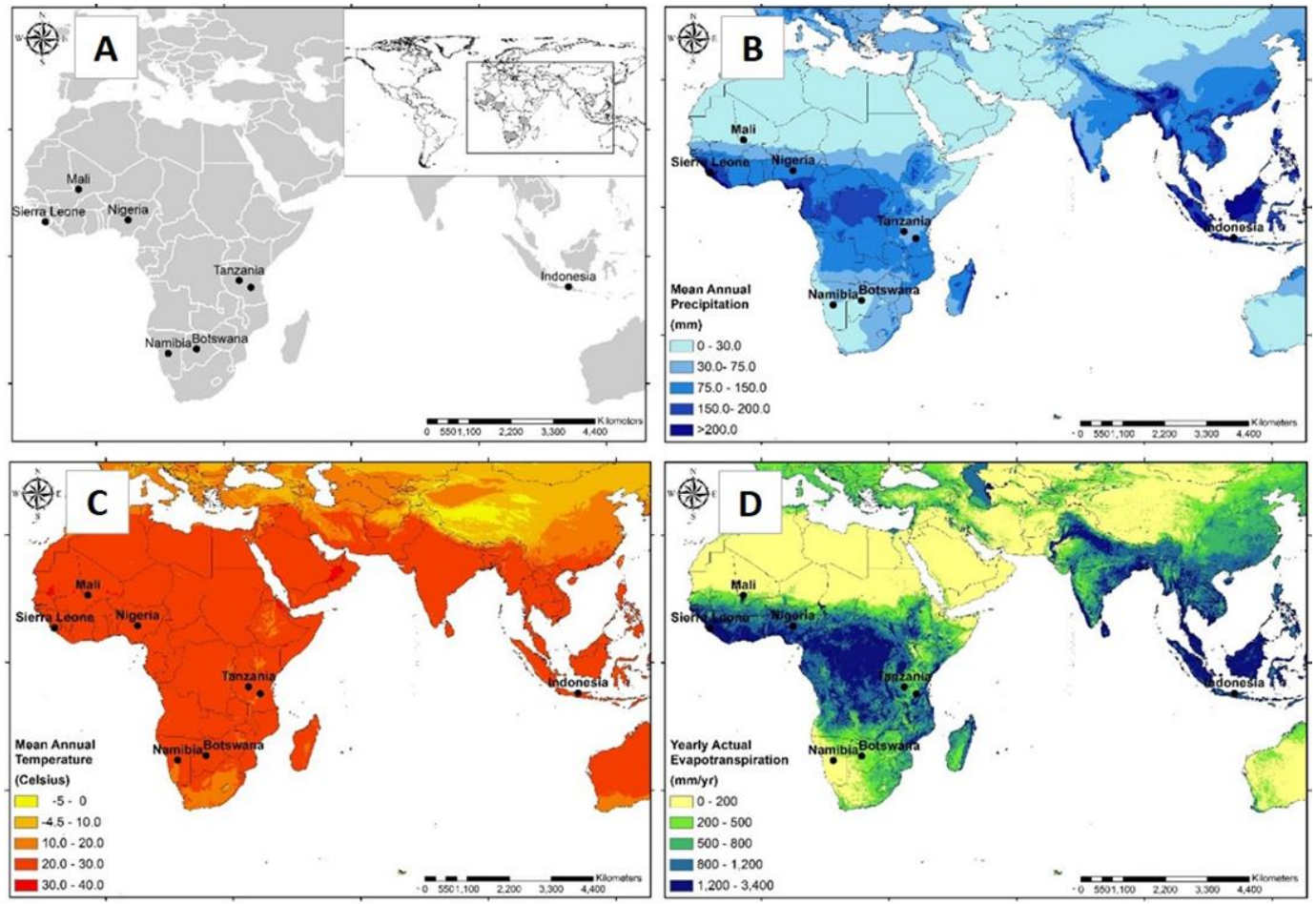

Figure 5-1 Location of origin for the eight bambara groundnut (*Vigna subterranea* (L.) Verdc.) genotypes [west Africa (*n = 3*), east Africa (*n = 2*), southern Africa (*n = 2*), and southeast Asia (*n = 1*)]. (**A**) Close-up of Africa and southeast Asia collection points (**B**) Mean annual precipitation (**C**) Mean annual temperature and (**D**) Mean annual evapotranspiration. Plotted with the package raster (Fick and Hijmans 2017).

5.3.2 Soil Substrate and Soil Water Treatments

The soil substrate, basal fertilizer, weed and pest control were carried out as described in Chapter 3; *3.2.2 Study Site and PVC Column Screening System*. For the two water treatments i.e., well-watered (WW) and drought stress (DS), a detailed description is provided in Chapter 3; *3.2.4 Water Treatments*. The DS treatment was terminated at 55 DAE (first destructive sample point), which also marked the end of the 30-d DS. Irrigation was resumed and all the plants i.e., WW and DS (recovery) were slowly irrigated once on alternate days until final harvest (105 DAE; second destructive sample point; Figure 5-2), which also marked 50-d of DS recovery.

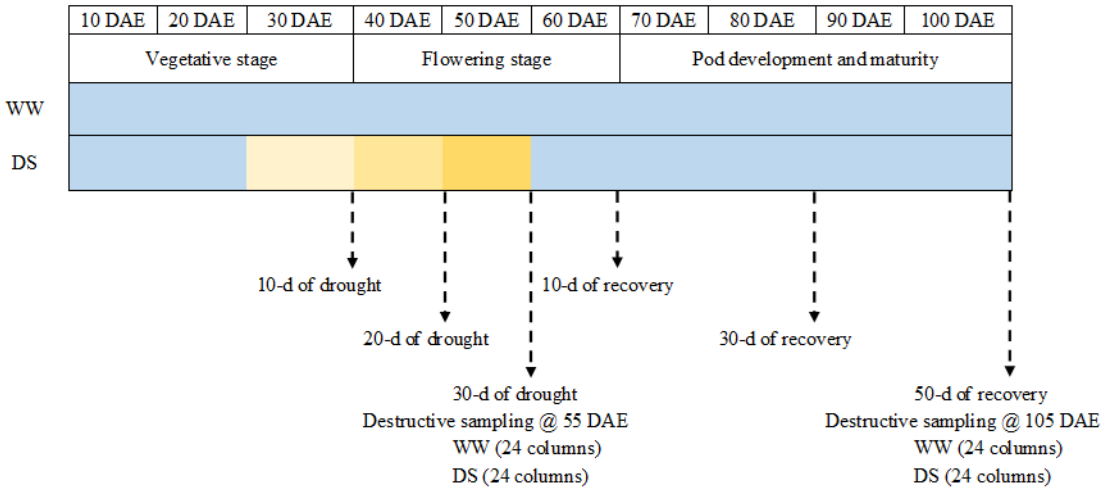

Figure 5-2 Design of the study: drought stress (DS) treatments were applied before and during the flowering stage by withholding irrigation. The DS treatment was maintained for 30-d followed by re-watering. Well-watered (control) was designated as WW treatment and received irrigation (to field capacity) throughout the growth period. The period of WW and DS treatment is represented by the solid ungraded blue colour and a graded brown colour scheme indicating an increasing DS intensity, respectively.

5.3.3 Root Traits

Root harvesting was conducted twice in each season, at 55 DAE (30-d of DS) and 105 DAE (50-d of DS recovery). Three biological replicates were used per treatment for root trait measurements per bambara groundnut genotype. Detailed description on data collection for total tap root length (TRL cm plant^{-1}), root fresh weight (RFW) g plant^{-1} and root length density (RLD) cm cm^{-3} is provided in Chapter 3; *3.2.5 Plant Sampling and Common Measurements.*

5.3.4 Shoot Traits

At 55 DAE (30-d of DS) and 105 DAE (50-d of DS recovery), shoot height (SH) and number of leaves (NoL) were measured as described in Chapter 3; *3.2.5 Plant Sampling and Common Measurements*. Pods were dried and shelled and the seeds weighed to determine grain yield (GY) converted in to g plant^{-1}. Three biological replicates were used per treatment for shoot traits and GY measurements per bambara groundnut genotype.

5.3.5 Reproductive Development

The description of reproductive development particularly: days to 50% flowering is presented in Chapter 3; *3.2.6 Developmental Traits*.

5.3.6 Stomatal Conductance

During DS, stomatal conductance (g_s) was measured in a time-course experiment at 35, 45 and 55 DAE after water was withheld using a dynamic diffusion AP4 cycling porometer (Delta-T Devices Ltd, Cambridge, UK). Three biological replicates for g_s were taken between 11:00h and 12 noon on the youngest, fully expanded trifoliate leaf and this was always on a sunny day in

Semenyih, Malaysia (average sunrise, sunset and day length: 07.02h, 19.15h and 12.11h, respectively).

5.3.7 Volumetric Water Content

Volumetric water content (VWC) was measured in a time-course experiment at 35, 45 and 55 DAE after water was withheld. Three biological replicates were measured using a handheld soil moisture ML2 Delta-T thetaProbe (ThetaProbe ML2, Delta-T Devices Ltd, Cambridge) from the top part of the soil profile (every 30cm) down to the bottom part of the soil profile via the pre-drilled holes in the sides of the PVC columns (Figure 3-2C). Similar to g_s, VWC readings were measured every week between 11:00h and 12 noon during the WW and DS treatment periods, with measurements for the former performed before irrigating to avoid reading fluctuations.

5.3.8 Statistical Analysis

Data on each measurable trait was subjected to two-way analysis of variance (ANOVA) to test the effects of the Genotype (G) and Water management (WM) and their interaction (G × WM) using Statistica Version 13.3 (TIBCO Software Inc, USA). Means were separated using Tukey's Honestly Significant Difference (HSD) at the 5% level of significance. Linear equations and correlation coefficients were calculated with SigmaPlot Version 12.5 software (Systat Software, Inc, USA).

5.4 Results

5.4.1 Vapor Pressure Deficit, Relative Humidity and Temperature Under Rainout Shelter

There were notable differences in weather patterns between the experiment seasons (Figure 5-3) that would have affected plant growth and development. Vapor pressure deficit (VPD), relative humidity (RH) and temperature during the 2018 and 2019 seasons are shown in Figure 5-3. In 2018, mean VPD (1.13kPa) during the experiment period (25th July–14th November) was 42% less than the 2019 season (8th February–3rd June; 1.96kPa). Mean RH in 2018 (79%) was 13% higher than the 2019 season RH (69%). The 2018 experiment season recorded 2°C lower temperatures than 2019, with the former experimental season recording a minimum and maximum of 27.5°C and 33.2°C, respectively.

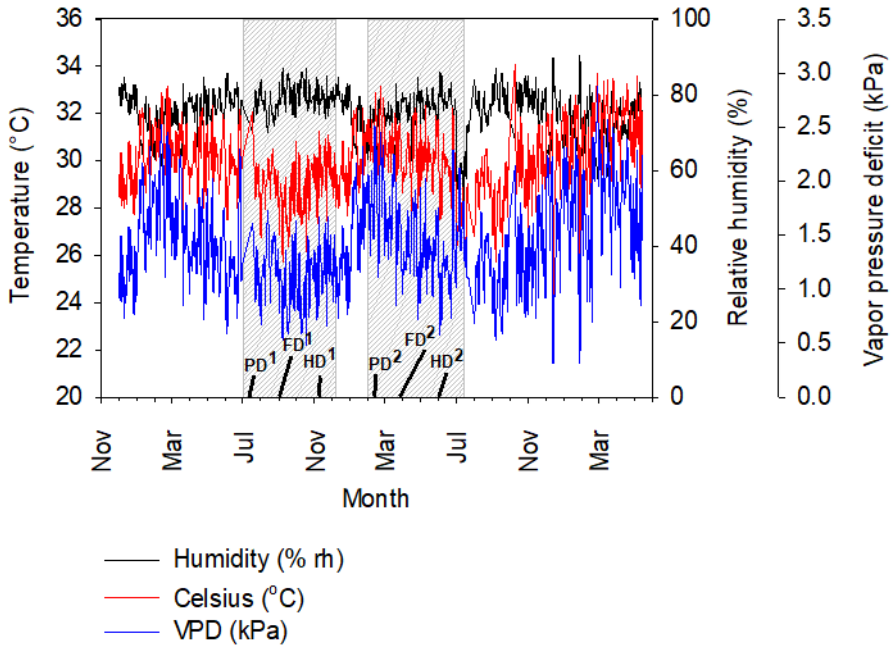

Figure 5-3 Summary of average monthly relative humidity (%; black line), temperature (°C; red line) and vapor pressure deficit (kPa; blue line). Planting dates (PD), flowering dates (FD) and harvesting date (HD) all with superscripts (1; 2), representing two seasons of study (2018 and 2019, respectively) conducted at the Crops For the Future Field Research Center (CFF-FRC).

5.4.2 Plant Flowering

According to the analysis of variance, days to 50% flowering was significantly affected by the interaction effect of genotype and water management for the 2018 ($P < 0.001$) season (Figure 5-4A, B). During the 2018 season, there was discrimination for days to 50% flowering between WW and DS treatments, the latter showing longer days to 50% flowering values for most genotypes except for LunT. Based on the mean values for the genotype, days to 50% flowering for WW plants ranged from 26 days (S19-3) to 44 days (Gresik), while that of the DS plants varied between 31 days (both S19-3 and Tiga nicuru) and 46 days (Gresik).

Similarly, in the 2019 season, days to 50% flowering was significantly affected by the interaction effect of genotype and water management ($P = 0.03$). However, in this particular season IITA-686, S19-3 and Tiga nicuru showed significantly ($P < 0.001$) longer days to 50% flowering under DS in 2019 as compared to the WW values. Generally, the discrimination between WW and DS plants in 2019, was consistent with the 2018 season — apart from LunT (which flowered 6 days earlier in 2018). Based on mean values for the genotype selections in 2019, days to 50% flowering of the WW plants ranged from 26 days (IITA-686) to 50 (Gresik) while that of the DS stressed plants varied from 44 days (DipC1) to 63 days (Gresik).

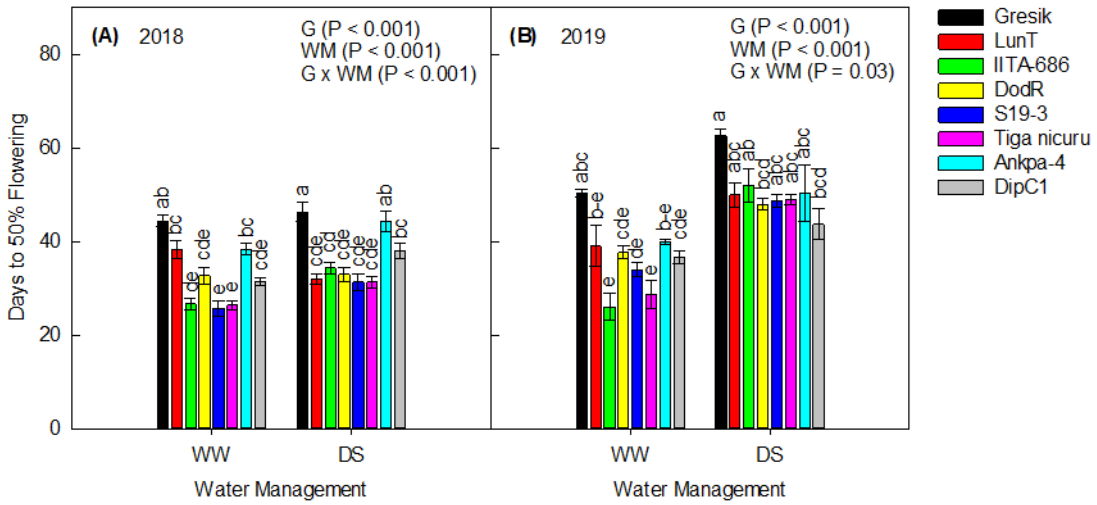

Figure 5-4 Interaction effect Genotype (G) × Water management (WM) on days to 50% flowering of eight bambara groundnut genotypes grown in a soil-filled PVC columns in a rainout shelter (**A**) WW and DS during 2018, (**B**) WW and DS during 2019. The data is mean ± se values (n = 3), with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

5.4.3 Plant Size and Number of Leaves

The interaction between genotypes and water management was not significant ($P > 0.05$) for shoot height (SH) and number of leaves (NoL) at 55 DAE in both 2018 and 2019 seasons (Table 5-1). However, SH showed significant differences between genotypes ($P < 0.01$ and $P < 0.001$ in 2018 and 2019, respectively) as well as highly significant differences between water management ($P < 0.001$; for both seasons). In 2018, the genotypes DodR and Ankpa-4 (both 23.4cm plant^{-1}) showed significantly higher SH, than Tiga nicuru (15.5cm plant^{1}). In 2019, the genotype DipC1 (33.2cm plant^{-1}) showed significantly higher SH, than Tiga nicuru (27.32cm plant^{-1}), Gresik (26.70cm plant^{-1}), and LunT (25.8cm plant^{-1}). Based on mean values for water management only, DS significantly reduced SH 21% and 12% in the 2018 and 2019 seasons, respectively (Table 5-1).

With respect to NoL at 55 DAE, there were significant differences between genotypes in 2018 and 2019 ($P < 0.01$ and $P < 0.05$, respectively) as well as highly significant differences between water management ($P < 0.001$; for both seasons). Based on mean values for genotype selections only, NoL in 2018 ranged from 20 (LunT) to 44 (Gresik). Similarly, in 2019 plants varied from 33 (LunT) to 55 (Gresik). Based on mean values for water management only, DS significantly reduced NoL by 57% in 2018 and 39% in 2019.

Table 5-1 Analysis of variance for shoot height (SH), and number of leaves (NoL) at 55 DAE of eight bambara groundnut genotypes, grown in soil-filled PVC columns in a rainout shelter under WW and DS in two seasons 2018 and 2019.

		SH (cm plant $^{-1}$)		NoL[2] (number)	
Treatment[1]	**N**	**55 DAE (2018)**	**55 DAE (2019)**	**55 DAE (2018)**	**55 DAE (2019)**
G					
Gresik	6	19.62 ± 1.27 ^{ab}	26.70 ± 1.46 ^{b}	44 ± 9.46 ^{a}	55 ± 10.41 ^{a}
LunT	6	17.40 ± 0.88 ^{ab}	25.81 ± 0.89 ^{b}	20 ± 5.60 ^{b}	33 ± 5.60 ^{b}
IITA-686	6	22.92 ± 2.63 ^{ab}	28.86 ± 0.56 ^{ab}	30 ± 6.86 ^{ab}	43 ± 6.86 ^{ab}
DodR	6	23.40 ± 1.38 ^{a}	30.87 ± 1.57 ^{ab}	27 ± 5.92 ^{ab}	40 ± 5.92 ^{ab}
S19-3	6	17.62 ± 1.96 ^{ab}	29.22 ± 0.95 ^{ab}	28 ± 5.03 ^{ab}	41 ± 5.03 ^{ab}
Tiga nicuru	6	15.47 ± 1.25 ^{b}	27.32 ± 2.30 ^{b}	39 ± 7.71 ^{ab}	52 ± 7.71 ^{ab}
Ankpa-4	6	23.40 ± 3.11 ^{a}	30.40 ± 1.79 ^{ab}	38 ± 9.69 ^{ab}	51 ± 9.69 ^{ab}
DipC1	6	21.77 ± 1.72 ^{ab}	33.19 ± 2.04 ^{a}	32 ± 8.49 ^{ab}	45 ± 8.49 ^{ab}
WM					
WW	24	22.59 ± 1.00 ^{a}	31.00 ± 0.86 ^{a}	44 ± 3.56 ^{a}	50 ± 3.13 ^{a}
DS	24	17.81 ± 0.91 ^{b}	27.10 ± 0.65 ^{b}	19 ± 1.15 ^{b}	30 ± 1.38 ^{b}
F probability					
G		0.01**	0.00***	0.01**	0.02*
WM		0.00***	0.00***	0.00***	0.00***
G × WM		0.58^{ns}	0.11^{ns}	0.52^{ns}	0.40^{ns}

[1] Treatments: G – genotype and WM – water management.

[2] NoL values rounded to the nearest integer because NoL represents discrete data.

The data is mean ± se values (n = 6), with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ns = not significant.

Rewatering bambara groundnuts plants after DS treatment resulted in a highly significant ($P < 0.001$) interaction effect between genotypes and water management for both SH and NoL at 105 DAE (50-d of DS recovery) in 2018 and 2019 seasons (Table 5-2; Figure 5-5). In both seasons, SH was mostly lower in plants at 105 DAE (50-d of DS recovery) compared to WW, except for the genotype LunT which recovered fully and also increased by 4% in DS (recovery) in 2018 and DodR (increased by 19%) in 2019. It is also worth noting that the genotypes DipC1, IITA-686, and DodR showed significant ($P < 0.001$) decrease in SH under DS (29%, 25% and 25%, respectively) than in WW in the 2018 season, while other genotypes showed no significant difference between WW and DS (50-d of DS recovery). In 2019, LunT and S19-3 also showed a significant ($P < 0.001$) decrease in SH under DS (49%, and 39%, respectively) than in WW.

Similarly, in both seasons, NoL was mostly lower in plants at 50-d of DS recovery compared to WW, except for the genotype DipC1 in the 2018 season which recorded 23% more NoL in the DS than the WW treatment (Table 5-2). The genotypes Ankpa-4 (2018 and 2019) and IITA-686 (2019), showed significant ($P < 0.001$) decrease in NoL under DS (51%, 51% and 39%, respectively) than in WW.

Table 5-2 Analysis of variance for shoot height (SH), and number of leaves (NoL) at 105 DAE of eight bambara groundnut genotypes, grown in soil-filled PVC columns in a rainout shelter under WW and DS in two seasons 2018 and 2019.

		SH (cm plant $^{-1}$)		NoL[2] (number)	
Treatment[1]	**N**	**105 DAE (2018)**	**105 DAE (2019)**	**105 DAE (2018)**	**105 DAE (2019)**
G × WM					
WW					
Gresik	3	28.13 ± 0.61 ^{abc}	37.15 ± 1.85 ^{abc}	118 ± 6.24 ^{b}	138 ± 7.75 ^{b}
LunT	3	24.10 ± 1.07 ^{cde}	43.98 ± 1.88 ^{ab}	47 ± 12.58 ^{cd}	67 ± 11.67 $^{c-f}$
IITA-686	3	32.83 ± 0.52 ^{a}	39.56 ± 0.89 ^{ab}	123 ± 26.12 ^{b}	133 ± 14.45 ^{b}
DodR	3	30.93 ± 0.93 ^{ab}	34.37 ± 3.40 $^{a-d}$	81 ± 16.86 ^{bc}	95 ± 13.02 ^{bcd}
S19-3	3	22.43 ± 2.03 ^{cde}	39.88 ± 2.44 ^{ab}	46 ± 5.77 ^{cd}	63 ± 2.60 ^{def}
Tiga nicuru	3	23.07 ± 0.55 ^{cde}	35.87 ± 1.13 $^{a-d}$	43 ± 6.66 ^{cd}	59 ± 5.86 ^{def}
Ankpa-4	3	26.10 ± 0.64 ^{bcd}	48.56 ± 1.28 ^{a}	225 ± 6.23 ^{a}	219 ± 5.86 ^{a}
DipC1	3	32.80 ± 1.86 ^{a}	45.51 ± 2.23 ^{ab}	63 ± 5.36 ^{bcd}	99 ± 6.57 ^{bcd}
DS					
Gresik	3	25.03 ± 1.93 $^{b-e}$	32.27 ± 2.34 ^{bcd}	86 ± 3.06 ^{bc}	99 ± 5.49 ^{bcd}
LunT	3	25.03 ± 1.43 $^{b-e}$	22.34 ± 0.75 ^{d}	20 ± 3.18 ^{d}	34 ± 4.51 ^{f}
IITA-686	3	24.60 ± 1.08 ^{cde}	33.14 ± 2.73 ^{bcd}	63 ± 8.84 ^{bcd}	81 ± 9.49 ^{cde}
DodR	3	23.33 ± 0.09 ^{cde}	42.46 ± 2.39 ^{ab}	50 ± 7.67 ^{cd}	62 ± 8.19 ^{def}
S19-3	3	19.93 ± 0.54 ^{e}	24.42 ± 1.80 ^{cd}	30 ± 0.33 ^{cd}	44 ± 2.03 ^{ef}
Tiga nicuru	3	21.60 ± 0.40 ^{de}	24.40 ± 1.93 ^{cd}	28 ± 2.19 ^{cd}	37 ± 3.76 ^{f}
Ankpa-4	3	21.03 ± 1.47 ^{de}	39.04 ± 7.74 ^{abc}	111 ± 24.38 ^{b}	108 ± 13.20 ^{bc}
DipC1	3	23.40 ± 0.82 ^{cde}	35.58 ± 2.60 $^{a-d}$	82 ± 6.57 ^{bc}	73 ± 2.33 $^{c-f}$
F probability					
G		0.00***	0.00***	0.00***	0.00***
WM		0.00***	0.00***	0.00***	0.00***
G × WM		0.00***	0.00***	0.00***	0.00***

[1] Treatments: G – genotype, WM – water management.

[2] NoL values rounded to the nearest integer because NoL represents discrete data.

The data is mean ± se values (n = 3), with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ns = not significant.

Figure 5-5 Differential plant shoot sizes at final harvest: 105 DAE (50-d of DS recovery) of eight bambara groundnut genotypes grown in soil-filled PVC columns in a rainout shelter under WW and DS treatment (at 50-d of recovery) in 2019. White bar = 30cm.

5.4.4 Root to Shoot Ratio

There was no significant interaction effect between genotype and water management during 2018 and 2019 seasons with respect to root to shoot (R:S) ratio at 55 and 105 DAE (50-d of DS recovery) (Figure 5-6; Figure 5-7). Results of R:S ratio at 55 DAE for the 2018 season showed significant differences ($P < 0.001$) between the genotypes. The eight genotypes showed substantial differences in biomass allocation with R:S ratio ranging from 0.15 (Ankpa-4) to 0.54 (DodR) in 2018 (Figure 5-6A). Differences in water management were also observed with R:S ratio significantly higher ($P < 0.001$; 22%) under DS in 2018 compared to WW (Figure 5-6B).

Despite the lack of statistical difference among genotypes ($P = 0.46$; Figure 5-6C) at 105 DAE (50-d of DS recovery) in the 2018 season, R:S ratio was lower in the genotype IITA-686 (0.36) and highest in DodR (0.62) — only 6% more than the second highest LunT (0.58). Differences in water management revealed higher R:S ratio in WW, although this was not statistically different ($P = 0.48$; Figure 5-6D) from the DS treatment.

Results of R:S ratio at 55 DAE for the 2019 season showed significant differences ($P = 0.03$) between the genotypes, ranging from 0.09 (Tiga nicuru) to 0.36 (DodR; Figure 5-7A). R:S ratio was significantly higher ($P < 0.01$; 42%) under WW compared to DS in the same season (Figure 5-7B). At 105 DAE (50-d of DS recovery) in the 2019 season, R:S ratio ranged from 0.10 (S19-3) to 0.90 (DodR; Figure 5-7C), with significantly higher (P = 0.04; 48%) R:S ratio under the WW treatment in 2019 compared to DS (Figure 5-7D).

