An integrated multi-omics approach to uncover drought tolerance biomarkers in two underutilised crops: sweet potato (*Ipomoea batatas* L.) and cassava (*Manihot esculenta* Crantz)

A Report

To the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

Drought stress poses a significant threat to global food security, particularly in arid and semi-arid regions where agriculture is increasingly vulnerable to climate variability. Root and tuber crops, specifically sweet potato (*Ipomoea batatas* L.) and cassava (*Manihot esculenta* Crantz), serve as essential staple crops due to their inherent resilience in marginal environments. However, their productivity is severely compromised by prolonged drought episodes, threatening the livelihoods of smallholder farmers. A meta-analysis conducted in this project revealed that drought stress reduces tuber yield by an average of 49.29% across major tuber crops, with sweet potato experiencing the highest reduction (61.08%) and cassava the lowest (32.45%). Despite cassava's relative resilience, all tuber crops face significant yield losses under water-limited conditions, emphasising the urgent need to enhance drought tolerance through dissecting the complex molecular mechanisms underpinning stress adaptation in locally adapted African germplasm.

A comprehensive understanding of the underlying molecular mechanisms regulating drought tolerance in these crops, especially in locally adapted African germplasm, remains limited. Recently, omics pipelines that include genomics, transcriptomics, proteomics, metabolomics, phenomics, etc. are highly useful in characterising and producing climate-smart future crops with a proper understanding of the molecular mechanisms of stress responses by the plant's genes, proteins, cellular metabolic circuits and resultant phenotype. Instead of mono-omics, two or more (hence 'multi-omics') integrated omics approaches can decipher the plant's stress tolerance response very well. The knowledge gained helps develop marker-assisted selection together with metabolic engineering and precision agriculture techniques to ensure crop improvement and food security under changing environmental circumstances.

PROJECT RATIONALE AND OBJECTIVES

This project was conceived in response to a pressing agricultural crisis: the escalating threat of drought and climate instability to food security in sub-Saharan Africa's arid and semi-arid regions. These areas are particularly vulnerable to shifts in rainfall patterns, which negatively impact crop productivity. In this challenging context, sweet potato and cassava are vital staple crops, renowned for their inherent resilience in marginal soils. However, prolonged drought significantly inhibits their yields, emphasising the urgent need to enhance their tolerance. This research addresses this need by moving beyond physiological observations to decode the fundamental molecular mechanisms of drought resilience.

The principal aim of this project was to integrate multi-omics techniques to identify metabolites, proteins and genes responsible for drought tolerance in sweet potato and cassava cultivars commonly grown in South Africa.

The specific objectives were:

- To characterise the inherent metabolic profiles of different sweet potato and cassava genotypes under optimal growth conditions to establish a biochemical baseline.
- To determine the changes in the leaf metabolome of these cultivars in response to varying intensities and durations of drought stress.
- To identify differentially expressed proteins in the leaves of sweet potato cultivars and elucidate their functional roles in drought response.
- To identify differentially expressed genes in the leaves of sweet potato through transcriptomic analysis to understand the broad genetic reprogramming under drought.
- To profile the temporal expression of key drought-responsive genes in cassava leaves, providing a detailed view of transcriptional dynamics over time.
- To integrate the metabolomic, proteomic and transcriptomic datasets to evaluate the relationships between molecular changes and their functional outcomes in drought adaptation.
- To build the capacity of postgraduate students and researchers in advanced bioinformatics, multi-omics data analysis and biomarker discovery.

METHODOLOGY

To achieve these objectives, the project employed a robust and integrated multi-omics experimental design across all phases. The foundation was a carefully selected panel of cultivars for both sweet potato and cassava, chosen for their contrasting agronomic traits, including their known differences in drought tolerance, yield and nutritional profile. These plants were grown under controlled greenhouse and rainout shelter conditions to minimise environmental variability. For the stress-response phase, a controlled stress imposition protocol was implemented, where selected cultivars were subjected to varying drought intensities (30%, 50% and 70% water depletion levels) across different durations (early and prolonged stress), allowing for a detailed analysis of the progression of their molecular responses.

The core analytical workflow combined three key methodologies: metabolomic profiling using liquid chromatography-mass spectrometry (LC-MS) and LC-MS quadruple time-of-flight (qTOF) to comprehensively measure small molecules; proteomic profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with data-independent acquisition (DIA) to identify and quantify protein changes; and transcriptomic analysis, which included RNA sequencing (RNA-seq) for global gene expression profiling in sweet potato (Chapter 4) and quantitative real-time PCR (qRT-PCR) for targeted analysis of four drought-responsive genes (MeZFP, MeALDH, MeRD28 and MeMSD) in cassava (Chapter 5). Sophisticated bioinformatics pipelines were essential for interpreting these complex datasets, including multivariate statistics (PCA, PLS-DA, OPLS-DA), pathway enrichment analysis and differential expression analysis.

KEY FINDINGS

Establishing the biochemical baseline: Inherent metabolic diversity

The initial metabolomic screening under optimal conditions was critical, as it revealed a landscape of significant inherent biochemical diversity, effectively providing a unique "metabolic fingerprint" for each genotype before any stress was applied. In sweet potato, the four cultivars were clearly separated based on their accumulation of specialised metabolites. Notably, sulphated flavonoids (e.g. kaempferol 7,4'-dimethyl ether 3-O-sulfate) and signalling lipids (e.g. lysophosphatidylethanolamine) were key

differentiators. A particularly significant discovery was withanolide in the cultivar Jane, a class of potent, stress-resilient compounds not previously reported in sweet potato, emphasising a massive and untapped reservoir of biochemical potential within sweet potato. Similarly, cassava genotypes exhibited distinctly different profiles, with the breeding line P4/10 showing the most unique separation from others. Key metabolites that acted as discriminants were involved in crucial pathways such as phenylpropanoid and flavonoid biosynthesis (e.g. 4-coumaroyl-CoA) and lipid metabolism (e.g. specific glycerolipids). This pre-existing metabolic blueprint suggested inherent, genetically encoded differences in stress preparedness and nutritional potential among the cassava lines.

Decoding drought response: Dynamic metabolic, proteomic and transcriptomic reprogramming

The stress-phase studies provided the most profound understanding, revealing that drought tolerance is not defined by the sheer magnitude of metabolic change, but by the timing, coordination and efficiency of molecular responses. In sweet potato metabolomics, the response was dominated by phenolic compounds. The drought-tolerant Atacama cultivar demonstrated a more stable and presumably more efficient metabolic profile under early stress, with key metabolic classes such as flavonoids and phenolic acids (e.g. chlorogenic acid, apigenin glycosides) being strongly associated with its tolerance, acting as potent antioxidants. Conversely, the susceptible Blesbok cultivar displayed a dysregulated metabolic response, characterised by a broad, non-specific upregulation of metabolites that points to a disorganised and energetically inefficient defence strategy.

In cassava metabolomics, a clear separation developed under the pressure of drought, revealing a tale of two distinct physiological strategies. The tolerant breeding line (P4/10) exhibited a proactive and energy-efficient strategy. It maintained remarkable metabolic homeostasis during early drought, only activating targeted, high-impact biochemical pathways under prolonged severe stress. Its resilience was reinforced by sustained energy production (via metabolites like acetyl-CoA), active membrane remodelling (through glycerophospholipids) and a robust antioxidant defence system that included specialised compounds like quercetin 3-arabinoside. In contrast, the moderately tolerant line (UKF4) demonstrated a reactive and

metabolically costly response. It showed minimal early adjustment but underwent significant, often disruptive reprogramming under prolonged stress, which ultimately led to a collapse in energy-related metabolites, indicating an unsustainable strategy that could lead to resource depletion.

The sweet potato proteomics study added a crucial functional layer to these findings, revealing divergent molecular investment strategies at the protein level. A comparison of Atacama and Jane showed that Jane upregulated proteins involved in secondary metabolism and biological defence, such as glycosyltransferases for flavonoid production and glutathione transferase for detoxification. Atacama, however, upregulated proteins related to photosynthesis and energy production (e.g. Rubisco, ribosomal proteins) and employed a different defence mechanism centred on the protease inhibitor sporamin, which is known to be induced by abiotic stress and wounding.

Transcriptomic profiling of two sweet potato cultivars, Atacama and Blesbok, under progressive drought stress revealed a fundamental divergence in their molecular strategies. The tolerant cultivar, Atacama, mounted a massive, early and sustained transcriptional response across all time points (2-, 7- and 14-weeks post drought imposition). Under severe stress, Atacama showed thousands of differentially expressed genes (DEGs), indicating a comprehensive rewiring of its physiology to cope with water deficit. In stark contrast, the susceptible cultivar, Blesbok, exhibited a delayed and muted response, with significantly fewer DEGs, particularly during the early and mid-stages of stress. This delayed reaction suggests that Blesbok is perpetually behind in activating the necessary defence and adaptation pathways. Venn diagram analyses further highlighted that while some stress responses are shared between cultivars, Atacama possesses a unique set of genes activated specifically for long-term drought adaptation. It is established in this chapter that the vigour and timeliness of the transcriptomic response are critical determinants of sweet potato drought tolerance.

A time-series gene expression analysis in cassava genotypes P4/10 (highly tolerant) and UKF4 (moderately tolerant) provided a granular view of transcriptional persistence. The tolerant P4/10 employed a proactive and sustained strategy: it initiated an early downregulation of metabolic genes (*MeALDH* and *MeMSD*) to

conserve resources, while simultaneously maintaining strong, persistent upregulation of key regulatory and protective genes (*MeRD28* and *MeZFP*). This indicates a programmed, long-term commitment to cellular homeostasis. Conversely, UKF4 displayed a reactive and ultimately deficient response. It showed minimal change initially, but under prolonged stress, significantly upregulated *MeALDH* and *MeMSD*, signalling a frantic, compensatory effort against accumulated damage. Crucially, UKF4 failed to sustain the expression of *MeRD28* and *MeZFP*, leading to a collapse in its core protective systems. This transcriptional persistence in P4/10, and its breakdown in UKF4, provides a direct mechanistic explanation for their contrasting metabolic performances.

