Understanding mechanisms and pathways underlying drought tolerance to identify climate-smart crops

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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For my sons: Brian Ethan, Eden Thanthwe and Benito Jabulani Enoch

My husband, Brain

and

My mum, Joyce

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Abstract

Drought stress is a major limiting factor for plant growth and development. With climate change and variability, droughts have become more frequent and intense, strongly influencing grain yield and nutritional quality. The current research investigated the mechanisms underpinning drought tolerance in the drought-resilient crop sorghum, to identify key traits that can be targeted in breeding programs, with a focus on root system architectural regulation and its impact on the nutritional quality of sorghum grown under drought conditions. However, plant breeding advances must align with understanding of farmers choices and nutritional impacts if we are to create meaningful solutions. Consequently, this study is highly interdisciplinary, incorporating social science, food science and plant biology. First, desirable traits for drought tolerance, which include grain early maturity, were identified in both sorghum and millets based on survey-based feedback from farmers in Malawi as a pathway to understanding wider context and inform laboratory research. Then screening of a large sorghum dataset comprising of genotypic and nutritional performance indicators, allowed identification of phenotypic and physiological traits linked to nutrition qualities during drought response. Finally, to understand the root phenotypic basis of this performance, screening of root traits was performed using high-throughput phenotyping followed by high-throughput transcriptomic analyses. Results reveal that root architectural traits vary dramatically across genotypes with differing drought adaptability and that the nodal root growth angle is a significant component of this variant. This study demonstrated that the root growth angle of seedlings can support later field performance predictions, and therefore a potential target for breeding. It further shows that drought influences steeper, deeper rooting in water stress-tolerant varieties. The study identified drought-dependent regulation of *LAZY, IAA-ARG7 (SAUR), LBD16* and other auxin-responsive genes not yet characterised in sorghum, that may play a crucial role in regulating root architecture in response to drought stress. Taken together, this work provides a mechanistic and practical framework for rapidly selecting germplasm as valuable pre-breeding material. The holistic approach used in this work is significant for designing nutritious, climate-resilient crops for future climate conditions and provides a monumental shift forward that our agricultural sector needs for sustainable food production.

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Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of Variance
AUX	Auxin
CSA	Climate-Smart Agriculture
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
GB	Glycine Betaine
GO	Gene Ontology
GSA	Gravitropic Setpoint Angle
IAA	Indole-3-acetic acid
IPCC	Intergovernmental Panel on Climate Change
KEGG	Kyoto Encyclopedia of Genes and Genomes
NPA	N-1-naphthylphthalamic acid
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RSA	Root system achitecture
RT-qPCR	Real time quantitative polymerase chain reaction

Chapter 1

Introduction

1.1 Impact of climate change and variability on crop productivity

Climate variability and change is increasingly impacting global agricultural productivity, through changes in temperature and water availability, limiting crop yield and grain nutrient content (Lynch, 2019, Ravi et al., 2020, MacAlister et al., 2020). Future climate projections predict more variable and extreme weather events due to rising temperatures, reduced precipitation, and frequent droughts (IPCC, 2019). At the same time, the global population is projected to grow to approximately 10 billion by 2050, which is predicted to require a 40-60% increase in global food production (Gerland et al., 2014, Falkenmark, 2020). It is also predicted that the number of undernourished people will increase by 150% in the Middle East and North Africa and by 300% in sub-Saharan Africa by the year 2080, compared to 1990 (Ceccarelli et al., 2010, Stein, 2014, Kumssa et al., 2015).

Sub-Saharan Africa has often been identified as one of the most vulnerable regions to climate variability and change (Challinor et al., 2007, Thornton et al., 2011, Ericksen et al., 2011, Davis and Vincent, 2017, Asmall et al., 2021). This is due to its heavy reliance on rainfed agriculture, which is highly sensitive to weather and climate variables such as temperature, precipitation, and extreme events coupled with limited capacity for adaptation (Kotir, 2011, IPCC, 2014, Gizaw and Gan, 2017, Mataya et al., 2019, MacAlister et al., 2020, Chaudhry and Sidhu, 2022). The climate in Malawi is already exhibiting significant changes, evidenced by changes in average temperatures, which have increased in all seasons and throughout the country (Sutcliffe et al., 2016). There is an overall drying trend and variability in rainfall amounts affecting seasonal patterns (Kotir, 2011, Mataya et al., 2019). These environmental changes will negatively impact agricultural crops and natural species (Bita and Gerats, 2013, IPCC, 2014).

In view of the associated impacts of climate change on food and nutrition security (IPCC, 2019), climate-resilient crop varieties and improved cropping systems are required to manage climate variability and to adapt to the increasing climate risks across Africa (Acevedo et al., 2020, Zimba et al., 2023). However, traditional crop breeding and varietal replacement cycles are slow processes that put farmers at risk in the developing world (Challinor et al., 2007, Atlin et al., 2017). Therefore, rapid crop improvement is critical for adapting cropping systems to climate change in sub-Saharan Africa (Sutcliffe et al., 2016). Findings from our review article highlighted that immediate action is required to enhance crops' resilience to climate change and sustainably improve crop yields to safeguard future food security (Benitez-Alfonso et al., 2023).

The following sub-sections provide an overview of climate-smart crops, with a focus on sorghum as a model for understanding drought tolerance and the effects of environmental (abiotic) stresses in plants.

1.1.1 Climate-smart crops

Climate-Smart Agriculture (CSA) is defined as agricultural practices that sustainably increase productivity and system resilience while reducing greenhouse gas emissions (Taylor, 2018, Venkatramanan and Shah, 2019, De Pinto et al., 2020, Zheng et al., 2024). CSA helps ensure that climate change adaptation and mitigation are directly incorporated into agricultural development planning and investment strategies (Matteoli et al., 2020, Hussain et al., 2022). ‘Climate-smart’ implies the development of a global solution to select stress-resilient crop species, including those bred for enhanced water and nutrient use efficiency, in combination with management techniques to reduce water use and preserve soil health (De Pinto et al., 2020). CSA is being widely promoted as the future of semi-arid and tropical agriculture and as a viable answer to climate change. The concept of nutrition-sensitive or nutrition-smart agriculture has recently been proposed by many stakeholders and it is significant for crop improvement (Balz et al, 2015). Although, the terms ‘Climate-smart’ and ‘climate-resilient’ are terms often used interchangeably, they have different contexts. According to IPCC glossary definitions of terms, in a broader context, the purpose of climate-smart agriculture is to implement farming techniques that target reducing greenhouse gas emissions, increasing agricultural productivity, and ensuring food security. CSA utilises adaptation methods to address the impacts of climate change on agriculture. On the other hand, climate resilience refers to the ability to foresee, prepare for, and recover from extreme climate-related events. Using resilience thinking can lead to long-term, holistic, and forward-looking planning. It is often blended with adaptation, and while it is related, it is distinct. Adaptation involves adjusting to the actual or expected climate and its effects (IPCC, 2018). Referring to crop production, ‘climate-smart’ implies the use of varieties to enhance adaptation to environmental change, while ‘climate-resilience’ refers to a combination of coping, adaptive and transformative capacities (Hellin et al., 2023).

1.1.2 Sorghum and millet as nutritious, climate-smart cereals

Sorghum and millets are among potential nutritious, smart-food cereals. They grow in harsh environments where other crops do not thrive (Kholová et al., 2013, Khalifa and Eltahir, 2023), and have low carbon footprints for production relative to other cereals (Dar et al., 2018). Sorghum and millets are C4 carbon cycle plants with high photosynthetic efficiency and productivity in low input conditions (Khalifa and Eltahir, 2023). Sorghum and millet are important cereal crops in arid and semi-arid regions where they contribute to food and nutrition security (Jiri et al., 2017, Zimba et al., 2023). These species are expected to have an increased importance in the future adaptation of agriculture to climate change globally (Kholová et al., 2013, Woomer and Adedeji, 2021). Sorghum uniquely fits production in such regions, due to high and stable water-use efficiency, drought and heat tolerance, high germplasm variability and comparative nutritional value (Hadebe et al., 2017). Sorghum and millets contain significantly higher amounts of nutrients especially micronutrients such as iron (Fe) and niacin among others when compared to other cereals (Saini et al., 2021). These grains are also increasingly important for industry, driven by the need for environmentally sustainable food crops and consumer demand for gluten-free products (Anunciacao et al., 2017, Alavi et al., 2019, Woomer and Adedeji, 2021). Sorghum and millets further provide a reliable source of phenolic compounds in a range of genetically distinct types and levels. Increased interest is due to their antioxidant activity and cholesterol-lowering properties, and they have the potential to have a significant impact on human health (Awika and Rooney, 2004). In addition, in Europe, sorghum has emerged as an alternative cereal for bioenergy production, due to its high biomass potential and tolerance to abiotic or environmental stresses (Hadebe et al., 2017, Parra-Londono et al., 2018, Khalifa and Eltahir, 2023). However, the crops have been neglected in terms of research and utilization, despite their potential benefits (Anunciacao et al., 2017).

1.1.3 Global production and consumption levels of sorghum and millet

In terms of global production and utilization, sorghum and millets are deemed to be the 5th and 7th most important cereals, respectively (Bhagavatula et al., 2013). Sorghum is widely grown covering an area of 44 million ha with production estimates of 62.5 million tonnes and productivity of 1.6 tons/ha globally. According to the world trend in production, sorghum and millet production has been relatively constant over the years, from 1994 to 2018, as shown below, figure 1.2 (FAO, 2019). On average top sorghum producer in the world is USA along with 12.1 million tons. Nigeria continues to be ranked as the second with 6.8 million tons of the production. The greatest share in the world's consumption of sorghum belongs to China. The top consumer of millet is India which is also the top producer of the said grain (FAO, 2019).

1.1.4 Sorghum production in Malawi

Sorghum is a significant crop in Malawi and plays a major role in ensuring food security for many farmers, particularly in the lower Shire valley region and many marginal areas where rainfall is a limiting factor for other major staples. Our study found that farmers have traditionally grown sorghum and pearl millet due to prolonged dry spells and frequent droughts experienced in most parts of Malawi which include Chikwawa and Nsanje (Zimba et al., 2023). 84% sorghum and millet are produced primarily for household food consumption. Despite sorghum being a preferred cereal in many African countries, Malawi still has a relatively underdeveloped value chain and limited production data. However, sorghum production forecast is projected to increase by 3.1% annually over the next five years, from 132,630 metric tons in 2021 to 159,210 metric tons by 2026 (https://www.reportlinker.com/dataset). This is due to an increase in demand for gluten-free foods, a number of products are now made from sorghum, including flatbread, muffins, tortillas, noodles, biscuits, and cakes. Beer, porridge, and stiff porridge are locally made sorghum products that enhance their current utilisation in urban populations (Taylor et al., 2006).

During our study in Malawi, stakeholders, including researchers, CGIAR representatives, and seed companies, outlined the potential for sorghum and millet as future crops in the context of community resilience to climate change (Zimba et al., 2023). However, the most commonly mentioned challenges include the accessibility of seeds for varieties with a wide range of desirable traits. It has been noted that sorghum and millet need formal seed producers and markets relative to major grains such as maize, which makes the seeds unavailable and costly (Munkombwe et al., 2020). These barriers to the availability of improved varieties impact the selection of a desirable variety that could enhance climate resilience. Sorghum and millet value chains have other challenges such as market availability, knowledge capacity on field and post-harvest technologies, and value-added skills and technologies. Enhancing the utilisation of these forgotten crops can be done by promoting sorghum and millet recipes and improving market accessibility. Another opportunity is the availability of natural genetic diversity within landraces, which can be used to select desirable traits for crop improvement for biotic and abiotic stress tolerance.

1.1.5 Abiotic stresses in plants

Plants encounter various abiotic stresses which include drought, nutrient deficiency, light intensity, salinity and extreme temperatures (Farooq et al., 2009a, Chaudhry and Sidhu, 2022). Climate change is profoundly exacerbating the impact of these stresses, by triggering physiological and biochemical responses in plants that affect growth and development and ultimately cause reduced yield and nutritional quality (Basu et al., 2016). As sessile organisms, plants sense and adapt to wide range of stresses to survive in their environment (Sharma et al., 2019, Kumar et al., 2019). Their complex responses involve alterations in their architectural plasticity, physiology, and molecular mechanisms. Exacerbated by shortage of water reserves, drought is the most serious risk to world food production (Halford et al., 2015, Farooq et al., 2009a, Gupta et al., 2020). Therefore, better insights and understanding of the impact of drought on plant growth and development and the mechanisms involved in plant response to drought are significant for developing strategies for crop adaptation.

1.2 Drought stress and its effects on plant growth and development

Agricultural drought can be interpreted as a deficiency of sufficient moisture needed by plants for growth and development (Farooq et al., 2012). In the more agronomic and physiological literature, drought is the water deficit that impairs plant growth and yield compared with the supply required for maximum or optimum growth (Lawlor, 2013). Drought stress affects plant growth and development, which include plant morphology and architecture, flowering, grain filling and maturation (Figure 1-1). These stages of development will be referred to in all other chapters of this thesis. Drought also affects important physiological and metabolic processes such as photosynthesis, enzyme activity, mineral nutrition, and respiration which ultimately result in yield quantity and quality loss (Maiti and Satya, 2014, Salehi-Lisar and Bakhshayeshan-Agdam, 2016).

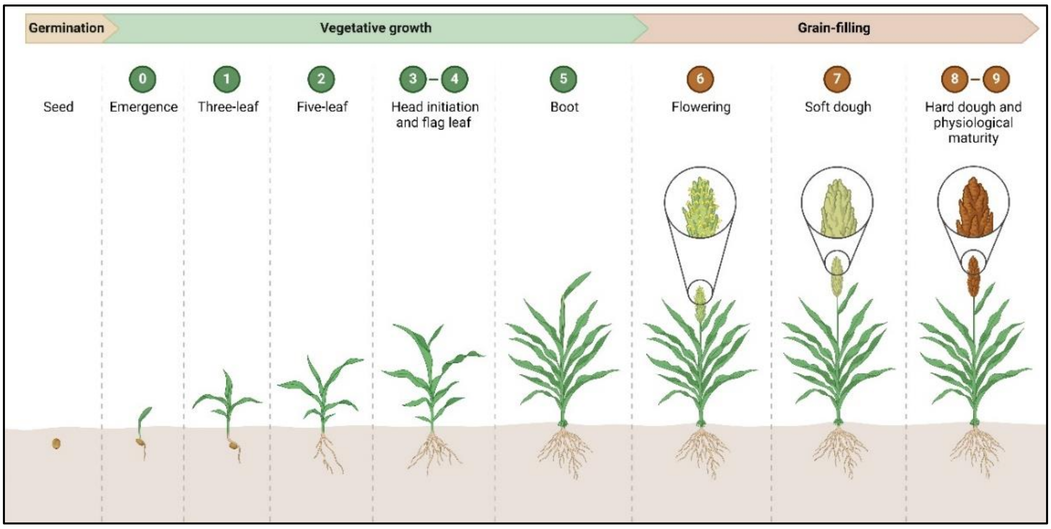


Figure 1-1: Summary of sorghum growth and developmental stages

Stage 0 represents the emergence stage, which takes approximately 0-10 days after seed sowing. Stage 1 takes approximately 10-20 days. Stage 2 to stage 4 takes approximately 20-45 days. Stage 5, booting and formation of flag leaf takes 45-60 days. Stage 6, the flowering or heading stage, takes 60-70 days. Stage 7 is grain filling and maturation (70-110 days). Stages 8-9 represent physiological and harvest maturation, which lasts approximately 110-140 days. Adapted from (Vanderlip and Reeves, 1972). Image created with BioRender.com.

The following section describes the biological processes that allow plants to detect and respond to drought. It will be focused on advances and knowledge gaps regarding drought stress effects on the root system architecture of sorghum compared to other cereals and on grain yield and nutrition quality. Further sections of this introduction will also provide insights into the selection of traits for improving drought tolerance in climate-smart crops.

1.2.1 Root system architecture in cereals and its role in drought sensing

Root system development is an important agronomic trait for crop improvement (Smith and De Smet, 2012, Lynch, 2013, Roychoudhry et al., 2013, Lynch, 2019). The Root System Architecture (RSA) for most cereal crops is composed of multiple embryonic (primary and seminal) and postembryonic (lateral, crown and brace) roots (Figure 1-2) The primary root is formed at the basal pole of the embryo, whereas seminal roots are formed at the scutella node (Rogers and Benfey, 2015). Many cereal species, such as rice, lack seminal roots entirely. Sorghum produces a sole seminal (primary) root and coleoptile nodal roots emerge at the 4th–5th leaf stage, whereas maize produces 3–7 seminal (primary and scutellum) roots and coleoptile nodal roots emerge at the 2nd leaf stage (Rogers and Benfey, 2015). The primary and seminal roots are important for early vigour and the exploration of the soil for nutrients and water (Rogers and Benfey, 2015, Del Bianco and Kepinski, 2018). Crown and brace roots (also called nodal roots) emerge from underground and aboveground shoot nodes, respectively, providing lodging resistance and playing an important role in the uptake of water and nutrients. Lateral roots are formed on all roots within the soil and also function to increase water and nutrient uptake (Smith and De Smet, 2012).

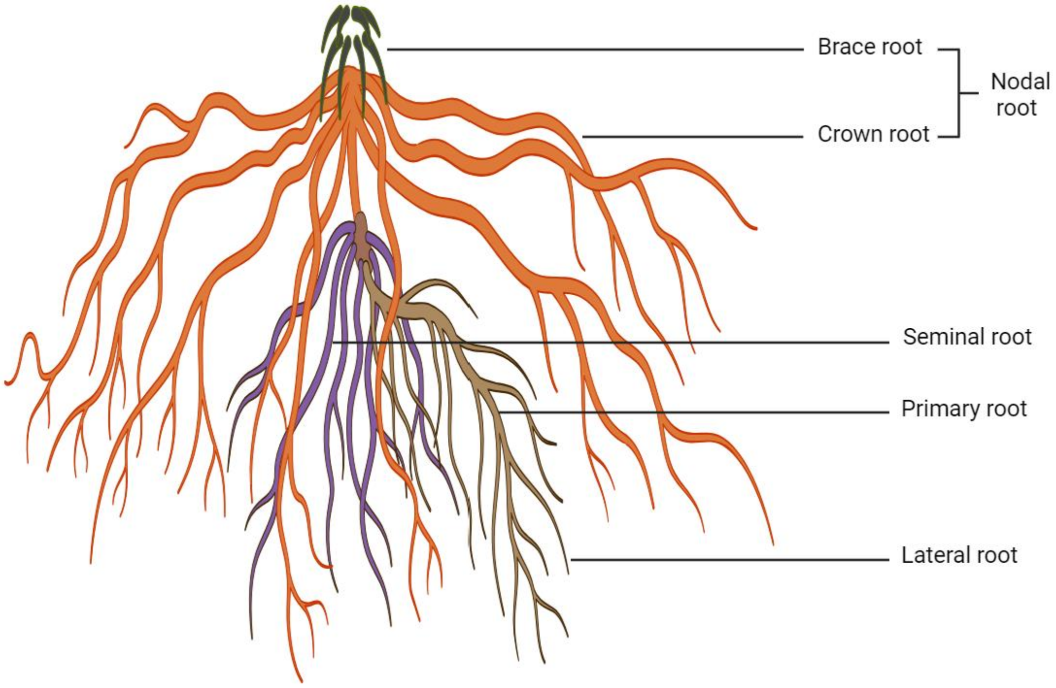


Figure 1-2: Schematic representation of root system architecture in cereals

Schematic representation of cereal RSA showing embryonic (primary and seminal) and postembryonic (lateral, crown and brace). Image created with BioRender.com.

Root system architecture, the spatial configuration of root system in the soil, plays a critical role in water acquisition and adaptation to drought but is poorly understood (Singh et al., 2012). Roots are the initial parts of the plant that encounter and sense drought stress and regulate water acquisition by penetrating deep into the soil (Singh et al., 2012, Comas et al., 2013, Amelework et al., 2015). RSA is influenced by interactions between genes, signaling molecules, and nutrient and water availability (Rogers and Benfey, 2015). Adaptation to drought-prone environments requires robust root architecture. Genotypes with a more vigorous root system have the potential to better adapt to soils with limited moisture content (Singh et al., 2012, Abdel-Ghani et al., 2019). However, root architecture is complex at both phenotypic and genetic levels. Previous studies have focused mainly on shoot systems, while root responses to drought have been largely ignored (Dolferus et al., 2011, Singh et al., 2012).

Root and shoot branches are major determinants of plant form and critical for the effective capture of resources below and above ground (Roychoudhry et al., 2017). The properties of root systems determine plant water access and can constrain shoot water status (Mokany et al., 2006). RSA can provide a growth advantage in specific environmental settings, for instance, drought, and directly influences the aerial parts of the plant that impact yield (Rogers and Benfey, 2015). Shoot traits may be related to root traits through genetic correlation or be dependent upon root traits through resource allocation trade-offs (Khasanova et al., 2019). The importance of the root system in supporting shoot growth has been studied and discussed under nutrient and/or water deficit conditions in most of major cereals such rice, wheat, and maize (Mir et al., 2012) but much less in the context of other cereals such as sorghum and millets.

1.2.2 Grain filling and nutrient accumulation in response to drought

The reduction of nutrient uptake leads to decreased plant growth and limits nutrient accumulation to achieve high nutritional value for human consumption (Soares et al., 2019). Increasing severity of drought stress affects pollen viability especially in self-fertilising cereals (wheat, rice, barley, sorghum) and developing florets abort prematurely during inflorescence development, leading to reduced grain yield (Dolferus et al., 2011). The impact of drought stress on grain quality is also important since it also affects the nutritional value, flavour, colour, and aroma of the food that is produced from it (Halford et al., 2015). It has been highlighted that the drought stress that affects grain composition and quality and how the stress interacts with physiological, molecular, and genetic factors needs more investigation in orphan cereal crops which include sorghum and millet (Halford et al., 2015). In particular, it is important to identify and focus on the most vulnerable stage of growth that affects yield formation under drought stress. Improving climate resilience could be achieved through a better understanding of the links between plant developmental status and drought stress effects and response (Dolferus et al., 2011, Ilyas et al., 2021).

1.3 Drought stress response mechanisms in climate-resilient crops

Plants in their natural habitats adapt to drought stress in the environment through various mechanisms, ranging from transient responses to low soil moisture to major survival mechanisms of escape by early flowering in the absence of seasonal rainfall (Farooq et al., 2014). However, economically important crops have been selected by humans to yield products such as grain, vegetable, or fruit in favourable environments with high inputs of water and fertilizer (Basu et al., 2016). For this reason, selected crops need to survive drought stress through mechanisms that maintain crop yield. Studies focusing survival of model plants under stress do not always take into consideration the effects on yield, the aspect of drought stress response that needs to be emphasized (Basu et al., 2016). The ability of a plant to sense the water-deficiency signal and initiate coping strategies in response is defined as drought resistance (Gupta et al., 2020, Liaqat et al., 2024).

Drought resistance (DR) is a complex trait or broader term applied to plant species with adaptive mechanisms that enable them to escape, avoid, or tolerate drought stress (Lawlor, 2013, Gupta et al., 2020). A drought escape mechanism is the ability of a plant to accelerate the plant's reproductive phase before the stress could hinder its survival. During drought escape, plants are able to modulate their vegetative and reproductive growth according to water availability, through two different mechanisms: rapid phenological development and developmental plasticity. Drought survival (DS) is a form of DR mechanisms in which cells, tissues, and organs that have ceased growing under drought (quiescent state) are able to maintain key cellular functions and recover rapidly to pre-deficit values with minimal damage, allowing resumption of activity once water becomes available again (e.g. photosynthesis) (Lawlor, 2013). Drought resistance and drought tolerance have a similar meaning, which is the ability to withstand and be unaffected by drought or water deficit. Although, the terms drought resistant and drought tolerant are poorly defined and are used interchangeably, Lawlor (2013) indicated that the term ‘drought tolerance’ is often used. In principle a drought-tolerant crop would have the capacity to withstand a water deficit without damage. Other scholars define, drought tolerance as a plant's ability to survive during drought. Drought resistant refers to plants that can survive long periods with no water at all (such as a cactus). In a more specific context, tolerance is the ability of plants to endure low internal water content while sustaining growth over the drought period (Basu et al., 2016, Gupta et al., 2020). Drought tolerance is a complex quantitative trait influenced by many genetic and environmental factors (Ceccarelli et al., 2010). In the last decade, although significant progress has been made in understanding the mechanisms of drought tolerance in model plants, it still remains an enormous challenge to translate fundamental knowledge into strategies to modify drought-tolerant crops in order to cope with future water shortages and the increasing demand for food production (Fang and Xiong, 2015). The following sections present the advancement in knowledge on some morphological, physio-biochemical, and molecular reactions of plants to drought stress.

1.3.1 Morphological mechanisms for drought tolerance

Root traits associated with maintaining plant productivity under drought include factors such as root length, branching and growth angle, which together determine the distribution of root surface area within the soil where nutrients and water are unevenly distributed (Comas et al., 2013, Lynch, 2013, Del Bianco and Kepinski, 2018). Sorghum roots may grow to depths of 1 to 2 m by the booting stage and can efficiently extract water to a lateral distance of 1.6 m from the plant (Routley et al., 2003). Root growth in sorghum terminates at the flowering stages. The optimal ‘steep, and deep’ root system ideotype, has been proposed to enable plants to exploit moisture from the deeper soil horizons by maximising soil exploration for optimum water and nutrient acquisition (Habyarimana et al., 2004, Malamy, 2005, Lynch, 2013, Lynch, 2019). Despite this, the optimisation of root phenotypes for improved water and nutrient capture under different drought conditions are still an underexploited opportunity for global agriculture (Lynch, 2019, Lynch, 2022).

1.4 Physiological and biochemical drought tolerance mechanisms

Several physiological mechanisms are linked to high performance of cereals, particularly sorghum under water deficit. At an early stage of water deficit, plants may partially close stomata to reduce water loss from transpiration and alter the metabolism to match the available carbon produced. (Figure 1-3) below summarise drought effects and physiological stress responses in plants. Sorghum uses C4 photosynthesis complex biochemical and physiological specialization that improves carbon assimilations (50 g/m2/day) at high temperatures compared to other major cereals (Badigannavar et al., 2018). As stress conditions increase, some osmolytes such as prolines, soluble sugars, spermines, and betaine accumulate in plant cells to maintain the cell turgor pressure (Fang and Xiong, 2015). Variations in the activities of numerous oxidation-protective enzymes such superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) are also frequently observed in drought-stressed plants (Fang and Xiong, 2015). The accumulation of free proline in leaves of water-stressed sorghum is linked to the plant's resilience to stress. It is suggested that proline is a source of respiratory energy when plants are recovering (Buchanan et al., 2005). According to Amelework et al. (2015), in sorghum, proline, glycine betaine (GB), and sugars function as osmolytes that protect cells from dehydration. The GB accumulation in cells can assist plants in either maintaining water within cells or protecting cellular components from dehydration (Chen and Murata, 2011). However, the genetic and metabolic basis of variation in GB accumulation is not well understood in sorghum (Amelework et al., 2015). Some studies have indicated that sucrose accumulation was observed in the stem of non-senescence (stay-green) sorghum genotypes at the flowering stage (Kouressy et al., 2008). This may delay the onset of senescence and maintain a high photosynthetic rate during the grain-filling stage. Under conditions of severe terminal drought, these sugar reserves in the stem may be translocated to the developing grain.

Stay-green is a post-anthesis drought resistance trait that provides resistance to pre-mature leaf senescence when the plant is under severe moisture stress conditions during grain filling stage. It contributes to an improved yield and yield stability under moisture stress condition (Tao et al., 2018). The stay-green trait is associated with a higher level of chlorophyll content, cytokinin, and leaf nitrogen concentration under moisture stress conditions (Amelework et al., 2015, Fang and Xiong, 2015). The underlying mechanisms that differentiate drought resistance from susceptibility within a species, especially for these under-researched minor cereals under this study, remain largely unclear.

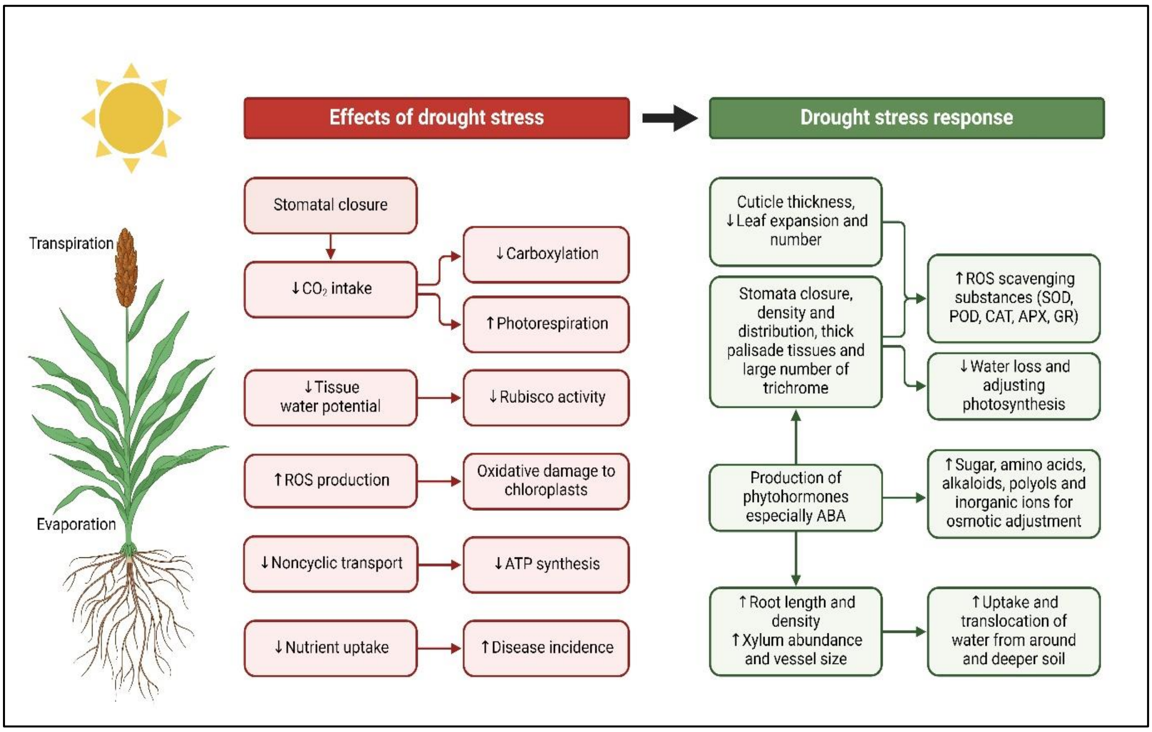


Figure 1-3: Summary of drought effect and responses

Drought affects the reduction of many metabolic processes for growth and development. In response, plants develop mechanisms aimed at survival and adaptation. Adapted from (Ilyas et al., 2021). Image created with BioRender.com.

1.5 Drought impacts on soils and strategies to build crop resilience

Soil is a crucial component of the biosphere and drought stress has negative consequences for the functioning of agricultural soil ecosystems (Geng et al., 2015). Extreme droughts have serious impacts on the pools, fluxes and processes of terrestrial carbon (C) and nitrogen (N) cycles. It has inconsistent effects on net N mineralization and fluxes in soils from various climates, soil and ecosystem types (Deng et al., 2021). Drought has an impact on soil carbon stocks, affecting both aboveground carbon processes and inputs from above- and belowground biomass and rhizodeposition, as well as outputs from soil organic matter (SOM) mineralization (Knapp et al., 2015, Canarini et al., 2017). A significant increase in atmospheric CO_2 content and impact on global warming can be caused by even small losses in the soil C pool, which is the largest in terrestrial ecosystems. Patterns of drought effects on soil carbon dynamics remain unclear despite extensive previous research (Deng et al., 2021).

Furthermore, drought water stress limits the availability of water and nutrients to the plants that develop on these soils and soil organisms activities. Soil management that can improve a soil's water retention capacity can be considered a high-priority demand in a changing climate (Benitez-Alfonso et al., 2023). For instance diversified cropping systems increase soil microbial biomass and activity (and the microbial population-microbiome, in the vicinity of the plant root system), leading to better soil structure, organic matter, and improved water retention (Benitez-Alfonso et al., 2023). Rotation with crops with different water requirements is a promising strategy that mitigates adverse effects in drought-prone areas. For example, cultivating crops with a low water requirement during seasons of high-water availability could conserve soil water for the next season’s crop. Crop diversification and intercropping also results in phenotypic plasticity, enabling plants to capture more available resources for example, through increases in root biomass for foraging nutrients. However, selecting the best suitable crop combinations in intercropping is crucial in drought-prone areas. selection of the best suitable crop combinations in intercropping is crucial in drought-prone areas. For example, the classic cereal–legume intercropping system, benefits the crops through improving soil fertility, increasing soil carbon and water infiltration, and a significant gain in soil moisture during drought periods, especially in sandy soils (Benitez-Alfonso et al., 2023). Mixing plants with deep and shallow roots (such as bean–maize, sorghum–red clover, and maize–grass pea) makes better use of the available soil moisture by enabling exploration of deep soil and subsoil. Such root combinations allow bio-drilling (where long tap-roots remain in the soil, providing channels for water drainage) which enhances soil structuring, increases soil organic matter and promotes soil aggregation, thereby improving the storage and availability of soil water in the root zone.

1.5.1 Root exudates and rhizosheath biology of drought-tolerant crops

A much-underexplored aspect of root biology relates to the root production of exudates and mucilage, a process that enables plants to influence their immediate surroundings and holds the potential to promote tolerance mechanisms to abiotic stresses (Brown et al., 2017, Galloway et al., 2020b). Work is beginning towards developing an in depth understanding of roots exudates or mucilage involved in the formation of rhizosheath. Rhizosheath is known to maintain the contact between roots and soil improving water and nutrient uptake (Haling et al., 2013, Pang et al., 2017). The rhizosheath provides a dynamic zone for water and nutrient interchange at the root–soil interface, which can improve drought resistance and can protect roots against other abiotic stresses (Brown et al., 2017). Although the rhizosheath is known to be beneficial for drought resistance in many plants, the regulation of rhizosheath formation in sorghum is unclear. Some recent studies have indicated that moderate water stress increased rhizosheath formation in foxtail millet, suggesting the rhizosheath is an important drought-adaptive trait (Zhang et al., 2020). The extent to which sorghum and millet rhizosheath formation can contribute to improve below-ground resource capture and drought adaptability in crop improvement programmes is not known.

1.6 Genetic and molecular mechanisms for drought tolerance

1.6.1 Genetic regulation of RSA under drought stress

General responses of plants to drought stress include the altered expression of numerous genes, such as those related to stress signal transduction, and the transcription and regulation of thousands of functional proteins, which collectively contribute to the molecular control of drought resistance (Fang and Xiong, 2015, Takahashi et al., 2020). Advances in molecular biology tools and the development of techniques such as microarray and RNASeq have enabled the analyses of transcriptomes in many species under wide range of environmental conditions (Dugas et al., 2011). This information has increased the understanding and identification of candidate genes under stress conditions. For instance, some regulators of drought-stress responsive genes have been identified in sorghum, belonging to the AP2/ERF and bZIP family of transcription factors (Joshi et al., 2017). However, it has also been shown that protein translation and post-translational regulation play a vital role in stress adaptation (Ghatak et al., 2017). Some studies observed a variation in the timing of induction and expression of drought related genes classifying the genes into two groups. The first group is responsible for proteins that function directly under stress tolerance, while the second one produces protein factors involved in the regulation of signal transduction and gene expression under drought (Yamaguchi-Shinozaki, 2002, Takahashi et al., 2020). The expression of genes related to water deficit in plants is found to be induced not only by water stress and desiccation, but also by the hormone abscisic acid (ABA). For example, the drought-induced inhibition of lateral root growth is partly mediated by ABA in the model plant Arabidopsis (Seo et al., 2009). In addition, plants that are sensitive to ABA are more drought tolerant than those insensitive to ABA (Xiong et al., 2006). It was also found that ABA-insensitive plants have higher transpiration rates and lose water much faster than ABA-sensitive plants (Yamaguchi-Shinozaki, 2002). Applying omics tools, pathways to drought mechanisms can be elucidated using the information on transcripts, proteins, and metabolites produced in response to stress. This information can outline the mechanisms of response to drought and thereby facilitate identification of potential targets for plant breeding.

RSA response to environmental extremes is determined by a combination of genetic and environmental components (Amelework et al., 2015). Regulation of root system architecture allows deeper root penetration and is intrinsic to water and nutrient capture, but the processes underlying these responses are poorly understood and remain underexploited in agriculture (Lynch, 2022, Kalra et al., 2024). Modifying the root system architecture of a plant can improve drought avoidance, for example, through the cloning and characterization of DEEPER ROOTING 1 (DRO1), a quantitative trait locus known to regulate root growth angle in rice (Uga et al., 2013, Uga et al., 2015). DRO1 is negatively regulated by auxin and is involved in cell elongation in the root tip, which causes the downward bending of the root in response to gravity (Uga et al., 2013, Roychoudhry and Kepinski, 2015, Ge and Chen, 2019). In maize, the ideotype for ‘steep, deep’ rooting, is characterized by steeper root growth angles, fewer axial roots, reduced lateral branching, and anatomical characteristics that reduce the metabolic cost of root tissue (Lynch, 2022, Lynch, 2013). Despite these efforts, our understanding of root structural changes, function and molecular responses to stresses is still limited (Zhang et al., 2022, Benitez-Alfonso et al., 2023). An open question is how root traits interact with the environment for optimized resource use efficiency (Maqbool et al., 2022).

1.6.2 The role of phytohormones in drought tolerance

Although auxin is known to modulates root architecture under stress (Gupta et al., 2020), some scholars indicate hydrotropic root responses function relatively independently of auxin and involve ABA signalling in root elongation zones. The overall role of phytohormones and their signalling pathways in drought stress are still unclear (Gupta et al., 2020). It is argued that upon exposure of plants to drought stress, ABA is the major hormone synthesized in roots and translocated to leaves to initiate adaptation of plants to drought stress through stomatal closure and reduced plant growth. ABA signaling genes (Wang et al., 2024), such as *OsNAP*, *OsNAC5*, and *DSM2*, promote improved yield under reproductive drought (Kim, 2014). However, modulating the ABA-induced drought adaptation of plants for better yield remains a greater challenge because of the potential inadvertent reduction in carbon gain upon stomatal closure and ABA-induced senescence, especially if the drought occurs at the reproductive stage. Brassinosteroid hormones also regulate drought response through signaling components linked to the ABA response pathway (Gupta et al., 2020)Similarly, scholars have further urged that auxin signaling enhances drought resistance by interacting with other phytohormones during drought stress. A decline in auxin levels under stress conditions can increase ABA levels in plants and induce growth modulation. Gupta et al. (2020) suggested that noncanonical auxin responses via EXO70A3 and PIN4 can modulate root architecture patterning and depth to boost water absorption from the soil, thereby improving drought tolerance. Despite this progress in knowledge, more work is still required to decipher the role of auxin coupled with other hormonal interactions in complex mechanisms and pathways of drought tolerance and its involvement in root plasticity. For this reason, this study aims to contribute to a better understanding of auxin-related developmental pathways during drought in climate-smart crops.

1.6.3 Quantitative Trait Loci for drought adaptability in sorghum

Several Quantitative Trait Loci (QTLs) identified in crops controlling RSA establishment suggest that there are biological trade-offs in root growth allocation that directly impact nutrient acquisition and yield (Rogers and Benfey, 2015). QTLs involved in controlling traits related to yield and yield components, root systems, stay-green traits, plant height, flowering, and maturity have been mapped on 10 linkage groups in sorghum (Sanchez et al., 2002). Additionally, several traits related to drought resistance have been identified and mapped (Amelework et al., 2015). However, the stay-green trait is recognized as the most crucial drought resistance trait in sorghum. Under terminal drought, the stay-green trait has a positive impact on grain yield. Several stay-green QTLs are identified and co-localized for grain yield, flowering time, and plant height. Four major stay-green specific QTLs viz., Stg1 (on SBI-03), Stg2 (SBI 03, Stg3 (SBI 02) and Stg4 (SBI 05) have been correlated to reduced leaf senescence and found to be stable across different environments (Amelework et al., 2015). A further four QTLs for nodal root angle (qRA1_5, qRA2_5, qRA1_8, qRA1_10), three QTLs for root dry weight (qRDW1_2, qRDW1_5, qRDW1_8) and eight QTLs for root volume, root fresh weight and root dry weight were identified by (Mace et al., 2012). One of the root angle QTLs is co-located with QTL for stay-green in sorghum and is associated with grain yield (Mace et al., 2012), which indicates a correlation. Two QTLs (qRT6 and qRT7) associated with brace roots have been mapped on sorghum Chromosome 6 and 7 (Amelework et al., 2015). Taken together, these studies have increased our knowledge on understudied crops like sorghum and built the basis for this study and future genetic studies.

1.7 The role of Phenomics: Integrating genomics and phenotyping to identify traits that enhance drought tolerance

Regulation of gene expression is an important mechanism against drought stress. Classical breeding strategies and biotechnological interventions (genetic engineering, gene editing) together can help in improving drought tolerance in plants by producing drought-tolerant genotypes. There is a need to identify the adaptation traits in different plants and molecular mechanisms behind genotypes that tolerate the adverse effect of drought stress without affecting productivity

(Bashir et al., 2021). Plant Phenomics is an approach to envision complex traits that are appropriate for selection and enable us to trace causal links between genotypes, environmental factors, and phenotypes (Pasala and Pandey, 2020). Plant phenotype is a complex interaction between the genotype and specific environmental conditions that has an impact on plant growth and development (Houle et al., 2010). The technique of plant phenotyping can be used in different dimensions, from the gene to the whole-plant level, under specific environments and management practices, which makes phenomics frequently justified (Pasala and Pandey, 2020).

As an analogy to genomics, phenomics has recently gained popularity among scientists and recommends using high-throughput phenotypic-driven tools to answer complex research questions (Chen et al., 2022, Zavafer et al., 2023).

1.7.1 Genomics approaches for trait identification

Genomics approaches with recently evolved sequencing platforms have enabled us to study the molecular basis of plants responses to drought (Tuberosa and Salvi, 2006). High throughput phenotyping platforms enable huge multi-trait, multi-environment data collection under different stress conditions. However, analysis and interpretation of these complex data is challenging (Atkinson et al., 2019). Therefore, genomics tools including gene editing, together with phenotypic and physiological experiment using controlled conditions can be integrated to elucidate genes governing complex traits under drought stress response (Cattivelli et al., 2008).

1.7.2 Phenotyping techniques for screening root traits

Apart from genotyping quantitative traits, high-throughput root phenotyping has been a major challenge for plant breeders in improving abiotic stress tolerance in crop plants. While this includes complex traits that are extremely difficult to measure, these data would be ideal for assisting plant breeders in developing new drought-tolerant varieties (Wasaya et al., 2018). Efficient screening methods coupled with high throughput phenotyping techniques are required for breeding climate-resilient crops (Badigannavar et al., 2018). Conventional methods of root phenotyping are time and labour consuming, and often destructive in nature. This leads to an under-explored opportunity to integrate genetics and physiology to understand the molecular basis of a wide range of plant processes under extreme climatic conditions. Furthermore, screening of root traits at early stages of plant development can be used as proxy for understanding traits at mature stages, but verification is needed on a case by case basis to be able to link the studied traits to increased crop productivity under drought (Comas et al., 2013).

Invasive or destructive methods of plant phenotyping are now replaced by the high-throughput precise and non-destructive imaging techniques able to quantify the shape, distribution, intrinsic root network size, and exploration of RSA (Badigannavar et al., 2018). For instance, rhizotrons, paper pouches, and plates are technologies for 2D root phenotyping. While, some technologies, including Magnetic Resonance Imaging (MRI), Positron Emission Tomography, and X-ray Computed Tomography, allow for 3D and 4D root phenotyping in soil (Shrestha et al., 2014, Atkinson et al., 2019). These methods have been used to identify any quantitative trait loci (QTL) controlling RSA as well as central genomic regions controlling root growth in juvenile plants. These methods have been used to identify many quantitative trait loci (QTL) controlling RSA as well as central genomic regions controlling root growth in immature plants. Research linking phenotypic and developmental traits of different genotypes and crop species during stress conditions is required. Overcoming the technical challenge of assessing root traits will allow breeding for optimal resource acquisition and plasticity to respond to adverse climate conditions (Zhu et al., 2011, Mir et al., 2012).

1.7.3 Relevance and shortfalls of genomics and phenomics as a route for trait identification

The climate resilience of agricultural crops could be improved by conventional breeding, but this is a relatively slow process. The integration of high-throughput technologies approaches could accelerate and increase the precision of trait selection in plant breeding programs (Benitez-Alfonso et al., 2023). These plant phenomics technique are very relevant in stress identification, study physiological processes, rapid and efficient screening, dissection and confirmation for understanding the genetic basis of different traits, genes and aspects. High-throughput phenomics technologies are essential to avoid human error and to reduce time consumption while phenotyping large germplasm populations, or for confirmation of gene or trait functional analysis (Pasala and Pandey, 2020).

Despite the outlined benefits, phenomics data collection remains costly and time-consuming, even harder in root biology where there are additional challenges in *in situ* root phenotyping (Atkinson et al., 2019). Data analytics and management is another concern where phenomic data, raise the possibility of addressing the 'many-to-many' relationships that are inherent and cause challenges for data interpretation (Yang et al., 2020, Houle and Rossoni, 2022). For instance, Transcriptomics and gene expression studies, nucleic acid-based measurements of transcriptomes, and epigenomes are the most widely known sources of extensive phenomic data (Pasala and Pandey, 2020).

1.8 Project overview and objectives

This project builds on ongoing sorghum and millet research aimed at improving yield and nutritional quality in dryland agricultural systems including sub-Saharan Africa. Despite some progress in breeding, the molecular, structural, and physiological basis of drought adaptation, particularly in relation to roots, is poorly understood (Fang and Xiong, 2015, Anders et al., 2021, Bashir et al., 2021). Similarly, the effects of drought stress on nutritional content are not well characterised (Soares et al., 2019). For many minor crops, including sorghum and millets, progress in breeding programmes could be significantly enhanced by a better understanding of their root biology. Phenotypic changes, physiological and molecular response to drought are species-dependent. It is therefore vital to understand specific crop responses to these stresses, to identify novel sources of genetic materials and rapidly develop climate-smart crops and varieties with greater resource use efficiency and climate resilience.

Interdisciplinary approaches together with the application of integrated high-throughput phenotyping, genotyping, crop management as well as stakeholder engagement (Benitez-Alfonso et al., 2023), would enhance our capacity to predict crop performance in future climates and also to identify traits that can potentially be improved or exploited to obtain higher and more stable crop yields under stressed environments (Prasad, 2020). It is against this background that the present research aims to integrate phenotyping and genomics approaches in understanding drought tolerance, and to contribute to the body of knowledge through identifying traits for current and future climate resilience. Understanding this holistic process in drought-prone regions will provide a practical framework for the targeted selection of germplasm as valuable pre-breeding material for incorporation in crop improvement programmes aimed at achieving sustainable food security in sub-Saharan Africa and many developing countries.

The interdisciplinary nature of this research is reflected in the specific objectives below, which are critical building blocks in addressing the previously identified gaps. Firstly, a survey was conducted to understand how farmers select the traits based on phenotypes in this era of climate change, and what could be the trade-offs in terms of the selected traits and economic yield with nutritional quality under drought stress. Secondly, investigating how drought influences root phenotypic changes and how this process is regulated at molecular level. These steps were reflected in the following specific objectives.

1. Identification of farmers' preferred sorghum and millet phenotypic traits and climate-smart cropping system in drought-prone areas of Malawi
2. Assessing natural variation and trade-offs in phenotypic traits and physiological functions linked to grain nutritional qualities in drought stress
3. Characterisation of root system architecture and polysaccharides root exudates across sorghum genotypes
4. Uncovering structural and molecular mechanisms underlying root growth angle regulation in response to drought stress.

Chapter 2

Materials and Methods

2.1 Plant material and growth conditions

2.1.1 Plant material sources

The main crop species used in this project are sorghum, millet, maize and rice. Table 2-1 shows the planting materials used in this project and their original sources.

Table 2-1: List of plant lines used in experiments in this project

Line	**References/source**
1. Sorghum seeds	
E36-1	ICRISAT, India
S35	ICRISAT, India
K214	ICRISAT, India
K258	ICRISAT, India
S35-SG-06-040	ICRISAT, India
S35-SG-06-016	ICRISAT, India
S35-SG-06-024	ICRISAT, India
S35-SG-06-040	ICRISAT, India
S35-SG-06-024	ICRISAT, India
S35-SG-06-026	ICRISAT, India
S35-SG-06-008	ICRISAT, India
S35-SG-07-001	ICRISAT, India
IS30400	ICRISAT, India
IS20727	ICRISAT, India
IS12697	ICRISAT, India
IS12706	ICRISAT, India
IS13893	ICRISAT, India
IS24503	ICRISAT, India
IS26749	ICRISAT, India

IS27786	ICRISAT, india
IS29627	ICRISAT, India
IS30508	ICRISAT, india
IS8774	ICRISAT-India
Thengeramanga	Seed/gene bank, Malawi, DARS
2. **Pearl millet *(landrace)***	Seed/gene bank Malawi, DARS
3. Maize seeds	
DKC 8033	Bayer, Malawi
4. Wheat	
Wt Cadenza	Kepinski lab, University of Leeds
5. Rice	
IR64	Kepinski lab, University of Leeds
6. Foxtail millet	
Cultivar Maxima	A. Baker lab, University of Leeds

2.1.2 Seed sterilisation

Seeds were surface sterilised using Chlorine gas or 20% v/v sodium hypochlorite solution (commercial bleach). For Chlorine gas, seeds contained in open 1.5 mL tubes were placed inside a desiccator jar under a fume hood and exposed to chlorine gas over a 2 to 3-hour exposure period. Chlorine gas was created with 3 mL of 37% hydrochloric acid and 100 mL of liquid bleach. After the exposure, the seeds were subsequently ventilated for 1 hour in a laminar flow hood before being directly sown onto their growth medium or left pre-germination on wet filter paper. For the bleach method, seeds were soaked in 20% v/v bleach solution for 20 minutes and washed with sterile deionized water 5 times under a laminar flow hood.

2.1.3 Plant growth media preparation depending on experimental design

Seeds were plated either on 120 mm or 245 mm square plates containing 45 mL and 100-200 mL of either Hoagland's No. 2 or Murashige and Skoog half-strength growth, containing 1% w/v sucrose and 0.8% w/v plant agar (Duchefa Biochemie B.V., Netherlands) The growth media were prepared according to the recipe shown in Table 2-2 for Hoagland and Table 2.3 for MS media. Ingredients were dissolved in autoclaved deionized water (dH_2O) and the pH for the media was adjusted to 5.8+/-0.2.

Table 2-2: Composition of Hoagland growth media

Ingredients	**Concentration (mg/L)**
Potassium Nitrate	606.6
Calcium Nitrate	656.4
Magnesium Sulphate	240.76
Ammonium Phosphate Monobasic (Ammonium Dihydrogen Phosphate)	115.03
Manganese Chloride Tetrahydrate	1.81
Boric Acid	2.86
Molybdenum Trioxide	0.016
Zinc Sulphate (Heptahydrate)	0.22
Copper Sulphate (Pentahydrate)	0.08
Ferric Tartrate	5

Table 2-3: Composition of MS media

Ingredients	g/L
MS-Salts (half-strength)	2.2
MES buffer (6mM)	4.2
Agar	15
Sucrose	10

2.1.4 Hormonal stock solution

The following stocks of plant hormones (Table 2-4) were used for the addition of hormones to plant growth media according to the experiment:

Table 2-4: Preparation of plant hormone stock solutions

Hormone	Solvent	Stock Concentration (mM)
IAA	70% EtOH	1,10 and 100
NPA	DMSO	25
2,4-D	70% EtOH	100

2.1.5 Plant growth conditions

Seeds were pre-germinated on wet filter paper (using sterile deionized water) in Petri dishes for 24 hours in the dark under controlled growth chambers. The temperature in the growth room was maintained at 20-25°C (+/- 2^{o}C). For auxin sensitivity experiments, seedlings were allowed to grow on solidified growth media in 120 mm square plates or liquid media in 100 mm growth pouches (CYG-Mega-International, Minnesota, USA) for 5 days and thereafter transferred to 240 mm plates or new 300 mm growth pouches (CYG- Mega-International, Minnesota, USA) containing media supplied with DMSO/70% EtOH or Indole-3acetic acid (IAA) dissolved in DMSO/70% EtOH. Plates or pouches were incubated in a plant growth room which was set and controlled under a long-day photoperiod (16 hr/ day) at 20-25^{o}C, with 60-75% humidity and light intensity of ~120umol/m2S-1 from white, fluorescent tubes.

2.2 Root phenotyping techniques and physiological experiments

Root phenotyping was carried out at several scales, varying in the throughput and granularity of analysis. All phenotyping techniques used in this thesis are as detailed below.

2.2.1 Screening of root seedling architecture in 2D System

Root phenotyping using a 2-dimensional growing system was carried out using pouches and plates. For pre-screening of a 2-dimensional system, pre-germinated seeds were placed in pouches and wrapped in aluminium foil to exclude light from the roots (Atkinson, Wingen et al. 2015). Depending on the experimental purpose, the pouches were placed in a walk-in growth chamber for 10-15 days. Similarly, 245 mm square plates were used for 2-D screening sorghum seedlings with emerging nodal roots.

2.2.2 Colander experiments for root angle quantification in 3D visualisation

To test the hypothesis of how drought influences root structural change, plants were grown in a colander in a controlled environment adapted from (Uga et al., 2013). The colander assay provides a simple tool for three-dimensional visualization and quantification of the nodal root angle. In this simple assay, 20 cm diameter kitchen colanders were buried in pots, with the upper edge of the colander level with the soil surface. The colander was filled with compost soils, a loam based compost which is a naturally reduced peat mix (John Innes No2 potting soil compost). One pre-germinated seed was then planted in the centre of the colander area and left to grow in the glasshouse according to the experimental plan. The temperature in the glasshouse was maintained at 25-27°C. Water was irrigated on top of the pots and measured to maintain field capacity.

For the drought experiment, plants were divided into two treatments: well-watered and water stress after 6 weeks of growth (approximately stage 2 to 3 of sorghum vegetative growth, Chapter 1, Figure 1-1). Severe drought stress treatment was applied to plants under stress treatment by withholding water for two weeks. Thereafter, the colanders were retrieved according to experimental purpose and a mark was made next to each hole from which each root emerged. The number of seminal roots emerging from each tier was counted and the root emergence angle from each tier was then calculated using trigonometry as shown in Figure 2-1 (Uga et al., 2013, Uga et al., 2011).

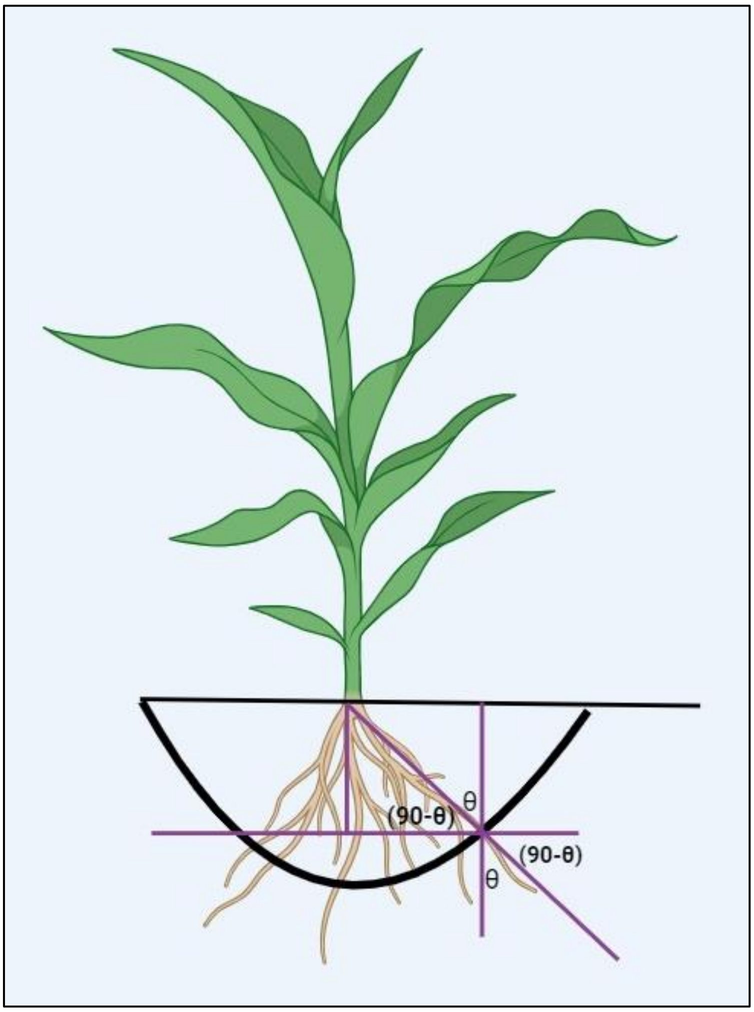


Figure 2-1: A schematic representation of a plant during the colander experiment

Diagram showing calculation of root angle using ‘colander’ method. The colanders had 16 tiers of holes in the sides and 8 concentric rings of holes in the base; a 3 mm border of plastic separated the holes. The diameter across the colander and the depth from the rim of the colander were measured for each row of holes. From this the radius of the colander could be calculated, this would act as the adjacent, the depth would act as the opposite. The length of the hypotenuse was calculated and used to work out (90-θ) in the equation “Adjacent/Hypotenuse= Cosine (90-θ)”. From this, the value of θ was calculated; this is the angle at which the roots emerged from the colander at the row of holes.

2.2.3 Calculation of deep rooting ratio in colander

The ratio of deep rooting was used for the quantification of deep rooting in sorghum grown from the colander method. The ratio of deep rooting (RDR) is defined as the number of roots that penetrated the lower part of the colander (≥50° from the horizontal, centred on the stem of the rice plant) divided by the total number of roots that penetrated the whole colander (Uga et al., 2011). A larger value for the ratio of deep rooting means that a greater proportion of the roots grew vertically downward.

2.2.4 Reorientation assay

For lateral root gravitropic setpoint angle (GSA) analysis, seeds plated on Hoagland or Half strength MS in square petri dishes were grown vertically in a growth room for 12 days. A reorientation of 30° with respect to the gravity was used to gravi-stimulate lateral roots so that upward bending roots were gravi-stimulated at 30° below their original angle, and downward bending roots were gravi-stimulated 30° above their original angle. After that, plates were scanned using an Epson Perfection V370 or V800 photo scanner or photographed using a RICOH GR II camera. Lateral root (~1 mm) tip angle was measured using FIJI/ImageJ.

2.2.5 Phenotypic and physiological measurements

For shoot phenotypes, observed and measured parameters were plant height, biomass, leaf count and stem diameter. For root phenotypes measured parameters were root length, lateral root count, root hull area, root volume, root growth angle. Grain parameters analysed include seed weight, panicle length, and seed size. Plants were imaged with a RICOH GR II camera for further analysis according to the experimental plan. For root Images were analysed using RootNav according to Pound et al. (2013) and WhinRhizo^{TM} Software (Regent Instrument, Canada). Chlorophyll content was determined using (SPAD values, chlorophyll meter, Hansatech Instrument Ltd) and chlorophyll fluorescent was measured using Chlorophyll Fluorimeter (Fluorometer, Hansatech Instrument Ltd).

2.3 Root exudates and rhizosheath quantification

2.3.1 Root exudates collections

This section outlines specific methods conducted on epitope profiling of root exudates in sorghum and compared to other cereals. Epitope mapping of sorghum high-molecular-weight root exudates was performed with monoclonal antibodies using ELISA (enzyme-linked immunosorbent assay), Nitrocellulose assay and immunolabelling methods of detecting polysaccharides (Galloway et al., 2020a, Akhtar et al., 2018).

Firstly, a four-hour assay of collecting exudates was used (Figure 2-1). For this method seedlings grown for 5-10 days on agar under controlled environment (as described in section 2.1.5 of this chapter) were removed and immersed in deionised water in 7 mL bottles. They were left to exude in de-ionised water for four hours. Since the seeds and shoots may also exude, they were prevented from touching the water in the bottle.

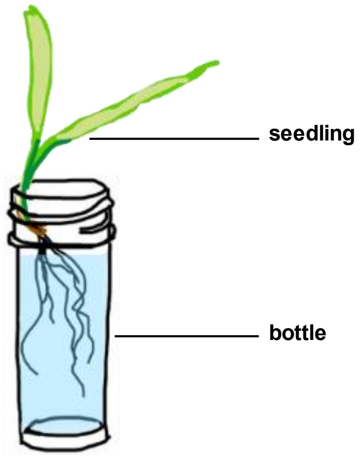


Figure 2-2: A schematic diagram of 4-hour root exudate method

A 7 to 10 days old seedling immersed in a sterilised 7ml bottle containing deionised water and left for four hours for roots to exude.

Hydroponic exudates collections systems makes it possible to control variables like nutrient concentrations, pH, and microbial populations with precision, which can be a source of inconsistency in soil studies. This enables the isolation of the effects of root exudates and studies their composition and functions with greater clarity and ability to quantify, providing new insights into Rhizosphere interactions. Hydroponic collections of exudates not only minimize root damage, thereby encapsulating almost all rhizodeposits, but also require fewer manipulations in downstream analysis (Oburger and Jones, 2018, Subrahmaniam et al., 2023).

However, exudates collected from hydroponics setting have limited representation of the soil reality complexities (Williams et al., 2021). Soils are complex matrices that are comprised of organic matter, minerals, and diverse microbial communities. The interaction of root exudates with these components results in adsorption, chemical transformation, and biodegradation. Hydroponic systems cannot replicate these processes, as exudates remain unaltered in the liquid medium (Oburger and Jones, 2018).

2.3.2 Enzyme-Linked-Immunosorbent-Assay (ELISA)

In order to profile the diversity of exudates produced from sorghum roots, epitope mapping of sorghum high-molecular-weight root exudates was performed with monoclonal antibodies using ELISA kit. This plate-based assay technique detects and quantifies soluble substances such as peptides, proteins, antibodies, and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. Monoclonal antibodies used in this study are listed in (Table 2-5). Following a protocol from (Galloway et al., 2018, Cornuault et al., 2018), exudates sample collected from seedlings in 4-hour exudate assay, were diluted in phosphate-buffered saline (PBS). The exudates samples (5-50 µg/mL), 100 µL per well were titrated down (5 fold dilution) to a 96 well microtitre plates, with the last row being left as a negative control containing no antigen. The plates were left in the refrigerator at 4°C overnight. The plates were then washed with water and patted dry. For blocking, 200 µL of 5% weight per volume of Bovine Serum albumin (BSA) or skimmed milk powder dissolved in PBS, (milk dissolved in PBS will be referred to as MP/PBS for the rest of the chapter), were added in the plates and left 1-2 hours at room temperature, followed by washing thoroughly and shake dry. The plates were then incubated with primary antibody with a 1:10 dilution of hybridoma supernatant in MP/PBS, 100 µL per well, for an hour followed by vigorous washing with water and pat dry. To aid detection of the target protein, a 1:1000 dilution of the secondary antibody, anti-rat HRP (Immunoglobulin G coupled with horseradish peroxidase; A9552 Sigma-Aldrich

USA) in MP/PBS, 100 µL per well, was used and it was left to incubate for an hour at room temperature. Followed by washing before adding 100 µL per well of the substrate containing (9 mL de-ionised water, 1 mL of 1 M sodium acetate buffer, 100 µL of 3,3',5,5'-tetramethylbenzidine 10 mg/mL in dimethyl sulphide (T-2885; Sigma-Aldrich, USA) and 10 µl of hydrogen peroxide) was added and left to react for 5 min. The reaction was then stopped by addition of 50 µL of 2.5 M sulphuric acid to each well. The absorbance values were read using a Multiskan plate reader with SkanIt software (Thermo Scientific, USA) at 450 nm.