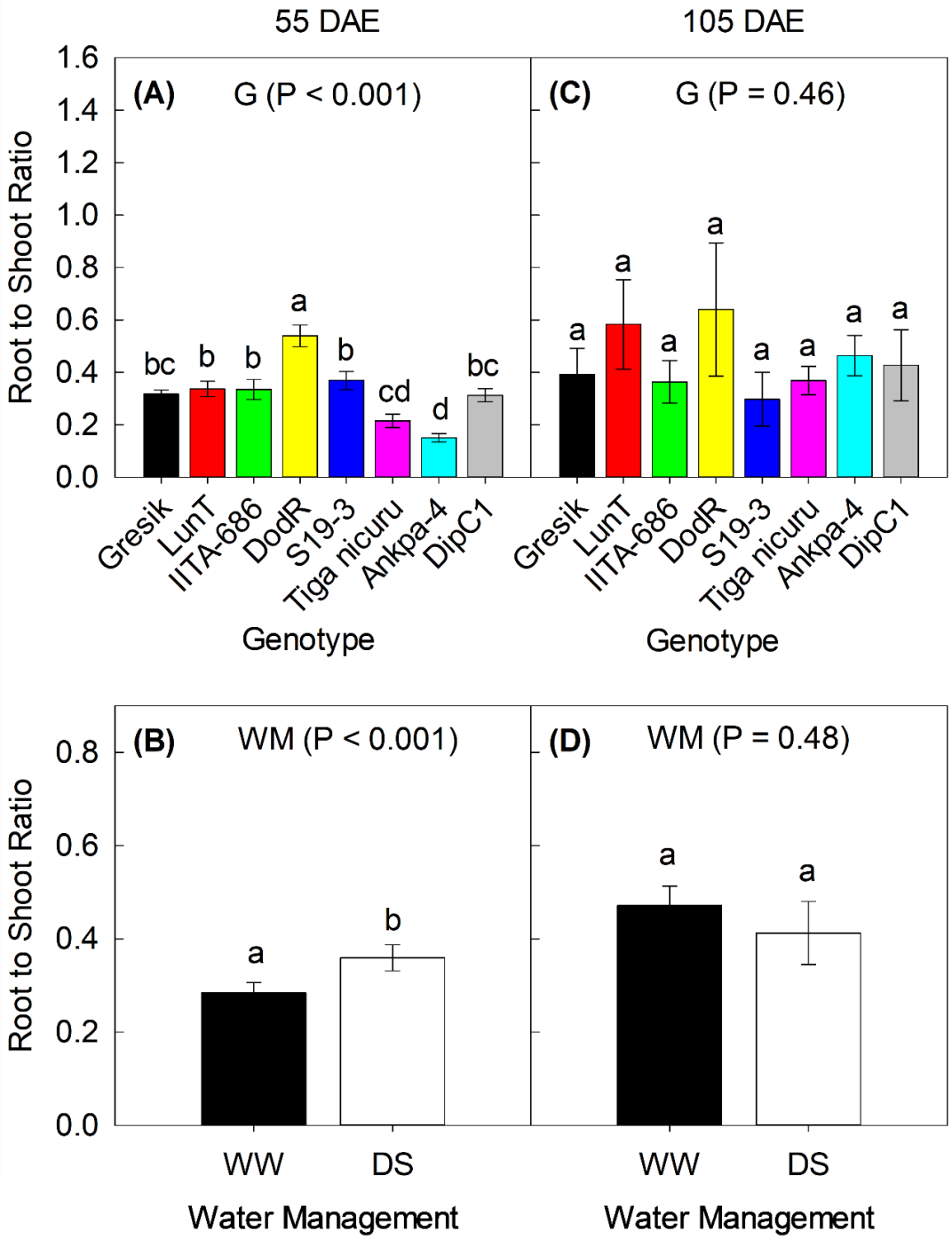

Figure 5-6 Effect of Genotype (G) — (**A, C**) at 55 and 105 DAE (50-d of DS recovery), respectively, the data is mean ± se values (n = 6) and Water management (WM) — (**B, D**) at 55 and 105 DAE (50-d of DS recovery), respectively, the data is mean ± se values (n = 24) on root to shoot ratio (R:S) of eight bambara groundnut genotypes during the 2018 season. Different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ns = not significant.

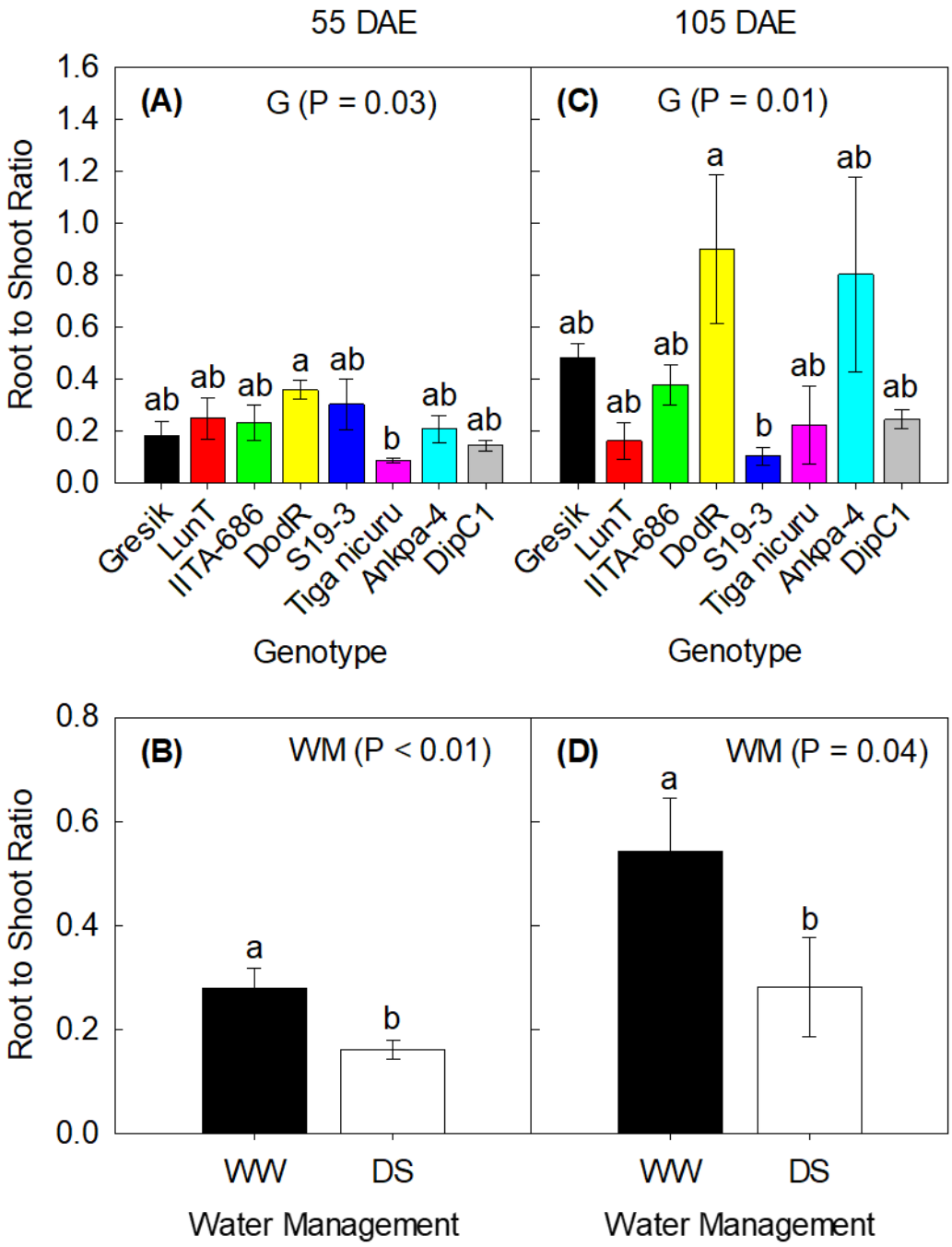

Figure 5-7 Effect of Genotype (G) — (**A, C**) at 55 and 105 DAE (50-d of DS recovery), respectively, the data is mean ± se values (n = 6) and Water management (WM) — (**B, D**) at 55 and 105 DAE (50-d of DS recovery), respectively, the data is mean ± se values (n = 24) on root to shoot ratio (R:S) of eight bambara groundnut genotypes during the 2019 season. Different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ns = not significant

5.4.5 Changes in Root Depth Profile

Root depth profile i.e., tap root length (TRL) was significantly affected by the interaction effect of genotype and water management at 55 DAE for the 2018 season ($P < 0.01$; Table 5-3). However, TRL was not significantly affected by the interaction effect of genotype and water management at 55 DAE for the 2019 season ($P = 0.94$; Table 5-3). TRL showed a significant decrease ($P < 0.001$) under the DS treatment by 14% and 22% in 2018 and 2019 (Figure 5-8), respectively. Based on mean values for the genotypes at 55 DAE in the 2018 season, LunT and IITA-686 showed significant ($P < 0.001$) decrease in TRL under DS (27% and 25%, respectively) than in WW, while DodR recorded an increase (1%) in TRL under DS.

At 55 DAE in the 2019 season, TRL ranged from 85.6cm (Gresik) to 117.1cm (DodR) with average TRL of 102cm (Table 5-3). Compared to DodR, the genotypes Gresik and LunT showed significantly ($P < 0.05$) less TRL, exclusively limited to the 60–90cm layer. The genotypes, S19-3 showed significantly higher TRL, recording the second largest TRL (113.5cm), although S19-3 was only 4cm shorter than the deepest rooting DodR genotype.

For TRL at 105 DAE (50-d of DS recovery), no interaction effect ($P = 0.55$) and genotype effects ($P = 0.25$) were observed in the 2018 season (Table 5-3). TRL showed a significant decrease under the DS treatment by 5% and 11% in 2018 and 2019, respectively. Genotypes Tiga nicuru and LunT showed highly significant ($P < 0.001$) decrease in TRL under DS (29% and 28%, respectively) than in WW in the 2019 season, with IITA-686, Ankpa-4, Gresik and DodR recording the least differences (1%, 2%, 3% and 8%, respectively) under DS (50-d of recovery).

Table 5-3 Analysis of variance for tap root length (TRL) at 55 DAE and 105 DAE (50-d of DS recovery) of eight bambara groundnut genotypes, grown in soil-filled PVC columns under a rainout shelter under WW and DS in two seasons 2018 and 2019.

		TRL (cm)			
Treatment [1]	**N**	**55 DAE (2018)**	**55 DAE (2019)**	**105 DAE (2018)**	**105 DAE (2019)**
G					
Gresik	6	93.30 ± 3.60 ^{c}	85.58 ± 11.03 ^{b}	100.76 ± 3.58	138.20 ± 2.50 ^{ab}
LunT	6	80.53 ± 5.62 ^{d}	86.19 ± 8.69 ^{b}	97.89 ± 2.78	118.40 ± 10.70 ^{b}
IITA-686	6	100.04 ± 6.61 ^{abc}	111.04 ± 7.80 ^{ab}	107.71 ± 2.98	121.90 ± 4.80 ^{ab}
DodR	6	107.09 ± 2.86 ^{ab}	117.76 ± 6.29 ^{a}	109.02 ± 2.69	140.90 ± 3.30 ^{a}
S19-3	6	100.76 ± 4.44 ^{abc}	113.52 ± 6.52 ^{a}	102.09 ± 4.94	124.00 ± 3.90 ^{ab}
Tiga nicuru	6	95.77 ± 3.90 ^{bc}	105.33 ± 4.25 ^{ab}	102.35 ± 3.17	120.60 ± 11.50 ^{ab}
Ankpa-4	6	106.32 ± 3.74 ^{ab}	92.52 ± 8.77 ^{ab}	109.50 ± 5.44	137.05 ± 2.10 ^{ab}
DipC1	6	108.25 ± 4.61 ^{a}	104.37 ± 7.66 ^{ab}	108.22 ± 4.61	124.00 ± 2.50 ^{ab}
WM					
WW	24	106.66 ± 1.76 ^{a}	114.56 ± 3.38 ^{a}	107.52 ± 2.11 ^{a}	135.90 ± 2.10 ^{a}
DS	24	91.35 ± 2.70 ^{b}	89.52 ± 3.69 ^{b}	101.86 ± 1.72 ^{b}	120.50 ± 3.70 ^{b}
G*WM					
WW					
Gresik	3	100.94 ± 1.60 $^{a-d}$	92.74 ± 5.66 ^{a}	105.21 ± 6.62 ^{a}	140.30 ± 2.80 ^{a}
LunT	3	93.10 ± 0.24 ^{bcd}	102.67 ± 4.81 ^{a}	95.00 ± 2.12 ^{a}	138.00 ± 3.80 ^{a}
IITA-686	3	114.06 ± 2.99 ^{a}	123.72 ± 11.68 ^{a}	114.00 ± 1.99 ^{a}	121.80 ± 10.60 ^{ab}
DodR	3	106.50 ± 5.51 ^{abc}	130.19 ± 5.17 ^{a}	112.50 ± 3.34 ^{a}	147.00 ± 3.90 ^{a}
S19-3	3	108.87 ± 3.73 ^{ab}	125.94 ± 6.27 ^{a}	109.65 ± 7.82 ^{a}	131.60 ± 3.10 ^{ab}
Tiga nicuru	3	103.20 ± 1.49 $^{a-d}$	113.98 ± 2.75 ^{a}	104.70 ± 5.10 ^{a}	141.30 ± 2.20 ^{a}
Ankpa-4	3	109.56 ± 4.08 ^{ab}	106.46 ± 13.72 ^{a}	108.32 ± 7.47 ^{a}	138.70 ± 1.60 ^{a}
DipC1	3	117.07 ± 2.85 ^{a}	120.77 ± 2.46 ^{a}	110.78 ± 8.86 ^{a}	128.70 ± 2.90 ^{ab}
DS					
Gresik	3	85.66 ± 1.92 ^{de}	78.43 ± 22.91 ^{a}	96.31 ± 0.69 ^{a}	136.10 ± 4.40 ^{a}
LunT	3	67.96 ± 0.29 ^{e}	69.72 ± 9.11 ^{a}	100.78 ± 5.07 ^{a}	98.70 ± 13.10 ^{b}
IITA-686	3	86.03 ± 3.65 ^{de}	98.36 ± 2.66 ^{a}	101.41 ± 0.91 ^{a}	122.00 ± 1.70 ^{ab}
DodR	3	107.68 ± 3.18 ^{ab}	105.33 ± 4.10 ^{a}	105.55 ± 3.60 ^{a}	134.80 ± 1.40 ^{a}
S19-3	3	92.66 ± 4.35 ^{bcd}	101.09 ± 4.35 ^{a}	94.52 ± 1.91 ^{a}	116.40 ± 3.10 ^{ab}
Tiga nicuru	3	88.34 ± 4.33 ^{cd}	96.68 ± 2.84 ^{a}	100.00 ± 4.34 ^{a}	100.00 ± 15.20 ^{b}
Ankpa-4	3	103.08 ± 6.53 $^{a-d}$	78.58 ± 1.34 ^{a}	110.68 ± 9.53 ^{a}	136.30 ± 4.30 ^{a}
DipC1	3	99.43 ± 4.49 $^{a-d}$	87.98 ± 4.28 ^{a}	105.66 ± 4.62 ^{a}	119.40 ± 0.50 ^{ab}
F probability					
G		0.00***	0.01*	0.25^{ns}	0.00***
WM		0.00***	0.00***	0.04*	0.00***
G*WM		0.01*	0.94^{ns}	0.55^{ns}	0.01*

[1] Treatments: G – genotype, WM – water management.

The data is mean ± se values with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ns = not significant.

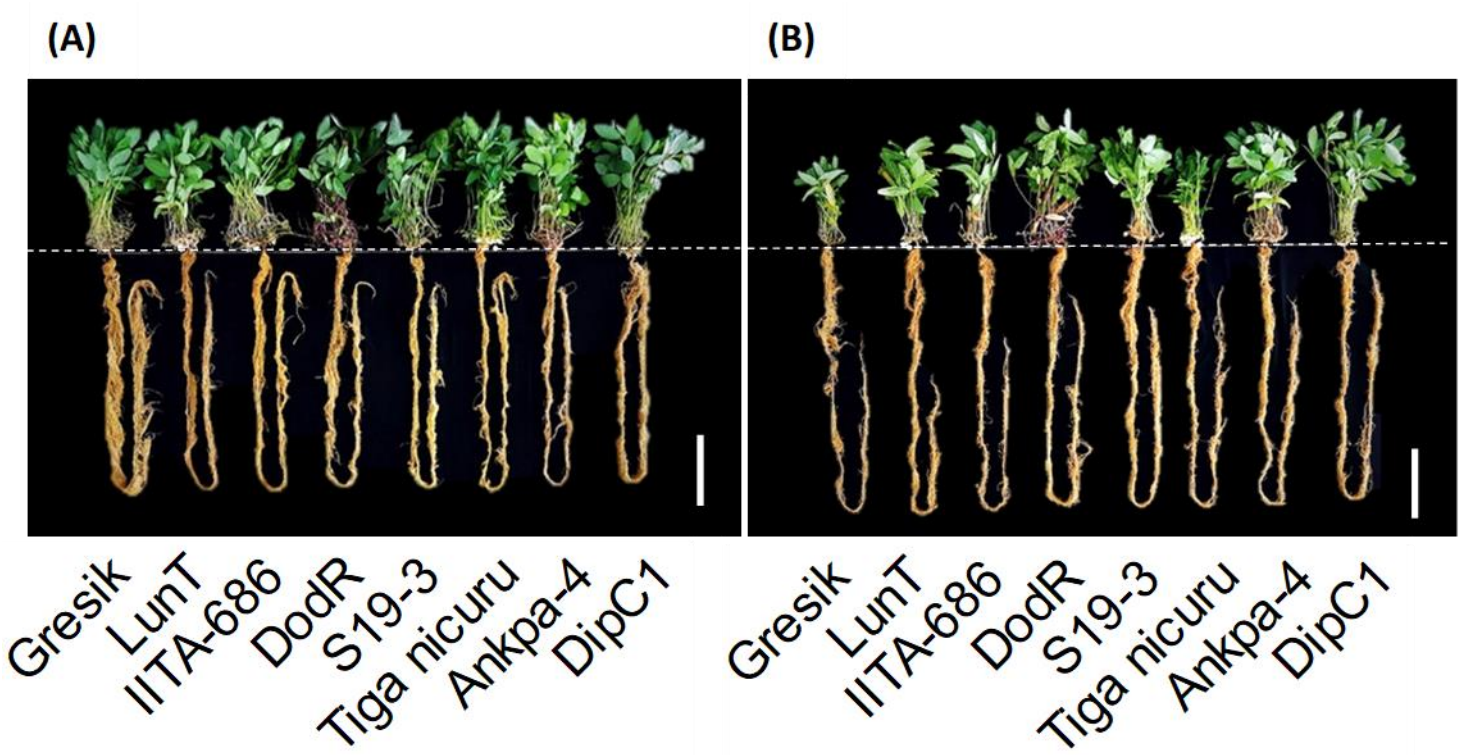

Figure 5-8 Example images of eight bambara groundnut genotypes grown in soil-filled PVC columns in a rainout shelter under (**A**) WW and (**B**) DS treatment at 55 DAE during 2019. White bar = 15cm.

5.4.6 Changes in Vertical Root Distribution

Vertical root distribution i.e., root length density (RLD; cm cm^{-3}) was measured at various soil depths (i.e., 0-30, 30-60, 60-90 and 90-110cm) and generally showed a decrease with soil depth. Within these soil depths, RLD at 55 DAE was significantly affected by the interaction effect of genotype and water management ($P < 0.001$; Figure 5-9), except at 90-110cm ($P = 0.41$) in the 2018 season and ($P < 0.001$; Figure 5-10A) and 30-60cm ($P = 0.88$; Figure 5-10A, B) soil depth in the 2019 season. RLD generally showed maximum distribution in the shallow 0-30cm soil depth for most of the studied genotypes under both WW and DS treatment at 55 DAE in both 2018 and 2019 seasons (Figure 5-9A, B; Figure 5-10A, B).

Under the WW treatment, the rainy-habitat genotype Gresik recorded the highest mean at the depth of 0-30cm, 0.73 and 0.44cm cm^{-3} in the 2018 and 2019 seasons, respectively (Figure 5-9A; Figure 5-10A). Under the DS treatment, RLD was reduced in almost all soil depths and all genotypes (Figure 5-9B; Figure 5-10B). RLD was lower in the deeper soil depths i.e., 60-90cm and 90-110cm, with the highest reduction observed in the 90-110cm layer (42 and 58%) in the 2018 and 2019 seasons, respectively. Deeper soil depths revealed marked differences among genotypes: with rainy-habitat genotype LunT having no roots in the 90-110cm soil depth in 2018 and recording the least (0.06 ± 0.013cm cm^{-3}) in 2019, whereas RLD of rainy habitat Gresik decreased progressively down to 110cm in both seasons. Dry-habitat S19-3 densely occupied the soil at depth, retaining a RLD of 0.16 ± 0.05 and 0.18 ± 0.01cm cm^{-3} in the deepest layer in both seasons, respectively (Figure 5-9B; Figure 5-10A).

In the absence of stress, all plants recovered to a similar degree as shown by the consistent lack of a significant interaction effect of genotype and water management ($P > 0.05$) at 105 DAE (50-d of DS recovery) in both seasons in all soil depths (Figure 5-9C, D; Figure 5-10C, D). However, significant differences

were noted among genotype in the 0-30, 30-60, and 60-90cm soil depths ($P < 0.001$; $P < 0.05$; and $P < 0.05$, respectively) and water management in the 30-60, 60-90 and 90-110cm soil depths ($P < 0.001$; $P < 0.001$ and $P < 0.05$, respectively) in the 2018 season. For the 2019 season, significant differences were noted among genotype selections in the 0-30, 30-60, and 90-110 soil depths ($P < 0.001$; $P < 0.001$ and $P < 0.05$, respectively) and water management in the 30-60, 60-90 and 90-110cm soil depths ($P < 0.001$; $P < 0.001$ and $P < 0.05$, respectively). The reduced RLD under DS had a tendency to recover when re-watered. Genotypes LunT and DodR (0-30cm), Tiga nicuru (30-60cm), LunT (60-90cm) and LunT and Ankpa-4 (90-110cm), maintained high RLD under drought recovery surpassing the density in the WW treatment in 2018 (Figure 5-9C, D). In the 2019 season, Gresik under DS surpassed the RLD density in the WW treatment for all the soil depths. Recovery was also observed in Tiga nicuru and DodR (0-30cm) and IITA-686 (90-110cm; Figure 5-10C, D).

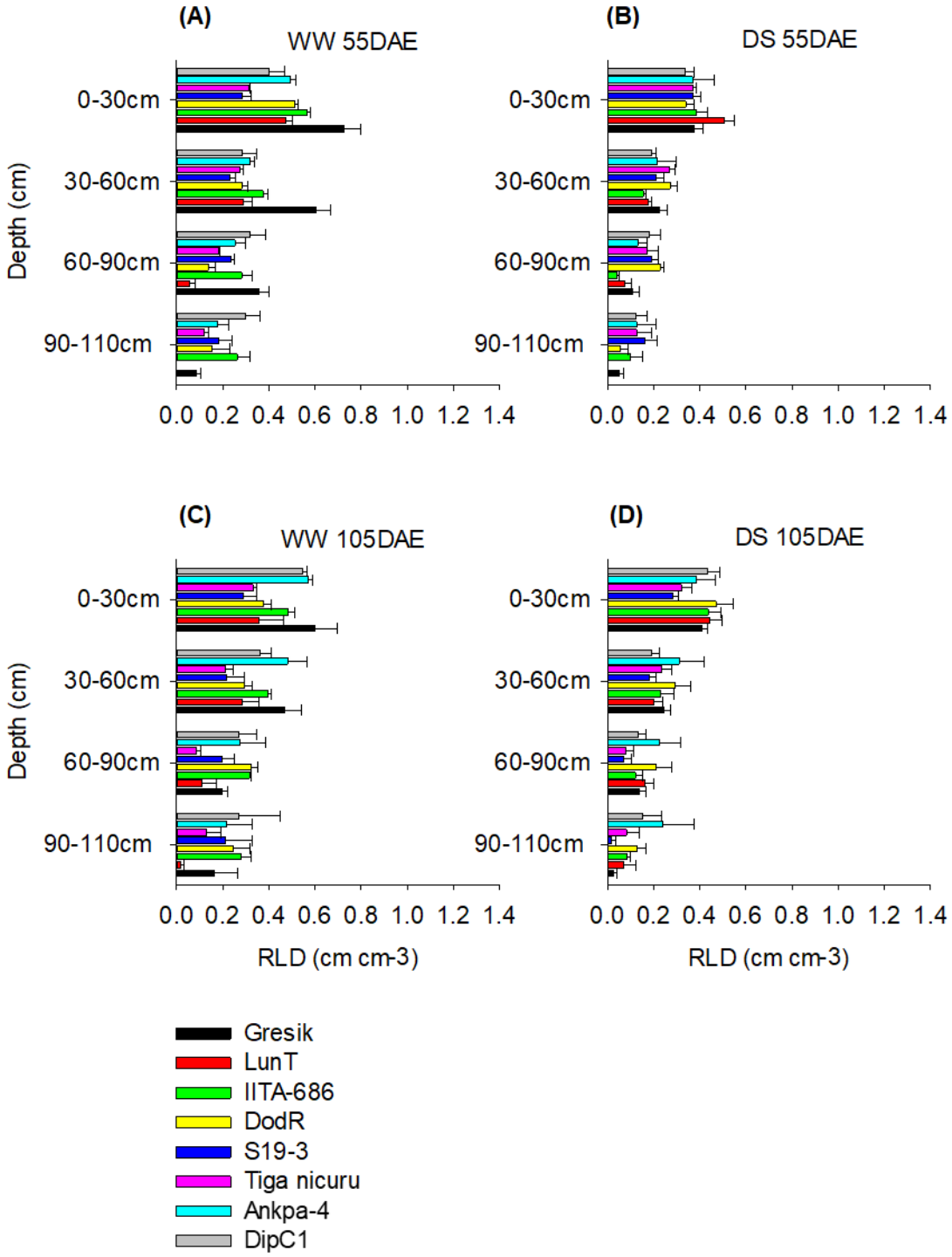

Figure 5-9 Root length density (RLD) of eight bambara groundnut genotypes at different soil depths grown in soil-filled PVC columns in the 2018 season (**A, B**) WW and DS at 55 DAE, respectively (**C, D**) WW and DS at 105DAE, respectively. The data is mean ± se values (n = 3) with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

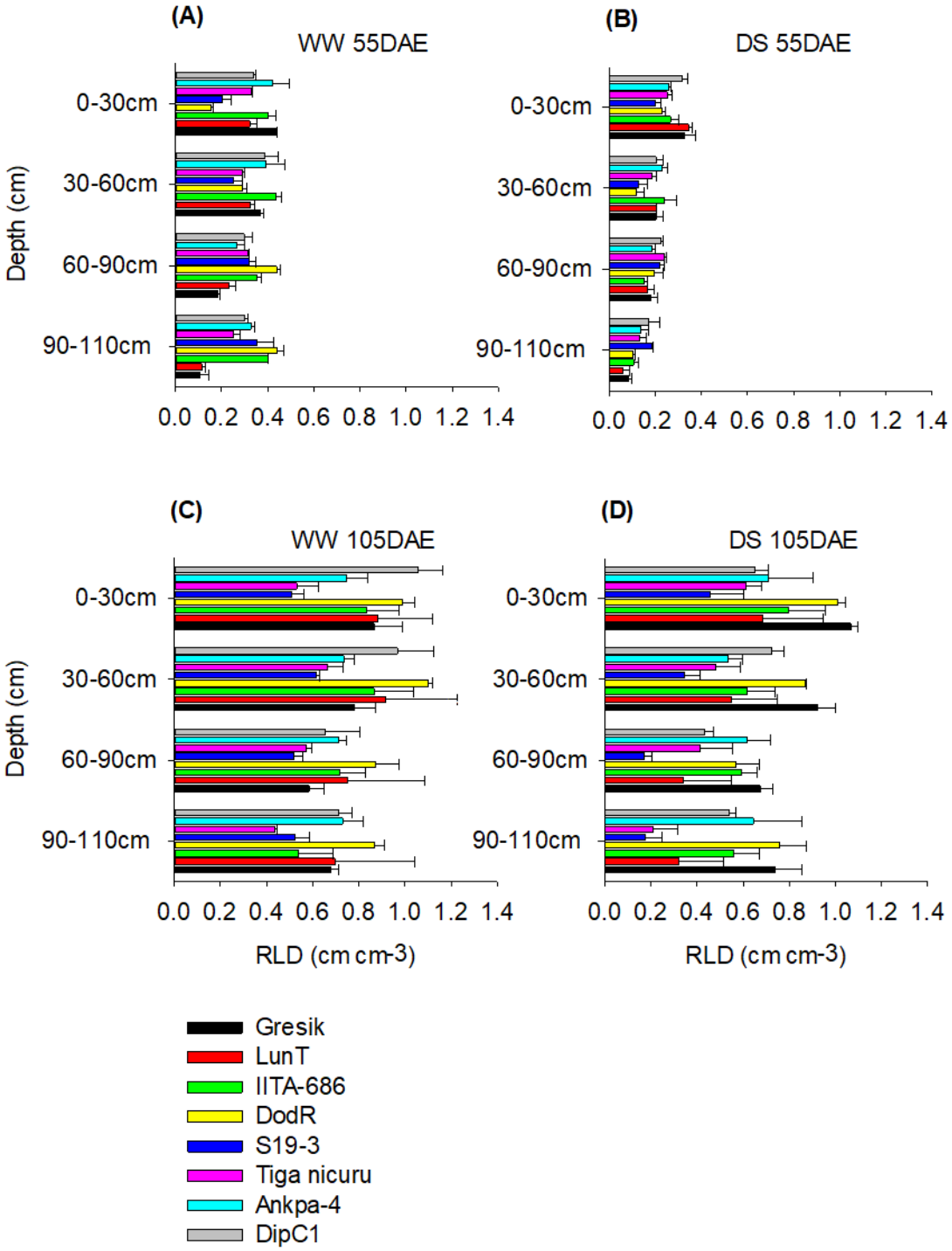

Figure 5-10 Root length density (RLD) of eight bambara groundnut genotypes at different soil depths grown in soil-filled PVC columns in the 2019 season (**A, B**) WW and DS at 55 DAE, respectively (**C, D**) WW and DS at 105 DAE, respectively. The data is mean ± se values (n = 3) with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$..