INTEGRATED CONCLUSION AND STRATEGIC IMPLICATIONS

This research provides a clear and actionable model for breeding more drought-tolerant crops. We have discovered that the most resilient varieties of sweet potato and cassava, such as Atacama and P4/10, do not just react to drought, but are proactively prepared, launching a swift, coordinated and sustained molecular defence that conserves water and energy. In contrast, susceptible varieties exhibit a delayed and disorganised response that leads to metabolic disorder and collapse. The practical value of this finding is that it provides breeders with a powerful new predictive model. Instead of relying solely on slow and unpredictable field trials, we can now select parent plants based on their internal molecular blueprint for success. By breeding for key biomarkers such as the sustained activity of specific regulatory genes in cassava or the robust accumulation of protective antioxidants in sweet potato, we can systematically and efficiently develop new, climate-resilient varieties. This strategy will directly accelerate the delivery of improved crops to farmers, enhancing productivity and food security in drought-prone regions.

STUDY RECOMMENDATIONS

This project delivers a validated suite of candidate metabolic and protein biomarkers for drought tolerance and nutritional quality, identified from both baseline and stress conditions. To translate these findings into applied breeding solutions, future work must prioritise field validation of the identified metabolic and protein biomarkers across diverse agro-ecologies. Subsequent integration into marker-assisted selection programmes will enable the rapid development of climate-resilient cultivars. Research

must also be extended to root tissues, the primary storage organs, to fully understand yield formation under water deficit. Finally, functional characterisation of key targets, such as the novel withanolide in sweet potato, through genetic and enzymatic assays is critical to confirm their mechanistic role in stress tolerance. This multi-pronged strategy will bridge the gap between foundational discovery and the development of drought-tolerant varieties, directly contributing to enhanced food security.

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ACRONYMS & ABBREVIATIONS

ARC	Agricultural Research Council
BD	Bulk density
cDNA	Complementary DNA
CSIR	Council for Scientific and Industrial Research
CV	Coefficient of variation
DEG	Differentially expressed genes
DEPS	Differentially expressed proteins
DIA	Data-independent acquisition
DS	Drought stress
EC	Exclusion criteria
FC	Field capacity
FDR	False discovery rate
GNPS	Global Natural Product Social Molecular Networking
GO	Gene ontology
Ha	Hectare
HAT	Hydrogen atom transfer
HI	Harvest index
HMDB	Human Metabolome Database
IC	Inclusion criteria
KEGG	Kyoto Encyclopaedia of Gene and Genomes
Kurt	Kurtosis
LC-MS	Liquid chromatography–mass spectrometry

MAP	Mean annual precipitation
MAT	Mean annual temperature
MDA	Mean decrease accuracy
mRNA	Messenger RNA
NR	Number of roots
NS	Non-stress
OPLS-DA	Orthogonal partial least squares discriminant analysis
PB	Plant biomass
PCA	Principal component analysis
PH	Plant height
pH	Soil pH
PLS-DA	Partial least squares discriminant analysis
PPR	Pentatricopeptide repeat
qRT	Quantitative real-time
RAF	Radical adduct formation
RCBD	Randomised complete block design
ROS	Reactive oxygen species
rRNA	Ribosomal RNA
RS	Root:shoot ratio
RSWC	Relative soil water content
SB	Shoot biomass
Skew	Skewness
SOC	Soil organic carbon
SP1	Early drought stress

SP2	Prolonged drought stress
Std	Standard deviation
TIMS	Trapped ion mobility spectrometry
TOF	Time of flight
TPY	Total plant weight
TY	Tuber yield
TYR	Tuber yield reduction
VIMP	Vegetable, Industrial and Medicinal Plants
VIP	Variable importance in projection
wpi	Weeks post imposition
$\bar{x}_1$	Mean of treatment
$\bar{x}_2$	Mean of control
Yp	Potential yield
Ys	Stress yield

CHAPTER 1: BACKGROUND

1.1 Background of and motivation for the project

Drought stress is a persistent abiotic stress that severely constrains global agricultural productivity, posing a substantial threat to food security, particularly in arid and semi-arid regions (Qader et al., 2021; Chaffai et al., 2024). Climate change projections indicate an increase in the frequency and severity of drought events, intensifying water scarcity and its impact on crop yields (Li et al., 2009). This scenario necessitates an urgent focus on developing climate-resilient staple crops capable of sustaining productivity under water-limited conditions. Among these, root and tuber crops, specifically sweet potato (*Ipomoea batatas* L.) and cassava (*Manihot esculenta* Crantz), are recognised for their inherent drought tolerance and nutritional importance, serving as crucial food security crops for millions of people in tropical and subtropical regions (Motsa et al., 2015; More et al., 2023). Sweet potato is valued for its nutritional density and shorter growing cycle (Tedesco et al., 2023), and cassava is renowned for its ability to yield under nutrient-deficient soils and erratic rainfall (Wei et al., 2025). Despite this resilience, prolonged drought significantly reduces the yield and quality of both crops, threatening the livelihoods of smallholder farmers who depend on them (Shiferaw et al., 2014; Ahmad et al., 2022). Traditional breeding efforts for drought tolerance have often been slow, disadvantaged by the complexity of the trait and a reliance on secondary physiological and yield parameters, which may not fully capture the underlying biochemical mechanisms.

1.2 The role of omics in plant stress

Plants respond to drought through a complex suite of physiological, molecular and biochemical adjustments aimed at maintaining cellular homeostasis (Bashir et al., 2021). Among these responses, metabolic reprogramming is a fundamental strategy, involving the accumulation of osmolytes, activation of antioxidant systems and restructuring of primary and secondary metabolic pathways (Patel et al., 2020; Xu & Fu, 2022). Metabolomics, the comprehensive analysis of small molecules, provides a direct functional readout of a plant's physiological state and can reveal the final

biochemical outcomes of genetic and enzymatic activity under stress (Ghatak et al., 2018; Satrio et al., 2024). Similarly, proteomics, the large-scale study of proteins, identifies the key enzymes and regulatory proteins that directly execute cellular functions and stress responses (Liu et al., 2019). While transcriptomics reveals the potential for a molecular response by showing which genes are activated, metabolomics and proteomics capture the actual functional outcome of that potential. Since metabolites and proteins are the molecules that directly influence physiology and stress tolerance, they provide a more direct and reliable measure for identifying robust biomarkers for breeding.

1.3 Knowledge gap and problem statement

Despite the importance of sweet potato and cassava, there is a critical knowledge gap regarding the specific molecular bases of their drought tolerance, especially in locally adapted South African germplasm. Previous research has focused largely on physiological traits (Karim et al., 2016) or transcriptomic changes (Olayide, 2022), with limited comprehensive, time-resolved metabolomic, proteomic and transcriptomic studies. Furthermore, most metabolomic research on sweet potato has centred on Asian genotypes (Wan et al., 2024), and comparative analyses of the temporal dynamics of metabolic responses between contrasting cultivars are scarce. A systematic, multi-omics characterisation is therefore required to decipher the conserved and species-specific biochemical strategies that confer drought resilience.

1.4 Project rationale and objectives

This project addresses the critical threat of drought to food security in South Africa and similar semi-arid regions. Focusing on two vital, drought-resilient staple crops, sweet potato and cassava, the research aimed to move beyond physiological observations and uncover the fundamental molecular mechanisms governing drought tolerance. The primary objective was to employ advanced omics technologies to create a detailed, molecular-level map of drought responses in locally relevant germplasm. This foundational knowledge is essential for developing scientific tools such as molecular biomarkers to systematically accelerate the breeding of climate-resilient varieties.

1.4.1 Main objective of the project

The main objective of the 2023/2024-01262 project was to identify potential metabolites, proteins and genes responsible for drought tolerance in sweet potato and cassava cultivars commonly grown in South Africa. This was achieved by using multi-omics tools such as metabolomics, proteomics and transcriptomics on sweet potato and cassava cultivars.

1.4.2 Project objectives

The specific objectives of the project included the following:

1. To determine metabolite changes in response to drought stress on the leaves of sweet potato and cassava plants.

2. To identify differentially expressed genes in sweet potato and cassava leaves and in response to drought stress.

3. To identify differentially expressed proteins in sweet potato and cassava leaves and in response to drought stress.

4. To evaluate the relationship between differentially expressed genes, metabolites and protein functions in response to drought stress.

5. To build capacity of postgraduate students and researchers in bioinformatics and biomarker discovery.

1.5 Significance of the study

This research provides the first detailed, comparative omic map of drought responses in South African sweet potato and cassava germplasm. The findings bridge a critical gap between observed drought tolerance and its underlying molecular determinants. The identification of key metabolites, proteins and genes serves as a foundational resource for developing molecular tools for marker-assisted selection, enabling the accelerated breeding of superior, drought-resilient varieties. Ultimately, this work contributes directly to enhancing crop productivity and securing food supplies in drought-prone regions of South Africa and similar agro-ecologies.

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CHAPTER 2: META-ANALYSIS OF THE EFFECTS OF DROUGHT STRESS ON YIELD-RELATED AGRONOMIC TRAITS OF MAJOR TUBER CROPS

Abstract

Increasing frequency of drought occurrence poses serious threats to tuber crop production. Several studies have examined the effects of drought stress on cassava, potato and sweet potato. Nonetheless, there is limited information on how soil properties and climatic conditions influence tuber yield (TY) under drought stress and on the response variation on the effect of drought stress due to differences in soil properties and climates. Therefore, this study aimed to evaluate the extent to which soil properties and weather conditions influence the effect of drought stress on TY. The metadata for this study was collected from 25 global studies, with 642 paired observations of field experiments evaluating the effect of drought stress on tuber crops. The recorded agronomic traits data include plant height (PH), shoot biomass (SB) and TY. To perform the meta-analysis, an effect size was calculated for each study based on TY under drought stress and non-stress. The percentage tuber yield reduction (%TYR) was computed based on TY under drought stress and non-stress. Effect sizes and confidence intervals showed consistent TY reduction under drought stress, though with varying magnitudes across studies. The mean for TY, PH and SB among all the experiments was reduced under drought stress by 37.71%, 11.14% and 3.37%, respectively. Cassava exhibited the lowest %TYR at 32.45%, compared to 47.63% in potato and 61.08% in sweet potato. Surprisingly, %TYR was highest in tropical (98.74%) and temperate (97.8%) climates and lowest in the savanna (52.32%) climate. The lower %TYR in savanna regions may be attributed to soil properties and seasonal rainfall patterns that support cassava's deep root development and access to subsoil moisture. Multivariate analysis revealed that higher %TYR was linked to rising temperatures and reduced rainfall. The impact of drought stress appeared to affect all crops and environments, especially on soils with lower clay content. Therefore, this study highlights that drought stress affects tuber crop performance across environments, with cassava showing better drought resilience. Further research should be conducted to investigate why the %TYR was higher under tropical and temperate environments.