Table 2-5: Monoclonal antibodies

Antibodies	**Epitope mapped**	**Source**
LM1	Extensin	Knox Lab, University of Leeds
LM2	Glucan	Knox Lab, University of Leeds
LM6-M	Arabinan	Knox Lab, University of Leeds
LM6	Arabinan	Knox Lab, University of Leeds
LM8	Xylogalacturonan	Knox Lab, University of Leeds
LM11	Heteroxylan	Knox Lab, University of Leeds
LM25	Xyloglucan	Knox Lab, University of Leeds
LM27	Heteroxylan	Knox Lab, University of Leeds
7E1:B11	Beta-glucan	Knox Lab, University of Leeds
LM30	Arabinogalactan	Knox Lab, University of Leeds

2.3.3 Nitrocellulose assay for seedling root printing

The nitrocellulose printing assay was followed using the protocol as described in Willats et al. (1998) and Galloway et al. (2018). 7-10 days old seedling grown on agar were placed on 9 cm^2 of nitrocellulose soaked with de-ionised water on sheets of Whatman 3MM paper. The seedlings were left on the nitrocellulose for 1-2 hours at room temperature and loosely covered. Then seedlings were removed, and nitrocellulose was left to dry out overnight at room temperature, covered loosely with aluminium to avoid direct light contact. The nitrocellulose sheet was then blocked with 22.5 mL of 5% weight per volume of MP/PBS with 0.025% volume/volume of sodium azide solution (to remove endogenous peroxidases) and incubated on a see-saw rocker with light rocking at room temperature for an hour. Primary antibody, a 1:10 dilution of hybridoma supernatant was then added to each nitrocellulose in weighing boat and left rocking for another hour. Followed by light rinsing in tap water and then with PBS thrice for 5 minutes each while rocking. This step was followed by adding secondary antibody, 1:1000 dilution of antibody anti-rat HRP in MP/PBS for another hour. The following substrate was then added; 25 mL of de-ionised water, 5 mL Chloronapthol (5 mg/mL in ethanol) and 30 µL of hydrogen peroxide and the print left to develop for 15 min, after which the sheets were rinsed with tap water and left to dry overnight between Whatman paper. The nitrocellulose sheets were then imaged on a scanner (Epson v800)

2.3.4 Immunofluorescence for localization

The aim for this assay was to detect and localise a polysaccharide at cellular level. This helps to lay a foundation for understanding the function and mechanisms of the root exudates. Following the detection of specific polysaccharide epitopes observed on ELISA and Nitrocellulose printing, the immunofluorescence labeling of excised root segments followed the protocol outlined by Willats et al. (2001) and Galloway et al. (2020a). Sorghum seedlings grown on agar plates were cut into 10 mm sections. The root segments were then left in 4% volume/volume paraformaldehyde in PEM buffer fixative solution overnight. They were removed the following day and rinsed twice in PBS. The root segments were then placed in 12 well cell culture plates and blocked with 2 mL per well of 5% weight per volume of milk powder dissolved in MP/PBS and incubated on a rocker with light rocking at room temperature for 30 min. After an hour had elapsed the MP/PBS solution was pipetted off and 2 mL of PBS solution was added to each well and rocked for 5 min and then pipetted off. This step was repeated twice. A subsequent 1 mL of primary antibody a 1:5 dilution in MP/PBS was added to each well and incubated on a rocker for 90 minutes. The primary antibody was then removed followed by three more 5 min washes with PBS solution. The secondary antibody was then added, 1 mL per well of anti-rat FITC (Immunoglobulin G coupled with fluorescein isothiocyanate) a 1:100 dilution in milk-PBS. The wells were washed a third time with 2 mL of PBS 5 min a time, three times and 1 mL per well of a 1:10 dilution of Calcofluor in PBS at 0.25 mg/ml was added.

Again, the wells were washed three times with PBS as before and then 100 µL of 0.1% Toluidine Blue solution, was added, making sure all the root segments were well covered, to counteract any autofluorescence from the root segments which is prevalent in many cereals and grasses. It was then immediately removed, and root segments were rinsed extensively by repeated pipetting and removal of PBS. The root segments were then stored at 4°C in their wells with 2 mL of PBS and adding a drop of Citifluor in PBS, to stop florescence fading. The root segments were then mounted on microscope cavity slides in a drop of Citifluor in PBS before viewing under a microscope (Olympus Optical GX; BX61: Olympus, USA). Images were taken with a Hamamatsu ORCA publisher camera (Hamamatsu, Japan).

2.4 RNA preparation and sequencing

2.4.1 RNA extraction

To understand the molecular mechanisms regulating the observed phenotype changes, the root tissues at different developmental stages and, depending on experiments, were collected, quickly washed, and flash-frozen in liquid nitrogen before being stored at -80^{o}C for RNA isolation for transcriptomic analysis or qPCR. Tissues from two to three different plants for each treatment were pooled together as one biological replicate. A total of three biological replicates were collected for each tested treatment. Total RNA was extracted using an Omega E.Z.N.A® plant RNA extraction kit following the manufacturer's instructions. On-column DNase I digestion was used to remove DNA contamination from RNA samples. RNA quantity and quality were assessed using a NanoDrop Spectrophotometer (Thermo Scientific). For transcriptomic analysis and quantitative real-time PCR, 2100 Agilent bioanalyzer (Agilent Technologies) was used to check that RNA had an RNA integrity number (RIN) above six and a purity (A260/280) value of above 1.8.

2.4.2 cDNA synthesis

According to the manufacturer's instructions, complementary DNA (cDNA) was synthesized from 1 µg of total RNA per sample using a SuperScript™ VILO™ cDNA Synthesis Kit Table 2-6. The components were mixed and incubated at 25°C for 10 minutes, then at 42°C for 60 minutes and terminate the reaction at 85°C for 5 minutes.

Table 2-6: Synthesize first-strand cDNA

Component	**Quantity**
5X VILO™ Reaction Mix	4 µl
10X SuperScript™ Enzyme Mix	2 µl
RNA (up to 2.5 µg)	1 µg
dH2O	To 20 µl

2.4.3 PCR and testing of primers

Primers were designed using Primer3 and BLAST (NIH). Table 2-7 shows the list of primers used in this project. For PCR reaction, GoTaq® G2 DNA polymerase kit (Promega) was used following manufacturer’s instructions. Table 2-8 shows the preparation of 10 mM dNTPs and Table 2-9 shows PCR reagents used.

Table 2-7: List of primers used in this project Oligo

Primer	**Sequence**
Sb beta-actin Forward	AATGGCTCTCTCGGCTTGC
Sb beta-actin Reverse	TGGCATCTCTCAGCACATTCC
GAPDH (Glyceraldehyde 3-phosphate) Forward primer	AAGGCCGGCATTGCTTTGAAT
GAPDH (Glyceraldehyde 3-phosphate) Reverse primer	ACATGTGGCAGATCAGGTCGA
LAZY4/2*: SORBI_3001G342000 Forward primer*	GTGGCTTCTACTGCTCCCAAC
LAZY4/2: SORBI_3001G342000 Reverse primer	GTCTCTTGTCTCTCTTGGGCT
LAZY4: SORBI_3002G373700 *Forward primer*	AGACAAACCGAGTGGGAGGA
LAZY4: SORBI_3002G373700 *Reverse primer*	CTGAGTCAAGGCCATCATCACTA

Table 2-8: Preparation of 10 mM dNTPs for PCR

Stock	Amount (µl)
100 mM dATP	10
100 mM dTTP	10
100 mM dCTP	10
100 mM dGTP	10
dH2O	60

Table 2-9: PCR reagents

GoTaq® G2 DNA polymerase kit reagents	**Vol for 1 x 10 ul**
GoTaq DNA polymerase	0.05
5X Green GoTaq reaction buffer	2
Forward primer (10 µM)	0.5
Reverse primer (10 µM)	0.5
dNTP (10 mM)	0.2
cDNA	0.5
dH_2O	6.25

2.4.4 Amplification/ thermocycling cycle

The Amplification and thermocycling cycle was carried out as shown in Table 2-10 below

Table 2-10: Thermocycling cycle

Step	**Time (min/seconds)**	**Temperature (ºC)**	
1. Initial denaturation	2 min	95	
2. Denaturation	1 min	95	Repeat steps 2-4 x 29 times
3. Annealing	1 min	X	
4. Extension	X	72	
5. Final extension	5 min	72	
6. Hold	∞	4	
7.			

2.4.5 Gel electrophoresis

About 50X Tris-acetate-EDTA (TAE) buffer for agarose gel electrophoresis was prepared according to Table 2-11 below. For use at 1 X (40 mM Tris-base, 20 mM acetic acid, pH 8.0, 1 mM EDTA), dH2O was used to dilute the 50 X stock. DNA size was analysed using agarose gels at 1% w/v concentration. Agarose was dissolved in 1X TAE buffer, and Gel Red Nucleic Acid Stain was added to a concentration of 1x. DNA samples were diluted with 6X Orange DNA Loading Dye, or with 5x Green Go Taq® Buffer if Go Taq G2 polymerase was used to amplify the amplicon in question. A voltage between 50 V and 95 V was applied in 1X TAE running buffer using Bio-Rad gel tanks. A UV trans-illuminator was used to visualize the bands.

Table 2-11: Composition of 50 X TAE buffer for agarose gel electrophoresis

Ingredient	Amount (prepare in 1L volumetric flask)
Tris Base	241g
Glacial acetic acid	57.1ml
0.5 M EDTA pH 8.0	100 ml
dH2O	Fill up to IL mark

2.4.6 Real-time quantitative PCR (RT-qPCR)

Real-time qPCR was performed using Bio-Rad's CFX96 Real-Time PCR system (Bio-Rad Laboratories Ltd. UK), in accordance with the cycling conditions instructed in the SSoFast Eva Green Supermix protocol. Where 1 µL of diluted cDNA, 7.5 µL SSoFast Eva Green Supermix, 500 nM forward and reverse primer concentration was used in a 15 µL total reaction volume per well/reaction in a sealed 96-well plate. A melt-curve was carried out for each amplicon at the end of every RT-qPCR. Based on the results of three technical repeats for three biological replicates, quantification of various mRNA expression levels was determined as described by (Taylor et al., 2019), comparing the expression of each gene of interest with the expression of two housekeeping genes, GAPDH and EFP using the geometric mean. These housekeeping genes were part of the recommended options for sorghum according to Sudhakar Reddy et al. (2016). To test the efficiency of each couple of primers, standard curves were generated using cDNA pooled from each sample and using 1:4 serial dilutions.

2.4.7 RNA sequencing and analysis

Total RNA samples containing 2 mg of RNA, with RNA integrity number (RIN) of > 7.0 and Nucleic Acid 260/280 ratio of 1.8-2.2 were submitted in triple biological replicate to Azenta/GENEWIZ for RNA next generation sequencing and data analysis. Table 2-12 shows quality control with respect to RIN integrity number and RNA quantity of the samples. (Figure 2-3) shows an image of gel electrophoresis and electropherograms of RNA samples extracted from well-watered and water-stressed plants following bionalyser quality check.

Table 2-12: Total RNA quantity and quality check

Sample ID	**Concentration ng/ul**	**RIN number**
WW-SG-rep1	962.01	8.9
WW-SG-rep2	1105.85	8.9
WW-SG-rep3	885.68	8.4
WS-SG-rep1	673.24	8.6
WS-SG-rep2	1268.25	9
WS-SG-rep3	538.89	9.1

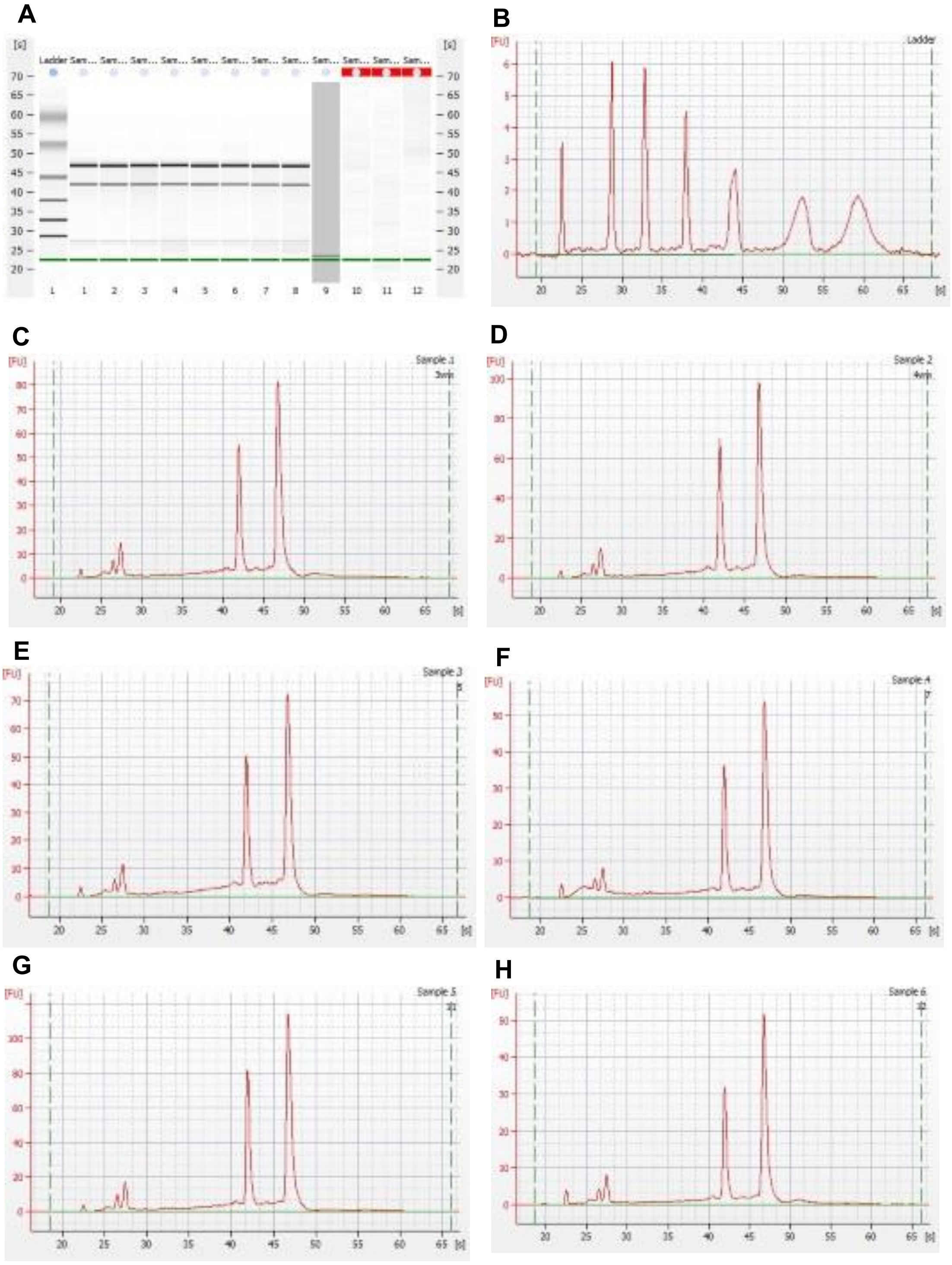


Figure 2-3: A gel electrophoresis image and electropherograms of RNA samples extracted from well-watered and water-stressed plants

RNA quality checks were analysed using Agilent Bioanalyzer 2100. A) Bands show RNA integrity from RNA samples as compared to an RNA ladder. B) Electropherogram of RNA ladder as a comparison for RNA integrity of RNA samples. C-E) Electropherogram from RNA samples (samples 1 to 3, well-watered-control). F-H) Electropherogram from root RNA samples (samples 4 to 6, water-stressed-treatment).

2.4.8 RNA sample sequencing statistics

RNA sequencing was carried out using Illumina NovaSeq™ platform system with 2x150 bp sequencing and 15-20M read pairs configuration. Data quality and analysis was performed by Azenta/GENEWIZ (Germany) applying the following steps. Quality Score was used to check the quality of sequencing. As shown in (Table 2-13) of sample sequencing statistics, all the biological replicates sent to sequence reach a quality score > 30, indicating 1 in 1000 probability of incorrect base call during the sequencing.

Table 2-13: Sample sequencing statistics

Sample		**Number of Reads**	**Mean Quality Score**
Drought water stress	WS-SG-rep1	36,478,773	35.48
	WS-SG-rep2	37,659,257	35.44
	WS-SG-rep3	33,092,323	35.54
Well-watered	WW-SG-rep1	34,941,633	35.55
	WW-SG-rep2	30,087,942	35.58
	WW-SG-rep3	41,208,689	35.58

2.4.9 Mapping sequence reads to the reference genome

Sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were mapped to the *Sorghum bicolor* reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. The STAR aligner is a splice aligner that detects splice junctions and incorporates them to help align the entire read sequences. BAM files were generated as a result of this step. (Table 2-14) below are the statistics of mapping the reads to the reference genome.

Table 2-14: Mapping statistics

Sample		**Total Mapped Reads**	**% Total Mapped Reads**	**% Unique Mapped Reads**
Drought water stress	WS-SG-rep1	28,832,656	81.28	79.16
	WS-SG-rep2	30,133,916	82.48	80.49
	WS-SG-rep3	26,630,704	83.07	81.14
Well-watered	WW-SG-rep1	27,983,946	82.20	80.27
	WW-SG-rep2	24,388,859	82.98	81.09
	WW-SG-rep3	32,289,505	80.35	78.38

2.4.10 Differential Gene Expression Analysis

DESeq2 was used to compare gene expression between the customer-defined groups of samples. P-values and log2 fold changes were generated using the Wald test. Genes with an adjusted p-value < 0.05 and absolute log2 fold change > 1 were referred to as differentially expressed genes for each comparison. Before performing differential transcriptomic analysis between well-watered and water stress samples, overall similarity among samples was assessed. (Figure 2-4) below provides a visual representation of raw (Figure 2-4A) and normalized (Figure 2-4B) expression values. The original values were normalized to adjust for different factors, including differences in sequencing amounts. These normalized values were used to accurately determine differentially expressed genes. The heat map (Figure 2-4C) shows the distances measured using expression values from each sample. The shorter the distance, the more closely related the samples are. This method is used to identify if the two groups are closely related or not. (Figure 2-4D) Principal component analysis was carried out to reveal the similarity within and between groups represented by colour codes. For water stress, one sample was different among the group and although it was used in the overall analysis, but this may bring variation to the data for some responsive genes.

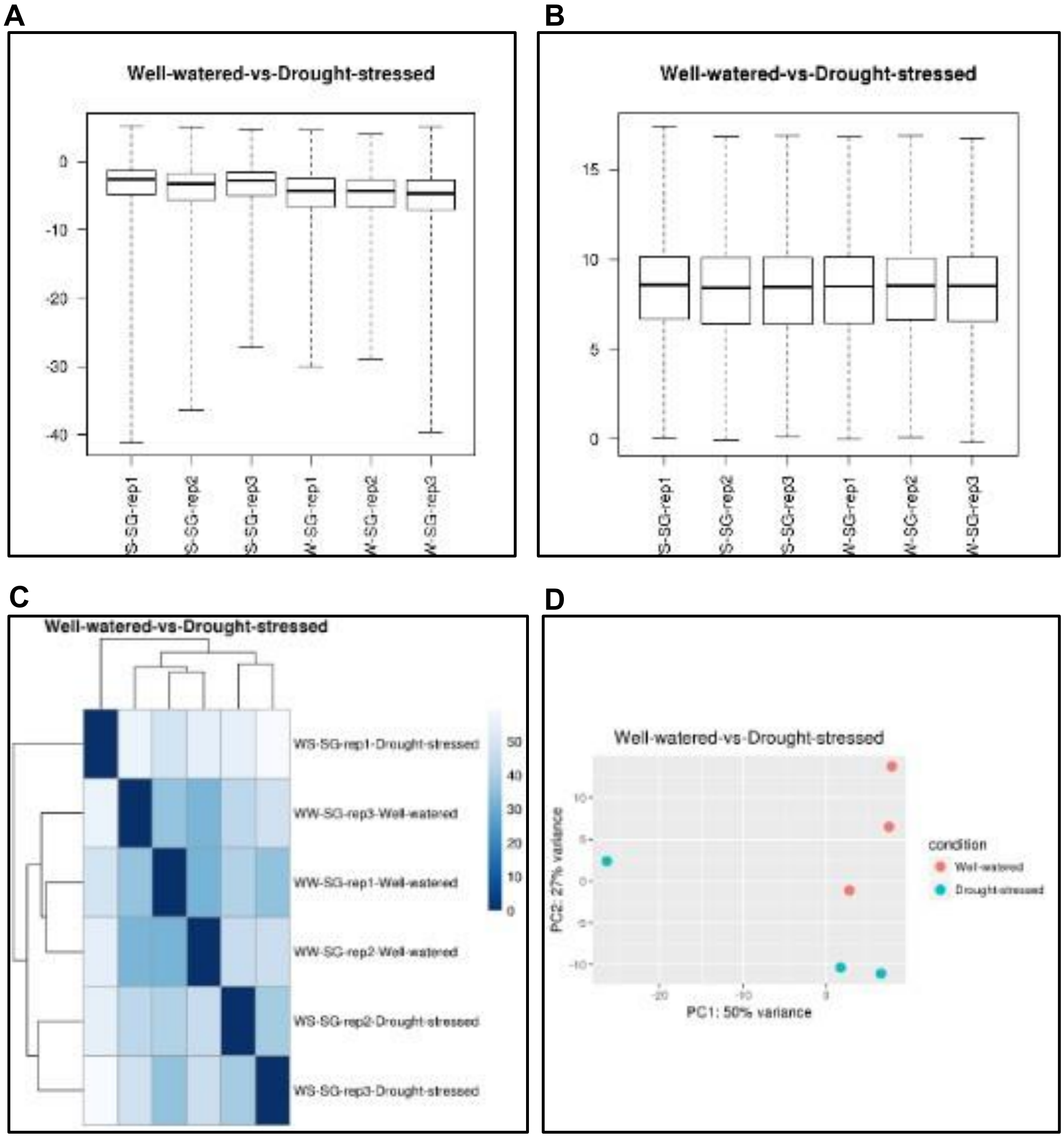


Figure 2-4: RNA-Seq data quality control

A-B) Box plot of the distribution of non-normalized and normalized (by sequencing yield) read counts. Box boundaries indicate the interquartile range (IQR). Black dots indicate outliers. Box whiskers represent data points 1.5x from the IQR. Read counts were transformed onto a log2 scale. ‘#’ indicates the number of replicates. C) Heat map of sample-to-sample distance. In the colour-code scale, shorter distances are represented by low numbers. The shorter the distance between samples, the more closely related the samples are. D) Principal component analysis was carried out to reveal the similarity between and within well-watered and water-stress sample groups.

2.5 Grain mineral analysis

2.5.1 Macronutrient quantification and analysis

This work used data shared from field research experiments that were carried out at ICRISAT's lysimetric platform, Hyderabad, India during 2017-2019 crop growing years. About 242 sorghum lines (representing 15 Landraces) with diverse phenology, morphology and shoot architectural characteristics were grown under two controlled treatments, well-watered (WS) and drought-water stress (WS). Landraces (*caudatum*, *bicolor*, *guinea*, *durra*, and *kafir*) and 10 intermediate races classified by (Harlan and de Wet, 1972). After harvesting, grain and stover macronutrient levels were quantified using near-infrared spectroscopy (NIRS) at ICRISAT-India and ILRI laboratories according to the protocol described in Choudhary et al. (2010). NIRS is a non-destructive, rapid and low-cost method to estimate the biochemicals of grains and differentiate them based on their chemical composition. It is a feasible alternative to the time-inefficient and resource-intense conventional methods of analysis such as Kjehldahl or high-performance liquid chromatography (HPLC) (Ejaz et al., 2021). Raw data comprised of phenotypic data and nutritional data (Starch, Fat and Protein) was shared for analysis (summary in Table 2-15 and 2-16).

Table 2-15: Summary of data comprised of physiological parameters observed and measured during experiments

Morphology and physiological parameters	**Year 1 (2017-2018)**		**Year 2 (2018-2019)**	
	WW	WS	WW	WS
Transpiration_preflowering (kg)	✓		✓	
Transpiration_postflowering (kg)	✓		✓	
Total transpiration (kg)	✓		✓	
Days to flowering	✓		✓	✓
Leaf dry weight (g)	✓	✓	✓	✓
Stem dry weight (g)	✓	✓	✓	✓
Panicle dry weight (g)	✓	✓	✓	✓
Total biomass (g)	✓		✓	✓
Total dry weight (g)		✓		
Total grain wt (g)	✓		✓	
100_grain wt (g)	✓		✓	
Transpiration efficiency (g/kg)	✓	✓	✓	✓
Transpiration (kg)_24DAS to 34DAS (pre-stress)		✓		✓
Transpiration (kg)_35DAS to 72DAS (pre-stress)		✓		✓
Water use efficiency (g/kg)	✓		✓	
Days to Maturity	✓	✓	✓	✓
Harvest Index (%)	✓		✓	
Panicle harvest index (%)	✓		✓	✓
Water extraction (kg)		✓		✓
No. of nodal tillers			✓	
No. of basal tillers			✓	
Total no. of tillers			✓	
No. of productive tillers			✓	
No. of non productive tillers			✓	
Main stem thickness (mm)			✓	
Plant height (cm)			✓	
Days to flowering			✓	✓

*DAS represents days after stress treatment, WS represents drought-water stress, WW represents well-watered.

Table 2-16: Summary of data comprised of nutritional parameters measured after grain harvest

Grain nutrient measured	**Year 1 (2017-2018)**		**Year 2 (2018-2019)**	
	WS	WW	WS	WW
Fat %		✓	✓	✓
Protein %		✓	✓	✓
Starch %		✓	✓	✓

2.5.2 Mineral element quantification

To complement macronutrient data from ICRISAT, controlled experiments were conducted under greenhouse conditions at Leeds, to generate grain yield for micro-mineral quantification from both water-stressed and well-watered plants. Seed pre-germination protocol and planting were followed as outlined in Chapter 2 of this thesis (General materials and methods). For this experiment, sorghum (variety S35) plants were exposed to well-watered and water-stressed conditions. The stress treatment was applied after 6 weeks of growth, prior to flowering.

For water stress treatment, reduced water application was maintained until harvesting. The grain was harvested from both treatments for mineral element analysis. The experiment was repeated with eight different sorghum genotypes to understand the natural variation in micro-nutrient content and levels exposed to similar treatment. The grain samples (1 g) were ashed in a furnace (Carbonlite) at 500°C until complete mineralization and the remainders dissolved in 1 mL concentrated HCL. Each sample was prepared in triplicate (Appendix 1 for detailed protocol).

2.5.3 ICP-OES analysis

The prepared samples were submitted for mineral analysis (School of Earth and Environment, University of Leeds) using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES), a standard method to determine minerals and trace elements in different samples offering high throughput and multielement options (Molina et al., 2019, Basri et al., 2019). For the analysis, a Thermo Scientific iCAP7400 ICP-OES instrument was used, with sample standardisation and calibration protocols followed as provided by the manufacturer (detailed protocol and calculations in Appendix 1). Table 2-17 below summarises what wavelength was used for each element.

Table 2-17: Wavelength and calibration for each element

Element	Wavelength / nm	Calibration Range
Magnesium	279.553 285.213	1-100 mg L-1 1-100 mg L-1
Iron	259.940 238.204	1-10 mg L-1 1-10 mg L-1
Manganese	257.610 279.482	1-10 mg L-1 1-10 mg L-1
Copper	324.754 327.396	10 – 100 µg L-1 10 – 100 µg L-1
Zinc	213.856 202.548	1-10 mg L-1 1-10 mg L-1

2.6 Statistical method and data analysis

Statistical analyses were performed using Origin Pro and RStudio (R programming language) software. Normality was tested visually by plotting histograms and Q-Q plots of data distribution; if ambiguous, a Shapiro–Wilk test was implemented. Unless otherwise stated in methods and if data was normal, ANOVA tests were performed to test for significance between groups. Tukey HSD post-hoc analysis was used following ANOVAs and Mann-Whitney post-hoc following non-parametric models. Examples of functions used for in Rstudio for testing signifcance: aov() – one-way ANOVA, TukeyHSD() – Tukey's HSD post-hoc test, t.test() – independent t-test, Wilcox.test() – Mann Whitney-Wilcoxon test, pairwise.wilcox.test() – pairwise Wilcox test with Benjamini-Hochberg correction. Data was visualized and graphs plotted using Excel, Origin Pro 2020, RStudio and GraphPad Prism.

Chapter 3

Identification of farmers' preferred sorghum and millet phenotypic traits and climate-smart cropping system by gender in drought-prone areas of Malawi

Originally Published in *Plants People Planet Journal* under the title: Gender differential in choices of crop variety traits and climate-smart cropping systems: insights from sorghum and millet farmers in drought-prone areas of Malawi

Chapter note

The following chapter is replicated here as it was in the original publication. Some sections of the chapters such as the chapter introduction, have been rewritten to retain consistency throughout this thesis. Although some sections in the methods, results, and discussion sections have been altered, some statements in these sections have remained unchanged. Figures are the same, but positioning in some places within the text has been altered to enhance clarity. Some of the supplementary figures that appeared in the publications are now included in the relevant position within the main text, rather than being placed collectively together at the end of the manuscript. However, this has not affected the main content of the work published.

3.1 Introduction

Climate-resilient crops and cropping systems are required to manage climate variability and to adapt to the increasing climate risks across Africa and globally (Acevedo et al., 2020). A cropping system refers to the type and sequence of crops grown and practices used for growing them. It encompasses all cropping sequences practiced over space and time based on the available technologies of crop production. Cropping systems have been traditionally structured to maximize crop yields. Management of cropping systems implies management of tillage, crop residue, nutrients, pests, and practices for soil conservation (Blanco-Canqui and Lal, 2010). Likewise, climate-resilient crops are crops and crop varieties that have enhanced tolerance to environmental stresses such as droughts, higher average temperatures, and other climatic conditions while maintaining crop yields (Acevedo et al., 2020). Rapid crop improvement is critical to adapting cropping systems to changing weather and climate in Malawi and globally (Sutcliffe et al., 2016). Continuously improving climate-smart crops could provide novel and bold solutions to increase crop productivity while reducing atmospheric carbon and nitrogen emissions (Jansson et al., 2018).

As previously stated in Chapter 1 of this thesis, the benefits of sorghum and millet as significant climate-smart cereals in arid and semi-arid regions are numerous. Lack of food diversification has caused malnutrition, which is one of the largest health impacts of climate change, affecting mostly poor women and children in Asia and Africa (Fao, 2011, Phalkey et al., 2015). Sorghum and pearl millets uniquely fits production in such regions and they are model crops for environmental stresses tolerance including drought and rising temperature. These crops have high and stable water-use efficiency, high germplasm variability and comparative nutritional value (Hadebe et al., 2017). Until recently, the crops have been forgotten in terms of conservation, utilization, and research, and progress in breeding programs has lagged behind (Anunciacao et al., 2017). There is a growing interest in the re-inclusion of these local crop species and varieties into food production systems and increased investment in crop improvement programmes to increase crop productivity (Jansson et al., 2018). In addition to limited research capacity for crop improvement, gender, social concerns, and farmer awareness are not exclusively utilized in current crop adaptation strategies.

Integrating farmers knowledge and gender perspectives on cropping technologies will assist in accelerating crop adaptation programs critical to achieving sustainable food production in Sub-Saharan Africa (Kakota et al., 2011, Murray et al., 2016, Djoudi et al., 2016, Teklewold, 2023). The literature on gender and agriculture has mainly focused on gender gaps in agricultural productivity and technology adoption (Joe-Nkamuke et al., 2019, Tufa et al., 2022, Teklewold, 2023), especially for major crops in Africa. In sub-Saharan Africa, there is increasing focus on identifying farmer's trait preferences within crop breeding to enable gender-responsive product development, for instance, in maize (Cairns et al., 2022). However, such innovations are also needed for climate-resilient crops such as sorghum and millet, which have been neglected. Traits that potentially enhance adaptive phenotypic plasticity or yield stability are significant in current and future variable climates (Haussmann et al., 2012). Farmer participation and perspectives on crop selection are useful pathways to assist in the development of new diverse germplasm adapted to specific production constraints and environments.

This study focussed on understanding the choices and decision for crop variety phenotypic traits and climate-smart farming practices of sorghum and millet producers as influenced by gender and climate risk awareness in drought-prone areas of southern Malawi. To further understand household farming resource allocation, the study examined the availability and accessibility of sorghum and millet seed, as well as farming resources among male and female farmers. Finally, the study aims to explore the existing challenges and future opportunities for sorghum and millet crop improvement programs, seed systems, production, and utilization.

This work provides new insights for the development of gender-responsive crop varieties and climate-smart technologies specific to community needs. It addresses the need for a greater gendered research focus on forgotten crops and their inclusion in African food systems. In addition to shaping breeding efforts, this work informs future government policy decisions and investment plans for nutritive and gender-sensitive, climate-smart cropping and adaptation strategies. Incorporating holistic gender analysis in cropping technology and crop improvement programs is a pathway to developing community resilience to climate change.

3.2 Research design and methods

3.2.1 Study area

The study was conducted in neighbouring districts of Nsanje and Chikwawa, which form part of Lower Shire Valley (Figure 3-1), in southern Malawi (a sub-Saharan Africa country). Both districts have tropical climate with strong seasonality. Mean maximum temperatures are between 37°C in October and 27°C in June (Ibrahim and Alex, 2008). Both districts have unreliable and variable rainfall with average annual precipitation of 800mm to 1200mm, with over 90%

occurring during the wet season from November to April (Bischiniotis et al., 2020). Chikwawa and Nsanje districts are drought-prone and represent the main traditional growing areas for sorghum and pearl millet in Malawi.

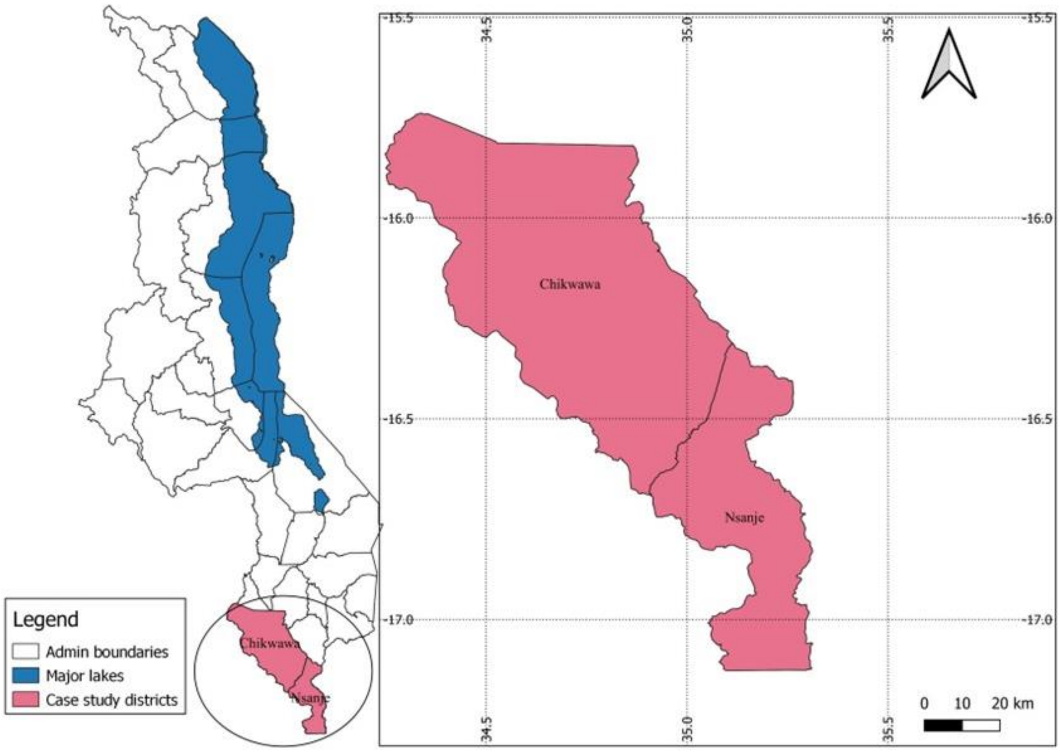


Figure 3-1: Map of Malawi showing the study locations (Chikwawa and Nsanje districts) denoted in red

Chikwawa and Nsanje districts form part of the Lower Shire Valley in the southern region of Malawi, a drought-prone area and the main growing sites of sorghum and millet.

3.2.2 Data collection tools and analysis

This study adopted a mixed-method approach that balances the examination of different phenomena including perceptions and statements with quantitative numerical data (Creswell et al, 2003). A combination of individual farmer semi-structured interviews, Focus Group Discussion (FGD) and expert stakeholder consultations.

Household Survey

The household survey employed a semi-structured questionnaire to collect both quantitative and qualitative data. A semi-structured questionnaire consists of both open-ended and closed questions providing greater depth than is possible with a structured questionnaire as respondents are not limited with their choice of answers (Gubrium and Holstein, 2002). The household survey was carried out in Nyachirenda Extension Planning Area (EPA) in Nsanje district and Mikalango EPA in Chikwawa district. Nyachirenda EPA has 12 sections with a population of 16,277 farming households, while Mikalango EPA has 29 sections with a population of 26,531 farming households. Two sections from each EPA were purposefully selected and one village from each section was randomly sampled in the selection of respondents. Therefore, the total sampled villages were 4 for both Nsanje and Chikwawa. The Agricultural Extension Development Coordinator (AEDC) provided the sorghum and millet farming household list for each study site, with it categorized as male-headed households and female-headed households. Through a probability proportionate to the size sampling approach (Skinner, 2014), a total of 179 households were sampled for this survey.

Due to Covid-19, new variants outbreak disruptions, a total of 152 interviews were conducted in March 2021, and an additional 27 interviews were conducted in March 2022. A household head was selected for the interviews for each sampled household. In the absence of the household head, the spouse, if available, would participate in the survey. The absence of men in households for other economic activities resulted in a higher number of female interviewees than males. In total Around 47.5% of male and 52.5% of female farmers participated in the survey (Table 3-1). It is worth noting that the female respondents are a mix of female household heads and spouses. Our study focused on the lived experiences of men and women in sorghum and millet production. These interviews generated data concerning the socio-economic characteristics of the households, climate-smart cropping systems and choices for sorghum and millet production, available sorghum and millet varieties, varietal trait preferences for sorghum and millet among male and female farmers, and gender differences and challenges in access to, control over and entitlement to resources among household members in sorghum and millet production.

Table 3-1: Demographic characteristics of sampled sorghum and millet farmers in Chikwawa and Nsanje districts, Malawi (n=179)

Variable	Category/Units	Mean	SD
Age	Respondents (Years)	44.3	14.36
Respondent Sex	Male (%)	46	
	Female (%)	54	
Household headship	Males (%)	64	
	Females (%)	36	
Marital Status	Single (%)	1	
	Married (%)	72	
	Separated (%)	5	
	Divorced (%)	7	
	Widower/widow (%)	16	
Farmer club membership	Males (%)	52	
	Females (%)	48	
Crops grown	Sorghum (% of farmers)	90	
	Pearl millet (% of farmers)	80	
	Maize (% of farmers)	49	
	Rice (% of farmers)	1	
	Cotton (% of farmers)	9	
	Groundnuts (% of farmers)	7	
	Beans (% of farmers)	9	
	Sesame (% of farmers)	23	

Key Informant Interviews

Two key informant interviews were conducted in each district, with the (AEDC) and Agricultural Extension Development Officer (AEDO) for the section selected. AEDC coordinates the activities for the Extension Planning Areas (EPA) while AEDO coordinates the activities of the farmers within a particular section within the EPA. These are knowledgeable government staff and are the ones who interact with the farmers in their area most frequently.

Key Informants were held prior to conducting the Focus groups and household survey. This was done to facilitate the development of questions or concepts for developing the questionnaires and interview guides. A flexible checklist with open-ended questions was used to interview them. This generated information on areas of climate-smart cropping systems and challenges farmers face in sorghum and millet production, including varieties grown in these areas. To gain detailed understanding of the challenges and opportunities for the sorghum and millet seed system, at least 10 individual expert stakeholder consultations were conducted comprising of seed companies, entrepreneurs, researchers and CGIAR centres who are working in sorghum value chain.

Focus Group Discussions

Generally, focus groups are considered as groups of people who share a similar type of experience and interest (Stewart and Shamdasani, 2014). People are collectively interviewed, and observations are made, however, emphasis is on the interaction within the group based on topics supplied by the researcher which is paramount for in-depth understanding of the topic. For this study, in each district, two focus groups were conducted which differentiated along gender lines, comprising of about 10 to 12 participants per discussion group. The groups were divided based on gender to allow both males and females to discuss gender issues separately among their peers. FGD participants were randomly selected from the survey participants for in-depth discussion. In female groups, we purposely selected representation of females as either a spouse under male-headed households or female household heads to capture a diversity of intra-gender dynamics. AEDO who works in the area, assisted in selecting the participants for the FDGs. A checklist with open-ended questions was used to evaluate the impact of climate change on sorghum and millet production over the years; climate smart agricultural technologies/practices that farmers are using to adapt to the impacts of climate change in sorghum and millet production; and varietal traits for sorghum and millet that male and female farmers prefer, and the reasons for their preferences. Each FGD lasted between 60 and 90 minutes.

3.2.3 Data analysis

Data collected from individual household interviews were subsequently digitised and processed in Microsoft Excel (2016 version) and Statistical Package for the Social Sciences (SPSS, version 26). The Chi-square test was used to interpret the association between gender and variables collected in this study. This test is appropriate for our study since our data is in the form of frequency counts that occur in two or more categorical (nominal) variables. Analysis of data from focus groups was based on the approach of content analysis using the thematic framework analysis (Ritchie and Spencer, 2002). This involved analysing the data by examining the underlying themes in the text material that contains information about particular themes of the research. In the analysis, data was sifted, charted and sorted according to the key research issues and themes.

3.3 Results

3.3.1 Climate risk awareness influence farmers' decisions for production and the specificity of varieties and response vary with gender

The role of household decision-making on sorghum and millet production

Our study found that farmers have traditionally grown sorghum and pearl millet due to prolonged dry spells and frequent droughts experienced in Chikwawa and Nsanje. Approximately 90% and 80% of farmers interviewed grow sorghum and millet respectively. Over 84% (n=126) of respondents indicated that sorghum and millet are produced primarily for household food consumption and that surplus, if available, is sold. During the focus group discussion, farmers emphasized that their experience on climate and weather past events shape their decision on crops to grow. It was observed that sorghum and pearl millet are part of their traditional crops, and the crops are preferred due to their tolerance to drought, heat, pests and infertile soils.

Data revealed that 80% (n=121) of respondents indicated that the head of the household decides what to cultivate and determines the land allocation for each crop. Under a quarter of respondents specified that the household head consults a spouse or decides together (Figure 3-2A). Due to the patrilineal marriage systems in this region, men typically have control over household decision-making and planning.

The results further revealed a gender imbalance on agricultural land entitlement and farming resources. The *p*-value ($p<0.004$) from the Pearson Chi-squared test indicates a significant association between gender and agricultural land entitlement and farming resources. About 60% (n=91) of farmers indicated that male farmers have agricultural land entitlement and control (Figure 3-2B). The entitlement to land broadly influences the selection of, and decision-making on cropping systems, with household head permission required for all household members to access the land. Similar trends are also observed in farm inputs and tools, where the male head of the household is mostly in control of farm inputs and associated tools. This implies that female farmers are disadvantaged by inequalities in control over and entitlement of production resources.

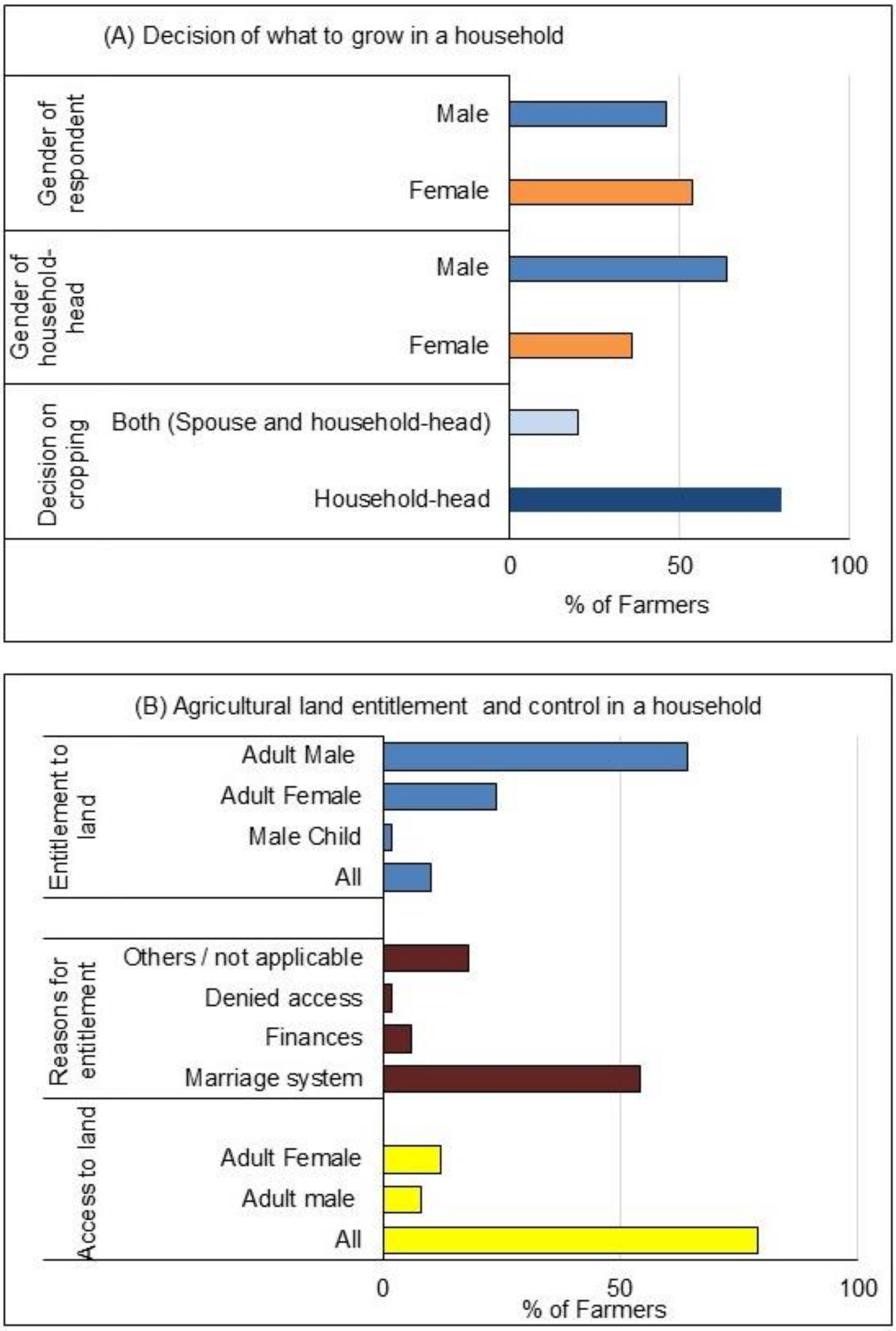


Figure 3-2: Gender differences in farmers' land control and decisions on which crops to grow in Malawi

A) Gender of respondents, the gender of the household head, and who controls general cropping decisions in a household among study participants. B) shows agricultural land entitlement, control and accessibility by males and females in a household. The results are collective responses from Chikwawa and Nsanje districts (n=179) of Malawi.

It was noted that although men make most of the decisions in married couples, women take a prominent role in the implementation of these decisions. Our study found that sorghum and millet is predominantly grown by female farmers. They are the ones present throughout farming seasons and experience the impacts of climate change and extreme weather events in the production of the food crops more than men. Women emphasized that men are most concerned when there is an economic benefit to the product. In contrast, male farmers believe that their female partners are the main decision-makers when it comes to sorghum and millet crops, and they may occasionally make joint decisions (Figure 3-2A). However, from the observation of this study women take a leading role in deciding specificity of the variety of sorghum and millet to grow. Focus group discussions reveal that occasionally, men leave the area for longer periods for casual employment, in some cases as a means for adaptation or coping mechanisms. They may also leave the household and reside on the islands in the Shire River for the cropping season to take advantage of fertile soils and moisture from marshes to grow crops such as maize, rice and vegetables intended for sale. This can be particularly problematic when men are absent at key times of the year and fail to see the realities of farming. Those lengthy periods of absence also have implications on availability of household family labour for food production and livelihoods. This was evident as over 94% (n=80 male and n=87 female), indicated reliance on family labour especially on food crops which women are mostly involved in.

3.3.2 Gendered differences in seed choices, variety ranking and phenotypic traits preferences

Significant variations were observed in preferred seed choices among male and female growers (Figure 3-3A). For example, females are more likely to grow improved varieties than landraces if they have desirable post-harvest traits. This is supported by the fact that 74% of farmers reported growing local varieties due to their availability and mainly from previous saved grain, while 43% reported choosing improved varieties (open pollination varieties) due to their higher yields and early maturity (Figure 3-3 B-C). A significant association was observed between crop variety preferences and the gender of the farmers (Figure 3-3D). In the case of sorghum, it was observed that both female and male farmers preferred a local variety called *Thengeramanga*, (38%) to other local landraces because of its drought tolerance and high yielding attributes, (Figure 3-3D) despite its late maturity and smaller grain size. While, another landrace, *Wayawaya* produces large grain size and, is early maturing but poor flavour. Among pearl millet varieties, around 37% of female and 40% of male farmers prefer *Mchewere,* a local Landrace for its stalk sweetness, grain flavour, grain reddish colour and palatability despite late maturation. An improved variety, *Nyakhombo,* is desirable due to its high yield, and early maturation despite its poor flavour.

For sorghum and millet, yield, maturity, and drought tolerance are primary desirable traits, whereas pests and storage stability are secondary traits. In terms of phenotypes ranking, expressed as a count of votes, was based on shoot characteristics (stem height and leaf branching), root depth, and root spreading. *Thengeramanga*, was highly rated due to its deep roots, but farmers do not like its relatively long stem and lodging challenges (Figure 3-4A). The major challenges of many local varieties are in their morphology, specifically very tall relative to the improved varieties, which are shorter and mature early. With a short rainy season coupled with windy seasons, farmers prefer dwarf varieties with thick stems to avoid lodging. However, they have other attributes, which include grain taste and palatability (Figure 3-4B), which may not be a breeding target in crop improvement, or the trait may have been lost unintentionally during improvement.

Interestingly, male farmers emphasized more on the agronomic traits linked to yield and were market-oriented while female farmers preferred postharvest characteristics including taste and long shelf life for household food intake sustainability. During the focus group discussion men emphasized that *Wayawaya* has relatively large grain size compared to other local varieties and so it is linked to higher yield gains. However, women pointed out the importance of flour quality after milling for which they prefer *Thengeramanga*. Women were also typically interested in grain colour, taste and palatability, attributes which are relevant for consumption of food and beverages.

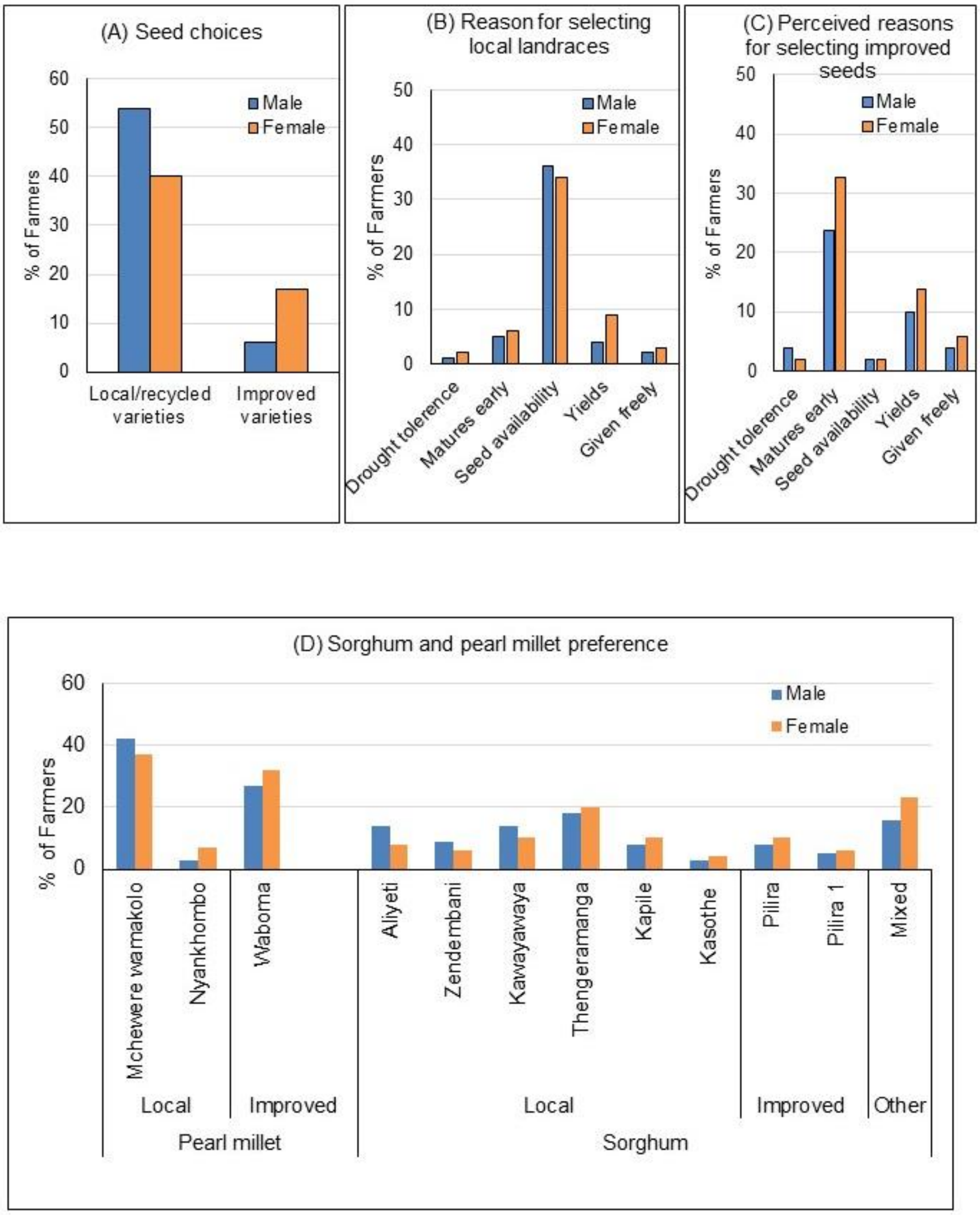


Figure 3-3: Gendered differences in seed choices and variety ranking by sorghum and pearl millet farmers in Malawi

A) Seed choices grown by gender 2019/2020 growing season. B) Farmers perceived reasons for the selection of seed of sorghum and pearl millet local landraces and C) Variation in perceived reasons for the selection of seeds of sorghum and pearl millet improved varieties. D) highest ranked sorghum and pearl millet varieties/landraces grown and farmers' preferences by gender. The results are collective responses from Chikwawa and Nsanje districts (n=179).

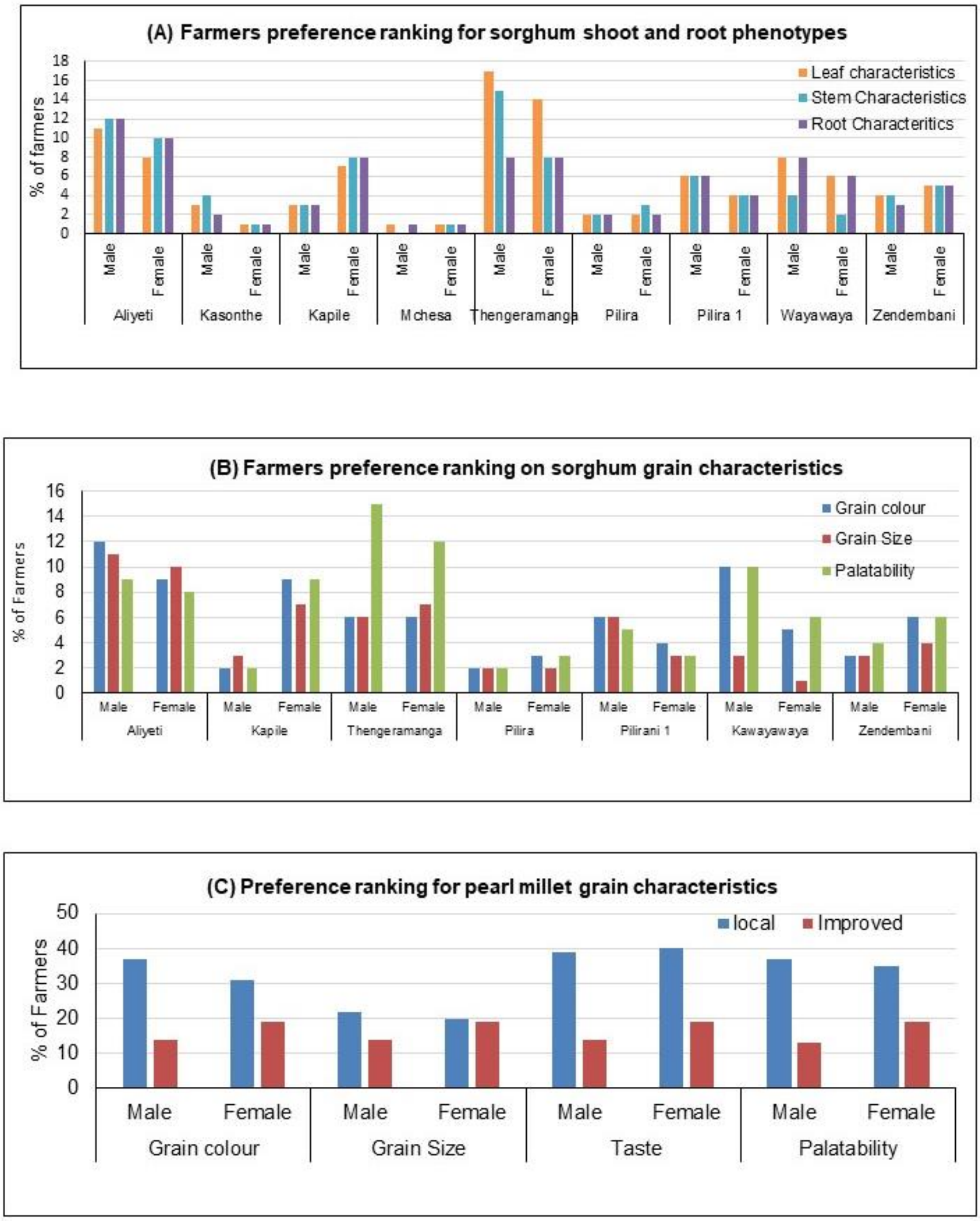


Figure 3-4: Malawi farmers preference ranking regarding sorghum and pearl millet phenotypic characteristics by gender

A) Farmers preferences on shoot and root phenotypes for commonly grown sorghum varieties. B) farmers preferred grain phenotypes among commonly grown sorghum local landraces and improved varieties. C) farmers preferred pearl millet grain phenotypes influenced by gender. The results are collective responses from Chikwawa and Nsanje districts (n=179).

3.3.3 Gender disparities affect sorghum and millet seed accessibility and availability among male and female farmers

The data show gender differences in access to quality sorghum and millet seeds, as well as availability challenges. A Chi-squared test was carried out to understand this association. A significant relationship was observed between seed accessibility and gender ($p<0.000$). Over 62% of men stated that they had the means to access seeds from seed distributors or local markets, compared to only 37% of female farmers. This disparity demonstrates that male farmers have greater access to high-quality seeds than female farmers. Most of the improved seed for sorghum and millets were sourced from organisations such as CGIAR centres (ICRISAT, CIMMYT) and Department of Agricultural Research Services (DARS), a government research institution through extension workers or farmer delivery programs. Others obtain improved seeds from informal local producers or multipliers whose seed quality is not paramount but nonetheless expensive which female farmers mostly cannot afford. About 64% of female farmers compared with 32% of male farmers indicated that friends and family were their main sources of sorghum and millet seeds of mainly local landraces.

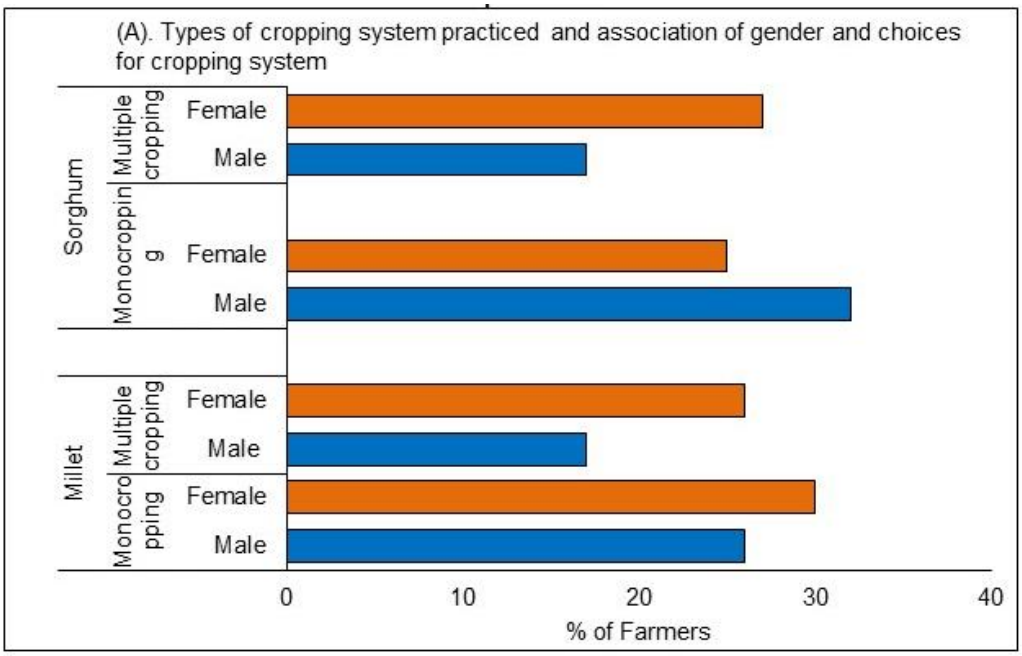
Regarding the availability of high-quality seed, there have been variations in the distance to access seed, as most local seed growers or sorghum distributors are further away from most farmers. While the average distance is 3.24km, men indicated they may travel more than 20km to look for good seeds from agro-dealers or seed distributors. The distance can affect female farmer's access to quality seeds, as most lack access to transportation. It has been noted that sorghum and millet lack formal seed producers and markets relative to major grains such as maize, which makes the seeds unavailable. These barriers to the availability of improved varieties impact the selection of a desirable variety that could enhance climate resilience.

3.3.4 Variation of choices and knowledge on climate-smart cropping systems and technologies

Association of gender and choices for cropping systems and technologies

Over the decades farmers have been practicing several cropping systems where other techniques have been learned but mostly passed on from generation to generation (Chivenge et al., 2015). This study revealed that at least 56% (n=85) of respondents practice monocropping for sorghum or pearl millet production, while 44% (n=66) of the respondents indicated multiple cropping (mixed cropping or intercropping) as their primary cropping practice. The results revealed a significant ($p<0.014$) association between the cropping system and gender in sorghum farming (Figure 3-5A). About 27% of female farmers prefer multiple cropping to monoculture, and in contrast, 32% of males choose monocropping. A similar pattern was also observed in millet, where 26% of female farmers practice multiple cropping compared to less than 20% males.

According to the farmers' perceptions (Figure 3-5B), the reasons for practicing monocropping include the following: 53% of respondents indicated that sorghum and pearl millet yield better and are easier crops to manage when grown as a sole crop. Nearly 12% of respondents pointed out that sorghum and millet crops are mainly grown on dry highlands or mostly infertile soils where most other crops would not perform well (Figure 3-5B). A further 7% of farmers explained that in most cases, sorghum growth habit and architecture included the crop's taller height, broader leaf shapes, and wider leaf angles, making it difficult for mixed cropping. It was evident that women utilize land as much as possible by having diversified crop enterprises for food self-reliance and diet diversification. It was observed that sorghum and millet are mainly intercropped with legumes such as common beans (red or white kidney beans, lima beans), cowpeas, and groundnuts. Farmers do recognize the benefits of mixed or intercropping for soil fertility improvement (21%, n=31) and moisture conservation (15%, n=23). However, most farmers in this study (59%) prefer intercropping sorghum with pearl millet or cowpeas, considering that both crops are adaptable to dry upland areas where it is not suitable for other food crops.