5.4.7 Soil Moisture Content and Stomatal Conductance

Volumetric water content (VWC) was measured only in 2019 at three time points i.e., 35, 45 and 55 DAE (Figure 5-11). At 35 DAE, this averaged 0.17 and 0.10m^3 m^{-3} in the surface soil (0-30cm) for WW and DS (Figure 5-11A, D), respectively, while at 30-60cm depth this in turn averaged 0.18 and 0.12m^3 m^{-3}. Subsequent soil depths i.e., 60-90 and 90-110cm averaged (0.19 and 0.14m^3 m^{-3}) and (0.23 and 0.14m^3 m^{-3}) for WW and DS, respectively. At 45 DAE, VWC averaged 0.13 and 0.06m^3 m^{-3} in the surface soil (0-30cm) for WW and DS (Figure 5-11B, E), respectively, while at 30-60cm depth this in turn averaged 0.13 and 0.06 m^3 m^{-3}. Subsequent soil depths i.e., 60-90 and 90-110cm averaged (0.13 and 0.08m^3 m^{-3}) and (0.17 and 0.08m^3 m^{-3}), respectively. Soil dried substantially by 55 DAE, with VWC dropping to 0.11 and 0.04m^3 m^{-3} in the surface soil (0-30cm) for WW and DS (Figure 5-11C, F), respectively, while at 30-60cm depth this in turn averaged 0.11 and 0.05m^3 m^{-3}. Subsequent soil depths i.e., 60-90 and 90-110cm averaged (0.12 and 0.07m^3 m^{-3}) and (0.15 and 0.05m^3 m^{-3}), respectively. The pattern of soil moisture depletion in the PVC columns was similar to the changes in stomatal conductance (g_s) (Figure 5-12).

Stomatal conductance (g_s) was measured in both the 2018 (Figure 5-12A-C) and 2019 (Figure 5-12D-F) seasons at three time points i.e., 35, 45 and 55 DAE. The effect of water deficit stress on plants was determined by stomatal conductance. Significant interaction effects of genotype and water management were observed across the three time points for both seasons, except at 35 DAE in 2018 ($P = 0.13$; Figure 5-12A). At this time point, significant differences were noted among genotype selections and water management (both $P < 0.001$; Figure 5-13A, B). In 2018, DS generally decreased g_s by 17%, 59% and 73% at 35, 45 and 55 DAE, respectively (Figure 5-12A-C), whilst a 26%, 51% and 68% decrease was observed in 2019 (Figure 5-12D-F). The eight genotypes at 55 DAE varied the most for g_s under DS in both seasons (2018 and 2019), ranging from

0.06mol m^{-2} s^{-1} (IITA-686) to 0.10mol m^{-2} s^{-1} (DodR) and 0.09mol m^{-2} s^{-1} (LunT) to 0.25mol m^{-2} s^{-1} (DodR), respectively (Figure 5-12C, F).

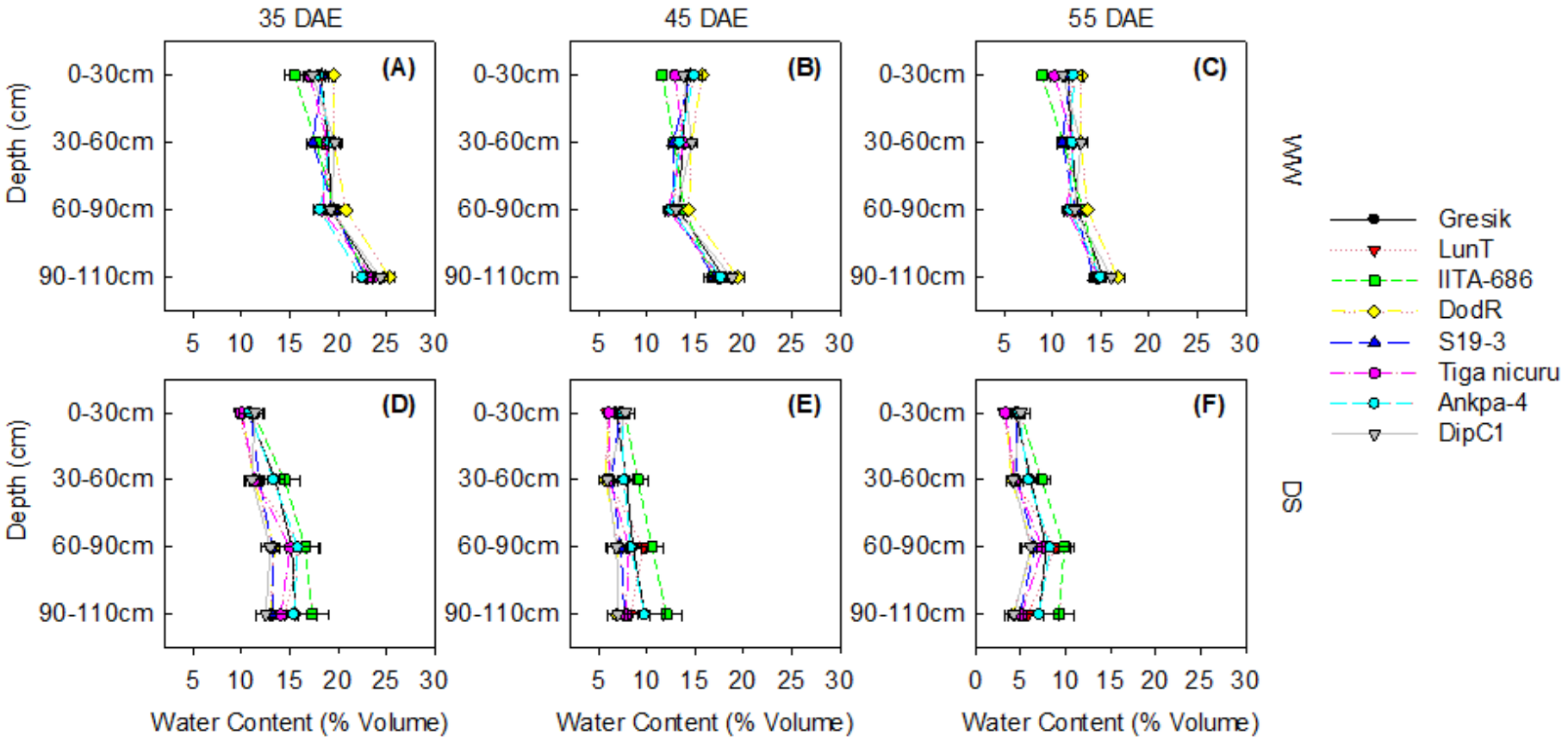

Figure 5-11 Soil volumetric water content measured in the soil-filled PVC columns under a rainout shelter at three time points (35, 45 and 55 DAE) in 2019. Measurements are of eight bambara groundnut genotypes grown under WW (**A-C**) and DS (**D-F**) treatments. The data is mean ± se values (n = 3) and horizontal bars represent (HSD, $P < 0.05$).

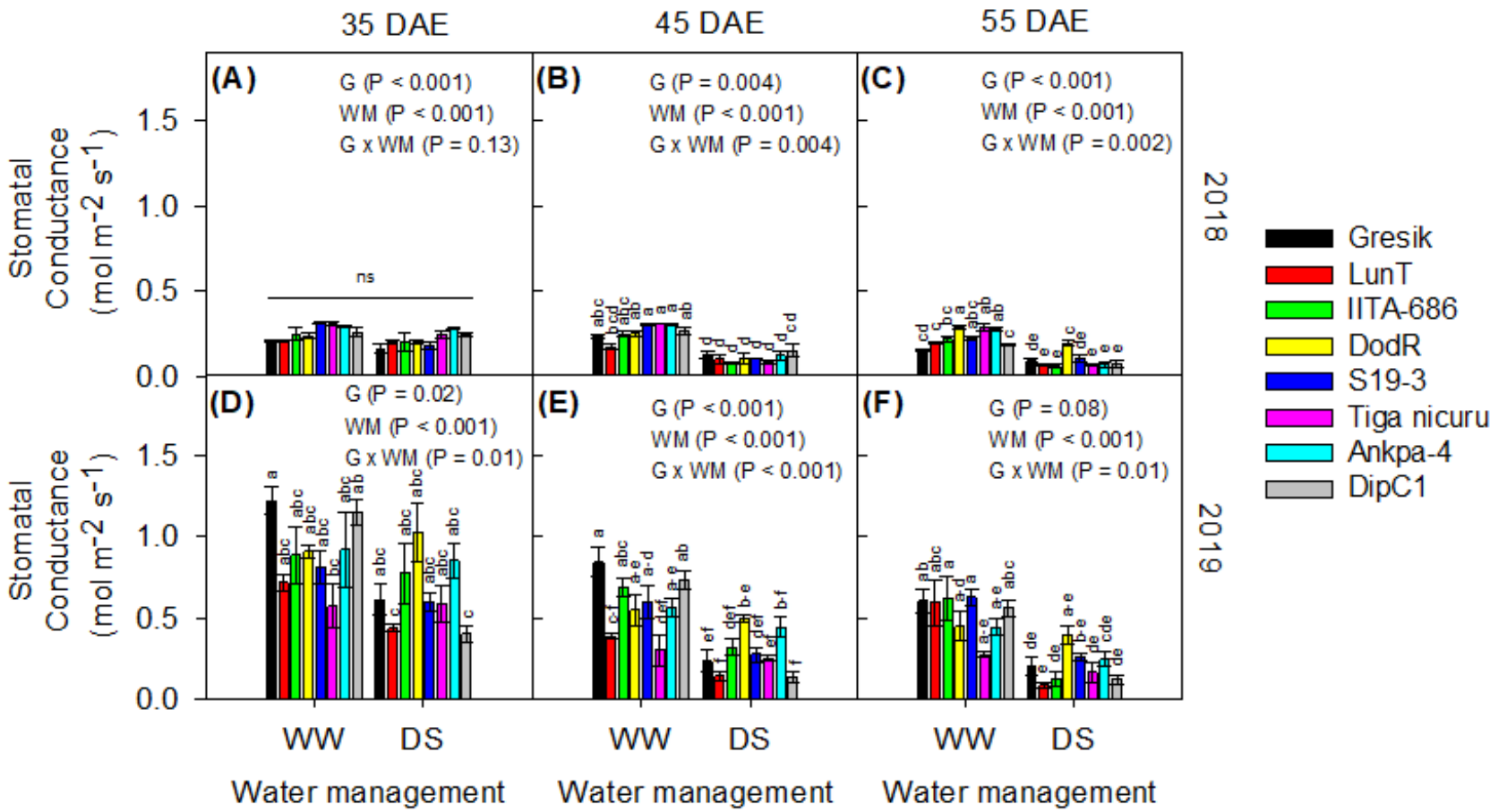

Figure 5-12 Interaction effect Genotype (G) × Water management (WM) on stomatal conductance, g_s (mol m^{-2} s^{-1}) of eight bambara groundnut genotypes grown in soil-filled PVC columns under a rainout shelter at (**A-C**) 35, 45 and 55 DAE, respectively in 2018 and (**D-F**) 35, 45 and 55 DAE, respectively in 2019. The data is mean ± se values (n = 3), with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and ns = not significant.

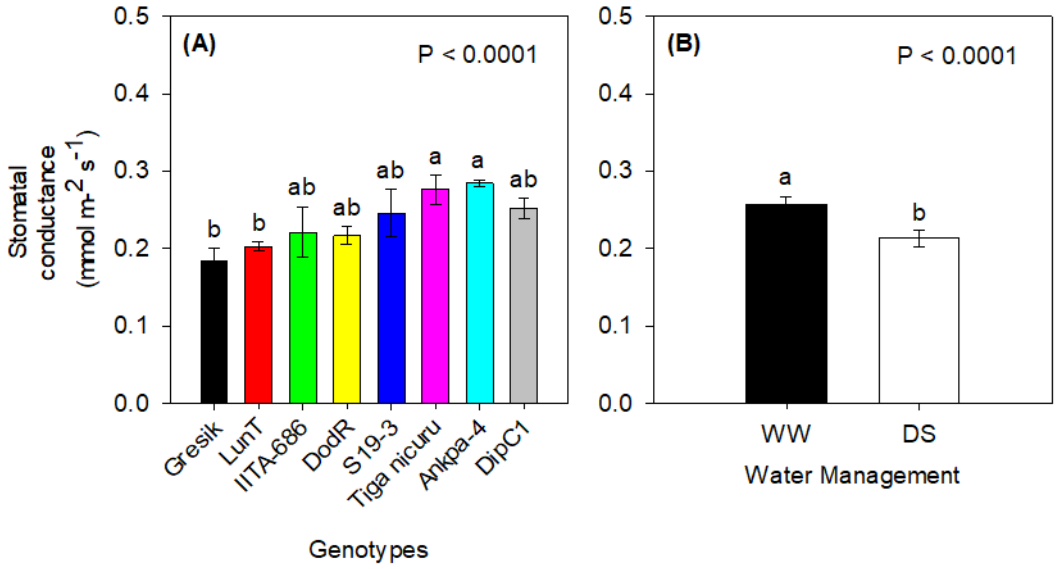

Figure 5-13 Effect of Genotype (G) — (**A**), the data is mean ± se values (n = 6) and Water management (WM) — (**B**), the data is mean ± se values (n = 24) at 35 DAE on stomatal conductance, g_s (mmol m^{-2} s^{-1}) of eight bambara groundnut genotypes during the 2018 season. The data is mean ± se values (n = 6) with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

5.4.8 Grain Yield

Significant interaction effects ($P = 0.04$; Figure 5-14A) between genotypes and water management were observed in the 2018 season and a highly significant interaction ($P < 0.001$; Figure 5-14B) in the following season (2019). Under the DS treatment, grain yield decreased by 76% in both the 2018 and 2019 seasons (Figure 5-14). IITA-686, DipC1 and DodR recorded the lowest reduction in grain yield in the 2018 season, whilst DipC1 and DodR had the lowest reduction in 2019. The genotype DodR was able to constantly produce the high grain yield under DS (31.7g $plant^{-1}$) in 2018 and (55.2g $plant^{-1}$) in 2019.

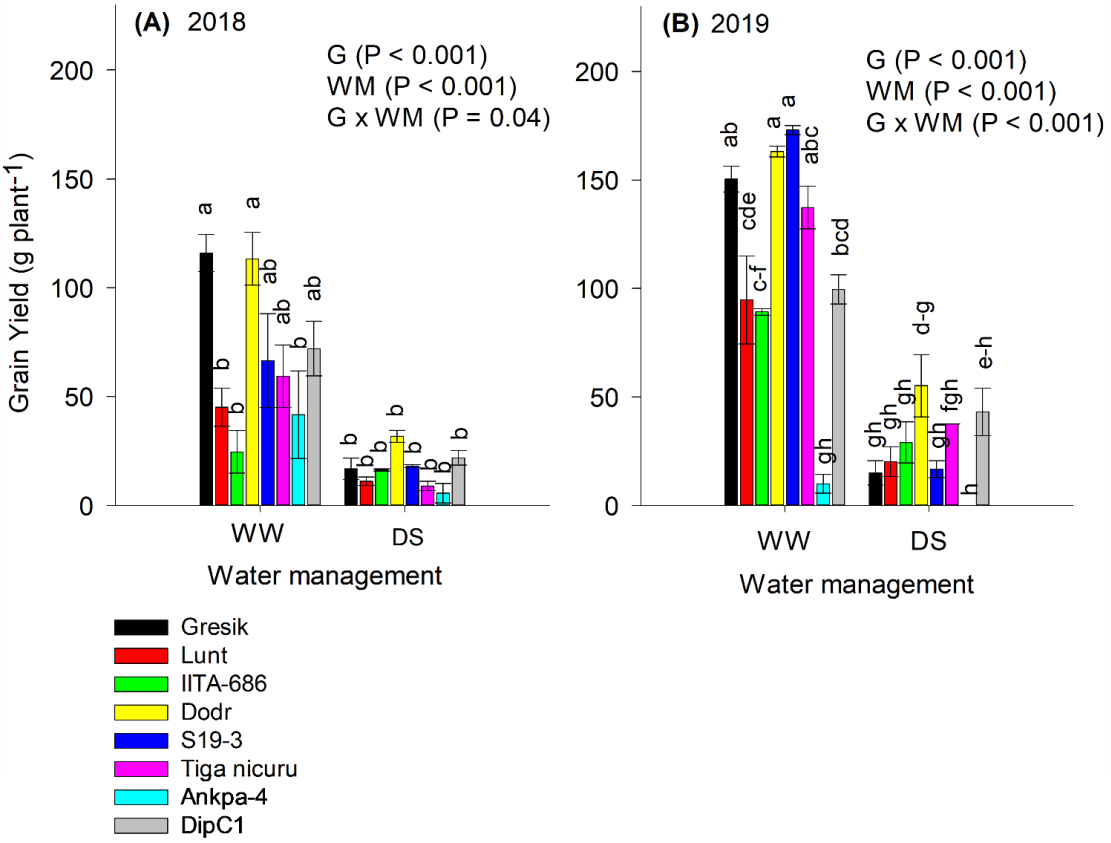

Figure 5-14 Analysis of variance for grain yield (g plant^{-1}) in two seasons (**A**) 2018 and (**B**) 2019 under well-watered conditions (WW) and drought stress (DS). The data is mean ± se values (n = 3) with different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

5.4.9 Correlations Between Root Traits and Grain Yield Under DS and WW treatment

Significant negative correlations ($P < 0.05$; Figure 5-15A, B) between topsoil RLD (0-30cm) at 55 DAE and grain yield were observed in the DS treatments in the 2018 and 2019 seasons (R^2 = 0.20 and 0.31, respectively). In contrast to the topsoil, subsoil RLD (60-90cm) at 55 DAE had significant and strong positive correlations ($P < 0.05$; Figure 5-16A, B) with grain yield in the DS treatments in the 2018 and 2019 seasons (R^2 = 0.27 and 0.49, respectively). Significant positive correlations ($P < 0.05$; Figure 5-16C, D) were also observed between TRL at 55 DAE and grain yield in the 2018 and 2019 seasons (R^2 = 0.19 and 0.36, respectively). The correlations between g_s and RLD (60-90cm) both at

55 DAE, were positive and significant ($P < 0.05$) under DS treatment ($R^2 = 0.45$ and $R^2 = 0.32$ for 2018 and 2019 seasons, respectively), with negative correlations under the WW treatment ($R^2 = 0.19$ and $R^2 = 0.28$ for 2018 and 2019 seasons, respectively) (Figure 5-16E, F). Under the DS treatment the genotypes DodR, S19-3, DipC1 and Tiga nicuru with high RLD (60-90cm) at 55 DAE in 2018 were strongly associated with high g_s also at the same stage i.e., 55 DAE (Figure 5-16E, F). In 2019 a somewhat similar trend was observed, in this particular instance with genotypes DodR, IITA-686, S19-3 and Tiga nicuru strongly associated with high g_s at 55 DAE (Figure 5-16E, F).

Shoot height (SH) was closely and positively correlated ($P < 0.05$) with RLD at 55 DAE in the deep 60-90cm of the soil in both WW ($R^2 = 0.22$ and $R^2 = 0.46$ for 2018 and 2019 seasons, respectively) and DS treatments ($R^2 = 0.08$ and $R^2 = 0.43$ for 2018 and 2019 seasons, respectively; Figure 5-17A, B).

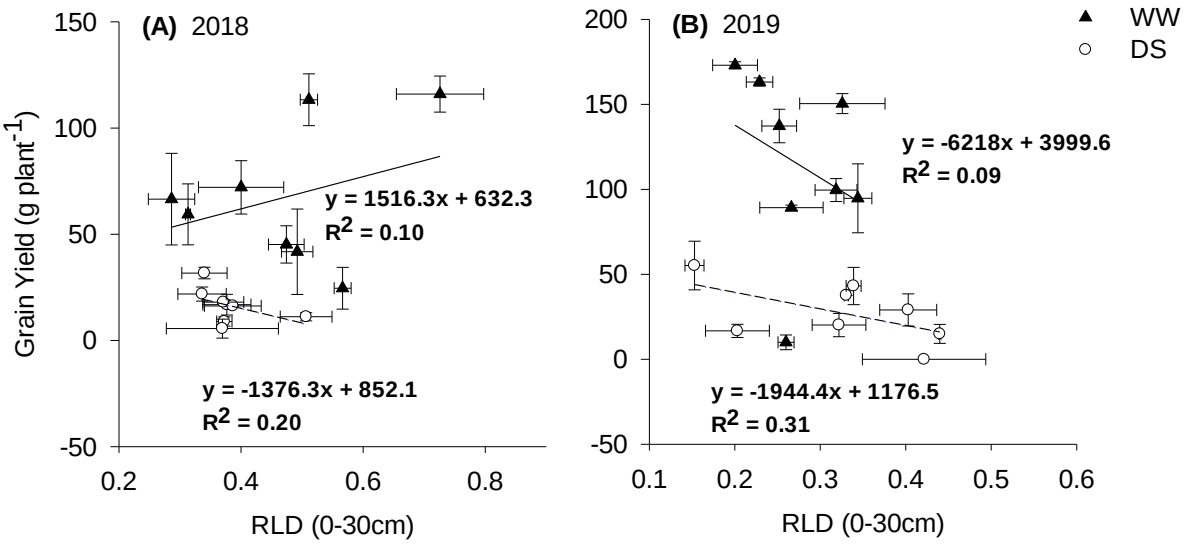

Figure 5-15 Relationship between root length density (RLD 0-30cm) and grain yield (g plant^{-1}) for two seasons: 2018 (**A**) and 2019 (**B**) under well-watered conditions (WW) and drought stress (DS). Coefficient of determination R^2 reported upon fitting with equation y = a*x + y0.

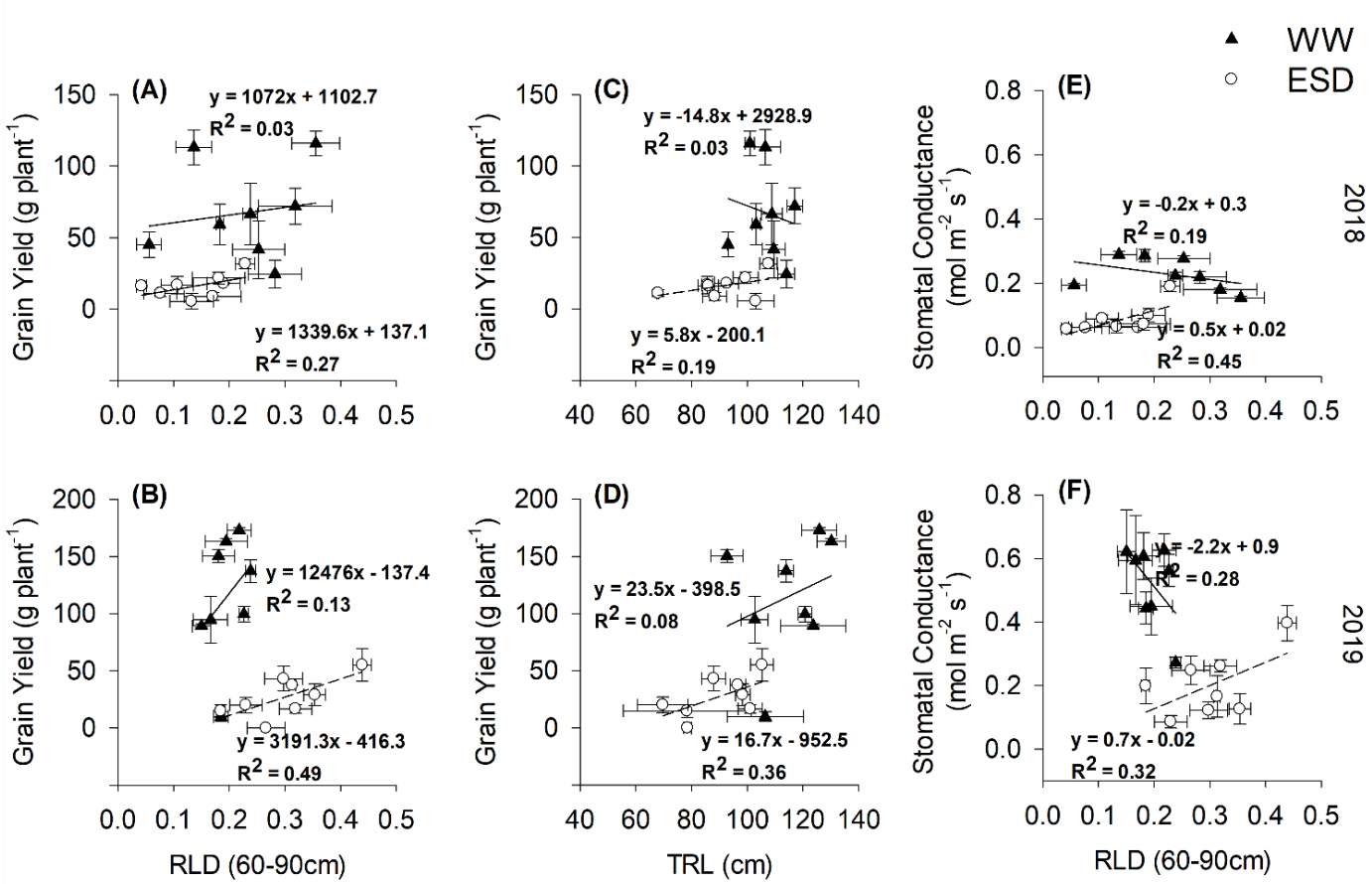

Figure 5-16 Relationship between root length density (RLD 60-90cm) and grain yield (g plant^{-1}), tap root length (TRL cm) and grain yield (kg ha^{-1}), and root length density (RLD 60-90cm) and g_s at 105 DAE, for two seasons: 2018 (**A, C, E**) and 2019 (**B, D, F**) under well-watered conditions (WW) and drought stress (DS). Coefficient of determination R^2 reported upon fitting with equation y = a*x + y0.