Keywords: cassava, percentage tuber yield reduction, savanna climate, sweet potato, potato, tuber yield

2.1 Introduction

Tuber crops rank third among food crops after cereals and legumes, highlighting their essential role in global agriculture (Chauhan et al., 2022). They constitute a substantial part of the world's food supply and serve as a key source of animal feed and processed products for human consumption and industrial use (FAO, 2013). These crops, including potato, cassava and sweet potato, are valued for their ability to produce high yields under diverse environmental conditions. Their capacity to thrive in low-input farming systems makes them particularly important for smallholder farmers in resource-limited regions (Nanbol & Namo, 2019).

Despite their adaptability, tuber crops are increasingly vulnerable to drought stress, which poses a significant threat to their yield potential, particularly in dryland farming systems (Daryanto et al., 2016). Overcoming this challenge requires the development and deployment of drought-tolerant varieties as well as the implementation of effective agronomic practices to support global food security in the face of changing climatic conditions. Climate change has intensified concerns among farmers and researchers due to rising temperatures and prolonged drought periods, making it more critical than ever to evaluate the effects of drought stress on agronomic traits (Zhang et al., 2018).

Agronomic traits play a fundamental role in determining crop productivity, as they directly influence plant growth, development and yield potential (Monneveux et al., 2013). These traits include plant height, biomass accumulation, root architecture and tuber yield, among others. In tuber crops, key agronomic traits such as tuber number, size and quality are particularly important because they determine market value, food security and processing potential (Fofana et al., 2024). Therefore, understanding these traits is essential because they help breeders and agronomists identify genotypes with superior drought tolerance and guide the selection of management practices that optimise yield under water-limited conditions. However, research has shown that drought stress adversely affects key agronomic traits (Stagnari et al., 2016; Meise et al., 2019; Fofana et al., 2024), leading to reduced tuber yield by disrupting physiological processes such as photosynthesis, cell expansion and division (Chaves et al., 2009; Qiao et al., 2024). Water availability is crucial during tuber initiation and

bulking, and drought stress during these stages can severely reduce tuber number, size and quality by limiting assimilate translocation (Monneveux et al., 2013). Nevertheless, tuber crops employ various drought adaptation mechanisms, including deep root systems and reduced leaf area, which enhance their resilience under water-limited conditions (Monneveux et al., 2013). These adaptive traits make tuber crops a valuable resource for ensuring agricultural sustainability and food security in regions prone to extreme weather conditions.

Furthermore, understanding the agronomic responses of tuber crops to drought stress is essential, particularly as drought events are projected to become more severe in sub-Saharan Africa. However, the impact of drought stress on agronomic traits, particularly tuber yield, varies significantly across different environments and crop types. For example, a study by Abiola and Oyetunji (2021) in Nigeria's tropical climate reported a 37% reduction in cassava tuber yield under drought stress, whereas Agili et al. (2012) observed a 69% reduction in sweet potato yield under similar conditions in Kenya. In Mozambique's temperate climate, Andrade et al. (2016) found that drought stress reduced sweet potato yield by 31%, whereas Laurie et al. (2015) reported an 85% reduction in sweet potato yield in South Africa's subtropical climate. However, Mthembu et al. (2022) indicated a lower percentage of tuber yield reduction in potatoes in the subtropical regions of South Africa which showed different tuber yields (16%).

Therefore, the disparities in the impact of drought stress on tuber yield reported in several studies can be attributed to variations in crop type, soil properties and climate conditions. Climate conditions play a substantial role in shaping drought responses, as factors such as temperature, rainfall distribution and evapotranspiration rates directly influence soil moisture availability and plant water uptake (Feng & Liu, 2015; Katul et al*.,* 2012; Wang et al., 2018). Likewise, soil textural classes determine the capacity of soils to retain water and supply nutrients, with sandy soils typically experiencing more rapid moisture depletion than loamy or clay soils (Scott, 2024). Despite the recognition of these factors, there is no clear consensus on the extent to which they contribute to variations in tuber yield under drought stress. However, the availability of multiple studies worldwide presents an opportunity for comprehensive analysis to gain a broader understanding of the factors controlling tuber yield responses to drought stress. Therefore, this study aimed to review and analyse

existing research on the effects of drought stress on tuber yield to assess and quantify variations due to crop type, soil properties and environmental conditions. The findings provide valuable insights into key agronomic traits associated with drought tolerance in tuber crops, support breeding programmes and management strategies aimed at improving drought resilience.

2.2 Materials and methods

2.2.1 Data collection

The established set of quality criteria drew upon the extensive work of numerous experienced researchers with specialised expertise in meta-analysis. The foundation for the quality criteria was the Checklist of Quality Criteria for Meta-Analysis for Research Synthesis, Peer Reviewers, and Editors developed by Koricheva and Gurevitch (2014). This checklist was informed by earlier contributions from other scientists who have defined quality standards in the fields of plant breeding and agronomy. Additionally, several key texts, including *Introduction to meta-analysis* by Borenstein et al. (2009), *Handbook to meta-analysis in ecology and evolution* by Koricheva et al. (2013) and *Handbook of research synthesis and meta-analysis* by Cooper et al. (2019), have further enriched the development of these criteria, offering readers comprehensive insights and detailed explanations. The quality criteria were organised into three categories: literature search and inclusion/exclusion criteria, meta-analysis, and results and database presentation. To enhance clarity, these quality criteria were further divided into sub-criteria to provide more specific guidance. If the article failed to meet these criteria, it did not fulfil critical aspects of this synthesis method.

2.2.2 Quality assessment of articles on effects of drought stress on major tuber crops

2.2.2.1 Inclusion criteria, exclusion criteria and search strategy

Firstly, inclusion criteria (IC) and exclusion criteria (EC) were defined to create a framework for the literature screening. Studies were included when they used the term “drought stress” and “limited water irrigation” in their title, abstract, or author keywords (IC1); “tuber crop name”, “tuber yield” and “field conditions” were also used (IC2); the

assessment of the effects of two water regimes (drought stress and non-stress conditions) on tuber crops' agronomic traits and yield was the aim of the study (IC3); field experiments conducted on all the continents were a part of the meta-analyses (IC4); the plants that experienced drought under field conditions (excluding pot studies) (IC5); the effect of water deficit was considered in comparison with well-watered condition and not in combination with other treatments (e.g. addition of fertilizers or growth hormones) (IC6); the reported plants were monoculture roots or tubers of potato, cassava and sweet potato (IC7); to minimise the impact of other agronomic factors (e.g. pests, nutrients, diseases) that might affect yield, only studies that examined the single effect of water reduction were included as other factors were controlled during the water treatment experiments (IC8). Articles were only included when they fulfilled all eight inclusion criteria. Articles were excluded when, for example, they were conducted under controlled environments to get the results for agronomic traits and tuber yield (EC1).

The second step was the collection of existing studies on the agronomic responses due to different water regimes. Therefore, the Web of Science Core Collection (time frame 2010–2024) and Scopus (time frame 2010–2024) databases were searched on 30 August 2024. Due to limited human resources, only these two scientific databases were searched. The following search string was used to retrieve relevant articles: (drought stress) AND (tuber yield) AND (field conditions) AND (cassava, potato, sweet potato, agronomic traits). A total of 372 articles were found (225 in Web of Science and 147 in Scopus) (Figure 2.1). A total of 269 articles were exported into Microsoft Excel and screened by title, abstract and full text according to the predefined inclusion and exclusion criteria. In total, 25 articles relevant to the scope of this study were found. Many articles (244) were excluded, as they did not contain the word "drought stress" or "water stress" in their title, abstract, or keywords. Figure 2.1 shows a flow diagram of the complete screening process.