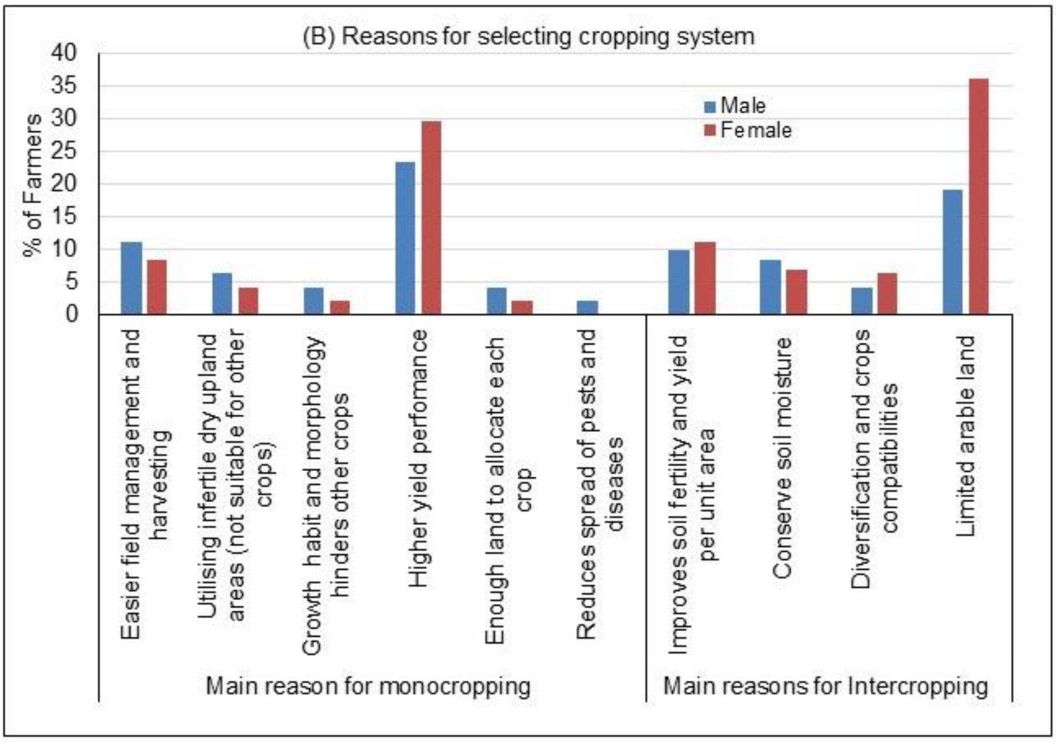


Figure 3-5: The association of gender and choices for cropping systems and technologies among sorghum and pearl millet farmers in Malawi

A) Types of cropping systems commonly practiced in sorghum and pearl millet farming, and how gender influences the choices. B) The reasons for the choices of sorghum and pearl millet cropping system by gender. The results are collective responses from Chikwawa and Nsanje districts (n=179).

3.3.5 Farmers' climate knowledge and gender influence on climate-smart cropping practices

Farmers are aware that it is becoming apparent that weather and climate have changed over time in Malawi and that these places are more vulnerable (Nkomwa et al., 2014). The knowledge has been observed through dramatic changes in weather conditions for a very long time now. Despite that, Nsanje has a long history of warmer climates, but it has now increased, irregular rainfall patterns, increased prevalence of pests, and frequent storms. Results from this study analysis revealed that there are many climate-smart cropping systems practices by farmers that provide multiple benefits in terms of productivity, resilience, and mitigation. However, most of the climate-smart technologies and associated practices are not well defined to farmers and in most cases, farmers self-define (Chinseu et al., 2019, Hermans et al., 2020, Dougill et al., 2021). From the farmers’ responses, two broad categories of practices were grouped namely conventional farming improved cropping practices which include good agronomical practices and conservation agriculture (Figure 3-6B). The most perceived climate-smart technologies by the farmers included compost manure application, mulching, pit planting, construction of terraces, selecting drought and early maturing varieties for drought adaptations. Our findings show that male farmers are likely to practice climate-smart technologies due to their access to farm inputs (manure and mulching) more than female farmers. Providing further evidence of gender-based disadvantages in the productive use of soil fertility inputs.

The study shows farmers significant variation on the knowledge and choices of climate-smart technologies in relation to gender. In this study, nearly 19% (n=34) of female respondents are not aware of climate smart technologies in sorghum and millet farming compared to less 10% (n=18) of male farmers. However, 37% and 32% of male and female respondents respectively are aware of CSA (Figure 3-6A). This could be caused by inequalities in accessing farming training and extension services with focus on cropping systems and climate-smart education. Men indicated that in past years most women were not participating/not involved in agricultural training but that equality on entitlement and accessibility to these training is improving at a slow pace. The other knowledge gap could be due to education level. According to our data 14% (n=26) of the male respondents left school at secondary school level whereas only 4% (n=8) of female respondents left school at that level. There was no significant association between gender and source of knowledge, with 22% (n=40) of male respondents and 16% females (n=30) learning more from extension officers followed by fellow farmers which implies the importance of technology delivery service and channels for farmers access and adoption (Figure 3-7).

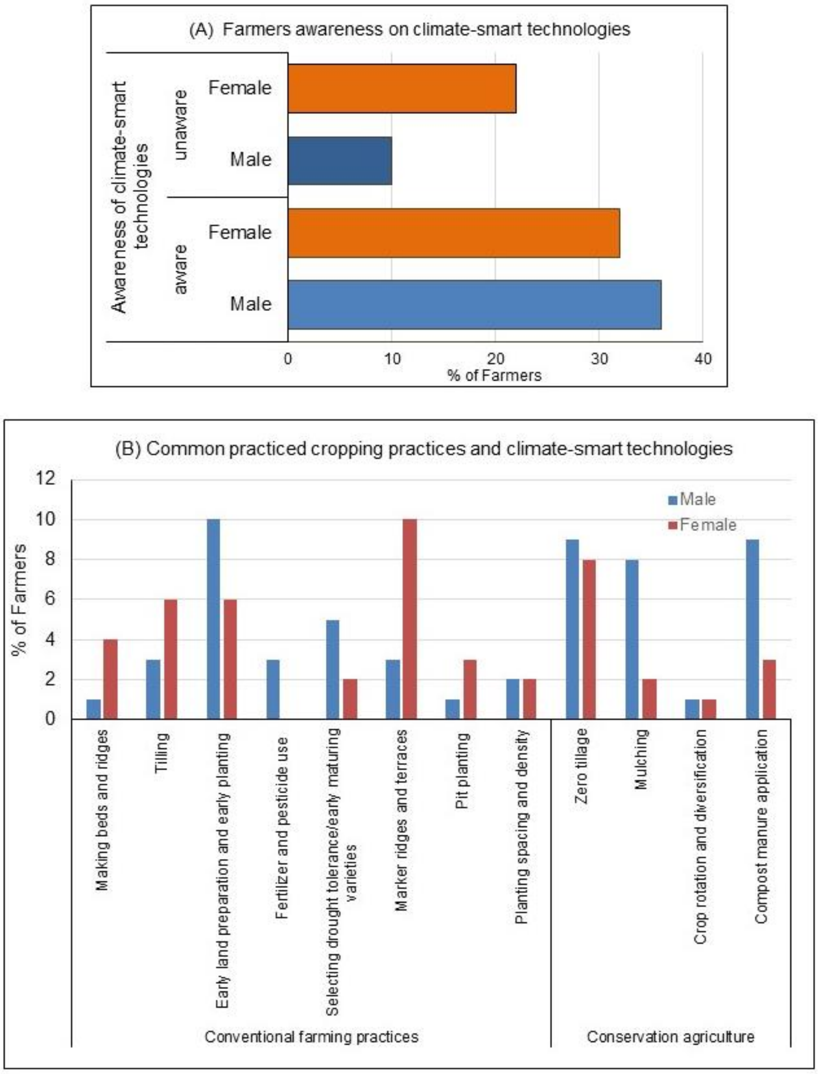


Figure 3-6: Farmers' climate knowledge and gender influence choices on climate smart cropping practices in Malawi

A) Shows sorghum and pearl millet farmers' awareness of climate-smart technologies and cropping practices. B) shows preferred climate-smart cropping practices for sorghum and pearl millet by gender. For farmers clarity the climate-smart practices were grouped as conventional farming practices and conservation agriculture. The results are collective responses from Chikwawa and Nsanje districts (n=179).

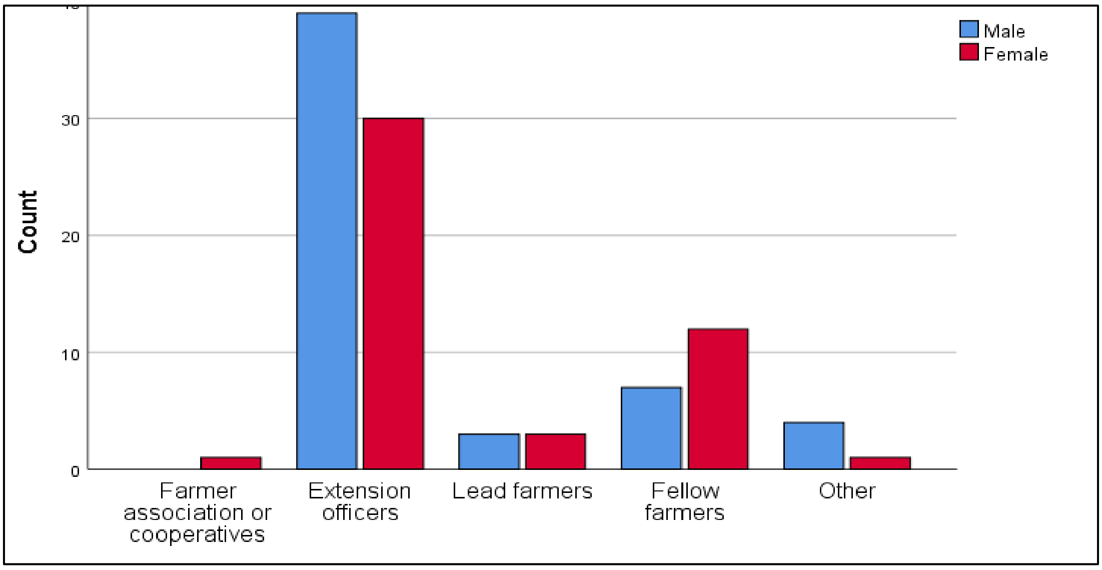


Figure 3-7: The association between gender and the source of information on climate-smart technology for sorghum and pearl millet farmers in Malawi

Results presents the collective responses for respondents in Chikwawa and Nsanje districts (n=179).

3.3.6 Farmers perceived challenges on CSAs

Nearly 15% of respondents reported a lack of sufficient knowledge as one of the drawbacks to implementing CSAs in their fields. The study shows that 36% of farmers have limited resources to practice climate-smart cropping systems, for instance, a lack of sufficient land for crop rotation. Almost 20% perceived climate-smart technologies as costs such as the construction of dams and pit planting. A further 30% indicated that most CSAs are labour-intensive and time-consuming. During focus group discussions with female farmers, it was stated that labour requirements for some of the cropping practices coupled with lack of entitlement and control for land affect their decisions on the technologies compared to male farmers. Other challenges include inaccessibility to improved sorghum and millet varieties, and conflicting farming systems, for example, crop residues for mulching and livestock feed (Table 3-2).

Table 3-2: Farmers' perceived challenges associated with climate-smart technologies practiced in sorghum and pearl millet production across gender in Chikwawa and Nsanje districts of Malawi.

Challenges	% of male Farmers	% of female females	% of total farmers
Labour intensive and time consuming	13	17	30
Lack knowledge and insufficient extension support	6	9	15
Yield loss due to reduced plant spacing/density	5	0	5
Increases pests' incidences when using mulching	3	0	3
Limited resources for adopting the technologies	20	16	36
Inaccessibility and unavailability of improved varieties	7	11	18
Extreme weather events (flooding, dry spells) ruin technologies	9	10	19

3.3.7 Differences in opportunities and challenges for sorghum and millet production among female and male: farmers and stakeholder perspectives

Stakeholders including researchers, the Consultative Group on International Agricultural Research (CGIAR) representatives, and seed companies outlined the potential of sorghum and millet as future crops in the context of community resilience to climate change. (Table 3-3 and Table 3-4), outlines summary of challenges and opportunities for sorghum and millets for climate adaptation and resilience that were gathered during household interviews and stakeholder consultations. The most mentioned challenges include seed accessibility for varieties with a wide range of desirable traits. Market availability and knowledge capacity on field and post-harvest technologies and value addition is still lacking. Therefore, market accessibilities and promotion of sorghum/millet recipes are one of the opportunities for commercialization of these forgotten crops and to improve their utilization.

Another opportunity is the availability of natural genetic diversity within landraces which can be used to select desirable traits for crop improvement. This was evident from the conservation unit both *in situ* and at the gene bank. According to Department of Agriculture Research Services (DARS), there is a growing concern on integrating gender needs during selection of sorghum breeding materials (verbal communication DARS representative on small grains (sorghum and millets). Consultations with other crop researchers indicated that sorghum improved varieties have been released in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), although availability is still low. According to our discussions with seed companies, for most of past breeding programs initially, farmers were not involved in selection of the plant and grain traits of the new varieties. Recent participation by farmers in trait selection has paid off in terms of varieties that perform well and are acceptable to farmers. Therefore, this is an opportunity for farmers to have desirable crop traits according to their priority needs.

Table 3-3: Farmers challenges on production, post-harvest handling, marketing, and access to support services for sorghum and pearl millet farmers in Malawi by gender

Specified Challenges	**Gender of the household head**									
	Sorghum					**Millet**				
	Male		Female			Male		Female		
Seed access	Freq.	Perc.	Freq.	Perc.	P-Value	Freq.	Perc.	Freq.	Perc.	P-Value
No	25.	29.1%	8.	11.1%	0.006	19.	26.8%	8.	10.7%	0.012
Yes	61.	70.9%	64.	88.9%		52.	73.2%	67.	89.3%	
Pest and Diseases										
No	26.	30.2%	9.	12.5%	0.008	31.	43.7%	25.	33.3%	0.200
Yes	60.	69.8%	63.	87.5%		40.	56.3%	50.	66.7%	
Production technologies										
No	34.	39.5%	25.	34.7%	0.533	31.	43.7%	33.	44.0%	0.967
Yes	52.	60.5%	47.	65.3%		40.	56.3%	42.	56.0%	
Post-harvest handling and storage										
No	33.	38.4%	16.	22.2%	0.029	28.	39.4%	24.	32.0%	0.348
Yes	53.	61.6%	56.	77.8%		43.	60.6%	51.	68.0%	
Markets										
No	44.	51.2%	36.	50.0%	0.884	39.	54.9%	44.	58.7%	0.649
Yes	42.	48.8%	36.	50.0%		32.	45.1%	31.	41.3%	
Support Services										
No	48.	55.8%	29.	40.3%	0.052	36.	50.7%	30.	40.0%	0.194
Yes	38.	44.2%	43.	59.7%		35.	49.3%	45.	60.0%	

Table 3-4: Stakeholder perspective on challenges and opportunities for sorghum and pearl millet crop production in Malawi

Challenges	Opportunities
Limited access and availability of improved sorghum and pearl millet seeds.	There is a growing interest and investment in research and crop improvement for under-utilised crop species by research centres such as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Universities. Availability of diverse landraces in-situ and local gene banks creates a platform as novel sources of traits for crop improvement
Limited knowledge and capacity related to climate-smart technologies.	Increasing investment in knowledge sharing platforms from government research services and the Consultative Group on International Agricultural Research (CGIAR) centres to farmers.
Unavailability of reliable formal markets for sorghum and millet grains and products.	Increased awareness of dietary diversification and the importance of sorghum and pearl millet-based products as gluten-free creates demand for food and feed.
Limited knowledge of post-harvest handling and value addition.	Potential diversity in recipes and products from sorghum and pearl millet.
Extreme and climate weather events including pests and disease incidences cause crop losses.	Growing interest to develop climate-resilient crops of desirable traits by breeders.

3.4 Discussion

3.4.1 Farmers’ knowledge and needs would guide the pre-selection of desired traits for crop improvement programs

This study demonstrates that gender and climate risk awareness influences farmer decisions and choices for sorghum and millet varieties and cropping systems in Malawi. Our findings show that the control of such decisions is primarily influenced by societal, cultural norms and systems. The evidence is provided that farmers experience on climate and weather events shape their decision on crops and varieties to grow. Due to patrilineal marriage systems in southern Malawi, male farmers have greater control over household decision making and planning which influences household food resilience and sustainability. Studies have indicated that in a household female farmers consult partners and were more likely to report joint decision-making than male farmers on most major agricultural crops (Acosta et al., 2020, Van Campenhout et al., 2023). According to our findings for sorghum and millet farming, male farmers believe that women are the sole decision-makers. However, the entitlement to land also influences the selection of and decision making on cropping systems. Our findings confirm results from other studies on gender inequalities on land ownership in Africa (Partey et al., 2020), indicating that female farmers’ choices on cropping practices and varieties are influenced by entitlement to land and other resource accessibility.

Gendered inequality in decision-making goes beyond cropping practices to socially constructed rules of resource accessibility and restricted mobility. This study confirms gender disparities affect sorghum and millet seed accessibility and availability. The data show gender differences in access to quality sorghum and millet seeds, as well as availability challenges. These barriers to the availability of improved varieties have an impact on their selection of a desirable variety that could be suitable for a specific cropping practice for climate resilience. The findings show trends in importance of gender differentiated choices on variety phenotypic preferences. Understanding phenotypes such as plant architectural traits has an implication on desirable cropping systems and plays a significant role in crop improvement selection. Farmers preference is mainly guided by yield and grain characteristics. Yield, in terms of weight and grain size is more preferred by men while women’s interest is beyond yield to post-harvest related attributes. Wanga et al., (2022) agrees that incorporating farmer-preferred traits is key to the adoption of new generation varieties in traditional sorghum growing areas. Farmers preference on local landraces over improved varieties confirm other studies that most landraces are well-adapted to low-input farming systems and possess essential quality traits (Orr et al., 2016). Despite farmers' desire for improved varieties each year, access to good seeds has been a significant challenge in both sorghum and millet growing areas across Africa (Okori et al., 2022). This is the case with many neglected local indigenous crops that do not have well-defined seed systems and value chains.

The findings indicate significant variation of choices and knowledge on climate-smart cropping systems and technologies among male and female farmers. Farmers are aware of the changes in weather and climate over time in Malawi and that some places are more vulnerable. The high awareness of climate change was evident on how they narrated their experience on the past and recent climate variability and the findings shows consistency with other studies (Nkomwa et al., 2014, Partey et al., 2020). Although local knowledge is a relatively unexploited resource, it is useful for understanding the impacts of climate change on crop production and choices on climate-smart cropping systems (Labeyrie et al., 2021).

This study adds to the findings of (Neufeldt et al., 2013) that most of these CSA cropping interventions are knowledge-intensive, location-specific, and require considerable capacity development. This current study provides evidence that the variation in knowledge on CSA between male and female farmers implies inequalities in accessing the information and trainings. The findings reveals that the increased knowledge on CSA and growing interest in under-researched crops species implies an opportunity for community climate adaptation, species conservation, enhancement and crop improvement. The vast natural genetic diversity within sorghum and millet landraces is important for selection of desirable traits and models for understanding climate resilient crop species for rapid crop improvement. Apart from genotyping challenges, screening for phenotypes associated with superior agronomic traits has been a major bottleneck and costly for plant breeders (Song et al., 2021). Therefore, farmers' participatory selection of traits according to their, climate change knowledge coupled with gender needs would guide the pre-selection of desired traits for crop improvement programs. Farmers along with a multi-disciplinary team should be involved in developing key traits for breeding pipelines. This is well aligned with the advancement of molecular biology and crop improvement technologies as an opportunity for Plant Breeders to identify and target desirable traits to meet societal family needs, thereby improving households' food and nutrition across low and middle-income nations.

3.4.2 Concluding remarks

This study focused on assessment of sorghum and pearl millet crop production and to understand varietal phenotypic trait and cropping system choices how they are influenced by gender and climate risk awareness in Malawi. These crops receive less attention but are very important as climate smart crops as adaptations to warmer climates and prolonged drought periods. It is shown that the integration of gender and climate risk awareness influences farmer decisions for production and varietal trait phenotypes choices for sorghum and millet. Understanding the diverse gender preferences for different varietal attributes is crucial for ensuring that farmers are involved in decision-making and selection.

The study discovers that new and crucial opportunities concerning sorghum and millet production should target female-headed households and women as they dominate sorghum and millet production. While recommending a more participatory, farmer-oriented approach to developing climate-smart technologies. We also recognize the need for more socioeconomic research studies to be conducted across underutilized crop species. It can be concluded that efforts to improve crop adaptation and resilience to climate change in vulnerable regions must pay greater attention to the processes and relations between gender and climate change knowledge bases. In order to achieve sustainable impacts, it is necessary to strengthen seed systems for underutilized climate-resilient crops, along with climate-smart cropping systems. The findings from this study offer intriguing considerations to inform government policy decisions and investment plans for gender-responsive, climate-smart adaptation strategies, and resilience programs in Malawi and sub-Saharan Africa.

Chapter 4

Natural variation and trade-offs in traits linked to sorghum grain yield and nutritional qualities under drought

4.1 Introduction

Drought is widely considered to be the most significant threat to global food production (Reddy et al., 2004, Farooq et al., 2009b, Halford et al., 2015). There is an urgent need to rapidly develop climate-resilient crops and sustain agricultural systems that produce more nutrient-rich food while simultaneously adapting to a changing climate. Although sorghum is generally considered drought tolerant, water stress still significantly hampers its productivity and nutritional quality across its major cultivation areas (Abreha et al., 2022). Drought stress changes the relationship between morpho-physiological traits and source-sink strength, which alters grain physico-chemical characteristics and reduces mineralization of nutrients (Stagnari et al., 2016, Impa et al., 2019). The molecular, biochemical, and physiological responses to drought integrate the effects of species genetics, environment, and management (G x E x M). As such, identifying mechanisms that allow plants to sustain crop production under drought is crucial for understanding drought tolerance and promoting drought resilience (Abreha et al., 2022, Vadez et al., 2024). Drought tolerance is characterised by traits that allow water to be available for grain filling and maturation or improve crop water utilisation efficiency. To maintain crop productivity, it is necessary to understand which traits optimise yield for varying drought environments (Vadez et al., 2024). The study in Chapter 3 of this thesis has a significant impact on the intentionality of designing crop adaptation strategies and solutions that address the needs of the community. The identification of robust, drought-tolerant, and elite varieties is accomplished through the linking of agricultural traits selected by farmers with physiology and molecular understanding of the trade-offs.

Different traits have distinct impact on plant performance during drought, and the interactions between genetic background and trade-offs in space and time are all significant (Abreha et al., 2022, Vadez et al., 2024). Trade-offs among traits refer to a condition in which an increase in the performance of one trait causes a decrease in the performance of another, given the limited amount of available resources (Ou et al., 2020, Fradgley et al., 2022). The most common trade-off considered in breeding is that between source and reproductive sinks, which ultimately determines seed yield for a given amount of total carbon assimilated.

Another obvious trade-off is the scare assimilation to allow roots grow deeper and access subsoil water or restrict root access to more easily available water, which is observed under drought conditions (Dwivedi et al., 2021). Modifying trade-offs is a breeding challenge since many genes of minor effect are also involved (Dwivedi et al., 2021). As a result, germplasm selection does not always lead to optimal solutions due to trade-offs which may often be unknown, vary across environment, plant development and genetics. According to previous studies, both trade-offs and environmental variation do not allow single factor optimisation, for instance in plant architecture and physiology, or use of inputs (Sadras and Denison, 2016). Trade-offs in the expression of plant traits are an invariable consequence of their plasticity, thus helping to maintain relative fitness under a wide range of unpredictable conditions and to optimise yield production.

While the current breeding programs and agricultural incentives are almost exclusively yield-based, comparatively little attention has been given to grain quality traits. This is a matter of great concern, because environmental stress will affect the relative abundance of starch, protein, and all other important minerals(Soares et al., 2019). The link between climate change, specifically drought and food quality remains one of the most ill-defined areas in global change studies yet is directly relevant to human health (Hummel et al., 2018, Soares et al., 2019). The impact of drought on mineral nutrition is still unclear, specifically for sorghum, an under-researched yet climate-resilient crop.

The diverse availability of sorghum races (commonly referred to as landraces) and genotypes within races provides a greater chance for discovering new novel sources of natural variability for selecting desirable traits. The classification of sorghum into races is based on its evolutionary divergence, resulting from genetic and morphological differentiation (Sapkota et al., 2020) due to the migration and adaptation of sorghum across Africa and Asia. Due to the significant interaction between genotypes and environments, it is crucial to evaluate germplasm for drought tolerance in a target environment when selecting genotypes for breeding programs. However, a poor understanding of sorghum natural variation, structural and physiological trade-offs is currently limiting pre-selection for stress tolerance, productivity, and nutritional quality in crop improvement (Araus et al., 2008, Dwivedi et al., 2021).

Therefore, the objectives of this study are to; i) determine the impact of drought stress on grain yield and nutritional qualities across and within sorghum landraces; ii) assess sorghum natural variation for grain nutritional quality in response to drought stress; iii) identify elite genotypes with higher yield potential and nutrition qualities under varying water conditions; iv) establish how phenotypic traits and physiological function linked to adaptations influences the grain nutrition qualities under drought stress conditions. The hypothesis was that the nutritional quality of grains is influenced by genetic variation in phenotype, physiological, and yield traits related to drought adaptations, which can be used to identify drought-tolerant genotypes.

The work is significant as large-scale data set of about 242 sorghum genotypes from a minicore collection was analysed, for a robust understanding of the drought effect on yield and nutrition quality in soil conditions. The work in this chapter sets a basis for identifying the key traits for rapid screening of climate-resilient, high-yielding, and nutritionally elite sorghum genotypes for the future. Breeding for improved crop quality has the potential to greatly contribute to improving nutritional security under increasingly challenging global conditions.

4.2 Results

4.2.1 Drought stress impact sorghum grain yield and the effect vary among landraces and genotypes

Sorghum grain nutrition lysimeter data set was used to investigate the effect of drought stress on grain yield and to establish natural variation across the collection of 242 sorghum genotypes grouped in landraces (*caudatum*, *bicolor*, *guinea*, *durra*, and *kafir*) and 10 intermediate races described in Chapter 2 (2.5.1) of this thesis. Grain size and grain weight are among the main components of grain economic yield based on previous work in cereals (Rattey et al., 2009, Mutava et al., 2011). However, for this data, grain weight was collected in both well-watered and drought stress while, grain size was only collected for well-watered conditions. Therefore, based on the data available and for comparison, total grain weight per plant was analysed to indicate grain yield in both drought stress and well-watered conditions across landraces. A comparison of well-watered and drought stress treatments shows highly significant ($p<0.001$) interaction in total grain weight among landraces (Figure 4-1). Overall, G*uinea bicolor* genotypes show higher performance on average grain dry weight (g) under drought, while the *Durra* landrace performs higher on average grain dry weight in well-watered compared to other landraces. It is worth noting that a large variability exists between genotypes within landraces in well-watered conditions, evidenced by the longer box plots than in water stress implying wider natural variation in normal environmental conditions. The findings further demonstrate the importance of tracing the landrace to screen for possible genetic variability and similarities across genotypes and as a valuable tool to screen for climate resilience that could be linked to past geographical location.

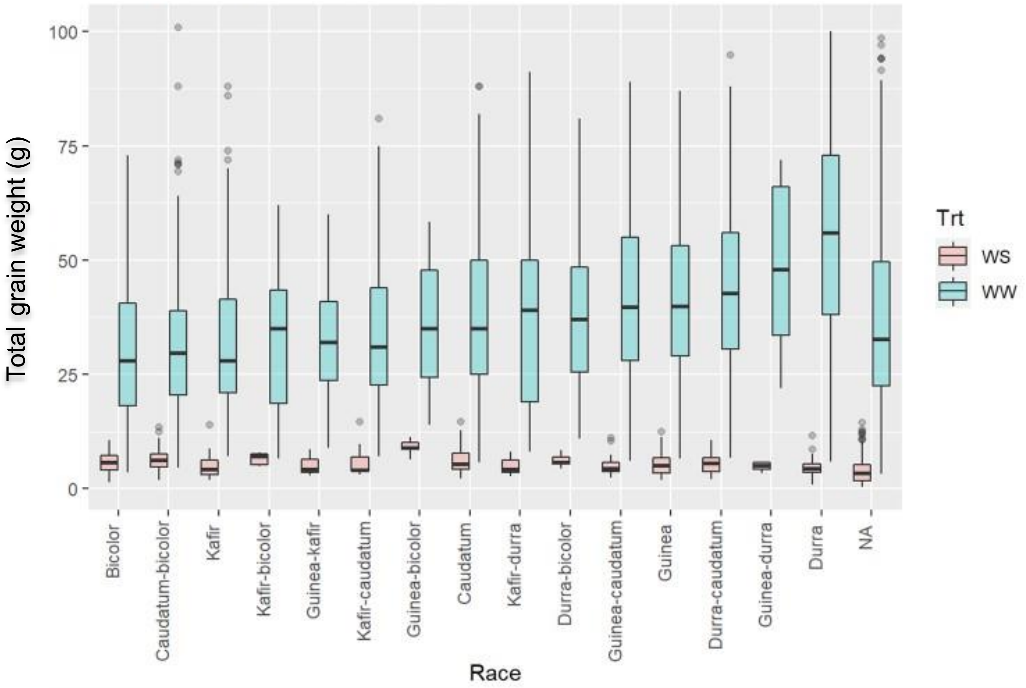


Figure 4-1: Variation in total grain weight in response to drought across the landraces and within landraces.

The effect of varying water conditions on total grain weight in grams per plant in both well-watered and water-stress conditions. Significant differences were observed across 15 land races and treatment on grain weight. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentiles. ANOVA was used to test for significance ($p<0.05$). NA represents the genotypes with unknown landrace category. ‘Trt’ represents treatment.

Further analysis was carried out to identify top-performing genotypes separately under well-watered and water-stress conditions. The analysis aids our understanding of genotypic variations in yield performance. This analysis identified the top 25 performing genotypes in drought (Figure 4-2A) and the top 25 performing genotypes in a control environment (Figure 4-2B). The findings clearly show that neither genotypes in the top-performing genotype under well-watered conditions are among the top-performing genotypes under drought stress. Indicating how drought impacts grain yield. Further analysis of total genotype-specific performance on grain weight for all 242 genotypes is attached in (Appendix).

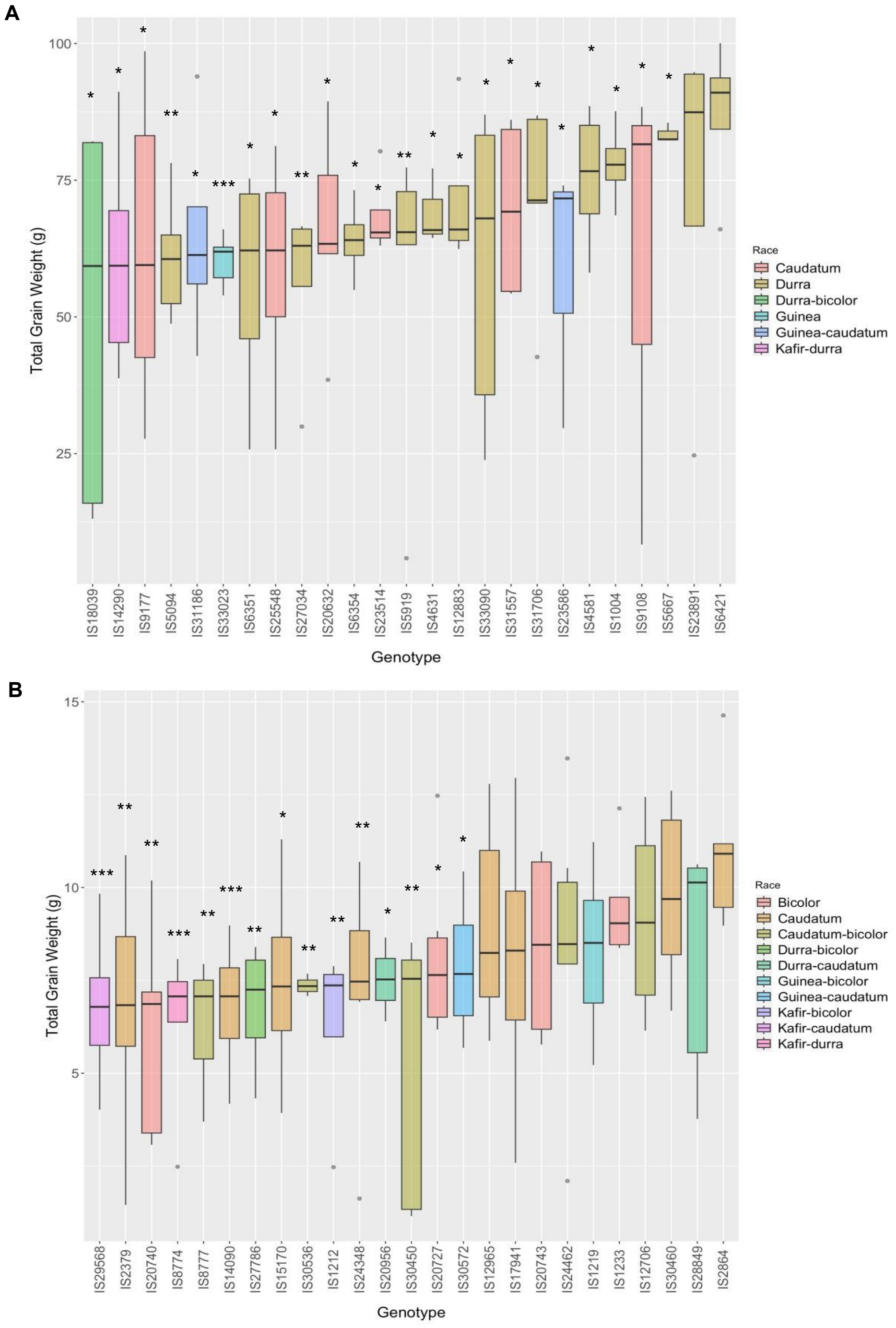


Figure 4-2: Top 25 genotypes with higher grain weight in varying water conditions

A) Variation in total grain weight for top-yield-performing genotypes in well-watered conditions. B) Variation in sorghum top-yield-performing genotypes in water stress conditions. Dark horizontal lines in the box plot represent the median, with the box representing the 25th and 75th percentiles. ANOVA was used to test for significance ($p<0.05$). Significance levels at $p<0.05$*,0.01**,0.001*** are based on the difference from the highest performing genotype for each graph (represented separately for each treatment, WW and WS).

4.2.2 Grain nutrient levels change with drought stress conditions and effects vary across landrace and genotypes

Macronutrient levels, protein, starch, and fat content were determined to understand the impact of drought stress treatment on grain nutritional composition. Overall, significant interactions were observed between treatments and across landraces. There are significant interaction effects ($p<0.001$) between the protein levels in well-watered and water-stress conditions (Figure 4-3A). Higher levels of protein were observed in water-stressed conditions compared to well-watered conditions. Further, there was significant relative variation in the response of races to protein levels. Higher protein content across landraces was observed in water stress conditions than in well-watered conditions (Figure 4-3B). Intermediate races genotypes, *Guinea-bicolor*, *Durra-bicolor*, *Kafir-bicolor*, and *Guinea-kafir* landraces exhibited higher protein levels in drought-stress conditions. As in protein starch content was higher in drought water-stress in comparison with well-watered (Figure 4-4A). Contrary to protein, for starch content, the main race *Kafir* shows a relatively higher accumulation of starch levels in drought water stress, whereas intermediate *Guinea-bicolor* has relatively lower starch levels in both well-watered and water-stress environments (Figure 4-4B). Finally, analysis of fat content (Figure 4-5A) shows a significant decline associated with the impact of drought stress compared to well-watered conditions. *Kafir-bicolor* exhibited relatively a significant higher fat content in well-watered conditions compared to other races (Figure 4-5B).

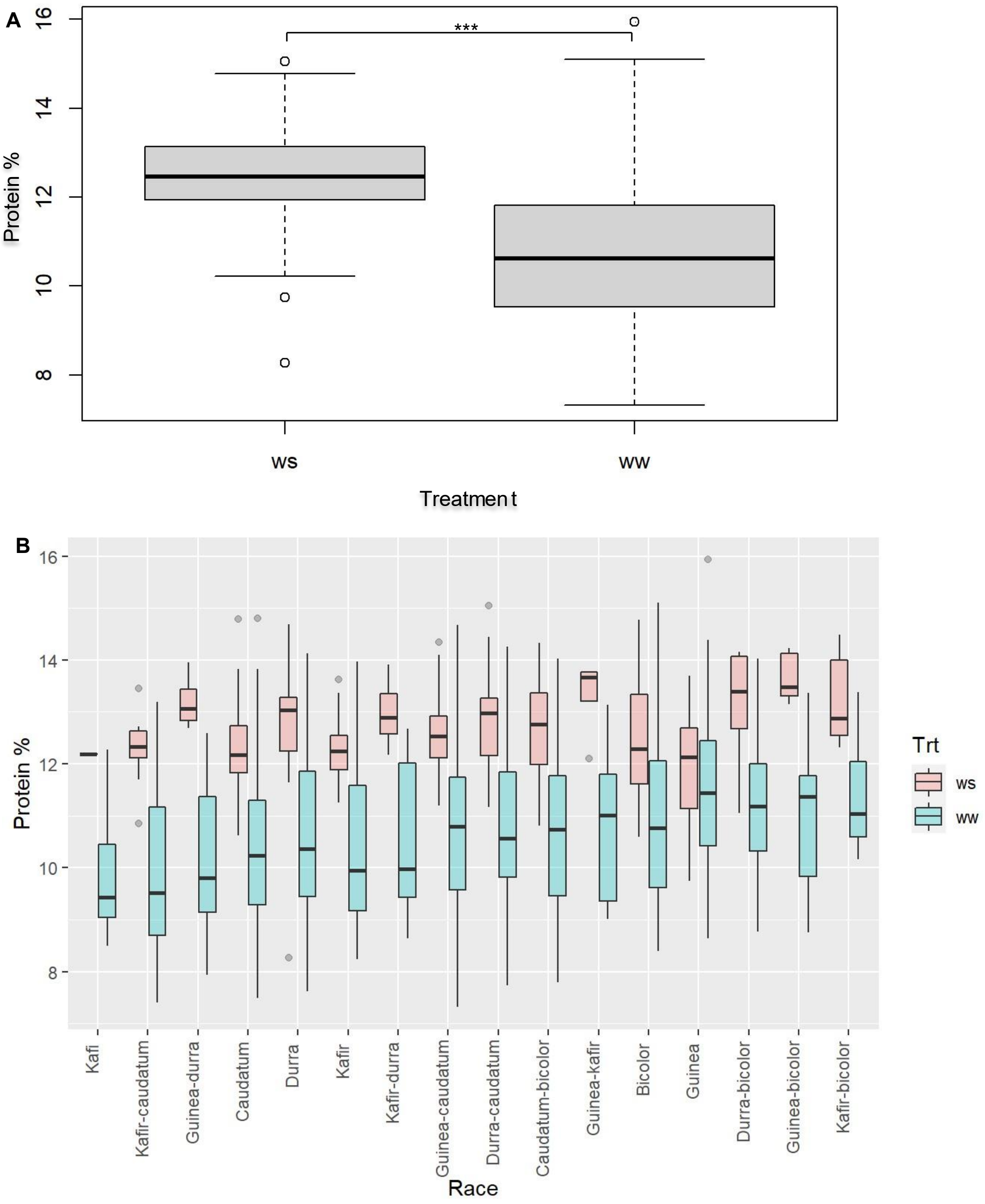


Figure 4-3: Total soluble protein content across sorghum landrace by treatment

A) Total grain protein content (%) in response to treatment (WS=water stress, WW=well-watered). B) Variation of sorghum landraces on total grain protein content % in response to treatment. Higher amounts were observed under water-stress conditions. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. 'Trt' on legends represent treatment.

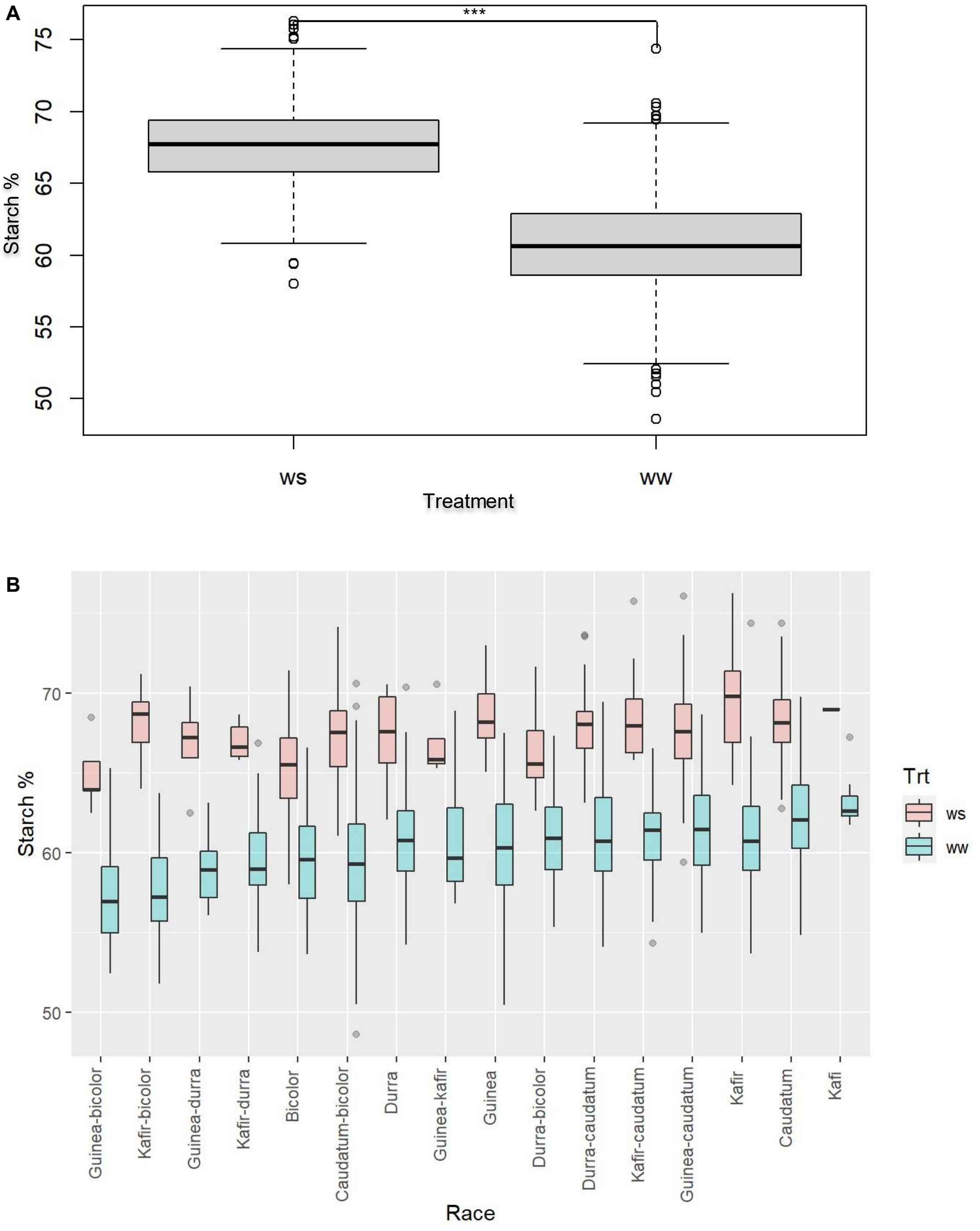


Figure 4-4: Total soluble starch content across sorghum landrace by treatment

A) Total grain starch content (%) in response to treatment (WS=water stress, WW=well-watered). B) Variation of sorghum landraces on total grain starch content % in response to treatment. Higher amounts were observed under water-stress conditions. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. 'Trt' on legends represent treatment.

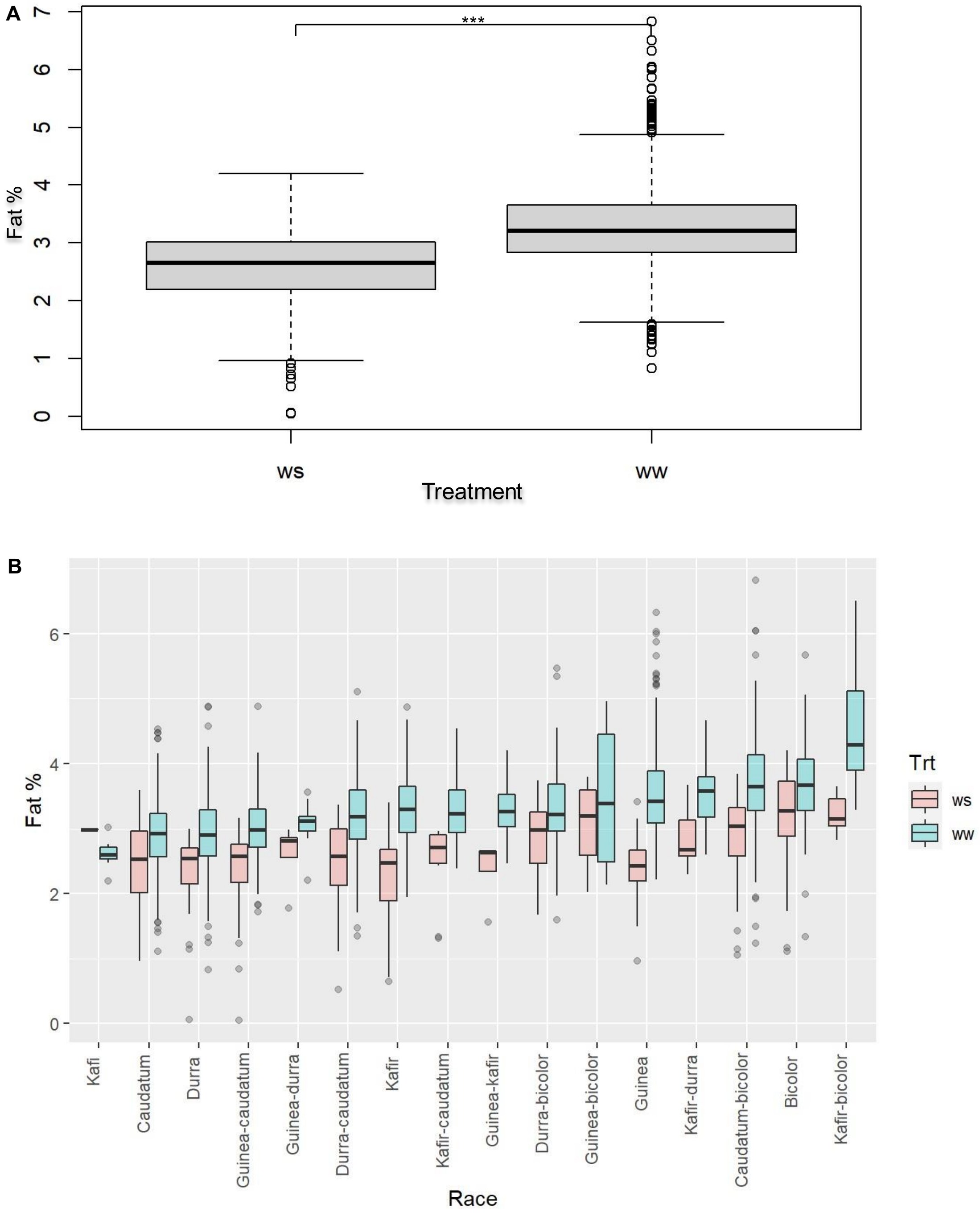


Figure 4-5: Total soluble fat content across sorghum landrace by treatment

A) Total grain fat content % in response to treatment (WS=water stress, WW=well-watered). B) Variation of sorghum landraces on total grain fat content % in response to treatment. Higher amounts were observed under well-watered conditions. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. ‘Trt’ on legends represent treatment.

4.2.3 Top performing genotypes for protein, starch and fat under varying water conditions

Further analysis was conducted to identify the top 10 percent (top 25) performing protein, starch, and fat genotypes in well-watered and water-stress conditions. This analysis helps to understand genotypic differences within and across the races and supports the identification of drought-resilient genotypes. Overall, a significant variation was observed in all the nutritional content influenced by treatment and genotypic differences. In terms of protein (Figure 4-6), IS20740 (mean of 14.3% protein) exhibited highest protein content in well-watered conditions (Figure 4-6A), while IS8348 and IS28849 exhibited highest mean values of 14.7% and 14.4% in water-stress conditions, respectively (Figure 4-6B). Genotype IS1219 is among the top 10% performing genotypes in both well-watered (13.1% protein content) and (13.8% protein content) in water-stressed conditions. Interestingly, genotype IS1219 is in the top 10% of high-yielding genotypes under drought stress as previously presented in (Figure 4-2B above on top 25 performing genotypes on gain weight per plant of yield performance).

For starch, IS28614 and IS25584, both with a mean of 67.6% starch, were among the top-performing genotypes in well-watered conditions (Figure 4-7A), while IS2397(75% starch) and IS29239 (73% starch) performed well in water stress (Figure 4-7B). In terms of fat content, IS30466 exhibited relatively higher fat content in well-watered, while IS608, IS22616, and IS12706, all from the same race and intermediate of *bicolor*, show higher mean fat content of 3.8% in water stress (Figure 4-8B). Again, IS1219 is among the top 10% containing higher mean fat content (3.6% and 3.4%), well-watered, and water stress, respectively (Figure 4-8). From the findings, it was further observed that genotypes of the race *bicolor* and their intermediate had high fat content in water stress, while *kafir* and their intermediate had high starch during drought. These performances imply associations with adaptative mechanisms under varying water supply conditions, geographical originality of these genotypes and how nutritional qualities are linked to both genetic and environmental factors. The current study confirms the previous findings in wheat by Nawaz et al. (2013) demonstrating that during terminal drought, tolerant genotypes yield significantly higher compared with other genotypes of the same species.

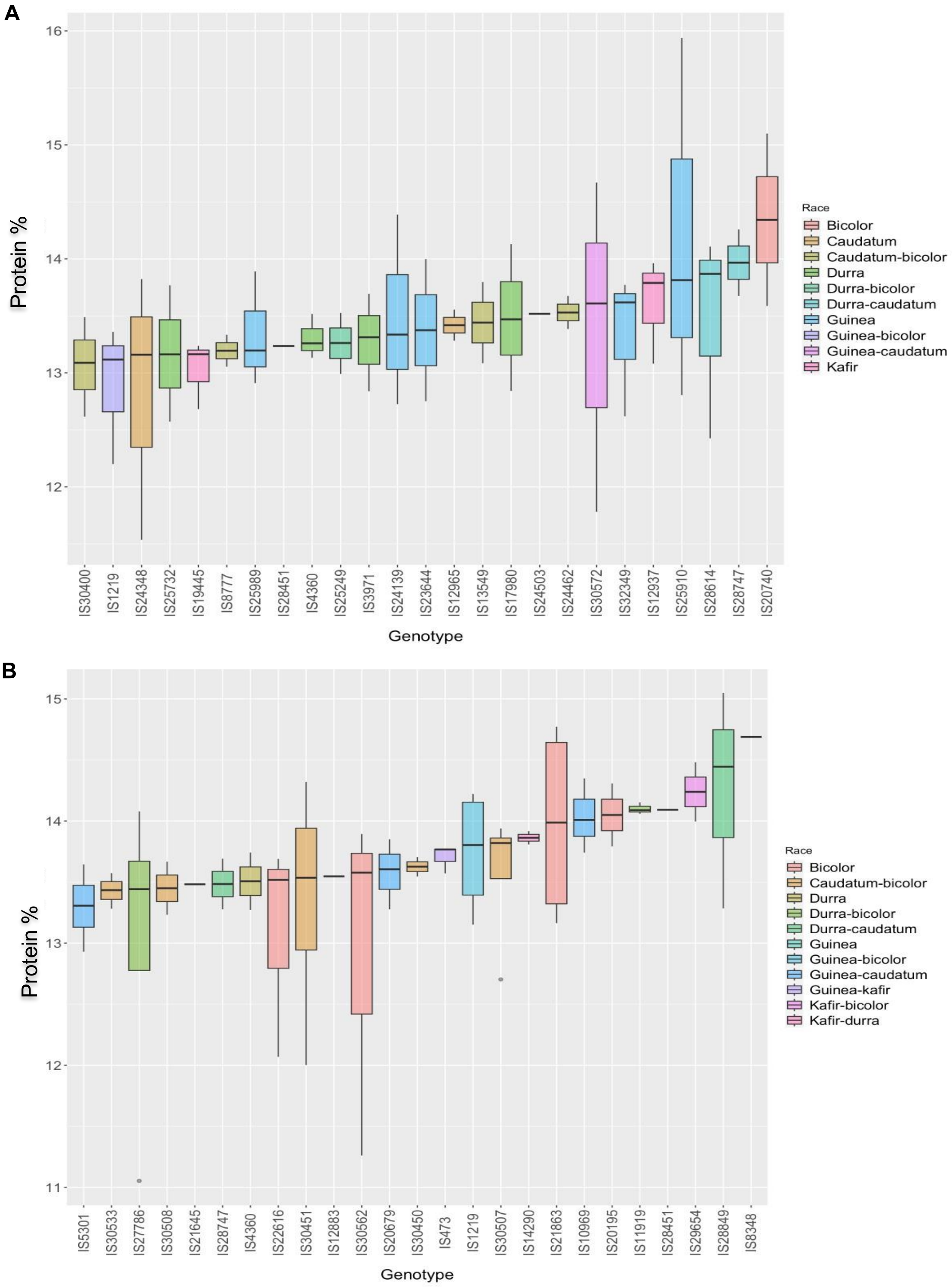


Figure 4-6: Top performing genotypes in grain protein content in well-watered and drought treatment

A) Top 10% of performing genotypes on grain protein accumulation in WW. B) Top 10% performing genotypes on grain protein accumulation in WS respectively. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile.

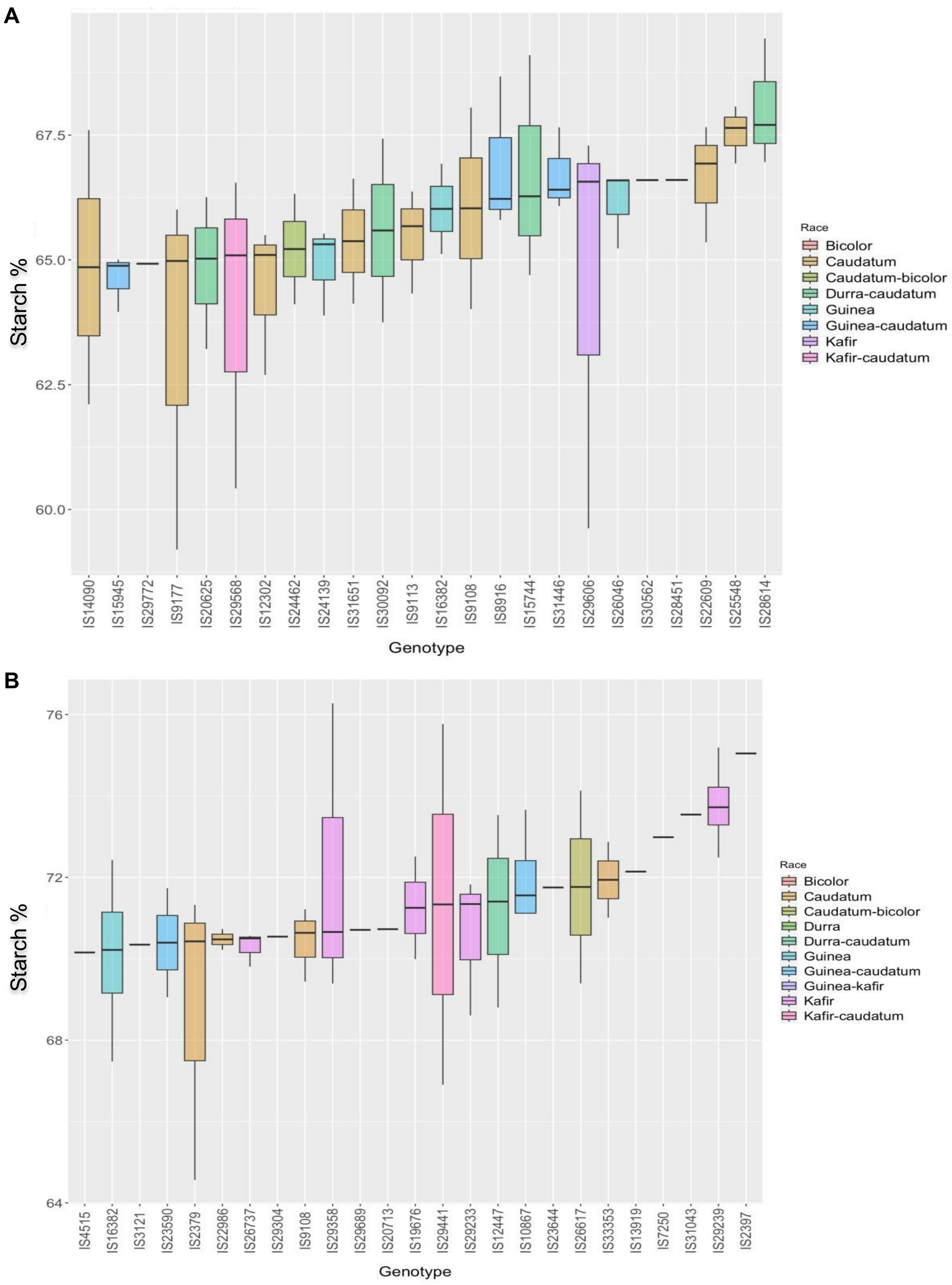


Figure 4-7: Top performing genotypes in starch content in well-watered and drought stress

A) Top 10% performing genotypes on grain starch content in WW. B) Top performing genotypes on grain starch content in WS. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile.

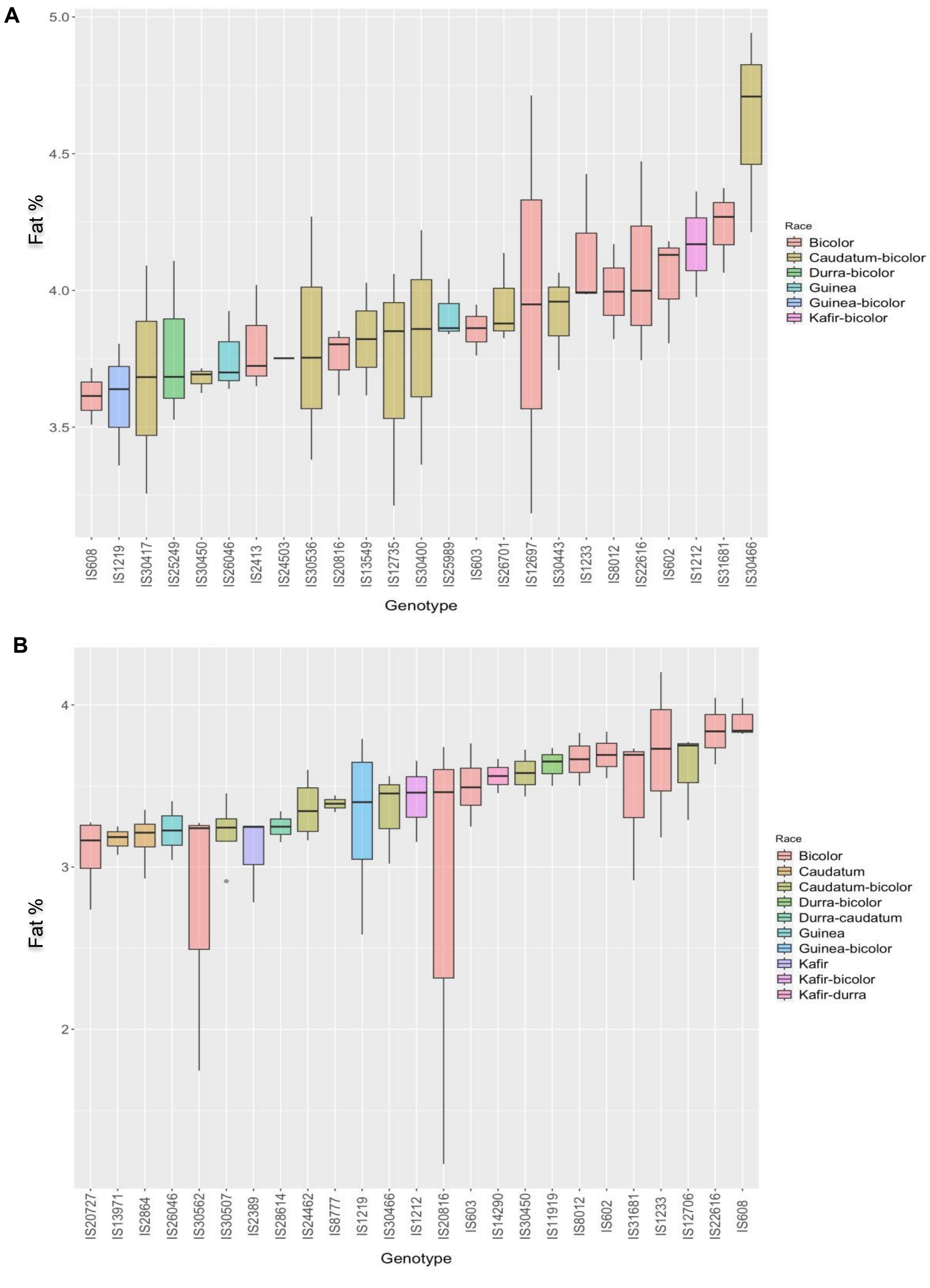


Figure 4-8: Top 10% performing genotypes in fat content in well-watered and drought treatment

A) Top 10% performing genotypes on total grain fat content in WW. B) Top 10% performing genotypes on total grain fat content in WS. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile.

4.2.4 Correlation analysis among protein, fat and starch under well-watered and drought water stress

Overall, significant variation exists across and within sorghum landraces on grain protein, starch and fat content as evidently presented (Table 4-1 below). To further analyse this effect, correlation analysis was carried out between protein, fat and starch (Figure 4-9). A significant but positive association (r=0.41, p<0.001) was observed between starch and protein levels and a weak significant negative association between protein and fat levels was shown (r=-0.16, p<0.001). The findings further show a significant strong inverse relationship between starch and fat (r=-0.61, p<0.001). For instance, among the landraces, it was observed that *Kafir-bicolor* exhibited significantly higher fat and lower starch content in well-watered conditions, while exhibited lower amounts of fat and higher starch content in drought stress conditions. This confirms previous studies in other cereals which have attributed this inverse relationship to metabolic trade-offs in carbon allocation during grain filling in most grain crops (Passioura and Angus, 2010). Conversely, genotypes with higher starch have lower fat content and overall lower yield. However, the balance between fat and starch can be further altered and widened by environmental stresses, in this context drought stress.