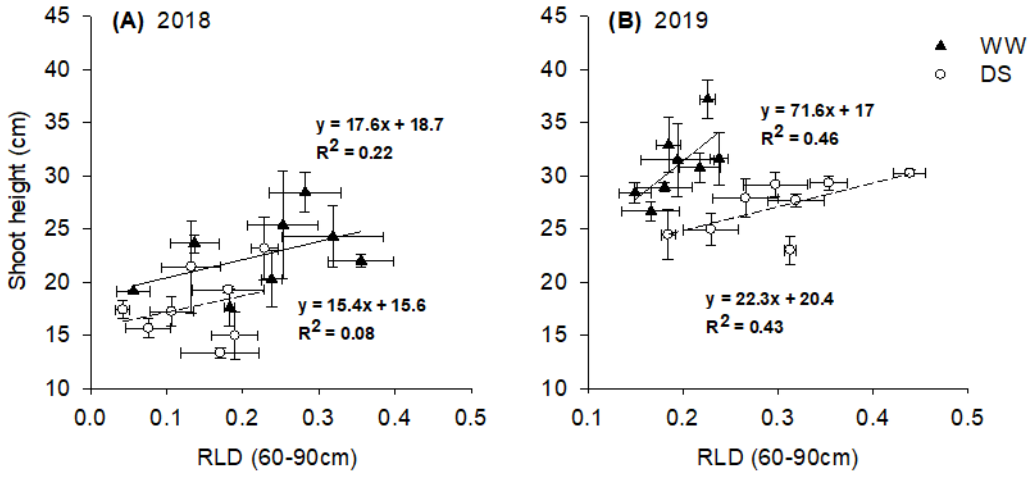

Figure 5-17 Relationship between root length density (RLD 0-30cm) and shoot height (cm) for two seasons: 2018 (**A**) and 2019 (**B**) under well-watered conditions (WW) and drought stress (DS). Coefficient of determination R^2 reported upon fitting with equation y = a*x + y0.

5.5 Discussion

The present results show strong genotype specific differences in root morphology, within eight diverse bambara groundnut core parental lines, i.e., single genotypes derived from landraces from various agroecologies. Moreover, the genotype specific differences were consistent with previous knowledge on the response of bambara groundnut to drought stress conditions (Jørgensen et al. 2010; Chai et al. 2016b). This study found enough differences to unequivocally identify the eight genotypes by their roots. In agreement with Serraj et al. (2004); Kashiwagi et al. (2006); Lalitha et al. (2015); Mateva et al. (2020); see *CHAPTER 4*, the most critical morphological features were (i) root depth profile, i.e., tap root length (TRL), which defines the soil volume that is exploitable, and (ii) the vertical root distribution, i.e., the root length distribution which regulates the effective capacity of foraging the soil volume.

5.5.1 Bambara Groundnut Core Parental Lines as Models for Natural Variation of Root System Architecture

Core parental lines i.e., single genotypes derived from landraces of contrasting geographic origin have been established for bambara groundnut. Landraces from multiple ecosystems may be helpful for increasing the range of production zones because they have become adapted to their local environments as a result of continuous growth and selection in the same location (Mayes et al. 2012). Therefore, the availability of core parental lines (i.e., Gresik, LunT, IITA-686, DodR, S19-3, Tiga nicuru, Ankpa-4, DipC1) and their judicious use will be critical for breeding and selection programmes. Core parental lines are an ideal resource to identify new sources of variation. For example, substantial variation in photoperiodic effect has been reported in the core parental lines of bambara groundnut (Kendabie et al. 2020), whereas broad diversity in drought and heat tolerance (Dhanaraj 2018; Rahmah et al. 2020) and phenotypic variability have been found (Gao et al. 2020). Clearly, the success of bambara groundnut drought breeding programmes is dependent on the extent of phenotypic variation present in the germplasm base. The ability to classify plant roots in these core parental lines allows for more in-depth research for resilience to localised stresses.

Most bambara groundnut experiments have extensive aboveground trait phenotyping (Collinson et al. 1997, 1999; Jørgensen et al. 2010; Sesay et al. 2010; Vurayai et al. 2011; Mabhaudhi and Modi 2013; Chibarabada et al. 2015a, 2015b; Chai et al. 2016a; 2016b; Muhammad et al. 2016). Below ground biomass, on the other hand, is aggregated into a single, black-box group and key questions to do with adaptation to water deficit stress remain unanswered.

To observe bambara groundnut root development, plants were grown in low-cost vertically oriented light weight polyvinyl chloride (PVC) columns filled with soil. Certain aspects of the research methodology adapted

in the present study aided in the identification of genotypes based on their origin. For example, because of the sandy clay classification of the soil, it was possible to extract and clean the individual root depth segments with little alteration to their morphology: a gentle wash of the roots separated them from all soil particles clinging to them. Moreso, with this low-cost PVC column phenotyping system and image analysis set-up (Figure 3-4), identification of variation in root depth profile and vertical root distribution was made possible, indicating inherent natural phenotypic diversity and the potential of the core parental germplasm collection to reveal quantitative trait loci (QTLs) involved in root development.

5.5.2 Reaching the Soil at Depth by Tap Root Length

In conditions of periodic drought, bambara groundnut is planted in moist soil either after or intercropped with major cereal crops (Chibarabada et al. 2015b). Due to drainage, evaporation, and plant water intake, the soil gradually dries from the surface, resulting in substantially increased water availability in deeper soil layers and progressively harder top soils (Lynch 2013). This dry-down scenario delays flowering leading to a decrease in grain yield (Pang et al. 2017). Bambara groundnut has to quickly develop a deep tap root system in order to explore deeper soil depths before surface soil layers dry out (Wasaya et al. 2018). Failure to do so minimises the ability to forage for stored deep soil moisture reserves which accumulate at the beginning of the rainy season. Results from a previous analysis of tap root length at the pre-flowering (35 DAE) stage (Mateva et al. 2020; see *CHAPTER 4*), showed average values of nearly 92cm as early as 35 DAE in the tap root of hot dry-habitat S19-3 and DipC1 (both from southern Africa) as well as dry-habitat DodR (east Africa) and Ankpa-4 (west Africa), against an average of nearly 66cm in the other four genotypes mostly sourced from rainy habitats. Indeed, the present

study also showed differential TRL among genotypes at 55 DAE (i.e., at the end of a 30-d drought stress).

Drought stress generally decreased TRL among the studied genotypes but it did not decrease in the genotype DodR (from Tanzania: tropical dry climatic conditions). Previous research has shown that DS decreases root length (Avramova et al. 2016; Durand et al. 2016), root biomass (Price et al. 2002), and RLD (Fang et al. 2017) in a variety of plant species. In the present study, maintenance of TRL with an increase in the RLD in the deeper soil depth i.e., 60-90cm under DS treatment was observed in DodR and this enabled consistent water foraging under dry soil conditions. Not only did DodR record the highest value for TRL as early as 55 DAE, but also demonstrated an intrinsic ability for early flowering about 3 and 2 days earlier than mean flowering (36 and 50 days) time in both 2018 and 2019 study seasons, respectively. The genotype DodR from arid areas of Tanzania is well-suited to dry environments because of its capacity to penetrate and extract available water from deep within the soil profile. Similar results were observed in dry-habitat DipC1, with early flowering in the DS treatment in 2019 basically providing two critical advantages i.e., low level-stress facilitating an extended reproductive duration and a better soil water availability and foraging which supported rapid rate in partitioning to grains (Figure 5-14B) (Krishnamurthy et al. 2013; Purushothaman et al. 2014). Several studies confirm that drought can induce plants to develop a deeper TRL as an adaptive response (Gregory 2006; Rellán-Álvarez et al. 2015; Wasaya et al. 2018). In the present study, an increase in TRL allowed the DodR plants to compensate for the gradually declining soil water availability by quickly exploring a much greater volume of soil as demonstrated by continued reduction of lower soil volumetric water content values and maintenance of stomatal conductance (Figure 5-11F). Moreover, the RLD in deeper soil depths showed that this was indeed the case (Figure 5-9),

which gave DodR, DipC1 and S19-3 a marked adaptive advantage over other genotypes. Therefore, these genotypes responded partly through drought escape and through drought avoidance and remained stable across seasons. These adaptation mechanisms explain genotype-dependent adaptation to the different agroecologies they were sourced from.

5.5.3 Foraging the Soil Volume by Root Length Distribution

Root branching density (BD) and branching intensity (BI) traits have a strong impact on water uptake. In this study both traits were not measured, in fact although useful, these traits can quickly become difficult to quantify as the plant nears maturity. In such cases RLD is a useful trait that can be used as a proxy to estimate both root BD and BI (Mateva et al. 2020; see *CHAPTER 4*). For successful plant establishment not only is a quick TRL of major importance but also a high RLD in deeper soil depths is considered an adaptive root trait (Kashiwagi et al. 2005; Palta et al. 2011). The present study showed RLD was significantly reduced in some of the studied genotypes, although to a lesser extent than aboveground shoot biomass. This resulted in increased differences among genotypes for R:S ratio. RLD was less affected by DS in rainy-habitat Tiga nicuru and LunT as well as dry-habitat S19-3 than in the other five genotypes, with DodR and S19-3 maintaining the highest RLD and R:S ratio. RLD generally decreased down the length of the soil column. The rainy-habitat Gresik had the most RLD in the topsoil layer (0-30cm) and used more water during the DS period (55 DAE), making less soil water available to plants after the flowering stage, which inevitably affected grain yield (Figure 5-14). Meanwhile, the lack of roots in the subsoil layer and inability to fully explore soil water in the deep layer, may further explain why Gresik had the lowest grain yield. The development and maintenance of root tissue require a substantial expenditure of resources. (Nielsen et al. 2001). Early in plant

development, the expenditure of carbon and nutrient resources in tissue construction and maintenance restricts the capacity to grow additional roots in various soil domains as resource availability changes. If roots proliferate early in the growth season in moist topsoil, for example, this decreases the potential for root development in deeper soil, where resources are more likely to be found later in the season. Furthermore, early root proliferation in topsoil may not be useful later in the season in hard, dry surface soils. Passioura (1983) indicated that less RLD would be advantageous in the topsoil layer only if more water could be used in deep soil layers. On the other hand, it is worth noting that roots in topsoil often are involved in scavenging phosphorus (Lynch and Brown 2001), so adapted soil conditions may also be important. If the genotype Gresik were more adapted to low pH soils, then it may have been selected in P scavenging — not needing deep rooting. As a result, in low-input cropping systems, strategic recombination of P-efficient genotypes may increase crop productivity (Wafula et al. 2021). Also, while anecdotal, DodR was found to be performing well among several parental lines grown in waterlogged conditions during the rainy season in Indonesia, demonstrating the genotype's robustness (E. Redjeki, personal communication, 2017).

Compared to the topsoil layer (0-30cm) of the column, RLD in deeper soil depths (60-90cm) gave a substantial positive contribution to grain yield and this was highly consistent across the two seasons. Accordingly, RLD in the topsoil layer (0-30cm) had a significant negative correlation with grain yield. These results are in agreement with Fang et al. (2011), who reported that greater RLD increases root competition and delays the effectiveness of roots in capturing water under DS conditions. In addition, this also aggravates abscisic acid (ABA) accumulation and subsequent stomatal closure (Tombesi et al. 2015). Previous work on wheat demonstrated that higher RLDs are critical for increased early vigour and pre-flowering water use, which would improve

grain yield (Liao et al. 2004; Rebetzke and Richards 1999). In our findings, DodR and S19-3 had relatively lower RLD in the topsoil layer compared to Gresik, but higher RLD in the subsoil layers in both seasons and this was positively associated with yield. Selecting for higher RLD in the subsoil layers is considered as an option for the adaptation of wheat to water stress, increasing the water extraction capacity in the subsoil profile for grain filling and increased grain yield (Palta et al. 2011), especially under terminal drought stress (Passioura 1983; Gaur et al. 2008). Angus and van Herwaarden (2001) argued that, if subsoil water can be fully exploited between anthesis and grain filling, then grain yield will be increased significantly under drought stress. Looking at the deepest soil depth (90-110cm), it is worth mentioning that the specific rooting pattern found in most dry-habitat genotypes might be due to an innate biological characteristic, but it could also be due to a methodological artefact. The majority of genotypes (especially at 105 DAE) reached the deepest soil depth (90-110cm) and the physical constraint (i.e., detachable perforated plate) faced by the growing roots might have stimulated the development of new lateral roots. Inaddition, a large standard error was observed, indicating that the sample means for some studied genotypes (at final harvest) were widely dispersed around the population mean. More replicates might be introduced to a future experiment to minimize the distribution of treatment means. However, while this high RLD could be artefactual (Gregory 2006), it still reveals the differential deep rooting vigour in the studied genotypes.

5.5.4 Integrating Root System Architecture with Below and Aboveground Plant Traits

Given that the root system is a hidden and complicated organ, the idea of indirect selection by utilising aboveground plant components seems quite appealing, although considerable errors have been reported (Casper and Jackson 1997). In the present study, shoot height was positively correlated with

the RLD in the 60-90cm of the soil. A study by Mateva et al. (2020); see *CHAPTER 4*) also found that shoot height was closely and positively correlated with lateral branching in the deep 60-90cm of the soil, and this was largely amongst genotypes originating from drier versus wetter agroecological environments. Furthermore, changes in root length has been observed in rice under water stress, and has been linked to increased shoot biomass and yield. (Niones et al. 2013). The trend agrees with intensive studies on chickpea (Chen et al. 2017), and wheat (Tolley and Mohammadi 2020), which suggest that there is a persistent tendency of a positive correlation between roots and shoots. Since a plant is a biological entity, the root system absorbs water and nutrients for the stem and leaves, which then provide food for the root system's maintenance. Therefore, shoot height is a good trait that can be used as a proxy to make estimations of difficult-to-access vertical root distribution especially when screening large populations. Also, when mapped, traits with higher genotype-to-genotype correlations (such as RLD at 60-90cm) are more likely to produce consistent QTLs.

5.6 Conclusion

Differences in root system and shoot responses to a drought treatment were observed among the eight bambara groundnut genotypes due to their different genetic background and buffering capacity (i.e., plasticity related to G×W). These responses encompassed morphological root and shoot traits, such as tap root length (TRL), root length density (RLD), shoot height (SH) and number of leaves (NoL) — in response to the DS treatment. These inherent characteristics govern root architecture and foraging dynamics, and hence have a direct impact on root system functionality. The present study found that on the basis of the closeness of their association with grain yield, drought resistance can be estimated through RLD in the 60-90cm in dry-habitat

genotypes such as DodR. SH is a good trait that could be used as a proxy to make estimations of RLD in bambara groundnut and these could be prioritized for screening large populations for dry-habitats. DodR and S19-3 can be used to map the genes and alleles responsible for root trait regulation and potential root system plasticity, shedding light on their evolution and ecological significance.

In the next experimental chapter, root traits such as tap root length and root length density in the 60-90cm soil layer were shown to be beneficial in screening and selecting superior lines from a bambara groundnut population.

CHAPTER 6 : Screening Promising Drought Resistant Early-Generation Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) Lines Based on Shoot and Root System Traits Under Drought Stress

6.1 Summary

Analyses of grain yield and other associated traits in breeding lines under different environments allow for a better understanding of their genetic yield potential and stress-resistance capability. The present study was carried out to evaluate performance of bi-parental segregating lines of bambara groundnut (*Vigna subterranea* (L.) Verdc.) under different water management (environments), determine the repeatability of the examined shoot and root system traits, and identify superior lines for their potential use in breeding for drought resistance. A total of 22 segregating lines and two parental single genotypes S19-3 (maternal) × DodR (paternal) were evaluated under well-watered and drought stress environments in a rainout shelter of the Crops For the Future-Field Research Center (CFF-FRC) at Semenyih, Malaysia (2019/2020). The preceding chapter findings revealed that the two single genotypes are fairly similar, with the primary difference being that DodR has more plasticity than S19-3. The experiment was laid out in a factorial treatment combination. The trait with the lowest repeatability i.e., broad-sense heritability was 100-seed weight (0.62), while high broad-sense heritability values were observed for root length density in the 60-90cm soil depth (0.99) which is significantly associated ($P < 0.05$ to $P < 0.001$) to shoot traits i.e., number of leaves (0.87), shoot dry weight (0.87) and shoot height (0.80). Grain yield showed a moderate level of heritability (0.64) across the two environments. A regression analysis revealed that 42%; $P < 0.001$ of the variation in grain yield is associated with root length density in the 60-90cm soil depth. The principal component analysis revealed that grain yield is positively associated with root length density in the 60-90cm soil depth and tap root length in the drought stress environment. Based on biplot analysis, 'Line12', 'Line35' and 'Line41' proved to be the best top three bambara groundnut lines in terms of yield under drought stress environments. These lines could be advanced as part of bambara groundnut breeding programme and potentially selected and registered as improved varieties for cultivation in drought prone areas.

Keywords: bambara groundnut (*Vigna subterranea* (L.) Verdc.), drought stress, genetic variability, heritability (H^2), principal component analysis (PCA), root length density, root system traits, selection.

6.2 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.), is a neglected African grain legume that flourishes under Southeast Asian environments with very high annual rainfall to very dry, Southern Africa environments relative to other grain legumes. Originating in West Africa, its production ranges across climatic gradients mostly covering rain-fed areas of the arid and semi-arid tropics, where unpredictable and insufficient rainfall is common. In southern Africa, owing to limited household and hired labour (Andlib et al. 2021), bambara groundnut is largely sown later than normal in order to make way for major crops such as sorghum and maize (Graham and Vance 2003) as the next crop. Consequently, the crop survives on residual soil moisture, exposing it to periodic drought stress.

The performance of bambara groundnut under drought stress has been a common starting point for the identification of traits related to drought tolerance and selection of genotypes for dry environments (Mabhaudhi and Modi, 2013; Chai et al. 2016a). Yield performance has been considered a reliable technique for evaluating large numbers of genotypes to identify those most suitable for cultivation under drought stressed conditions (Tuberosa 2012). Selection in segregating populations is one of the main tasks of plant breeders for exploiting genetic variations to improve stress resistance (Bhargava and Srivastava 2019). There has been appreciable evaluation of various above ground (shoot) physiological and morphological traits and their association with drought tolerance in bambara groundnut (Mwale et al. 2007; Al Shareef et al. 2014; Chibarabada et al. 2015b; Nautiyal et al. 2017; Mabhaudhi et al. 2018). These studies have proved fruitful, revealing the potential in selecting individual lines with greater drought resistance. However, below ground (root) adaptation mechanisms remain undocumented, particularly with respect to the functional significance of deep root length distribution which has been

reported as important in a number of cereal and grain legume crops, including bambara groundnut (Mateva et al. 2022; see *CHAPTER 5*).

Studies by Mateva et al. (2020); see *CHAPTER 4* and Mateva et al. (2022); see *CHAPTER 5*, reported that bambara groundnut is well adapted to growing on residual soil moisture due to its intrinsic deep rooting characteristics. The authors postulate that further drought improvement is possible considering the number of natural genotypic differences in genotypes such as DodR and S19-3, that have shown superior shoot (Gao et al. 2020) and root system architecture (RSA) traits (Mateva et al. 2020; see *CHAPTER 4*). Moreover, the advantage of S19-3 and DipC1 (sourced from Namibia and Botswana, respectively) is not so much its deep-rooting capability, but instead an ability for greater root length density in deeper (60-90cm) in the soil profile, which is positively correlated to an increase in grain yield during drought stress — features directly related to the genotypes source/country of origin (Mateva et al. 2022; see *CHAPTER 5*). Association studies identify traits that have a positive significant correlation with yield in bambara groundnut, which is useful information in a breeding programme (Khan et al. 2021b). Therefore, including, evaluating and estimating the extent of variation for important root traits such as root length density (RLD) under drought stress conditions in segregating bambara groundnut genotypes should be one of the prerequisite for future breeding programmes.

The present study was undertaken to: (1) evaluate performance of bi-parental segregating lines of bambara groundnut under different water management i.e., environments and determine the heritability of the examined shoot and root system traits and (2) identify superior lines better adapted to drought stress environments for their potential use in breeding for drought resistance.

6.3 Materials and Methods

6.3.1 Plant Materials

This study was conducted under fixed rainout shelter during the 2019/2020 cropping season at Crops for the Future-Field Research Center (CFF-FRC) in Malaysia. Based on monthly climatic data, CFF-FRC rainout shelter had mean relative humidity (RH; %), vapor pressure deficit (kPa), maximum and minimum temperature (^{o}C) of 71.7%, 1.8kPa, 33.1^{o}C and 27.0^{o}C, respectively. Weather data for specific months is presented in Table 6-1.

Table 6-1 Average monthly relative humidity (%), vapor pressure deficit (kPa) and temperature (°C) of the study conducted at the Crops For the Future-Field Research Center (CFF-FRC), Semenyih, Malaysia.

Month	Relative humidity (%)	Vapor pressure deficit (kPa)[1]	Temperature (^{o}C)	
			Maximum	Minimum
December 2019	73.3	1.6	32.2	24.2
January 2020	71.9	1.7	32.8	25.6
February 2020	68.7	1.8	33.7	26.9
March 2020	71.2	1.9	33.5	29.1
April 2020	73.1	1.9	33.3	29.2

This study used an F_4 segregating population (a selection of 22 lines) drawn from a much larger population (114 lines). The selection was based on lines with high and low values for stomatal conductance (g_s) a trait previously found to be useful and related to root length density in the 60-90cm soil depth (Mateva et al. 2022; see *CHAPTER 5*). The population created at the University of Nottingham, arose from a controlled cross between two distinct parental genotypes — contrasting for drought stress plasticity, to produce population SD: S19-3 (maternal) × DodR (paternal). The 22 lines and two

parental genotypes used are listed and shown in (Table 6-2; *Appendix 1*, respectively).

Table 6-2 List of lines (22) and parental genotypes (two), used for the soil-filled PVC column experiment to screen promising bambara groundnut breeding lines for drought resistance at the Crops For the Future-Field Research Center (CFF-FRC), Semenyih, Malaysia.

Designation[1]	Group	Source country[2]	Genotype/Line
SD11	Line	UNM-Malaysia	Line11
SD12	Line	UNM-Malaysia	Line12
SD35	Line	UNM-Malaysia	Line35
SD39	Line	UNM-Malaysia	Line39
SD41	Line	UNM-Malaysia	Line41
SD45	Line	UNM-Malaysia	Line45
SD46	Line	UNM-Malaysia	Line46
SD49	Line	UNM-Malaysia	Line49
SD50	Line	UNM-Malaysia	Line50
SD64	Line	UNM-Malaysia	Line64
SD65	Line	UNM-Malaysia	Line65
SD69	Line	UNM-Malaysia	Line69
SD70	Line	UNM-Malaysia	Line70
SD82	Line	UNM-Malaysia	Line82
SD86	Line	UNM-Malaysia	Line86
SD90	Line	UNM-Malaysia	Line90
SD93	Line	UNM-Malaysia	Line93
SD94	Line	UNM-Malaysia	Line94
SD95	Line	UNM-Malaysia	Line95
SD96	Line	UNM-Malaysia	Line96
SD101	Line	UNM-Malaysia	Line101
SD107	Line	UNM-Malaysia	Line107
6	Parental genotype	Tanzania	DodR[3]
7	Parental genotype	Namibia	S19-3

[1] SD - S19-3 (maternal) × DodR (paternal)

[2] UNM-Malaysia – University of Nottingham Malaysia

[3] Dodoma Red (DodR), the national capital of Tanzania.

S19-3 is a dark coloured (*Appendix 1*) early flowering, semi-bunched type bambara groundnut with quantitative long day (more pods under 16 hrs than 12 hrs) photoperiod (Kendabie et al. 2020). It is sourced from a dry habitat and as such possesses a deep tap root system with high root length distribution for efficient water foraging in deeper soil depths (Mateva et al. 2020; see *CHAPTER 4*). In addition, the genotype is characterised by fewer number of leaves per plant, high harvest index and shelling percentage (Gao et al. 2020). On the other hand, the genotype DodR is a high yielding, red coloured (*Appendix 1*), spreading type bambara groundnut with high numbers of leaves and classified as quantitative short day — showing a decline in pod and seed yield with increasing photoperiod; (Kendabie et al. 2020). Sourced from a dry-habitat, DodR flowers early and has a quick and deep tap root system with high lateral root distribution in deeper soil depths (Mateva et al. 2020; see *CHAPTER 4*).

6.3.2 Experimental Design and Trial Management

The PVC column, soil substrate, basal fertilizer, weed, pest control and experimental design were carried out as described in Chapter 3; *3.2.3 Experimental Design and Layout*. For a detailed description of the two water managements i.e., well-watered (WW) and drought stress (DS), refer to Chapter 3; *3.2.4 Water Treatments*. For the purposes of this chapter WW and DS from here on are referred to as environments instead of treatments as defined in the previous Chapter 5. Plant samples were harvested at 25 DAE (first destructive sample point). Remaining plants were subjected to drought stress for a period of 30-days (Figure 6-1). The DS environment was terminated at 55 DAE (second destructive sample point). Irrigation was resumed bringing the soil moisture in the columns back to field capacity. All the plants i.e., WW and DS (recovery) were then slowly irrigated once on alternate days until final harvest (105 DAE; third destructive sample point).

Figure 6-1 Trial set-up at the Crops For the Future-Field Research Center (CFF-FRC), Semenyih, Malaysia: the drought stress (DS) environment was imposed during the flowering stage by withholding irrigation. The DS environment was maintained for 30-d followed by re-watering. Well-watered (control) was designated as WW environment and received irrigation throughout the growth period.