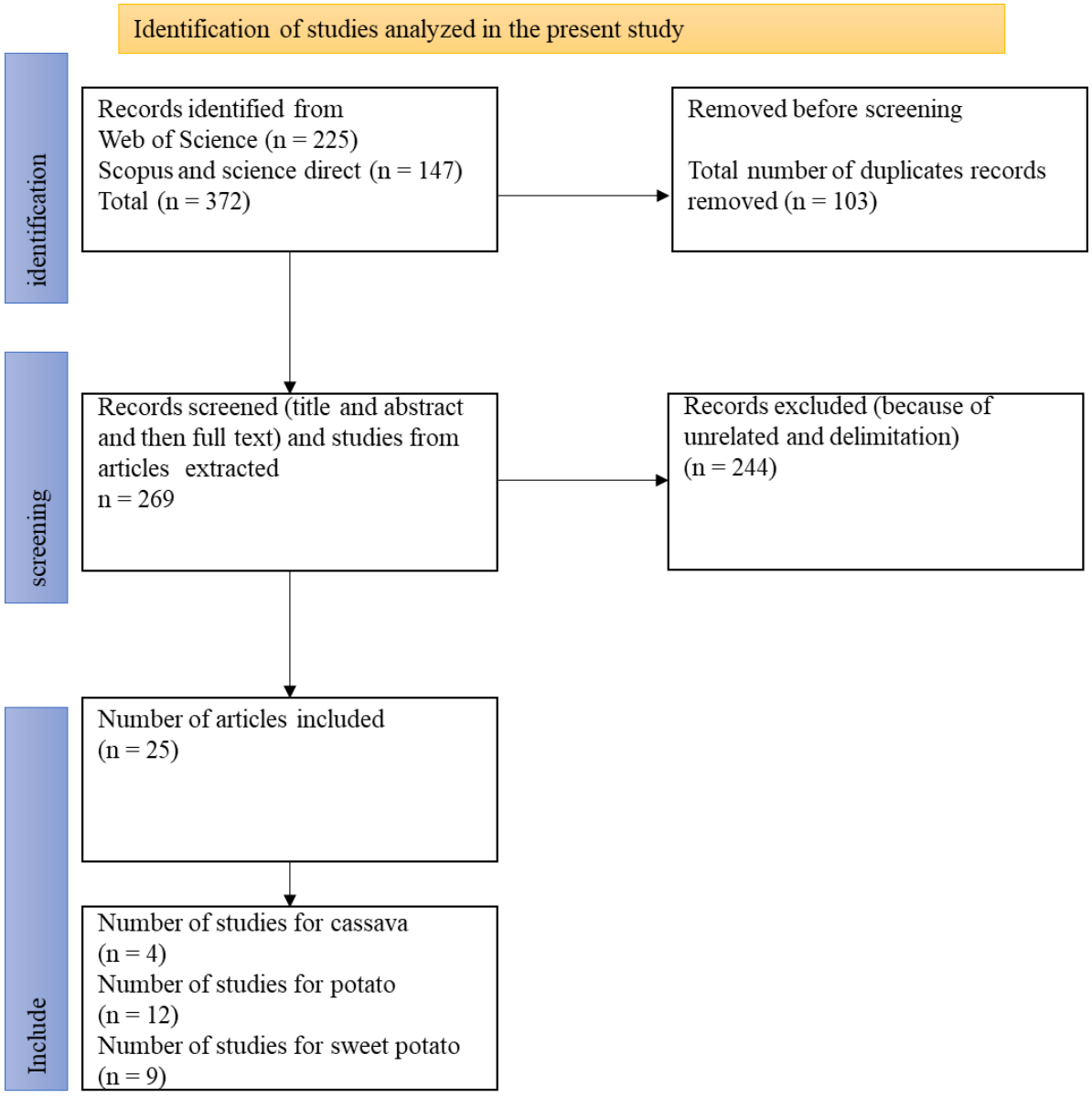

Figure 2.1: Literature selection process for meta-analysis

The complete reference list of the 25 is presented in Table 2.1 and Figure 2.2. The data retrieved offered the possibility to analyse the state of knowledge on studies evaluating the effects of drought stress on agronomic traits and tuber yield. This information will aid future research by guiding researchers in the drought-tolerance breeding programmes. Therefore, the studies were grouped according to crop type, soil texture, climate and continent where they were studied. A total of 3 crops, 6 soil textural classes and 6 climatic regions were formed based on the information provided in the subsections below. The aim of these categories was to structure the collected drought stress articles and allow a simplified investigation.

Table 2.1: Summary of database of references included in study

No.	Crop name	Climate	Country	Reference
1	Cassava	Tropical	Nigeria	Abiola et al., 2021
2	Cassava	Savanna	Ghana	Adjebeng-Danquah et al. 2016
3	Sweet potato	Tropical	Kenya	Agili et al., 2012
4	Sweet potato	Temperate	Mozambique	Andrade et al., 2016
5	Potato	Semi-arid	Poland	Boguszewska-Mańkowska et al., 2020
6	Potato	Subtropical	Peru	Cabello et al., 2014
7	Potato	Subtropical	Japan	Deguchi et al., 2010
8	Potato	Subtropical	South Korea	Dong et al., 2018
9	Sweet potato	Tropical	Nigeria	Esan et al., 2018
10	Potato	Subtropical	Ethiopia	Gelmesa et al., 2023
11	Sweet potato	Tropical	Ethiopia	Gitore et al., 2021
12	Potato	Arid	Iran	Hajibarat et al., 2021
13	Sweet potato	Tropical	India	Hanume et al., 2023
14	Potato	Tropical	Bangladesh	Hasan et al., 2012
15	Potato	Tropical	Ethiopia	Kebede et al., 2019
16	Sweet potato	Semi-arid	Kenya	Kivuva et al., 2014
17	Sweet potato	Subtropical	South Africa	Laurie et al., 2015
18	Potato	Subtropical	Nepal	Luitel et al., 2015
19	Sweet potato	Tropical	Indonesia	Mau et al., 2019
20	Potato	Subtropical	South Africa	Mthembu et al., 2022
21	Potato	Semi-arid	Iran	Nouri et al., 2016
22	Cassava	Semi-arid	Kenya	Orek et al., 2020
23	Sweet potato	Temperate	Mozambique	Ricardo, 2011
24	Cassava	Semi-arid	Kenya	Silim et al., 2020
25	Potato	Subtropical	Egypt, Oman, Japan	Zaki et al., 2022

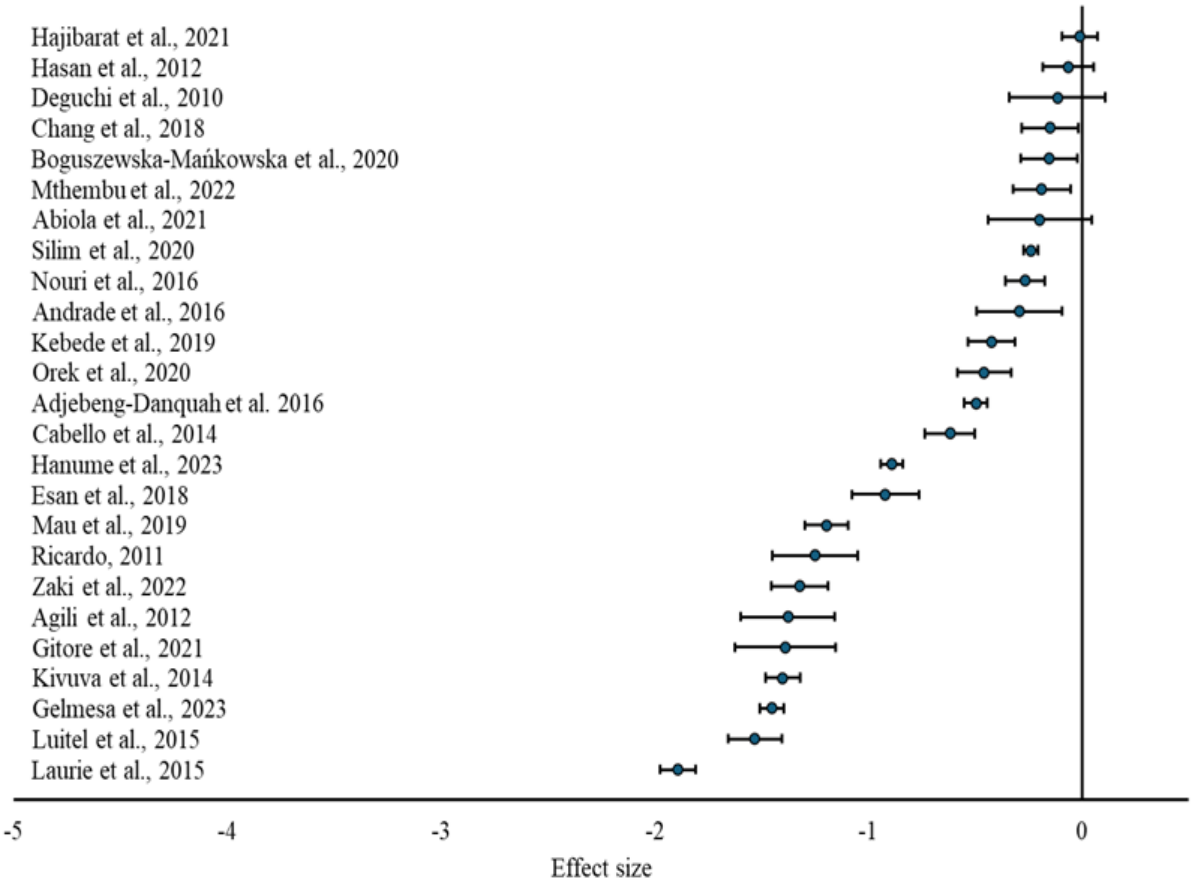

Figure 2.2: Effect sizes for 25 independent studies examining the effect of drought stress on major tuber crops compared to non-stress (control). Black squares are the effect estimates for each study with lower and upper 95% Confidence Intervals.

Lastly, the total number of articles per category was calculated and the relevant articles were identified. Simultaneously, information on the drought stress and non-stress conditions and environmental conditions was extracted. After all the relevant studies meeting the selection criteria were selected, data was extracted and compiled into a Microsoft Excel database. The database captured information including each assigned study's unique ID, names of authors, year of publication, treatment and the names of genotypes used in the studies. The final database comprised 642 paired observations from 25 peer-reviewed journal articles. The overall effect sizes of the studies was calculated only when both of these elements were fulfilled. See Figure 2.2.

2.2.2.2 Definitions of environmental factors

The climatic regions were categorised based on the mean annual precipitation (MAP) and mean annual temperature (MAT) and do not necessarily comply with the Koppen (1936) system. Those climatic categories were subtropical, tropical, temperate, arid, semi-arid and savanna. Subtropical depicts warm (MAT: 10–30 °C $year^{-1}$) and dry to wet (MAP: 100–1 000 mm $year^{-1}$) climate; tropical represents hot (MAT: > 20 °C $year^{-1}$) and wet (MAP: > 1 000 mm $year^{-1}$) climate; temperate represents cool (MAT: < 5 °C $year^{-1}$) and dry (MAP: 0–800 mm $year^{-1}$) climatic zones; arid represents hot (MAT: > 20 °C $year^{-1}$) and dry (MAP: 0–300 mm $year^{-1}$); semi- arid represents cool (MAT: < 20 °C) and dry (MAP: 0–300 mm $year^{-1}$) climate; savanna represents warm to hot (MAT: 8 – 20 °C) and wet (MAP: 1 000-1 300 mm $year^{-1}$) climatic zones. When the information about MAP and MAT was not provided in the peer-reviewed article, the MAP and MAT of the last 30 years for that particular area were used based on data gathered from climate-data.org (2021). The definitions of all environmental factors and soil properties considered in the study are presented in Table 2.2.

Table 2.2: Description of environmental factors and soil properties used in this analysis

Environmental factors	Symbol	Units	Definitions
Mean annual precipitation	MAP	mm yr^{-1}	Mean precipitation per year for the study location
Mean annual temperature	MAT	°C yr^{-1}	Mean temperature per year for the study location
Soil organic carbon	SOC	Mg ha^{-1}	Soil organic carbon of the topsoil layer (0-30 cm)
Soil bulk density	BD	g cm^{-3}	Bulk density of the topsoil layer (0-30 cm)
Soil pH	pH		pH of the topsoil layer (0-30 cm) as given in papers at that location
Clay content	Clay	%	Average clay content (or fine-textured soil particles) of the topsoil (0-30 cm)
Sand content	Sand	%	Average sand content (or coarse-textured soil particles) of the topsoil (0-30 cm)
Silt content	Silt	%	Average silt content (or medium-textured soil particles) of the topsoil (0-30 cm)
Nitrogen	N	mg kg^{-1}	Nitrogen content of the topsoil (0-30 cm) as given in papers
Phosphorus	P	mg kg^{-1}	Phosphorus content of the topsoil (0-30 cm) as given in the papers
Potassium	K	mg kg^{-1}	Potassium content of the topsoil (0-30 cm) as given in the papers

The soil textural classes are categorised into three classes: clay loam, loam and sandy soil, based on the percentage of clay, sand and silt in the soil, in that order. When the data for the soil properties was not given, the data from other studies conducted in the same area was used. The classification of all environmental factors and soil properties is summarised in Table 2.3.