Table 4-1: Summary table on the interaction between nutrient mean levels and landraces

Race	Fat.m	Fat.sd	Protein.m	Protein.sd	Starch.m	Starch.sd
Bicolor	3.5	0.7	11.3	1.5	60.9	3.8
Caudatum	2.8***	0.6	10.8***	1.5	63.5***	3.7
Caudatum-bicolor	3.5	0.8	11.2	1.6	61.2	4.6
Durra	2.8***	0.7	10.8**	1.5	61.4	3.4
Durra-bicolor	3.2**	0.7	11.6	1.4	61.9	3.3
Durra-caudatum	3.1***	0.7	11.1	1.4	62.1**	4.0
Guinea	3.3**	0.8	11.5	1.2	61.9*	4.5
Guinea-bicolor	3.3	1.0	11.7	1.7	59.2	4.4
Guinea-caudatum	2.9**	0.6	11.1	1.6	62.8***	3.8
Guinea-durra	3.0**	0.4	10.8	1.9	60.6	4.1
Guinea-kafir	3.2*	0.6	11.2	1.7	61.7	3.8
Kafir	3.1	0.7	10.8**	1.5	63.0	4.6
Kafir-bicolor	4.2***	0.9	11.8	1.3	60.2***	5.6
Kafir-caudatum	3.1***	0.6	10.4***	1.7	62.5*	4.1
Kafir-durra	3.4	0.5	11.0	1.6	60.9	3.9

m=mean, sd= standard deviation. Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

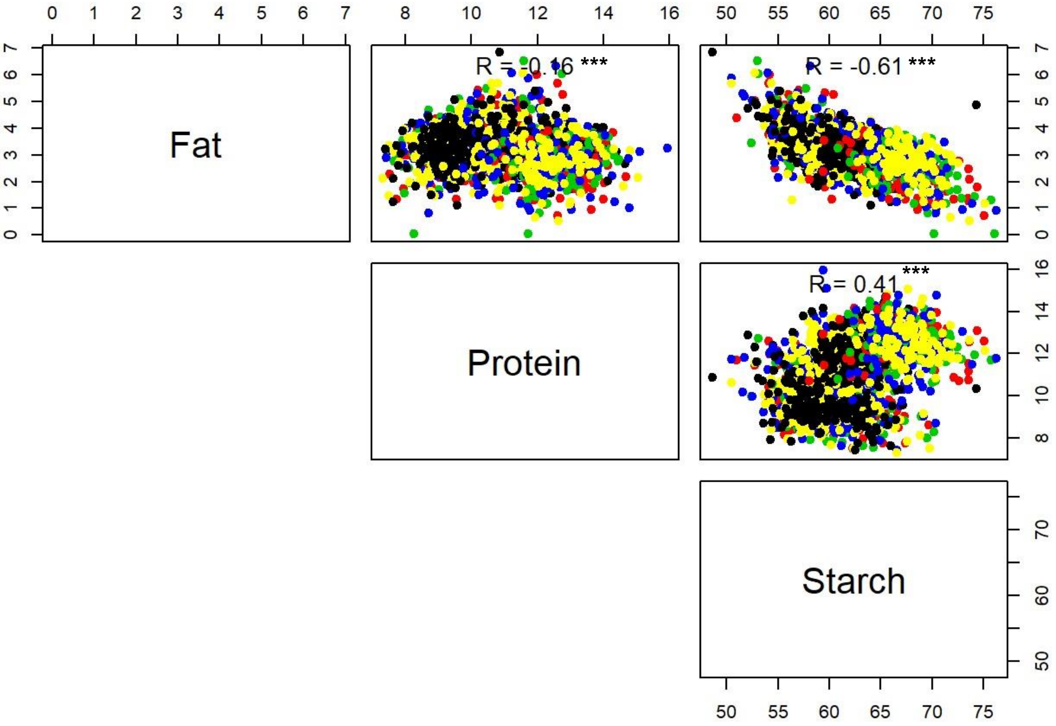


Figure 4-9: Correlation analysis among protein, fat and starch under well-watered and drought water stress

Strong significant negative association was observed between starch and fat levels. ANOVA was used to test for significance ($p<0.05$) of the correlation coefficient, which is denoted as r. Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

4.2.5 Principal component analysis reveals the trade-offs between grain nutritional qualities and physiological parameters in drought stress

The association between physiological functions can be linked with grain yield and nutrition qualities through principal component analysis (PCA) (Figure 4-10), and this was performed to understand the trade-offs under varying water conditions. As indicated by Lever et al. (2017) the purpose behind employing PCA is to reduce the complexity of the data while maintaining the trends, clusters or patterns and making it easier to transform into fewer dimensions. The first component shows 39% variables that are closely linked together. From the observation of transpiration pre-flowering, days to maturity, harvest index, days to flowering variables are clustered together implying high correlation. On the other hand, total transpiration, total grain weight, panicle dry weight, transpiration post flowering, water efficiency, total biomass, stem dry weight are clustered together. This implies the correlation of these variables, mainly physiological functions linked to yield. According to PCA on nutritional content, starch and fat are on opposite sides of the PCA indicating that these variables tend to change in opposite directions.

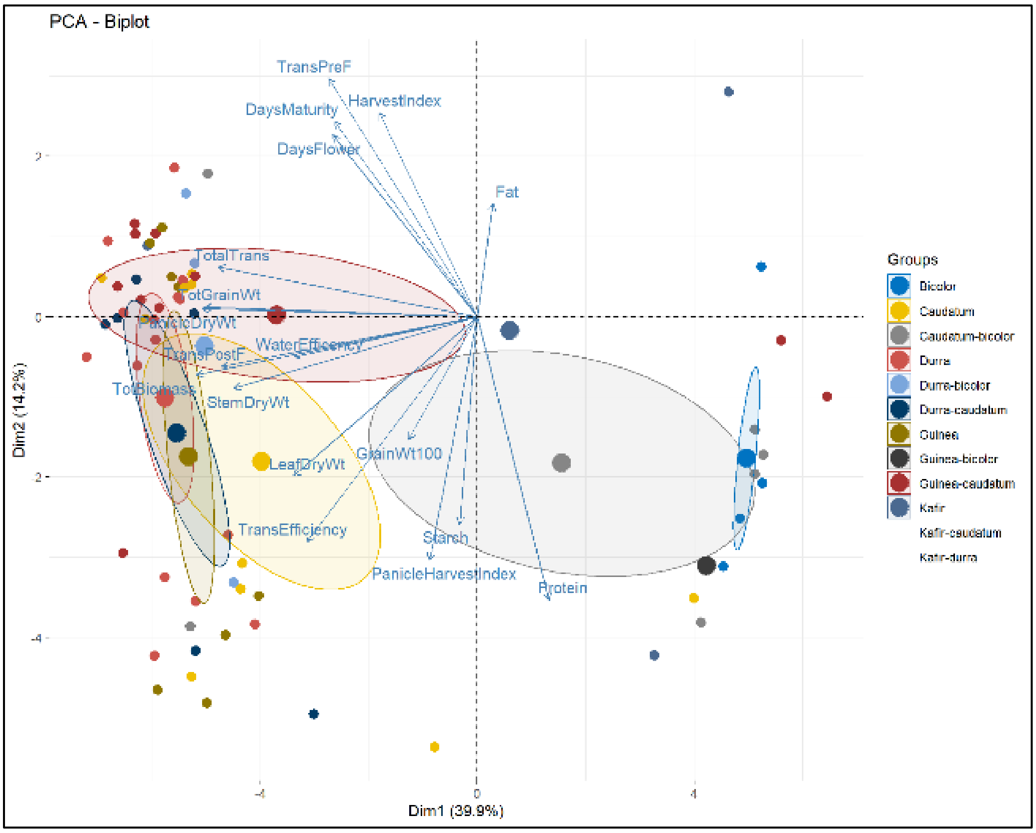


Figure 4-10: Correlation of nutritional content and physiological factors

PCA for available corrected physiological parameters with protein, starch and fat content across sorghum races.

4.2.6 Transpiration correlates strongly with high biomass and yield

Further analysis investigated how the changes to transpiration and biomass in response to drought may influence nutrition qualities (Figure 4-11). These parameters have been used for decades as proxy indicators for pre-selection of genotypes in plant breeding (Vadez et al., 2011). According to the findings of the analysis, transpiration efficiency and grain weight are highly correlated under well-watered and compared to water stress. Results demonstrate higher correlation of total grain weight and biomass in well-watered conditions ($r=0.93$, $p<0.001$), (Figure 4-11A) while inverse weak insignificant correlation between the same parameters in drought stress (r=-0.42) (Figure 4-11B). In terms of nutrition concentration levels, the findings from the analysis show variations in response to well-watered and drought water stress (Figure 4-11). For grain protein, weakly associated findings indicate a non-significant relationship with both biomass and total transpiration in both treatment conditions. Similar patterns were also observed with starch and fat content levels in drought stress. Interestingly, significant (r=-0.53, p<0.05) inverse correlation was observed between fat content and transpiration efficiency in well-watered conditions. This implies that biomass and transpiration do not directly link to changes in grain nutrition qualities. However, they may interact with other traits to alter nutrient changes in grains.

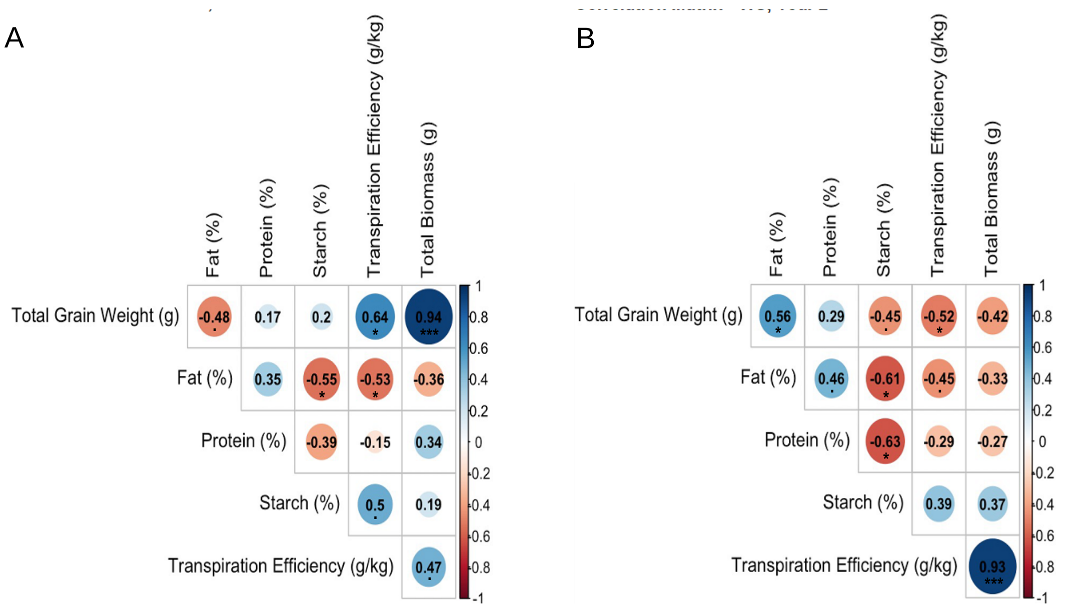


Figure 4-11: Correlation matrices comparing linear relationships between variables

A) Mean total grain weight, mean grain fat ratio, mean grain protein ratio, mean grain starch ratio, mean transpiration efficiency, and mean total biomass in WW treatment. B) Mean total grain weight, mean grain fat ratio, mean grain protein ratio, mean grain starch ratio, mean transpiration efficiency, and mean total biomass in WS treatments. R-values and significance are annotated as p<0.001(***), p<0.01(**), p<0.05(*), p<0.1(.). Colour denotes r-value (annotated) of the linear model (-1<R<1), where -1 is dark red, 0 is white, 1 is dark blue; size of circle denotes R^2 value.

4.2.7 Grain phenotypes and nutrition qualities are linked to genotypic differences in flowering timing

Analysis was carried out to understand the effect of drought on flowering time and maturation across sorghum races (Figure 4-12). The findings demonstrate significant variation in the timing of flowering. Genotypes from Guinea-bicolor and Durra-bicolor exhibited early flowering and consequently early maturing. To investigate the link between grain phenotypes and nutrition qualities, regression analysis was performed to establish the association between these variables (Figure 4-13). Flowering and maturation are associated with protein accumulation (Figure 4-13). To further understand the association of nutrient elements with flowering and maturation, regression analysis was performed. The findings indicate that protein is significantly inversely associated with days to flowering and maturation in both well-watered and drought response although the relationship is weak due to genotypic natural diversity in adaptability and response. However, it was noted that protein content in response to well-watered conditions was inversely related to these parameters. Interestingly, in drought stress, fat shows significant inverse correlation with number of days to flowering while, starch shows significant positive correlation. The relationship between fat and starch with panicle dry weight is not significant for both well-watered and drought water stress (Figure 4-14). Further analysis indicates a weak positive correlation that is significantly different suggesting that grain size impacts nutrition but that there are other important traits/determinants as well. In this study some of the genotypes in water stress did not yield and very challenging to analyse correlation with 100 seed weight, which is a caveat to this study. However, grain weight was recorded and could be similarly used to determine how grain phenotypes influence nutrition qualities.

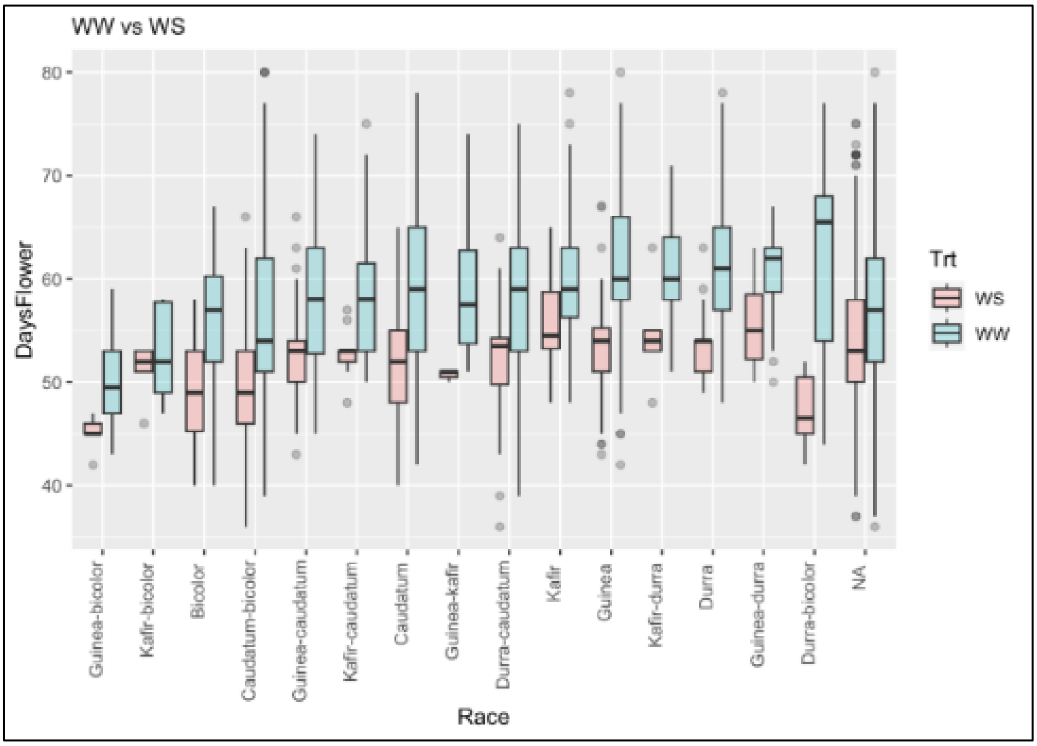


Figure 4-12: Variation in days to flowering in response across sorghum landraces

Genotypic natural variation for days to flowering in response to well-watered and drought water stress conditions. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile.

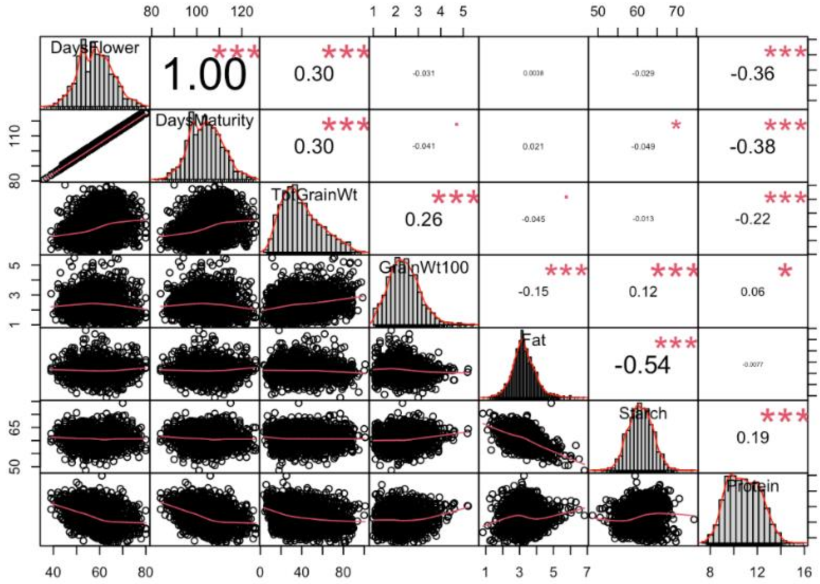


Figure 4-13: Correlation between grain phenotypes and nutrient element

The variables include mean total grain weight, days to maturity and days to flowering, mean grain protein, fat and starch, r-values and significance are annotated as p<0.001(***), p<0.01(**), p<0.05(*), p<0.1(.).

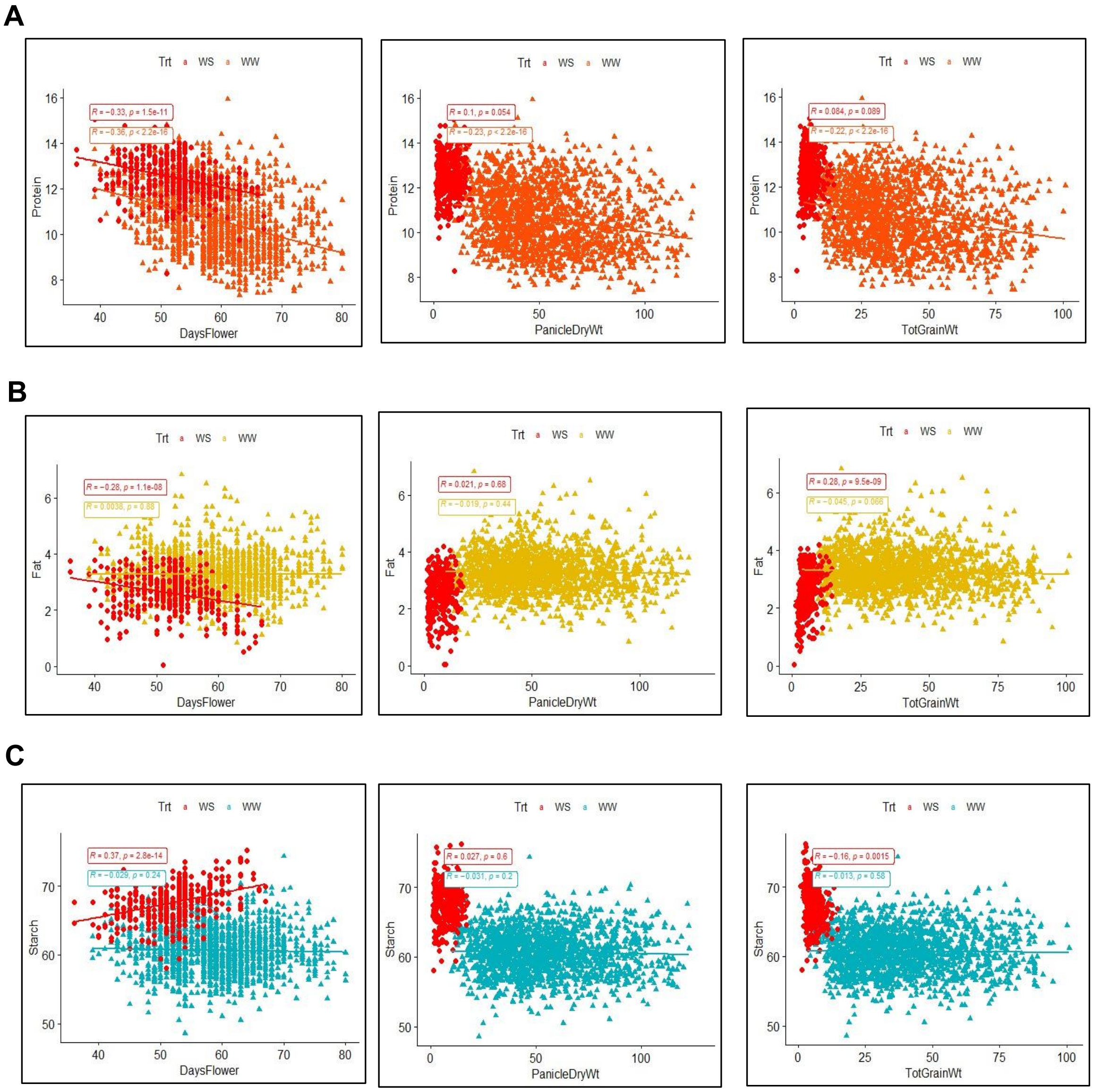


Figure 4-14: Correlation between nutrition qualities and flowering and grain phenotype in well-watered and water stressed plants

A) Protein content (%) correlated with days to flowering, panicle dry weight and yield. B) Fat content (%) correlated with days to flowering, panicle dry weight and yield. C) Starch content (%) correlated with days to flowering, panicle dry weight and yield.

4.2.8 Drought stress impact on micronutrient levels and the response varies with genotypes

To explore the effect of drought stress on the microelement content under drought stress, sorghum grain magnesium, zinc, iron, manganese and calcium concentration were quantified for both well-watered and water stress plants. Initially the experiment used one genotype S35 and to understand genotypic differences across sorghum lines, the experiment was repeated with eight genotypes with different in drought adaptability. The findings show relative variation across genotypes in levels of these microelement under drought-stress conditions. Genotypes IS20727 shows a significant increase in Zn and Mg and Cu in drought stress, while insignificant change in Fe and Mn levels. However, Impa et al. (2019) reported declining of sorghum grain micronutrient content in dry conditions (Zn, Fe, Mn and Cu). Another study reported higher grain K and Fe concentrations in drought-tolerant genotypes compared to the susceptible ones. The variation in results could be due to different genotypes responding differently and other environmental and technical variations.

(Figure 4-17) concentration of Iron content (Fe), Zinc (Zn), Magnesium (mg) and Manganese (Mn) in well-watered and drought stress across genotypes. F) concentration of minerals in one variety and first experiment. Significant variation exists in the increase and decrease in the mineral elements.

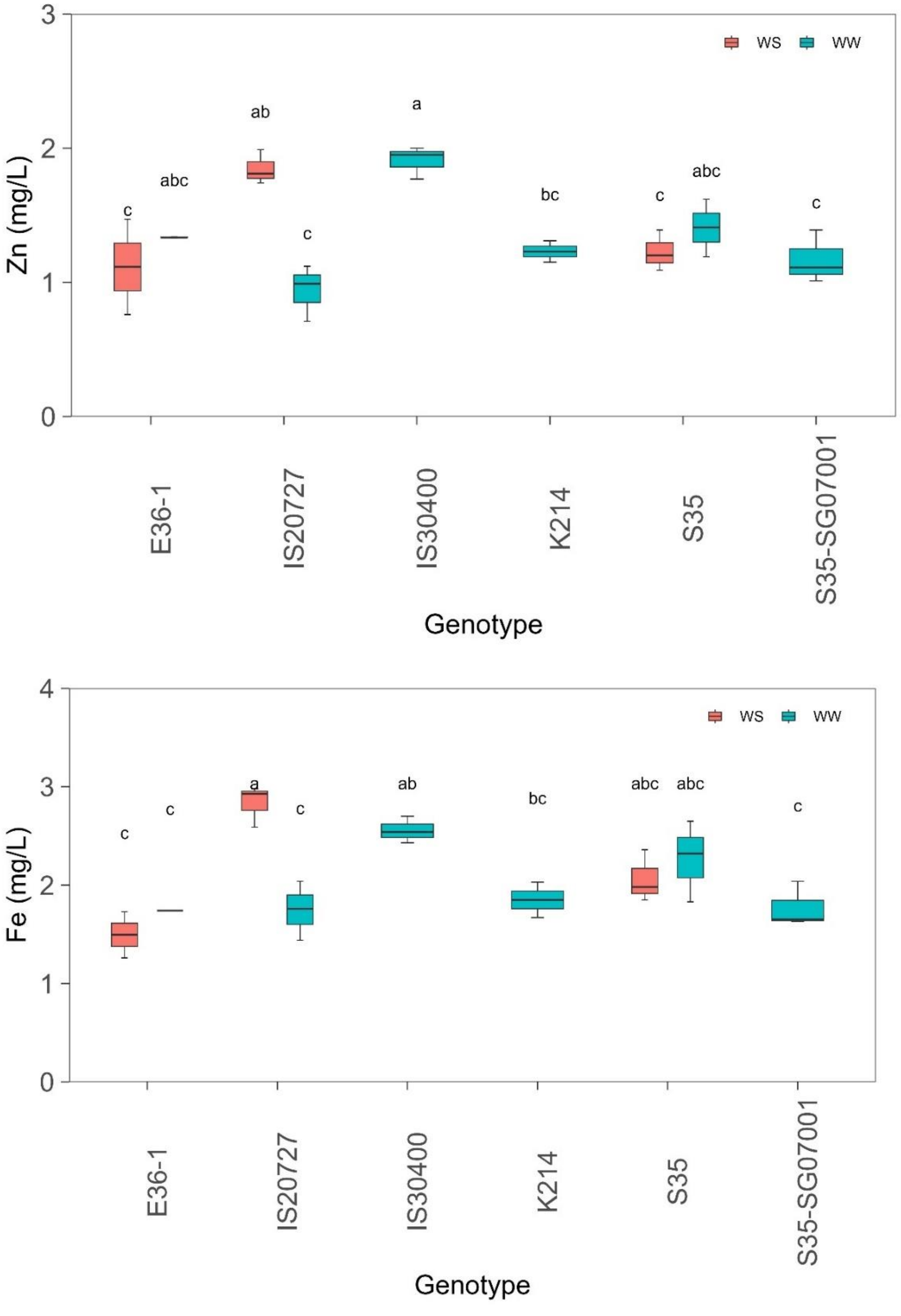


Figure 4-15: Zinc (Zn) and Iron (Fe) concentration among sorghum genotypes in well-watered and water stress conditions

Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Interaction between genotypes and treatment was significant ($p<0.001$). Treatments with the same letter are not significantly different

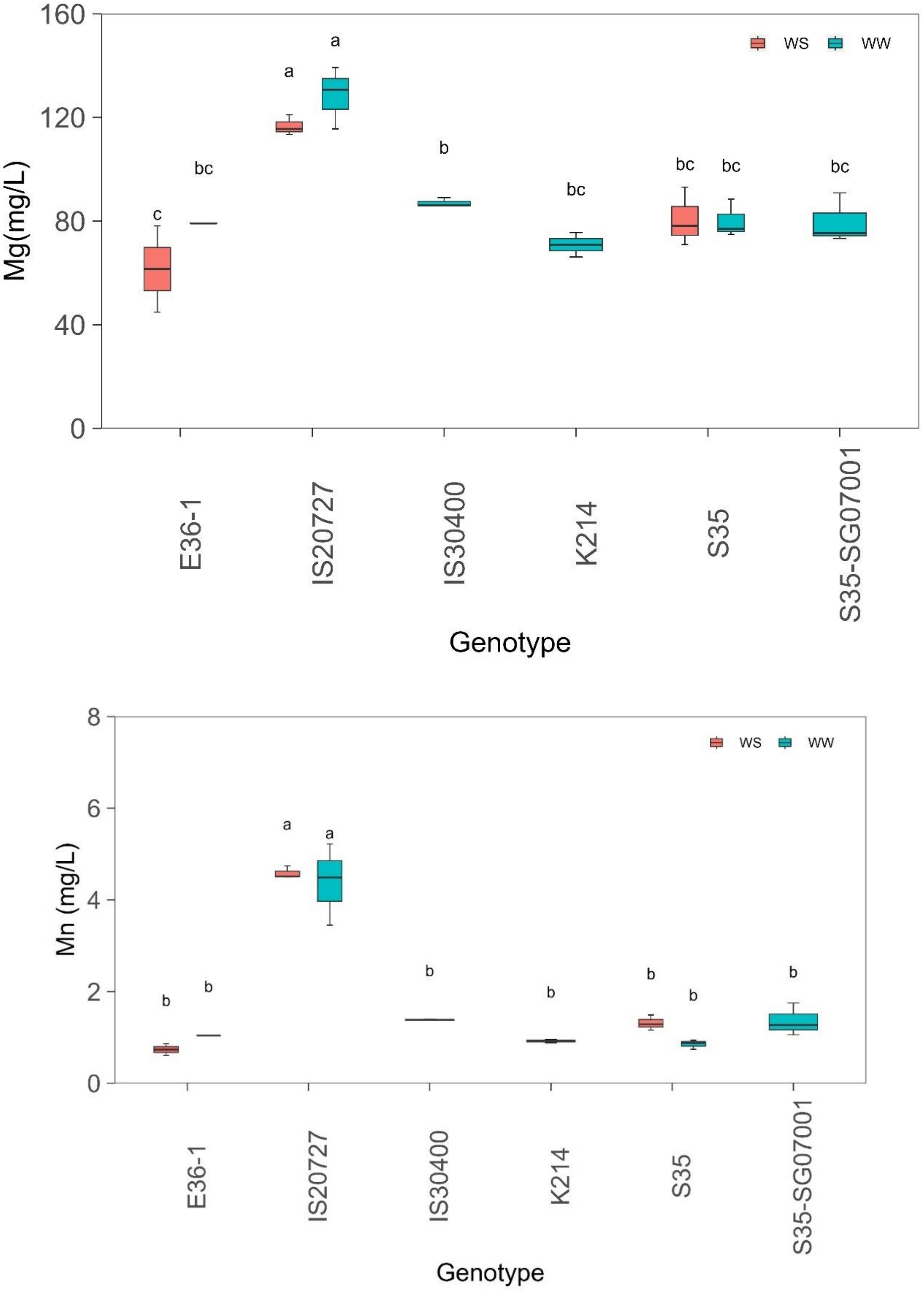


Figure 4-16: Magnesium (Mg) and Manganese (Mn) concentration among sorghum genotypes in well-watered and water stress conditions

Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Interaction between genotypes and treatment was significant ($p<0.001$). Treatments with the same letter are not significantly different

4.2.9 Phenotypic traits underlying high performance in grain yield and grain microelement during drought stress.

A controlled small-scale experiment was conducted to identify phenotypic traits that are associated with yield and micro-element levels. This work was carried out to add value and to support the on the field data from ICRISAT that focused on protein, fat and starch on large-scale trials. There was significant variation in both plant height and grain phenotypes associated with grain yield. Interestingly, genotypes with highest height, IS30400 never produced in water stress. While IS20727 was able to produce grains in both conditions compared to all genotypes. In addition, IS20727 was the earliest to flower compared to other genotypes. Referring to ICRISAT large scale data set, IS20727 was among top 25 genotypes in high yielding in water stress conditions among 243 genotypes, while IS30400 was among top 20 high yielding genotypes in well-watered conditions. The trade-offs observed through this experiment was the plant height and grain yield partitioning.

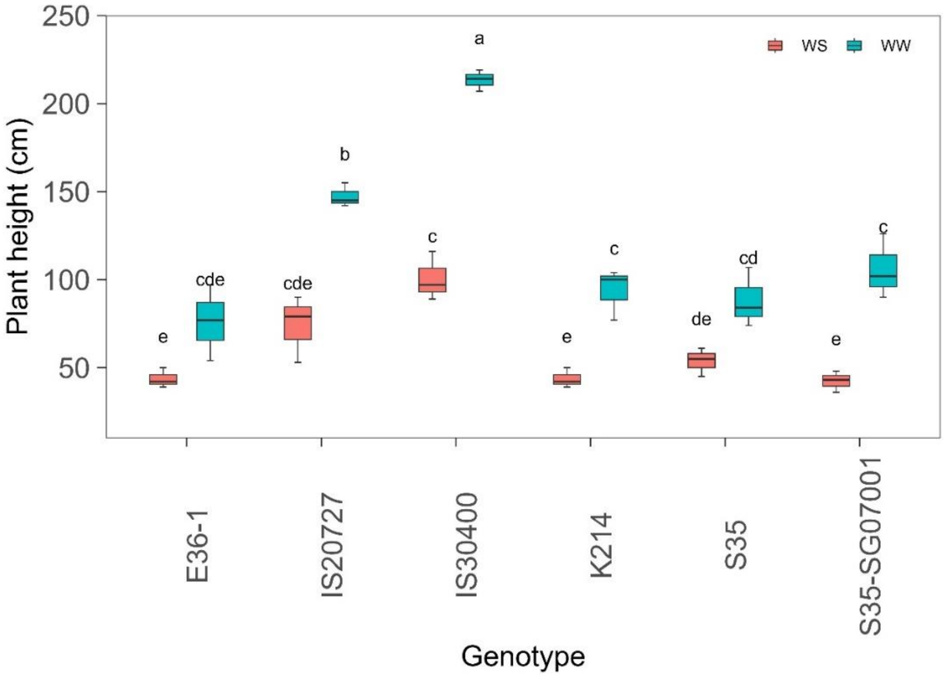


Figure 4-17: Plant height among sorghum among sorghum genotypes in well-watered and water stress conditions

Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Interaction between genotypes and treatment was significant ($p<0.001$). Treatments with the same letter are not significantly different.

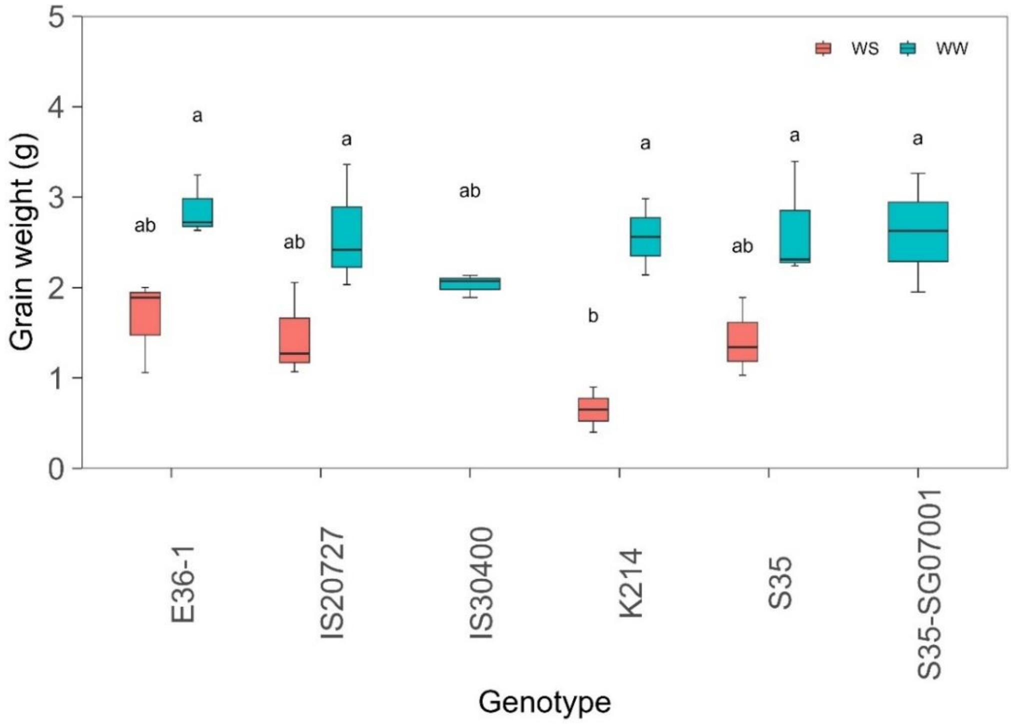


Figure 4-18: Grain weight among sorghum genotypes in among sorghum genotypes in well-watered and water stress conditions

Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentile. ANOVA was used to test for significance ($p<0.05$). Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Interaction between genotypes and treatment was significant ($p<0.001$). Treatments with the same letter are not significantly different

4.3 Discussion

4.3.1 Genotypes’ adaptive mechanisms influence variation in yield and nutrition qualities levels in response to drought

There is an urgent need to develop climate-resilient crops and sustain agricultural systems that produce more nutritious food while simultaneously adapting to a changing climate. Although complex, a greater understanding of both macro- and micronutrient content and its associated mechanisms under stress conditions is significant to fast-track selection, and contributing to the development of well-adaptable, productive, nutritious, climate-smart varieties for the future. The findings from this study suggest that drought stress impacts on grain yield and nutritional qualities across and within sorghum landraces differently. This adds to the evidence that crop yield depends on the source-sink relation, which is influenced by environmental changes (Yu et al., 2015). The findings indicate significant natural variation within the landraces and across genotypes in terms of yield. The findings genotype-specific variation implies that the genetic and metabolic pathways governing the synthesis of these macronutrients are optimized differently. Results are not surprising as limited research studies suggests that there exist resilient genotypes that function differently under drought stress at various levels, including molecular and physiological (Abreha et al., 2022). However, this study adds value on a systematic understanding of this knowledge gap especially for underutilised crop species and the trade-offs between yield and nutrition qualities in response to drought stress.

Drought stress alters the relationship between morpho-physiological traits and the source activity and sink strength (Yu et al., 2015), alters grain physico-chemical characteristics (Impa et al., 2019, Stagnari et al., 2016) and reduces mineralization of nutrients and impair membrane permeability. In this study, we show that drought influences the increase in grain starch. The observation is supported by (Yang et al., 2004) in wheat urges that drought during grain filling may accelerate the rate of grain filling, thus mainly attributed to increase in sink activity through key enzymes involved in the change of sucrose to starch, particularly sucrose synthase soluble starch synthase, and the starch branching enzyme. Unlike, study findings from Bing et al. (2014) drought stress induced during flowering stage led to reduced total starch, amylase and amylopectin accumulation, which is related to compromised activities on sugar nucleotide precursors by enzymes, such as starch synthase (SSS), granule-bound starch synthase (GBSS), starch branching enzyme (SBE), and starch debranching enzymes (DBE) during grain filling. These finding differences could be due to irregular variations in the environment and genotypes interactions (Farooq et al., 2014). On protein, the findings shows positive increase in drought stress as in line with other studies (Impa et al., 2019, Sarshad et al., 2021) showed that drought stress increased the total protein content and positively affected the total soluble carbohydrate, crude protein, and proline contents. Although Stagnari et al. (2016) indicated that protein digestibility could also be compromised due to increased level of starches, as a response to the drought stress. As in wheat, the protein content of crops, especially grains, significantly determines the quality of food and feed by influencing various characteristics, such as the rheological properties of dough and processing quality (Shewry, 2007). Although beyond this study's scope, our analysis from a large-scale data set of about 242 sorghum genotypes (15 landraces) grown for two seasons in lysimeter facilities under open field adds value to this knowledge that is increasingly becoming important in understanding water limitations and its interaction with crops quality. A study by Fischer et al. (2019) demonstrated that nutrient concentrations decreased in severe drought but increased in mild drought. This suggests that the effects of nutrient accumulation are determined by the intensity level, as evidenced by this present and previous studies.

4.3.2 Phenotypic and physiological traits correlation with grain yield and nutrition quality

Plants respond and adapt to drought stress through the induction of various morphological and physiological responses (Ngara et al., 2021). There is an urgent need to find affordable and trustworthy physiological indicators that can help in the selection of drought-adaptive genotypes. Here we investigated the physiological basis of genotypic variation in drought adaptation and characterise the physiological traits that may indicate plant capacity for drought adaptability (Chen et al., 2016). Grain nutrition qualities are linked to genotypic differences to early flowering and grain maturation in drought stress. Early maturing traits are the most desirable for farmers (Chapter 3 of this thesis) as they indicate plant drought escape. However early maturation is a trade-off to high yielding but could be a great indicator for high nutritious genotypes for a specific breeding selection. The findings further show that performance variability and correlation found among protein, starch and fat levels across landraces during well-watered and drought stress indicate high germplasm diversity potential for selection.

4.3.3 Micronutrient elements are highly elevated in drought and are linked to biological processes in response mechanisms

From our findings, environment and genotype interaction plays a crucial role in micronutrient (Cu, Fe, K, Mg, Mn, and Zn) levels in sorghum grain. We observed that genotypes with high tolerant abilities have high increased levels of micronutrient. Significant differences among sorghum accessions for nutrients exists (Motlhaodi et al., 2018) reported (Ca, Fe, K, Mg, Mn, Na, P and Zn) and these traits have a strong broad-sense heritability ranging analysis of 336 sorghum recombinant inbred lines (RILs) showed large variability and high heritability for Fe and Zn content (Abreha et al., 2022). These studies did not investigate the effect of drought stress on the concentrations of these nutrients in different sorghum genotypes (Abreha et al., 2022). With few studies on sorghum, (Abu Assar et al., 2002) reported that sorghum genotypes showed considerable variation in mineral composition (K and Fe), with drought-tolerant genotypes containing higher K and Fe content compared to susceptible ones when grown under drought stress conditions. Thus, the tolerant genotypes could maintain optimal mineral and other nutrient compositions even when grown under water deficit conditions.

The increased amount of these molecules indicates their role in drought stress tolerance, whereas the differences in the level of their accumulation among genotypes points to genotype-specific tolerance mechanisms. Tavanti et al. (2021) findings indicate that micronutrient applied at low concentration has the ability to increase the defence systems of plants in response to abiotic stresses. Zinc (Zn) plays a role in the detoxification of superoxide radicals, membrane integrity, as well as the synthesis of proteins and the phytohormone AUX/IAA. Zn is essential element for human health, playing a critical role in numerous biological functions including improving immune systems (Krężel and Maret, 2016). Likewise, bioavailability of Fe in seeds or grains is an important issue for nutritional quality and human nutrition, Iron (Fe) plays a crucial role in redox systems in cells and in various enzymes (Broadley et al., 2012). Foliar or soil application of fertilizers containing Cu, Fe, Mn, Mo, Ni, Se and Zn at low concentrations has the ability to elicit and activate antioxidative enzymes, non-oxidizing metabolism, as well as sugar metabolism to mitigate damage by oxidative stress. Plants treated with micronutrients show higher tolerance to abiotic stress and better nutritional status (Tavanti et al., 2021). The presence of genotypes with higher concentration of Fe and Zn and stable heritability of the nutrient content (Motlhaodi et al., 2018) suggest sorghum genotypes with higher concentration of these nutrients can be utilized for enhancing micronutrient composition in elite sorghum materials but considering trade-offs as well. Despite the nutritional compound that has been analysed in this study, drought may increase the presence of anti-nutritional compounds, in particular phytic acid and polyphenols, which can inhibit digestibility, bioavailability and the level of uptake of such micronutrients (Hummel et al., 2018) and therefore requires further research attention.

4.3.4 Concluding remarks

The link between climate change and food quality remains one of the most ill-defined areas in global change studies, yet is directly relevant to human health. Our analysis revealed that drought stress impacts nutrient levels in grain sorghum. A significant interaction was observed between sorghum landraces and water conditions influencing macronutrient content in grain. Results demonstrate variable grain nutrient content with water conditions, with grain protein and starch content relatively higher under drought stress conditions, while the fat content declines. The findings further show that performance variability and correlation between protein, starch, and fat levels across landraces during well-watered and drought stress indicate high germplasm diversity potential for selection. The study further shows that micronutrients Mg, Zn, Fe, and Ca levels were significantly higher under drought stress in drought-adaptable genotypes. A greater understanding of both macro- and micronutrient content and its associated phenotype under stress conditions is significant to fast-track selection, and contributing to the development of well-adaptable, productive, nutritious, climate-smart varieties. This study was limited by a scarce dataset due to severe drought exposure, with some varieties failing to produce grains for nutrient assessment. Future research should explore varying drought severity, starting with mild stress to assess its impact on nutrition. Further work is needed to investigate nutrient assimilation at the molecular level to identify nutrient transport mechanisms and pathways linked to drought stress in varying species.

Chapter 5

Characterisation of root system architecture and root exudates under varying environments

5.1 Introduction

Root system architecture (RSA) is a significant and potentially programmable component for crop improvement due to its developmental and environmental plasticity (Jung and McCouch, 2013, Sarkar et al., 2013). Shaping root architecture into optimal topologies for maximal resource capture and resilience to environmental stresses is important for sustainable food production in the context of climate change and global warming (Lynch, 2019). The ability of plants to access soil moisture in drought-prone areas is largely determined by RSA, a complex trait that is especially relevant for sorghum, which is commonly grown in drought-stress environments (Demissie et al., 2023). While much progress has been made in elucidating the molecular and physiological mechanisms that underpin the regulation of RSA in a model plants such as Arabidopsis, these pathways have been uncharacterised in sorghum. This is partially because roots are often considered the hidden and neglected half of plant architecture and have not been a direct target for breeding selection (Waisel et al., 2005, Waines and Ehdaie, 2007, Maqbool et al., 2022). Secondly, to date, root system phenotyping and quantification of their plastic response to the uneven distribution of nutrients and water in the soil is challenging (Singh et al., 2012, Atkinson et al., 2019). However, recent root phenotyping advances have enabled the imaging of mature root system under realistic soil conditions, presenting unique opportunities for crop improvement through high-throughput phenotyping (Bucksch et al., 2014).

Soil is a crucial component of the biosphere and is a major sink for organic carbon. Plant roots release a wide range of carbon-based compounds into soils, including polysaccharides, but their functions are not known in detail (Preece and Peñuelas, 2020). Root exudation releases a vast array of compounds into the rhizosphere, including sugars, organic acids, amino acids, secondary metabolites, and structural carbohydrates (Walker et al., 2003). Sasse et al. (2018) stated that root exudates, include a great complexity of both low- and high-molecular-weight components, often contain polysaccharide-rich viscoelastic gels and in many cases have adhesive properties influence zones of soil at root surfaces known as rhizospheres (Baetz and Martinoia, 2014). The wider term ‘exudates’ encompasses mucilage but can also include compounds of lower molecular weight and more soluble high-molecular-weight polysaccharides and protein components that may not contribute to gel-like structures (Galloway et al., 2020b).

Exudates and mucilage enable plants to exert influences on their immediate surroundings, creating microenvironments that can be favourable for their growth. The release of exudates from root tips, for instance, may lubricate roots to ease penetration through deeper layers of soil and may also be involved in forming a protective barrier, specifically in dry soils (Pang et al., 2017). In recent years, significant progress has been made in analysing root exudate composition and its interactions within rhizosphere. The mechanism by which root exudates are secreted is still poorly understood. Root-derived compounds are typically constitutive, but can be triggered by either biotic or abiotic stress (Field et al., 2006, Badri and Vivanco, 2009, Preece and Peñuelas, 2020). The mechanism by which plant roots secrete compounds is primarily thought to be a passive process mediated through three separate pathways: diffusion, ion channels, and vesicle transport (Badri and Vivanco, 2009). Further investigation of polysaccharide exudate chemistry and molecular mechanisms, especially for drought-tolerant crops, will be useful for the screening of genetic populations and the identification of underground traits directly relevant to crop breeding (Galloway et al., 2020a). The rhizosheath is known to be beneficial for drought resistance in many plants, but the regulation of rhizosheath formation in sorghum is unclear (Galloway et al., 2020b). Some recent studies have indicated that moderate water stress increased rhizosheath formation in cereals, an important drought-adaptive trait (Liu et al., 2019, Zhang et al., 2020). The rhizosheath provides a dynamic zone for water and nutrient interchange at the root–soil interface, which can improve drought resistance and can protect roots against other abiotic stresses (Brown et al., 2017). Previous work has speculated that some grasses can increase the thickness of their rhizosheath, potentially to secure water uptake during periods of drought (Liu et al., 2019). The extent to which sorghum rhizosheath formation can contribute to improving below-ground resource capture and understanding of drought adaptability in crop improvement programs is not known. Only a small number of studies have recently focused on unravelling the polysaccharide secretions from root tips and the possible secretion from root hairs that promote rhizosheath formation (Galloway et al., 2020b), in diverse genotypes within the same crop species and across species. (Bucksch et al., 2014, Singh et al., 2010, Singh et al., 2012, Parra-Londono et al., 2018, Dowd et al., 2022, Demelash et al., 2021, Lynch, 2022, Chandnani et al., 2023)

Therefore, to begin to elucidate the molecular and physiological links between root system architecture and rhizosheath formation in sorghum, we focussed on the following objectives in this chapter: i) characterising root system architectural variation in multiple sorghum genotypes using high throughput phenotyping techniques. ii) identifying potential root ideotypes in sorghum in response to drought stress and iii) profiling root exudates and rhizosheath formation in different sorghum genotypes. Collectively, these findings holds significant potential to enhance our understanding of the natural variation in root system architecture in sorghum, linking these to drought adaptability and the composition of exudates in sorghum roots.

5.1 Results

5.1.1 Investigating natural variation in sorghum root seedling architectural traits using 2D seed germination pouches

Pouch growth systems using layers of filter paper saturated with Hoaglands nutrient solution and covered with a transparent film was used for 2D visualisation of sorghum roots of multiple genotypes with differing seed size and morphology. Genotypes used in this study include IS30400, E36-1, IS20727,K214, S35, K258, S35SG06008, S35SG06016, S35SG06024, S35SG06026, S35SG06040, S35SG07001. A 2D root phenotyping has the advantage of allowing for rapid and much higher-throughput phenotyping of large populations (Chandnani et al., 2023). According to the results in (Figure 5-1A) below, there was no significant difference in seedling primary root length across sorghum genotypes screened in this manner. This is possible because, generally, during initial growth, sorghum invests in primary root growth, and lateral nodal roots appear at later stages of development (Stage 2, Figure 1-2, Chapter 1). Thus, these data are comparable to the findings of (Singh et al., 2010), who observed that the natural ability of sorghum's vertical primary root growth favours exploration of water at deeper soil strata, which potentially enhances survival during drought stress.

In these experiments, lateral root branching and density were quantified across sorghum genotypes. Lateral root branching and growth are important traits because root branching largely determines the size, shape, and area of the plant root system (Nibau et al., 2008). Quantificatuion of these parameters revealed that that numbers of lateral roots were significantly different ($p<0.05$) across the eight genotypes screened (Figure 5-1C). Intriguingly, most of the stay-green trait genotypes (defined in Chapter 1 of this thesis), for instance, S35-SG06016 and S35-SG06040, had the highest number of lateral roots compared to the other genotypes. Root hull area (Figure 5-1E) was also significantly different across the screened sorghum seedling root systems.The other significant trait used to screen for root traits in crop improvement is lateral root growth angle, which is also referred to as Gravitropic Setpoint Angle (GSA) (Uga et al., 2013, Roychoudhry et al., 2013). It has been previously reported that the lateral root growth angle is linked to the depth of the mature root system in the field for several cereal species (Rich and Watt, 2013). Here, lateral root growth angles were also quantified of sorghum root genotypes. Significant variation was observed in root growth angles in the sorghum lines screened (Figure 5-2 A).

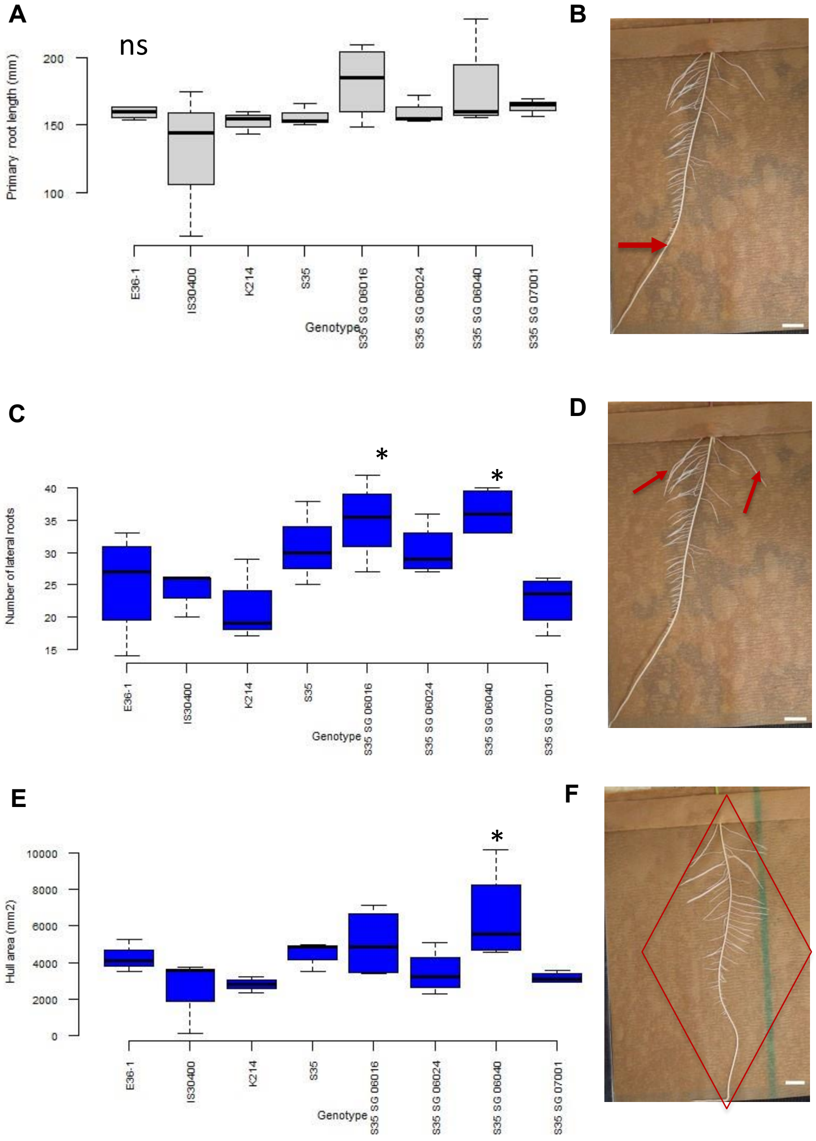


Figure 5-1: Seedling root traits variation in sorghum grown in pouches

A) Variation in sorghum seedling primary root length (mm). B) Images showing primary root, pointer showing the part measured (in RootNav). C) Variation in sorghum seedling lateral roots count. D) Image of sorghum in pouches with a pointer on sample lateral roots counted. E) Variation in convex root hull area (mm^2) representing area covered by whole RSA. For box plots, dark horizontal lines represent the median, with the box representing the 25th and 75th percentiles, dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance ($p<0.05$). n=>35 lateral roots. The scale bar on images represents 1 cm.

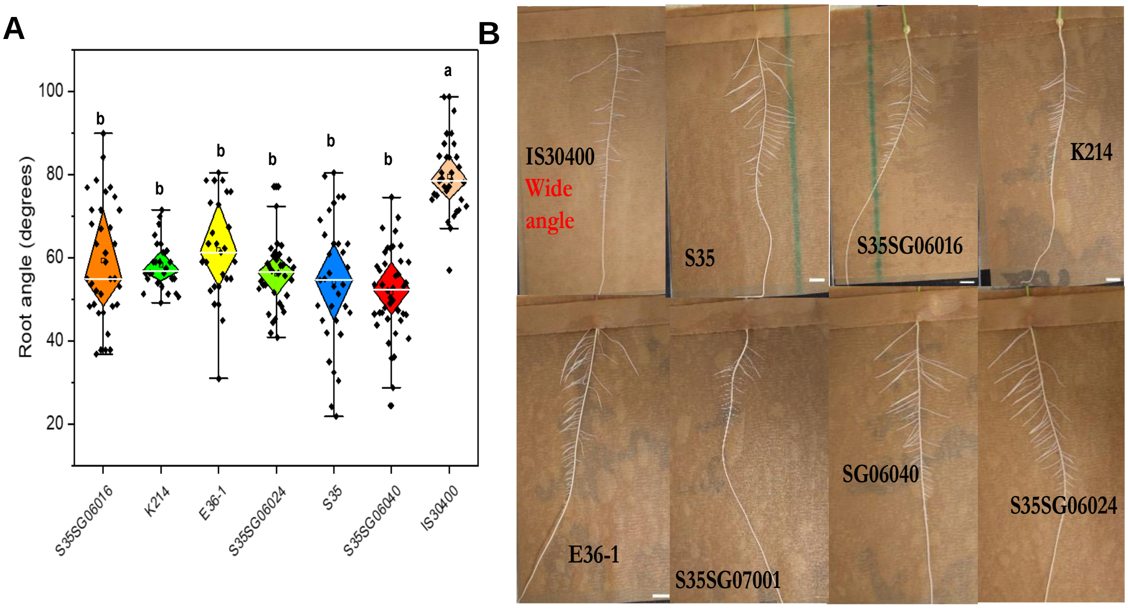


Figure 5-2: Sorghum seedlings have a variation in their root growth angle

A) Seedling lateral root growth angle variation (GSA). B) Images of sorghum seedling roots grown in pouches showing seed germination pouches (7 days old) having primary root and lateral roots for a 2D visualisation. ANOVA was used to test for significance ($p<0.05$). N=20 plants for each genotype. The scale bar represents 1 cm.

5.1.2 Characterising natural diversity of root growth angles in mature sorghum roots using 3D (Colander) method in soils

Sorghum nodal roots appear relatively late and lack seminal roots compared to other cereals such as maize, rice, or wheat (Chapter 1, introduction). The nodal roots usually appear after 5 leaves fully open (Stage 2 of sorghum growth Figure 1-1). Therefore, phenotyping root traits in sorghum cannot be achieved by utilising phenotyping tools for other species. Rather, its species-specific phenotypic platforms need to be developed. A colander-based method was used to quantify root traits at later root development, especially the nodal root branching angle, a major component of the root system in sorghum. Results are shown in the form of variation in root angle, indicated by differences in the root system percentage at given angles between genotypes.

In these experiments, the IS30400 genotype exhibited shallower nodal root angles compared to other genotypes. As previously observed, most of the stay-green varieties had deeper rooting angles.

These findings led to a hypothesis to understand whether this can be noted at the seedling stage to assist in the rapid pre-selection of performing genotypes, especially in drier environments, to reduce crop breeding generation time.

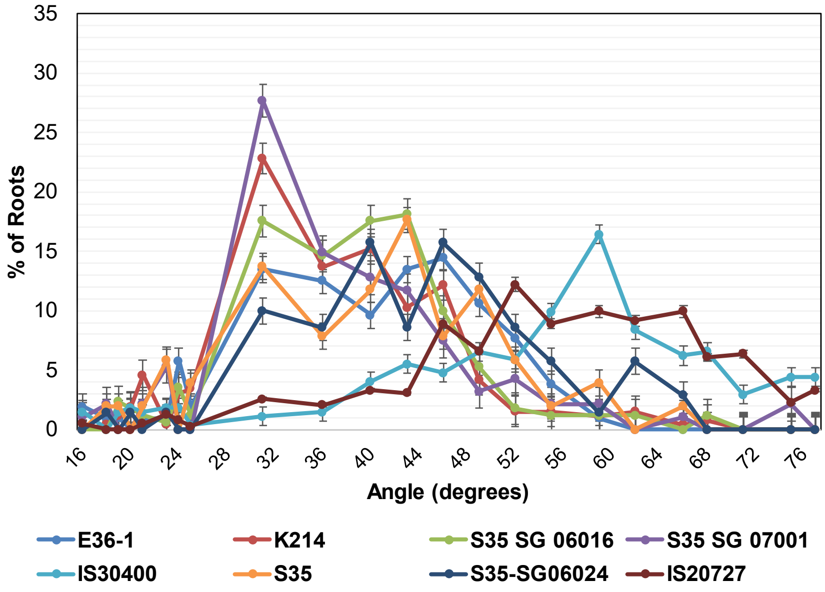


Figure 5-3: Root growth angle variations among sorghum genotypes in colander method

Points towards 16° show more vertical angles, while points towards 76° show less vertical angles. Results show variation in root angle, indicated by difference in the root system percentage at given angles between genotypes.

5.1.3 Lateral root growth angle in sorghum seedlings are proxy traits to later root development in sorghum

To investigate if the phenotyping techniques used at the seedling stage could assist in root pre-selection of sorghum lines for breeding programs, two genotypes with differing drought adaptability was used to test if root angles could be proxy for later root development to assist in rapid selection of the root traits. These were, IS30400, a drought-sensitive genotype, and E36-1 post-flowering-drought tolerant cultivar with parental source (Ochieng et al., 2021). Pouches were used to assess root traits at the seedling stage (10-12 days old), and then plates were used to quantify nodal root angle (after 5 leaves were fully grown) Finally, the colander based experimental method was used to assess later stages of root growth and development at the pre-flowering stage.

The findings show significant variation ($p<0.05$) in root angle across these tested genotypes (Figure 5-4). Interestingly, the genotypes maintained their consistency in terms of root angle in all the phenotyping techniques. Genotype IS30400 has a wider lateral root angle at the seedling stage phenotypes shown in (Figure 5-4 A) and the quantification of angle changes in (Figure 5-4B). Intriguingly, the phenotype at seedlings leads to a wider nodal root angle later in growth, as shown in the root phenotype of plants grown on plates (Figure 5-4C) and quantification of angle variation in (Figure 5-4D). Surprisingly, this was maintained when the colander method was used, and the root angle phenotype was wider in IS30400 than in E36-1. The findings suggest that data obtained from phenotyping root traits at early stages can be extrapolated to predict root architectural parameters in later stages of development. The findings are also fascinating because they link root architecture, and particularly root growth angle to drought adaptability. The shallow rooting IS30400 variety is drought-sensitive, especially in the pre- and post-flowering stages. As discussed in Chapter 4 of this thesis, this is demonstrated by its inability to flower and produce grain during drought conditions. This suggests that root growth angle may be related to drought susceptibility in sorghum.

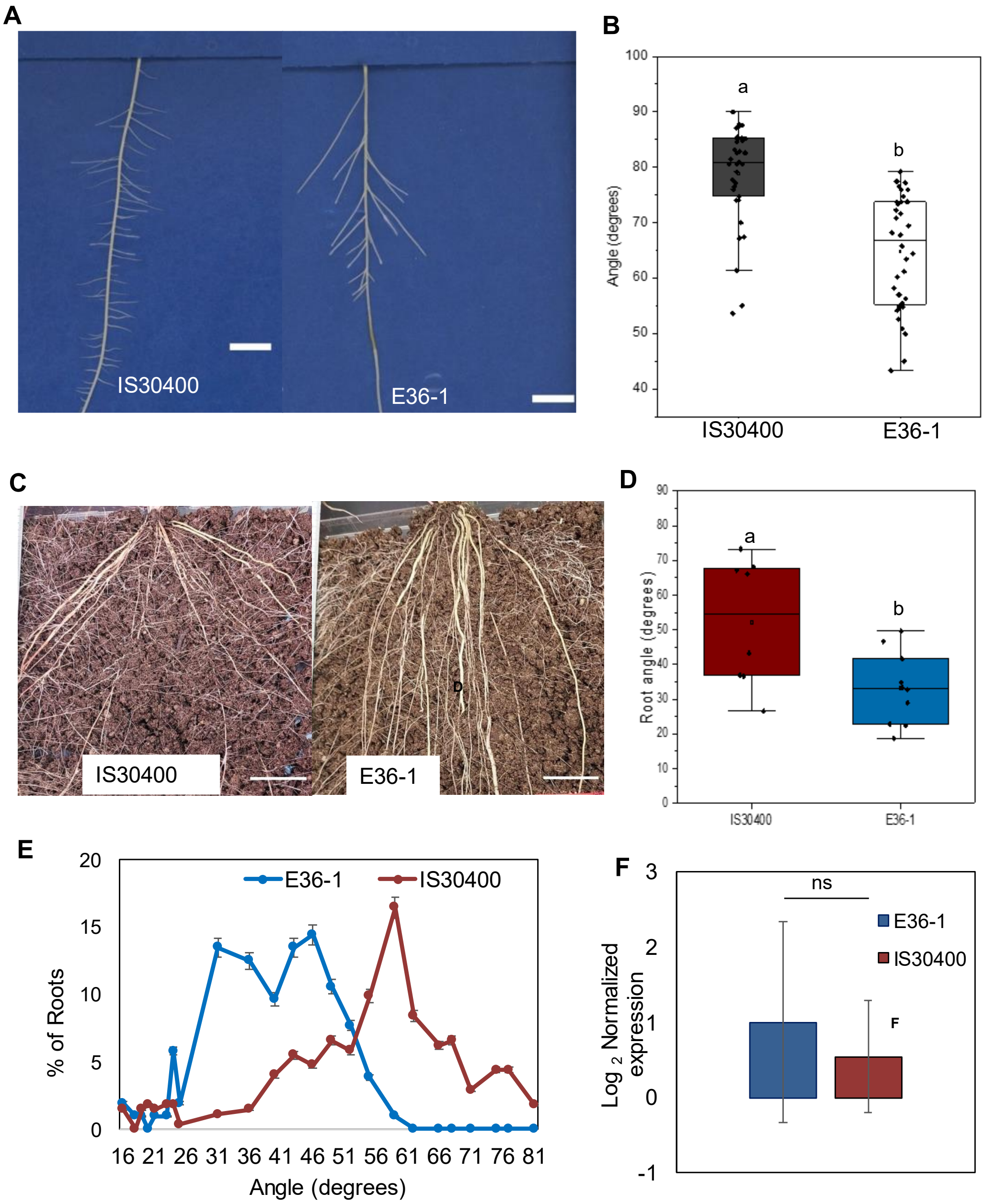


Figure 5-4: Root angle in seedlings are proxy trait to later development

A) Images of seedling roots in pouches, roots with wider angle (IS30400), and genotype E36-1 with vertical GSA in pouches (10 days old). B) GSA variation in seedlings C) images of nodal roots in plates D) Variation in lateral growth angles in rhizotrons plates and E) Images of two distinct varieties with variation in root angle phenotypes in colander. F) *LAZY* expression between 2 genotypes. E) Graph showing root angle variation, in degrees variations between two treatments, well-watered (ww) and drought water-stress (ws) grown in pots using the 'colander' method of calculating angles in wheat. Points towards 18° show more vertical angles, while points towards 81° indicate less vertical angles. F) *LAZY2/4* expression between IS30400 and E36-1, not significant. Oneway-ANOVA was used to test for significance ($p<0.05$). The scale bar in images represents 1 cm.