6.3.3 Plant Measurements

6.3.3.1 Shoot Traits

In a time-course experiment at 25 DAE, 55 DAE (at the end of the 30-d DS) and 105 DAE (50-d DS recovery), measurements of shoot height (SH), number of leaves (NoL), shoot fresh weight (SFW), and shoot dry weight (SDW) were taken. Three biological replicates were used per treatment for shoot trait measurements per bambara groundnut lines and genotypes. Detailed description on data collection is provided in Chapter 3; *3.2.5 Plant Sampling and Common Measurements*. Pods were dried and shelled and the seeds weighed to determine grain yield column^{-1}. The weight of 100 seeds (100-SW), was determined using the following formula:

$$\text{100-SW} = \text{grain yield column}^{-1} / \text{total number of seeds plant}^{-1} \quad (1)$$

6.3.3.2 Gas Exchange Measurements

Gas exchange measurements including net photosynthetic rate (P_n), rate of transpiration (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were taken at CO_2 concentration of 400µmol mol^{-1} using a LI-6400 portable photosynthesis system infrared gas analyser (LiCor, Lincoln, NE, USA) with an automatic cuvette of up to 6cm^2 leaf area. Measurements were taken at 25, 35, 55 (at the end of the 30-d DS) and 105 DAE (50-d DS recovery). Three biological replicates were used per treatment per bambara groundnut lines and genotypes. The gas exchange measurements were taken on the well-watered and drought stressed plants. Measurements were taken between 11:00h and 12 noon and this was always on a sunny day in Semenyih, Malaysia (average sunrise, sunset and day length: 07.02h, 19.15h and 12.11h, respectively). Readings were taken from the youngest, fully expanded trifoliate

leaf at photosynthetic photon flux densities (PPFD) of ca 1000μmol m^{-2} s^{-1} for three minutes.

6.3.3.3 Reproductive Development

The description of reproductive development particularly: days to 50% flowering is presented in Chapter 3; *3.2.6 Developmental Traits*.

6.3.3.4 Root Traits

Root harvesting was conducted three times i.e., 25 DAE, 55 DAE (at the end of the 30-d DS) and 105 DAE (50-d DS recovery). Detailed description on data collection for total tap root length (TRL cm $plant^{-1}$), root fresh weight (RFW) g $plant^{-1}$ and root length density (RLD) cm cm^{-3} is provided in Chapter 3; *3.2.5 Plant Sampling and Common Measurements*. Three biological replicates were used per treatment for root trait measurements per bambara groundnut lines and genotypes.

6.3.3.5 Statistical Analysis

Data on each measurable trait was subjected to two-way analysis of variance (ANOVA) to test the effects of the lines and parental genotypes from here collectively referred to as lines (L) and Environments (E) and their interaction (L × E) using Statistica Version 13.3 (TIBCO Software Inc, USA). Means were separated using Tukey's Honestly Significant Difference (HSD) at the 5%. Linear mixed models (Residual Maximum Likelihood, REML) were used to estimate variance components and their interactions. Lines and environments were considered fixed effects and replicates within environments as random effects in the model.

Estimation of heritability values in the in broad sense (H^2) were calculated as defined by (Falconer 1960) using the equation:

$$H^2 = \sigma^2_g / [\sigma^2_g + (\sigma^2_{ge} / m) + ((\sigma^2_e / (r)(e))] \quad (2)$$

where H^2 indicates the broad-sense heritability, σ^2_g is the genotypic i.e., Lines variance, σ^2_{ge} is the lines × environment interaction variance, σ^2_e is the residual error variance, r is the number of replicates and e is the number of environments. Mean squares (MS) from the analysis of variance were used to compute the variances in the heritability formula. These are given below:

$$\sigma^2_e = MS_e \quad (3)$$

$$\sigma^2_{ge} = (MS_{ge} - MS_e) / r \quad (4)$$

$$\sigma^2_g = (MS_g - MSg_e) / (r)(e) \quad (5)$$

where MS_e is the error mean square, MS_{ge} the mean square for genotype-by-environment interaction, e the number of environments, and MS_g the genotypic mean square.

Pearson's correlation coefficients (r) were calculated per environment using Statistica Version 13.3 (TIBCO Software Inc, USA). The principal component analysis (PCA) and PCA biplots were used to determine the relationship between multiple traits.

6.4 Results

6.4.1 Physiological responses to drought stress

Significant decline of stomatal conductance (g_s), transpiration (E), photosynthesis (P_n) and intercellular carbon (C_i) were observed in all bambara groundnut lines from 25 DAE (start of DS) right up to 55 DAE (30-d of DS) (Figure 6-2A-D). Line50, Line45, DodR and Line107 exhibited small differences between drought and recovery values after re-watering for P_n, g_s, C_i and E

control, respectively. C_i remained unchanged, dipping slightly at 50-d of DS recovery (Figure 6-2D).

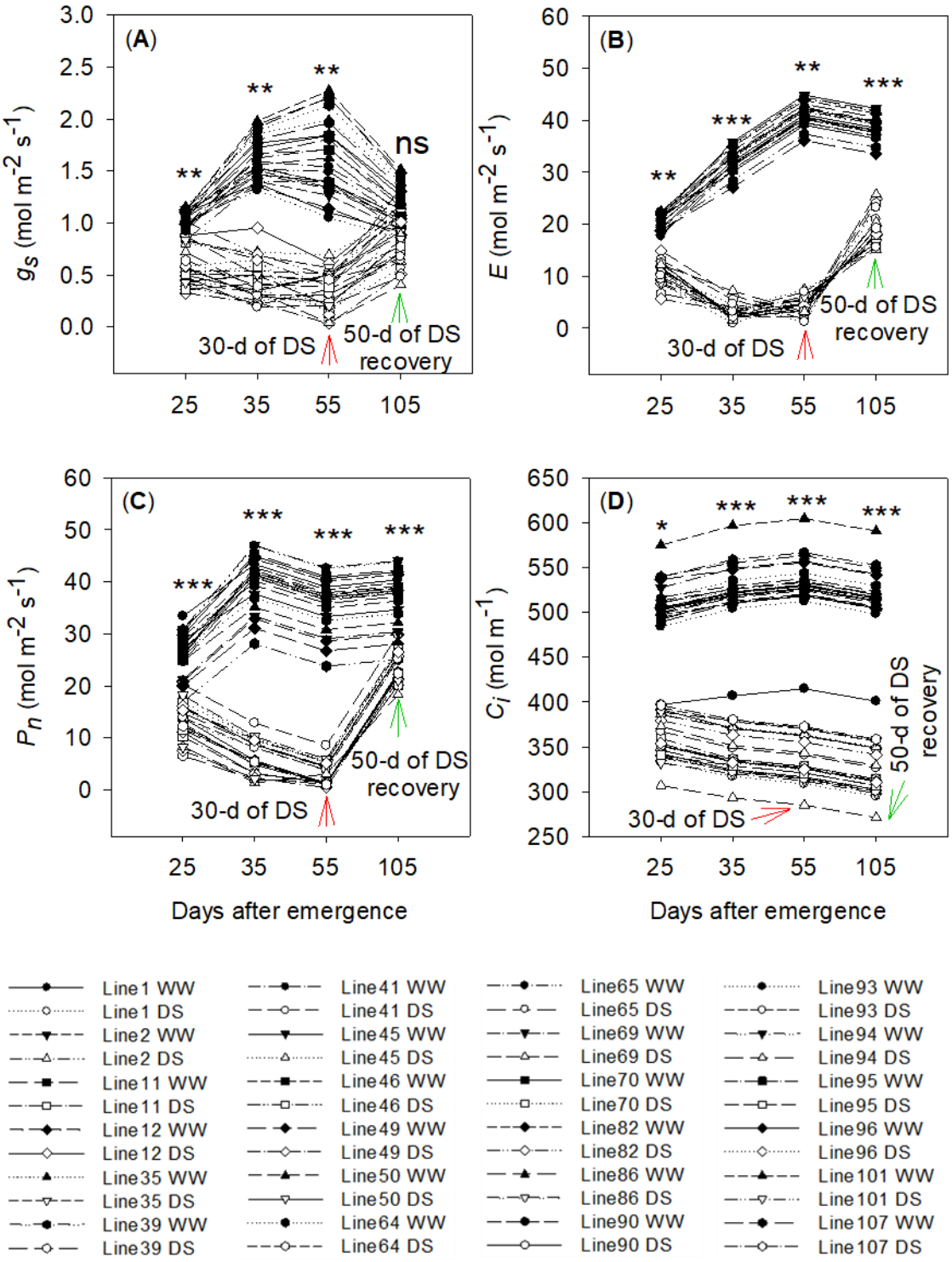

Figure 6-2 Effects of drought stress on physiological parameters of bambara groundnut. (**A**) the stomatal conductance, (**B**) leaf transpiration, (**C**) photosynthesis and (**D**) intercellular carbon were measured from day 25–55 of drought stress and once at 50-d of drought stress recovery. Red (↗) and Green (↗) arrows represent (30-d of drought stress and 50-d of drought stress recovery, respectively). The data is mean values (n = 3), with significant

interaction difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$; ns - non-significant difference.

6.4.2 Effect of Lines and Environment on Shoot, Root and Grain Yield Traits at 50-d of Drought Stress Recovery

ANOVA analysis revealed highly significant main effects of lines, environment and their interactions among the lines for 11 traits [SH, NoL, SDW, P_n, g_s, C_i, E, TRL, RLD (60-90cm), GY and 100-SW] especially at 50-d of drought stress recovery (Table 6-3). The frequency distribution of the 11 traits displayed an approximately normal distribution (*Appendix 2* to *Appendix 12*). The highly significant interaction indicates that there was a differential response between lines and environment. However, stomatal conductance (g_s), was non-significantly affected by the interaction of the line × environment, with significant differences only observed for main effects of lines (Figure 6-3A) and environment with DS decreasing g_s by 71% (Figure 6-3B). Line12 recorded the highest g_s of 1.4mol m^{-2} s^{-1} and this was statistically different from Line49, Line93, S19-3 and Line65 (0.79, 0.81, 0.83 and 0.85mol m^{-2} s^{-1}, respectively) which recorded the lowest values. Across the two environments, the heritability estimates ranged from 0.99 for RLD (60-90cm) and 0.62 for 100-SW (Table 6-3). NoL and SDW had the same heritability value of 0.87.

Table 6-3 Mean squares, significant tests and broad sense heritability after analysis of variance for shoot, root and yield traits of 22 bambara groundnut lines and two parental genotypes evaluated in two environments.

Traits	Sources of variation				CV	H^2
	L	E	L × E	Residual Error		
SH	70.5***	4298.4***	33.2***	4.1	23.1	0.80
NoL	4274.1***	17117.4***	1205.8***	288.7	28.1	0.87
SDW	209.5***	13803.2***	58.2***	20.5	53.5	0.87
P_n	60.1***	6444.4***	39.0***	8.2	26.9	0.74
g_s	0.2**	47.9***	0.1^{ns}	0.1	62.3	0.71
C_i	3254***	1408263***	1514***	23	24.6	0.81
E	26.8***	13373.7***	22.2***	8.1	35.7	0.68
TRL	879.7***	61525.5***	771.8***	296.2	31.3	0.67
RLD (60-90cm)	0.1***	3.8***	0***	0	87.5	0.99
GY	62.3***	11866.7***	64.2***	18.1	116.5	0.64
100-SW	133.7*	66066.6***	136.6*	74	100.3	0.62

L – line; E – environment; CV – coefficient of variation; H^2 – broad sense heritability.

The data is mean ± se values (n = 3), with significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$; ns - non-significant difference. SH – shoot height; NoL – number of leaves; SDW – shoot dry weight; P_n – photosynthesis; g_s – stomatal conductance; C_i – intercellular carbon; E – transpiration; TRL – tap root length; RLD (60-90cm) – root length density in the 60-90cm soil depth; GY - grain yield column^{-1}; 100-SW – weight of 100 seeds.

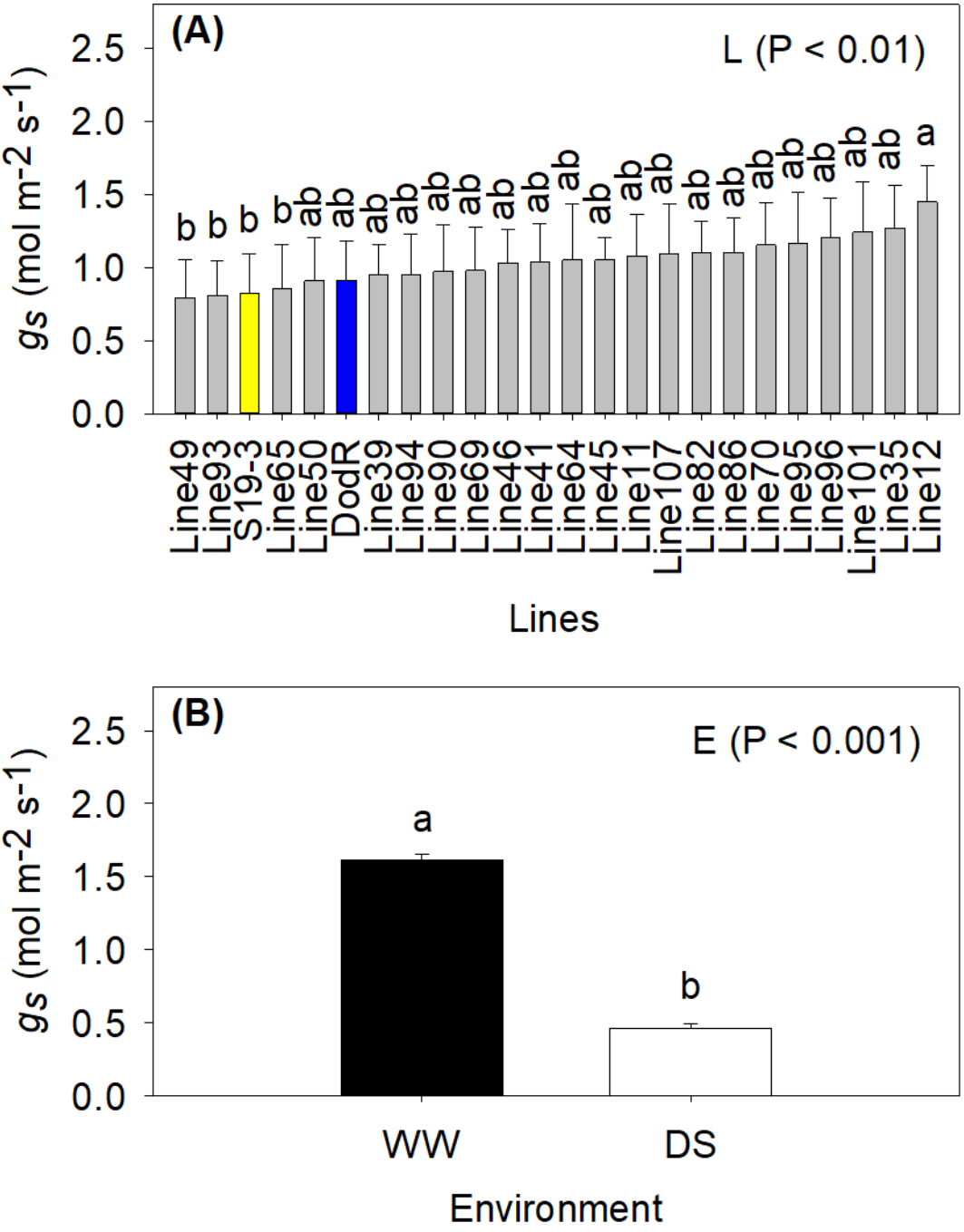

Figure 6-3 Effect of (**A**) lines (L), the data is mean ± se values (n = 6), ordered from smallest to largest value. (**B**) well-watered (WW) and drought stress (DS) constitute environments (E), the data is mean ± se values (n = 72). Different letters showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Table **6-4** summarizes the mean values; standard error (Std. Err), and coefficients of variation (CVs) obtained for all traits recorded in the two environments. The table shows the best five and bottom five lines in terms of grain yield (GY) under drought stress (50-d of DS recovery).

The mean shoot heights (SH) under DS (50-d of DS recovery) and WW environment were 25.0 and 35.9cm, respectively. Under the DS environment (50-d of DS recovery), the shortest line was Line96 (17.3cm), while the tallest was Line11 (34.3cm). Line94, and Line69 were the tallest under WW environment with average shoot heights of 39.5cm, while Line86 was the shortest (29.8cm).

A 16% reduction in the number of leaves (NoL) was observed on average between WW (134) and DS (112) environments due to DS. Line35 and DodR developed the heaviest shoot dry weight (SDW) i.e., 27.9 and 44.1g, under DS (50-d of DS recovery) and WW environment, respectively; while Line70 and Line86 had the least SDW i.e., 4.6 and 22.1g, under DS (50-d of DS recovery) and WW environment, respectively (Table 6-4; Figure 6-4).

Means of photosynthesis (P_n), stomatal conductance (g_s), intercellular carbon (C_i) and transpiration (E) under DS environment were 36%, 71%, 38% and 50% lower than the values recorded under WW environment, respectively.

Under DS (50-d of DS recovery), Line35 managed to penetrate the soil the deepest (118cm), while Line11 was the deepest (134.3cm) under WW environment. Although a 75% reduction in root length density in the 60-90cm (RLD 60-90cm) was observed at DS (50-d of DS recovery), Line35 and Line11 developed and had the most (both 0.5cm cm^{-3}) RLD (60-90cm) in the DS environment.

The average grain yield column^{-1} and seed weight based on 100 seeds (100-SW) were reduced by more than 90% under the DS (50-d of DS recovery) environment as compared to the WW environment.

Table 6-4 Means for 11 traits of 22 bambara groundnut lines and two parental genotypes top five best and five bottom performing lines when evaluated under drought stressed (50-d of DS recovery) and well-watered environments. Ranks are according to grain yield under drought stressed environment.

	SH (cm plant $^{-1}$)		NoL[1] (number plant $^{-1}$)		SDW (g plant^{-1})		*Pn* (*µmol m^{-2} s^{-1}*)		*gs* (*mol m^{-2} s^{-1}*)		*Ci* (*µmol m^{-1}*)		*E* (*mol m^{-2} s^{-1}*)		TRL (cm plant^{-1})		RLD (60-90cm) (cm cm^{-3})		GY (g column^{-1})		100-SW (number plant^{-1})	
	WW	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**	**WW**	**DS**
Top 5 lines																						
Line12	38.0	33.7	158	174	37.5	25.6	29.9	20.1	1946.4	951.8	549.4	348.7	39.9	22.0	116.7	96.0	0.5	0.4	15.6	0.9	49.2	9.5
Line35	37.0	29.8	138	151	41.0	27.9	38.2	21.7	1853.9	685.7	517.0	356.1	43.2	19.9	120.3	118.0	0.6	0.5	15.1	0.8	28.8	4.9
Line41	37.8	30.8	144	146	38.4	20.6	38.3	29.8	1588.9	493.9	515.3	326.7	37.6	24.8	126.0	103.0	0.5	0.3	16.2	0.5	40.9	5.5
Line107	34.3	23.7	118	129	30.2	12.2	37.2	22.4	1797.5	391.6	520.6	358.5	37.6	25.7	119.7	65.0	0.6	0.0	15.2	0.5	50.0	3.7
Line45	36.5	26.8	114	96	29.1	13.7	34.7	26.6	1382.1	721.1	513.2	349.8	39.6	21.6	104.3	82.7	0.2	0.1	14.5	0.3	47.0	3.9
Bottom 5 lines																						
Line90	32.0	19.7	110	108	24.4	8.1	36.3	22.6	1651.9	294.7	510.6	311.6	38.1	20.4	131.7	63.3	0.4	0.0	23.5	0.0	41.6	0.0
Line93	36.8	20.0	145	61	32.8	4.8	43.9	25.1	1316.4	303.4	498.4	300.2	38.5	17.1	129.0	53.0	0.6	0.0	14.1	0.0	29.0	0.0
Line95	37.3	20.7	126	100	30.0	10.8	38.9	22.4	1917.2	418.5	519.6	306.9	40.5	16.4	105.0	61.3	0.3	0.0	8.5	0.0	26.2	0.0
Line96	30.5	17.3	90	82	25.3	5.3	41.9	26.5	1769.6	641.6	516.7	310.5	41.9	19.3	95.3	56.3	0.2	0.0	19.8	0.0	43.5	0.0
Line101	36.0	19.7	96	84	29.3	7.9	39.8	21.1	1979.6	501.6	590.7	357.1	39.1	15.6	109.7	60.3	0.4	0.0	21.3	0.0	40.8	0.0
Mean [a]	35.9	25.0	134.1	112.3	32.9	13.4	37.0	23.7	1618.1	464.5	517.5	319.8	38.6	19.3	116.4	75.0	0.4	0.1	18.3	0.1	44.9	2.1
Min [a]	29.8	17.3	89.7	59.7	22.1	4.6	25.1	18.3	1316.4	188.9	401.0	271.0	31.9	15.1	76.3	53.0	0.2	0.0	5.8	0.0	26.2	0.0
Max [a]	39.5	34.3	200.7	175.3	44.1	27.9	44.0	29.8	1979.6	951.8	590.7	358.5	43.2	25.7	134.3	118.0	0.6	0.5	37.5	0.9	60.7	10.8
Std.Err [a]	0.4	0.6	3.7	4.1	0.9	0.9	0.7	0.4	0.04	0.03	3.8	2.8	0.4	0.4	2.5	2.6	0.0	0.0	1.0	0.0	1.5	0.6
CV [a]	8.6	21.8	23.2	30.8	23.6	55.2	15.7	13.0	40.6	44.6	6.2	7.4	9.4	19.0	18.5	29.0	42.5	147.8	44.2	236.9	28.6	229.9

[1] NoL values rounded to the nearest integer because NoL represents discrete data; [a] – Values calculated from all the lines and parental lines. The data is mean values (n = 3); SH – shoot height; NoL – number of leaves; SDW – shoot dry weight; P_n – photosynthesis; g_s – stomatal conductance; C_i – intercellular carbon; E – transpiration; TRL – tap root length; RLD (60-90cm) – root length density in the 60-90cm soil depth; GY - grain yield column^{-1}; 100-SW – weight of 100 seeds; WW – well-watered and DS – drought stress.

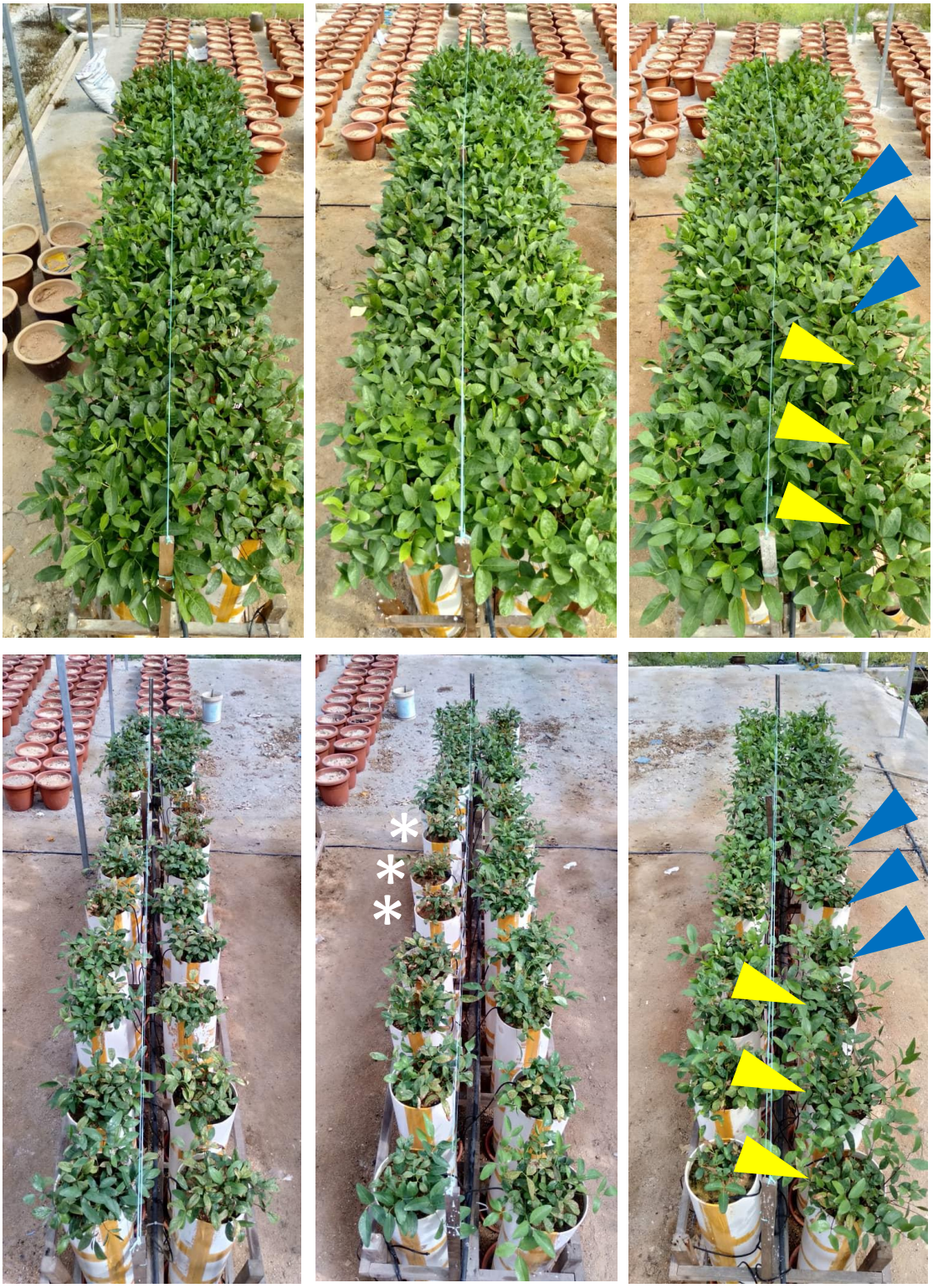

Figure 6-4 Bambara groundnut under well-watered environment (top) and drought stress (50-d of DS recovery; bottom) with parental genotypes DodR (yellow arrows) and S19-3 (blue arrows) marked. Under drought stress (50-d of DS recovery), the Line70 with the least shoot dry weight (SDW) is marked with (asterisk).

6.4.3 Changes in Tap Root Length (TRL)

Tap root length (TRL) was significantly affected by line effect only at 25 days after emergence (DAE) ($P < 0.01$; Figure 6-5A). TRL ranged from 31.6cm (Line95) to 54.3cm (Line107) with average TRL of 43.8cm (Figure 6-5A). Compared to Line107, all the other lines showed slow TRL growth —with TRL exclusively limited to the 30-60cm layer. As expected, it appeared that TRL reflected changes in RLD in the 60-90cm soil depths, with a much stronger relationship under DS compared to WW environment (r = 0.95, $P < 0.05$ and r = 0.80, $P < 0.05$, respectively; Table 6-5).

Figure 6-5B, C shows that DS induced a decline in tap root length (TRL) as compared to WW environment. At 55 DAE, TRL was significantly affected by the interaction effect of the line × environment ($P < 0.001$). DS generally reduced TRL by 38%. Line94 recorded the highest TRL reduction (66%) with the least reduction observed in Lines12 and Line95 (both 19%). Interestingly, Line101 recorded an 18% increase in TRL under DS.

After re-watering, the plants showed only partial recovery of TRL, with Line49 showing full recovery with 3% greater TRL in the DS (50-d of DS recovery) environment (Figure 6-5D, E). Line93 still showed a significant decrease (59%) under the DS (50-d of DS recovery) (Figure 6-5E). Line35 almost recovered fully with the least decrease (2%) in TRL under DS (50-d of DS recovery) compared to the WW environment.