Table 2.3: List of classes describing the environmental factors, crop types, soil properties and continents used in the analysis

Environmental factors	Remarks	Class range	Name
Soil pH	Soil pH of the topsoil horizon (0-30 cm) as given in the articles	< 5 5.1-6.5 7.0-8.0 >8	Highly acidic Acidic Basic Highly basic
Clay %	Average clay content of topsoil horizon (0-30 cm)	0-20% 21-40% > 40%	Low Medium High
Sand %	Average sand content of the topsoil horizon (0-30 cm)	0-25% 26-50% > 50%	Low Medium High
Silt %	Average silt content of the topsoil horizon (0-30 cm)	0-20% 21-40% > 40%	Low Medium High
Soil bulk density	Density of the topsoil (0-30 cm)	< 1.4 g cm^{-3} >1.4 g cm^{-3}	Low High
Climatic regions	Hot and wet	MAP: > 1 000 mm yr^{-1} MAT: > 20 °C yr^{-1}	Tropical
	Dry to wet	MAP:300-1 000 mm yr^{-1} MAT: 10-20 °C yr^{-1}	Subtropical
	Cool and moist	MAP: < 800 mm yr^{-1} MAT: 1.2-18.6 °C yr^{-1}	Temperate
	Warm and dry	MAP: 100-300 mm yr^{-1} MAT: 3.5-31.9 °C yr^{-1}	Arid
	Cool and dry	MAP:300-550 mm yr^{-1} MAT: -2.3-25 °C yr-1	Semi-arid

2.2.3 Definitions of experimental environmental conditions

The studies consisted of observations carried out under different soil moisture conditions and were duly divided between drought stress (DS) and non-stress (NS) treatments. The observations that were grouped under the non-stress treatment were considered to be observations made on tuber crop cultivars that received adequate moisture without any significant moisture stress as reported in the respective studies. On the other hand, the observations grouped under drought stress treatment were made on tuber crop cultivars grown with limited soil moisture availability that induced significant drought stress compared to the non-stress treatment, as reported by the respective studies. Some studies reported multiple water regimes, and in such cases, only the maximum and minimum levels were regarded as non-stress and drought stress conditions, respectively.

2.2.4 Definitions of agronomic traits

The definitions of agronomic traits used in the analysis are presented in Table 2.4. A total of eight agronomic traits were extracted from peer-reviewed articles and captured in the database. Plant height (PH) was the average height of the plants from the base to the tip of the plant in a particular plot, and units were normalised to cm. The shoot biomass (SB) was recorded as given in the papers and normalised to grams per plant (g plant^{-1}), using the available plant population density. The number of roots (NR) was captured and standardised to the number of roots per plant. The tuber yield (TY) was recorded as given in the papers, and the units were normalised to g plant^{-1} using the available plant population density. The root:shoot ratio (RS) was recorded as the ratio of root biomass to shoot biomass. Harvest index (HI) was recorded as given in the articles, and in cases where it was not provided, it was calculated using the following formula:

$$HI = \frac{TY}{TPW} \times 100 \qquad (1)$$

Where TY = tuber yield produced in g plant^{-1}, TPW = total plant weight produced in g plant^{-1}.

The percentage of tuber yield reduction was calculated using the following equation:

$$\%TYR = \frac{Y_p - Y_s}{Y_p} \times 100 \quad (2)$$

Where %TYR = percentage tuber yield reduction; Y_p = tuber yield produced under non-stress conditions in g plant^{-1}; Y_s = tuber yield produced under drought stress conditions in g plant^{-1}.

Table 2.4: Definition of agronomic and yield-related traits used in this study

Variable	Symbol	Unit	Description
Plant height	PH	cm	Height from the base of the plant at the soil surface to the tip
Shoot biomass	SB	g plant^{-1}	Total weight of aboveground biomass produced per plant
Tuber yield	TY	g plant^{-1}	Total weight of tubers produced per plant
Number of roots	NR		Number of tubers produced per plant
Harvest index	HI	%	Percentage of tuber yield to total plant biomass
Root:shoot ratio	RS		Ratio of root biomass to shoot biomass produced per plant
Total plant biomass	PB	g plant^{-1}	Total weight of aboveground and belowground biomass produced per plant

2.2.5 Data analysis

The data collected for the yield and yield-related traits was tested for normality, outliers, linearity and homoscedasticity before statistical analyses. Summary statistics describing mean, median, minimum, maximum, quartile 1 (Q1, 25%), quantile 3 (Q3, 75%), standard deviation, coefficient of variation (%CV), skewness (skew) and kurtosis (Kurt) were generated for yield and yield-related traits, soil properties and quantitative environmental factors using IBM SPSS statistical software (version 29). The boxplots showing the distribution of mean values of agronomic traits based on minimum, maximum, median and the first and third quartile values after removing outliers were generated using IBM SPSS (version 29). Pearson correlation was performed to examine the relationship between agronomic traits under drought stress and non-stress conditions using IBM SPSS (version 29). Furthermore, the Pearson correlation coefficient between agronomic traits, environmental factors and crop variables with %TYR was done using IBM SPSS (version 29). A multivariate analysis, using uncentred principal component analysis (PCA), was conducted to show the multiple relationships between %TYR, MAP, MAT, clay, sand, silt, pH, bulk density,

crops, soil textural classes and climates using R statistical software. The metadata and relevant results for effect size were extracted from each study. The effect size of each study (log response ratio) was calculated using the following equation:

$$lnR = \ln(R) = \ln\left(\frac{\bar{x}_1}{\bar{x}_2}\right) = \ln(\bar{x}_1) - \ln(\bar{x}_2) \quad (3)$$

Where $\bar{x}_1$ = mean of treatment (drought stress) and $\bar{x}_2$ = mean of control (non-stress). The forest plots were generated using MetaWin3 software.

2.3 Results

2.3.1 Variation of mean values of soil properties, environmental factors and agronomic traits

The variations among the soil properties and environmental factors are presented in Table 2.5. Among the soil properties, bulk density, soil pH and sand content had negative skewness values, showing that the values for those variables were skewed to the left. The MAP shows a relatively higher %CV (49.46%) than MAT (21.27%), indicating that mean temperature values had low variations. Among all the soil properties variables, only sand (-1.47) and silt (- 0.97) had negative kurtosis values. The variations among the recorded agronomic traits (under drought stress and non-stress conditions) are presented in table 5b. All means for all the agronomic traits were lower under drought stress than non-stress conditions, except for dry matter content and root:shoot ratio (table 2.5). Among the recorded traits, only plant height had a negative skewness and kurtosis under different water regimes, indicating that most plant height observations were around the mean (Table 2.6).

Table 2.5: Summary statistics of environmental factors and soil properties used in the analysis

Variables	BD	pH	N	P	K	Sand	Silt	Clay	MAT	MAP
Mean	1.43	6.93	0.29	23.25	1.56	43.59	33.32	22.95	22.84	771.92
Median	1.51	7.13	0.11	11.23	0.64	50	22	18	24.75	675
Min	0.85	3.94	0.02	0.16	0.02	7.5	12	5.67	7.5	230
Max	1.66	8.89	10	125	7.22	73.3	66	62	28	2435
Q1	1.3	6.57	0.03	3.5	0.22	20	21.75	15	18.3	537
Q3	1.53	7.24	0.23	30	0.8	60	53	22	27	800.9
Std	0.17	0.76	0.89	29.45	2.35	21.67	18.28	13.23	4.86	381
cv%	12.1	10.94	304.73	126.68	151.26	49.71	54.87	57.62	21.27	49.36
Skew	-1.49	-1.42	8.58	1.81	1.69	-0.35	0.79	2.04	-0.91	1.53
Kurt	2.16	3.69	86.38	2.99	1.13	-1.47	-0.97	3.19	-0.02	2.41

BD = bulk density (g cm^{-3}); N = nitrogen (mg kg^{-1}); P = phosphorus (mg kg^{-1}); K = potassium (mg kg^{-1}); CV = coefficient of variation; Kurt = kurtosis; MAP = mean annual precipitation; MAT = mean annual temperature; Max = maximum; Min = minimum; Q1 = lower quartile; Q3 = upper quartile.

Table 2.6: Summary statistics of recorded agronomic traits and percentage yield reduction of tuber crops under drought stress and non-stress conditions

Variables	PH		TY		NR		SB		HI		RS		PB		%TYR
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	
Mean	186.86	166.05	1637.37	1019.87	10.02	8.05	3489.65	3200.99	21.81	16.62	0.8	0.89	6305.54	6093.2	49.29
Median	223.8	190.6	580.3	275.5	8	7.23	722.66	756.7	19.4	2.69	0.66	0.77	1162.58	1033.7	50.04
Min	47.5	37.5	1.03	0	1	0.13	1.07	1.73	0.25	0.26	0.07	0.05	6.87	7.15	0.53
Max	302.5	290	17060	15190	39	35	20840	14560	79	83	5.06	4.81	37500	33940	98.74
Q1	83	77.4	280.86	113.8	5.63	4.63	310.56	145	0.49	0.47	0.38	0.4	568.82	260.1	26.68
Q3	258.8	239.6	1590	830.75	12	10.53	6970	5810	39.3	28.69	0.96	1.06	13232.5	11170	71.93
Std	85.41	80.38	2440.12	1925.26	6.54	5.67	4558.48	3507.13	20.38	20.37	0.73	0.78	8274.45	6772.07	26.98
cv%	45.71	48.41	149.03	188.78	65.25	70.37	130.63	109.56	93.47	122.55	90.74	87.61	131.23	111.14	54.93
Skew	-0.46	-0.22	2.51	3.3	1.78	1.33	1.32	0.85	0.49	1.15	3.3	2.59	1.29	0.94	-0.08
Kurt	-1.41	-1.52	6.98	13.18	3.56	2.88	0.88	-0.12	-0.86	0.55	14.13	8.18	0.93	0.56	-1.17

PH = plant height (cm); TY = tuber yield (g plant^{-1}); NR = number of roots per plant; SB = shoot biomass (g plant^{-1}); HI = harvest index (%); RS = root:shoot ratio; PB = plant biomass (g plant^{-1}); %TYR = percentage tuber yield reduction; NS = non-stress conditions;

DS = drought stress conditions; CV = coefficient of variation; Kurt = kurtosis; MAP = mean annual precipitation; MAT = mean annual temperature; Max = maximum; Min = minimum; Q_1 = lower quartile; Q_3 = upper quartile.