5.1.4 Root phenotypic characterisation of architectural traits shows genotypic diversity in soil-based screening method under varying water conditions

To investigate variation in additional root traits under drought water stress, seedlings were grown in pots containing soil to quantify root length. In these experiments, 12 genotypes (IS30400, E36-1, IS20727,K214, S35, K258, S35SG06008, S35SG06016, S35SG06024, S35SG06026, S35SG06040, S35SG07001) were tested to assess the variation in root length in water-stressed and well-watered conditions. The figure shows significant variation in root length across genotypes (Figure 5-5 A-B) and treatment. IS30400, S35SG07001 (stay-green genotype) and S35SG06016 exhibited significant longer primary root in water stress compared to other genotypes which have insignificant changes in primary root length. However, for lateral root count, IS30400 had relatively significant least number of lateral roots. This implies the trade-offs in the primary root length compared to lateral roots. To confirm that drought stress was induced the shoot traits were also analysed (Figure 5-6 A-B). Interestingly, genotype IS30400 exhibited longer shoot height (Figure 5-6A) while shortest leaf size (Figure 5-6B). While a dwarf variety E36-1 displayed a shorter shoot and compensated with larger leaf size. The study links to Chapter 4, on grain size where E36-1 has relatively large grains compared to IS30400 which exhibited smaller grain size. The results could imply the trade-offs in the sink activity between reproductive parts and biomass allocation. Large size suggests genotype competitive advantage on optimising photosynthesis compared to the other genotypes. Noteworthy, to indicate that most stay-green trait genotypes have large leaf sizes.

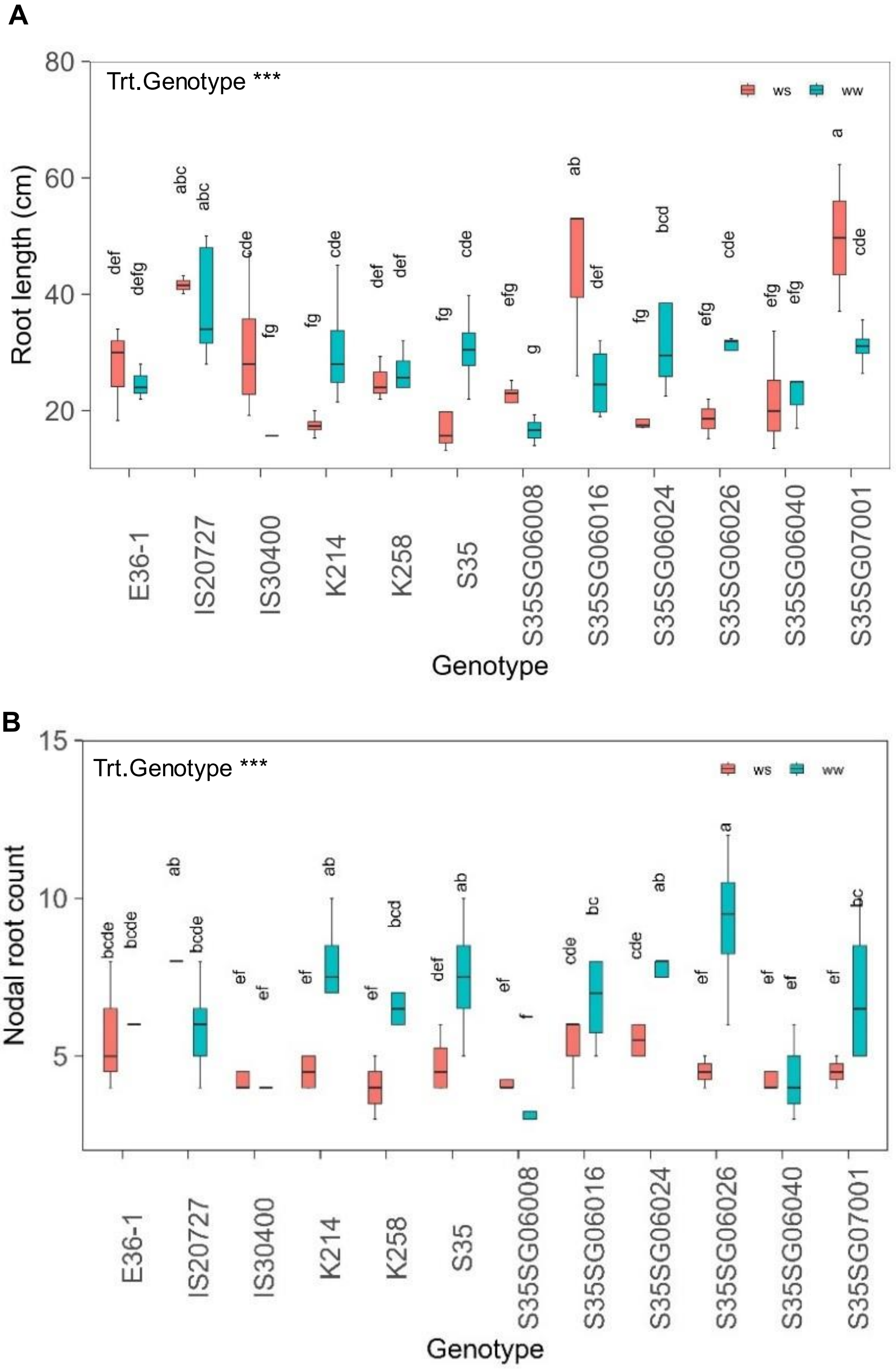


Figure 5-5: Sorghum root traits in response to drought

A) Box plot showing root length among sorghum genotypes in well-watered (ww) and water-stressed (ws) conditions. B) Box plot showing nodal roots count per plant across genotypes in well-watered (ww) and water-stressed (ws) conditions. n=8-10 plants per genotype per treatment. For box plots, dark horizontal lines represent the median, with the box representing the 25th and 75th percentiles, dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance ($p<0.05$)

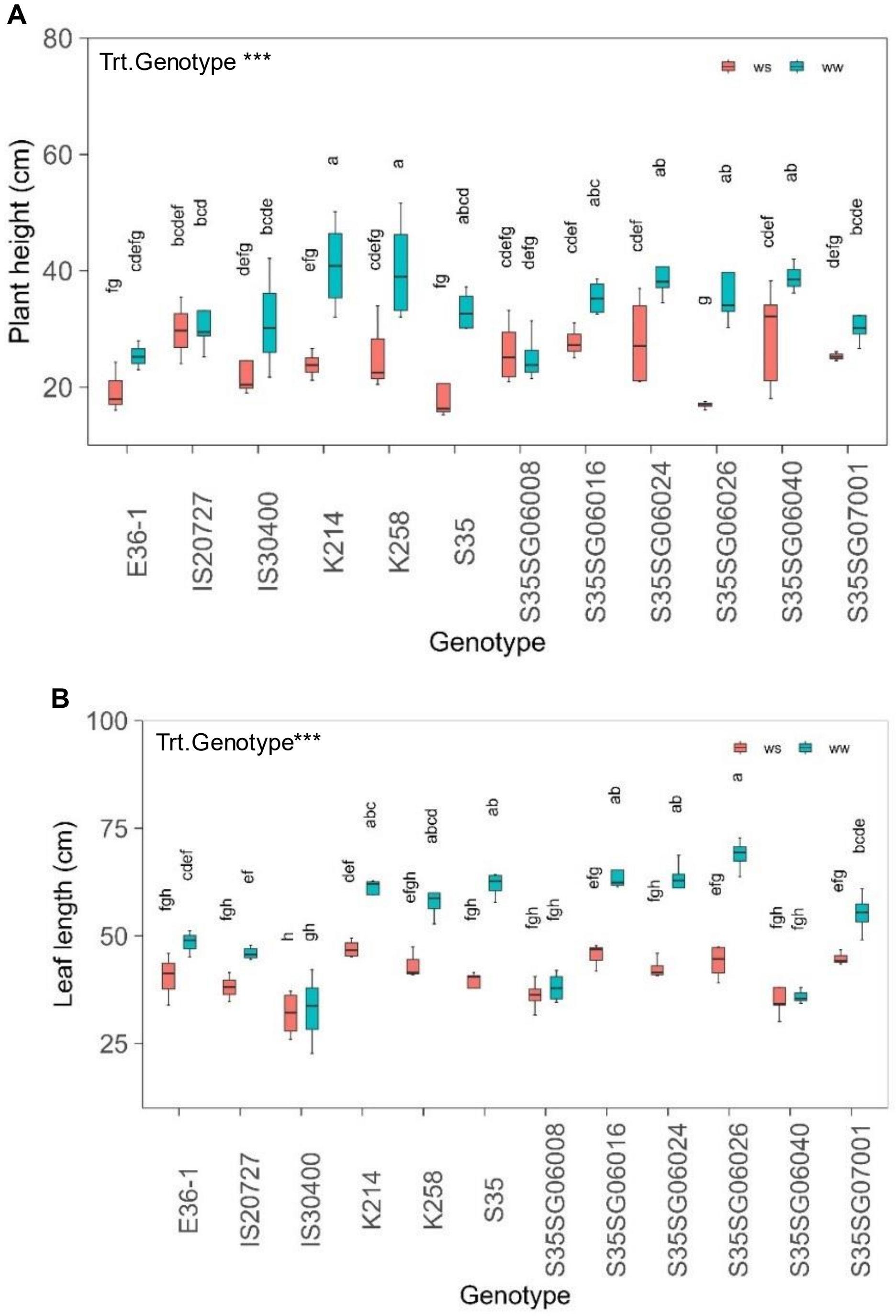


Figure 5-6: Shoot phenotypes linked to seedling root traits in well-watered and water-stressed plants

A) Variation on plant height across sorghum genotypes B) Leaf length per plant across genotypes in well-watered (ww) and water-stressed (ws) conditions. Leaf length was quantified by average measurement of 3 middle leaves per plant. n=8-10 plants per genotype per treatment. For box plots, dark horizontal lines represent the median, with the box representing the 25th and 75th percentiles, dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance ($p<0.05$).

5.1.5 Sorghum seedling roots release a diverse array of polysaccharide exudates

An ELISA (enzyme-linked immunosorbent assay) was performed to determine the composition of the root exudates released from sorghum in comparison with other cereals. A 4-hour root exudate collection assay described in (Chapter 2 of this thesis) was carried out using 7-day-old sorghum and maize seedlings. Higher signals of xylogalacturonan was observed in sorghum than other cereals. xyloglucan and heteroxylan were also relatively higher compared to in rice and foxtail millet.

Antigen	**Anti-body**	**Sorghum**		**Rice**		**Foxtail millet**		**Scale**
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Extensin	LM1	0.359	0.213	0.105	0.053	0.074	0.003	2
Glucan	LM2	1.069	0.271	0.083	0.022	0.078	0.006	1.5
Arabinan	LM6-M	0.127	0.022	0.118	0.757	0.089	0.002	1
Xylogalacturonan	LM8	1.643	0.048	0.095	0.305	0.218	0.096	0.5
Xyloglucan	LM25	1.636	0.042	0.316	0.161	0.376	0.069	0.1
Heteroxylan	LM11	0.520	0.375	0.116	0.096	0.107	0.060	
Heteroxylan	LM27	1.489	0.205	0.171	0.051	0.120	0.010	
Beta-glucan	7E:B11	0.652	0.205	0.473	0.100	0.083	0.059	

Figure 5-7: Exudate profiling in sorghum roots in comparison with other cereals

A heatmap comparison of the polysaccharide epitopes detected per unit weight of the isolated high-molecular-weight (HMW) root exudate of sorghum, rice and foxtail millet. For each crop three exudate biological samples were collected and analysed by indirect ELISA. Values shown are from wells coated with 10 µg mL-1 of HMW exudate. Values are the absorbance at 450 nm. Values are the means of 3 biological replicates (n= 9 plants per biological replicate). The heatmap scale gradient is shown on the right. SD; standard deviation.

5.1.6 Variation in root exudate composition across sorghum genotypes with differing RSAs

ELISA was carried out to characterise natural variation in sorghum in sorghum seedlings grown on germination pouches supplied with Hoagland media. Based on previous signal detection strength, xylogalacturonan and heteroxylan (high signal polysaccharides), and extensin (low strength) were selected to explore the variations across genotypes. There was no significant variation in the amount of root exudates produced by sorghum genotypes. Consistently, all genotypes screened exhibited high signal in xylogalacturonan and heteroxylan, while low in extensin had a low signal.

A

Sorghum Genotypes	**Mabs**					
	LM8		**LM27**		**LM1**	
	Mean	SD	**Mean**	SD	**Mean**	SD
K214	**0.46**	0.04	**0.28**	0.03	**0.06**	0.00
SG-S3507001	**0.47**	0.07	**0.37**	0.09	**0.07**	0.02
S35	**0.46**	0.21	**0.35**	0.10	**0.07**	0.01
IS20727	**0.42**	0.10	**0.25**	0.02	**0.06**	0.00
SG-S3506016	**0.45**	0.01	**0.20**	0.03	**0.07**	0.02
E36-1	**0.49**	0.06	**0.24**	0.10	**0.07**	0.02
IS30400	**0.45**	0.03	**0.27**	0.02	**0.06**	0.00
SG-S3506040	**0.46**	0.04	**0.38**	0.06	**0.60**	0.00

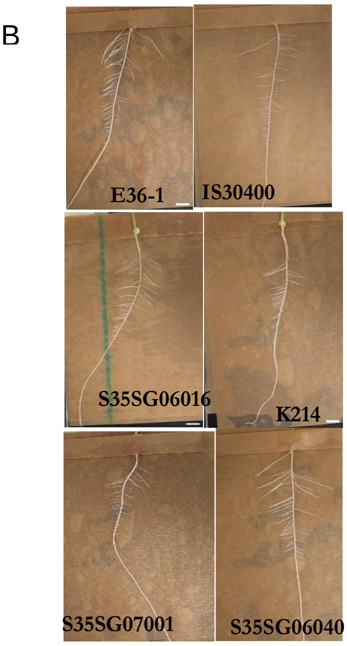


Figure 5-8: The variation in root exudate composition across sorghum genotypes

A) heatmap comparison of the polysaccharide epitopes detected per unit weight of the isolated high-molecular-weight (HMW) root exudate of sorghum cultivars. For each cultivar three exudate biological samples were collected and analysed by indirect ELISA. Values shown are from wells coated with 10 µg mL-1 of HMW exudate. Values are the absorbance at 450 nm. Values are the means of 3 biological replicates (n= 9 plants per biological replicate). B) Images showing root phenotypes grown in Hoagland growing media on pouches. The scale bar in images represents 1 cm.

5.1.7 Xylogalacturonan epitope mapping in sorghum and maize

Through ELISA, a 4-hour root exudate assay was carried out to collect high-molecular weight exudates from 5 days old sorghum and maize seedlings were collected. The findings reveal, that xylogalacturonan signal is stronger in sorghum roots than in maize roots. Indicating the higher amount and concentration produced from sorghum roots. xylogalaturonan is specifically associated with root cap cells in a range of angiosperm species (Willats et al., 2001). Surprisingly, the finding of xylogalacturonan higher signal level in sorghum root and not found or in very low amount in other cereals such as barley and wheat (Akhtar, J., PhD Thesis 2021), wheat and maize, by Galloway et al. (2020a) suggest unique functional role in sorghum. As indicated in the results sorghum exhibited low amount of Glucan and Xyloglucan compared to maize.

A

Antigen	**Antibody**	**Sorghum**		**Maize**		**Scale**
		Mean	SD	Mean	SD	2
Xylogalacturonan	LM8	0.88*	0.11	0.27	0.14	1.5
Glucan	LM2	0.26	0.02	0.54*	0.04	1
Xyloglucan	LM25	0.22	0.07	0.55*	0.12	0.5
						0.1

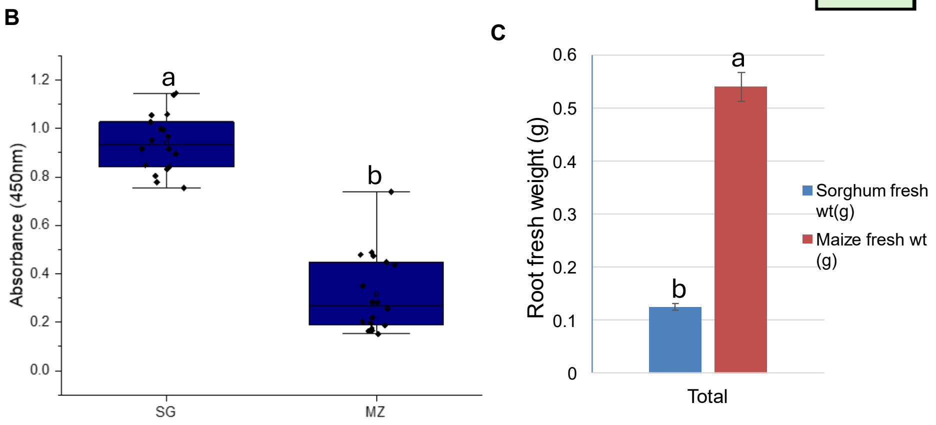


Figure 5-9: Detection of Xylogalacturonan in Sorghum and Maize 5 day old seedlings grown in liquid media (Hoagland media)

A) Variation in detection signal of xylogalacturonan, xyloglucan and Glucan between sorghum and maize. B) The variation in xylogalacturonan binding between sorghum and maize. C) Root fresh weight of sorghum and maize. ANOVA was used to test for significance at $p<0.05$. Letters in the graphs indicate significant differences.

5.1.8 Nitrocellulose root printing for exudates

Because other cereals have weak signals for the LM8 epitope (xylogalactouranan), that prompted a next question to investigate the localisation of this epitope through a root printing assay. The assay uses nitrocellulose sheets to assess where along the roots, different components are exuded. Nitrocellulose sheets were probed with glucan epitope (LM2) (representing weak signal in sorghum roots), xylogalacturonan epitope (strong signal), along with a control (without any antibody). The findings from this assay demonstrated that root prints were more visible in xylogalacturonan epitope compared to glucan and control. The strong prints on the nitrocellulose sheet indicates where along the seedling root surface xylogalacturonan exudes.

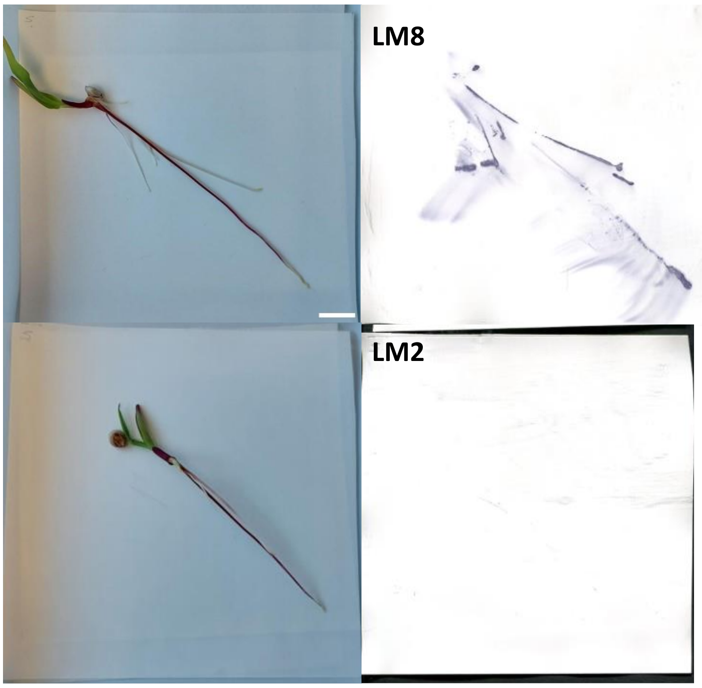


Figure 5-10: Root printing on Nitrocellulose binding sheet tracking the release of polysaccharide epitopes

Paired images of prints of 7-day old sorghum seedlings placed on nitrocellulose sheets and photographs of the seedling on the nitrocellulose *in situ*. Sheets w probed with monoclonal antibodies. LM8, xylogalacturonan in comparison with LM2 (negative control). Each image is representative of three prints per antibody. The scale bar in images represents 1 cm

5.1.9 Localisation of Xylogalacturonan (XGA) in sorghum root using immunofluorescence

To confirm that the high signal of LM8 targeting xylogalacturonan observed highly in sorghum root tips, Immunofluorescence microscopy to investigating in closer detail. The localising xylogalacturonan (LM8) epitope in sorghum roots, as the localisation has significant potential in uncovering the functionality of the polysaccharide. The findings reveal and confirm the hypothesis that LM8 signal for xylogalacturonan was particularly strong at the root cap in 5-day old sorghum root, the strength of the signal level decreasing as you move further away from the root tip, as evidenced by (Figure 5-9).

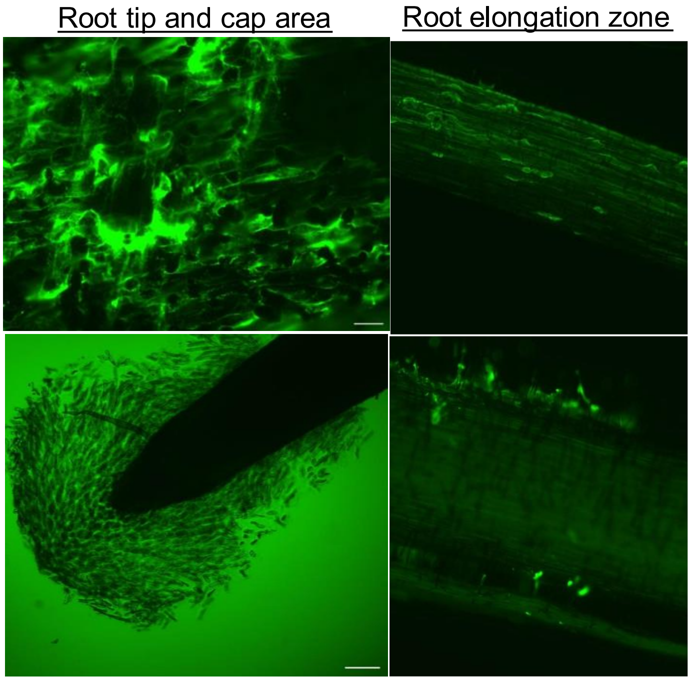


Figure 5-11: Immunolabelling for xylogalacturonan localisation in sorghum seedling root

LM 8 signal is strong at root cap especially in detached cells than away from the root cap. Root segments (~ 1 cm) were probed with LM8 xylogalacturonan monoclonal antibodies. Labelling indicated abundant at root tips and cap, especially in detached cells of a root cap than along the elongation zone and away from the root tip. Each image is representative of micrographs of three different root segments. Root segments of ~ 1 cm in length were taken 3 cm up from the root apex. Scale bar = 50 µm.

5.2 Discussion

Work undertaken in this chapter demonstrated the diversity of RSA among sorghum genotypes and lays the groundwork for exploring the composition of sorghum root exudations with various polysaccharide epitope mapping. Root system traits are often complex and relatively understudied because of their growth in opaque, heterogeneous, and complex soils. But the importance of the root system to the plant is undisputed due to its essential roles in nutrient and water acquisition, abiotic and biotic stress response signaling, and anchoring and stabilizing the plant in the soil. Characterising sorghum root system architecture, root exudates, and rhizosheath composition have a potential impact on understanding drought tolerance. This is necessary to develop crop varieties with more efficient root systems that can lead to enhanced yields in both favourable and water-stressed environments (Chandnani et al., 2023).

5.2.1 Root phenotyping of architectural traits shows variation in various screening method

Root phenotypic characterisation of architectural traits shows genotypic diversity across various screening methods. Generally, the sorghum root system comprises a primary root (sole seminal root), which is important for the initial water and nutrient uptake during seedling establishment. Next, nodal roots emerge and dominate during the later stages of growth. Changes in RSA can therefore have huge impacts on the final yield of a crop (Chandnani et al., 2023). Of the factors that control total RSA, LR formation and growth is one of the most important Nibau et al. (2008) . Variations in the size, shape and surface area of plant root systems are brought about largely by variations in root branching. Insignificant differences in seedling primary root length across genotypes tested using growth pouches imply difficulty in using this trait for pre-selection in sorghum. However, root growth angle in young seedlings can be used as a proxy trait predict root system depth and may be linked to and performance under water stress conditions in later developmental stages in sorghum. Collectively, our work shows for the first time, that root architectural traits i.e. root growth angle quantified using a 2D method, can be extrapolated to overall root architecture i.e. rooting depth quantified in soil using 3D methods, such as colander-based phenotyping. This study we also show significant differences in root growth angle of the sorghum genotypes with differing drought adaptability.

5.2.2 A variety of polysaccharides exudates are released from sorghum seedling roots

Though complex, understanding the composition of root exudation is essential to shed light on rhizosphere biology and can have potential implications in predicting soil carbon sequestration capacity of individual varieties. Thus, leading to a better understanding of plant roots-soil interactions (Rhizosphere), especially in response to increasingly common disturbances such as drought stress. The type and amount of root exudates produced varies with the plant species, cultivar, the age of the plant, and substrate and stress factors (Uren, 2000, Inderjit and Weston, 2003). Although many functions of exudates are being covered, further work is required to elucidate exudate's role in drought adaptability, and its contribution to rhizosphere in general.

5.2.3 Xylogalacturonan in sorghum root tips

Xylogalacturonan (XGA) is a class of pectic polysaccharides found in plant cell walls (rhamnogalacturonan, homogalacturonan) -XGA, abundant in reproductive tissues –and also associated with root caps of angiosperm species (Willats et al., 2004). The presence of XGA in plants has been reported in storage tissues or reproductive organs, such as the cell walls of peas, soybeans, watermelons, apples, pears, onions, potatoes, pine pollen, and cotton seeds (Zandleven et al., 2007). What is already known is that pectins mediate cell adhesion, cell expansion, cell wall porosity, and defense responses. They also play diverse roles in maintainence of cell wall architecture and cell development. Therefore, the significant strong signal of XGA in sorghum roots suggests its unique functionality. Further work is needed to characterise the specific role that XGA plays within sorghum root tip exudates.

5.2.4 Concluding remarks

In this study, a significant portion of the large genetic diversity underlying the phenotypic variation for root architectural and growth/morphological traits was captured. Our results suggest that phenotypic variation across sorghum genotypes in varying water environments may provide an opportunity for breeding selection and identification of novel resources for drought tolerance. The findings from this study contribute suggest that high-throughput phenotyping protocols for screening of existing large germplasm collections seedling screening can rapidly accelerate pre-selection of breeding materials and could predict the performance in the field. Additionally, a more in-depth understanding of the genetic and molecular regulation of root growth, development, and functional traits is of utmost importance to improve crop adaptation to marginal soils and climate change. Therefore, data on the molecular regulation of root architecture is covered in the next chapter (Chapter 6) of thesis.

Chapter 6

Uncovering structural and developmental pathways underlying root growth angle regulation in response to drought stress

6.1 Introduction

A key feature of plant architectural regulation is that the growth angle of lateral branches is often set and maintained with respect to gravity. Such growth angles are also known as gravitropic setpoint angles (GSA) (Digby and Firn, 1995). The GSA is one of the most important but least understood components of the wonderful diversity of plant forms observed throughout nature (Roychoudhry and Kepinski, 2015). Root growth angle is a significant agronomic trait and a major component of root system architecture (Smith and De Smet, 2012, Roychoudhry et al., 2013, Roychoudhry and Kepinski, 2015). Improving architectural traits through genetic improvement can have a significant impact on crop resilience to environmental stresses and optimise productivity (Waite and Dardick, 2021). Studies have focused on understanding how a given genotype or cultivar's organ orientation or GSA, is determined (Digby and Firn, 1995, Waite and Dardick, 2021, Yoshihara and Spalding, 2020). It has been discovered that many of these genes belong to the IGT (*LAZY/DRO1/TAC1)* family. They share a conserved *IGT*-like motif in domain II of their coding gene sequences and have been shown to regulate gravitropism and lateral organ growth angle in the shoots and roots (Yoshihara and Spalding, 2017). Additionally, the *IGT* gene family appears to be conserved across all land plants, as evidenced by genome sequencing. The *LAZY* trait was initially described in rice and maize in the 1930s as an ageotropic mutation, resulting in impaired lateral development (Overbeek, 1936, Jones and Adair, 1938, Waite and Dardick, 2021).

Since the initial *LAZY* discovery, six Arabidopsis *LAZY* (*ATLAZY*) gene sub-family was identified, *ATLAZY1/2/3/4/5/6*. Previous studies in Arabidopsis mutations have demonstrated that the *LAZY2/3/4* gene family are all expressed in the root tips of primary roots, including the columella cells, and contribute to the regulation of root GSA, with LAZY4 and LAZY2 additively promote more vertical lateral root angles (Yoshihara and Spalding, 2017, Furutani and Morita, 2021, Jiao et al., 2021). Studies on *AtLAZY4* have also shown it to be predominantly expressed in the cortical and endodermal tissue of the root elongation zone, as well as in columella cells. (Taniguchi et al., 2017). The discovery of IGT genes has led to significant understanding of gravitropism, such as a rice study demonstrating that steeper and deeper root systems, determined by DRO1 homologue expression, enhance drought tolerance. (Uga et al., 2013). Although the mechanisms underlying this performance are still unclear. Studies of *LAZY* proteins and molecular mechanisms in protoplast systems have begun deciphering their molecular function in Arabidopsis root and shoot gravitropism and confirming they have a functional role in both gravity-dependent PIN membrane polarity and auxin transport (Jiao et al., 2021, Furutani and Morita, 2021, Taniguchi et al., 2017).

Until recently, root growth angle has received relatively little attention in a range of cereals as potentially a useful selection trait for breeding programs targeting adaptation to specific environmental and management conditions. Although the knowledge of its role in drought adaptation has recently been studied (Singh et al., 2010, Singh et al., 2012, Uga et al., 2013), knowledge of the molecular mechanisms underlying the effect of drought on root growth angle during drought remains limited. Understanding the molecular mechanisms that regulate root growth angle is key to enhancing productivity and applications in crop improvement (Kirschner et al., 2024).

Despite similarities in early root system development and some shared mechanisms of genetic regulation between sorghum and other model plants, there are major anatomical differences in their root systems. Therefore, It is crucial to understand the mechanisms that govern root angle regulation in cereals because of their complex root system, which consists of distinct root types formed at different stages of development (Kirschner et al., 2024). Moreover, sorghum as a naturally drought-adapted crop is a good model for investigating drought-mediated RSA changes.

The lack of a direct link between genetic regulators and drought stress-induced RSA development, especially for lateral root growth, is unclear (Ranjan et al., 2022). In addition to understanding molecular mechanisms that control the angle of root growth regulation. The structural angle changes caused by the interaction between root growth and their surrounding dry soil environment are not well understood. Root phenotyping can play a role in understanding (Wasaya et al., 2018, Atkinson et al., 2019). In addition, the effect of water availability on overall RSA has not been extensively studied. It is also important to recognise the environmental regulation of RSA can be species-specific. While many molecular mechanisms are being uncovered from laboratory-grown plants in controlled environments (Maurel and Nacry, 2020), it will be fascinating to understand how tropic responses mutually interact and operate during the growth of roots in real, drying soils and how they impact soil foraging and water uptake. Translational research looking at our fundamental understanding of the RSA from model species to sorghum and the potential role of *LAZY* genes and other uncharacterised regulators in drought will likely lead to the discovery of novel breeding targets for yield-increasing root system traits in dry conditions (Roychoudhry et al., 2013, Roychoudhry et al., 2017).

Considering these knowledge gaps, the main aim of this work was to investigate the molecular pathways underlying sorghum root growth angle regulation under drought conditions. Specifically, the objectives of this chapter were to: i) discover root angle structural changes during drought; ii) understand the potential role of *LAZY2/4* genes in sorghum root angle regulation and how they are expressed in drought; iii) identify novel developmental pathways underlying root structural changes during drought. Exploration of these objectives would answer how structural changes in root angle are regulated during drought.

6.2 Results

6.2.1 Sorghum lateral roots maintain GSAs

The distribution of lateral organs and their characteristically non-vertical growth orientation are critical for the determination of plant form (Mullen and Hangarter, 2003). Although much work on GSA maintenance has been done in model plants, less is known in sorghum and many minor cereals. Angle dependence can be defined as the relationship between the stimulation angle of a gravi-responding organ with respect to gravity and the rate at which this organ will bend towards or away from the gravity vector due to its stimulation angle (Mullen and Hangarter, 2003, Mullen et al., 2000). The strength of the gravitropic response increases with stimulation angle at which an organ is reoriented. For this reason, re-orientation assays were conducted under a controlled environment to test if sorghum lateral roots maintain GSA. Growing seedling roots are rotated at 30° with respect to gravity according to the method described in Roychoudhry and Kepinski (2015). To achieve this aim, the initial attention was focused on understanding the changes in lateral roots as they determine the angle of root branching. The findings in (Figure 6-1) show that sorghum roots maintain their original GSA once re-oriented. However, the kinetics of reorientation are significantly slower than those of Arabidopsis. Sorghum lateral roots attained their previous GSAs after approximately 24 hours post reorientation, while Arabidopsis lateral roots can return to their original GSAs in approximately 6 hours. These results demonstrate that similar to rice and wheat lateral roots (Kaye, RS, 2018 PhD thesis), sorghum lateral roots actively maintain GSAs following gravity.

The findings from this initial assay were significant for the next investigation, which sought to understand the effect of drought on root growth angle in the soil environment.

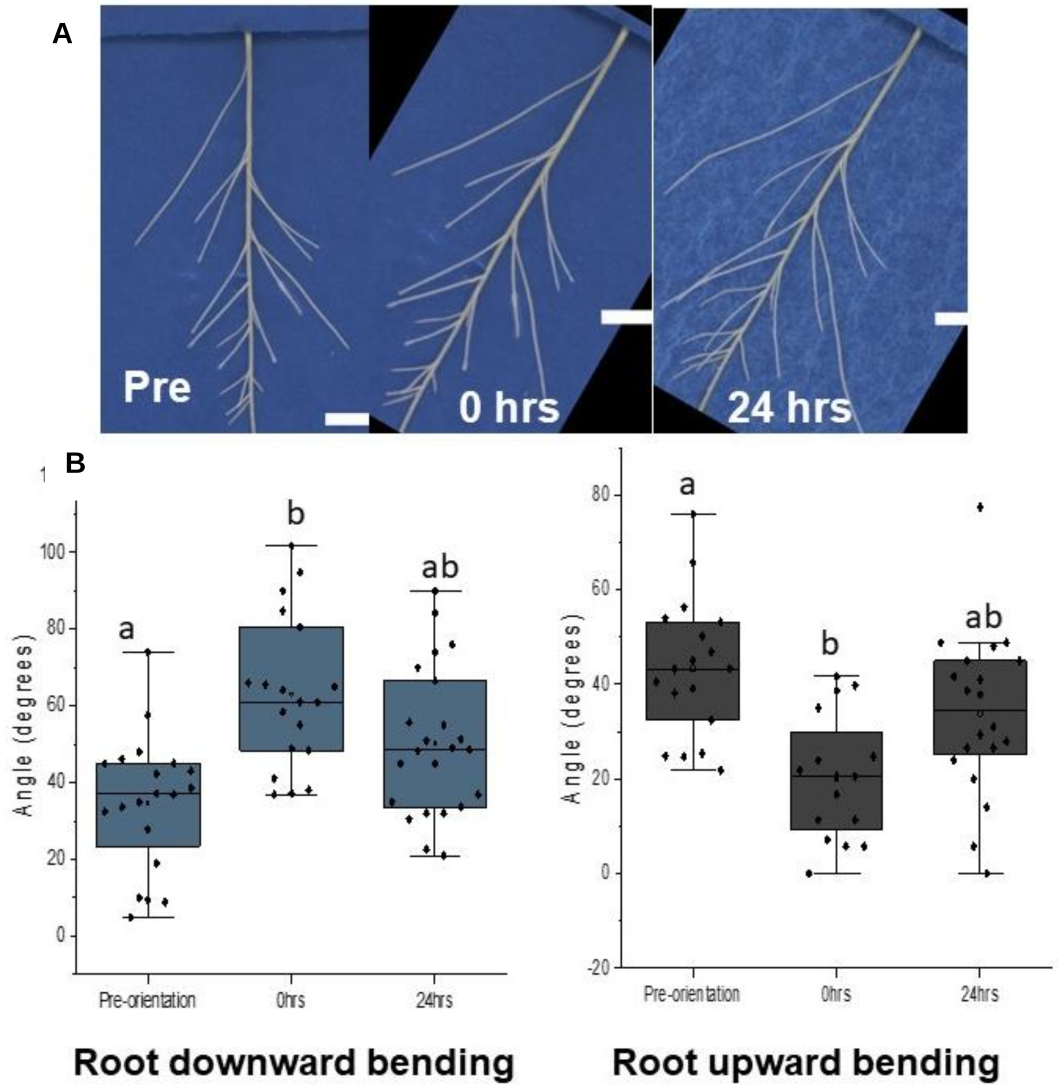


Figure 6-1: Maintenance of sorghum root gravitropic set-point angle (GSA)

A) Images of 10-day-old sorghum seedlings grown in germination pouches, showing lateral root reorientation assay. Pre-reorientation= plants were imaged and quantified before reorientation by 30^o, 0 hrs = the plants were imaged and quantified after tilting at 30^o angle and 24hrs = plants were imaged and quantified at 24 hours after reorientation. n>20 plants. The scale bar represents 10 mm. B) Quantification of sorghum lateral root growth angle after reorientation by 30° after both upwards and downwards bending. Dark horizontal lines in the box plot represent the median, with the box representing the 25th and 75th percentiles, while dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance ($p<0.05$).

6.2.2 Drought-water stress influences structural changes of root angle towards verticality

To explore root angle plasticity in sorghum, Phenotypic changes in the growth angle of nodal roots during drought stress were investigated. In monocots, nodal roots play a dominant role in determining the architecture of root systems. Because of that, they are a major focus area for research into understanding how different ideal phenotypes affect tolerance to water deficit (Lynch, 2013, Lynch, 2019). A colander-based phenotyping method was used as a tool to visualise the nodal roots in three dimensions and quantify their root growth angles in the soil, both in well-watered and water-stressed (drought) conditions. The root colander phenotyping method (Chapter 2), has been previously reported by (Uga et al., 2013). The root angle was calculated by observing the number of roots that emerged per tier of the colander representing a particular angle degree (refer to Chapter 2 for the colander's root angle calculation).

The ratio of deep rooting (RDR), defined as the number of roots that penetrated the lower part of the colander (<50º) divided by the total number of roots that penetrated the whole colander) was used to indicate the steepness of the root angles between well-watered and drought water-stress treatments. A larger value for the ratio of deep rooting means that a greater proportion of the roots grew downward (Uga et al., 2013). Findings from these experiments show that the RDR was significantly higher in drought-treated plants compared to well-watered plants, suggesting that drought induces steeper rooting in sorghum (Figure 6-2 A). This is also confirmed by root angle percentage change (Figure 6-2 B) and representative images of root phenotypic changes (Figure 6-2 C), which show significant changes to the roots towards verticality under drought (Figure 6-2 B). As shown in (Figure 6-2 D-F), significant differences in root volume, root area and number of nodal roots suggest phenotypic changes strongly demonstrate the drought effect on root traits and the trade-offs during limited soil moisture.

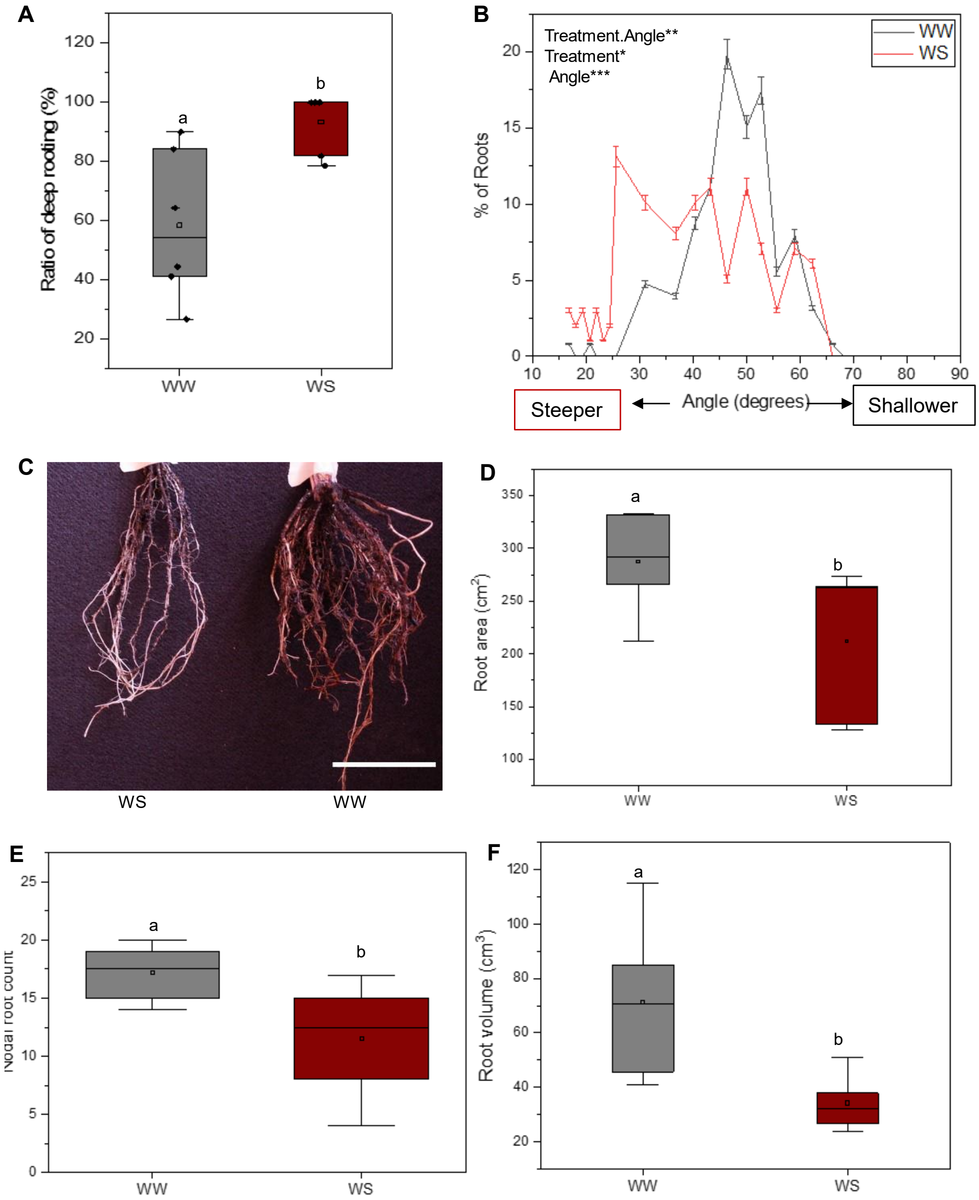


Figure 6-2: Phenotypic changes in root angle and associated root traits in sorghum

A) The ratio of deep rooting (RDR), defined as the number of roots that penetrated the lower part of the colander ($\leq 50^{o}$) divided by the total number of roots that penetrated the whole colander. A larger value for the ratio of deep rooting means that a greater proportion of the roots grew at steeper angle B) Root angle variation, between two treatments, well-watered (ww) and drought water-stress (ws) grown in pots using the 'colander' method of calculating angles. Points towards 10^{o} show more vertical angles, while points towards 90^{o} indicate less vertical angles. C) Representative images of a colander experiment comparing ww and ws treatments n>18 plants per treatment. D-F) Root phenotypic changes in root area, root volume, number of nodal roots respectively. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentiles while, dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance ($p \leq 0.05$). Significant codes represent: '***' p=0.0001,'**' p=0.001, '*' 0.05. The scale bar represents 10 mm.

6.2.3 Maize and wheat change their root angles under drought stress

To understand if root growth angle responses to drought stress are conserved across species, colander experiments of wheat and maize plants were conducted as described in Chapter 2 of this thesis. Significant differences in root phenotypes were observed in both species' roots (Figure 6-3). Drought significantly induces steeper rooting architectures and deeper rooting ratios in both species. A higher percentage of roots grew towards the vertical, as shown by the deep rooting ratio, confirming the hypothesis that drought changes the root angle towards a deeper vertical angle.

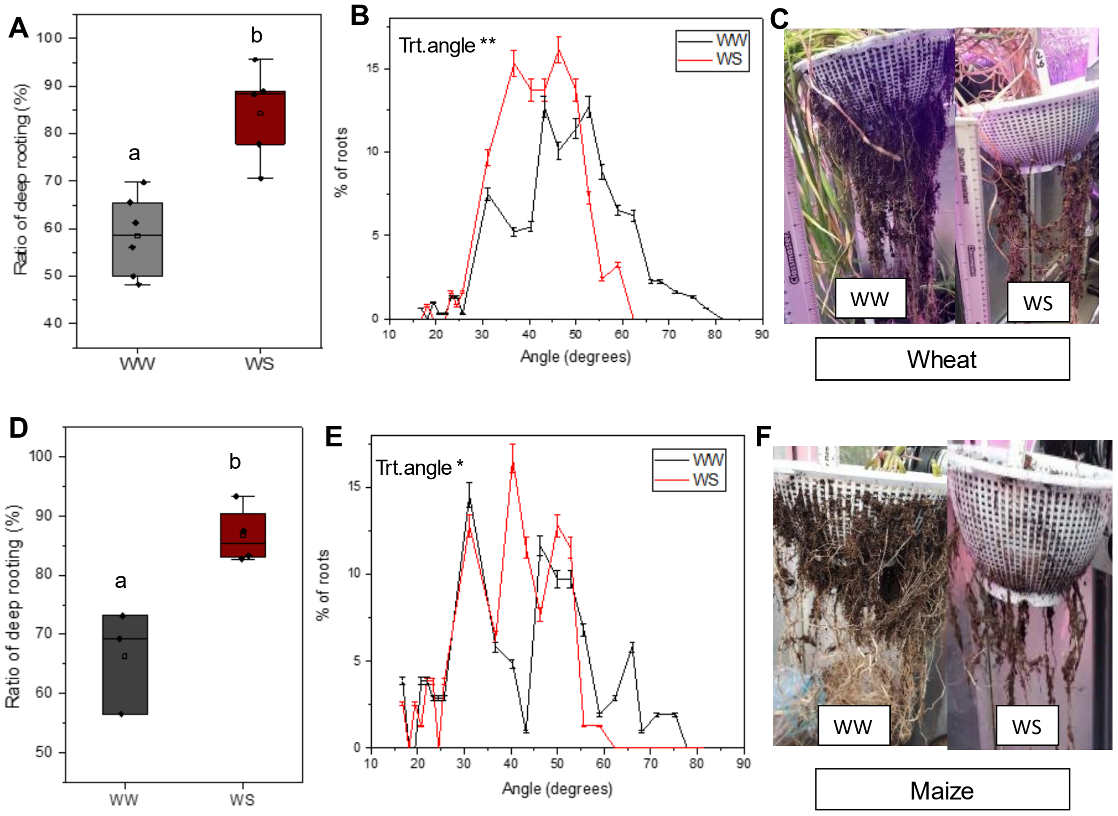


Figure 6-3: Root angle phenotypic changes in maize and wheat

A) The ratio of deep rooting (RDR) in roots. B) Root growth angle variation, in degrees variations between two treatments, well-watered (ww) and drought water-stress (ws) grown in pots using the 'colander' method of calculating angles in wheat. Points towards 10^o show more vertical angles, while points towards 81^o indicate less vertical angles. C) Representative images of a colander experiment comparing ww and ws treatments n>12 plants per treatment of wheat. D) RDR in maize roots. E) Root angle variation, in degrees variations between two treatments, ww and ws grown in pots using the 'colander' method of calculating angles in maize. Points towards 10^o show more vertical angles, while points towards 90^o indicate less vertical angles. F) Representative images of a colander experiment comparing ww and ws treatments n>12 plants per treatment of maize plants. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentiles while, dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance (p≤0.05).

6.2.4 Shoot phenotypic changes are linked to root growth angle changes in drought

Shoot as other root traits were analysed to understand changes in drought response and as confirmation of drought-stress in plants. Root areas and the number of nodal roots were significantly higher in well-watered compared to drought-stress conditions (Figure 6-4). While, root diameter and root dry weight were not significantly different between the treatments (Figure 6-4 A and B). As a caveat to this study, shoot traits such as primary root length could not be determined when roots were extracted from the pots with a colander, which is one of the limitations of the colander method. Regarding quantified shoot traits, plant height, shoot biomass and chlorophyll content (Figure 6-4 E-F) were significantly higher in well-watered conditions compared to drought water stress. As expected, the findings on shoot phenotypic and physiology changes demonstrate the negative effects of drought stress on plant physiological mechanisms, which reduce growth and development. Therefore, the visible effects in the shoots' results indicate that stress was induced in the plants.

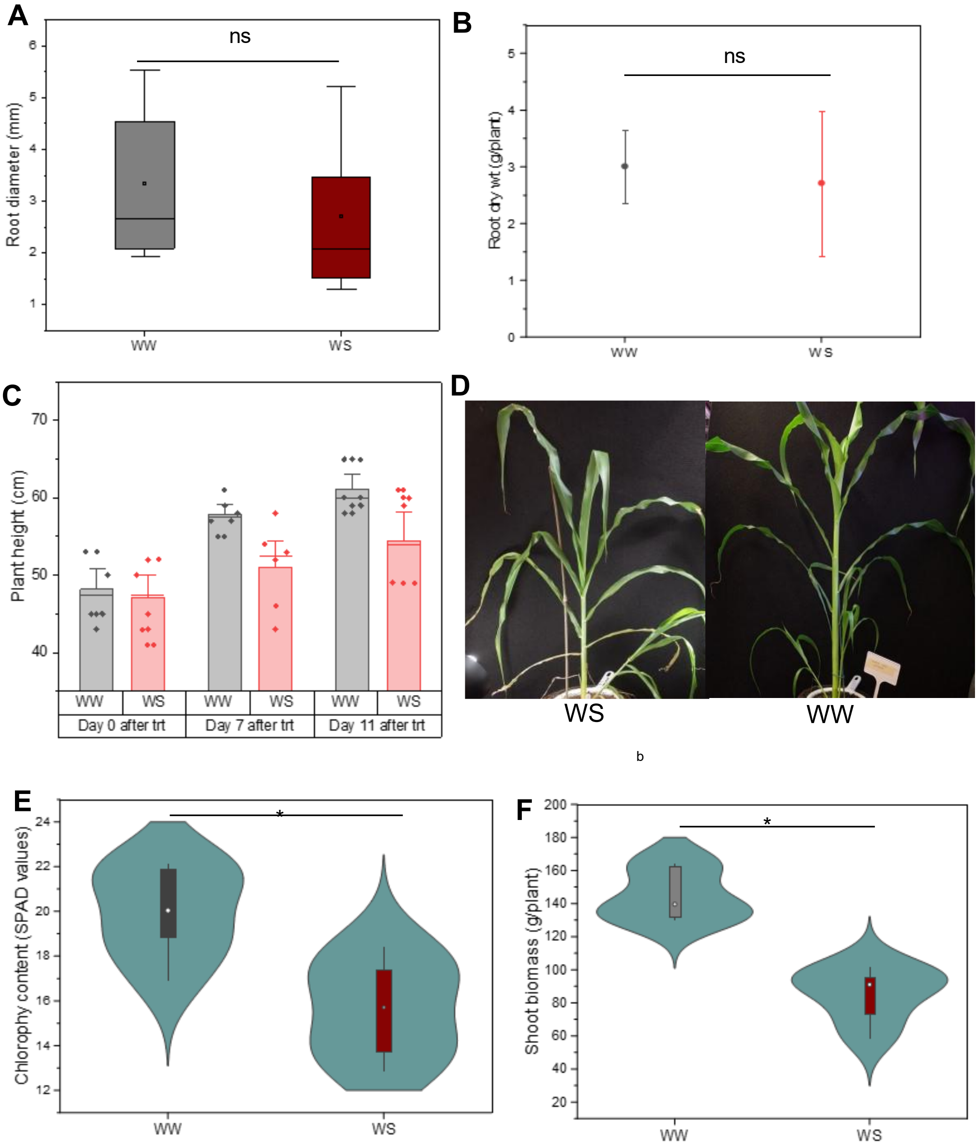
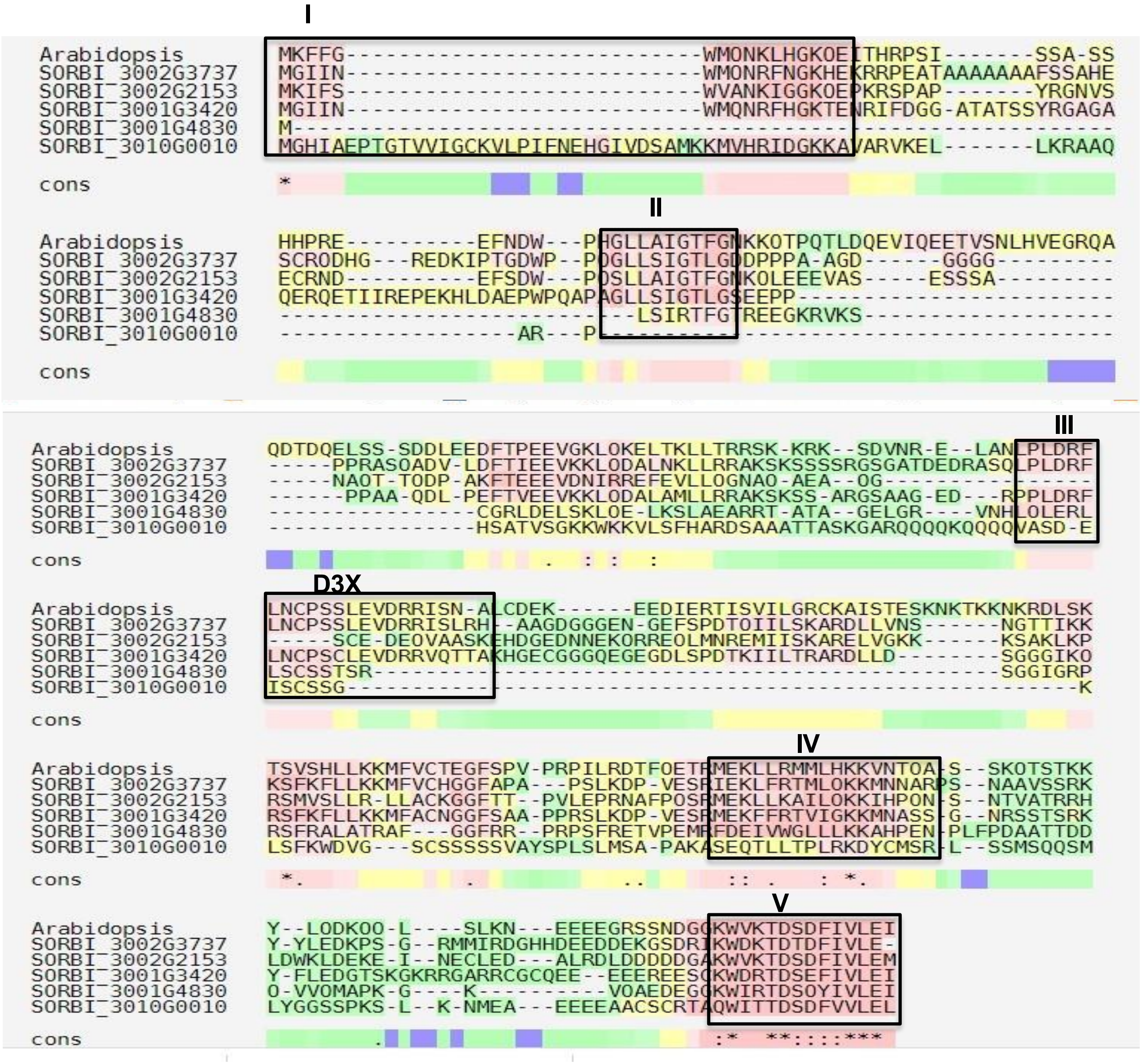


Figure 6-4: Effect of drought on shoot and other root phenotypes

A) Variation in root diameter (cm) quantified using WHINRhizo software for both treatments, well-watered (ww) and drought water-stress (ws). B) Comparison of root dry weight, measured on weighing balance, expressed as grams per plant sample. C) Comparison of plant height before drought and days after drought stress was induced. D) Representation of images of plants of E36-1 cultivar exposed to ww and ws treatment. E) Violin graph representing chlorophyll content values (quantified using Soil Plant Analysis Development (SPAD) chlorophyll meter). F) Comparison of soot biomass (in grams) for ww and ws treatment. Dark horizontal lines in box plot represent the median, with the box representing the 25th and 75th percentiles while, dotted lines outside the boxes represent values within 1.5 times interquartile range (IQR). ANOVA was used to test for significance (p≤0.05).