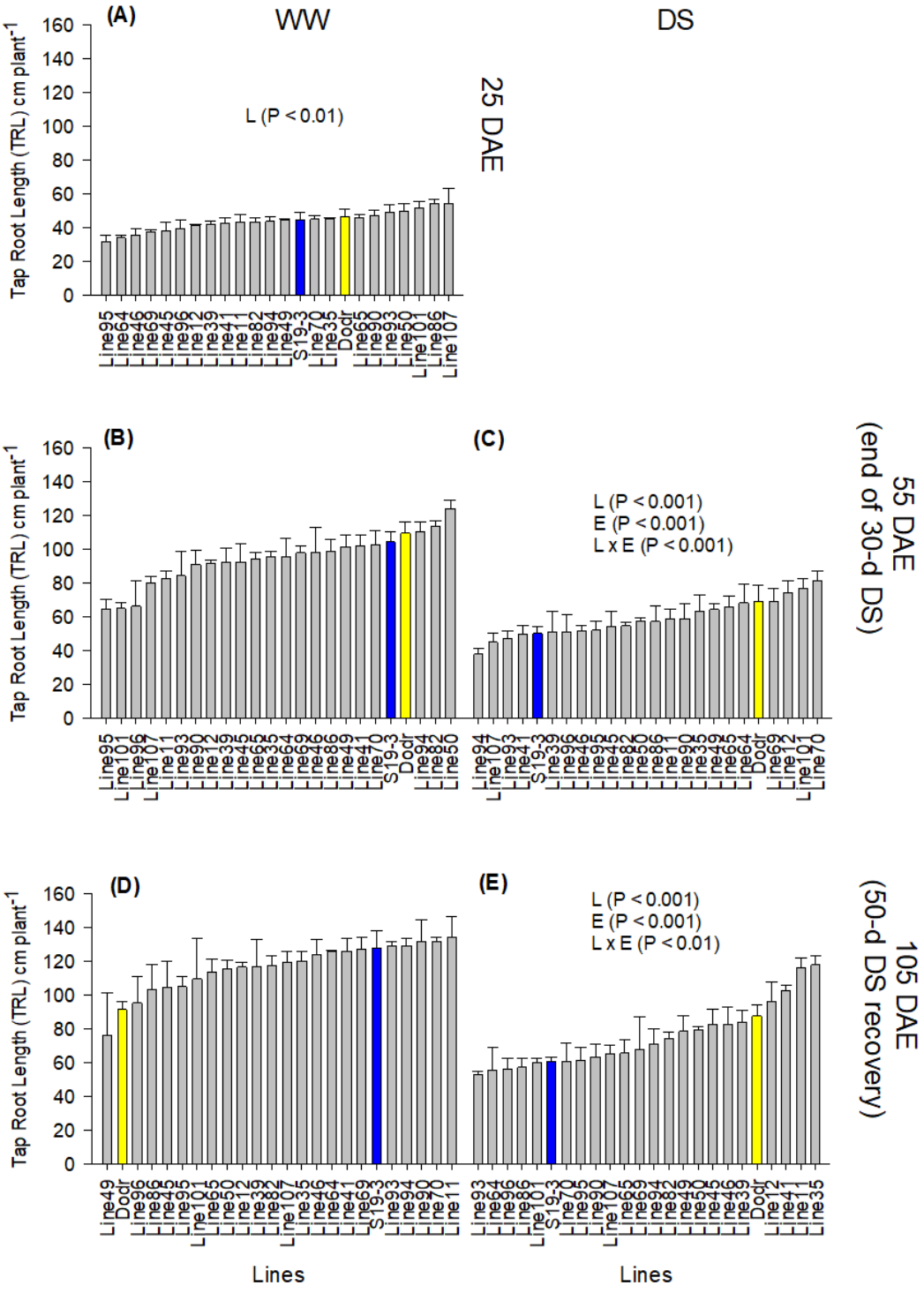

Figure 6-5 Tap root length (TRL) of 22 bambara groundnut lines and two parental genotypes ordered from smallest to largest value at (**A**) 25 DAE, (**B**) 55 DAE for well-watered, (**C**) 55 DAE for drought stress (**D**) 105 DAE for well-watered, (**E**) 105 DAE (50-d DS recovery). The DS treatment was intentionally left blank at 25 DAE. The two parental genotypes DodR and S19-3 are represented by yellow and blue coloured bars. The data is mean ± se values (n = 3), with errors bars showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

6.4.4 Changes in Root Length Density (RLD) in the 60-90cm Soil Depth

Root length density in the 60-90cm soil depth (RLD 60-90cm) was not significantly affected by line effect at 25 days after emergence (DAE) (*P = 0.43*; Figure 6-6A). Similar to the data on TRL, compared to Line107 (0.002cm cm^{-3}), all the other lines showed somewhat slow root distribution i.e., RLD (60-90cm) — exclusively limited to the 30-60cm layer after 25 days of growth.

At 55 DAE, RLD (60-90cm) was significantly affected by the interaction effect of the line × environment (*P < 0.001*). Figure 6-6B, C shows that DS induced a decline in RLD (60-90cm) as compared to WW environment. DS generally reduced RLD (60-90cm) by 95%. S19-3, Line41, Line46, Line50, Line82, Line93, Line94, Line95 and Line107 seem to have suffered the most in the DS environment, all recording no RLD (60-90cm), whilst the least reduction was observed in Lines70 (80%).

After re-watering, some plants showed only partial recovery of RLD (60-90cm), at 105 DAE (50-d of DS recovery; Figure 6-6D, E). Line101, Line86, Line93, and S19-3 showed the least recovery, with plants still failing to establish roots and colonise a greater soil volume in the 60-90cm soil depth (Figure 6-6E). However, DodR, Line35, Line11, Line12, Line45 and Line41 showed partial recovery with only 10%, 14%, 15%, 30%, 39% and 43% decrease in RLD (60-90cm) under DS (50-d of DS recovery) environment compared to the WW environment.

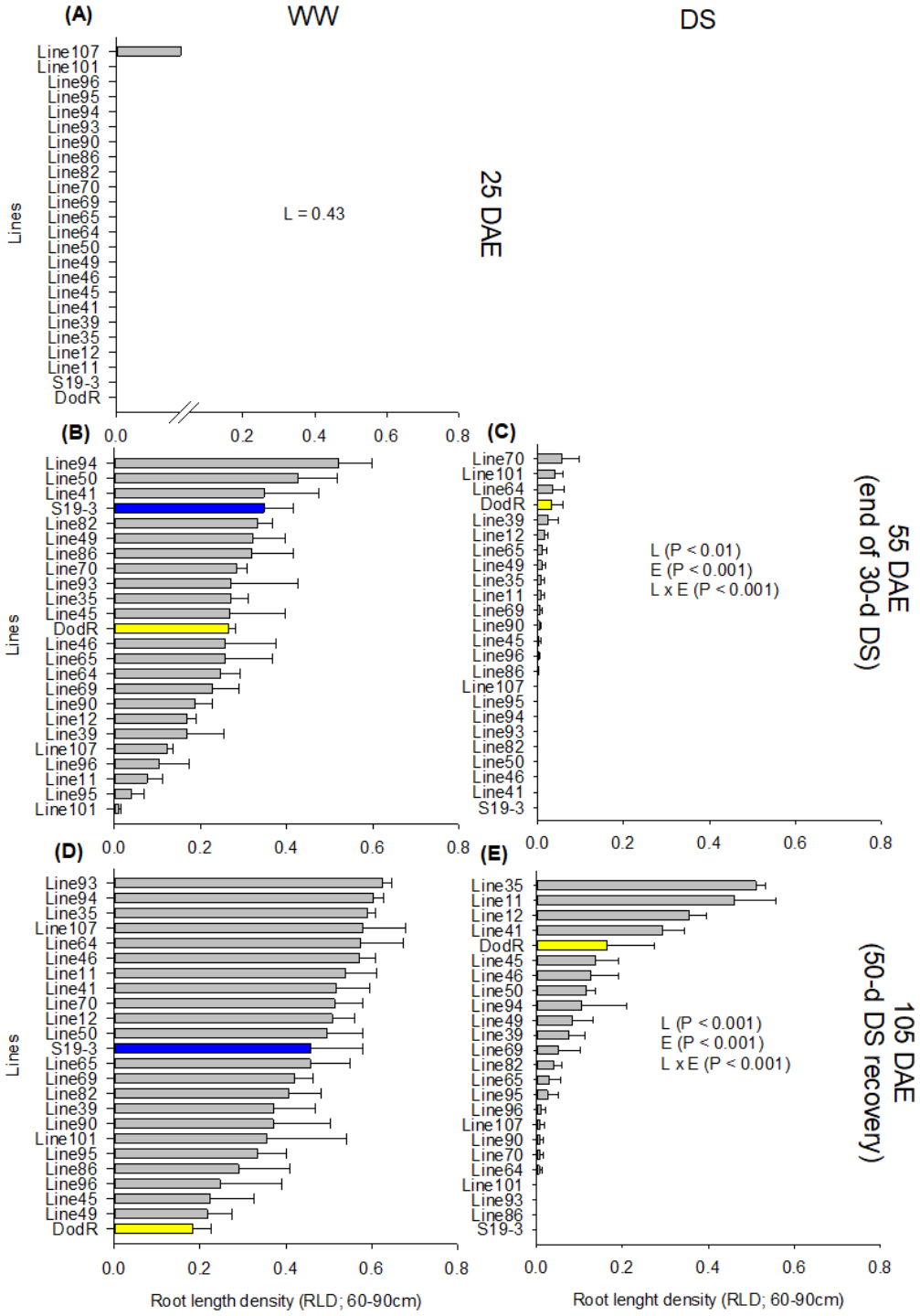

Figure 6-6 Root length density in the 60-90cm soil depth (RLD 60-90cm) of 22 bambara groundnut lines and two parental genotypes ordered from smallest to largest value at (**A**) 25 DAE, (**B**) 55 DAE for well-watered, (**C**) 55 DAE for drought stress (**D**) 105 DAE for well-watered, (**C**) 105 DAE (50-d DS recovery). The DS treatment was intentionally left blank at 25 DAE. The two parental genotypes DodR and S19-3 are represented by yellow and blue coloured bars. The data is mean ± se values (n = 3), with errors bars showing significant difference (HSD) as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

6.4.5 Genetic Correlations Among Traits

Table 6-5 summarizes Pearson correlations coefficients (*r*) describing the degree of correlations among measured traits. Under well-watered (WW) environment, associations were obtained between **SH** and NoL (r = 0.57), SDW (r = 0.53), TRL (r = 0.34), RLD (60-90cm) (r = 0.32); **NoL** and SDW (r = 0.73), RLD (60-90cm) (r = 0.24); $\boldsymbol{P_n}$ and E (r = 0.56) and **GY** and 100-SW (r = 0.54) all ($P < 0.001$). TRL showed a strong correlation with RLD (60-90cm) (r = 0.69; $P < 0.001$; Table 6-5), which obviously reflects the relationship between deep tap rooting and subsequent root distribution in order to efficiently colonise the soil volume and forage for resources. RLD (60-90cm) was associated ($P < 0.01$) with above ground shoot traits i.e., SH, NoL, and SDW (r = 0.32, 0.24 and 0.26, respectively). P_n was positively associated with C_i (r = 0.32; $P < 0.01$).

Under drought stress (DS) environment (50-d of DS recovery), distinct and stronger associations ($P < 0.001$) were obtained compared to the WW environment. Associations were obtained between **SH** and NoL (r = 0.81), SDW (r = 0.88), TRL (r = 0.72), RLD (60-90cm) (r = 0.67); **NoL** and SDW (r = 0.84), TRL (r = 0.63), RLD (60-90cm) (r = 0.78); **SDW** and TRL (r = 0.73), RLD (60-90cm) (r = 0.78), Ci and GY (r = 0.43); **TRL** and RLD (60-90cm) (r = 0.83); **RLD (60-90cm)** and GY (r = 0.39) and **GY** and 100-SW (r = 0.71) (Table 6-5). There was a general trend of greater C_i with the increase in TRL and RLD (60-90cm) r = 0.31 and 0.24, respectively ($P < 0.05$), with an even stronger relationship between C_i and GY (r = 0.43; $P < 0.001$). NoL and SDW were positively correlated with P_n (r = 0.36; $P < 0.01$ and 0.24; $P < 0.05$, respectively). The regression between RLD (60-90cm) had large standard errors (indicating means for some lines were widely dispersed around the population mean). However, these were acceptable, significant and positively associated with

GY (R^2 = 0.42; P < 0.001; Figure 6-7), indicating that 42% of the variation in GY was explained by RLD (60-90cm).

Table 6-5 Pearson's correlation coefficients (r) describing association of 11 traits in 22 bambara groundnut lines and two parental genotypes evaluated under well-watered (top) and drought stress (50-d DS recovery; bottom). Bold correlation are significant at * P < 0.05, ** P < 0.01, and *** P < 0.001.

Well-watered (WW)	Traits Abbreviation	SH	NoL	SDW	P_n	g_s	C_i	E	TRL	RLD (60-90cm)	GY	100-SW
Shoot height [a]	SH											
Number of leaves [a]	NoL	0.57 ***										
Shoot dry weight [a]	SDW	0.53 ***	0.73 ***									
Photosynthesis [a]	P_n	-0.04	-0.09	-0.02								
Stomatal conductance [a]	g_s	-0.04	-0.12	-0.05	-0.16							
Intercellular carbon [a]	C_i	0.01	-0.16	-0.12	0.32**	0.13						
Transpiration [a]	E	-0.12	-0.15	0.00	0.56 ***	-0.04	0.02					
Tap root length [a]	TRL	0.34 ***	0.14	0.10	0.08	0.16	0.06	0.02				
Root length density (60-90cm) [a]	RLD (60-90cm)	0.32 **	0.24 *	0.26 *	0.11	0.09	0.12	0.15	0.69 ***			
Grain yield column^{-1} [b]	GY	-0.13	-0.14	0.01	0.08	-0.13	-0.01	0.12	0.00	0.09		
Weight of 100 seeds [b]	100-SW	-0.05	-0.01	-0.02	-0.11	-0.09	-0.09	0.01	0.07	0.00	0.54 ***	
Drought stress (DS)[1]	Traits	SH	NoL	SDW	P_n	g_s	C_i	E	TRL	RLD (60-90cm)	GY	100-SW
Shoot height [a]	SH											
Number of leaves [a]	NoL	0.81 ***										
Shoot dry weight [a]	SDW	0.88 ***	0.84 ***									
Photosynthesis [a]	P_n	-0.17	0.36**	0.24*								
Stomatal conductance [a]	g_s	0.06	0.09	0.12	-0.08							
Intercellular carbon [a]	C_i	0.26 *	0.26 *	0.32 **	-0.22	0.27 *						
Transpiration [a]	E	0.24 *	0.21	0.23	0.37 **	0.07	0.29 *					
Tap root length [a]	TRL	0.72 ***	0.63 ***	0.73 ***	-0.07	0.22	0.30 *	0.31 **				
Root length density (60-90cm) [a]	RLD (60-90cm)	0.67 ***	0.69 ***	0.78 ***	-0.15	0.24 *	0.27 *	0.24 *	0.83 ***			
Grain yield column^{-1} [b]	GY	0.32 **	0.36 **	0.45 **	-0.06	0.14	0.43 ***	0.23	0.31 **	0.39 ***		
Weight of 100 seeds [b]	100-SW	0.24 *	0.31 **	0.29 *	-0.18	0.03	0.31 **	0.06	0.15	0.20	0.71 ***	

[1] – DS (50-d of drought stress recovery); [a] – 55 DAE and [b] – 105 DAE

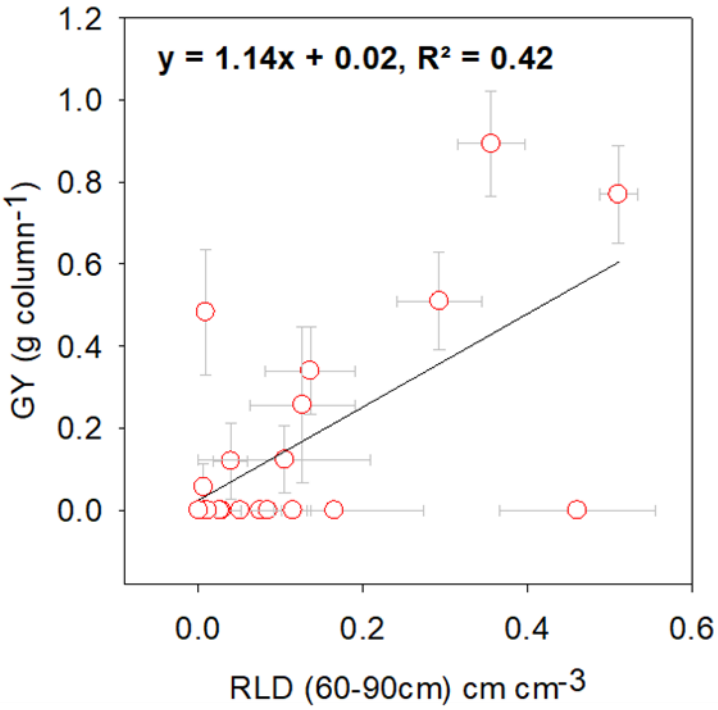

Figure 6-7 Regression of bambara groundnut lines root length density (RLD 60-90cm) and grain yield column^{-1} (GY) at 50-d of DS recovery grown in a soil-filled PVC column of 20 × 110cm (diameter and length, respectively). The data represents mean (n = 3). Coefficient of determination R^2 reported upon fitting with equation y = a*x + y0.

6.4.6 Principal Component Analysis (PCA)

The rotated component matrix (Table 6-6) shows the proportion of total variance explained by different principal components and their correlations with different traits. From the WW environment, three principal components were important, contributing 62.3% of the total variation observed. The first two principal components were the most influential with a cumulative contribution to the total variation of 47%. Variables GY had high positive loading into the first principal component while P_n, g_s, E, TRL and RLD (60-90cm) had high positive loading into the second principal component. These were followed by P_n, GY and 100-SW which had high positive loading into the third principal components, respectively.

Similarly, three principal components were important under the DS environment, accounting for 79.1% of the total variation of which 66.9% was accounted for by the first two components. All traits except P_n, had high positive loading into the first principal component while P_n, g_s, C_i, E, GY and 100-SW had high positive loading into the second principal component.

Table 6-6 Rotated component matrix of 11 traits of 22 bambara groundnut line and two parental genotypes evaluated in WW (well-watered) and DS (drought stress) environments.

		Well-watered (WW)				Drought stress (DS)[1]		
	Trait Abbreviation	PC 1	PC 2	PC 3	Trait Abbreviation	PC 1	PC 2	PC 3
Shoot height [a]	SH	-0.53	0.05	-0.01	SH	0.35	-0.31	0.15
Number of leaves [a]	NoL	-0.50	-0.22	0.17	NoL	0.36	-0.29	-0.02
Shoot dry weight [a]	SDW	-0.47	-0.16	0.17	SDW	0.39	-0.25	0.04
Photosynthesis [a]	P_n	0.06	0.36	0.43	P_n	-0.11	0.28	0.71
Stomatal conductance [a]	g_s	0.04	0.38	-0.29	g_s	0.21	0.32	-0.24
Intercellular carbon [a]	C_i	0.03	0.12	-0.60	C_i	0.23	0.39	-0.35
Transpiration [a]	E	0.09	0.48	0.18	E	0.21	0.34	0.49
Tap root length [a]	TRL	-0.23	0.43	0.02	TRL	0.38	-0.14	0.14
Root length density (60-90cm) [a]	RLD (60-90cm)	-0.30	0.46	0.03	RLD (60-90cm)	0.38	-0.11	0.06
Grain yield column^{-1} [b]	GY	0.26	0.04	0.38	GY	0.32	0.36	0.01
Weight of 100 seeds [b]	100-SW	0.17	-0.11	0.36	100-SW	0.26	0.38	-0.18
	Explained variance (eigenvalue)	2.78	2.39	1.68	Explained variance (eigenvalue)	5.76	1.60	1.34
	Proportion of total variance (%)	25.30	21.73	15.25	Proportion of total variance (%)	52.33	14.58	12.17
	Cumulative variance (%)	25.30	47.00	62.30	Cumulative variance (%)	52.33	66.90	79.10

[1] – DS (50-d of drought stress recovery);

[a] – 55 DAE

[b] – 105 DAE

Principal components with eigenvalues >1 are presented and considered significant.

6.4.7 Principal Component Biplot Analysis

The relationships between the different traits and the bambara groundnut lines with respective principal components are further illustrated by the principal component biplots in Figure 6-8 and Figure 6-9 for the WW and DS environments (50-d of DS recovery), respectively. Smaller angles between dimension vectors in the same direction indicated high correlation of the different traits in terms of discriminating the bambara groundnut lines. Additionally, correlation coefficients among traits and environment are also presented in Table 6-5. Lines excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull. Most traits were positively correlated in both environments except for *Pn* and its association with NoL and SDW (r = -0.36 and -0.24, respectively; Table 6-5; Figure 6-9) under the DS environment.

Under the WW environment, a seven-sided convex hull was formed from markers Line96, Line86, Line 49, Line39, Line94, Line35, and Line46 (Figure 6-8). The convex hull was produced by linking markers of lines that are the furthest away from the biplot origin such that all other lines are contained within the convex hull. The lines were equally concentrated on the positive and negative side of the first principal component with Line82 and Line64 being more inclined in the direction of GY, g_s, C_i and P_n (Figure 6-8; *Appendix 13*).

Under the DS environment (50-d of DS recovery), the convex hull was formed from markers Line12, Line35, Line11, Line49, Line69, Line93, Line86, Line96 and Line82 (Figure 6-9). Similar to the WW environment, most of the bambara groundnut lines were equally scattered in the positive and negative side of the first principal component, however with lines such as Line12, Line35 and Line41 excelling in grain yield which was contributed mostly by high RLD (60-90cm) and TRL, as well as optimum values for shoot traits such as SH, SDW and NoL (Figure 6-9; *Appendix 13*).

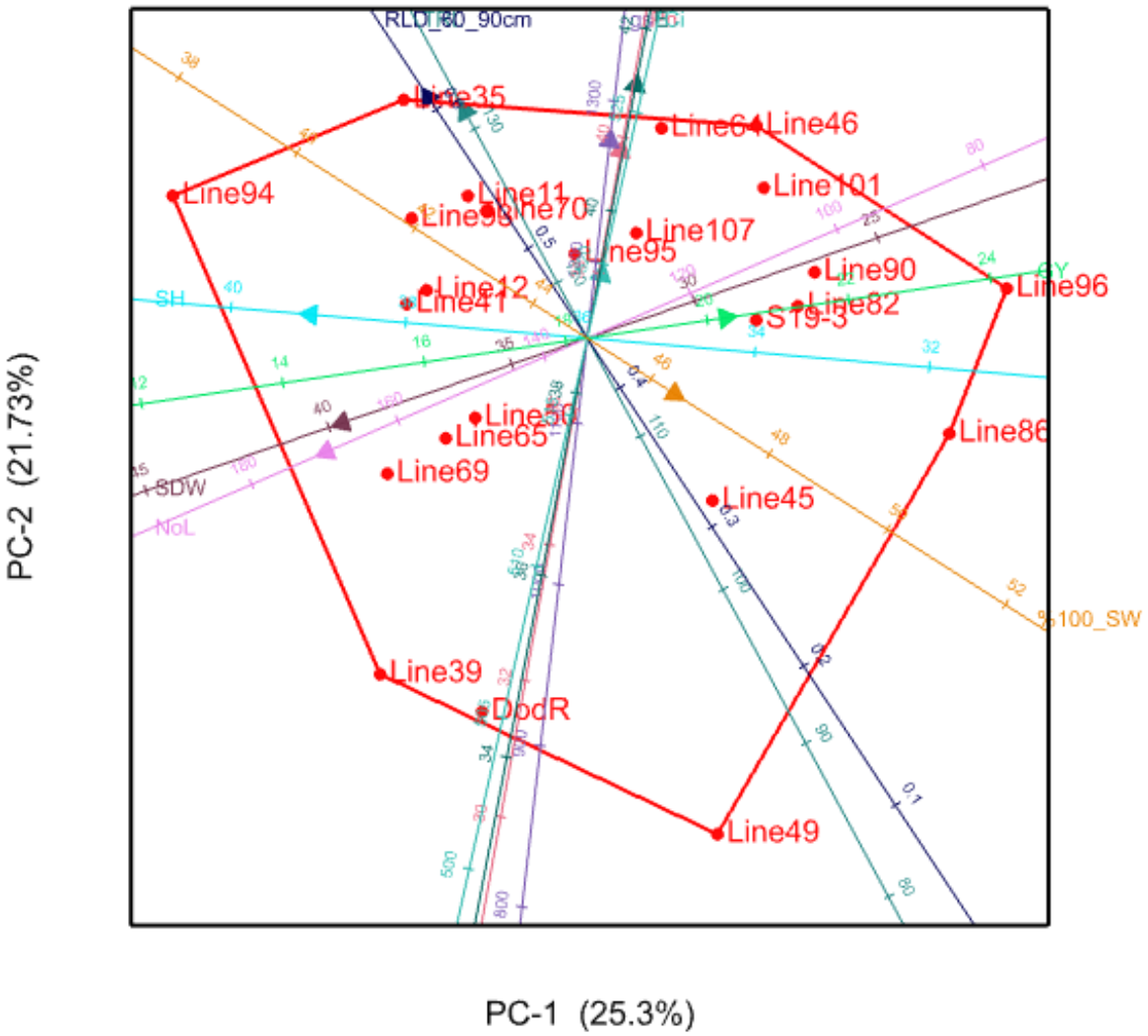

Figure 6-8 Principal component biplot showing line and parental genotype grouping under WW (well-watered) environment. Arrows pointing in opposite directions mean negative correlations. SH – shoot height; NoL – number of leaves; SDW – shoot dry weight; P_n – photosynthesis; g_s – stomatal conductance; C_i – intercellular carbon; E – transpiration; TRL – tap root length; RLD (60-90cm) – root length density in the 60-90cm soil depth; GY - grain yield column^{-1}; 100-SW – weight of 100 seeds. Trait names might overlap due to the statistical package used.