2.3.2 Effect of the crop on percentage tuber yield reduction

The mean percentage tuber yield reduction of the crops evaluated in the study was 49.29%. Among the tuber crops evaluated, cassava had a significantly lower mean %TYR (32.45%) with values ranging from 0.53% to 87.54%, followed by potato (47.63%), which ranged from 1.64% to 94.83%, and sweet potato (61.08%), which ranged from 1.79% to 98.74% (Figure 2.3). The %TYR in sweet potato was shown to be more than two times higher than the %TYR in cassava. Figure 2.4 confirms that cassava experienced the least %TYR under drought stress, with a relatively narrow confidence interval, and sweet potato exhibited the most negative effect size, indicating the highest %TYR and a wider confidence interval. The %TYR was significantly different on cassava, potato and sweet potato at $p < 0.05$ (Figure 2.3), and there was a significant difference between tuber yield under drought stress and non-stress conditions in all the crops.

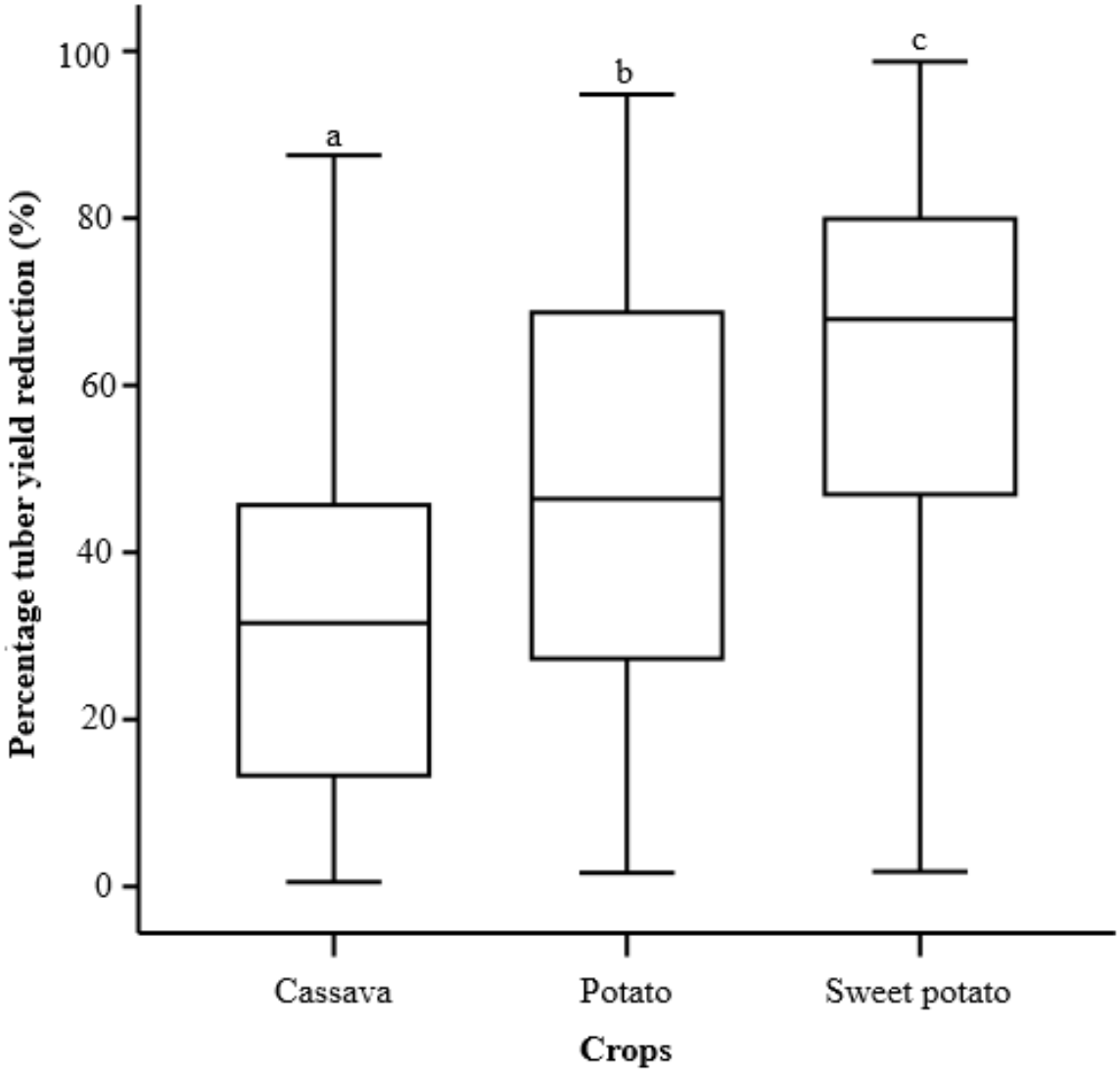

Figure 2.3: %TYR in different tuber crops. Each boxplot presents the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%) for %TYR. Boxplots accompanied by similar letters were not significantly different at $p < 0.05$.

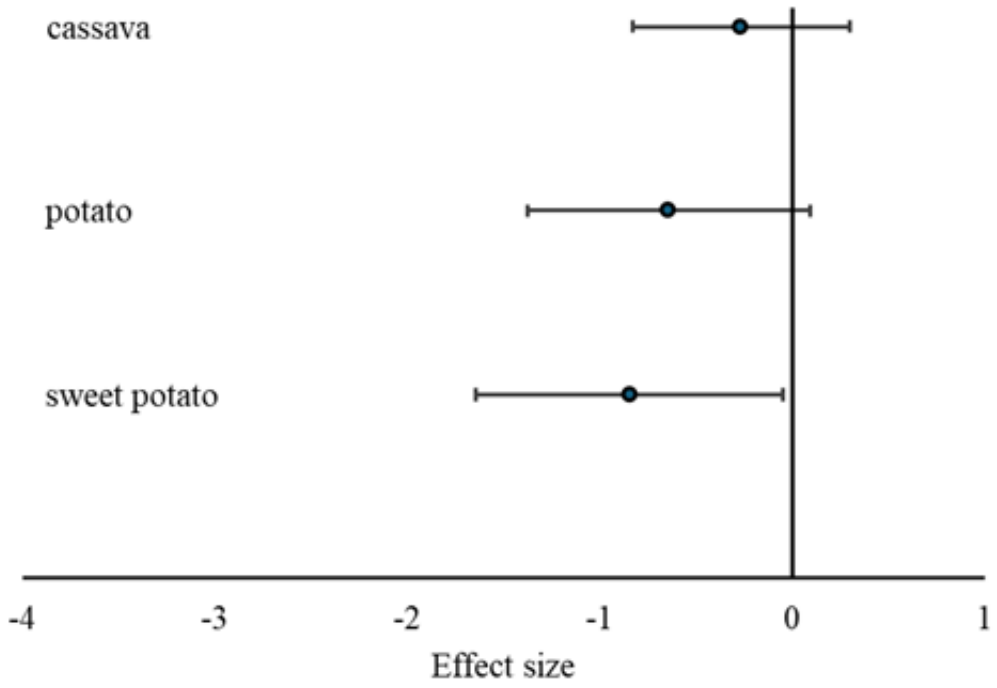

Figure 2.4: Effects of drought stress conditions on %TYR in different crops. Values are means ± 95% confidence intervals.

2.3.3 Impact of environmental conditions on percentage of tuber yield reduction

2.3.3.1 Soil texture

The boxplots (Figure 2.5) highlight that the %TYR in each soil texture differed, with clay having the lowest mean %TYR of 38.14%, followed by sandy clay loam with a mean %TYR of 39.42%; the highest mean %TYR was observed in loam soil. The variation of %TYR was high for loam soils (Figure 2.5), with a minimum of 1.79% and a maximum of 98.74%, and the least for %TYR on clay soils (min = 5.35%, max = 81.02%). There was no significant difference between the %TYR on clay and sandy clay loam soils, clay loam and sandy, and loam and sandy loam (Figure 2.5), but the median %TYR on loam soils was higher than on other soils.

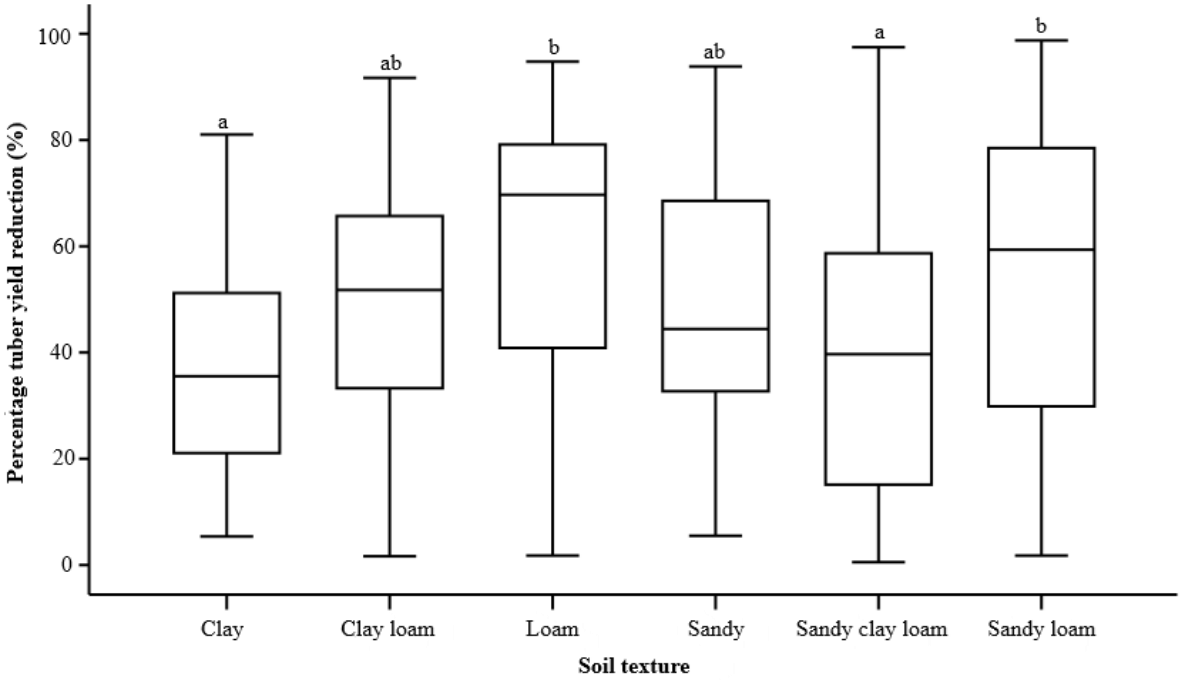

Figure 2.5: %TYR in different soil textural classes. Each boxplot presents the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%) for %TYR. Boxplots accompanied by similar letters were not significantly different at $p < 0.05$.