6.2.5 Identification of orthologue of sorghum *LAZY 2/4* genes as regulators of root growth angle

LAZY genes are key regulators of gravitropism in shoot and root branch angle (Taniguchi et al., 2017, Yoshihara and Iino, 2007, Yoshihara and Spalding, 2017, Yoshihara et al., 2013). To identify *LAZY* homologs in sorghum, EnsemblPlants (https://plants.ensembl.org/) was interrogated to conduct an *in silico* analysis aligning the protein sequence of known *LAZY2* and *LAZY4* (here collectively referred to as *LAZY2/4*) in Arabidopsis and sorghum genome. From this alignment, two candidate genes with *LAZY2/4* conserved domains were identified in SORBI_3002G373700 and SORBI_3001G34200 (Figure 6-5). *LAZY2/4* protein has IV domains, and previous work has shown that mutations in the extended domain 3 (D3X) lead to steep rooting angles in Arabidopsis and wheat (Kaye, RS, 2018 PhD thesis; Binns, AJ, 2023 PhD thesis). With this background knowledge, the possible roles of *LAZYs* in drought-dependent regulation of root architecture in sorghum was investigated. During primer testing for two aligned candidate genes, one shows higher levels of expression (*SORBI_3001G342000*) while SORBI_3002G373700 expression levels were low. For this reason, the expression pattern of *SORBI_3001G342000*, hereafter annotated as *LAZY2/4* was investigated during drought stress using Quantitative real-time PCR. Tissue from root tips of sorghum from plants grown in colanders (these are the same plants phenotyped in Figure 6-2, section 6.2.2 of this Chapter), exposed to drought and well-watered, were used for RNA extraction. The expression of *LAZY2/4* in drought is significantly ($p<0.03$) higher compared to well-watered treatment (Figure 6-6).



```
Arabidopsis      MKFFG---------------------------WMONKLHGKOEITHRPSI-------SSA-SS
SORBI_3002G3737  MGIIN---------------------------WMONRFNGKHEKRRPEATAAAAAAAFSSAHE
SORBI_3002G2153  MKIFS---------------------------WVANKIGGKOEPKRSPAP-------YRGNVS
SORBI_3001G3420  MGIIN---------------------------WMQNRFHGKTENRIFDGG-ATATSSYRGAGA
SORBI_3001G4830  M--------------------------------------------------------------
SORBI_3010G0010  MGHIAEPTGTVVIGCKVLPIFNEHGIVDSAMKKMVHRIDGKKAVARVKEL-------LKRAAQ

cons             *

Arabidopsis      HHPRE----------EFNDW---PHGLLAIGTFGNKKOTPQTLDQEVIQEETVSNLHVEGRQA
SORBI_3002G3737  SCRODHG---REDKIPTGDWP--PDGLLSIGTLGDDPPPA-AGD------GGGG---------
SORBI_3002G2153  ECRND----------EFSDW---PDSLLAIGTFGNKOLEEEVAS-----ESSSA---------
SORBI_3001G3420  QERQETIIREPEKHLDAEPWPQAPAGLLSIGTLGSEEPP------------------------
SORBI_3001G4830  ---------------------------LSIRTFGTREEGKRVKS-------------------
SORBI_3010G0010  ------------------AR---P---------------------------------------

cons

Arabidopsis      QDTDQELSS-SDDLEEDFTPEEVGKLOKELTKLLTRRSK-KRK--SDVNR-E--LANLPLDRF
SORBI_3002G3737  -----PPRASOADV-LDFTIEEVKKLODALNKLLRRAKSKSSSSRGSGATDEDRASQLPLDRF
SORBI_3002G2153  -----NAOT-TODP-AKFTEEEVDNIRREFEVLLOGNAO-AEA--OG----------------
SORBI_3001G3420  ------PPAA-QDL-PEFTVEEVKKLODALAMLLRRAKSKSS-ARGSAAG-ED---RPPLDRF
SORBI_3001G4830  ----------------CGRLDELSKLOE-LKSLAEARRT-ATA--GELGR----VNHLOLERL
SORBI_3010G0010  ----------------HSATVSGKKWKKVLSFHARDSAAATTASKGARQQQQKQQQQVASD-E

cons                                  .  : :  :

Arabidopsis      LNCPSSLEVDRRISN-ALCDEK------EEDIERTISVILGRCKAISTESKNKTKKNKRDLSK
SORBI_3002G3737  LNCPSSLEVDRRISLRH--AAGDGGGEN-GEFSPDTOIILSKARDLLVNS------NGTTIKK
SORBI_3002G2153  -----SCE-DEOVAASKEHDGEDNNEKORREOLMNREMIISKARELVGKK------KSAKLKP
SORBI_3001G3420  LNCPSCLEVDRRVQTTAKHGECGGGQEGEGDLSPDTKIILTRARDLLD--------SGGGIKO
SORBI_3001G4830  LSCSSTSR------------------------------------------------SGGIGRP
SORBI_3010G0010  ISCSSG--------------------------------------------------------K

cons

Arabidopsis      TSVSHLLKKMFVCTEGFSPV-PRPILRDTFOETRMEKLLRMMLHKKVNTOA-S--SKOTSTKK
SORBI_3002G3737  KSFKFLLKKMFVCHGGFAPA---PSLKDP-VESRIEKLFRTMLOKKMNNARPS--NAAVSSRK
SORBI_3002G2153  RSMVSLLR-LLACKGGFTT--PVLEPRNAFPOSRMEKLLKAILOKKIHPON-S--NTVATRRH
SORBI_3001G3420  RSFKFLLKKMFACNGGFSAA-PPRSLKDP-VESRMEKFFRTVIGKKMNASS-G--NRSSTSRK
SORBI_3001G4830  RSFRALATRAF---GGFRR--PRPSFRETVPEMRFDEIVWGLLLKKAHPEN-PLFPDAATTDD
SORBI_3010G0010  LSFKWDVG---SCSSSSSVAYSPLSLMSA-PAKASEQTLLTPLRKDYCMSR-L--SSMSQQSM

cons              *.            .           ..      :: .   : *.

Arabidopsis      Y--LODKOO-L----SLKN---EEEEGRSSNDGGKWVKTDSDFIVLEI
SORBI_3002G3737  Y-YLEDKPS-G--RMMIRDGHHDEEDDEKGSDRIKWDKTDTDFIVLE-
SORBI_3002G2153  LDWKLDEKE-I--NECLED---ALRDLDDDDDGAKWVKTDSDFIVLEM
SORBI_3001G3420  Y-FLEDGTSKGKRRGARRCGCQEE--EEEREESCKWDRTDSEFIVLEI
SORBI_3001G4830  O-VVOMAPK-G----K----------VOAEDEGGKWIRTDSOYIVLEI
SORBI_3010G0010  LYGGSSPKS-L--K-NMEA---EEEEAACSCRTAQWITTDSDFVVLEL

cons                     .                         :*  **::::***
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Figure 6-5: Homologue of *LAZY4* genes identified in sorghum

Protein sequence alignment of sorghum homologue to Arabidopsis *LAZY2/4*. '*', **, ***, represents significantly conserved amino acid in the proteins. Domain III is specific to *LAZY2/4* which both contain the PLQ motif and the additional extended D3X domain (PLDRFLNCPSSLEVDRR). Sequence alignment was carried out using T-coffee.

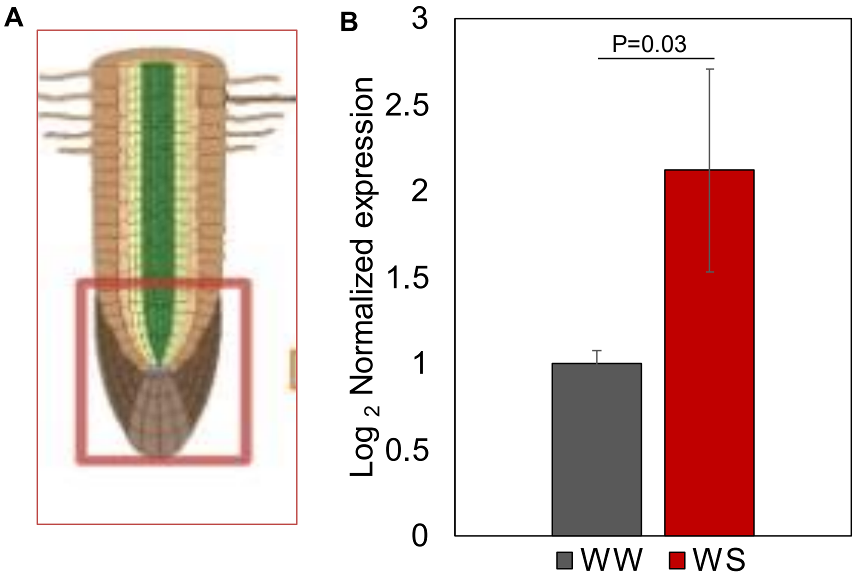

Figure 6-6: *LAZY2/4* SORBI_3001G342000 is highly expressed during drought in root tips

A) Schematic representation of the nodal root tip tissue length collected for RNA extraction for *LAZY 2/4* expression and subsequent transcriptomic analysis. B) Relative transcript expression of SORBI_3001G342000 quantified by RT-qPCR in nodal root tips. Student t-test was performed to test for significance between ww and ws treatment.

6.2.6 Transcriptome analysis unveils differentially expressed genes in sorghum root tips in varying water conditions

To better elucidate the mechanisms underlying root regulation and to identify novel developmental pathways implicated with changes in the root growth angle phenotype during well-watered and drought, a transcriptomic analysis was performed on root tips obtained from the soil colander experiments through RNA-sequencing (RNA-seq) data, to identify differentially expressed genes (DEGs). This process allows for the elucidation of differentially expressed genes across two or more conditions and is widely used in many applications of RNA-seq data analysis (McDermaid et al., 2019) .

Among 21182 differentially expressed genes (DEGs) genes identified between well-watered (WW) and drought/water stress (WS) treatments, 276 were significantly different ($p<0.05$); 133 genes were down-regulated, while 143 genes were up-regulated (Figure 6-7). The findings from this transcriptome analysis help in the identification of novel drought-dependent responsive genes and pathways in root tips.

Heat maps were used to explore and visualise the expression of significant DEGs (Figure 6-8A). This analysis is useful for identifying co-regulated genes across the treatment conditions. Overall, the analysis showed that root tips exposed to drought experience significant changes in their transcriptome compared to well-watered roots. However, DEG heatmaps do have one distinct downfall related to redundancy. This is where some genes belong to families with multiple members that have similar sequences and functions. If such genes are included in the analysis, the heat map may display clusters of genes with similar expression profiles, contributing to redundancy (McDermaid et al., 2019). Redundancy can indicate a robust biological signal, but if excessive, it may obscure unique patterns, making it difficult to identify specific genes or smaller differentially expressed pathways. For this reason, a second plot was created that only included the top statistically significant differentially expressed genes if two or more were discovered (Figure 6-8B).

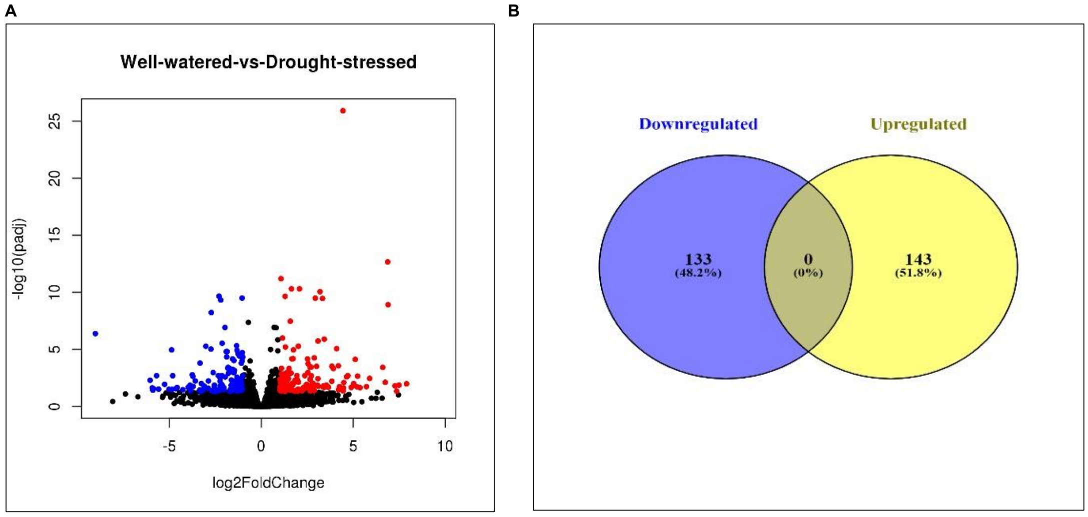

Figure 6-7: Transcriptomic analysis in water-stressed and well-watered sorghum root tips

A) Volcano plot of DEGs in well-watered vs drought stress treatments. The volcano plot shows the global transcriptional change across the groups compared. All the genes are plotted and each data point represents a gene. The log2 fold change is on the x-axis and the log10 of the adjusted p-value is on the y-axis. Red and blue dots indicate genes with an adjusted p-value less than 0.05 and a log2 fold change greater than +/-1. These represent, respectively, upregulated and downregulated genes. B) Venn diagram of significantly expressed genes. The Venn diagram illustrates the number of significant DEGs both upregulated and downregulated.

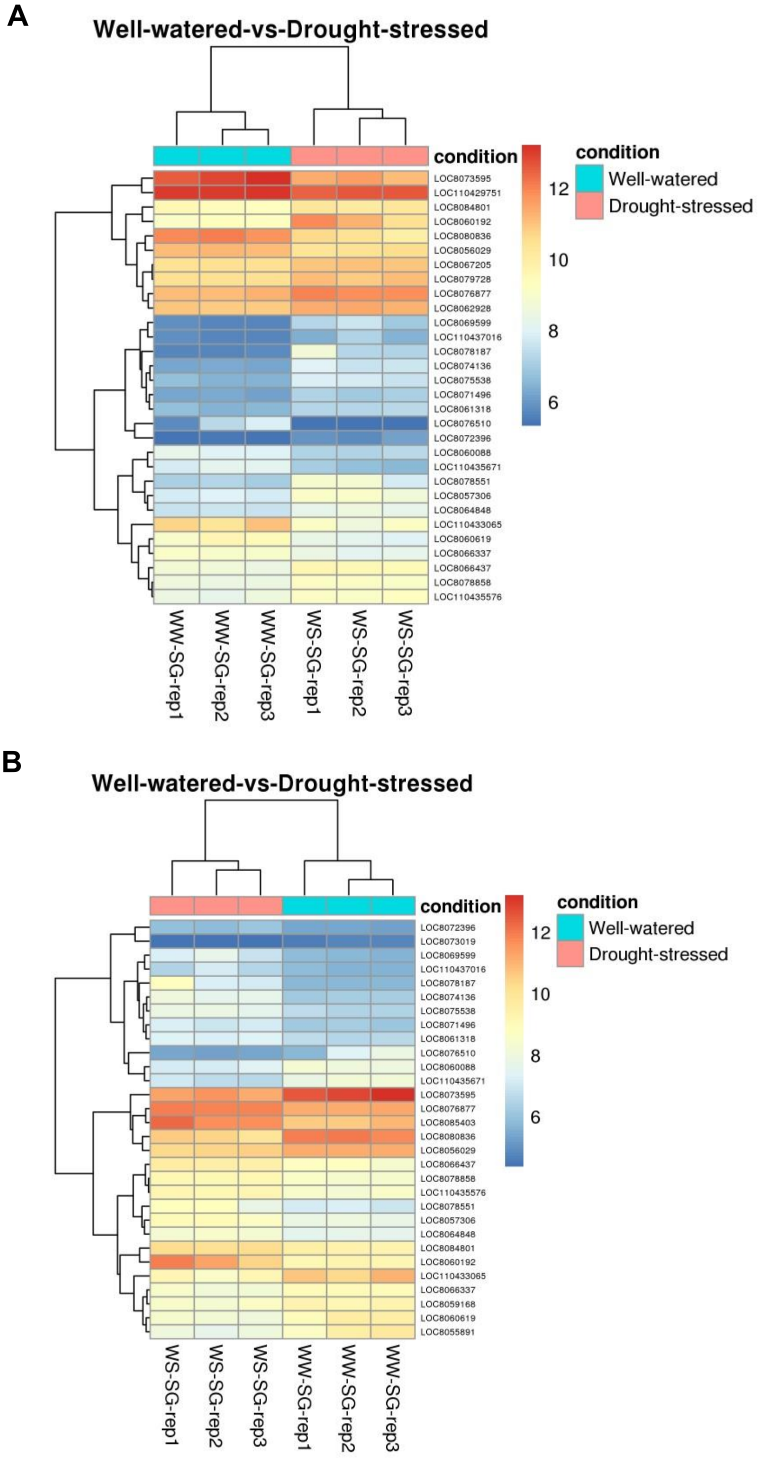

Figure 6-8: Differentially expressed genes bi-clustering heat maps for well-watered (WW) and water-stress in sorghum root tips

A) The expression profile of the top 30 genes sorted by their adjusted p-values (0.05). B) A second plot was generated to include only the top statistically significant differentially expressed genes if two or more were identified. Each cell represents the expression levels of a gene for the respective intersecting row and column. The placement along the chosen colour spectrum visually indicates the magnitude according to the scale.

6.2.7 Enrichment analysis of differentially expressed genes reveals developmental pathways in root tips under drought stress

To comprehend the biological processes implicated with root angle phenotypic changes in drought stress, gene ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis was conducted. Because many genes in sorghum are not yet characterized and functional annotation of individual genes is still incomplete, EnsemblPlants, BLAST2GO and SHINNYGO bioinformatic tools were used to associate gene ontology identifiers to each differentially expressed gene in root tips between well-watered and drought water-stress.

KEGG is aimed at connecting genomic information with higher-order functional information by computerization of current knowledge on cellular processes and standardization of gene annotations (Kanehisa and Goto, 2000). The biological function of cells and tissues is a result of many interacting molecules; it cannot be attributed to just a single gene or a single molecule. Therefore, KEGG process links a set of genes in the genome with a network of interacting molecules in the cell, such as a pathway or a complex representing higher order biological function. Using this method, the most common Gene Ontology (GO) terms describing biological processes are linked to stress and defence response, polysaccharide metabolic processes and cell wall biogenesis response (Figure 6-9A). Whilst GO identifiers describing cellular component (Figure 6-9B) categorise genes as acting in extracellular regions, plasma membrane, and cell wall . Finally, the only GO terms describing molecular functions (Figure 6-9C) found in the analysis are linked to hydrolase activity acting on glycosyl bonds.

Gene Ontology (GO) enrichment analysis revealed that the GO categories related to drought tolerance include terms related to response to stimulus especially response to water deprivation. In (Appendix 1 all significant DEGs GO Terms) show that the major transcription factors responsive to drought stress include heat stress transcription factor (HSF), ethylene-responsive transcription factor (ERF), WRKY transcription factor (WRKY), homeodomain leucine zipper transcription factor (HD-ZIP), basic helix-loop-helix transcription factor (bHLH), and V-myb myeloblastosis viral oncogene homolog transcription factor (MYB). Functional protein genes for heat shock protein (HSPs), late-embryogenesis-abundant protein (LEAs), chaperones, aquaporins, and expansins These genes' higher expression in drought suggests that they might play an important role in sorghum drought tolerance. The observation further confirms the transcriptional responses of field-droughted sorghum seedlings reported by Varoquaux et al. (2019). Overall, KEGG enrichment analysis from the findings of this study (Figure 6-10) suggests that drought induces a significant increase in metabolic pathways and the biosynthesis of secondary metabolites in the root tips of sorghum.

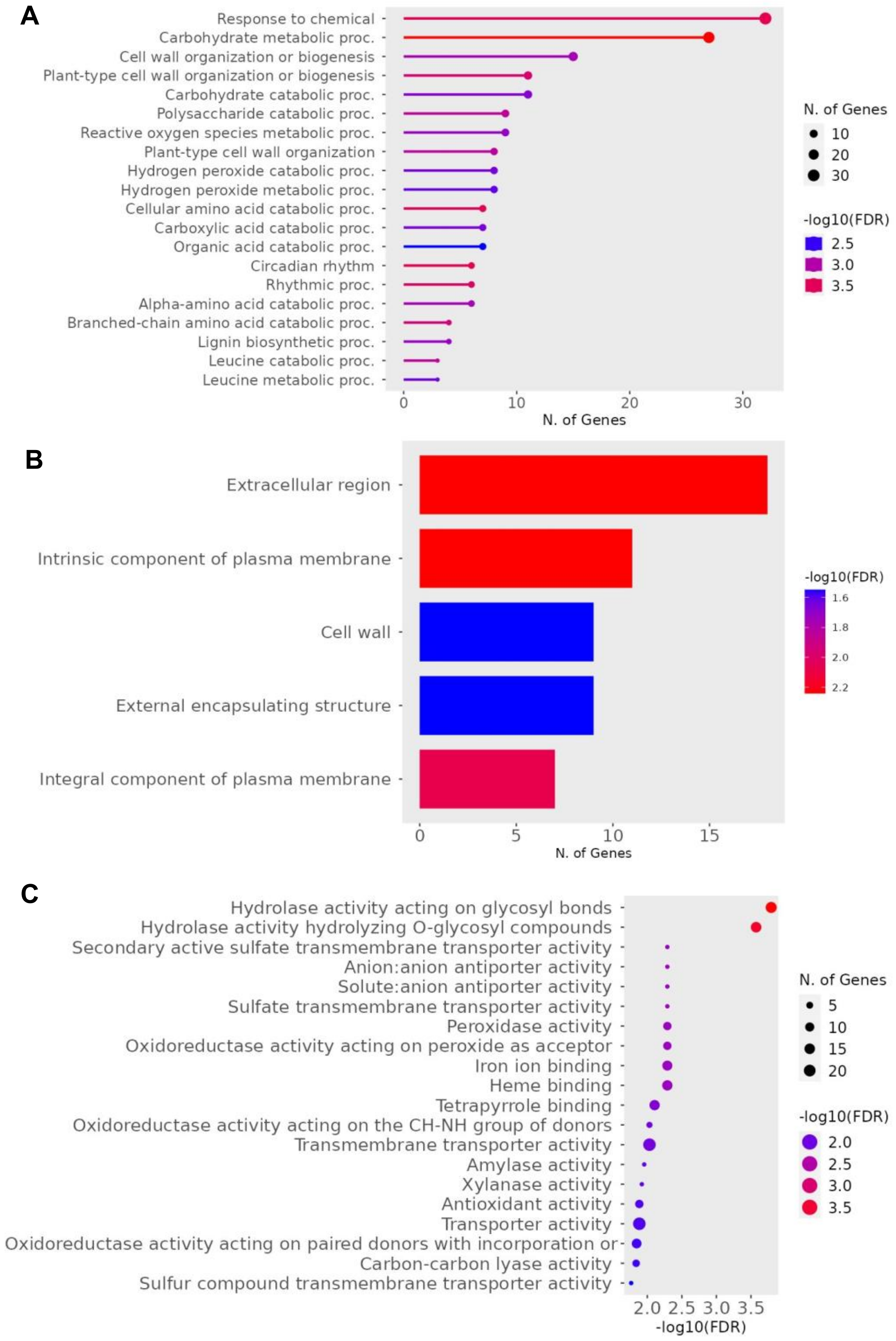

Figure 6-9: Gene ontology identifiers in differentially expressed genes between well-watered and water-stress in sorghum root tips

Gene ontology (GO) IDs for biological process, retrieved using Blast2GO, of differentially expressed gene either up- or down-regulated (FDR 2) between Well watered and Drought water stress. A) Biological processes. B) Cellular component and C) Molecular functions.

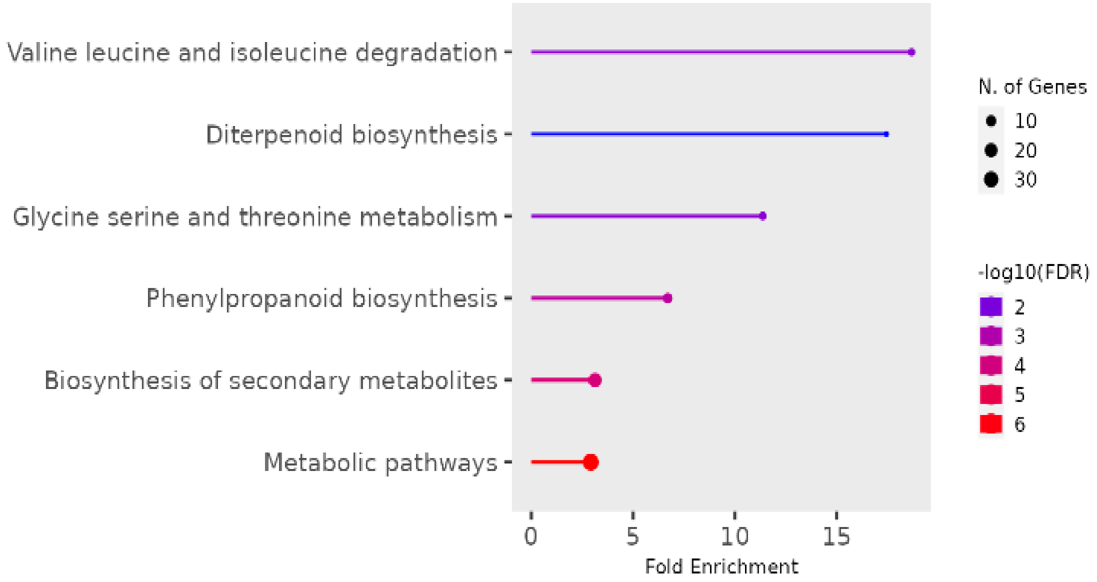

Figure 6-10: KEGG pathway analysis between well-watered and drought stress in sorghum root tips

Pathways with larger bubbles at the end represent high enrichment.

6.2.8 Novel developmental pathways in root growth angle regulation in sorghum root

A large number of studies have identified auxin transport and signalling as key determinants of growth angle regulation and induction (Roychoudhry et al., 2013, Roychoudhry and Kepinski, 2022). A gene ontology approach was again used to understand broader hormonal changes occurring following drought stress treatment. From the Gene Ontology and KEGG analysis, drought-dependent regulation of potentially auxin-responsive genes, together with growth and developmental pathways, were identified. Uncharacterised genes potentially related to auxin signalling and responsive to drought were uncovered from this analysis. These are *IAA-ARG7(SAUR)*, *LBD16* (an auxin-regulated gene involved in organ size), *WAT1*-related protein, and a homolog of *LAZY2/4* candidate genes (*SORBI_3001G342000*) (Figure 6-11A) and *LAZY4 SORBI_3001G373700.* All these genes cluster into developmental growth and endogenous stimulus GO categories. This confirms their involvement in regulation as hypothesized. These genes are not well characterised in sorghum and have not been studied for their involvement in drought. For example, WAT1-related protein identified in Arabidopsis and recently been reported in Foxtail millet (Wang et al., 2022), is an auxin-regulated transmembrane transporter with

ATP hydrolysis activity involved in growth and developmental processes. From this study on sorghum root tips, the higher expression of theWAT1-related protein in response to drought (Figure 6-11A) suggests a critical role in root regulation in drought, as evidenced by the KEGG enrichment analysis (Figure 6-11B). Although the expression levels were generally lower for *LAZY2/4* (*SORBI_3001G342000*) for both conditions, its higher expression levels in water-stress conditions and its involvement in growth and developmental pathways confirmed by KEGG analysis suggest a significant role during drought, which may still need further functional analysis.

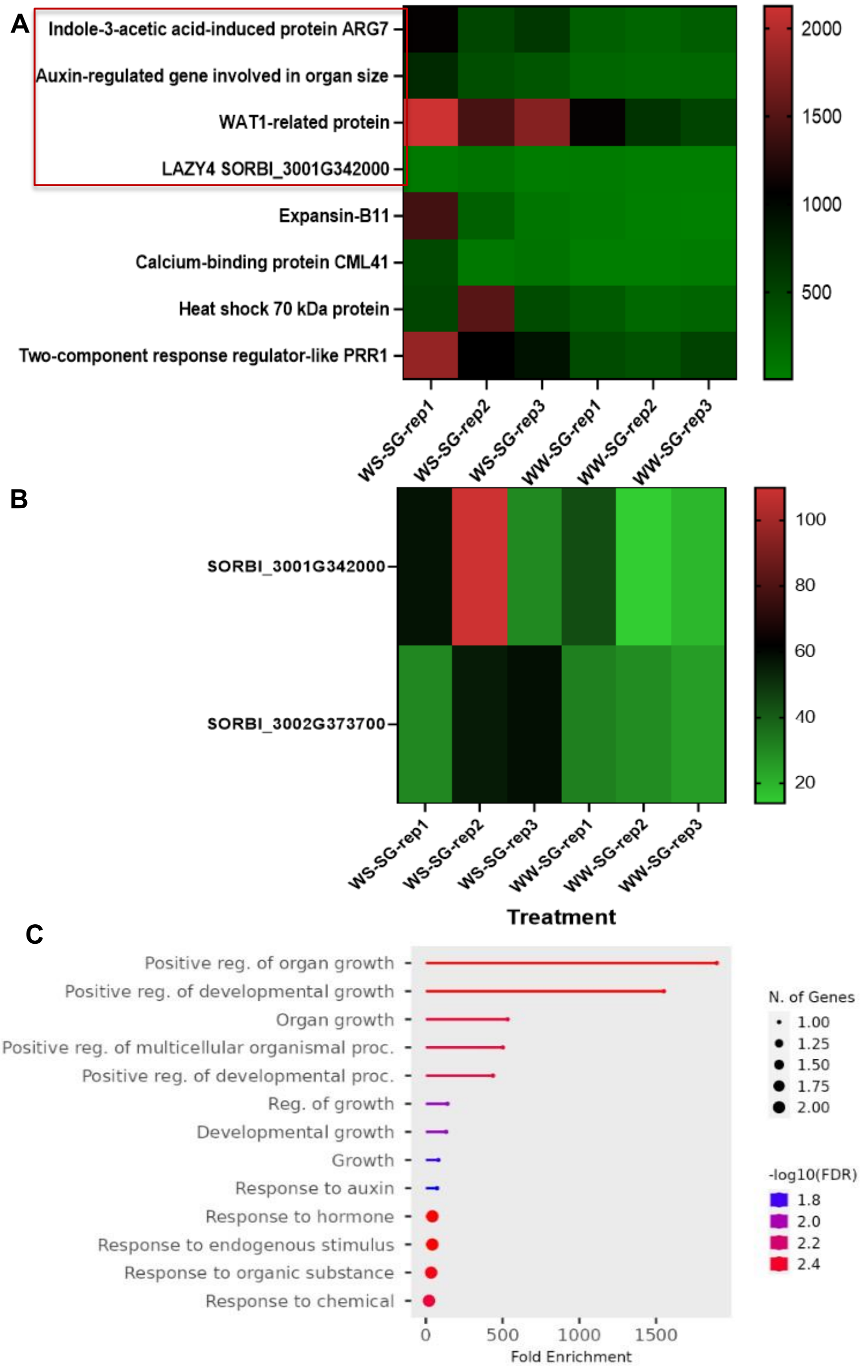

Figure 6-11: Drought-dependent regulation of potential auxin-responsive genes expressed in well-watered and water-stressed sorghum root tips

A) A heat map showing the comparison of expression levels in selected DEGs in well-watered (ww) and water-stressed sorghum root tips. The genes blocked in the orange mark are auxin-responsive genes. B) Heat map of *LAZY2/4* genes homologs in sorghum root tips. Each cell represents the expression levels of a gene for the respective intersecting row and column. The placement along the chosen colour spectrum visually indicates the magnitude according to the scale. C) Heat map of transcription factors in response to drought. D) KEGG pathway analysis created from marked auxin-responsive genes to identify the pathways enriched.

6.3 Discussion

Work undertaken in this chapter has demonstrated that root branching angle changes in response to drought stress. Prior work has shown that in sorghum, similar to other cereal crops, the growth angle of nodal roots is one of the major components of root system architecture (Singh et al., 2010, Uga et al., 2013). This trait is crucial in the content of drought tolerance mechanisms in crops and possibly for breeding selection. Understanding the molecular mechanisms underlying root angle control is essential for incorporating the root angle trait as a target into breeding programs.

6.3.1 Phenotypic architectural plasticity is useful in drought tolerance mechanisms in roots

Through screening and quantifying nodal root traits using high throughput phenotyping protocols in sorghum during drought stress, this study found that steeper roots are observed in drought-stressed plants compared to well-watered plants. Due to the dense nature of soil makes phenotyping of root systems in situ challenging (Atkinson et al., 2019). The majority of studies (Mace et al., 2012, Singh et al., 2012) looking at root angle changes in crop plants have been done in seedlings growing in the growth media on pouches or plates. This method of observation of root growth allows only a 2-D visualisation of the roots at early stages of development and poses a restriction specifically for sorghum, where nodal roots develop at a very late stage of development compared to other major cereals (Singh et al., 2012). This study lays a foundation for using a simple colander assay as a holistic 3-dimensional visualisation of root growth, especially at later stages of development in soil. This is crucial for investigating and predicting root physical changes due to environmental stimuli.

From this study the changes to root phenotype induced by water deprivation, stimulating drought adds to our understanding of root angle plasticity. This plasticity contributes substantially to their capacity to explore the soil for water and nutrient uptake. Lateral roots growing at more vertical angles, reaching deeper in the soil help the plant not only with the capture of nutrients like nitrogen but provides a mechanism for enhancing drought tolerance. Another significant aspect to consider is understanding the interaction between root-soil dynamics and cultivation strategies which remains underexplored in many contexts. Models and experimental data have begun to shed light on these dynamics, but there is still a need for more region-specific studies. The key questions that were beyond this research scope but critical for further exploration include: What impact does water stress have on root architecture in different soil textures, and how does that influence root growth angle? How to optimise cultivation practices and technologies to influence deeper rooting of crops in various soil conditions. Though complex, root plasticity, dependent on internal and external signals, makes it a promising trait for genetic engineering.

6.3.2 Transcriptional profiling in sorghum root tips in response to drought stress

Following the phenotypic changes observed in this study, we explore some potential internal regulators that determine the verticality of lateral roots during drought stress response. This was done through transcriptomic and gene expression analysis using RNA-Seq and quantitative PCR methods. Molecular components that contribute to tolerance or resistance in plants can be utilized as biomarkers or indicators to phenotype a plant's response to drought easily. The homologue of genes identified according to GO terms, coupled with uncharacterized genes discovered in sorghum exposed to drought stress, could be used as powerful genetic targets for the improvement of drought tolerance in sorghum and novel sources for other drought susceptible major cereals. These findings support recent observations in sorghum seedlings exposed to drought (ZHANG et al., 2019) and some genes being observed in sorghum exposed to drought in the field (Varoquaux et al., 2019). This indicates that is indicates that these DEGs might function in crosstalk responses to drought. Constitutive differences between the treatment responses could potentially be important in understanding differential responses to drought.

6.3.3 Novel developmental pathways involved in root angle regulation in sorghum in drought response

Through KEGG enrichment analysis, most implicated pathways are valine leucine. Diterpenoid biosynthesis, glycine serine and threonine metabolism, phenylpropanoid biosynthesis, metabolic pathways. Most of these pathways are for stress defence response mechanisms (Zhao et al., 2022, Naoumkina et al., 2010). Previous studies on developmental pathways have demonstrated that genotypes are expressed in different ways depending on environmental conditions thus, confirming plasticity (Sultan, 2017). Turning to developmental regulators this study found that

SORBI_3002G207700, A homologue of WAT1-related protein is auxin-regulated (Transmembrane transport pathways or ATP hydrolysis coupled transmembrane transport), SORBI_3001G34200 a homolog of LAZY2/4 genes, SORBI_3007G015600 (Auxin regulated gene, organ size) and SORBI_3006G252466 (indole-3-acetic acid-induced protein ARG7) were enriched in positive regulation of root organ growth and development. Due to the complexities of higher plants such as sorghum and challenges in transformation protocols for such crops, it could be suggested that some uncharacterised genes discovered here could be tested in the model plant through mutation of the gene to confirm its function. It is also suggested exploring the genetic diversity, natural variation and mutations within the *LAZY* locus in sorghum sequenced lines (both landraces and in the available mutant lines) will help to identify significant variation in the single nucleotide polymorphism (SNPs) and aid into understanding the functionality of these genes and engineer roots for resource optimisation.

6.3.4 The role of hormonal regulators of root angle in drought

Mechanisms that may regulate root plasticity during drought are still unclear owing to the interaction between hydrotropism and gravitropism. Our findings support auxin and *LAZY2/4* gene related mechanisms in regulating function in root angle changes in drought. Some studies indicate that species vary in their hydrotropic responsiveness, which can be masked by the dominant effects of gravitropism (Morris et al., 2017). The development of hydrotropic curvature is primarily observed in the middle of the elongation zone, so ablation of the entire meristem has little impact on it. Additionally, during drought, positive hydrotropism is observed, and the bending of roots toward patches of water is another factor that influences RSA and drought tolerance. ABA was shown to be involved in the hydrotropism response of roots. While gravitropism, primarily affects cell elongation in the root tip–proximal part of this zone (Karlova et al., 2021, Dinneny, 2019), which are the root tips that were used for this transcriptomic study. This study supports previous findings by Uga et al. (2013) showed that certain alleles of *DEEPER ROOTING 1* (*DRO1*) and its homologs increase the gravitropism of rice (*Oryza sativa* L.) roots, effectively benefitting drought stress avoidance. This present study lays a foundation for the first time in identifying and characterizing sorghum *LAZY2/4* and the uncharacterised genes presented in this study and its role in contributing to root structural changes during drought.

6.3.5 Concluding remarks

A greater understanding of the root architectural traits, such as growth angle will lead to an ability for optimisation to produce improved crop cultivars that are robust in limited conditions. Therefore, understanding root plasticity will be instrumental for the development of crops that are resilient in the face of abiotic stress. Through screening and quantifying nodal root traits using high-throughput genotyping protocols in sorghum in drought water-stress and control conditions. followed by high-throughput transcriptomic and gene expression analyses. and molecular mechanisms underlying sorghum RSA regulation

This study has shown that drought influences structural changes of root angle towards verticality in water stress. I identified drought-dependent regulation of potentially auxin-responsive genes including homologs of the LAZY2/4 family of genes known to regulate the gravitropic angle in several plant species, but not yet characterized in sorghum to date. To my knowledge the differences to drought stress from specific root tips at vegetative growth stage has not been covered in sorghum. The transcriptomic data from this study root tip tissues in drought stress further points towards a complex dynamic system which needs further understanding. Although the main focus of this study is on genes that are homologous to known biological functions, a significant number of DEGs are not fully characterized or annotated. This is due to the relatively poor level of annotation of the sorghum genome which is a caveat to this study. Trait engineering efforts in sorghum and other drought-prone species can benefit from the exciting potential of these novel genes and pathways.

Chapter 7

General Discussion

Defining mechanisms and pathways underpinning drought tolerance in climate-smart crops is key to expanding our understanding of knowledge gaps and complex research questions in plant development biology. The interdisciplinary work presented in this thesis provides novel information that integrates into a wider research effort to develop a comprehensive understanding of phenotypic variation, molecular regulation of root plasticity, and its effect on crop yield performance and nutritional quality in response to drought stress. From this, we can identify novel traits and molecular targets to rapidly develop high-yielding, nutritious, climate-resilient crops to keep pace with current and future climate projections, particularly in dryland regions that are the most vulnerable to climate change.

The main findings from Chapter 3 of this thesis are the following;

- Climate risk awareness influences farmers' decisions for production, and the specificity of varieties and responses vary with gender.
- Gendered differences in seed choices, variety ranking and phenotypic traits preferences.
- Gender disparities affect sorghum and millet seed accessibility and availability among male and female farmers.
- Variation of choices and knowledge on climate-smart cropping systems and technologies.
- Farmers' climate knowledge and gender influence on climate-smart cropping practices.
- Farmers and stakeholder perspectives on the differences in opportunities and challenges for sorghum and millet production among female and male farmers.

The main findings from Chapter 4 of this thesis are the following;

- Drought impact on nutrition qualities and response varies with genotype
- Genotypes adaptive mechanisms influence variation in yield and nutrition quality levels in response to drought

- Micronutrient elements are highly elevated in drought and are linked to biological processes in response mechanisms
- Transpiration correlates highly with biomass and yield but weakly correlates with nutrition qualities
- Grain phenotypes and nutrition qualities are linked to genotypic differences to flowering time

The main findings from Chapter 5 of this thesis are the following;

- Root phenotyping of architectural traits shows variation in various high throughput root traits screening method
- Lateral root growth angle in sorghum seedlings are proxy traits to later root development in sorghum
- A variety of polysaccharide exudates are released from sorghum seedling roots

The main findings from Chapter 6 of this thesis are the following;

- Sorghum lateral roots maintain GSA, as it returns from its original angle following gravity upon reoriented
- Drought-water stress influences structural changes of root angle towards verticality
- Phenotypic architectural plasticity is useful in drought tolerance mechanisms in roots
- Identification of orthologue of sorghum LAZY 2/4 genes as regulators of root growth angle
- Transcriptomic analysis unveils differentially expressed genes in sorghum root tips in varying water conditions
- Enrichment analysis of differentially expressed genes reveals novel developmental pathways in root tips under drought stress
- The role of hormonal regulators of root angle in drought stress

This Chapter 7 therefore aims to synthesize the main findings from the work presented in this thesis. The work demonstrates a participatory farmer approach to identifying traits linked to high performance in drought-prone areas of Malawi as a pathway to providing insights for crop improvement. The work has further elucidated the impact of drought on the grain nutrition of sorghum and identified the phenotypic traits and genetic diversity of sorghum genotypes that could be linked to the trade-offs in grain yield and nutritional quality performance in drought stress response. The finding from this work demonstrates that characterising root traits at the seedling level could be significant in speeding up the pre-selection of climate-resilient crops and for other breeding purposes. The findings from this present study advances our knowledge in understanding root angle regulation mechanisms and environmental plasticity for root optimisation. While, the integration of root exudates quantification in this study paves the way to a better understanding of root lubrication in dry soils (Galloway et al., 2020b) and how this may relate to variation in root system architecture across crop varieties for drought resilience.

7.1 Farmers' participation in crop trait pre-selection offers a promising pathway to achieving breeding targets

This work provides evidence that farmers' experience of climate and weather events shapes their decision on crops and varieties to grow. Although local knowledge is a relatively unexploited resource, it is useful for understanding the impacts of climate change on crop production and choices of varieties to fit their current local and projected future climate patterns (Labeyrie et al., 2021). As highlighted in Chapter 3, the farmer's primary selection of crop trait is optimal yield, seconded by maturation period and drought tolerance, while post-harvest traits are secondary needs. The vast natural genetic diversity within sorghum and millet landraces is important for selecting desirable traits and models for understanding climate-resilient crop species for rapid crop improvement.

Therefore, farmers' participatory selection of traits according to their knowledge and needs, while factoring in climate change projection models would guide the pre-selection of desired traits for crop improvement programs. Importantly, these findings significantly impact the intentionality of designing crop adaptation strategies and solutions that respond to the community's needs and provide climate resiliency. In this context, the findings in Chapter 3 are valuable to linking agronomic traits selected by farmers with physiology and molecular understanding of the trade-offs to identify robust, drought-tolerant, and elite varieties. For instance, farmers' insights on sorghum and millet plant architecture and, specifically, their preferences for dwarf varieties with vertical leaf angles can inform improved agricultural practices to fit into those existing farming systems. The findings unveil sorghum and millet forgotten local landraces and local varieties the communities prefer based on different attributes. Thus, these data offer researchers and breeders' new insights into the identification of novel diverse genetic sources that can advance innovation in sorghum and millet crop improvement.

7.2 Genotypic diversity in drought adaptation mechanisms influences grain performance

Natural genetic variation in sorghum crop varieties is vital for understanding traits and discovering new sources for crop improvement programs. Historically, the elite crops used in modern agriculture have been developed through domestication and selection by farmers and plant breeders over hundreds of years (Paux et al., 2022). The elite germplasm's genetic variability has decreased because of domestication, genetic drift, and modern selection. Notably, it is becoming more evident that the commonly utilised elite gene pool in many crops cannot provide the necessary genetic gain for yield and quality in the context of current global change (Haudry et al., 2007). Therefore, as described in Chapter 4, using a large data set of sorghum minicore collection offers an opportunity to screen for diversity and understand how drought adaptation mechanisms affect yield quantity and quality to identify elite climate-resilient crops for both the current and predicted future. Our findings in Chapter 4 supported by transcriptome data in Chapter 6 (Appendix on list of significant DEGs in well-watered and drought water stress) reveals that the micronutrient levels are linked to elevated biological processes in response to drought stress. It implies these elements' biosynthesis could be involved in drought response and defence mechanisms. The nutritional variation across genotype reflects a complex interplay of genetic, biochemical and environmental factors including soil types (Guwela et al., 2024) of which further studies are required to understand genetic regulation for these dynamic environmental responses and mechanisms.

7.3 Root phenotypes and exudates for drought-resilience

While genotyping capacity has increased rapidly, phenotyping has become the major limitation (Atkinson et al., 2019, Lynch, 2013), in research programs aiming at characterising the genetic diversity for crop response to climate changes and reduced input (Furbank and Tester, 2011). High-throughput phenotyping methods to reveal the underlying phenotypic and molecular mechanisms are crucial to advancing our knowledge gaps. The development of phenotypic tools presents opportunities to identify genetic and environmental patterns and mechanisms in a range of phenotypes, species, and environments (Des Marais et al., 2013). To date, classical non-destructive 2D techniques agar based plates or rhizotrons have been integral to our understanding of root development(Atkinson et al., 2015, Pound et al., 2013). However, it has been challenging to extrapolate root performance in 3D (Topp et al., 2013, Clark et al., 2011). Despite progress in visualising roots using advanced imaging software and the tomographic technique developed for medical use (Atkinson et al., 2019) its application is limited due to cost. This work, therefore, has demonstrated the use of a simple high-throughput colander method to quantify root system architecture in 3D. This method can extrapolate root traits such as root density, root angle, and rooting depth in soil.

The work has further shown a unique pattern of exudates produced in sorghum roots. It is argued that root exudates help reduce cell damage during root movement, specifically in dry soils. Additionally, root exudates alter soil pH, organic matter content, and microbial community in the rhizosphere, impacting soil health and fertility. Root exudates help in the formation of the rhizosheath (root-adhering soil) that could help plants better cope with water stress, nitrogen and phosphorus deficiencies, and soil acidity (Ndour et al., 2020). This present study provides a ground-breaking discovery on the sorghum root excretion of xylogalacturonan in abundance not found or limited in other major cereals according to previous work (Galloway et al., 2018, Galloway et al., 2020a, Willats et al., 2001, Willats et al., 2004). Considering this finding, it is suggested that follow-up work be conducted on the functionality of these exudates profiled in sorghum and how they are linked to drought stress and more generally, their involvement in soil health and fertility in the context of climate change. It will also be significant to screen root exudate variation in a large germplasm collection for possible selection of elite varieties.

7.4 Root growth angle, a potential breeding target for drought tolerance in sorghum

Root architecture collectively demonstrates a high degree of plasticity, which enables the root systems to adapt to changing environments. The findings in Chapter 6 demonstrate a notable increase in the ratio of deeper rooting in response to drought stress, causing a shift in root angle towards steeper architectures. This work confirms that root phenotypic changes confer critical characteristics contributing to a plant's ability to endure drought stress. It can be argued that insignificant differences exhibited in primary root growth within sorghum seedlings of different genotypes cannot be used to predict yield performance. Conversely, through this study in Chapter 5, the consistency of the shape of the root angle between the E36-1 and IS30400 cultivars with differing drought adaptability revealed interesting findings that could potentially utilised to predict later developmental performance.

Notably, previous research on wheat (Manschadi et al., 2006), maize (Lynch, 2013), rice (Uga et al., 2013) unveiled a compelling correlation association that links steeper root angles and deeper rooting depth to yield, particularly under salinity stress in rice. In this study, the findings from previous research are consistent with sorghum. Genotypes with steeper root angles exhibited a significant increase in yield when compared to genotypes with wider angles in drought stress conditions. This is evident from the findings in Chapters 4 and 5, of grain yield performance and RSA characterisation, genotype IS20727 was among the best high-yielding varieties in drought conditions and displayed drought adaptability. Conversely, IS30400, a genotype with a wider lateral root angle, exhibited a significant reduction in yield in drought conditions. Conversely, IS30400 is among the highest-yielding lines under normal water conditions compared to IS20727. This discovery emphatically underscores the pivotal role played by the nodal root angle in facilitating the access of resources to deeper soil layers. Therefore, this root trait can be potentially utilised to rapidly screen diverse germplasm for pre-breeding selection aiming for different water conditions.

7.5 Novel molecular developmental mechanisms and pathways in root angle regulation underlying drought stress response

Lateral (nodal) root growth angle has recently received increased attention as an important trait for breeding drought tolerance in sorghum (Demelash et al., 2024, Elias et al., 2024, Demelash et al., 2021). Significant progress is being made in understanding auxin's role in root development (Roychoudhry et al., 2023, Roychoudhry and Kepinski, 2022, Kepinski, 2007). However, recent literature acknowledges that the genetic regulation underpinning growth angle regulation remains poorly understood (Guo et al., 2024). The study in Chapter 6 contributes to addressing this gap in the literature by uncovering structural and molecular mechanisms underlying root growth angle regulation in response to drought stress. The molecular regulation of root growth angle is a multi-faceted process that involves the perception of gravity, signal transduction through calcium signalling and auxin redistribution (Siqueira et al., 2022, Roychoudhry et al., 2023). The present study involving sorghum transcriptomic analysis in root tips under drought stress presents identifies novel genes that are highly expressed in drought and are involved in root growth angle regulation. The growth and developmental pathways confirmed by the KEGG analysis suggest a significant contribution to root developmental processes during drought, which still need further functional analysis. For instance, this research identifies some auxin-regulated genes as key regulators of changes to the root phenotype in drought including *SORBI_3006G252466 (IAA-ARG7)*, SORBI_3007G015600 (Arabidopsis, *LBD16), SORBI_3002G207700 (WAT1*-related protein), and homologs of *LAZY2/4* candidate genes (*SORBI_3001G342000*) and *LAZY4 SORBI_3001G373700*. This discovery confirms the role of SAUR and AUX/IAA genes as auxin-responsive genes that control various aspects of cell elongation and growth in drought. However, these candidate genes have not yet been characterised in sorghum, and the findings from this study would help annotate them to characterise their function further and contribute to precision breeding. A further pathways analysis unveils that drought induces a significant increase in metabolic pathways and the biosynthesis of secondary metabolites in the root tips of sorghum. However, findings from these mechanisms raise an equally interesting question of how drought defence metabolic pathways and developmental pathways interact. The interplay between diverse signalling pathways, feedback loops, and hormonal crosstalk underscores how complex and adaptable root development can be in response to environmental cues. By comprehending these molecular mechanisms, we can gain valuable insights into plant biology and develop possible strategies for optimising RSA and improving crops' resilience to stress.

7.6 Optimising root system architecture to improve drought tolerance for sustainable crop production

Optimising root system architecture (RSA) to improve drought tolerance involves modifying root traits to enhance water uptake and efficiency under water-limited conditions. This includes both, genetic and management strategies aimed at developing root systems that are more efficient in accessing deep soil moisture and using available water more effectively (Dodd et al., 2011). Optimising RSA to improve above and below-ground resource capture and downstream grain yield and nutrient content is an attractive strategy for the biotechnological improvement of crop varieties. The work has provided an understanding of root traits desirable for drought tolerance using simple assays and high-throughput phenotyping methods to screen for desirable root traits leading to the identification of tools to select for genotypes with optimal RSA for drought conditions. This research sheds new light on integrating phenotypic traditional breeding selection and genomics to identify and select complex traits associated with RSA and drought tolerance**.** For most crops, breeding for deeper rooting depth is a primary target for improving drought tolerance (Lynch 2013, 2019). Our findings on drought-dependent regulation of sorghum root angle could potentially be pre-selection trait for steeper rooting to promote deeper root growth and improve water uptake in dry-regions.

The molecular and genetic data from this study could support rapid precision breeding by targeting candidate genes discovered here and genetic engineering of root steeper deeper rooting angles. The candidate genes discovered here can inform breeders and be utilised as CRISPR/Cas9 editing targets to control root development and response to drought. From the pathways identified, manipulating auxin transport and signalling pathways can modify root architecture to improve drought tolerance. Further, engineering roots to release exudates that can alter soil properties and microbial communities, influencing water availability and uptake significantly improves drought tolerance.

Based on our findings, a hypothetical model is proposed to summarise traits for selecting and developing a sorghum variety with optimised root angle, greater rooting at depth and root density for balanced water uptake (Figure 7-1). This proposed ideotype would include i) roots that will have enhanced nutrient uptake efficiency and effective nutrient partitioning to improve grain nutrient content while ii) maximising total biomass production while ensuring sufficient allocation to grains. It is hypothesized that the shallow rooting variety (e.g., IS30400) in this model is drought-sensitive, as demonstrated by the findings in this thesis. However, it was shown to be a high-yielding variety under well-watered conditions. While a deep-rooted variety (e.g., E36-1) in this model represents high-yielding drought-tolerant lines. Shallow-rooted lines could be well suited for optimal water availability, while deeper-rooted lines are well suited for dry environments.

The model demonstrates the integration of multiple traits through careful selection and breeding strategies to develop crop varieties that are drought-tolerant, high-yielding, and nutrient-rich. As for the proposed ideotypes selected for maize targeting nutrient uptake by Lynch (2013), Selecting root phenotypes will be better with a clear environmental target. Recognising, that breeding in a limited resource environment is more intricate because of multiple stresses. The selection of drought-resilient variety proposed here is a simple model of a selection system since one resource is targeted. Though complex, managing the trade-offs between traits is crucial to achieving crop improvement goals. The limitation remains that integrating genotype, environment including soil, phenotypic data and management into meaningful knowledge reflecting the plant stress response profile is challenging. It is mostly due to the difficulty of comparing dynamic changes in the environment to phenotypic plasticity in a comparative manner for many plants simultaneously (Harfouche et al., 2019). This thesis, therefore, contributes towards advancing our understanding of root traits that could be potentially prioritised for rapid crop breeding selection.

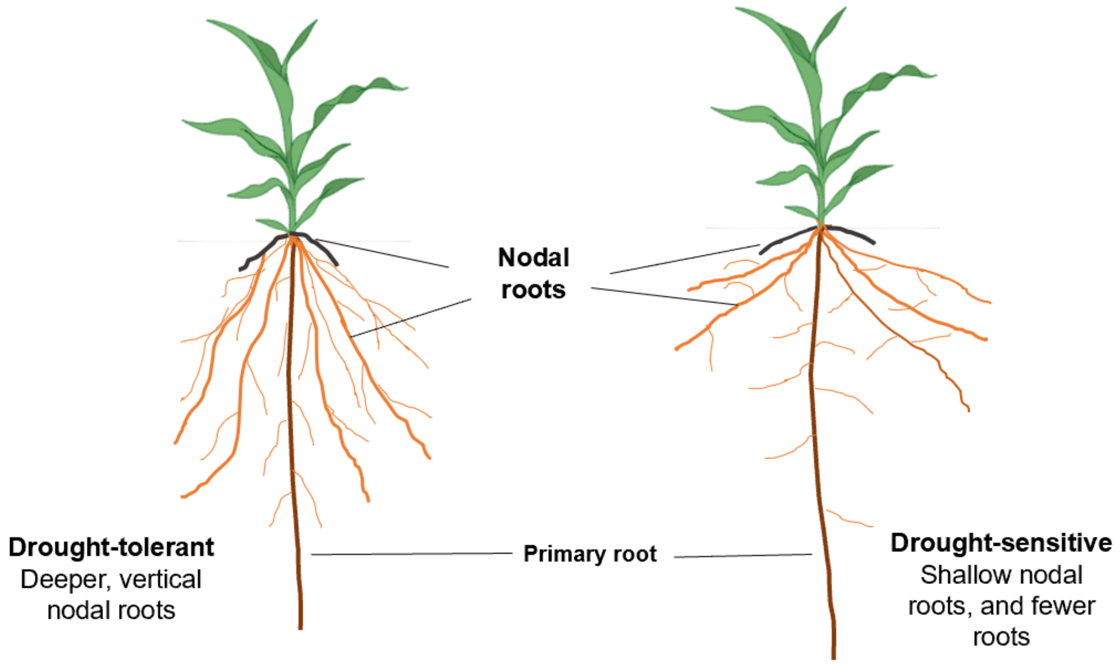

Figure 7-1: A schematic representation of the hypothetical model to identify a drought-resilient variety

The proposed root ideotype for post-flowering drought tolerance is displayed by sorghum genotypes with deeper, steeper rooting, such as E36-1, S35, and IS20727. While the post-flowering drought-sensitive genotype is displayed by a genotype with a shallow rooting system, as demonstrated by genotype IS30400.

7.7 The role of AI and predictive modelling approaches in the selection of drought tolerant traits

The integration of genomics and phenomics studies will accelerate the development of climate-resilient crops, but these multi-omics technologies are generating large, heterogeneous, and complex datasets that are not currently able to be analysed. (Harfouche et al., 2019). AI and predictive modeling are reshaping the selection of drought-tolerant crop traits by making it possible to make efficient, data-driven decisions (Njah et al., 2019, Harfouche et al., 2019). The use of these technologies speeds up genomic analysis, uncovering genes and alleles associated with drought resilience, while integrating multidimensional data, such as soil moisture, plant physiology, and environmental factors, to predict trait performance. AI-enhanced crop models and machine learning simulate interactions between plants and their environment, which guides the selection of traits tailored to the specific environment (Hegde et al., 2024). Additionally, machine learning prioritizes key traits, such as deep rooting or water use efficiency, optimising breeding strategies and resource allocation. AI also improves field trials by designing optimal setups and analysing data to isolate environmental effects, enhancing trial precision. Some of the limitations of AI include data accessibility, model interpretability, and scaling. In relation to plant breeding, AI is conceptually designed not only to predict breeding values for complex traits across environments and time scales, but also to learn and improve iteratively. The intelligence and efficiency of mining data that accurately represents the underlying systems biology and environment is necessary, along with human interaction on both the input and output ends (Harfouche et al., 2019). Despite these challenges, AI-driven approaches offer breeders novel insights into trait-environment interactions, expediting the development of resilient, high-performing crops. To accelerate breeding outcomes, the continued development and optimisation of AI-driven methodologies and interdisciplinarity approach would be essential to keep pace with the changing climate and ensure global food security and safety.