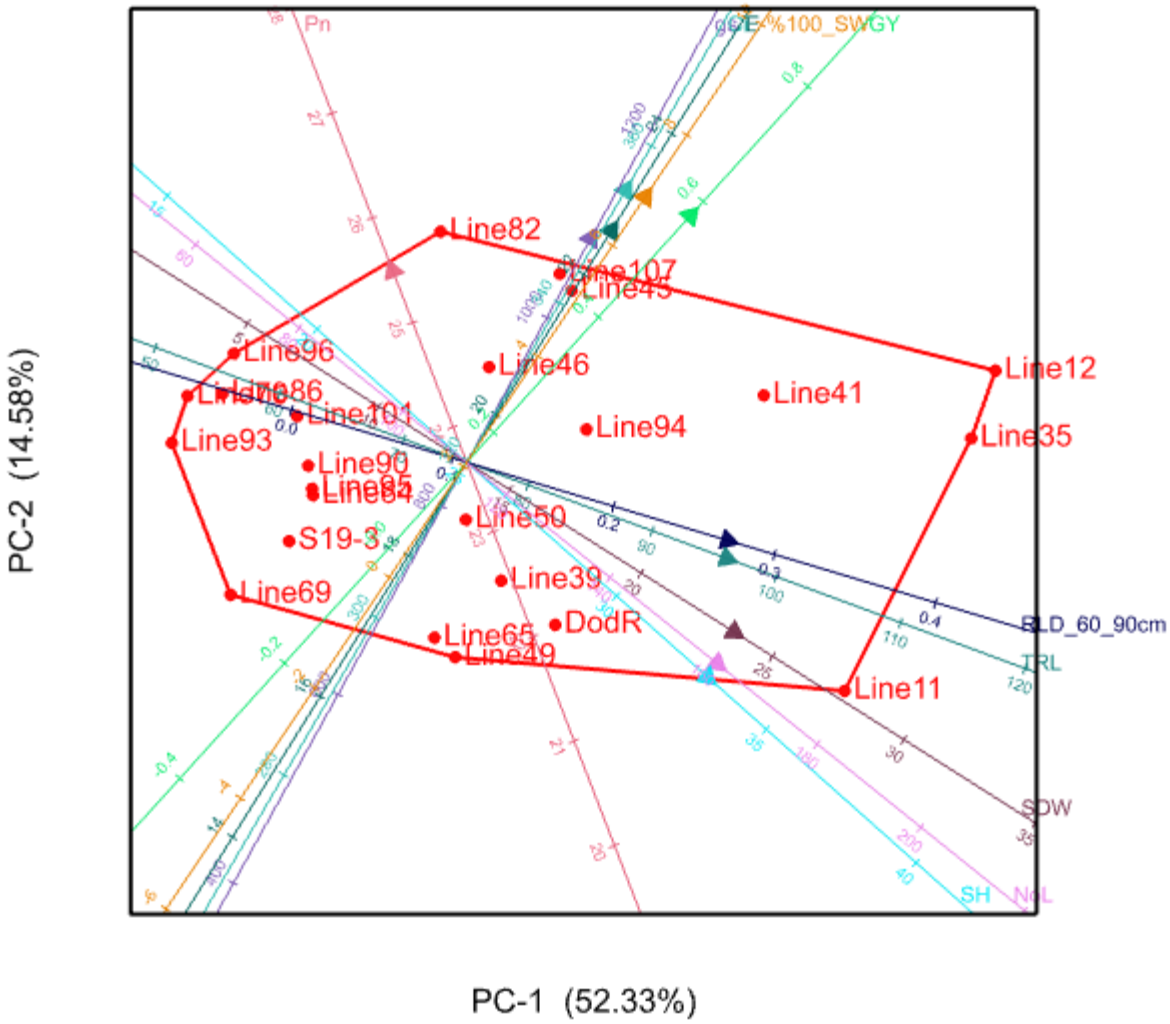

Figure 6-9 Principal component biplot showing line and parental genotype grouping under DS (drought stress) environment 105 DAE (50-d of DS recovery). Arrows pointing in opposite directions mean negative correlations. SH – shoot height; NoL – number of leaves; SDW – shoot dry weight; P_n – photosynthesis; g_s – stomatal conductance; C_i – intercellular carbon; E – transpiration; TRL – tap root length; RLD (60-90cm) – root length density in the 60-90cm soil depth; GY - grain yield column^{-1}; 100-SW – weight of 100 seeds. Trait names might overlap due to the statistical package used.

6.5 Discussion

Development and release of drought resistant bambara groundnut varieties is the goal of advocates of this underutilized crop. In the present study, grain yield performance under drought stress environment was used as a primary index for drought resistance. A detailed early generation population

screening utilising key adaptive traits is a good way to find materials for advanced breeding programmes. The observed significant effects of the bambara groundnut lines, environment and line × environment interaction were expected since both parental genotypes i.e., S19-3 (maternal) × DodR (paternal), utilized for the cross were selected based on previous evaluations of root system traits.

Broad-sense heritability (H^2) offers an estimation of how much progeny/trait expression can be advanced through selection. In this current study, high RLD (60-90cm) and above ground shoots (i.e., NoL and SDW) broad sense heritability values (>0.80) were observed, suggesting selection could be used effectively and bambara groundnut improvement possible using these significantly correlated traits under drought stress ($P < 0.001$) and well-watered ($P < 0.05$; Table 6-5) conditions. Although estimates of heritability in controlled trials can be exaggerated due to greater experimental regulation of conditions (Gahoonia and Nielsen 2004; Khan et al. 2021b) than in actual farmer fields. Similar high estimates have been observed for wheat ranging between 0.62 and 0.93 (Maccaferri et al. 2016), and between 0.45 and 0.81 in maize (Colombi et al. 2015). Although values in the current study are generally high, the estimates clearly indicate useful targets for bambara groundnut improvement.

6.5.1 Association of Shoot Traits Under Drought Stress

Bambara groundnut lines were subjected to progressive drought stress for a period of 30-d and then the soil was rewetted, and leaf gas exchange evaluated. Plants will usually prevent water scarcity by minimising water loss by stomata closure. Bambara groundnut can sense water stress around their root system (Mateva et al. 2022; see *CHAPTER 5*) and possibly respond by sending chemical signals to the above ground shoot parts to induce various

adaptive responses, including increased stomatal closure (Collinson et al. 1997; Mabhaudhi and Modi 2013; Chai et al. 2016a, 2016b). Mateva et al. (2022); (see *CHAPTER 5*) reported that the magnitude and pattern of water stress was determined by g_s in eight bambara groundnut parental genotypes sourced from diverse agroecologies.

In this study, a reduction of g_s due to the 30-d drought stress was observed and this reduction of g_s led to reduced E, P_n and Ci in all tested bambara groundnut lines on the last day of the drought stress i.e., 55 DAE (Figure 6-2A-D). Interestingly it was observed from the present results that the perception of water shortage was rapid in shallow rooted lines and that they showed reduced g_s and E more rapidly than the deep rooting lines. In a study by Mateva et al. (2022); (see *CHAPTER 5*) testing eight bambara groundnut single genotypes under similar severe drought stress environment, g_s of genotypes DodR and S19-3 (with deep root length density) responded slower to drought stress treatment than that of LunT (shallow root length density). The two deep root length density genotypes were used to produce the population SD: S19-3 (maternal) × DodR (paternal) and subsequently used in this study under drought stress followed by recovery and exhibited similar tendencies with respect to g_s. It was clear in the present study that the decrease of E in the drought stress environment was due mainly to decreased g_s. A similar response was observed in studies of bambara groundnut (Chai et al. 2015) and vegetable amaranth (*Amaranthus sp*.) (Liu and Stützel 2002).

6.5.2 Deep Root Distribution is Beneficial for Enhanced Resource Acquisition and Higher Grain Yield

Elongation of the tap root precedes root distribution in bambara groundnut (Mateva et al. 2020; see *CHAPTER 4*). In the current study, tap root

length (TRL) and root length density in the 60-90cm soil depth (RLD 60-90cm) was affected by drought stress (Figure 6-5 and Figure 6-6). Based on the drought responses in leaf g_s, it can be suggested that some lines were able to regulate stomatal openings in response to chemical signals from roots, and maintain P_n through temporal water access facilitated by large TRL and RLD that penetrated the 60-90cm soil depth (Figure 6-7). This has also been shown in studies by (Comstock 2002; Yan et al. 2017). Recently Yan and colleagues (Yan et al. (2017), reported that stomatal closure may be induced by ABA and zeatin-riboside (ZR), and that the ABA/ZR ratio shows a substantial connection with gs, suggesting that the combined chemical signal plays a role in coordinating stomatal activity. However, during the late stages of crop growth i.e., DS recovery, TRL and RLD (60-90cm) showed marked recovery when compared to well-watered plants (Figure 6-5 and Figure 6-6). This revealed that when the stress is removed, the programmed genotypic patterns are substantially restored/recovered. The highest grain yield was obtained in lines exhibiting partial to full RLD (60-90cm) recovery, suggesting that greater RLD (60-90cm) enhanced the soil volume colonised and thus improved extraction of soil resources distributed in deeper soil depths particularly leached nitrogen (N). Studies by Dayoub et al. (2017) and Carton et al. (2018) utilised nine legumes and three lupins species, respectively, to demonstrate that N uptake was connected to growth rate of (here, RLD).

Whether it be mineral soil N or N_2 from biological fixation, legume crops are generally notorious for poor soil absorption early in their life cycle, which results in excessive leaching (Dayoub et al. 2017). This is primarily owing to legume seeds carrying a considerable quantity of N and are potentially capable of sustaining seedling development for a major portion of the plant cycle (Herdina and Silsbury 1990). However later on in their development as these reserves are depleted and the root system extends into greater and deeper soil

volume, N uptake is greatly improved. This specific legume feature most likely leads to the late use of exogenous N (mineral soil N and N_2 from biological fixation). During the late growth observed in this current study, it is worth noting that water could not have been a limiting factor to attaining higher yields and so the underlining speculation is that lines with deep roots especially higher RLD (60-90cm) may have benefited from enhance root N foraging (Dayoub et al. 2017).

Crop yields in many developing countries are significantly lower than in developed countries, owing in large part to inherently poor soil nutrients and much less use of chemical fertilisers. Furthermore, in arid and semi-arid agroecologies, deeper soil layers contain higher concentrations of a variety of leached nutrients (McCulley et al. 2004). Particularly noteworthy, nitrate (N) are rather mobile in the soil and as the present study progressed during recovery, the speculation is that N and other exchangeable ions e.g., Ca^{2+} and Mg^{2+}, leached deep into the soil, thereby altering concentrations in both the shallow and deeper soil depths. This could have potentially made nutrients critical for full pod development available exclusively to bambara groundnut lines that penetrated and developed deep RLD enough to efficiently take up the mobile resources. Indeed, efficient N and cation uptake depends on the root distribution in the soil profile. This observation is consistent with reports by (Lynch 2013; White et al. 2013; Bordron et al. 2019), who state that greater RLD in the 0–60cm soil profile leads to deep N acquisition and enhanced nitrogen use efficiency (NUE). Similarly, (Dayoub et al. 2017) evaluated divergent legume species i.e., peanut, fenugreek (*Trigonella foenum-graecum* L.), faba bean (*Vicia faba* L.), winter lentil (*Lens culinaris* L.), alfalfa (*Medicago sativa* L.), pea (*Pisum sativum* L.), chickpea, soybean and common vetch (*Vicia. sativa* L.) for N acquisition. The authors reported that soil N uptake among the studied species was correlated with the root distribution. While this result may not be as

obvious in this present study, it is evident that because N is soluble, the capacity to take up water may well correspond with N absorption when there is drought stress. More research will be required to demonstrate this.

6.6 Conclusion

In summary, exposure of drought stress brought severe negative effects on shoot, root growth and the grain yield of the bambara groundnut lines and the population SD: S19-3 (maternal) × DodR (paternal) could be used as a rich source of genetic diversity for drought breeding purposes. Overall, the root system growth and grain yield performance of 'Line12', 'Line35' and 'Line41' was better than the other lines. The greater resistance of Line12', 'Line35' and 'Line41' to drought stresses was attributed to strong recovery of RLD (60-90cm) — enough to colonise a greater soil volume for efficient water uptake and possibly nutrient resource foraging. Moreso, RLD (60-90cm) (0.99), NoL (0.87) and SDW (0.87) had higher estimates of broad sense heritability. Therefore, these traits, particularly RLD (60-90cm) are transmissible and could be utilised to improve the development of superior bambara groundnut varieties through cross breeding and phenotypic selection. Furthermore, the significant differences among the studied lines observed for RLD (60-90cm) showed there was sufficient genetic variation within the population enough to warrant future quantitative trait loci (QTL) studies.

CHAPTER 7 : General Discussion, Conclusions, Implications and Further Work

7.1 General discussion

The objective of this work was to explore the natural occurring genetic differences of the root system architecture (RSA) in bambara groundnut — a known drought resistant grain legume, under the hypothesis that genotypes sourced from dry agroecologies with periodic drought stress have throughout the years developed root system traits (selective pressure — conferring deep rooting phenotype survival benefits) that improve water foraging in deep soil depths.

The research experiments utilised a low-cost soil-filled polyvinyl chloride (PVC) column phenotyping system (Mateva et al. 2020) and WinRhizo Pro image analysis software v2009 (Regent Instruments, Montreal, QC, Canada). A team of three was enough to extract the bambara groundnut plants from the PVC column and wash ~24 columns day^{-1}. After moving the sample to the lab for further analysis, ~20 scans day^{-1} were taken with two users. Improvements to the root washing (e.g., use of a larger pressure adjustable watering head) and possibly automating movement of the soil-filled PVC columns would greatly improve throughput. This would result in larger populations and more replicates for greater statistical power. Nevertheless, from the current study a detailed examination of the morphology and development of bambara groundnut root system at different growth stages spanning from the flowering stage to pod development and maturity was possible. The soil-filled PVC column system allowed for the replication of natural soil and physical properties such as bulk density, which are often

overlooked in these sorts of studies in order to save on time. Using this phenotyping system, previously unstudied root variation in bambara groundnut genotypes and biparental lines was made possible. This provided proof of concept, enough to warrant future improved experimental setups to further the study of bambara groundnut root systems.

7.1.1 Variations of Tap root and Root Length Density in Bambara groundnut: ***An Agroecological Perspective***

The root systems of bambara groundnut, like those of many dicotyledons possess a well-defined tap root structure with several first-order lateral roots (Mateva et al. 2020; see *CHAPTER 4*). These lateral roots further branch into second- and third-order laterals. According to Fitter et al. (1991) and Taub and Goldberg (1996), high exploitation efficiency is associated with plants that possess a deep herringbone topology than those with a dichotomous root system. This deep topology, according to Paula and Pausas (2011), will be especially important in the early stages of plant growth. The differences in root system architecture and rooting distribution observed when the eight bambara groundnut genotypes (Gresik, LunT, IITA-686, DodR, S19-3, Tiga nicuru, Ankpa-4, DipC1) were grown under non-limiting conditions indicates a genotypic distinction linked to selection in environments with different levels of resources, in the case of cultivated landraces, with recurrent selection by farmers. Bambara groundnut genotypes (i.e., Gresik, LunT, and IITA-686) exhibited relatively shallow and highly branched root growth closer to the soil surface. In contrast, genotypes S19-3, DipC1 and DodR sourced from relatively drier regions of sub-Saharan Africa generally had longer tap roots and greater root length distribution in deeper (60-90cm) soil depths.

Genotypes S19-3, DipC1 and DodR at the pre-flowering growth stage showed differential root foraging patterns and branching habits i.e., deep-

cheap rooting. The appearance of these traits in the absence of drought stress implies that these single genotypes which are derived from landraces are intrinsically adapted to dry regions, and that they have been selected by the interaction of environmental resource limitations and agricultural domestication. In fact, a deep-cheap rooting offers decisive advantages in the cultivation conditions of subtropical deserts, semi-arid, and tropical dry areas in Africa. The annual rainfall in these areas, which ranges from 350-570mm depending on the country, in most cases is insufficient to meet the needs of an entire bambara groundnut cultivation period. Farmers address this dilemma by planting small sole bambara groundnut plots that are covered in crop residues to minimize evaporation –one of the cornerstones of conservation agriculture (Ranaivoson et al. 2017). Since the sandy soils in these arid climates are heavily filtering, these cropping strategies assume that the plants have temporary access to valuable soil moisture, even if the rains at the start of the growing season are irregular and inadequate on their own to ensure successful crop establishment. Several researchers have researched the hydrodynamic functioning of soils and plants in rain-fed grain legume habitats in detail, including Austin et al. (2004) and León et al. (2011). They emphasise the importance of rapid root access to the soil's deep layers for seedling survival, as well as the densest possible colonisation of these horizons, which hold water and mobile nutrients including nitrogen. Bambara groundnut genotypes' well-defined deep tap root system, with numerous first-order lateral branches will then be optimal since they combine rooting depth and root length distribution. These attributes were indeed observed in genotypes from dry regions (DipC1 and S19-3) which produced limited lateral roots in the shallow soil depths but had long deep tap roots >90cm depth at 35 days after emergence (DAE), when none of the other genotypes had reached that depth yet. These root growth traits observed in the dry region DipC1 and S19-3 are not present in tropical

wet region genotypes Gresik and LunT, which, in case of drought stress treatment, showed lower grain.

7.1.2 Bambara Groundnut Morphophysiological and Root Analyses: ***Responding to Drought Stress***

Stomata play a critical role of balancing water conservation and enabling photosynthesis via regulating gaseous exchange (Haworth et al. 2011). When drought stress was imposed on the plants during this study, bambara groundnut plants with deep TRL and extensive RLD, adaptation mechanisms to dry environmental conditions, seemed to produce the highest grain yield. Bambara groundnuts ability to forage for water was estimated using the TRL and RLD, parameters that measure the plant ability to penetrate deeper soil depths and colonise a large volume of soil (Mateva et al. 2020; see *CHAPTER 4*). The observed high TRL and RLD in S19-3, DipC1 and DodR was partly attributable to elongation dynamics associated with the genotypes quick and deep rooting system, adaptation mechanisms to dry environmental conditions. Indeed, the genotypes from dry regions developed particularly few lateral roots in shallow soil depths but had long TRL. These adaptation mechanisms played a pivotal role in helping retain significantly higher levels of leaf stomatal conductance than the rainy habitat Gresik and LunT genotypes. S19-3 was described as a "water-spender" in a study by Jørgensen et al. (2010), with late stomatal closure and, as a result, a slow decline in transpiration rate during drought. The rainy habitat Gresik and LunT genotypes were less successful in regulating stomatal responses than the dry region S19-3 and DodR genotypes, as evidenced by the prolonged delay in stomatal conductance reduction or the extensive TRL and RLD (60-90cm), parameters that represent stomatal conductance (Collinson et al. 1997; Jørgensen et al. 2012; Tombesi et al. 2015). Since stomatal regulation is linked to water use efficiency by balancing water

lost during transpiration and carbon absorbed for photosynthesis (Haworth et al. 2011), the low stomatal conductance and substantial TRL and RLD (60-90cm) in S19-3 and DodR illustrates the two genotypes' superior drought phenotype relative to Gresik and LunT. As soil water levels drop due to progressively drying, the osmotic potential across the plasma membrane of the root cell will reverse and prevent water absorption by the roots. Synthesis and aggregation of organic solutes such as proline and glycine betaine for osmoregulation against drought-induced oxidative damage is one method for maintaining water absorption in progressively drying soil (McNeil et al. 1999; Ashraf and Foolad 2007). Under drought stress, proline accumulates in bambara groundnut (Muhammad et al. 2016; Kundy 2019). Kundy (2019) observed that S19-3 had significantly higher proline accumulation on day 49 after plant emergence than DodR, which led to the preservation of optimal water status and, eventually, plant development. The weight of roots and shoots was used to monitor changes in plant growth trends. However, although no differences were observed for the interaction of genotype and water management, the differences in root-to-shoot (R:S) ratio observed among genotypes were distinct, with the genotype DodR consistently accumulating more root biomass and subsequently more R:S ratio. While the differences in the interaction of genotype and water management may not be apparent in the current study, future work on the traits as well as root anatomical measurements are required.

7.1.3 Bambara groundnut future-fit varieties: ***Searching for Combinations of Traits for Specific Environments***

Evolutionary knowledge of the root morphological and developmental traits used by bambara groundnut leads to the quest for trait variants that are more frequently present in certain agroecological

environments and are therefore likely to have an adaptive benefit on these environments. These syndromes have been defined on the basis of root and shoot characteristics, with an adaptive benefit for quick foraging of water under drought stress (Kundy 2019). The present results on root system architecture and rooting distribution of different single genotypes, two extremes of root foraging and branching pattern occur: one characterizing plants from dry regions with deep soils, the other more specific to plants from regions of higher rainfall with shallow soils (Mateva et al. 2020; see *CHAPTER 4*). In the first situation, deep-cheap rooting ensures a significant capture and storage of the resources of the environment to the detriment of competing species. In the arid to semi-arid regions, quick deep rooting, with reduced root surface area, volume, and diameter and limited surface root branching, make up a series of traits that significantly increase the deep foraging capacity and give a selective advantage in dry agroecologies with poor soil. The dry regions genotypes DipC1, S19-3 and DodR show this combination, which gives it a decisive superiority over other genotypes. In regions of higher rainfall with shallow soils, plants with shorter tap root length, with high root branching density and intensity values in the shallow soil layer 0-30cm do not need to forage for deep water reserves. On these shallow soils, shallow roots allow for potentially rapid absorption of phosphorus. This set of traits is found united by Gresik, and corresponds to the conditions encountered in humid climates such as Indonesia.

7.1.4 Bambara groundnut root system breeding pipeline: ***identifying the ROOT for the problem***

Ideotype formation is the first step in any bambara groundnut breeding pipeline in order to hypothesize the ideal phenotype for a given environment. An ideotype is the presumed optimal phenotype for a specific environment

(Donald, 1968), as outlined in " see *1.2 Ideotype Development*". The capacity for phenotypic and genotypic variation within bambara groundnut genotypes and lines, especially for tap root length and root length density in the 60-90cm soil depth, has demonstrated great potential for a breeding pipeline to offset climate change and lead to yield stability in drought-prone regions. Second, the phenotyping method outlined in "*CHAPTER 3*", can be used and, if necessary, updated in certain aspects to increase the throughput of root trait measurements for that ideotype. Third, as stated in "*CHAPTER 6*", biparental populations of genotypes should be screened for target root and shoot traits that are correlated to bambara groundnut performance, such as grain yield. The biparental population can also be used for forward genetics allowing genetic mapping. On the basis of these results, genotypes/lines with opposing phenotypes can be chosen for smaller yet more intense trials, including on farm field studies, to better grasp root trait–environment interactions (York 2019).

7.2 Implications and Further work

In the course of this work, I provide a novel report on root system architecture and its contribution to bambara groundnut water foraging and drought resistance. This is accomplished through the use of a low-cost PVC column phenotyping system and image analysis. As a result of molecular techniques, the decoded bambara groundnut genome as well as greater understanding of root system morphology and foraging patterns, progress in drought tolerance breeding for bambara groundnut is expected to accelerate. Selected lines from the S19-3 (maternal) × DodR (paternal) cross will be advanced as part of the bambara groundnut BamBREED breeding programme (Future Food Beacon). This breeding program's elite lines could be registered as improved varieties and distributed to the general public for use in drought-

prone areas, offering food diversity and significantly increasing the nutritious content of people's diets.

The phenotyping system utilised, on the other hand, did not have a high throughput, but despite this restriction, it allowed plants to flourish in natural soil with realistic soil physical characteristics such as bulk density. The use of high-throughput three-dimensional visualization using X-ray computed tomography would be useful in future studies to enable the rhizosphere of bambara groundnut to be visualised and quantified non-destructively at a microscopic resolution. This would provide a better representation of rhizosphere processes that are at play.

Considering that the rooting capacity and amount of growing season rainfall, play a role in soil water extraction. Future research should also focus on determining the average and maximum differences in soil water extraction by depth in the soil profile for bambara groundnut parental lines. Inaddition, large standard errors were observed, indicating that the sample means for some studied genotypes (at final harvest) were widely dispersed around the population mean. More replicates could be introduced to future studies to minimize the spread of treatment means.

It is also important to equate the present results to studies conducted in situ under standard farmer practice in order to provide more precise biological and ecological explanations. However, substantially greater replication would be required to account for the greater variation in soil physical and chemical properties found in heterogeneous soil in different agroecologies where bambara groundnut is currently cultivated.

Lastly, the present thesis focuses only on soil water as a resource and the contribution of root traits to drought resistance. Further studies tackling root system architecture and nutrient (particularly nitrogen and phosphorus)

uptake would give further insight into how roots operate under soils more or less poor in nutrients and under different pedoclimatic conditions. Molecular basis of drought resistance in bambara groundnut and in particular molecular genetics study of root traits and development of molecular markers for indirect selection of roots in breeding programmes would be of great value going forward. The identification of markers - for example identified by Gao et al. (2021), could be further studied and used in marker-assisted selection. This would be essential in speeding up the characterization of root system architecture for the selection of preferred phenotypes.