2.3.3.2 Climate

The overall mean of the %TYR was higher under tropical (98.74%), temperate (97.8%), subtropical (97.45) and semi-arid (93.50%) climates, and lower under arid (76.11%) and savanna (52.32%) climates (Figure 2.6). The variation of %TYR was higher under tropical climates with a minimum of 1.78% and a maximum of 98.74%, followed by subtropical (minimum = 1.79% and maximum = 97.8%), and lower variation was observed under the savanna (min = 26.62%, max = 52.32%) climate (Figure 2.6). The median for %TYR under the subtropical climate was higher than any other climate (Figure 2.6). There was a significant difference between Y_p and Y_s under all the climates.

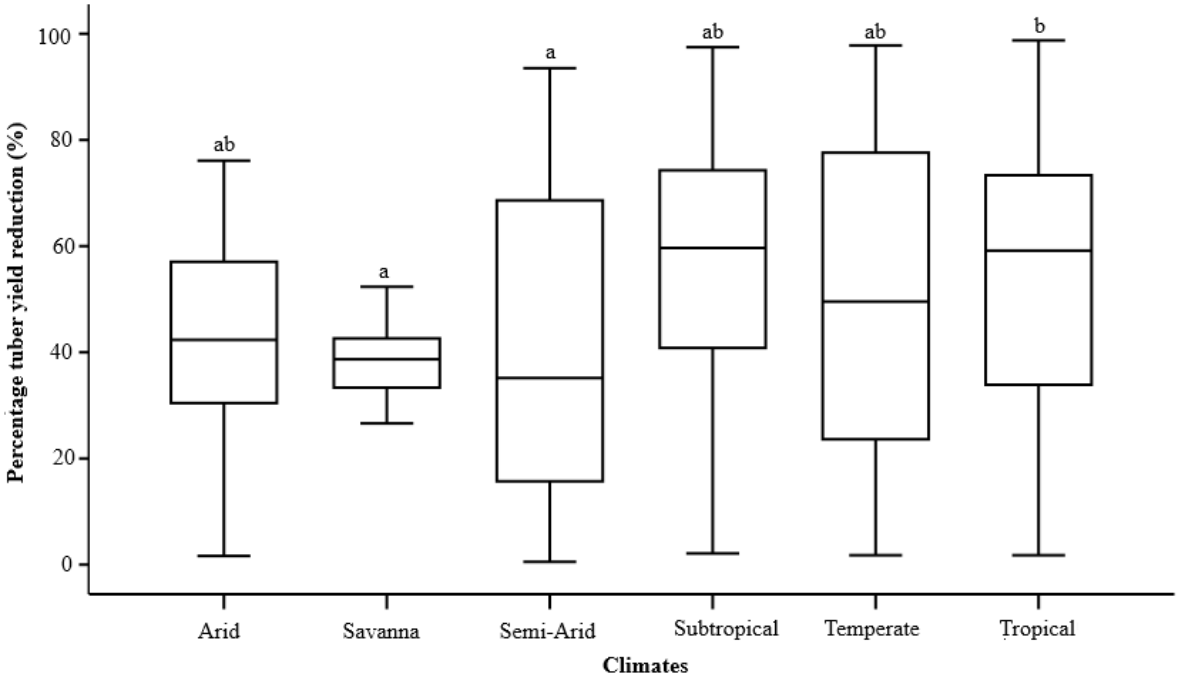

Figure 2.6: %TYR in different climates. Each boxplot presents the minimum, maximum, median, quartile 1 (25%) and quartile 3 (75%) for %TYR. Boxplots accompanied by similar letters were not significantly different at $p < 0.05$.

2.3.4 Correlation analysis

The Pearson coefficient showed the relationship between agronomic traits of tuber crops under drought stress and non-stress conditions (Table 2.7). Under drought stress conditions, tuber yield was high and positively correlated with PH ($r = 0.72$; $p < 0.01$), SB ($r = - 0.82$; $p < 0.01$) and PB ($r = 0.90$; $p < 0.01$) and negatively correlated with HI ($r = 0.67$; $p < 0.01$) (Table 6). Under non-stress conditions, TY was strongly and positive correlated with PH ($r = 0.76$; $p < 0.01$), SB ($r = -0.87$; $p < 0.01$) and PB ($r = 0.96$; $p < 0.01$) and strongly negative correlated with HI ($r = -0.70$; $p < 0.01$) (Table 6). Under both treatments, RS was positively correlated with TY (R = 0.12 under NS; r = 0.19 under DS). Plant height exhibited non-significant correlation with PB under drought stress and non-stress treatments (Table 2.7).

Table 2.7: Pearson correlation coefficient between agronomic traits under drought stress (below diagonal) and non-stress (above diagonal) conditions

Variables	PH	TY	NR	SB	HI	RS	PB
PH		**0.76****	**-0.30****	**0.17**	**-00.16**	**-0.12**	**0.21**
TY	**0.72****		**-0.15***	**0.87****	**-0.70****	**0.12**	**0.96****
NR	**-0.10**	**0.05**		**0.37****	**-0.25****	**0.18***	**0.45****
SB	**0.24***	**0.82****	**0.66****		**-0.71****	**-0.04**	**0.93****
HI	**0.03**	**-0.67****	**-0.43****	**-0.69****		**0.18****	**-0.71****
RS	**-0.001**	**0.19***	**0.50****	**0.02**	**0.24****		**0.03**
PB	**0.19**	**0.90****	**0.71****	**0.94****	**-0.69****	**0.17***	

*p < 0.01; **p < 0.05 PH = plant height (cm); TY = tuber yield (g plant^{-1}); NR = number of roots per plant; SB = shoot biomass (g plant^{-1}), HI = harvest index (%), RS = root:shoot ratio; PB = plant biomass (g plant^{-1}).

Furthermore, the relationship between (%TYR with agronomic traits, environmental factors and crops was explored using a Pearson correlation (Table 2.8). The %TYR was negatively correlated with tuber yield under drought stress (r = - 0.30; p < 0.01) and non-stress (r = -0.21; p < 0.01) conditions (Table 2.7). Among the soil nutrients, only soil phosphorus was negatively correlated with %TYR (r = -0.22; p < 0.01). In addition, sand content was positively correlated with %TYR (r = 0.18; p < 0.01) (Table 2.8), indicating that the impact of drought stress conditions was higher on soils with high sand content. In addition, the %TYR was negatively correlated with PB and SB (Table 2.7), exhibiting the importance of biomass production in reducing %TYR.

Table 2.8: Pearson correlation coefficient between agronomic traits, environmental factors, crop variables with %TYR

Variable	%TYR
Crop name	-0.40**
Climate	0.17**
Continent	0.15**
BD	0.05
pH	0.01
N	0.11**
P	-0.22**
K	0.03
Sand	0.18**
Silt	-0.18**
Clay	-0.06
Soil texture	0.16**
MAT	-0.16**
MAP	0.43

*p < 0.05; **p < 0.01 BD = bulk density (g cm^{-3}); N = nitrogen (mg kg^{-1}); P = phosphorus (mg kg^{-1}); K = potassium (mg kg^{-1}); MAP = mean annual precipitation; MAT = mean annual temperature, Y_p = tuber yield under non-stress (g $plant^{-1}$); Y_s = tuber yield under drought stress (g $plant^{-1}$).

2.3.5 Multivariate analysis

The PCA biplot in Figure 2.7 illustrates the inter-relationship between crops (cassava, potato and sweet potato), climate types (tropical, subtropical, semi-arid, arid, temperate and savanna) and soil textural classes (sandy, sandy loam, loam, clay, clayey, sandy clay loam and clay loam). The analysis reveals that cassava is closely associated with clayey soils and savanna climates, whereas potato is closely associated with arid environments and phosphorus-rich soils. Soil texture and climate interactions show that sandy soils are associated with subtropical climates and negatively correlated with nitrogen (N), whereas clay and loamy soils are more prevalent in semi-arid and savanna climates. Additionally, sandy clay loam and clay loam soils are related to high bulk density (BD) and mean annual temperature (MAT), whereas loamy soils are associated with potassium (K) and appear favourable for cassava cultivation. Arid regions exhibit a strong correlation with phosphorus (P), and tropical regions are linked to sweet potato and high %TYR (Figure 2.7).