7.8 Implication and impact of interdisciplinarity research approach to identify drought tolerance traits

This thesis utilises multiple disciplines to create meaningful solutions to our approach to agri-food research, designed to address the global agricultural challenges. This is evidenced by the relevance of this holistic, systematic research approach in the context of the scientific gaps identified in the background. Though it is a challenging concept to combine different methodologies of multiple disciplines (Horton et al., 2017), the holistic approach in this work has been unique, and a wide variety of research approaches were used to ensure a relationship between them. We conducted a scoping study to explore how gender and climate risk awareness influence sorghum and pearl millet farmers' crop varietal trait choices and cropping practices in drought-prone areas of Malawi. Farmers' choices reflected their long knowledge of drought effects and provided insights on drought-tolerant crop mechanisms, including early maturation (a drought avoidance mechanism) and drought-resilient. The subsequent laboratory work benefitted from these insights and practical knowledge, which led to an informative research-lead focus that resulted in finding tools to rapidly screen for the traits proxies and trade-offs. For instance, a large dataset of drought effects on sorghum grain was used to explore the trade-offs between those agronomic traits farmers perceived as ideal for grain yield and nutritional qualities under drought stress.

Much about the effect of climate on crop qualities is still unclear. This thesis has advanced our scientific knowledge by linking drought effects to nutrition qualities and elucidating how drought-tolerant crops maintain grain nutrition qualities as climate patterns directly impact human health and nutrition whilst taking advantage of our previous understanding of root system architecture as a part of the plant that has been neglected due to challenges in screening methods. The findings raised an important question on how drought from the roots influences important trait grain yield that farmers indicate as a primary need in breeding and nutrition qualities, which is perceived as a secondary need to the farmers but very critical in solving malnutrition challenges, also referred to as 'hidden hunger' in vulnerable regions. The screening of the RSA traits informed us of the need for breeders to pay attention to below-ground root system architectural traits that initially perceive drought. The selection of nutritious varieties, that are resilient to environmental stresses helps promote sustainable agricultural practices, thereby supporting food security sustainably.

Finally, the integration of work presented in this thesis has the potential to advance scientific knowledge, leading to more robust discoveries and applicable research outcomes.

7.9 Conclusion

The work integrated high-throughput phenotyping protocols, genomics, transcriptomic analysis, and bioinformatics to screen natural genetic variation in root architecture to optimise growth and improve yield and micronutrient content under drought. The work presented in this thesis advances our understanding on mechanisms and pathways underlying drought tolerance to identify climate-smart crops**.** The knowledge obtained from this work has the potential for developing rapid high-throughput phenotyping protocols for rapid screening of existing wider germplasm collections with desirable traits. Understanding these phenomena will help establish a practical framework for the targeted manipulation of plant architecture to improve above and below-ground resource capture and downstream grain yield and nutrient content. It will further be crucial in the breeding of high-yielding crop cultivars that are robust to climate change and have optimised growth in low-input and limited conditions.

Chapter 8 Appendix

8.1 Summary Table of Yield by Genotype in drought-stress

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Caudatum	IS2864	10.9	2.2
Durra-caudatum	IS28849	10.1	3.2
Caudatum	IS30460	9.7	2.4
Caudatum-bicolor	IS12706	9.1	2.9
Bicolor	IS1233	9.0	1.5
Guinea-bicolor	IS1219	8.5	2.2
Caudatum-bicolor	IS24462	8.5	3.8
Bicolor	IS20743	8.5	2.7
Guinea-caudatum	IS19153	8.3	NA
Caudatum	IS17941	8.3	4.3
Caudatum	IS12965	8.2	2.8
Guinea	IS7250	8.2	NA
Guinea-caudatum	IS30572	7.7	2.1
Bicolor	IS20727	7.6	2.3
Caudatum-bicolor	IS30450	7.5	3.7
Durra-caudatum	IS20956	7.5	1.6
Caudatum	IS24348	7.5	3.1
Kafir-bicolor	IS1212	7.4	2.6
Caudatum-bicolor	IS30536	7.3	0.3
Caudatum	IS15170	7.3	3.0
Durra-bicolor	IS27786	7.2	1.6
Caudatum	IS14090	7.1	1.7
Caudatum-bicolor	IS8777	7.1	2.2
Kafir-durra	IS8774	7.1	2.2
Guinea-caudatum	IS20713	6.9	3.1
Bicolor	IS20740	6.9	3.0
Caudatum	IS2379	6.8	3.2
Kafir-caudatum	IS29568	6.8	2.0
Caudatum-bicolor	IS30507	6.7	0.9
Durra-caudatum	IS28313	6.7	2.5
Guinea-caudatum	IS10969	6.7	2.0
Caudatum	IS10302	6.6	4.3
Caudatum-bicolor	IS20298	6.6	2.3
Caudatum	IS13971	6.6	1.4
Kafir	IS19445	6.4	1.4
Caudatum-bicolor	IS30383	6.4	1.5
Guinea	IS16382	6.3	0.9
Guinea	IS25089	6.3	2.8
Bicolor	IS20816	6.3	2.9
Kafir	IS30231	6.2	0.3
Guinea-caudatum	IS29565	6.2	1.4
Durra-caudatum	IS12447	6.0	1.2
Caudatum-bicolor	IS2872	6.0	4.4
Caudatum-bicolor	IS24453	5.9	1.1
Caudatum	IS20697	5.9	1.7
Durra-bicolor	IS4060	5.9	2.0
Guinea	IS21512	5.9	1.8
Caudatum	IS14861	5.9	2.3
Bicolor	IS20195	5.9	2.9
Guinea	IS26484	5.8	2.1
Caudatum-bicolor	IS14010	5.8	1.6

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Durra-caudatum	IS28614	5.7	1.4
Guinea-caudatum	IS26222	5.7	1.1
Caudatum	IS29914	5.7	2.2
Caudatum-bicolor	IS30466	5.6	2.7
Bicolor	IS608	5.5	1.2
Guinea-bicolor	IS7957	5.5	4.7
Kafir	IS29392	5.5	1.8
Kafir	IS26737	5.5	1.2
Caudatum	IS31651	5.5	NA
Kafir	IS26749	5.5	1.5
Guinea-caudatum	IS29950	5.4	1.1
Kafir	IS24492	5.4	1.2
Guinea-caudatum	IS23521	5.3	1.3
Durra-bicolor	IS11919	5.3	0.3
Caudatum	IS23992	5.3	1.8
Caudatum-bicolor	IS30443	5.2	1.5
Caudatum-bicolor	IS30508	5.2	0.6
Kafir-caudatum	IS29441	5.1	6.0
Caudatum	IS22986	5.1	2.8
Guinea	IS15931	5.0	3.7
Guinea	IS27557	5.0	1.9
Caudatum	IS9745	4.9	1.8
Guinea	IS24218	4.9	NA
Guinea-caudatum	IS29187	4.9	4.4
Durra	IS22720	4.9	1.4
Kafir	IS22239	4.9	4.7
Caudatum	IS14779	4.9	0.4
Bicolor	IS603	4.8	1.2
Guinea-durra	IS29733	4.8	1.6
Durra-bicolor	IS25249	4.7	NA
Caudatum-bicolor	IS29326	4.7	2.5
Caudatum-bicolor	IS30417	4.7	1.0
Caudatum-bicolor	IS30451	4.7	1.4
Caudatum-bicolor	IS2902	4.7	3.6
Guinea-caudatum	IS15945	4.7	2.1
Caudatum	IS7305	4.6	0.2
Caudatum	IS26694	4.6	2.2
Guinea	IS32349	4.6	0.5
Guinea-caudatum	IS28449	4.5	2.0
Caudatum	IS10757	4.5	1.1
Caudatum	IS4092	4.5	1.4
Guinea-caudatum	IS20679	4.4	1.4
Durra	IS3971	4.4	1.7
Bicolor	IS602	4.3	1.6
Durra	IS4360	4.3	4.3
Guinea	IS21645	4.3	2.4
Guinea	IS25910	4.3	1.5
Kafir-durra	IS29714	4.2	1.7
Bicolor	IS31681	4.2	1.6
Kafir-bicolor	IS29654	4.2	1.6
Durra-caudatum	IS32245	4.2	2.7
Guinea-caudatum	IS29269	4.1	0.5
Bicolor	IS30562	4.1	1.4
Guinea	IS16528	4.0	2.2
Caudatum	IS29335	4.0	0.7
Bicolor	IS8012	4.0	2.5

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Kafir-caudatum	IS29241	3.9	2.2
Guinea-caudatum	IS29468	3.9	1.7
Kafir	IS29519	3.9	2.5
Kafir	IS29582	3.9	3.3
Kafir	IS29358	3.9	1.5
Kafir	IS2389	3.8	1.6
Guinea-caudatum	IS15478	3.8	1.8
Caudatum-bicolor	IS16151	3.8	1.4
Kafir	IS3158	3.8	0.5
Durra-caudatum	IS15744	3.8	0.9
Caudatum-bicolor	IS12735	3.7	1.1
Bicolor	IS21863	3.7	1.2
Kafir	IS29606	3.7	1.8
Durra	IS19859	3.7	3.3
Kafir-caudatum	IS13893	3.7	0.8
Guinea-caudatum	IS10867	3.6	0.7
Durra	IS32787	3.6	3.5
Caudatum	IS19389	3.6	1.5
Caudatum	IS9113	3.6	1.6
Guinea	IS7310	3.6	0.2
Caudatum	IS9108	3.6	1.1
Caudatum	IS11473	3.6	2.9
Durra-caudatum	IS29627	3.5	0.8
Kafir	IS22294	3.5	0.5
Durra	IS1041	3.5	1.6
Bicolor	IS22616	3.5	0.6
Guinea-caudatum	IS23590	3.5	1.6
Guinea-caudatum	IS23579	3.4	0.5
Bicolor	IS12697	3.4	1.4
Guinea-caudatum	IS28451	3.4	1.6
Guinea-caudatum	IS19262	3.4	2.2
Guinea-kafir	IS473	3.4	2.2
Durra-caudatum	IS28747	3.4	1.4
Kafir	IS24463	3.3	2.1
Kafir	IS29233	3.3	2.1
Durra	IS25732	3.3	1.3
Caudatum	IS2382	3.3	1.4
Durra	IS5386	3.2	1.5
Guinea	IS7679	3.2	1.3
Durra	IS25836	3.1	2.5
Durra	IS12883	3.1	1.0
Guinea	IS30838	3.1	0.2
Durra	IS27034	3.0	NA
Durra	IS22799	3.0	0.9
Durra-caudatum	IS7131	3.0	NA
Durra-caudatum	IS30092	3.0	2.0
Caudatum-bicolor	IS26701	3.0	0.5
Kafir	IS29239	3.0	0.4
Durra	IS5919	3.0	1.5
Kafir-durra	IS14290	2.9	1.1
Caudatum	IS31043	2.8	0.6
Kafir	IS12945	2.8	0.9
Caudatum-bicolor	IS30533	2.8	2.4
Kafir-caudatum	IS13919	2.8	1.5
Guinea	IS5295	2.8	1.2
Kafir	IS19676	2.7	1.0

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Durra	IS4613	2.7	1.0
Guinea-caudatum	IS5301	2.7	0.5
Durra-caudatum	IS28141	2.7	2.5
Durra-caudatum	IS28389	2.6	2.1
Kafir	IS29689	2.4	1.6
Guinea-caudatum	IS8916	2.4	2.7
Caudatum	IS22609	2.3	1.8
Guinea	IS24139	2.2	1.3
Durra	IS4581	2.2	NA
Guinea	IS26046	2.1	2.3
Durra	IS8348	2.1	1.5
Guinea	IS23644	2.1	1.7
Guinea	IS25989	2.0	1.2
Durra	IS31706	2.0	0.1
Guinea	IS7987	2.0	NA
Kafir	IS2397	1.9	3.3
Kafir-durra	IS13782	1.9	1.4
Caudatum-bicolor	IS26617	1.9	2.1
Guinea-caudatum	IS29772	1.8	0.3
Caudatum	IS12302	1.8	1.4
Kafir	IS12937	1.8	0.8
Guinea	IS32439	1.8	1.0
Durra-caudatum	IS31714	1.7	0.4
Guinea-kafir	IS19450	1.7	2.2
Caudatum	IS33353	1.7	0.9
Durra	IS6351	1.6	NA
Bicolor	IS24503	1.4	0.6
Guinea-durra	IS4372	1.4	1.4
Caudatum-bicolor	IS30400	1.3	0.8
Caudatum-bicolor	IS995	1.3	NA
Guinea	IS4951	1.2	0.9
Guinea	IS24175	1.2	NA
Guinea	IS19975	1.2	1.0
Guinea-caudatum	IS23586	1.0	NA
Durra	IS17980	0.9	NA
Durra	IS4515	0.9	NA
Caudatum-bicolor	IS2426	0.8	1.4
Durra-caudatum	IS29314	0.8	0.4
Bicolor	IS2413	0.7	NA
Bicolor	IS3121	0.6	0.8
Durra	IS4631	0.6	NA
Durra	IS33090	0.5	NA
Bicolor	IS12804	NA	NA
Bicolor	IS24939	NA	NA
Caudatum	IS15466	NA	NA
Caudatum	IS20632	NA	NA
Caudatum	IS21083	NA	NA
Caudatum	IS23514	NA	NA
Caudatum	IS25548	NA	NA
Caudatum	IS31557	NA	NA
Caudatum	IS9177	NA	NA
Caudatum-bicolor	IS13294	NA	NA
Caudatum-bicolor	IS13549	NA	NA
Caudatum-bicolor	IS23216	NA	NA
Caudatum-bicolor	IS27887	NA	NA
Durra	IS1004	NA	NA

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Durra	IS11026	NA	NA
Durra	IS23891	NA	NA
Durra	IS4698	NA	NA
Durra	IS5094	NA	NA
Durra	IS5667	NA	NA
Durra	IS6354	NA	NA
Durra	IS6421	NA	NA
Durra-bicolor	IS11619	NA	NA
Durra-bicolor	IS18039	NA	NA
Durra-bicolor	IS25301	NA	NA
Durra-caudatum	IS20625	NA	NA
Durra-caudatum	IS29091	NA	NA
Durra-caudatum	IS29100	NA	NA
Durra-caudatum	IS30079	NA	NA
Guinea	IS23684	NA	NA
Guinea	IS26025	NA	NA
Guinea	IS27697	NA	NA
Guinea	IS33023	NA	NA
Guinea-caudatum	IS24953	NA	NA
Guinea-caudatum	IS31186	NA	NA
Guinea-caudatum	IS31446	NA	NA
Guinea-kafir	IS29304	NA	NA
Kafir-caudatum	IS27912	NA	NA

8.2 Summary Table of total grain weight by Genotype in well-watered

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Durra	IS6421	91.0	14.6
Durra	IS23891	87.4	33.3
Durra	IS5667	82.5	1.8
Caudatum	IS9108	81.6	44.4
Durra	IS1004	77.8	7.8
Durra	IS4581	76.6	12.3
Guinea-caudatum	IS23586	71.7	25.0
Durra	IS31706	71.3	17.9
Caudatum	IS31557	69.2	17.5
Durra	IS33090	68.0	28.4
Durra	IS12883	66.0	14.5
Durra	IS4631	65.9	7.0
Durra	IS5919	65.5	29.1
Caudatum	IS23514	65.4	7.9
Durra	IS6354	64.0	7.5
Caudatum	IS20632	63.4	18.9
Durra	IS27034	63.0	15.3
Caudatum	IS25548	62.2	21.7
Durra	IS6351	62.1	22.7
Guinea-caudatum	IS28451	62.0	34.7
Guinea	IS33023	61.9	4.8
Guinea-caudatum	IS31186	61.3	21.3
Durra	IS5094	60.6	11.6
Caudatum	IS9177	59.5	28.9
Kafir-durra	IS14290	59.3	20.8
Durra-caudatum	IS31714	59.3	26.3
Durra-bicolor	IS18039	59.3	34.1
Durra	IS4515	59.2	18.4
Guinea-caudatum	IS23579	59.2	23.8
Durra	IS22720	58.8	7.6
Durra	IS25732	58.3	20.8
Guinea	IS26046	57.5	18.3
Durra	IS11026	56.2	8.0
Caudatum	IS21083	55.7	19.4
Guinea	IS7987	55.4	18.1
Bicolor	IS24939	54.8	12.2
Durra	IS4698	54.0	16.5
Durra-caudatum	IS20625	53.8	22.2
Guinea-caudatum	IS8916	53.8	5.3
Guinea-bicolor	IS7957	53.1	18.5
Guinea	IS4951	52.8	14.0
Bicolor	IS22616	52.1	15.0
Durra	IS8348	51.0	8.5
Guinea	IS5295	50.9	15.9
Guinea	IS24218	50.4	21.1
Durra	IS3971	50.4	19.6
Caudatum-bicolor	IS13294	50.0	14.4
Durra-caudatum	IS28141	49.8	31.7
Guinea	IS16528	49.5	13.3
Guinea-durra	IS4372	49.4	10.0
Bicolor	IS12804	49.0	12.0
Guinea	IS32349	48.9	26.1

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Durra-caudatum	IS20956	48.3	26.1
Durra-bicolor	IS25249	47.6	8.7
Durra-bicolor	IS25301	47.5	27.6
Guinea	IS21512	47.3	17.7
Caudatum	IS9113	46.6	10.1
Durra-caudatum	IS7131	46.3	13.5
Caudatum-bicolor	IS13549	45.8	21.5
Guinea-caudatum	IS5301	45.8	15.6
Guinea-caudatum	IS23590	45.7	32.4
Caudatum	IS7305	45.3	15.4
Guinea-caudatum	IS23521	45.3	18.0
Guinea	IS30838	45.2	32.0
Guinea	IS26484	45.1	12.0
Caudatum	IS14779	45.1	17.4
Durra-caudatum	IS29091	43.6	25.2
Durra	IS22799	43.4	31.2
Caudatum-bicolor	IS27887	43.4	21.8
Guinea	IS7679	43.3	22.1
Kafir	IS26737	42.4	6.6
Durra-caudatum	IS15744	42.3	13.2
Caudatum	IS11473	42.2	11.1
Caudatum	IS15466	41.9	27.8
Durra-bicolor	IS27786	41.7	21.8
Kafir-caudatum	IS13893	41.7	16.2
Durra-caudatum	IS28747	41.7	21.0
Guinea-caudatum	IS10969	41.6	17.2
Durra-bicolor	IS11619	41.5	20.1
Caudatum	IS10757	41.4	12.5
Bicolor	IS20743	40.4	17.0
Guinea-durra	IS29733	39.6	7.9
Caudatum	IS2864	39.5	14.0
Caudatum	IS33353	39.5	14.2
Guinea	IS23644	39.4	14.5
Guinea-caudatum	IS29772	39.3	8.7
Caudatum	IS15170	39.3	12.9
Caudatum-bicolor	IS2902	38.8	12.5
Caudatum-bicolor	IS30533	38.7	4.1
Guinea	IS7310	38.6	24.6
Caudatum	IS24348	38.5	16.9
Guinea	IS7250	38.5	20.9
Bicolor	IS31681	38.4	13.7
Durra	IS1041	38.2	14.9
Guinea	IS24175	37.7	13.5
Kafir	IS12945	37.4	10.2
Guinea	IS32439	37.3	6.6
Durra-caudatum	IS29314	37.2	24.5
Durra	IS4360	36.7	20.5
Durra-caudatum	IS28614	36.7	17.6
Kafir	IS30231	36.5	6.9
Guinea	IS19975	36.4	12.1
Caudatum	IS10302	36.3	14.8
Guinea-kafir	IS29304	36.3	3.1
Bicolor	IS20195	36.2	11.4
Durra-caudatum	IS29100	36.2	20.0
Bicolor	IS12697	36.0	22.6
Guinea	IS15931	35.9	13.4

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Bicolor	IS21863	35.7	6.7
Guinea-caudatum	IS31446	35.4	23.6
Bicolor	IS2413	35.2	14.4
Durra	IS32787	35.0	21.3
Caudatum	IS9745	34.9	11.7
Caudatum	IS2379	34.8	10.2
Kafir	IS29689	34.8	9.7
Durra-bicolor	IS4060	34.8	9.5
Guinea-kafir	IS473	34.8	10.7
Caudatum	IS2382	34.3	6.0
Guinea-caudatum	IS20713	34.3	16.0
Caudatum-bicolor	IS26617	34.3	8.8
Caudatum	IS4092	33.9	13.9
Durra-caudatum	IS28849	33.8	14.0
Caudatum	IS22609	33.7	22.0
Durra	IS17980	33.1	5.7
Caudatum	IS26694	32.7	15.2
Caudatum	IS22986	32.5	10.2
Guinea-caudatum	IS24953	32.4	10.1
Durra	IS4613	32.0	5.1
Caudatum	IS19389	31.9	15.2
Kafir-bicolor	IS29654	31.9	18.2
Durra-caudatum	IS30079	31.8	10.1
Guinea-caudatum	IS10867	31.7	6.5
Guinea-caudatum	IS15945	31.7	19.6
Caudatum-bicolor	IS30508	31.7	16.1
Durra-caudatum	IS32245	31.7	17.2
Guinea-caudatum	IS19153	31.5	7.3
Guinea	IS25910	31.5	22.8
Caudatum	IS31043	31.2	8.7
Caudatum	IS29914	31.2	8.3
Guinea	IS16382	31.1	10.9
Kafir	IS12937	30.9	15.0
Caudatum-bicolor	IS26701	30.6	10.6
Caudatum	IS20697	30.5	23.7
Caudatum-bicolor	IS2426	30.4	15.5
Guinea-caudatum	IS29565	30.3	6.6
Durra-caudatum	IS29627	30.1	5.6
Guinea	IS27697	30.0	8.7
Guinea	IS25089	29.9	15.0
Guinea-caudatum	IS28449	29.9	14.9
Guinea-caudatum	IS20679	29.7	8.1
Durra	IS19859	29.5	7.3
Kafir	IS22294	29.5	9.4
Caudatum-bicolor	IS30383	29.2	6.7
Guinea-caudatum	IS30572	29.1	15.4
Caudatum-bicolor	IS16151	29.0	13.7
Kafir	IS29239	28.4	10.1
Guinea-caudatum	IS29468	28.1	5.9
Guinea	IS25989	28.1	14.4
Caudatum	IS13971	28.0	9.9
Kafir	IS29358	27.9	6.2
Kafir-durra	IS29714	27.8	29.6
Guinea	IS24139	27.7	13.2
Caudatum-bicolor	IS29326	27.6	10.6
Guinea	IS21645	27.3	6.5

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Guinea-caudatum	IS29187	27.1	8.6
Bicolor	IS30562	26.9	0.7
Caudatum-bicolor	IS30451	26.7	8.2
Caudatum	IS12965	26.5	10.6
Kafir	IS29606	26.5	10.1
Durra	IS5386	26.2	8.6
Caudatum	IS30460	26.1	5.6
Guinea	IS23684	26.0	22.9
Caudatum	IS12302	25.9	13.2
Caudatum-bicolor	IS995	25.6	7.5
Durra-caudatum	IS30092	25.5	6.4
Caudatum-bicolor	IS30507	25.5	36.1
Caudatum-bicolor	IS30443	25.3	4.2
Caudatum	IS14090	25.1	26.9
Kafir-caudatum	IS29568	25.1	5.5
Caudatum	IS17941	24.9	5.9
Kafir-caudatum	IS29241	24.8	5.6
Caudatum-bicolor	IS30400	24.6	10.7
Bicolor	IS3121	24.5	25.8
Caudatum-bicolor	IS24453	24.3	9.8
Caudatum-bicolor	IS12735	24.3	19.9
Guinea-caudatum	IS29950	24.3	6.0
Kafir	IS29519	24.2	7.2
Guinea	IS26025	24.2	29.5
Caudatum-bicolor	IS2872	23.9	23.9
Durra-caudatum	IS12447	23.9	4.7
Guinea-caudatum	IS29269	23.6	8.8
Durra-bicolor	IS11919	23.5	5.4
Caudatum-bicolor	IS14010	23.4	6.1
Kafir-durra	IS8774	23.2	7.5
Caudatum	IS29335	22.5	7.2
Kafir	IS19676	22.0	4.1
Guinea-caudatum	IS15478	21.9	18.4
Caudatum-bicolor	IS30536	21.4	7.8
Bicolor	IS8012	21.3	8.2
Kafir	IS2389	21.1	6.4
Kafir-bicolor	IS1212	21.0	3.1
Durra	IS25836	21.0	11.3
Kafir	IS24463	21.0	13.6
Caudatum-bicolor	IS30466	21.0	2.3
Durra-caudatum	IS28313	20.8	12.0
Kafir-caudatum	IS13919	20.8	18.2
Caudatum	IS31651	20.5	11.4
Guinea	IS27557	20.3	18.2
Guinea-caudatum	IS26222	20.3	14.1
Guinea-kafir	IS19450	20.2	7.3
Kafir	IS26749	20.2	18.5
Kafir	IS29233	20.0	5.7
Bicolor	IS20727	19.8	8.3
Caudatum-bicolor	IS24462	19.8	7.6
Caudatum-bicolor	IS20298	19.6	5.1
Caudatum-bicolor	IS12706	19.6	7.4
Kafir	IS29392	19.4	8.0
Caudatum-bicolor	IS8777	19.3	12.6
Guinea-bicolor	IS1219	19.2	6.0
Kafir	IS24492	19.2	4.0

Race	Genotype	TotGrainWt.md	TotGrainWt.sd
Bicolor	IS608	18.8	10.4
Bicolor	IS603	18.2	10.8
Kafir	IS19445	17.8	16.3
Kafir-durra	IS13782	17.8	9.7
Bicolor	IS602	17.7	6.9
Kafir-caudatum	IS27912	17.6	10.0
Caudatum-bicolor	IS23216	17.2	8.9
Kafir	IS22239	17.2	7.6
Bicolor	IS20740	16.9	7.6
Bicolor	IS1233	16.8	6.1
Kafir	IS2397	16.7	6.0
Caudatum	IS23992	16.7	9.6
Caudatum-bicolor	IS30417	15.9	16.4
Kafir-caudatum	IS29441	15.6	5.6
Caudatum-bicolor	IS30450	14.7	5.8
Bicolor	IS20816	14.2	9.4
Kafir	IS3158	14.1	15.3
Caudatum	IS14861	12.6	1.3
Kafir	IS29582	12.5	8.5
Durra-caudatum	IS28389	11.1	2.4
Guinea-caudatum	IS19262	6.4	9.7
Bicolor	IS24503	6.0	3.4

8.3 Summary Table of Fat content by Genotype in drought stress

Race	Genotype	Fat.md	Fat.sd
Bicolor	IS608	3.8	0.1
Bicolor	IS22616	3.8	0.2
Caudatum-bicolor	IS12706	3.8	0.3
Bicolor	IS1233	3.7	0.4
Bicolor	IS31681	3.7	0.5
Bicolor	IS602	3.7	0.2
Bicolor	IS8012	3.7	0.2
Durra-bicolor	IS11919	3.7	0.1
Caudatum-bicolor	IS30450	3.6	0.2
Kafir-durra	IS14290	3.6	0.1
Bicolor	IS603	3.5	0.2
Bicolor	IS20816	3.5	1.4
Kafir-bicolor	IS1212	3.5	0.3
Caudatum-bicolor	IS30466	3.5	0.3
Guinea-bicolor	IS1219	3.4	0.5
Caudatum-bicolor	IS8777	3.4	0.1
Caudatum-bicolor	IS24462	3.3	0.2
Durra-caudatum	IS28614	3.2	0.1
Kafir	IS2389	3.2	0.3
Caudatum-bicolor	IS30507	3.2	0.2
Bicolor	IS30562	3.2	0.9
Guinea	IS26046	3.2	0.3
Caudatum	IS2864	3.2	0.2
Caudatum	IS13971	3.2	0.1
Bicolor	IS20727	3.2	0.2
Caudatum-bicolor	IS20298	3.1	0.2
Caudatum	IS30460	3.1	0.0
Caudatum-bicolor	IS26701	3.1	0.7
Caudatum-bicolor	IS24453	3.1	0.6
Durra-caudatum	IS32245	3.1	NA
Guinea-caudatum	IS28449	3.1	0.1
Caudatum-bicolor	IS30451	3.1	0.4
Caudatum	IS19389	3.0	0.2

Race	Genotype	Fat.md	Fat.sd
Caudatum-bicolor	IS30533	3.0	0.4
Caudatum-bicolor	IS30443	3.0	0.6
Caudatum	IS9745	3.0	0.1
Guinea-caudatum	IS29565	3.0	0.2
Kafir	IS29519	3.0	NA
Guinea	IS7310	3.0	0.1
Bicolor	IS20740	3.0	0.4
Durra-caudatum	IS28313	2.9	0.6
Durra-caudatum	IS28389	2.9	NA
Kafir-bicolor	IS29654	2.9	0.2
Bicolor	IS20195	2.9	0.1
Caudatum-bicolor	IS30383	2.9	0.4
Caudatum-bicolor	IS16151	2.9	0.6
Caudatum	IS7305	2.9	0.1
Kafir-caudatum	IS29568	2.9	0.2
Kafir	IS26737	2.9	0.5
Caudatum-bicolor	IS30417	2.9	0.5
Durra-caudatum	IS20956	2.8	0.3
Kafir-caudatum	IS29241	2.8	NA
Guinea	IS25910	2.8	0.5
Durra	IS19859	2.8	0.3
Guinea-durra	IS29733	2.8	0.6
Caudatum	IS11473	2.8	0.5
Guinea	IS33023	2.8	NA
Guinea	IS27557	2.8	0.2
Guinea-caudatum	IS29187	2.8	0.1
Caudatum-bicolor	IS29326	2.8	0.0
Caudatum	IS4092	2.8	0.4
Guinea-caudatum	IS26222	2.8	0.1
Guinea-caudatum	IS29950	2.8	1.6
Kafir-caudatum	IS13893	2.7	0.3
Durra-bicolor	IS27786	2.7	0.6
Caudatum-bicolor	IS14010	2.7	0.3
Guinea-caudatum	IS19262	2.7	0.7

Race	Genotype	Fat.md	Fat.sd
Durra	IS22720	2.7	0.2
Durra	IS5919	2.7	NA
Guinea-caudatum	IS28451	2.7	NA
Kafir	IS29392	2.7	0.2
Guinea-caudatum	IS10867	2.7	0.4
Kafir-durra	IS8774	2.7	0.0
Guinea-caudatum	IS5301	2.7	0.2
Durra	IS32787	2.7	NA
Guinea-kafir	IS473	2.7	0.0
Caudatum	IS20697	2.6	0.8
Bicolor	IS20743	2.6	0.6
Caudatum	IS15170	2.6	0.3
Guinea-caudatum	IS29269	2.6	0.3
Caudatum	IS2382	2.6	NA
Caudatum	IS29335	2.6	0.5
Caudatum-bicolor	IS30536	2.6	0.7
Durra	IS4360	2.6	0.1
Guinea-caudatum	IS23521	2.6	0.3
Durra	IS5386	2.6	0.5
Kafir	IS29582	2.6	0.2
Guinea-caudatum	IS15478	2.6	0.2
Durra-caudatum	IS29627	2.6	0.2
Kafir	IS22294	2.6	0.4
Caudatum	IS14861	2.6	NA
Guinea	IS26484	2.6	0.1
Durra	IS12883	2.6	NA
Kafir	IS22239	2.6	0.3
Kafir	IS29606	2.5	0.1
Guinea	IS23644	2.5	NA
Kafir-durra	IS29714	2.5	0.4
Caudatum	IS9113	2.5	NA
Guinea	IS21512	2.5	0.5
Kafir	IS24492	2.5	0.2
Durra-caudatum	IS28747	2.5	0.2

Race	Genotype	Fat.md	Fat.sd
Kafir	IS19676	2.5	0.3
Guinea	IS25089	2.5	0.2
Guinea-caudatum	IS29468	2.5	0.1
Kafir-durra	IS13782	2.5	NA
Guinea-caudatum	IS15945	2.5	0.2
Guinea	IS7679	2.5	NA
Caudatum	IS10302	2.5	0.2
Durra-caudatum	IS12447	2.5	0.5
Kafir	IS24463	2.5	0.7
Durra-caudatum	IS28849	2.5	0.3
Kafir	IS26749	2.5	0.2
Durra-bicolor	IS4060	2.4	0.4
Caudatum	IS23992	2.4	0.4
Durra	IS25836	2.4	0.4
Guinea	IS16528	2.4	0.2
Guinea	IS30838	2.4	NA
Guinea	IS16382	2.3	0.2
Bicolor	IS21863	2.3	0.7
Caudatum	IS26694	2.3	0.7
Caudatum	IS12965	2.3	0.9
Guinea	IS32439	2.3	NA
Caudatum	IS10757	2.3	0.3
Guinea	IS24139	2.3	NA
Guinea-caudatum	IS30572	2.2	0.2
Guinea	IS4951	2.2	NA
Caudatum	IS14779	2.2	0.4
Caudatum	IS22609	2.2	0.2
Durra	IS8348	2.2	NA
Guinea-caudatum	IS20679	2.2	0.3
Caudatum-bicolor	IS2902	2.2	NA
Guinea	IS5295	2.2	NA
Guinea	IS32349	2.2	0.2
Caudatum-bicolor	IS12735	2.2	0.6
Caudatum	IS29914	2.1	0.4

Race	Genotype	Fat.md	Fat.sd
Kafir	IS30231	2.1	0.3
Durra	IS1041	2.1	0.6
Guinea	IS15931	2.1	0.1
Bicolor	IS24503	2.1	NA
Kafir	IS3158	2.1	0.3
Caudatum-bicolor	IS30508	2.1	0.5
Caudatum	IS2379	2.0	0.6
Caudatum	IS22986	2.0	0.0
Guinea-bicolor	IS7957	2.0	NA
Guinea-caudatum	IS8916	2.0	NA
Durra-caudatum	IS30092	1.9	0.1
Caudatum	IS9108	1.9	0.1
Kafir-caudatum	IS29441	1.9	0.8
Guinea-caudatum	IS23590	1.8	0.2
Caudatum	IS24348	1.8	0.7
Guinea	IS21645	1.8	NA
Caudatum	IS14090	1.8	0.5
Caudatum	IS17941	1.7	0.4
Kafir	IS29358	1.7	0.9
Caudatum-bicolor	IS2872	1.6	0.7
Guinea	IS25989	1.6	NA
Caudatum	IS33353	1.6	0.3
Guinea-kafir	IS29304	1.6	NA
Guinea	IS19975	1.5	NA
Kafir	IS29689	1.5	NA
Caudatum	IS31043	1.4	NA
Guinea-caudatum	IS10969	1.3	0.4
Kafir-caudatum	IS13919	1.3	NA
Guinea-caudatum	IS20713	1.3	NA
Caudatum-bicolor	IS26617	1.2	0.3
Kafir	IS29239	1.2	0.2
Durra	IS22799	1.2	NA
Kafir	IS29233	1.2	1.0
Durra	IS25732	1.1	NA

Race	Genotype	Fat.md	Fat.sd
Bicolor	IS3121	1.1	NA
Durra-caudatum	IS15744	1.1	0.6
Guinea	IS7250	1.0	NA
Guinea-caudatum	IS23579	0.8	NA
Kafir	IS2397	0.7	NA
Durra	IS4515	0.1	NA
Bicolor	IS12697	NA	NA
Bicolor	IS12804	NA	NA
Bicolor	IS2413	NA	NA
Bicolor	IS24939	NA	NA
Caudatum	IS12302	NA	NA
Caudatum	IS15466	NA	NA
Caudatum	IS20632	NA	NA
Caudatum	IS21083	NA	NA
Caudatum	IS23514	NA	NA
Caudatum	IS25548	NA	NA
Caudatum	IS31557	NA	NA
Caudatum	IS31651	NA	NA
Caudatum	IS9177	NA	NA
Caudatum-bicolor	IS13294	NA	NA
Caudatum-bicolor	IS13549	NA	NA
Caudatum-bicolor	IS23216	NA	NA
Caudatum-bicolor	IS2426	NA	NA
Caudatum-bicolor	IS27887	NA	NA
Caudatum-bicolor	IS30400	NA	NA
Caudatum-bicolor	IS995	NA	NA
Durra	IS1004	NA	NA
Durra	IS11026	NA	NA
Durra	IS17980	NA	NA
Durra	IS23891	NA	NA
Durra	IS27034	NA	NA
Durra	IS31706	NA	NA
Durra	IS33090	NA	NA
Durra	IS3971	NA	NA

Race	Genotype	Fat.md	Fat.sd
Durra	IS4581	NA	NA
Durra	IS4613	NA	NA
Durra	IS4631	NA	NA
Durra	IS4698	NA	NA
Durra	IS5094	NA	NA
Durra	IS5667	NA	NA
Durra	IS6351	NA	NA
Durra	IS6354	NA	NA
Durra	IS6421	NA	NA
Durra-bicolor	IS11619	NA	NA
Durra-bicolor	IS18039	NA	NA
Durra-bicolor	IS25249	NA	NA
Durra-bicolor	IS25301	NA	NA
Durra-caudatum	IS20625	NA	NA
Durra-caudatum	IS28141	NA	NA
Durra-caudatum	IS29091	NA	NA
Durra-caudatum	IS29100	NA	NA
Durra-caudatum	IS29314	NA	NA
Durra-caudatum	IS30079	NA	NA
Durra-caudatum	IS31714	NA	NA
Durra-caudatum	IS7131	NA	NA
Guinea	IS23684	NA	NA
Guinea	IS24175	NA	NA
Guinea	IS24218	NA	NA
Guinea	IS26025	NA	NA
Guinea	IS27697	NA	NA
Guinea	IS7987	NA	NA
Guinea-caudatum	IS19153	NA	NA
Guinea-caudatum	IS23586	NA	NA
Guinea-caudatum	IS24953	NA	NA
Guinea-caudatum	IS29772	NA	NA
Guinea-caudatum	IS31186	NA	NA
Guinea-caudatum	IS31446	NA	NA
Guinea-durra	IS4372	NA	NA

Race	Genotype	Fat.md	Fat.sd
Guinea-kafir	IS19450	NA	NA
Kafir	IS12937	NA	NA
Kafir	IS12945	NA	NA
Kafir	IS19445	NA	NA
Kafir-caudatum	IS27912	NA	NA

8.4 Summary Table of fat content by genotype in well-watered

Race	Genotype	Fat.md	Fat.sd
Caudatum-bicolor	IS30466	4.7	0.4
Bicolor	IS31681	4.3	0.2
Kafir-bicolor	IS1212	4.2	0.3
Bicolor	IS602	4.1	0.2
Bicolor	IS22616	4.0	0.4
Bicolor	IS8012	4.0	0.2
Bicolor	IS1233	4.0	0.3
Caudatum-bicolor	IS30443	4.0	0.2
Bicolor	IS12697	3.9	1.1
Caudatum-bicolor	IS26701	3.9	0.2
Bicolor	IS603	3.9	0.1
Guinea	IS25989	3.9	0.1
Caudatum-bicolor	IS30400	3.9	0.4
Caudatum-bicolor	IS12735	3.9	0.4
Caudatum-bicolor	IS13549	3.8	0.3
Bicolor	IS20816	3.8	0.1
Caudatum-bicolor	IS30536	3.8	0.4
Bicolor	IS24503	3.8	NA
Bicolor	IS2413	3.7	0.2
Guinea	IS26046	3.7	0.2
Caudatum-bicolor	IS30450	3.7	0.0
Durra-bicolor	IS25249	3.7	0.3
Caudatum-bicolor	IS30417	3.7	0.4
Guinea-bicolor	IS1219	3.6	0.2
Bicolor	IS608	3.6	0.1
Caudatum-bicolor	IS30507	3.6	0.2
Kafir-bicolor	IS29654	3.6	0.2
Durra	IS3971	3.6	0.1
Durra-caudatum	IS31714	3.6	NA
Durra-caudatum	IS32245	3.6	0.2
Durra-bicolor	IS11919	3.5	0.3
Caudatum-bicolor	IS30383	3.5	0.3
Caudatum-bicolor	IS30508	3.5	0.4

Race	Genotype	Fat.md	Fat.sd
Caudatum	IS23992	3.5	0.1
Guinea	IS23644	3.5	0.7
Durra	IS27034	3.5	0.2
Guinea	IS19975	3.5	0.1
Caudatum-bicolor	IS2426	3.5	0.1
Kafir	IS12937	3.5	0.2
Bicolor	IS3121	3.5	0.9
Caudatum-bicolor	IS13294	3.5	0.2
Caudatum-bicolor	IS27887	3.5	0.3
Guinea	IS27557	3.5	0.3
Kafir-caudatum	IS27912	3.5	0.2
Guinea	IS25910	3.4	0.1
Caudatum-bicolor	IS995	3.4	0.1
Caudatum-bicolor	IS30533	3.4	0.1
Guinea-caudatum	IS23579	3.4	0.0
Caudatum-bicolor	IS12706	3.4	0.1
Guinea-caudatum	IS23586	3.4	0.1
Caudatum	IS30460	3.4	0.2
Durra	IS5919	3.4	0.4
Caudatum-bicolor	IS26617	3.4	0.5
Caudatum-bicolor	IS14010	3.4	0.1
Bicolor	IS30562	3.3	NA
Caudatum	IS9745	3.3	0.1
Durra	IS8348	3.3	0.2
Guinea-caudatum	IS29772	3.3	NA
Caudatum-bicolor	IS30451	3.3	0.4
Bicolor	IS20743	3.3	0.2
Caudatum-bicolor	IS24462	3.3	0.3
Caudatum-bicolor	IS16151	3.3	0.2
Durra-caudatum	IS28614	3.3	0.2
Kafir	IS19445	3.3	0.1
Durra-caudatum	IS28141	3.3	0.2
Bicolor	IS12804	3.3	0.4
Durra-caudatum	IS28389	3.3	0.4

Race	Genotype	Fat.md	Fat.sd
Guinea	IS16382	3.3	0.1
Guinea	IS26025	3.3	0.4
Caudatum	IS12302	3.3	0.3
Caudatum-bicolor	IS8777	3.2	0.5
Caudatum	IS20632	3.2	0.2
Kafir-durra	IS14290	3.2	0.3
Kafir	IS29689	3.2	0.4
Durra	IS5094	3.2	0.2
Durra	IS6421	3.2	0.1
Kafir	IS24492	3.2	0.1
Bicolor	IS24939	3.2	0.1
Guinea	IS15931	3.2	0.0
Durra	IS4631	3.2	0.1
Durra-bicolor	IS4060	3.2	0.3
Durra-caudatum	IS29627	3.2	0.2
Durra	IS19859	3.2	0.2
Guinea-caudatum	IS23521	3.2	0.1
Durra-caudatum	IS28849	3.2	0.1
Guinea	IS30838	3.2	0.1
Guinea-caudatum	IS19153	3.2	0.3
Durra	IS4515	3.2	0.2
Kafir-durra	IS8774	3.2	0.4
Guinea-caudatum	IS24953	3.2	0.2
Kafir	IS29239	3.2	0.1
Durra-bicolor	IS27786	3.2	0.2
Bicolor	IS21863	3.2	0.0
Kafir-durra	IS13782	3.2	0.0
Durra	IS31706	3.2	0.2
Guinea-caudatum	IS26222	3.2	0.3
Caudatum	IS20697	3.2	0.4
Caudatum	IS31557	3.1	0.3
Caudatum-bicolor	IS23216	3.1	0.2
Bicolor	IS20727	3.1	0.5
Bicolor	IS20740	3.1	0.0

Race	Genotype	Fat.md	Fat.sd
Caudatum-bicolor	IS20298	3.1	0.1
Bicolor	IS20195	3.1	0.3
Kafir	IS29233	3.1	0.4
Caudatum	IS11473	3.1	0.2
Caudatum-bicolor	IS24453	3.1	0.2
Durra-caudatum	IS20625	3.1	0.3
Caudatum	IS19389	3.1	0.1
Caudatum	IS13971	3.1	0.0
Kafir	IS26749	3.1	0.3
Caudatum	IS26694	3.1	1.0
Guinea	IS7987	3.1	0.0
Durra-caudatum	IS29314	3.1	0.1
Guinea-durra	IS4372	3.1	NA
Kafir-caudatum	IS29568	3.1	0.2
Kafir	IS24463	3.1	0.1
Guinea	IS5295	3.1	0.2
Caudatum	IS29335	3.1	0.1
Guinea-kafir	IS473	3.1	0.1
Guinea	IS7310	3.1	0.2
Caudatum-bicolor	IS29326	3.1	0.1
Kafir	IS22294	3.1	0.1
Guinea	IS27697	3.1	0.2
Kafir	IS26737	3.0	0.2
Durra	IS4360	3.0	0.2
Kafir-durra	IS29714	3.0	0.2
Guinea-caudatum	IS5301	3.0	0.2
Guinea-caudatum	IS19262	3.0	0.1
Guinea	IS24139	3.0	0.3
Guinea-caudatum	IS29269	3.0	0.1
Kafir	IS2389	3.0	0.3
Guinea-caudatum	IS29468	3.0	0.4
Durra	IS4698	3.0	0.2
Kafir	IS29582	3.0	0.0
Kafir	IS19676	3.0	0.4

Race	Genotype	Fat.md	Fat.sd
Guinea	IS7250	3.0	0.1
Caudatum	IS23514	3.0	0.4
Guinea	IS24175	3.0	0.2
Kafir	IS12945	3.0	0.2
Durra-caudatum	IS28313	3.0	0.1
Guinea-caudatum	IS28449	3.0	0.2
Caudatum	IS2382	3.0	0.3
Durra-caudatum	IS28747	3.0	0.2
Caudatum	IS10302	3.0	0.3
Caudatum	IS2864	3.0	0.2
Guinea-caudatum	IS20713	3.0	0.1
Durra	IS12883	3.0	0.3
Durra-caudatum	IS12447	3.0	0.2
Guinea-caudatum	IS10867	3.0	0.8
Kafir-caudatum	IS13893	3.0	0.3
Guinea	IS24218	3.0	0.4
Durra	IS6351	3.0	0.1
Guinea-kafir	IS29304	2.9	0.2
Durra-caudatum	IS20956	2.9	0.1
Durra-caudatum	IS30092	2.9	0.3
Caudatum	IS7305	2.9	0.0
Durra-bicolor	IS25301	2.9	0.1
Guinea-durra	IS29733	2.9	0.5
Kafir-caudatum	IS29241	2.9	0.1
Caudatum	IS33353	2.9	0.4
Guinea-caudatum	IS31186	2.9	0.3
Kafir	IS3158	2.9	NA
Guinea-caudatum	IS29565	2.9	0.1
Durra-caudatum	IS30079	2.9	0.2
Guinea-caudatum	IS29187	2.9	0.2
Durra	IS33090	2.9	0.4
Caudatum	IS22986	2.9	0.2
Guinea	IS16528	2.9	0.3
Guinea-caudatum	IS8916	2.8	0.0

Race	Genotype	Fat.md	Fat.sd
Guinea	IS23684	2.8	0.3
Caudatum	IS9177	2.8	0.1
Durra	IS22720	2.8	0.3
Guinea	IS4951	2.8	0.2
Durra	IS25732	2.8	0.1
Kafir	IS22239	2.8	0.4
Kafir	IS29358	2.8	0.4
Guinea	IS32439	2.8	0.5
Durra	IS5667	2.8	0.1
Durra	IS5386	2.8	0.3
Caudatum	IS4092	2.8	0.3
Guinea	IS33023	2.8	0.4
Caudatum-bicolor	IS2872	2.7	0.8
Caudatum	IS29914	2.7	0.4
Kafir	IS2397	2.7	0.1
Caudatum	IS15170	2.7	0.3
Guinea-caudatum	IS10969	2.7	0.1
Kafir-caudatum	IS13919	2.7	0.3
Guinea-caudatum	IS28451	2.7	NA
Guinea	IS26484	2.7	0.1
Caudatum	IS31651	2.7	0.0
Caudatum	IS15466	2.7	0.2
Durra	IS4581	2.7	0.1
Caudatum	IS24348	2.7	0.1
Caudatum	IS22609	2.7	0.5
Caudatum	IS25548	2.7	0.2
Guinea-caudatum	IS30572	2.7	0.4
Caudatum	IS21083	2.7	0.2
Durra	IS17980	2.7	0.4
Guinea-kafir	IS19450	2.7	0.3
Kafir	IS29392	2.7	0.1
Caudatum	IS17941	2.6	0.2
Guinea-caudatum	IS23590	2.6	0.2
Guinea	IS21512	2.6	0.3

Race	Genotype	Fat.md	Fat.sd
Durra	IS6354	2.6	0.6
Caudatum	IS10757	2.6	0.1
Guinea-caudatum	IS15945	2.6	0.6
Caudatum	IS9108	2.6	0.7
Guinea	IS25089	2.6	0.3
Guinea-caudatum	IS20679	2.6	0.5
Durra	IS1041	2.6	0.3
Guinea	IS21645	2.6	0.5
Caudatum	IS14861	2.5	0.3
Caudatum	IS9113	2.5	0.3
Durra-caudatum	IS7131	2.5	0.1
Caudatum	IS12965	2.5	0.4
Kafir	IS30231	2.5	0.4
Guinea-caudatum	IS29950	2.5	0.3
Kafir	IS29606	2.5	0.5
Durra-bicolor	IS18039	2.5	0.7
Kafir	IS29519	2.5	0.4
Caudatum	IS14090	2.5	0.4
Guinea	IS32349	2.5	0.1
Caudatum-bicolor	IS2902	2.5	0.1
Durra	IS25836	2.5	0.0
Durra	IS11026	2.4	0.5
Guinea	IS7679	2.4	0.2
Kafir-caudatum	IS29441	2.4	0.2
Durra	IS1004	2.4	0.2
Durra-caudatum	IS29100	2.4	0.5
Caudatum	IS14779	2.4	0.3
Durra-caudatum	IS15744	2.4	0.4
Caudatum	IS2379	2.3	0.2
Caudatum	IS31043	2.3	0.4
Guinea-caudatum	IS15478	2.3	0.0
Durra-bicolor	IS11619	2.3	0.4
Guinea-caudatum	IS31446	2.2	0.3
Guinea-bicolor	IS7957	2.2	0.1

Race	Genotype	Fat.md	Fat.sd
Durra	IS22799	2.1	0.4
Durra-caudatum	IS29091	2.1	0.4
Durra	IS4613	2.0	0.6
Durra	IS32787	1.9	NA
Durra	IS23891	1.9	0.5

Summary Table of Starch by Genotype in drought stress

Race	Genotype	Starch.md	Starch.sd
Kafir	IS2397	75.0	NA
Kafir	IS29239	73.7	1.1
Caudatum	IS31043	73.5	NA
Guinea	IS7250	73.0	NA
Kafir-caudatum	IS13919	72.1	NA
Caudatum	IS33353	71.9	1.3
Caudatum-bicolor	IS26617	71.8	3.3
Guinea	IS23644	71.8	NA
Guinea-caudatum	IS10867	71.6	1.2
Durra-caudatum	IS12447	71.4	2.4
Kafir	IS29233	71.3	1.7
Kafir-caudatum	IS29441	71.3	6.3
Kafir	IS19676	71.3	1.8
Guinea-caudatum	IS20713	70.7	NA
Kafir	IS29689	70.7	NA
Kafir	IS29358	70.7	3.7
Caudatum	IS9108	70.6	0.9
Guinea-kafir	IS29304	70.5	NA
Kafir	IS26737	70.5	0.4
Caudatum	IS22986	70.5	0.4
Caudatum	IS2379	70.4	3.7
Guinea-caudatum	IS23590	70.4	1.9
Bicolor	IS3121	70.3	NA
Guinea	IS16382	70.2	2.1
Durra	IS4515	70.2	NA
Guinea-caudatum	IS29468	69.9	0.2
Kafir	IS29582	69.9	0.8
Guinea	IS19975	69.9	NA
Guinea	IS32439	69.9	NA
Durra	IS22799	69.8	NA
Guinea	IS7310	69.8	2.9
Durra	IS25732	69.7	NA
Kafir	IS3158	69.7	2.5

Race	Genotype	Starch.md	Starch.sd
Caudatum	IS9113	69.7	NA
Caudatum	IS4092	69.6	1.5
Guinea-caudatum	IS23579	69.6	NA
Caudatum-bicolor	IS24462	69.6	1.4
Guinea	IS15931	69.6	2.4
Guinea-caudatum	IS15945	69.6	1.0
Durra	IS25836	69.5	0.8
Caudatum	IS14090	69.5	0.4
Kafir	IS22239	69.5	1.8
Kafir-bicolor	IS1212	69.4	1.3
Guinea	IS27557	69.4	2.8
Guinea	IS32349	69.4	1.6
Guinea-caudatum	IS28451	69.3	NA
Caudatum	IS10757	69.2	2.5
Caudatum	IS24348	69.1	5.8
Durra-caudatum	IS30092	69.1	0.3
Guinea-caudatum	IS26222	69.1	0.7
Bicolor	IS21863	69.0	1.9
Caudatum	IS17941	69.0	0.5
Guinea	IS25089	69.0	1.0
Kafir	IS29519	69.0	NA
Bicolor	IS30562	68.9	1.3
Caudatum	IS29335	68.9	2.0
Caudatum-bicolor	IS30533	68.8	0.8
Caudatum	IS7305	68.8	1.2
Caudatum-bicolor	IS2872	68.8	3.1
Caudatum	IS29914	68.8	1.0
Caudatum	IS26694	68.8	1.5
Durra-caudatum	IS15744	68.8	4.2
Guinea	IS24139	68.7	NA
Caudatum-bicolor	IS14010	68.7	1.5
Kafir-durra	IS13782	68.7	NA
Durra-caudatum	IS29627	68.6	1.9
Kafir	IS2389	68.6	1.9

Race	Genotype	Starch.md	Starch.sd
Durra	IS5386	68.6	1.8
Guinea-caudatum	IS8916	68.6	NA
Caudatum-bicolor	IS30536	68.5	1.3
Durra	IS22720	68.5	2.6
Caudatum-bicolor	IS30507	68.5	1.1
Guinea-bicolor	IS7957	68.5	NA
Guinea	IS16528	68.4	1.3
Caudatum-bicolor	IS30508	68.4	1.6
Guinea-caudatum	IS29950	68.3	4.5
Kafir	IS26749	68.3	3.5
Guinea	IS7679	68.2	NA
Caudatum-bicolor	IS30451	68.2	2.2
Caudatum	IS13971	68.2	0.6
Guinea	IS30838	68.1	NA
Guinea-caudatum	IS29565	68.1	1.2
Caudatum-bicolor	IS30417	68.0	0.0
Durra-caudatum	IS28313	68.0	0.4
Caudatum	IS19389	67.9	1.4
Durra-caudatum	IS32245	67.9	NA
Kafir	IS22294	67.9	5.2
Durra-bicolor	IS4060	67.9	2.6
Guinea	IS21645	67.8	NA
Kafir-caudatum	IS13893	67.8	1.7
Guinea	IS25989	67.8	NA
Guinea	IS4951	67.6	NA
Guinea	IS26046	67.5	0.1
Kafir-caudatum	IS29568	67.5	2.1
Caudatum	IS30460	67.4	2.1
Caudatum-bicolor	IS20298	67.4	1.7
Bicolor	IS31681	67.4	2.8
Caudatum	IS2382	67.4	NA
Durra	IS4360	67.4	2.4
Guinea-caudatum	IS28449	67.4	0.8
Caudatum-bicolor	IS29326	67.3	1.4

Race	Genotype	Starch.md	Starch.sd
Caudatum	IS14779	67.3	2.2
Kafir	IS29606	67.3	2.5
Caudatum	IS12965	67.2	2.3
Guinea-durra	IS29733	67.2	3.3
Caudatum	IS23992	67.2	0.2
Kafir-durra	IS29714	67.2	0.8
Caudatum-bicolor	IS12735	67.2	0.9
Guinea	IS25910	67.2	0.2
Kafir-durra	IS8774	67.1	1.3
Caudatum	IS14861	67.0	NA
Caudatum	IS22609	67.0	1.7
Durra-caudatum	IS20956	67.0	2.4
Caudatum	IS9745	67.0	1.8
Caudatum	IS20697	66.9	0.7
Kafir	IS24492	66.9	2.0
Guinea-caudatum	IS19262	66.9	3.9
Caudatum	IS11473	66.8	0.9
Kafir	IS29392	66.8	2.0
Caudatum	IS15170	66.8	2.3
Durra-caudatum	IS28614	66.8	0.4
Caudatum	IS2864	66.8	1.6
Guinea	IS26484	66.8	0.8
Caudatum-bicolor	IS30383	66.8	1.9
Durra	IS1041	66.8	1.6
Durra	IS12883	66.6	NA
Caudatum-bicolor	IS30443	66.6	1.8
Durra-caudatum	IS28849	66.6	2.4
Guinea-caudatum	IS29187	66.6	1.3
Guinea-caudatum	IS10969	66.5	1.2
Durra	IS32787	66.4	NA
Guinea	IS33023	66.3	NA
Bicolor	IS20740	66.3	0.8
Guinea	IS5295	66.2	NA
Kafir-caudatum	IS29241	66.2	NA

Race	Genotype	Starch.md	Starch.sd
Guinea-caudatum	IS29269	66.1	1.2
Kafir	IS24463	66.0	3.3
Guinea-caudatum	IS23521	65.9	1.0
Bicolor	IS20743	65.8	1.3
Kafir-durra	IS14290	65.8	0.0
Caudatum	IS10302	65.8	3.3
Guinea-caudatum	IS30572	65.7	1.2
Durra-bicolor	IS27786	65.7	1.3
Guinea-caudatum	IS15478	65.7	1.9
Guinea-kafir	IS473	65.7	0.4
Caudatum-bicolor	IS30466	65.7	2.4
Bicolor	IS603	65.6	2.7
Durra	IS19859	65.6	3.8
Durra-caudatum	IS28747	65.5	1.2
Caudatum-bicolor	IS24453	65.5	1.8
Guinea	IS21512	65.5	0.5
Durra	IS8348	65.5	NA
Bicolor	IS608	65.5	2.1
Kafir-bicolor	IS29654	65.5	2.1
Caudatum-bicolor	IS26701	65.4	1.5
Bicolor	IS20195	65.4	0.6
Kafir	IS30231	65.4	1.6
Caudatum-bicolor	IS8777	65.3	6.1
Durra-caudatum	IS28389	65.3	NA
Bicolor	IS20816	64.7	2.2
Caudatum-bicolor	IS2902	64.7	NA
Durra-bicolor	IS11919	64.6	1.5
Caudatum-bicolor	IS30450	64.5	1.1
Guinea-caudatum	IS5301	64.4	3.9
Caudatum-bicolor	IS12706	64.4	1.1
Caudatum-bicolor	IS16151	64.3	2.1
Bicolor	IS8012	64.3	3.1
Bicolor	IS1233	64.1	1.3
Guinea-caudatum	IS20679	64.1	2.7

Race	Genotype	Starch.md	Starch.sd
Guinea-bicolor	IS1219	63.9	1.3
Bicolor	IS22616	62.4	3.1
Bicolor	IS20727	62.4	1.3
Durra	IS5919	62.1	NA
Bicolor	IS602	61.7	3.2
Bicolor	IS24503	58.0	NA
Bicolor	IS12697	NA	NA
Bicolor	IS12804	NA	NA
Bicolor	IS2413	NA	NA
Bicolor	IS24939	NA	NA
Caudatum	IS12302	NA	NA
Caudatum	IS15466	NA	NA
Caudatum	IS20632	NA	NA
Caudatum	IS21083	NA	NA
Caudatum	IS23514	NA	NA
Caudatum	IS25548	NA	NA
Caudatum	IS31557	NA	NA
Caudatum	IS31651	NA	NA
Caudatum	IS9177	NA	NA
Caudatum-bicolor	IS13294	NA	NA
Caudatum-bicolor	IS13549	NA	NA
Caudatum-bicolor	IS23216	NA	NA
Caudatum-bicolor	IS2426	NA	NA
Caudatum-bicolor	IS27887	NA	NA
Caudatum-bicolor	IS30400	NA	NA
Caudatum-bicolor	IS995	NA	NA
Durra	IS1004	NA	NA
Durra	IS11026	NA	NA
Durra	IS17980	NA	NA
Durra	IS23891	NA	NA
Durra	IS27034	NA	NA
Durra	IS31706	NA	NA
Durra	IS33090	NA	NA
Durra	IS3971	NA	NA

Race	Genotype	Starch.md	Starch.sd
Durra	IS4581	NA	NA
Durra	IS4613	NA	NA
Durra	IS4631	NA	NA
Durra	IS4698	NA	NA
Durra	IS5094	NA	NA
Durra	IS5667	NA	NA
Durra	IS6351	NA	NA
Durra	IS6354	NA	NA
Durra	IS6421	NA	NA
Durra-bicolor	IS11619	NA	NA
Durra-bicolor	IS18039	NA	NA
Durra-bicolor	IS25249	NA	NA
Durra-bicolor	IS25301	NA	NA
Durra-caudatum	IS20625	NA	NA
Durra-caudatum	IS28141	NA	NA
Durra-caudatum	IS29091	NA	NA
Durra-caudatum	IS29100	NA	NA
Durra-caudatum	IS29314	NA	NA
Durra-caudatum	IS30079	NA	NA
Durra-caudatum	IS31714	NA	NA
Durra-caudatum	IS7131	NA	NA
Guinea	IS23684	NA	NA
Guinea	IS24175	NA	NA
Guinea	IS24218	NA	NA
Guinea	IS26025	NA	NA
Guinea	IS27697	NA	NA
Guinea	IS7987	NA	NA
Guinea-caudatum	IS19153	NA	NA
Guinea-caudatum	IS23586	NA	NA
Guinea-caudatum	IS24953	NA	NA
Guinea-caudatum	IS29772	NA	NA
Guinea-caudatum	IS31186	NA	NA
Guinea-caudatum	IS31446	NA	NA
Guinea-durra	IS4372	NA	NA

Race	Genotype	Starch.md	Starch.sd
Guinea-kafir	IS19450	NA	NA
Kafir	IS12937	NA	NA
Kafir	IS12945	NA	NA
Kafir	IS19445	NA	NA
Kafir-caudatum	IS27912	NA	NA

8.5 Summary Table of Starch by Genotype in well-watered

Race	Genotype	Starch.md	Starch.sd
Durra-caudatum	IS28614	67.7	1.3
Caudatum	IS25548	67.6	0.6
Caudatum	IS22609	66.9	1.2
Guinea-caudatum	IS28451	66.6	NA
Bicolor	IS30562	66.6	NA
Guinea	IS26046	66.6	0.8
Kafir	IS29606	66.6	4.2
Guinea-caudatum	IS31446	66.4	0.8
Caudatum	IS15466	66.3	4.1
Durra-caudatum	IS15744	66.3	2.2
Guinea-caudatum	IS8916	66.2	1.6
Caudatum	IS9108	66.0	2.9
Guinea	IS16382	66.0	1.3
Caudatum	IS9113	65.7	1.0
Durra-caudatum	IS30092	65.6	2.6
Caudatum	IS31651	65.4	1.8
Guinea	IS24139	65.3	0.9
Caudatum-bicolor	IS24462	65.2	1.6
Caudatum	IS12302	65.1	1.5
Kafir-caudatum	IS29568	65.1	3.2
Durra-caudatum	IS20625	65.0	1.5
Caudatum	IS9177	65.0	3.7
Guinea-caudatum	IS29772	64.9	NA
Guinea-caudatum	IS15945	64.9	0.6
Caudatum	IS14090	64.9	3.9
Durra-caudatum	IS31714	64.8	NA
Kafir	IS29582	64.6	1.0
Durra-caudatum	IS28747	64.6	0.3
Caudatum	IS20632	64.6	2.3
Durra	IS22799	64.6	2.1
Caudatum	IS14861	64.6	0.7
Kafir	IS19445	64.6	1.7
Caudatum-bicolor	IS30533	64.6	1.1

Race	Genotype	Starch.md	Starch.sd
Caudatum	IS21083	64.5	1.1
Caudatum	IS17941	64.5	1.9
Durra	IS6421	64.5	1.1
Caudatum	IS31043	64.5	2.4
Guinea-caudatum	IS29950	64.5	1.6
Durra-caudatum	IS28313	64.4	1.3
Durra	IS22720	64.4	2.7
Caudatum	IS23514	64.4	1.8
Caudatum	IS4092	64.3	1.6
Caudatum	IS22986	64.3	2.4
Kafir	IS29519	64.3	2.2
Caudatum	IS31557	64.2	2.1
Caudatum-bicolor	IS23216	64.2	1.2
Guinea	IS33023	64.2	0.7
Durra	IS27034	64.2	1.7
Guinea	IS7310	64.2	1.4
Durra-bicolor	IS18039	64.2	3.4
Kafir	IS26737	64.1	3.6
Caudatum-bicolor	IS29326	64.1	0.9
Guinea	IS23684	64.0	1.1
Durra-bicolor	IS11619	63.9	0.1
Guinea-kafir	IS19450	63.9	1.5
Caudatum	IS20697	63.8	2.5
Guinea	IS27557	63.7	3.7
Guinea	IS16528	63.7	4.0
Guinea	IS15931	63.7	0.6
Durra-caudatum	IS30079	63.7	1.8
Guinea-caudatum	IS19262	63.6	3.0
Caudatum	IS26694	63.6	1.5
Bicolor	IS1233	63.6	2.3
Durra-bicolor	IS27786	63.5	0.7
Caudatum	IS12965	63.5	2.1
Guinea	IS7679	63.5	1.3
Kafir	IS22294	63.5	1.5

Race	Genotype	Starch.md	Starch.sd
Guinea-caudatum	IS30572	63.4	2.4
Kafir	IS29233	63.4	1.2
Durra-bicolor	IS25301	63.4	0.3
Kafir-caudatum	IS13919	63.4	1.5
Caudatum-bicolor	IS8777	63.4	0.2
Bicolor	IS24503	63.3	NA
Durra	IS11026	63.3	0.8
Caudatum	IS19389	63.3	1.8
Kafir	IS12937	63.3	4.0
Bicolor	IS24939	63.3	2.0
Guinea-caudatum	IS20679	63.3	1.9
Kafir	IS24492	63.2	2.5
Caudatum	IS29914	63.2	2.5
Guinea-caudatum	IS24953	63.2	0.9
Guinea	IS32349	63.2	1.9
Durra	IS5386	63.2	1.4
Caudatum	IS2379	63.1	1.1
Durra-caudatum	IS29314	63.0	0.3
Guinea-caudatum	IS15478	63.0	1.6
Durra	IS25732	63.0	2.7
Caudatum-bicolor	IS13294	63.0	3.3
Durra	IS5919	63.0	1.2
Guinea	IS25989	62.9	2.8
Guinea	IS7250	62.9	1.1
Caudatum-bicolor	IS30417	62.9	2.8
Kafir	IS24463	62.9	2.6
Durra-caudatum	IS29627	62.9	3.1
Guinea-caudatum	IS5301	62.8	2.7
Kafir	IS2397	62.8	0.6
Durra	IS31706	62.8	1.0
Guinea	IS19975	62.8	0.7
Guinea-caudatum	IS23521	62.8	2.2
Guinea	IS30838	62.7	0.8
Durra	IS1004	62.7	1.9

Race	Genotype	Starch.md	Starch.sd
Guinea-caudatum	IS31186	62.7	0.3
Guinea	IS21645	62.7	3.1
Caudatum-bicolor	IS30450	62.7	2.4
Kafir	IS30231	62.7	2.5
Durra	IS8348	62.6	1.8
Durra-caudatum	IS28141	62.6	1.8
Guinea-caudatum	IS26222	62.5	2.4
Guinea	IS25089	62.5	1.5
Guinea-caudatum	IS29187	62.5	1.5
Guinea-durra	IS4372	62.5	NA
Kafir	IS29392	62.4	1.4
Durra	IS5667	62.4	1.1
Kafir-caudatum	IS29441	62.4	1.3
Caudatum-bicolor	IS2902	62.3	3.0
Guinea	IS27697	62.3	3.1
Caudatum	IS13971	62.3	2.6
Durra	IS4581	62.3	1.8
Caudatum-bicolor	IS16151	62.3	0.4
Guinea	IS5295	62.3	1.8
Caudatum	IS33353	62.2	0.3
Kafir-bicolor	IS29654	62.2	2.6
Durra	IS4360	62.2	2.1
Guinea-caudatum	IS29468	62.2	1.9
Guinea-caudatum	IS23586	62.2	0.9
Guinea-caudatum	IS10969	62.2	0.5
Guinea-caudatum	IS10867	62.2	0.1
Caudatum	IS9745	62.2	0.0
Bicolor	IS12697	62.2	4.8
Durra	IS3971	62.2	2.8
Kafir	IS29689	62.2	2.2
Kafir	IS29239	62.1	1.3
Guinea	IS26025	62.1	0.4
Caudatum-bicolor	IS20298	62.1	2.8
Bicolor	IS20195	62.1	1.2

Race	Genotype	Starch.md	Starch.sd
Caudatum	IS30460	62.1	1.6
Kafir-durra	IS13782	62.1	0.1
Caudatum	IS14779	62.0	1.6
Guinea-caudatum	IS29565	62.0	3.0
Guinea-caudatum	IS23579	62.0	2.7
Kafir	IS2389	62.0	0.3
Bicolor	IS20816	61.9	4.3
Caudatum	IS2382	61.9	1.5
Kafir	IS26749	61.9	0.3
Guinea	IS4951	61.9	0.4
Bicolor	IS12804	61.9	0.7
Guinea-caudatum	IS23590	61.9	1.5
Durra-bicolor	IS4060	61.9	1.4
Durra-caudatum	IS29100	61.9	1.2
Caudatum	IS29335	61.9	0.5
Bicolor	IS20743	61.9	2.1
Caudatum-bicolor	IS30451	61.8	0.4
Caudatum	IS2864	61.8	3.2
Guinea-kafir	IS473	61.8	2.4
Caudatum-bicolor	IS2872	61.8	2.9
Kafir-caudatum	IS13893	61.8	1.7
Durra-caudatum	IS28849	61.8	0.7
Caudatum-bicolor	IS14010	61.7	1.6
Bicolor	IS3121	61.7	1.8
Bicolor	IS20740	61.7	2.8
Kafir-caudatum	IS29241	61.7	1.9
Kafir-caudatum	IS27912	61.7	1.8
Guinea	IS25910	61.7	1.9
Caudatum	IS24348	61.7	4.1
Durra	IS12883	61.7	0.3
Guinea	IS32439	61.6	0.9
Guinea-caudatum	IS20713	61.6	0.2
Bicolor	IS602	61.5	4.2
Durra	IS33090	61.5	1.0

Race	Genotype	Starch.md	Starch.sd
Guinea	IS21512	61.5	1.7
Caudatum-bicolor	IS995	61.5	1.2
Durra	IS6351	61.5	0.9
Durra	IS1041	61.5	2.1
Caudatum	IS15170	61.5	4.7
Durra-caudatum	IS29091	61.4	0.7
Kafir	IS29358	61.4	2.6
Durra	IS4515	61.4	0.3
Durra	IS25836	61.4	0.3
Guinea	IS24175	61.4	1.1
Caudatum	IS11473	61.4	1.0
Caudatum-bicolor	IS30507	61.2	0.7
Kafir	IS12945	61.2	0.8
Durra	IS4631	61.2	3.4
Durra	IS5094	61.2	0.3
Caudatum-bicolor	IS30536	61.2	0.4
Kafir-durra	IS14290	61.1	2.8
Kafir	IS19676	61.1	3.2
Kafir	IS22239	61.1	2.9
Bicolor	IS21863	61.1	0.7
Bicolor	IS22616	61.1	2.2
Guinea-caudatum	IS29269	61.0	3.4
Durra-caudatum	IS28389	61.0	0.7
Caudatum	IS23992	61.0	0.5
Caudatum-bicolor	IS26617	61.0	4.4
Caudatum-bicolor	IS12735	61.0	2.8
Durra	IS19859	60.9	2.6
Caudatum	IS7305	60.9	1.4
Guinea	IS24218	60.9	0.7
Caudatum-bicolor	IS26701	60.8	3.5
Durra	IS4613	60.8	1.9
Kafir	IS3158	60.6	NA
Bicolor	IS603	60.6	1.1
Guinea	IS7987	60.5	2.4

Race	Genotype	Starch.md	Starch.sd
Caudatum-bicolor	IS13549	60.5	2.3
Caudatum-bicolor	IS30383	60.5	1.1
Kafir-durra	IS8774	60.5	3.8
Durra	IS17980	60.5	1.1
Caudatum-bicolor	IS30443	60.5	3.9
Guinea-bicolor	IS1219	60.5	1.1
Durra-caudatum	IS20956	60.5	1.1
Guinea-caudatum	IS28449	60.4	2.0
Caudatum	IS10757	60.4	2.5
Caudatum-bicolor	IS30400	60.4	2.7
Guinea-caudatum	IS19153	60.3	0.4
Caudatum-bicolor	IS27887	60.3	1.7
Bicolor	IS608	60.2	1.0
Durra	IS6354	60.2	0.3
Caudatum-bicolor	IS12706	60.1	0.8
Durra	IS32787	60.1	NA
Caudatum-bicolor	IS30508	60.0	1.7
Bicolor	IS31681	60.0	2.4
Durra-bicolor	IS11919	60.0	1.1
Bicolor	IS20727	59.9	2.4
Caudatum-bicolor	IS2426	59.8	1.5
Durra-bicolor	IS25249	59.8	2.2
Guinea	IS26484	59.6	1.6
Durra-caudatum	IS32245	59.4	0.3
Guinea-durra	IS29733	59.4	2.2
Guinea	IS23644	59.4	1.5
Durra-caudatum	IS7131	59.3	2.2
Bicolor	IS8012	59.1	1.3
Durra	IS4698	59.0	1.6
Guinea-kafir	IS29304	59.0	2.5
Durra-caudatum	IS12447	58.8	0.5
Guinea-bicolor	IS7957	58.8	5.2
Kafir-durra	IS29714	58.7	1.2
Kafir-bicolor	IS1212	58.6	2.0

Race	Genotype	Starch.md	Starch.sd
Durra	IS23891	58.6	1.7
Caudatum	IS10302	58.6	2.3
Caudatum-bicolor	IS24453	57.9	1.1
Caudatum-bicolor	IS30466	57.3	3.2
Bicolor	IS2413	57.3	4.4

8.6 Summary Table of protein content by Genotypes in drought stress

Race	Genotype	Protein.md	Protein.sd
Durra	IS8348	14.7	NA
Durra-caudatum	IS28849	14.4	0.9
Kafir-bicolor	IS29654	14.2	0.3
Guinea-caudatum	IS28451	14.1	NA
Durra-bicolor	IS11919	14.1	0.0
Bicolor	IS20195	14.1	0.4
Guinea-caudatum	IS10969	14.0	0.3
Bicolor	IS21863	14.0	0.8
Kafir-durra	IS14290	13.9	0.1
Caudatum-bicolor	IS30507	13.8	0.6
Guinea-bicolor	IS1219	13.8	0.5
Guinea-kafir	IS473	13.8	0.1
Caudatum-bicolor	IS30450	13.6	0.1
Guinea-caudatum	IS20679	13.6	0.3
Bicolor	IS30562	13.6	1.4
Durra	IS12883	13.5	NA
Caudatum-bicolor	IS30451	13.5	1.0
Bicolor	IS22616	13.5	0.9
Durra	IS4360	13.5	0.3
Durra-caudatum	IS28747	13.5	0.3
Guinea	IS21645	13.5	NA
Caudatum-bicolor	IS30508	13.4	0.3
Durra-bicolor	IS27786	13.4	1.3
Caudatum-bicolor	IS30533	13.4	0.1
Guinea-caudatum	IS5301	13.3	0.3
Guinea-bicolor	IS7957	13.3	NA
Caudatum-bicolor	IS30443	13.3	0.6
Durra-caudatum	IS29627	13.3	0.5
Caudatum-bicolor	IS2872	13.2	0.4
Bicolor	IS20740	13.2	0.3
Durra	IS1041	13.2	0.1
Durra	IS19859	13.2	0.5

Race	Genotype	Protein.md	Protein.sd
Caudatum	IS11473	13.2	0.3
Guinea	IS24139	13.2	NA
Durra	IS5386	13.2	1.1
Durra-caudatum	IS28614	13.2	0.1
Kafir	IS29392	13.2	0.5
Durra-caudatum	IS28389	13.2	NA
Caudatum-bicolor	IS14010	13.1	0.5
Caudatum-bicolor	IS30417	13.1	0.0
Caudatum	IS15170	13.1	0.9
Caudatum	IS24348	13.1	1.2
Guinea	IS21512	13.1	0.8
Guinea-durra	IS29733	13.1	0.6
Guinea	IS25089	13.0	0.5
Kafir	IS29606	13.0	0.1
Kafir	IS30231	13.0	0.2
Guinea	IS5295	13.0	NA