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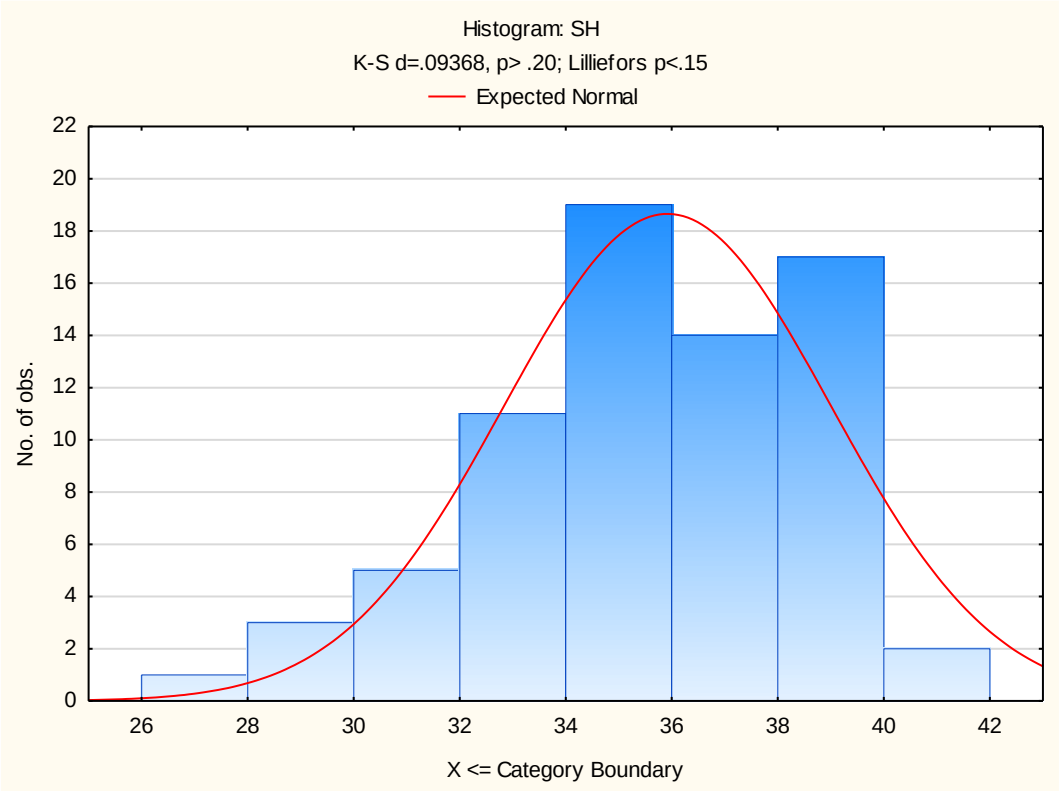
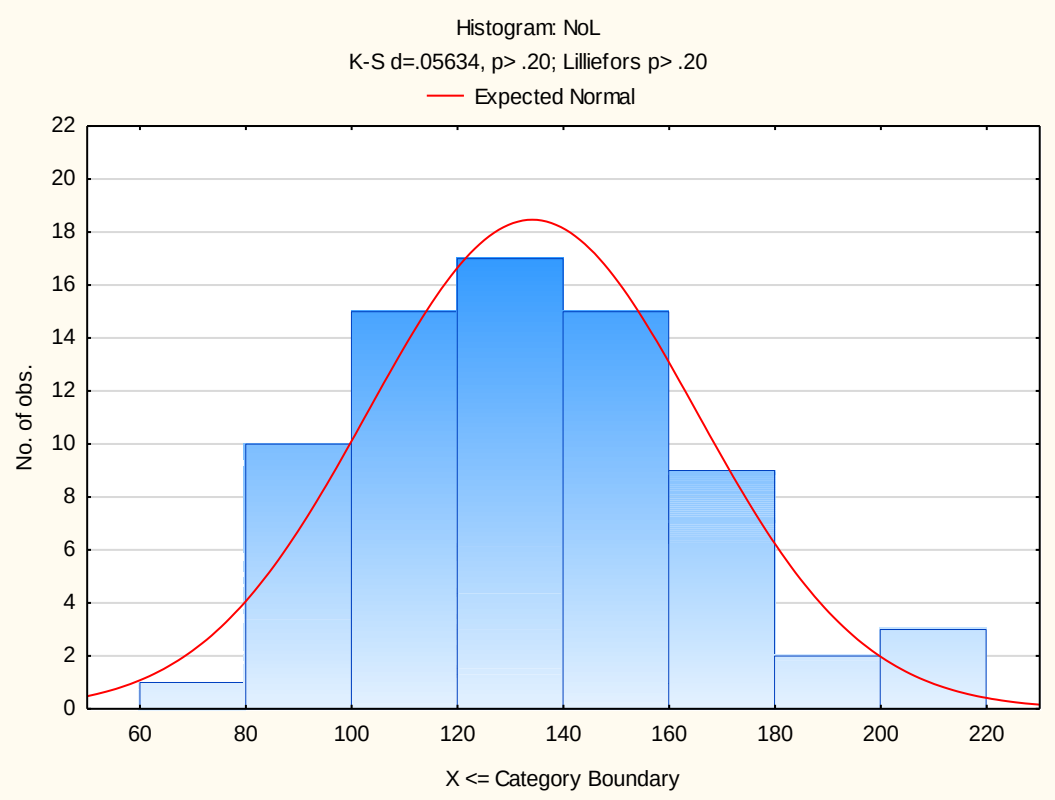
Appendices

Appendix 1 Parental genotypes S19-3 (maternal) × DodR (paternal), used to generate the F_4 segregating population. Black bar = 1cm

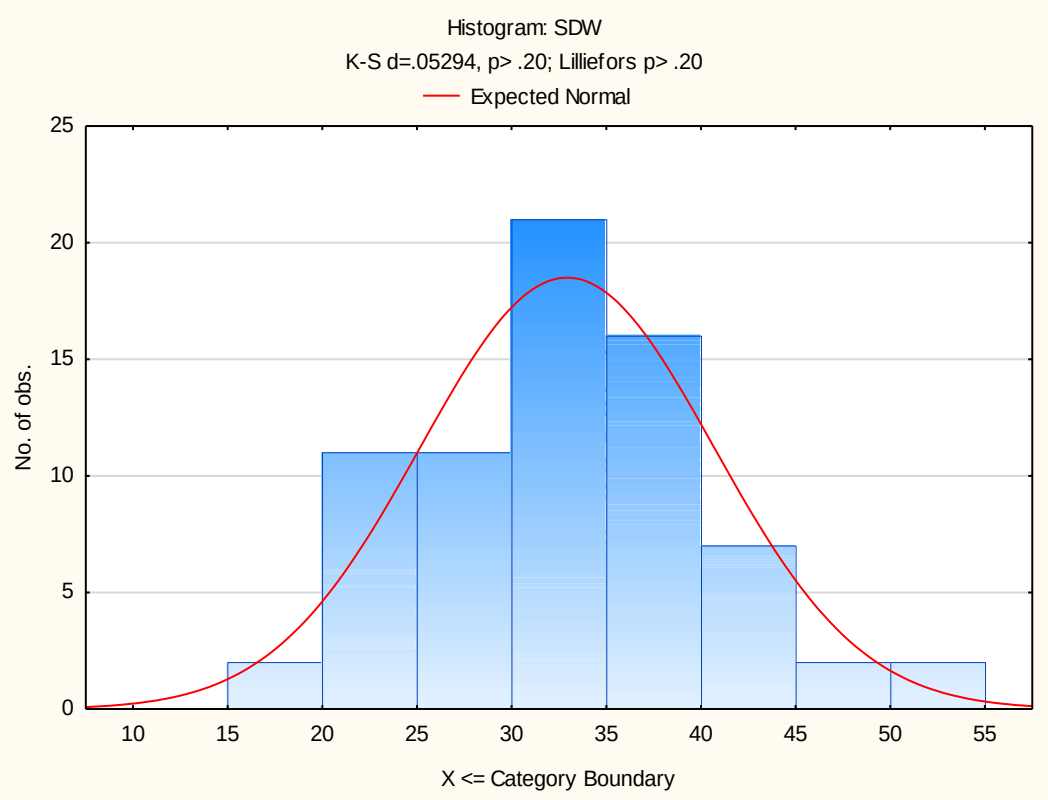
Appendix 2 Phenotypic variations of the SH – shoot height trait in a bambara groundnut bi-parental segregating population.



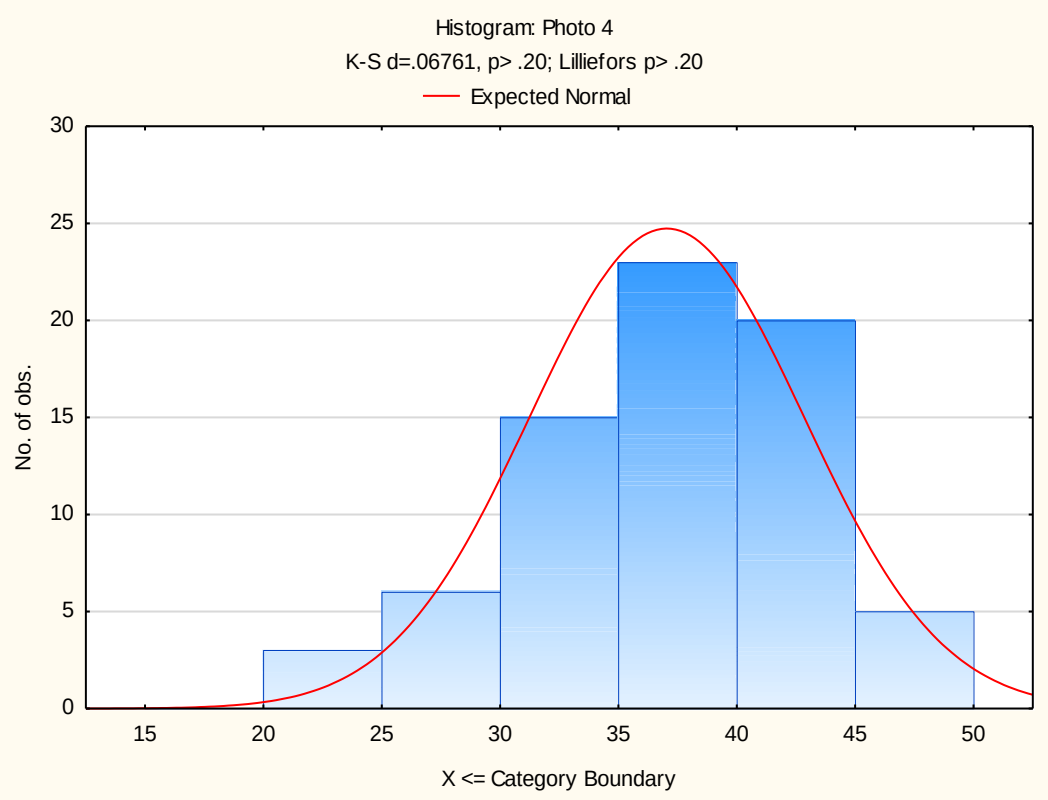
Appendix 3 Phenotypic variations of the NoL – number of leaves trait in a bambara groundnut bi-parental segregating population.



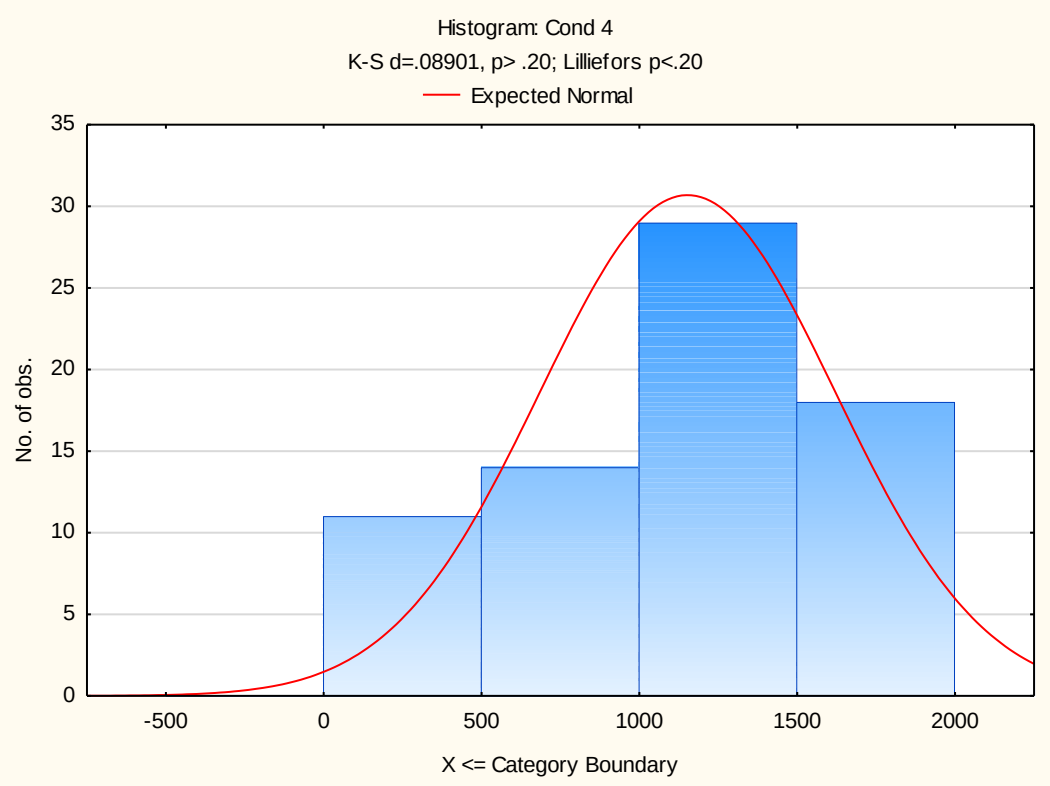
Appendix 4 Phenotypic variations of the SDW – shoot dry weight trait in a bambara groundnut bi-parental segregating population.



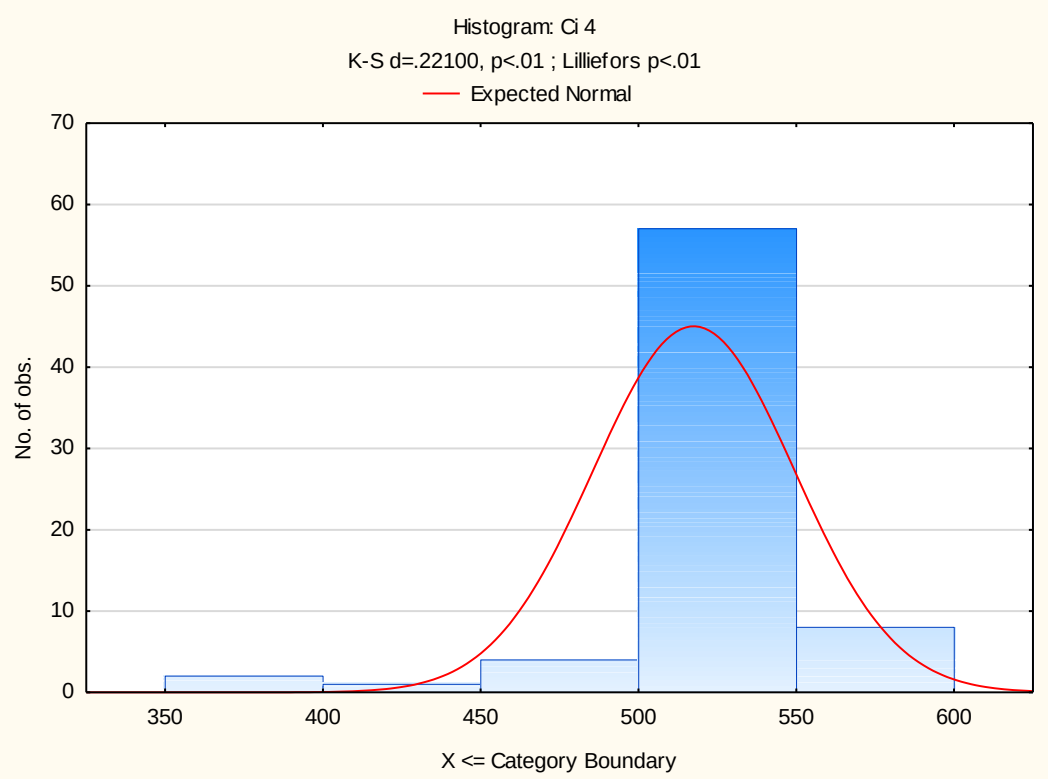
Appendix 5 Phenotypic variations of the P_n – photosynthesis trait in a bambara groundnut bi-parental segregating population.



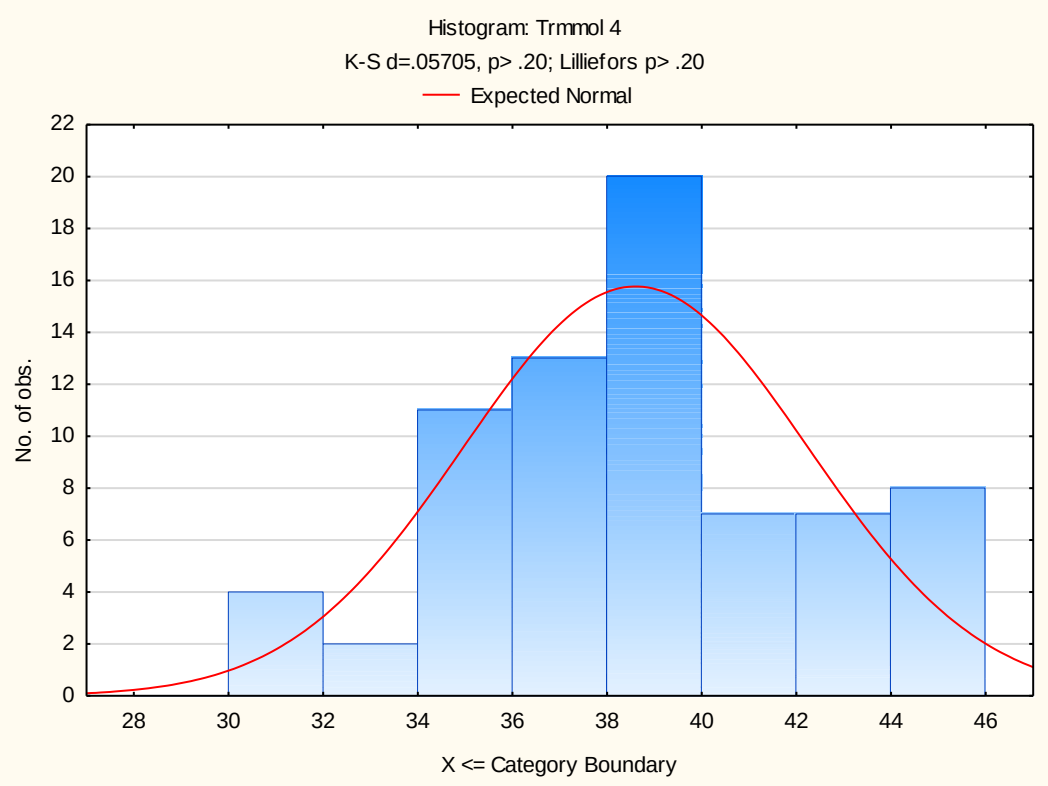
Appendix 6 Phenotypic variations of the g_s – stomatal conductance trait in a bambara groundnut bi-parental segregating population.



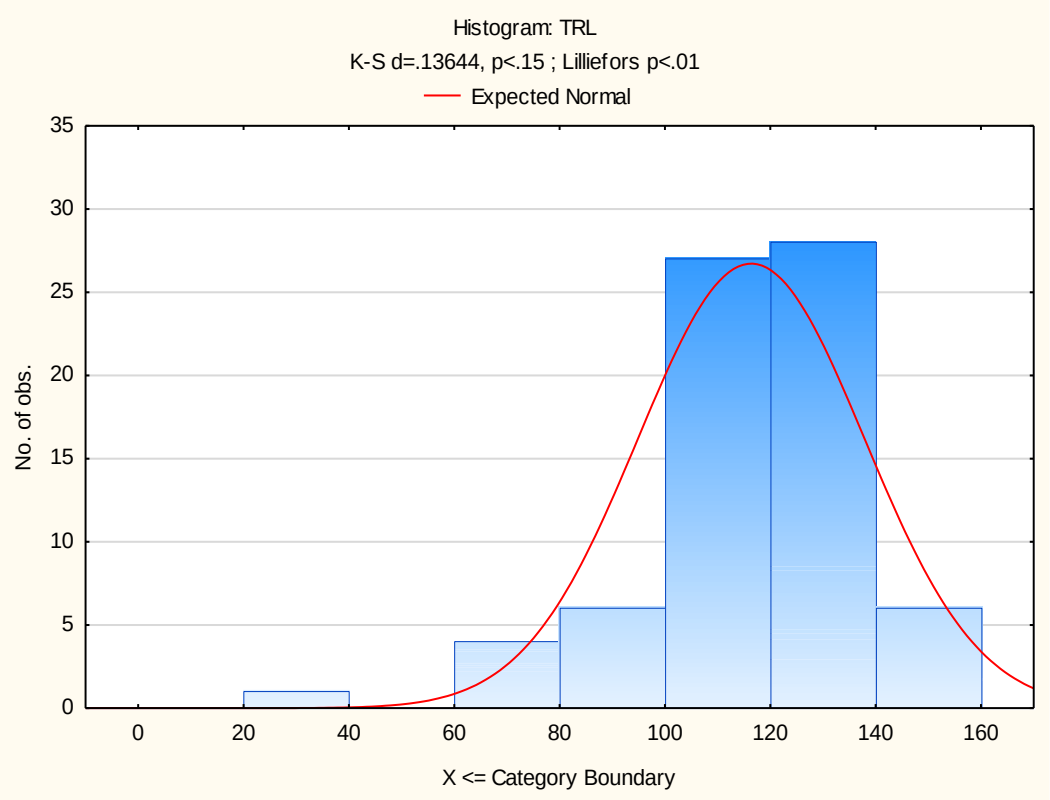
Appendix 7 Phenotypic variations of the C_i – intercellular carbon trait in a bambara groundnut bi-parental segregating population.



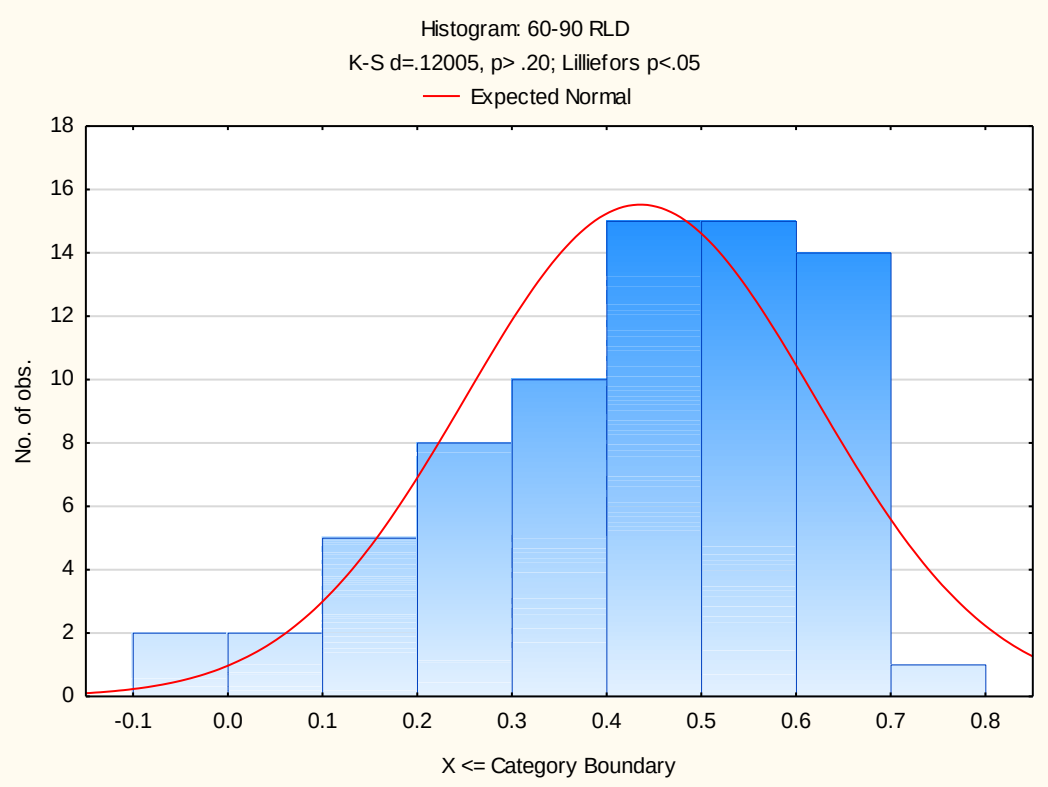
Appendix 8 Phenotypic variations of the *E* – transpiration trait in a bambara groundnut bi-parental segregating population.



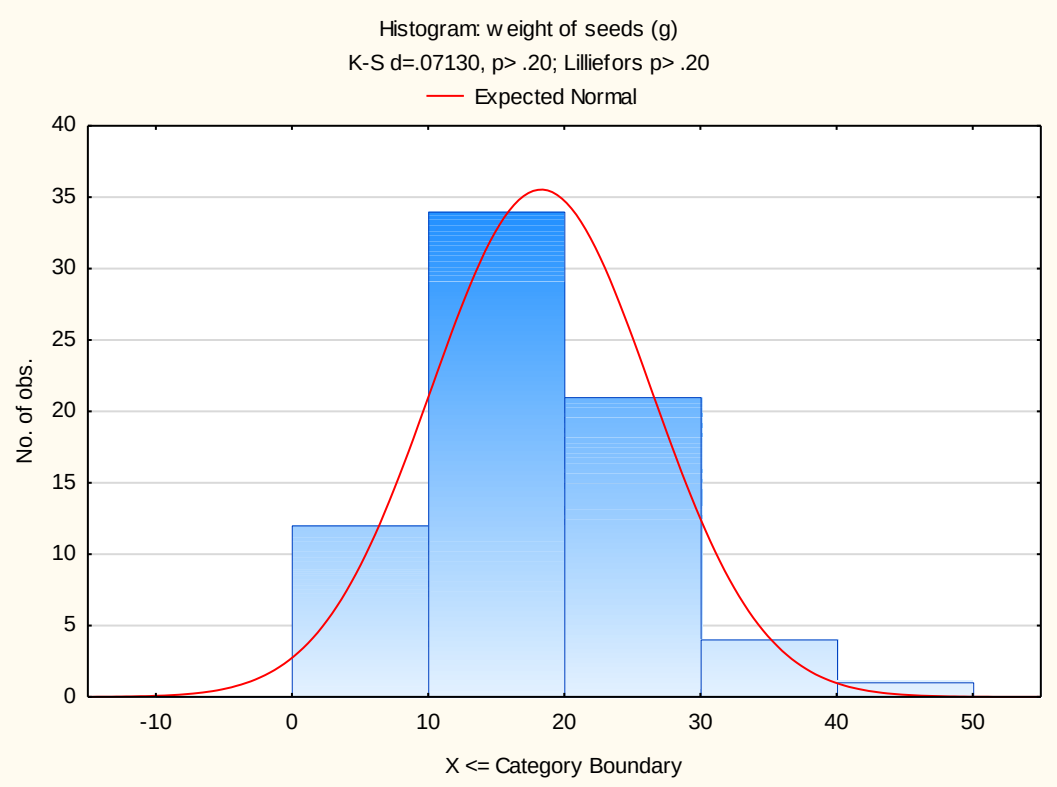
Appendix 9 Phenotypic variations of the TRL – tap root length trait in a bambara groundnut bi-parental segregating population.



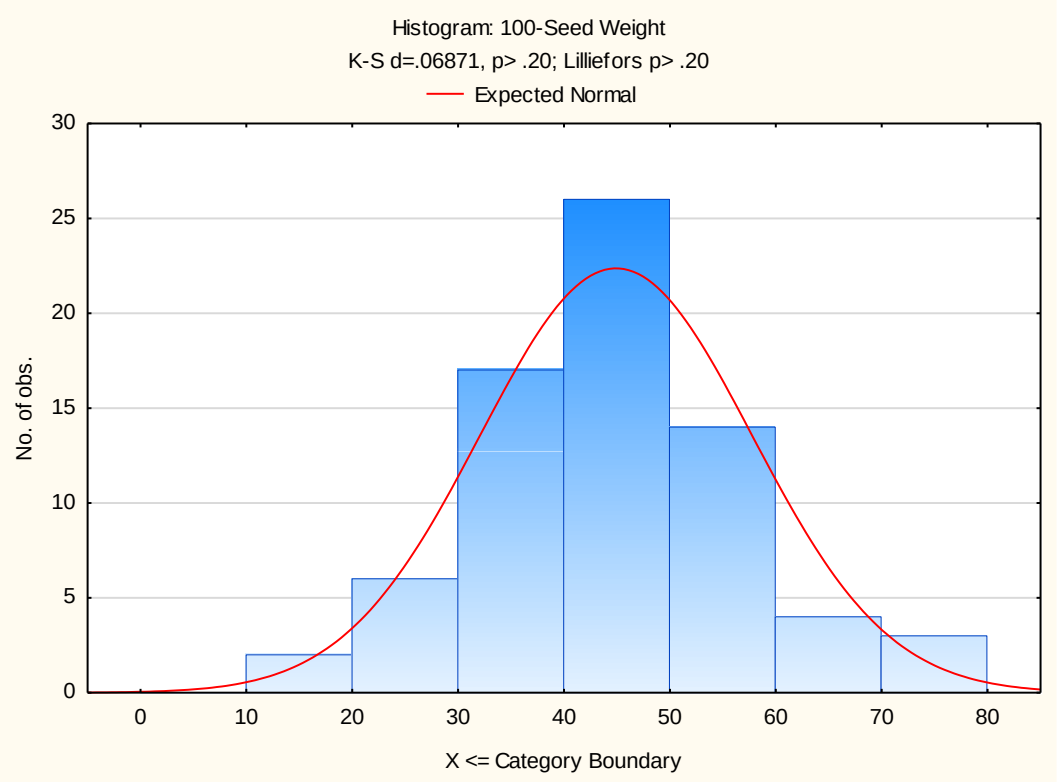
Appendix 10 Phenotypic variations of the RLD (60-90cm) – root length density in the 60-90cm soil depth trait in a bambara groundnut bi-parental segregating population.



Appendix 11 Phenotypic variations of the GY – grain yield column^{-1} trait in a bambara groundnut bi-parental segregating population.



Appendix 12 Phenotypic variations of the 100-SW – weight of 100 seeds trait in a bambara groundnut bi-parental segregating population.



Appendix 13 Genotype and genotype by environment interaction biplot based on "which-won-where". Assess which Line performed well in which environment. The convex hull was formed from Line12, Line39, Line49 and Line82. Four perpendicular lines were drawn starting from the origin and extended beyond the convex hull, dividing the biplot into four sectors with environments in them. Environments well-watered (WW) and drought stress (DS) in sector 1 (SC1) and sector 2 (SC2), respectively.