Guinea	IS7250	13.0	NA
Durra-caudatum	IS28313	13.0	0.1
Durra	IS32787	13.0	NA
Bicolor	IS31681	13.0	0.7
Caudatum-bicolor	IS8777	13.0	0.9
Caudatum-bicolor	IS24462	13.0	0.6
Caudatum	IS9745	13.0	0.2
Caudatum	IS14779	12.9	0.4
Caudatum	IS10302	12.9	1.3
Guinea-caudatum	IS28449	12.9	0.3
Caudatum	IS14861	12.9	NA
Durra-bicolor	IS4060	12.9	0.6
Durra	IS22720	12.8	0.3
Guinea-caudatum	IS23590	12.8	0.2
Guinea	IS23644	12.8	NA
Guinea-caudatum	IS26222	12.8	0.4
Kafir-durra	IS13782	12.7	NA
Caudatum-bicolor	IS30383	12.7	0.4

Race	Genotype	Protein.md	Protein.sd
Kafir-caudatum	IS29241	12.7	NA
Caudatum-bicolor	IS30536	12.7	0.1
Guinea-caudatum	IS29187	12.7	0.0
Kafir-durra	IS8774	12.7	0.3
Guinea	IS4951	12.6	NA
Durra-caudatum	IS15744	12.6	0.4
Caudatum-bicolor	IS12706	12.6	0.9
Caudatum	IS2379	12.6	1.4
Kafir-caudatum	IS13893	12.6	0.1
Caudatum	IS9113	12.6	NA
Caudatum	IS23992	12.6	0.0
Kafir	IS2397	12.6	NA
Bicolor	IS608	12.6	0.6
Guinea-caudatum	IS30572	12.6	0.7
Kafir-bicolor	IS1212	12.5	0.3
Guinea	IS16382	12.5	0.9
Kafir-durra	IS29714	12.5	0.5
Guinea-caudatum	IS29565	12.5	0.4
Guinea-caudatum	IS29950	12.5	0.6
Kafir	IS24492	12.5	0.6
Guinea	IS32439	12.5	NA
Guinea-caudatum	IS10867	12.5	0.3
Caudatum	IS17941	12.4	0.4
Caudatum	IS7305	12.4	0.6
Kafir	IS3158	12.4	0.1
Bicolor	IS602	12.4	1.4
Caudatum	IS14090	12.4	0.7
Caudatum	IS29335	12.4	0.3
Guinea-caudatum	IS29468	12.4	0.1
Caudatum	IS9108	12.4	0.3
Kafir-caudatum	IS29568	12.3	0.6
Bicolor	IS1233	12.3	0.2
Caudatum-bicolor	IS20298	12.3	0.4
Guinea	IS32349	12.3	0.2

Race	Genotype	Protein.md	Protein.sd
Caudatum	IS12965	12.3	1.0
Guinea-caudatum	IS23521	12.3	0.3
Kafir	IS29358	12.3	0.3
Kafir	IS2389	12.3	0.2
Guinea-caudatum	IS29269	12.2	0.2
Caudatum-bicolor	IS30466	12.2	0.6
Durra	IS25732	12.2	NA
Guinea	IS7679	12.2	NA
Kafir	IS29689	12.2	NA
Guinea	IS30838	12.2	NA
Kafir	IS29519	12.2	NA
Kafir	IS22294	12.2	0.1
Kafir	IS22239	12.2	0.4
Durra-caudatum	IS32245	12.2	NA
Durra	IS22799	12.2	NA
Caudatum	IS22609	12.2	0.4
Kafir	IS26737	12.2	0.4
Kafir	IS26749	12.2	0.4
Caudatum	IS30460	12.1	0.6
Caudatum	IS10757	12.1	0.1
Bicolor	IS20816	12.1	0.6
Guinea-kafir	IS29304	12.1	NA
Guinea-caudatum	IS23579	12.1	NA
Guinea-caudatum	IS19262	12.0	0.5
Caudatum	IS20697	12.0	0.9
Kafir	IS29582	12.0	0.1
Guinea-caudatum	IS8916	12.0	NA
Bicolor	IS20743	12.0	0.4
Guinea	IS26484	12.0	0.6
Caudatum	IS29914	12.0	0.3
Guinea-caudatum	IS15478	12.0	1.0
Kafir	IS29233	11.9	0.5
Durra-caudatum	IS30092	11.9	0.4
Durra-caudatum	IS20956	11.9	0.2

Race	Genotype	Protein.md	Protein.sd
Caudatum	IS22986	11.9	0.2
Guinea-caudatum	IS20713	11.9	NA
Kafir-caudatum	IS29441	11.9	0.3
Durra	IS25836	11.9	0.2
Caudatum-bicolor	IS12735	11.9	0.2
Caudatum	IS33353	11.9	0.2
Caudatum	IS13971	11.9	0.1
Kafir	IS24463	11.9	0.7
Caudatum	IS19389	11.8	0.3
Caudatum-bicolor	IS26617	11.8	0.2
Durra-caudatum	IS12447	11.8	0.5
Caudatum-bicolor	IS2902	11.8	NA
Caudatum	IS2382	11.8	NA
Guinea-caudatum	IS15945	11.8	0.8
Caudatum	IS2864	11.7	0.8
Caudatum	IS26694	11.7	0.3
Kafir	IS29239	11.7	0.3
Bicolor	IS603	11.7	0.6
Caudatum-bicolor	IS29326	11.6	0.3
Durra	IS5919	11.6	NA
Guinea	IS16528	11.6	0.0
Caudatum	IS4092	11.6	0.2
Kafir	IS19676	11.5	0.4
Caudatum-bicolor	IS26701	11.4	0.1
Guinea	IS15931	11.4	1.0
Guinea	IS26046	11.4	1.0
Bicolor	IS20727	11.4	0.9
Caudatum-bicolor	IS16151	11.3	0.6
Caudatum-bicolor	IS24453	11.1	0.5
Guinea	IS7310	11.0	1.1
Bicolor	IS8012	11.0	0.0
Kafir-caudatum	IS13919	10.9	NA
Bicolor	IS3121	10.8	NA
Guinea	IS25989	10.8	NA

Race	Genotype	Protein.md	Protein.sd
Guinea	IS27557	10.8	0.2
Caudatum	IS31043	10.7	NA
Bicolor	IS24503	10.7	NA
Guinea	IS33023	10.5	NA
Guinea	IS25910	10.5	1.1
Guinea	IS19975	10.2	NA
Durra	IS4515	8.3	NA
Bicolor	IS12697	NA	NA
Bicolor	IS12804	NA	NA
Bicolor	IS2413	NA	NA
Bicolor	IS24939	NA	NA
Caudatum	IS12302	NA	NA
Caudatum	IS15466	NA	NA
Caudatum	IS20632	NA	NA
Caudatum	IS21083	NA	NA
Caudatum	IS23514	NA	NA
Caudatum	IS25548	NA	NA
Caudatum	IS31557	NA	NA
Caudatum	IS31651	NA	NA
Caudatum	IS9177	NA	NA
Caudatum-bicolor	IS13294	NA	NA
Caudatum-bicolor	IS13549	NA	NA
Caudatum-bicolor	IS23216	NA	NA
Caudatum-bicolor	IS2426	NA	NA
Caudatum-bicolor	IS27887	NA	NA
Caudatum-bicolor	IS30400	NA	NA
Caudatum-bicolor	IS995	NA	NA
Durra	IS1004	NA	NA
Durra	IS11026	NA	NA
Durra	IS17980	NA	NA
Durra	IS23891	NA	NA
Durra	IS27034	NA	NA
Durra	IS31706	NA	NA
Durra	IS33090	NA	NA

Race	Genotype	Protein.md	Protein.sd
Durra	IS3971	NA	NA
Durra	IS4581	NA	NA
Durra	IS4613	NA	NA
Durra	IS4631	NA	NA
Durra	IS4698	NA	NA
Durra	IS5094	NA	NA
Durra	IS5667	NA	NA
Durra	IS6351	NA	NA
Durra	IS6354	NA	NA
Durra	IS6421	NA	NA
Durra-bicolor	IS11619	NA	NA
Durra-bicolor	IS18039	NA	NA
Durra-bicolor	IS25249	NA	NA
Durra-bicolor	IS25301	NA	NA
Durra-caudatum	IS20625	NA	NA
Durra-caudatum	IS28141	NA	NA
Durra-caudatum	IS29091	NA	NA
Durra-caudatum	IS29100	NA	NA
Durra-caudatum	IS29314	NA	NA
Durra-caudatum	IS30079	NA	NA
Durra-caudatum	IS31714	NA	NA
Durra-caudatum	IS7131	NA	NA
Guinea	IS23684	NA	NA
Guinea	IS24175	NA	NA
Guinea	IS24218	NA	NA
Guinea	IS26025	NA	NA
Guinea	IS27697	NA	NA
Guinea	IS7987	NA	NA
Guinea-caudatum	IS19153	NA	NA
Guinea-caudatum	IS23586	NA	NA
Guinea-caudatum	IS24953	NA	NA
Guinea-caudatum	IS29772	NA	NA
Guinea-caudatum	IS31186	NA	NA
Guinea-caudatum	IS31446	NA	NA

Race	Genotype	Protein.md	Protein.sd
Guinea-durra	IS4372	NA	NA
Guinea-kafir	IS19450	NA	NA
Kafir	IS12937	NA	NA
Kafir	IS12945	NA	NA
Kafir	IS19445	NA	NA
Kafir-caudatum	IS27912	NA	NA

8.7 Summary Table of Protein by Genotypes in Well watered

Race	Genotype	Protein.md	Protein.sd
Bicolor	IS20740	14.3	1.1
Durra-caudatum	IS28747	14.0	0.4
Durra-caudatum	IS28614	13.9	0.9
Guinea	IS25910	13.8	1.6
Kafir	IS12937	13.8	0.5
Guinea	IS32349	13.6	0.6
Guinea-caudatum	IS30572	13.6	1.5
Caudatum-bicolor	IS24462	13.5	0.2
Bicolor	IS24503	13.5	NA
Durra	IS17980	13.5	0.6
Caudatum-bicolor	IS13549	13.4	0.5
Caudatum	IS12965	13.4	0.2
Guinea	IS23644	13.4	0.9
Guinea	IS24139	13.3	0.8
Durra	IS3971	13.3	0.4
Durra-bicolor	IS25249	13.3	0.3
Durra	IS4360	13.3	0.2
Guinea-caudatum	IS28451	13.2	NA
Guinea	IS25989	13.2	0.5
Caudatum-bicolor	IS8777	13.2	0.2
Kafir	IS19445	13.2	0.3
Durra	IS25732	13.2	0.6
Caudatum	IS24348	13.2	1.2
Guinea-bicolor	IS1219	13.1	0.6
Caudatum-bicolor	IS30400	13.1	0.4
Guinea	IS19975	13.1	0.5
Guinea	IS33023	13.1	0.8
Kafir-bicolor	IS29654	13.1	0.3
Guinea-caudatum	IS19153	13.0	0.4
Bicolor	IS21863	13.0	0.9
Guinea	IS24175	13.0	0.2
Guinea	IS21512	12.9	0.6

Race	Genotype	Protein.md	Protein.sd
Durra-caudatum	IS7131	12.9	0.4
Guinea-caudatum	IS10969	12.9	0.4
Guinea-kafir	IS473	12.9	0.2
Guinea	IS26046	12.8	0.2
Kafir	IS12945	12.8	0.8
Durra-bicolor	IS27786	12.8	1.0
Durra	IS22799	12.8	0.3
Guinea	IS27557	12.7	1.2
Durra	IS11026	12.7	0.3
Durra-caudatum	IS20625	12.7	1.3
Kafir	IS3158	12.7	NA
Guinea-caudatum	IS28449	12.7	0.7
Bicolor	IS20195	12.7	0.7
Caudatum-bicolor	IS12706	12.7	1.5
Durra	IS1041	12.7	0.5
Guinea-caudatum	IS20679	12.7	0.8
Guinea	IS4951	12.7	0.2
Bicolor	IS12697	12.6	0.8
Caudatum-bicolor	IS14010	12.6	0.9
Guinea-caudatum	IS20713	12.6	0.5
Bicolor	IS1233	12.6	0.2
Bicolor	IS22616	12.6	0.8
Durra-caudatum	IS30092	12.6	0.2
Durra-bicolor	IS25301	12.6	0.6
Durra-caudatum	IS28141	12.6	0.6
Durra	IS22720	12.5	0.5
Guinea	IS30838	12.5	0.5
Durra-bicolor	IS11619	12.5	1.0
Bicolor	IS602	12.5	0.8
Durra	IS5094	12.5	0.2
Guinea	IS5295	12.5	0.4
Guinea-durra	IS29733	12.5	0.4
Durra-caudatum	IS28313	12.5	0.8
Kafir	IS29606	12.5	1.4

Race	Genotype	Protein.md	Protein.sd
Kafir	IS2389	12.5	0.4
Bicolor	IS31681	12.5	0.3
Caudatum	IS20697	12.5	1.5
Durra	IS25836	12.4	0.0
Durra-bicolor	IS4060	12.4	0.5
Kafir-durra	IS14290	12.4	0.2
Durra	IS4515	12.4	0.7
Caudatum-bicolor	IS30507	12.4	0.6
Kafir-durra	IS8774	12.4	0.0
Guinea	IS24218	12.3	0.2
Caudatum	IS11473	12.3	0.4
Durra-caudatum	IS12447	12.3	0.3
Caudatum-bicolor	IS30466	12.3	0.5
Guinea	IS21645	12.3	0.0
Caudatum	IS12302	12.3	1.2
Bicolor	IS20816	12.3	0.4
Durra-caudatum	IS20956	12.3	0.5
Guinea-caudatum	IS5301	12.3	0.3
Durra	IS27034	12.3	0.5
Caudatum-bicolor	IS20298	12.3	0.5
Caudatum-bicolor	IS2872	12.3	0.7
Caudatum	IS31651	12.3	0.5
Caudatum	IS23992	12.3	0.8
Caudatum-bicolor	IS30443	12.3	0.6
Caudatum-bicolor	IS30533	12.3	0.0
Guinea-caudatum	IS23521	12.3	0.6
Guinea-caudatum	IS15478	12.3	0.8
Durra-caudatum	IS28389	12.3	0.3
Caudatum	IS29914	12.2	0.6
Durra	IS31706	12.2	0.3
Kafir	IS26737	12.2	0.5
Caudatum-bicolor	IS30417	12.2	0.5
Guinea	IS16382	12.2	0.8
Guinea-caudatum	IS23590	12.2	0.9

Race	Genotype	Protein.md	Protein.sd
Durra-bicolor	IS11919	12.2	0.2
Caudatum	IS22986	12.2	0.9
Guinea-caudatum	IS23586	12.2	0.1
Caudatum-bicolor	IS30450	12.2	0.3
Caudatum	IS14090	12.2	0.5
Kafir-caudatum	IS29441	12.1	0.8
Bicolor	IS603	12.1	0.8
Caudatum	IS9108	12.1	1.0
Durra-caudatum	IS28849	12.1	0.6
Guinea	IS27697	12.1	2.1
Guinea	IS26025	12.1	0.8
Kafir	IS22239	12.1	1.2
Guinea	IS7250	12.1	0.4
Kafir	IS29392	12.1	0.3
Guinea-caudatum	IS23579	12.1	0.4
Bicolor	IS20743	12.1	0.4
Durra	IS5386	12.0	0.7
Guinea	IS7310	12.0	0.5
Guinea	IS23684	12.0	0.2
Durra	IS33090	12.0	0.0
Durra	IS4613	12.0	0.1
Caudatum	IS7305	12.0	0.2
Kafir-caudatum	IS29568	12.0	0.3
Caudatum	IS2379	12.0	0.0
Guinea	IS16528	12.0	0.5
Caudatum	IS15170	12.0	0.7
Guinea-caudatum	IS24953	11.9	0.3
Guinea	IS32439	11.9	0.7
Bicolor	IS30562	11.9	NA
Durra	IS4698	11.9	0.3
Caudatum	IS25548	11.9	0.5
Kafir-durra	IS29714	11.9	0.3
Caudatum	IS2382	11.9	0.3
Guinea	IS15931	11.9	0.3

Race	Genotype	Protein.md	Protein.sd
Guinea	IS25089	11.9	0.3
Durra-caudatum	IS29627	11.9	0.6
Caudatum	IS2864	11.9	0.8
Kafir	IS24492	11.9	0.5
Durra	IS5667	11.9	0.8
Caudatum-bicolor	IS27887	11.9	0.2
Kafir	IS2397	11.9	0.7
Caudatum-bicolor	IS30536	11.9	0.3
Caudatum-bicolor	IS29326	11.8	0.5
Guinea	IS26484	11.8	0.7
Kafir	IS29689	11.8	0.2
Caudatum	IS4092	11.8	0.5
Durra	IS6421	11.8	0.5
Guinea-kafir	IS19450	11.8	0.5
Guinea-kafir	IS29304	11.8	0.6
Guinea-caudatum	IS26222	11.8	0.6
Durra	IS23891	11.8	0.2
Durra	IS5919	11.8	1.0
Guinea-caudatum	IS10867	11.8	0.0
Kafir	IS30231	11.8	0.3
Bicolor	IS3121	11.8	0.3
Caudatum	IS10302	11.8	0.3
Caudatum-bicolor	IS26617	11.8	0.4
Caudatum	IS17941	11.7	1.2
Caudatum-bicolor	IS12735	11.7	0.9
Guinea-bicolor	IS7957	11.7	0.1
Kafir-bicolor	IS1212	11.7	0.7
Durra	IS19859	11.7	0.8
Guinea-caudatum	IS19262	11.7	0.3
Guinea	IS7987	11.7	0.3
Durra	IS1004	11.7	0.4
Kafir-durra	IS13782	11.7	0.6
Guinea	IS7679	11.7	0.3
Kafir	IS26749	11.7	0.6

Race	Genotype	Protein.md	Protein.sd
Durra	IS8348	11.7	0.4
Durra-caudatum	IS30079	11.7	0.5
Kafir	IS29358	11.6	0.7
Caudatum-bicolor	IS13294	11.6	0.1
Caudatum	IS14779	11.6	0.2
Guinea-caudatum	IS29950	11.6	0.1
Caudatum	IS31557	11.6	1.0
Bicolor	IS608	11.6	0.3
Caudatum-bicolor	IS30451	11.5	0.2
Guinea-caudatum	IS31446	11.5	1.0
Caudatum-bicolor	IS2426	11.5	0.1
Caudatum	IS10757	11.5	0.2
Durra-caudatum	IS32245	11.5	0.3
Kafir-caudatum	IS27912	11.5	0.4
Caudatum	IS26694	11.5	1.5
Kafir	IS24463	11.5	1.5
Caudatum	IS23514	11.5	0.4
Caudatum-bicolor	IS30508	11.5	0.4
Guinea-caudatum	IS29187	11.4	1.0
Caudatum	IS20632	11.4	0.9
Durra-bicolor	IS18039	11.4	0.5
Durra-caudatum	IS29314	11.4	1.0
Durra	IS4631	11.4	0.3
Guinea-caudatum	IS29565	11.4	0.2
Kafir	IS22294	11.3	1.0
Durra-caudatum	IS29100	11.3	0.7
Guinea-caudatum	IS8916	11.3	0.8
Bicolor	IS2413	11.3	0.7
Kafir	IS19676	11.3	0.2
Caudatum-bicolor	IS30383	11.3	0.3
Durra-caudatum	IS31714	11.3	NA
Kafir-caudatum	IS13919	11.3	0.3
Caudatum-bicolor	IS2902	11.3	0.8
Kafir	IS29233	11.2	0.4

Race	Genotype	Protein.md	Protein.sd
Caudatum-bicolor	IS16151	11.2	0.3
Durra	IS32787	11.2	NA
Caudatum	IS14861	11.2	0.7
Guinea-caudatum	IS29269	11.2	0.3
Caudatum-bicolor	IS24453	11.1	0.3
Durra	IS12883	11.1	1.4
Guinea-caudatum	IS29468	11.1	0.7
Bicolor	IS24939	11.1	0.4
Kafir	IS29582	11.1	0.6
Caudatum-bicolor	IS26701	11.0	0.7
Bicolor	IS12804	11.0	0.6
Guinea-durra	IS4372	11.0	NA
Caudatum	IS31043	11.0	0.9
Caudatum-bicolor	IS995	11.0	0.3
Kafir	IS29239	11.0	0.0
Bicolor	IS20727	10.9	0.4
Caudatum	IS21083	10.9	0.2
Guinea-caudatum	IS15945	10.9	0.2
Caudatum	IS9113	10.9	0.4
Caudatum	IS29335	10.8	0.8
Caudatum-bicolor	IS23216	10.8	0.8
Caudatum	IS33353	10.8	0.3
Caudatum	IS13971	10.8	0.5
Guinea-caudatum	IS31186	10.8	0.6
Durra	IS6351	10.8	0.6
Kafir-caudatum	IS29241	10.8	0.4
Durra	IS6354	10.7	0.3
Caudatum	IS19389	10.6	0.4
Kafir	IS29519	10.6	1.1
Caudatum	IS9177	10.6	1.3
Durra-caudatum	IS15744	10.6	0.5
Kafir-caudatum	IS13893	10.6	0.2
Durra	IS4581	10.6	0.8
Caudatum	IS30460	10.4	0.5

Race	Genotype	Protein.md	Protein.sd
Caudatum	IS9745	10.4	0.3
Durra-caudatum	IS29091	10.4	0.4
Caudatum	IS22609	10.4	0.2
Guinea-caudatum	IS29772	10.3	NA
Bicolor	IS8012	10.2	0.0
Caudatum	IS15466	9.4	0.4

8.8 List of Significant Differentially expressed genes in sorghum roots between well-watered (WW) and drought water-stress (WS)

ID	log2FoldChange	WS-SG-rep1	WS-SG-rep2	WS-SG-rep3	WW-SG-rep1	WW-SG-rep2	WW-SG-rep3	Biological processes	Locus tag/synonym
LOC8057813	7.8978028	97.6584	29.1529	1.0991	0	0	0	chlorophyllase-1	SORBI_3007G168000
LOC8065192	7.4867337	1.00679	352.75	15.3874	0	2.14386	0	low-temperature-induced 65 kDa protein	SORBI_3001G200700
LOC8061116	7.3629738	0	155.482	16.4865	0.99508	0	0	probable non-specific lipid-transfer protein 2	SORBI_3009G222000
LOC8077956	7.2698239	24.1629	56.3623	2.1982	0	0	0	putative NAD kinase 3	SORBI_3003G173700
LOC8069599	6.891603	294.989	461.588	179.153	7.96063	0	0	methylsterol monooxygenase 1-1	SORBI_3003G173700
LOC8078187	6.8708413	1141.7	222.534	197.838	3.98031	3.21579	6.03046	thiamine thiazole synthase 1, chloroplastic	SORBI_3003G191000
LOC8060280	6.7361561	56.3801	50.5317	4.39639	0	1.07193	0	protein ECERIFERUM 3	SORBI_3002G207900

LOC8060647	6.6008622	61.414	107.866	16.4865	0.99508	0	0.86149	vegetative cell wall protein gp1	SORBI_3002G365700
LOC8059830	5.8918186	8.0543	428.548	30.7747	5.97047	1.07193	0.86149	ABA-inducible protein PHV A1	SORBI_3001G492000
LOC8073244	5.7178294	5.03394	231.28	18.6847	3.98031	0	0.86149	11 kDa late embryogenesis abundant protein	SORBI_3007G088300
LOC8073071	5.3636346	3.02036	378.988	48.3603	6.96555	0	3.44598	uncharacterised	SORBI_3009G168800
LOC8083675	5.2335833	13.0882	412.999	85.7297	9.95078	1.07193	2.58448	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	SORBI_3001G384100
LOC8077913	5.2005718	99.6719	4579.92	379.189	76.621	38.5895	22.3988	dehydrin DHN1	SORBI_3009G116700
LOC8070042	5.1060346	686.629	416.887	35.1711	19.9016	10.7193	2.58448	COBRA-like protein 5	SORBI_3001G336700
LOC8078746	5.023312	489.299	7.77411	32.9729	8.95571	2.14386	5.16896	phospholipase A1-Igamma1, chloroplastic	SORBI_3003G038000
LOC8076229	4.8871422	1405.47	268.207	90.126	46.7687	8.57544	4.30747	expansin-B11	SORBI_3004G294400

LOC8085057	4.7049936	213.439	53.447	19.7838	5.97047	4.28772	0.86149	uncharacterized LOC8085057	SORBI_3003G108000
LOC8055826	4.6369811	15.1018	549.046	93.4233	12.936	7.50351	6.03046	eukaryotic peptide chain release factor subunit 1-2	SORBI_3009G118600
LOC8070506	4.484199	11.0747	144.793	87.9279	7.96063	2.14386	0.86149	beta-amylase 1, chloroplastic	SORBI_3001G226600
LOC8062599	4.4700311	16.1086	367.327	96.7206	18.9065	1.07193	1.72299	uncharacterized LOC8062599	SORBI_3002G143300
LOC8072395	4.4525397	171.154	22.3506	3.29729	1.99016	5.35965	1.72299	uncharacterized LOC8072395	SORBI_3003G111200
LOC8074136	4.4371217	518.495	293.473	344.018	13.9311	21.4386	18.0914	transcription factor PCL1	NA
LOC8076953	4.2522492	170.147	62.1929	13.1892	9.95078	2.14386	0.86149	protein NRT1/ PTR FAMILY 3.1	SORBI_3009G136500
LOC8062302	4.1918639	2327.69	271.122	137.387	87.5669	22.5105	39.6287	glucan endo-1,3-beta-glucosidase GII	SORBI_3003G422100
LOC110437142	4.1883788	329.219	123.414	40.6666	5.97047	5.35965	15.5069	uncharacterized LOC110437142	SORBI_3001G480400

LOC110437016	4.0949951	114.774	350.807	134.09	22.8868	5.35965	6.89195	glutamate decarboxylase-like	SORBI_3007G152800
LOC110434162	3.9160937	35.2375	37.8988	30.7747	2.98524	3.21579	0.86149	protein NRT1/ PTR FAMILY 6.2	SORBI_3003G185100
LOC8070256	3.8388243	342.308	334.287	57.1531	30.8474	7.50351	12.9224	linoleate 9S-lipoxygenase	SORBI_3006G248300
LOC8081532	3.8328806	436.946	116.612	35.1711	6.96555	26.7983	7.75344	uncharacterized LOC8081532	SORBI_3001G426400
LOC110435396	3.6470257	432.918	51.5035	27.4775	20.8966	9.64737	10.3379	uncharacterized protein At3g49140-like	SORBI_3005G142900
LOC8074585	3.5440201	23.1561	39.8423	17.5856	2.98524	3.21579	0.86149	transcription factor LG2	SORBI_3009G230000
LOC8072396	3.4214142	101.686	75.7976	151.676	8.95571	16.079	6.03046	expansin-A9	SORBI_3003G112100
LOC8054836	3.412387	1046.05	191.437	54.9549	33.8327	69.6755	18.0914	protein TsetseEP	SORBI_3007G164750
LOC8069067	3.3926287	36.2443	52.4752	14.2883	4.97539	3.21579	1.72299	NAC domain-containing protein 43	SORBI_3010G022400
LOC110434089	3.3504203	310.09	147.708	17.5856	19.9016	18.2228	8.61494	lysine-rich arabinogalactan protein 19-like	SORBI_3003G100900
LOC8060192	3.3254866	6243.09	3446.85	1557.42	331.361	411.621	379.057	endochitinase A	SORBI_3006G132300

LOC8075538	3.1905008	396.674	378.988	267.081	60.6998	27.8702	25.8448	heat stress transcription factor C-1b	SORBI_3003G286700
LOC8083963	3.0981497	458.088	65.1082	109.91	14.9262	13.9351	44.7977	probable calcium-binding protein CML41	SORBI_3001G076300
LOC8078551	3.0830693	817.511	764.778	222.018	70.6506	90.0421	52.5511	peroxidase 70	SORBI_3002G416500
LOC8086147	3.0765832	653.405	601.522	352.811	143.291	31.086	16.3684	tropomyosin-1	SORBI_3008G087500
LOC8085514	3.0580581	841.674	197.268	147.279	78.6112	12.8632	50.8281	noroxomaritidine synthase	SORBI_3001G510400
LOC8056949	3.0029864	864.83	481.023	148.378	76.621	40.7333	68.9195	uncharacterized LOC8056949	SORBI_3006G002900
LOC8071496	2.9338452	259.751	176.861	228.612	28.8573	40.7333	18.0914	cation/calcium exchanger 1	SORBI_3008G179200
LOC8085757	2.8816065	175.181	113.696	69.2432	22.8868	12.8632	12.9224	anti-sigma-I factor RsgI6	SORBI_3006G032800
LOC8070491	2.8800124	110.747	34.9835	30.7747	11.9409	9.64737	2.58448	long-chain-alcohol oxidase FAO1	SORBI_3001G221200
LOC8067828	2.851623	97.6584	56.3623	25.2793	5.97047	13.9351	5.16896	transcription factor MYB41	SORBI_3007G039100
LOC8060193	2.8138334	2912.64	1093.23	464.919	110.454	413.765	111.994	endochitinase A	SORBI_3006G132400

LOC8055281	2.775732	1707.51	2360.41	204.432	172.149	158.646	292.908	pathogenesis-related protein	SORBI_3003G331700
LOC8083539	2.7610915	244.649	91.3458	69.2432	41.7933	8.57544	9.47643	alpha-amylase isozyme 3B	SORBI_3007G156400
LOC8061402	2.7174122	1281.64	333.315	320.937	147.272	60.0281	87.0109	ankyrin repeat domain-containing protein 2A	SORBI_3001G010100
LOC8068762	2.7038282	135.916	89.4022	51.6576	10.9459	16.079	15.5069	probable sulfate transporter 3.4	SORBI_3010G033800
LOC8057046	2.6806942	66.448	230.308	65.9459	9.95078	17.1509	29.2908	actin-depolymerizing factor 3	SORBI_3001G034900
LOC8078880	2.6607957	237.602	84.5434	26.3784	22.8868	15.007	17.2299	aldehyde dehydrogenase family 3 member F1	SORBI_3004G300800
LOC8060875	2.6586739	131.889	238.082	130.793	40.7982	26.7983	12.0609	uncharacterized protein At1g28695	SORBI_3001G012700
LOC8065615	2.6529589	361.437	143.821	120.901	27.8622	55.7404	16.3684	probable methyltransferase PMT27	SORBI_3010G261900
LOC8069000	2.5431947	313.111	140.906	132.991	46.7687	24.6544	29.2908	myb-related protein Hv33	SORBI_3009G216900

LOC8056582	2.5318596	94.638	125.357	285.766	35.8228	31.086	20.6759	expansin-A9	SORBI_3003G059900
LOC8075122	2.5105268	715.826	515.035	209.928	71.6456	108.265	73.227	fasciclin-like arabinogalactan protein 11	SORBI_3003G062000
LOC8070077	2.464868	31.2104	83.5717	95.6215	16.9163	11.7912	9.47643	uncharacterized LOC8070077	SORBI_3007G125600
LOC8059531	2.4633982	1880.68	381.903	257.189	163.193	148.998	144.731	14 kDa proline-rich protein DC2.15	SORBI_3001G541300
LOC8073951	2.4509554	951.414	250.715	174.757	88.562	99.6895	63.7505	expansin-B11	SORBI_3004G294300
LOC110431693	2.3644132	984.638	449.927	217.621	128.365	94.3299	98.2103	protein IN2-1 homolog B-like	SORBI_3001G412800
LOC8054592	2.3253373	573.869	309.993	125.297	46.7687	113.625	41.3517	putrescine hydroxycinnamoyltransferase 1	SORBI_3002G175600
LOC8081576	2.3211464	57.3869	54.4188	23.0811	11.9409	6.43158	8.61494	potassium transporter 22	SORBI_3002G001800
LOC8081333	2.3088282	478.224	91.3458	197.838	71.6456	47.1649	36.1827	probable glutathione S-transferase	SORBI_3003G425850
LOC8077410	2.2849132	66.448	70.9387	214.324	27.8622	28.9421	15.5069	serine decarboxylase 1	SORBI_3003G437900
LOC8078573	2.2744258	451.041	271.122	136.288	110.454	30.014	37.0442	ricin B-like lectin R40C1	SORBI_3002G420800

LOC8155716	2.2494023	637.296	373.157	163.766	116.424	100.761	30.1523	probable beta-D-xylosidase 7	SORBI_3005G110436
LOC8066586	2.2296088	360.43	110.781	84.6306	39.8031	55.7404	23.2603	uncharacterized LOC8066586	SORBI_3009G001800
LOC8057306	2.0771483	747.036	741.456	492.396	146.277	188.66	135.255	4-hydroxyphenylacetaldehyde oxime monooxygenase-like	SORBI_3002G110200
LOC8065163	2.023844	156.052	88.4305	65.9459	23.8819	20.3667	31.8753	cytochrome P450 89A2	SORBI_3001G195500
LOC8061318	2.0138393	224.514	204.07	249.495	77.6161	37.5176	52.5511	uncharacterized LOC8061318	SORBI_3009G100200
LOC110430724	2.0100548	112.76	76.7693	49.4594	17.9114	11.7912	29.2908	catalase isozyme 2	SORBI_3001G517700
LOC8065156	1.9724087	203.371	331.371	102.216	39.8031	91.1141	31.8753	uncharacterized LOC8065156	SORBI_3001G193400
LOC110436298	1.9633962	145.984	206.014	87.9279	29.8524	51.4527	31.8753	vegetative cell wall protein gp1-like	
LOC8064914	1.9545144	237.602	136.047	58.2522	37.813	37.5176	36.1827	3-ketoacyl-CoA synthase 20	SORBI_3001G247700
LOC8066848	1.9346959	236.595	107.866	236.306	63.685	40.7333	47.3822	glycine-rich protein 2	SORBI_3006G228000

LOC8060268	1.8716949	1192.04	679.263	250.594	241.804	227.249	111.133	cellulose synthase A catalytic subunit 9 [UDP-forming]	SORBI_3002G205500
LOC8054565	1.8365672	590.984	238.082	355.009	149.262	109.337	73.227	probable protein phosphatase 2C 68	SORBI_3002G172000
LOC8057963	1.8001305	1700.46	494.628	460.522	318.425	266.911	177.468	expansin-B11	SORBI_3002G124400
LOC8079518	1.7973654	75.509	64.1364	49.4594	21.8917	17.1509	15.5069	uncharacterized LOC8079518	SORBI_3003G018000
LOC8085403	1.748263	6642.78	3805.43	3609.44	1169.22	1220.93	1793.63	expansin-A2	SORBI_3003G338801
LOC8071096	1.7289892	192.296	139.934	209.928	66.6703	45.0211	51.6896	uncharacterized LOC8071096	SORBI_3006G199900
LOC8057776	1.7153546	492.319	1510.12	440.738	311.46	198.307	234.326	heat shock 70 kDa protein	SORBI_3003G350700
LOC8054251	1.7086937	1002.76	757.004	619.891	348.277	274.414	105.964	putative polyol transporter 1	SORBI_3001G469600
LOC8060334	1.69363	91.6176	58.3058	79.1351	16.9163	28.9421	24.9833	uncharacterized LOC8060334	SORBI_3003G146300
LOC8066008	1.6801556	136.923	93.2893	69.2432	27.8622	31.086	34.4598	thaumatin-like protein 1b	SORBI_3006G015900

LOC8076128	1.6626686	1589.72	1081.57	986.99	526.396	286.205	342.875	uncharacterized LOC8076128	SORBI_3004G059600
LOC8064848	1.6377088	388.62	496.571	401.171	137.321	150.07	126.64	lysine-specific demethylase JMJ30	SORBI_3002G247200
LOC8063551	1.6161535	854.762	455.757	290.162	160.208	217.602	144.731	uncharacterized LOC8063551	SORBI_3009G223500
LOC8057100	1.6135379	923.224	1173.89	682.54	377.135	349.449	182.637	probable galactinol--sucrose galactosyltransferase 2	SORBI_3001G044800
LOC8066437	1.5773316	1000.75	951.356	846.306	371.164	337.658	230.019	aquaporin NIP2-1	SORBI_3004G238100
LOC8062620	1.5592216	892.013	429.519	429.747	173.144	278.702	143.008	probable glutathione S-transferase GSTU6	SORBI_3001G317300
LOC8085503	1.5503858	774.219	534.47	338.522	296.533	130.775	135.255	transcription factor MYB2	SORBI_3001G508400
LOC8072479	1.508342	1828.33	1057.28	911.152	441.815	381.607	510.866	two-component response regulator-like PRR1	SORBI_3004G216700
LOC8082543	1.4736193	1081.29	482.966	607.801	269.666	226.177	286.016	indole-3-acetic acid-induced protein ARG7	SORBI_3006G252466

LOC8085173	1.427879	5718.55	12366.7	13501.3	4330.58	3966.14	3443.39	sucrose:sucrose 1-fructosyltransferase	SORBI_3004G004800
LOC8064968	1.4103923	6760.58	3300.11	3078.57	1493.61	1309.9	2139.09	arogenate dehydrogenase 2, chloroplastic	
LOC8061378	1.3896987	927.251	353.722	497.891	204.986	196.163	277.401	gibberellin 20 oxidase 1-D	SORBI_3001G005300
LOC8055296	1.3892395	180.215	118.555	190.144	68.6604	50.3807	67.1965	protein LURP-one-related 8	SORBI_3003G335900
LOC8077270	1.3756202	685.622	420.774	518.774	171.153	186.516	267.925	CDGSH iron-sulfur domain-containing protein NEET	SORBI_3002G297900
LOC8077060	1.3619981	57.3869	109.809	29.6757	43.7834	13.9351	18.9529	lazy gene	SORBI_3001G342000
LOC8063571	1.318128	1758.86	1007.72	1034.25	494.554	597.065	433.331	cytochrome b561 and DOMON domain-containing protein At3g61750	SORBI_3009G226900
LOC8057377	1.3094097	1635.02	828.914	635.279	339.322	486.656	424.716	floral homeotic protein APETALA 2	SORBI_3001G036800

LOC110435576	1.3091475	695.69	646.223	728.702	279.617	214.386	340.29	two-component response regulator ORR9-like	SORBI_3005G030500
LOC8077100	1.3074579	241.629	222.534	319.838	91.5472	130.775	94.7643	uncharacterized LOC8077100	SORBI_3001G347400
LOC110437683	1.3061636	1313.86	756.032	730.9	366.189	289.421	476.406	uncharacterized LOC110437683	SORBI_3008G149450
LOC8084801	1.2938167	1508.17	1275.93	1435.42	661.727	558.476	501.389	glucuronoxylan 4-O-methyltransferase 1	SORBI_3008G072600
LOC8063131	1.2926791	828.586	554.877	342.919	308.474	216.53	180.052	probable pectinesterase/pectinesterase inhibitor 51	SORBI_3003G376900
LOC8062757	1.2912877	159.072	179.776	282.468	96.5226	56.8123	99.9333	transmembrane protein 45B	SORBI_3002G219000
LOC8066090	1.2865014	718.846	413.971	353.91	214.937	188.66	205.897	protein AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE	SORBI_3007G015600

LOC8066090	1.2865014	718.846	413.971	353.91	214.937	188.66	205.897	protein AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE	SORBI_3007G015600
LOC8085586	1.2721013	800.396	520.865	430.846	203.991	246.544	274.817	potassium channel KAT1	SORBI_3004G107500
LOC8085191	1.2657187	548.699	706.472	419.855	278.622	199.379	218.819	uncharacterized LOC8085191	SORBI_3004G009300
LOC8055798	1.2597696	2074.99	1014.52	1037.55	591.077	555.26	577.201	probable cinnamyl alcohol dehydrogenase 8D	SORBI_3002G195700
LOC8085131	1.2572247	544.672	584.03	692.432	356.238	201.523	204.174	protein NRT1/ PTR FAMILY 3.1	SORBI_3009G136700
LOC8060278	1.2541263	2125.33	1428.49	1748.67	1086.63	638.87	497.943	WAT1-related protein At4g30420	SORBI_3002G207700
LOC8059245	1.2450494	96.6516	117.583	146.18	43.7834	49.3088	58.5816	uncharacterized LOC8059245	SORBI_3001G004000
LOC8078123	1.2290258	524.536	949.413	796.846	311.46	370.888	286.877	lamin-like protein	SORBI_3003G134900
LOC8082152	1.2271756	492.319	359.552	355.009	177.124	214.386	124.917	sulfate transporter 3.1	SORBI_3001G496800

LOC110436819	1.2030743	353.382	649.138	431.946	196.03	167.221	259.31	uncharacterized LOC110436819	ncRNA
LOC8073401	1.2026771	4647.33	3415.75	6877.06	1778.21	2511.53	2201.12	arabinogalactan protein 1	SORBI_3006G100501
LOC8082113	1.1732197	2444.48	1693.78	1358.49	745.314	925.076	767.591	sucrose transport protein SUT1	SORBI_3001G488700
LOC8063405	1.1580517	517.489	265.291	353.91	202.001	157.574	149.9	protein FAF-like, chloroplastic	SORBI_3001G121700
LOC8078858	1.1543778	673.541	659.827	629.783	351.263	264.767	266.202	anti-sigma-I factor RsgI6	SORBI_3001G439400
LOC8076954	1.1181129	348.348	276.953	229.712	121.4	176.868	96.4873	protein NRT1/ PTR FAMILY 3.1	SORBI_3009G136600
LOC8076120	1.111255	144.977	164.228	105.513	65.6752	60.0281	66.335	uncharacterized LOC8076120	SORBI_3004G056300
LOC8086393	1.1091518	1139.68	1482.91	1655.24	753.274	480.225	748.638	cytochrome P450 84A1	SORBI_3002G029500
LOC8081145	1.1054652	654.412	540.301	1034.25	303.499	385.895	346.321	flavonoid 3'-monooxygenase	SORBI_3002G040500
LOC110434657	1.1049895	3342.53	1466.39	3131.33	1249.82	1188.77	1252.61	stearoyl-[acyl-carrier-protein] 9-desaturase 1, chloroplastic-like	SORBI_3001G280700

LOC8076245	1.0946516	16468	11975	16532.6	5307.75	8714.79	7037.54	vegetative cell wall protein gp1	SORBI_3004G297950
LOC8085850	1.0930598	991.685	1252.6	1815.71	692.575	470.577	739.162	two-component response regulator ORR6	SORBI_3006G263300
LOC110429657	1.0929785	266.799	283.755	357.207	130.355	145.783	149.038	protein-lysine 6-oxidase-like	SORBI_3008G055000
LOC8082934	1.090368	6678.02	9737.07	12461.6	4411.18	3860.02	5289.57	uncharacterized LOC8082934	SORBI_3004G086500
LOC110434224	1.0806413	634.276	360.524	448.432	257.725	196.163	228.296	clathrin interactor EPSIN 3-like	SORBI_3003G351701
LOC8083262	1.0756844	16546.5	22336	34031.4	9508.97	13404.5	11680.1	arabinogalactan protein 1	none
LOC8076877	1.0723091	4646.32	4185.39	3969.94	1959.31	1904.82	2222.65	uncharacterized LOC8076877	SORBI_3009G130900
LOC8072375	1.058769	196.323	136.047	129.694	78.6112	77.179	66.335	uncharacterized LOC8072375	SORBI_3003G108500
LOC8069792	1.0560944	783.28	543.216	458.324	257.725	290.493	310.138	alpha-amylase 3, chloroplastic	SORBI_3003G276400
LOC8066250	1.0445988	667.5	770.608	502.288	250.76	351.593	338.567	peroxidase 70	SORBI_3001G328100

LOC8067204	1.0350491	118.801	131.188	142.883	64.6801	51.4527	74.95	heptahelical transmembrane protein 4	SORBI_3008G110400
LOC8058966	1.0288938	453.054	366.355	392.378	209.962	129.704	253.279	UDP-glucuronate 4-epimerase 6	SORBI_3002G256100
LOC8079099	1.0159724	4698.68	2516.87	3448.97	2108.57	1667.92	1497.28	patatin-like protein 2	SORBI_3005G186400
LOC8060874	1.0120084	51274.7	24862.6	38476.1	20776.2	15682.3	20372.6	cyanohydrin beta-glucosyltransferase	SORBI_3001G012400
LOC8072397	1.0061561	532.59	606.38	707.819	343.302	340.874	236.049	germin-like protein 5-1	SORBI_3003G112200
LOC8079182	0.7468919	30.2036	55.3905	58.2522	31.8425	28.9421	24.9833	lazy gene	SORBI_3002G373700
LOC8073048	-1.0110959	946.38	860.982	1116.68	1712.53	1887.67	2290.71	vacuolar amino acid transporter 1	SORBI_3010G146700
LOC8054875	-1.0136997	552.726	437.294	482.504	883.63	1248.8	841.679	E3 ubiquitin ligase BIG BROTHER	SORBI_3002G270300
LOC110432906	-1.026917	1137.67	607.352	1034.25	1574.21	1790.12	2297.6	2-oxoisovalerate dehydrogenase subunit beta 1, mitochondrial	SORBI_3002G046800

LOC8083888	-1.0338912	507.421	550.018	650.666	1022.94	1332.41	1141.48	U1 small nuclear ribonucleoprotein 70 kDa	SORBI_3001G274200
LOC8056029	-1.0451275	1115.52	1174.86	1395.85	2511.58	2640.16	2453.53	GEM-like protein 4	SORBI_3006G154500
LOC8059991	-1.0460087	41801.8	31009.9	19673.9	54758.2	63427.2	72780.7	phytochrome a	SORBI_3001G111500
LOC8071347	-1.0526507	351.369	304.162	295.657	537.342	727.841	708.148	long chain acyl-CoA synthetase 4	SORBI_3010G045400
LOC8083352	-1.0530587	1264.52	640.392	934.233	1541.38	1913.4	2435.44	nudix hydrolase 8	SORBI_3006G168300
LOC110436331	-1.0576544	1707.51	1015.49	1792.63	2940.46	2691.62	3765.59	SNF1-related protein kinase regulatory subunit gamma-1	SORBI_3006G047000
LOC8065159	-1.083146	667.5	482.966	401.171	1036.87	776.077	1474.02	electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	SORBI_3001G194900
LOC8067521	-1.0842975	623.201	780.326	1362.88	2200.12	1703.3	1960.76	LOB domain-containing protein 42	protein coding

LOC8065116	-1.086211	121.821	134.103	160.468	304.494	273.342	304.969	tyrosine-sulfated glycopeptide receptor 1	
LOC8071258	-1.087248	1994.45	1691.84	1769.55	2876.77	3290.83	5423.1	isovaleryl-CoA dehydrogenase, mitochondrial	SORBI_3009G027000
LOC8067338	-1.0937095	404.728	441.181	295.657	809.994	984.032	644.397	ACT domain-containing protein ACR9	SORBI_3009G015100
LOC8065128	-1.1058616	60.4072	89.4022	94.5224	181.104	141.495	202.451	peroxidase 45	SORBI_3010G245400
LOC8083911	-1.1424995	65.4412	68.0234	89.027	170.158	186.516	134.393	receptor kinase-like protein Xa21	SORBI_3001G064700
LOC8056046	-1.1583813	278.88	177.833	224.216	359.223	642.086	518.619	L-type lectin-domain containing receptor kinase IV.1	SORBI_3006G158266
LOC8074416	-1.1651553	179.208	179.776	300.054	414.948	411.621	649.566	lipase	SORBI_3002G409500
LOC8081891	-1.1963106	284.921	353.722	251.693	582.121	773.934	685.749	low affinity sulfate transporter 3	SORBI_3001G470900

LOC8082882	-1.2089119	240.622	196.296	360.504	519.431	762.142	560.832	L-type lectin-domain containing receptor kinase IV.1	SORBI_3006G158200
LOC8085626	-1.2195702	731.934	1096.15	696.828	1398.09	2571.56	1911.65	probable calcium-transporting ATPase 9, plasma membrane-type	SORBI_3005G028400
LOC8076918	-1.2214258	114.774	127.301	180.252	223.893	368.744	391.118	glutathione S-transferase zeta class	SORBI_3005G086100
LOC110433469	-1.2485571	2435.42	4637.26	2274.03	5224.16	7285.91	9698.7	CTP synthase-like	SORBI_3003G241000
LOC8068803	-1.2520605	60.4072	68.0234	91.2251	123.39	231.537	167.991	uncharacterized LOC8068803	SORBI_3008G121900
LOC8067611	-1.2625288	465.136	460.616	672.648	1079.66	1422.45	1331.01	CBL-interacting protein kinase 15	SORBI_3005G012000
LOC8085674	-1.2720272	265.792	152.567	276.973	373.154	627.079	677.996	probable acyl-activating enzyme 1, peroxisomal	SORBI_3001G514900
LOC8078579	-1.2784644	587.964	252.659	327.531	885.62	1055.85	892.508	cyclic dof factor 2	SORBI_3002G421900

LOC8083058	-1.2905162	243.642	176.861	172.558	265.686	662.453	522.927	5'-3' exoribonuclease 4	SORBI_3005G022200
LOC8079630	-1.3014242	636.289	480.051	387.982	870.694	911.141	1925.44	lipoamide acyltransferase component	SORBI_3003G148800
LOC110433518	-1.3045112	3913.38	1914.37	1882.76	4459.94	7672.88	6912.63	dormancy-associated protein 1	SORBI_3001G191200
LOC8072150	-1.3230704	51.3461	68.0234	51.6576	109.459	204.739	114.579	putative disease resistance protein RGA4	SORBI_3005G053800
LOC8059168	-1.3239892	293.982	229.336	363.801	779.146	649.59	789.99	probable cytokinin riboside 5'-monophosphate phosphoribohydrolase LOGL1	SORBI_3003G273700
LOC8078835	-1.32504	60.4072	54.4188	68.1441	119.409	170.437	167.991	uncharacterized LOC8078835	SORBI_3001G433000
LOC8066337	-1.3427996	318.145	200.183	233.009	652.771	629.223	623.722	calmodulin-binding protein 60 A	SORBI_3008G123100
LOC8056370	-1.3445256	171.154	110.781	128.594	277.627	489.872	275.678	organic cation/carnitine transporter 7	protein coding

LOC8069618	-1.3457546	357.409	309.993	226.414	449.775	727.841	1094.1	putative glycerol-3-phosphate transporter 1	SORBI_3007G045500
LOC8078619	-1.3478398	509.434	190.466	281.369	740.338	947.586	809.804	IQ domain-containing protein IQM1	SORBI_3003G193800
LOC8069347	-1.3568603	104.706	65.1082	131.892	240.809	244.4	286.016	putative anthocyanidin reductase	SORBI_3010G210700
LOC8060395	-1.3647785	33.224	32.0682	29.6757	62.6899	108.265	74.0885	ABC transporter E family member 2	SORBI_3004G128100
LOC8082007	-1.3788572	2099.15	1054.36	2886.23	4368.39	6324.39	5013.03	ethylene-responsive transcription factor 1	protein coding
LOC8076943	-1.4037975	90.6108	130.216	142.883	491.569	251.904	218.819	small EDRK-rich factor 2	SORBI_3009G134450
LOC8083453	-1.4129171	41.2783	34.9835	28.5766	66.6703	105.049	107.687	putative disease resistance protein RGA3	SORBI_3006G027800
LOC8077221	-1.4334926	86.5837	105.922	103.315	282.602	356.953	160.238	uncharacterized LOC8077221	SORBI_3002G289000
LOC8071113	-1.4366588	80.543	51.5035	63.7477	138.316	250.832	141.285	cinnamoyl-CoA reductase 1	SORBI_3007G063300

LOC110431002	-1.4603502	5775.94	4302	3133.53	8363.63	15252.5	12739.8	probable inositol oxygenase	SORBI_3010G168800
LOC8058116	-1.4877791	1008.8	1940.61	2220.18	4692.79	3956.49	5847.82	proline dehydrogenase 2, mitochondrial	SORBI_3001G304700
LOC8084619	-1.4903592	153.032	98.1481	178.054	368.179	392.326	443.669	putative aldehyde oxidase-like protein	SORBI_3002G107200
LOC110434091	-1.4906438	251.697	127.301	384.684	514.456	830.746	799.466	coiled-coil domain-containing protein 25-like	SORBI_3003G296600
LOC8084901	-1.5331363	34.2308	24.2941	26.3784	71.6456	93.2579	80.9804	receptor-like protein kinase HERK 1	SORBI_3001G414000
LOC8070856	-1.5441177	177.195	98.1481	108.811	211.952	373.032	534.988	nudix hydrolase 13, mitochondrial	SORBI_3006G059700
LOC110435648	-1.5526312	2244.13	1161.26	1027.66	5021.17	3939.34	4044.71	protein LNK1-like	SORBI_3001G352400
LOC8084962	-1.5636352	89.6041	68.9952	68.1441	273.647	215.458	181.775	CBL-interacting protein kinase 31	SORBI_3002G424500
LOC8066191	-1.5741787	105.713	115.64	105.513	175.134	347.305	450.561	GEM-like protein 7	SORBI_3004G225900

LOC110432530	-1.5847012	107.726	48.5882	48.3603	161.203	168.293	284.293	calcium-binding protein 5-like	SORBI_3002G376700
LOC8083305	-1.5894531	106.719	94.2611	96.7206	218.917	258.335	417.824	beta-glucosidase 25	SORBI_3010G233900
LOC8073348	-1.6037265	549.706	312.908	290.162	1105.53	1798.7	600.461	protein SAR DEFICIENT 1	SORBI_3003G083200
LOC8059264	-1.6095556	242.636	219.619	200.036	427.884	383.751	1208.68	uncharacterized LOC8059264	SORBI_3001G227800
LOC8057862	-1.6100429	816.504	433.407	658.36	1220.96	2218.9	2384.61	sorbitol dehydrogenase	SORBI_3007G181600
LOC8078930	-1.623197	79.5362	99.1199	193.441	464.702	453.426	227.434	ethylene-responsive transcription factor ERF014	SORBI_3004G310600
LOC8081925	-1.6292636	48.3258	35.9552	30.7747	91.5472	152.214	112.856	metal tolerance protein 3	SORBI_3004G315400
LOC8085878	-1.6402738	6012.53	2212.71	4331.55	7551.65	12901.8	18688.4	stem-specific protein TSJT1	SORBI_3006G269200
LOC8075285	-1.6900309	32.2172	28.1811	32.9729	53.7342	137.207	110.271	putative hydrolase C777.06c	SORBI_3006G072000
LOC8058996	-1.6937397	87.5905	97.1764	76.9369	178.119	371.96	297.215	solute carrier family 35 member F1	SORBI_3002G262400

LOC8086028	-1.7049966	135.916	37.8988	91.2251	184.089	323.723	355.797	putative disease resistance protein RGA3	SORBI_3005G228250
LOC8064171	-1.7402027	514.468	489.769	438.54	1005.03	1288.46	2525.9	SNF1-related protein kinase regulatory subunit beta-1	SORBI_3009G180200
LOC8069381	-1.7900843	761.131	692.867	729.801	1539.39	1787.98	4223.9	basic leucine zipper 9	SORBI_3010G218500
LOC8072102	-1.8062099	663.473	317.767	759.477	1756.31	1637.91	2691.31	dynein light chain LC6, flagellar outer arm	SORBI_3003G303100
LOC8058651	-1.8227045	695.69	457.701	507.783	1393.11	1377.43	3104.82	uncharacterized protein At2g27730, mitochondrial	SORBI_3004G166400
LOC110433415	-1.8617277	546.685	407.169	397.874	1171.21	1300.25	2440.61	hydroxymethylglutaryl-CoA lyase, mitochondrial-like	SORBI_3003G126500
LOC8077281	-1.8814558	1423.6	1137.94	1000.18	2520.53	4146.23	6456.03	serine/threonine-protein kinase STY17	SORBI_3002G300400

LOC8059588	-1.9062068	3893.25	2236.03	3264.32	7312.83	11357.1	16538.1	glycerophosphodiester phosphodiesterase GDPD1, chloroplastic	SORBI_3004G157300
LOC8066710	-1.9627182	154.038	128.273	81.3333	242.799	696.755	478.991	probable LRR receptor-like serine/threonine-protein kinase At5g10290	SORBI_3005G182400
LOC8060088	-1.9786374	83.5633	85.5152	123.099	491.569	335.514	323.06	ultraviolet-B receptor UVR8	SORBI_3003G354900
LOC8083217	-2.0320783	45.3054	12.6329	29.6757	169.163	111.481	77.5344	ethylene-responsive transcription factor ERF109	SORBI_3002G225700
LOC110430626	-2.0473318	126.855	128.273	69.2432	229.863	270.126	840.818	fructose-1,6-bisphosphatase, cytosolic-like	SORBI_3009G152700
LOC8060619	-2.1286587	267.805	197.268	152.775	548.288	1145.89	1008.81	uridine kinase-like protein 2, chloroplastic	SORBI_3007G194700

LOC8073431	-2.1370321	314.118	113.696	114.306	511.47	827.53	1045.85	vacuolar amino acid transporter 1	SORBI_3006G106700
LOC8077059	-2.1402155	39.2647	22.3506	24.1802	59.7047	133.991	184.36	serine/threonine-protein kinase RIPK	SORBI_3001G341600
LOC8065398	-2.2002843	50.3394	77.7411	54.9549	77.6161	486.656	277.401	uncharacterized LOC8065398	SORBI_3010G062100
LOC8073595	-2.2059678	1992.43	2624.73	1612.38	6634.19	9232.54	12876.7	probable galactinol--sucrose galactosyltransferase 2	SORBI_3006G122400
LOC8070635	-2.2355087	142.964	73.854	37.3693	279.617	673.172	245.526	uncharacterized LOC8070635	SORBI_3005G038300
LOC8054108	-2.235602	621.188	675.376	330.829	1346.34	4817.25	1502.45	probable WRKY transcription factor 70	SORBI_3008G060300
LOC8082057	-2.2374207	91.6176	30.1247	34.072	193.045	358.025	184.36	G-type lectin S-receptor-like serine/threonine-protein kinase At1g61480	SORBI_3005G203200

LOC110431653	-2.2539549	5.03394	11.6612	5.49549	28.8573	39.6614	37.9057	uncharacterized LOC110431653	ncRNA
LOC8080836	-2.3008882	1343.05	1057.28	596.81	4866.93	5522.58	4382.42	protein CCA1	SORBI_3007G047400
LOC8057568	-2.3257428	59.4004	68.0234	58.2522	58.7096	338.73	533.265	uncharacterized LOC8057568	SORBI_3006G177600
LOC8063819	-2.3341662	610.113	337.202	237.405	928.408	1567.16	3478.71	choline monooxygenase, chloroplastic	SORBI_3010G248301
LOC8085397	-2.4135048	58.3937	10.6894	56.054	206.976	360.169	99.0718	WRKY transcription factor WRKY24	SORBI_3003G337900
LOC8085227	-2.4455523	48.3258	60.2493	68.1441	112.444	140.423	709.009	serine/threonine-protein kinase STY46	SORBI_3004G016800
LOC8061838	-2.4678733	26.1765	23.3223	15.3874	36.8179	96.4737	225.711	putative receptor protein kinase ZmPK1	SORBI_3003G251600
LOC8084920	-2.4866314	76.5158	23.3223	10.991	149.262	350.521	122.332	uncharacterized LOC8084920	pseudo
LOC8060030	-2.5501422	8.0543	3.88705	8.79279	20.8966	67.5316	32.7368	uncharacterized LOC8060030	SORBI_3005G094900

LOC8054720	-2.5519945	9.06108	10.6894	7.69369	23.8819	67.5316	69.781	receptor-like serine/threonine-protein kinase SD1-8	SORBI_3003G319400
LOC8078740	-2.5691737	7.04751	11.6612	20.8829	101.498	33.2298	99.0718	cytokinin dehydrogenase 1	SORBI_3003G036700
LOC8078722	-2.5892327	16.1086	43.7294	16.4865	185.085	34.3018	240.357	cysteine proteinase inhibitor 4	SORBI_3003G400400
LOC110430139	-2.6006764	76.5158	133.132	32.9729	87.5669	573.483	811.527	uncharacterized LOC110430139	ncRNA
LOC8074867	-2.6178927	167.127	211.844	235.207	463.707	820.027	2485.41	CBL-interacting protein kinase 15	SORBI_3008G032000
LOC8082557	-2.6744966	9.06108	7.77411	7.69369	14.9262	81.4667	60.3046	beta-fructofuranosidase, insoluble isoenzyme 6	SORBI_3006G255400
LOC8060174	-2.7028034	7.04751	21.3788	14.2883	37.813	51.4527	188.667	transcription factor bHLH129	SORBI_3006G127900
LOC110433065	-2.7206433	431.912	184.635	430.846	2224	1602.54	3074.67	phosphoenolpyruvate carboxykinase (ATP)	SORBI_3001G432800
LOC8055891	-2.7370135	173.167	104.95	170.36	400.022	1100.87	1487.8	probable sarcosine oxidase	SORBI_3002G253500

LOC8083660	-2.8769975	110.747	127.301	54.9549	142.296	672.1	1338.76	CBL-interacting protein kinase 10	SORBI_3001G379600
LOC8073082	-2.8958597	51.3461	6.80234	92.3242	474.652	493.088	150.761	peroxidase 2	SORBI_3002G391200
LOC110435671	-3.0154016	90.6108	38.8705	23.0811	291.558	484.512	459.176	uncharacterized LOC110435671	SORBI_3005G206300
LOC8082323	-3.0979132	2.01357	17.4917	12.0901	43.7834	75.0351	151.623	alanine--glyoxylate aminotransferase 2 homolog 3, mitochondrial	SORBI_3001G379000
LOC8083976	-3.1142238	3.02036	1.94353	7.69369	32.8376	56.8123	18.9529	probable N-acetyltransferase HLS1	SORBI_3001G079600
LOC8082558	-3.192008	6.04072	22.3506	9.89188	13.9311	165.077	171.437	beta-fructofuranosidase, insoluble isoenzyme 5	SORBI_3006G255500
LOC8067351	-3.2602438	13.0882	3.88705	14.2883	62.6899	198.307	37.9057	putative disease resistance RPP13-like protein 1	SORBI_3009G018300
LOC8069196	-3.3059063	10.0679	12.6329	4.39639	7.96063	116.84	143.869	uncharacterized LOC8069196	SORBI_3010G267200

LOC8057181	-3.3315438	37.2511	14.5765	12.0901	82.5915	285.133	276.54	fe(2+) transport protein 1	SORBI_3001G142800
LOC8084087	-3.3520453	34.2308	11.6612	6.59459	23.8819	126.488	385.949	cysteine-rich receptor-like protein kinase 10	SORBI_3002G329100
LOC8080214	-3.5917079	25.1697	136.047	32.9729	147.272	493.088	1702.31	uncharacterized LOC8080214	SORBI_3001G028800
LOC8085119	-3.709957	3.02036	10.6894	6.59459	9.95078	132.919	123.194	uncharacterized LOC8085119	SORBI_3009G133900
LOC8079922	-3.7273189	3.02036	2.91529	4.39639	20.8966	37.5176	77.5344	photosystem II 10 kDa polypeptide, chloroplastic	SORBI_3002G032700
LOC8067309	-3.7523339	70.4751	9.71764	40.6666	324.396	147.926	1155.26	uncharacterized LOC8067309	
LOC8068090	-3.8533032	2.01357	0.97176	2.1982	6.96555	22.5105	44.7977	uncharacterized LOC8068090	
LOC8078340	-3.8750544	4.02715	2.91529	1.0991	10.9459	67.5316	40.4902	UPF0481 protein At3g47200	SORBI_3004G112400
LOC8081492	-3.9769191	38.2579	10.6894	1.0991	47.7638	177.94	563.417	cysteine-rich repeat secretory protein 55	SORBI_3001G418200

LOC8078035	-4.2681739	3.02036	2.91529	1.0991	2.98524	73.9632	59.4431	putative transporter arsB	SORBI_3001G539200
LOC8086013	-4.5783162	2.01357	3.88705	0	7.96063	96.4737	38.7672	putative disease resistance RPP13-like protein 3	
LOC8065633	-4.6528173	1.00679	9.71764	0	39.8031	21.4386	211.066	potassium transporter 21	SORBI_3001G183700
LOC8071230	-4.8175923	1.00679	0.97176	2.1982	13.9311	61.1	41.3517	uncharacterized LOC8071230	SORBI_3009G021400
LOC8073798	-4.8595062	4.02715	0.97176	0	5.97047	110.409	30.1523	receptor-like protein kinase 5	SORBI_3004G099700
LOC8073019	-4.8766338	1.00679	1.94353	2.1982	33.8327	58.9562	57.7201	G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130	SORBI_3006G077000
LOC8055147	-5.0258261	3.02036	0.97176	0	2.98524	70.7474	57.7201	lecithin-cholesterol acyltransferase-like 1	SORBI_3001G288200
LOC8071200	-5.2589832	8.0543	0.97176	0	7.96063	228.321	111.133	peroxidase 5	SORBI_3009G055300
LOC8063818	-5.5833525	13.0882	54.4188	3.29729	56.7195	339.802	3001.44	uncharacterized LOC8063818	SORBI_3010G248200

LOC8075150	-5.6948767	4.02715	0.97176	2.1982	2.98524	231.537	137.839	iron-phytosiderophore transporter yellow stripe 1	SORBI_3004G299500
LOC8059311	-5.854689	0	5.83058	0	2.98524	69.6755	268.786	peroxidase N	SORBI_3001G235800
LOC110432221	-5.8988906	2.01357	0	0	1.99016	63.2439	55.9971	glucan endo-1,3-beta-glucosidase 3-like	SORBI_3002G327900
LOC8055530	-5.9284233	1.00679	0	0	7.96063	15.007	43.9362	hydroquinone glucosyltransferase	SORBI_3002G173900
LOC8062746	-6.0453051	1.00679	0	0	15.9213	25.7263	31.0138	cytochrome P450 71A1	SORBI_3002G216200
LOC8076510	-9.0237018	2.01357	0	0	26.8671	364.456	668.519	peroxidase 12	SORBI_3006G277500

8.9 Mineral element sample standardisation and calibrations

Samples were diluted 1:10 in ~2 % HNO_3 before analysis. Appropriate internal standard was added to each dilution (outline below). The following dilution schedule was used: 1.0 ml sample + 8.9 ml 2 % HNO_3 + 0.1 ml yttrium and lutetium internal standard solution. Direct calibration was used for the analysis using 2% HNO_3 as diluent.

For internal standardisation, 0.1 ml Yttrium (371.030 nm) and lutetium (261.542 nm) at a concentration of 100 mg L^{-1} was added to all standards and samples for use as an internal control. This results in an internal standard concentration of 1 mg L^{-1}.

Limit of Detection

Limit of Detection was estimated as 3 times the standard deviation of 5 blank measurements multiplied by 10 to account for sample dilution

$$LOD = 10 \times 3\sigma_{5\ Blanks}$$

Precision and accuracy

Precision was estimated by calculating the 95% confidence interval of 5 repeated sample measurements.

$$\bar{x} \pm \frac{t_{n-1}s}{\sqrt{n}}$$

Where n = number of measurements
n-1 = degrees of freedom (5)
t = t value (2.78 for 4 degrees of freedom)
s = calculated standard deviation of 5 measurements
x = calculated mean of 5 measurements

Accuracy was estimated by Spiking a sample with a known amount of analyte and measuring the analyte recovery. Sample and Sample + Spike were analysed 5 times.

$$\%\ Recovery = 100 \times \frac{M_{Spike-}M_{Sample}}{C_{spike}}$$

Where M_{spike} = Measured concentration of spiked sample.
M_{Sample} =Measured concentration of sample.
C_{Spike} = Actual concentration of spike.

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