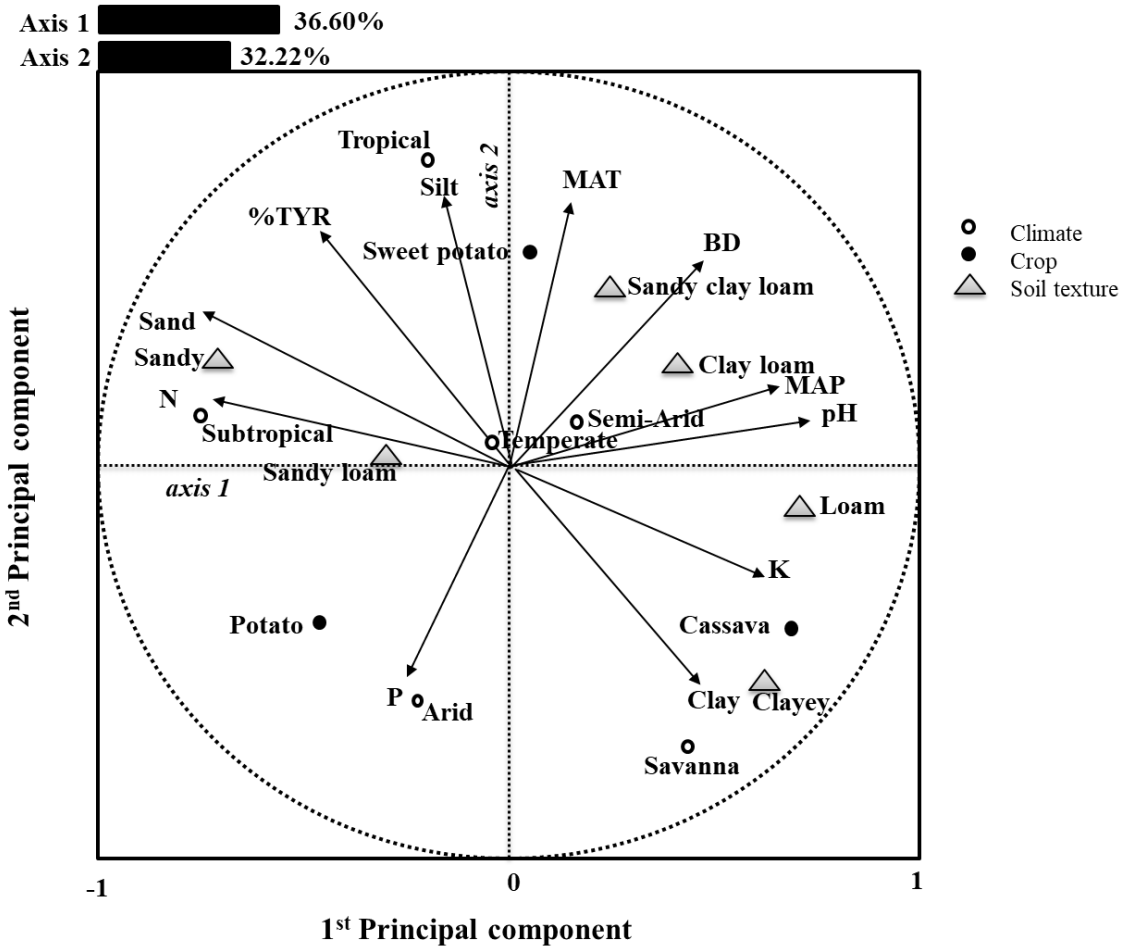

Figure 2.7: PCA showing the inter-relationship between crops, climate and soil textural classes

2.4 Discussion

2.4.1 Impact of drought stress on agronomic traits of major tuber crops

The results of the study show the detrimental effects of drought stress on the agronomic traits of major tuber crops, especially on tuber yield (Table 2.6). Tuber crops grown under drought stress conditions exhibited a reduced plant height, tuber yield, number of tubers per plant, shoot biomass, total plant biomass and harvest index, compared to non-stress conditions (Table 2.6). The reduction of these agronomic traits under drought stress conditions is due primarily to restricted photosynthetic rate, reduced carbon assimilation and limited nutrient uptake (Ahluwalia et al., 2021). Drought stress often induces stomatal closure, leading to a decline in CO_2 influx and photosynthetic efficiency, thereby limiting energy production essential for plant growth and development (Flexas & Medrano, 2002; Parkash et al., 2024).

These findings are in line with those of Batool et al. (2020), who highlighted that drought stress conditions reduced agronomic traits, including plant height, tuber yield and shoot biomass in potato cultivars evaluated under drought stress and non-stress conditions. Interestingly, the root:shoot ratio was higher under drought stress (0.89) than non-stress conditions (0.8). This indicates an adaptive response in which plants allocate more resources towards root growth to enhance water uptake. This shift in biomass partitioning is a survival mechanism well documented in several studies, enabling plants to explore deeper soil layers for moisture under drought stress conditions (Seleiman et al., 2021; Kalra et al., 2024; Ke et al., 2025). These findings highlight the importance of focusing on aboveground agronomic traits and below-ground traits that contribute to drought resilience. The observed increase in root:shoot ratio suggests that root architectural traits, such as root length density, root depth and root surface area, play a critical role in enhancing water uptake under limited moisture conditions (Huang et al., 2023). Future breeding efforts should prioritise the identification and incorporation of root traits associated with improved water foraging capacity and resource use efficiency. Therefore, integrating high-throughput phenotyping tools and genomic selection approaches targeting root traits could accelerate the development of drought-tolerant tuber crop varieties.

2.4.2 Impact of crop type on the variations in %TYR

The %TYR varied significantly among the evaluated crops, with an average %TYR of 49.29% (Table 2.6). This highlights the variability in drought tolerance among the evaluated crops (cassava, potato and sweet potato) (Daryanto et al., 2016; Daryanto et al., 2017) and the need to select resilient crops and understand how they adapt to water stress, including their response mechanisms, to minimise yield losses in related crop species. Among the tuber crops, cassava exhibited the lowest %TYR (32.45%), followed by potato (47.63%) and sweet potato (61.08%). This highlights that cassava is more drought tolerant than potato and sweet potato due to its deep root system, efficient water use, ability to maintain photosynthesis under water stress and strong osmotic adjustment mechanisms, which help it sustain growth and yield under drought conditions (Okogbenin et al., 2013; Muiruri et al., 2021). In contrast

to common assumptions regarding the drought resistance of tuber crops, our findings highlight that yield reduction was higher in sweet potato than potato. This suggests that drought tolerance in sweet potato could be related more to survival rather than tuber yield production. The findings are in line with Daryanto et al. (2016), who reported that potato had lower yield reduction due to drought stress conditions compared to sweet potato. The %TYR in sweet potato was more than twice that of cassava (Figure 2.3), highlighting its vulnerability to drought stress. This suggests that targeted breeding efforts should focus on enhancing tuber yield production and drought tolerance of sweet potato. Conversely, cassava's inherent drought adaptability positions it as a strategic crop for drought-prone regions (Okogbenin et al., 2013). Furthermore, the results show that cassava is highly different in its drought response compared to the two other crops (potato and sweet potato) (Figure 2.4). This suggests that cassava may be utilising unique regulatory mechanisms that allow it to tolerate drought stress more effectively than the other two crops (Muiruri et al., 2021). Therefore, the use of high-throughput phenotyping technologies such as transcriptomics, metabolomics and root imaging are essential for understanding the molecular and physiological traits behind drought resilience of cassava.

2.4.3 Impact of environmental factors on %TYR

2.4.3.1 Soil texture

The findings of the study highlight that soil texture plays a crucial role in modulating %TYR under drought stress. Among the soil types evaluated, clay soil exhibited the lowest mean %TYR (38.14%) compared to other textural classes (Figure 2.5). This trend is attributable to its superior water-holding capacity (Libohova et al., 2018), indicating that this characteristic effectively mitigates drought-induced yield reductions by maintaining soil moisture availability for longer durations. In contrast, loam soils displayed the highest variation in %TYR (ranging from 1.79% to 98.74%) (Figure 2.5). This is possibly because additional interacting factors, such as genotype-specific responses and localised soil heterogeneity, significantly influenced drought resilience in this soil type. Furthermore, clay soils demonstrated lower %TYR and exhibited minimal variability, reinforcing their capacity to provide a stable moisture

environment that buffers crops against severe yield losses. This stability contrasts with loam and sandy soil types, where fluctuations in water availability may exacerbate yield inconsistencies. Although no statistically significant differences in %TYR were observed between specific soil types (e.g. clay, sandy clay loam, clay loam, sandy, loam and sandy loam), the consistently higher median %TYR in loam soil suggests its reduced efficiency in sustaining productivity under water-limited conditions. This further emphasises the advantage of clay-rich soils in promoting crop resilience, as their superior moisture retention properties create a more uniform agronomic environment, thereby reducing the severity of drought-induced yield reduction. However, clay-rich soils also present certain challenges such as poor drainage, compaction and reduced aeration (TAT, 2024), which can hinder root development and nutrient uptake under prolonged wet conditions. Therefore, while clay soils can buffer against short-term drought stress, effective management practices such as maintaining good soil structure and drainage are essential to optimise crop performance.

2.4.3.2 Climate

The data from this study highlights the significant influence of climatic conditions on the %TYR (Figure 2.6). Overall, the mean %TYR was higher in tropical (98.74%), temperate (97.8%) and subtropical (97.45%) climates (Figure 2.6), indicating that these regions are highly impacted by drought stress in terms of yield loss. In contrast with common assumptions, the impact of drought stress conditions on tuber yield production was lower under semi-arid, arid and savanna climates compared to tropical, temperate and subtropical climates. This suggests that crops grown in semi-arid, arid and savanna climates may have developed adaptive mechanisms that enhance their resilience to drought stress (Irob et al., 2023; Rehman et al., 2024). These adaptations include deeper root systems, enhanced water use efficiency, osmotic adjustments and improved stomatal regulation, allowing them to produce optimum yield under drought stress (Muiruri et al., 2021). Conversely, tuber crops in tropical, temperate and subtropical climates may be more sensitive to drought stress due to their reliance on higher and more consistent moisture levels. This highlights the importance of breeding and selecting drought-resilient genotypes suited to different climatic conditions to

ensure sustainable tuber production. Furthermore, the significantly lower %TYR was observed in savanna climates compared to other climates. That may be attributed to the traditional preference for growing cassava in these regions. Cassava's deep root system enables it to survive and maintain productivity under limited water availability and in low fertility soils (Moore & Lawrence, 2024).

The significant differences observed between potential yield (Yp) and stress yield (Ys) across all climates highlight the adverse impact of drought stress on tuber yields. These findings are consistent with the broader literature on drought stress in tuber crops (Daryanto et al., 2016; Daryanto et al., 2017). This gap between Yp and Ys highlights the necessity for breeding programmes aimed at enhancing drought tolerance. The considerable variation in %TYR within tropical and subtropical climates suggests that there is potential for selecting cassava varieties with improved drought tolerance tailored to specific environmental conditions. Therefore, targeted breeding, improved agronomic practices and climate-specific cultivar development should be prioritised by plant breeders to ensure sustainable tuber yield production under increasing drought risk.

2.4.4 Association between %TYR, crops, climates and soil texture classes

The analysis provides valuable insights into how environmental and edaphic factors influence the performance and resilience of major tuber crops such as cassava, potato and sweet potato across different regions and diverse soils. The PCA shows distinct groupings of crops based on their associations with specific climatic conditions and soil properties. Notably, cassava appeared to be in the same direction as semi-arid and savanna climates (Figure 2.7), indicating that it thrives in these regions with moderate to low rainfall and relatively warm temperatures. This aligns with existing knowledge that cassava is a drought-tolerant crop capable of growing in less fertile soils with minimal water availability (Okogbenin et al., 2013; Zhao et al., 2015; Anikwe & Ikenganyia, 2018; Devi et al., 2022; Koundinya et al., 2024). Moreover, cassava’s deep rooting system and capacity to store carbohydrates in roots for extended periods enable it to sustain growth and productivity during prolonged dry spells. In contrast, sweet potato appeared to be in the same direction as %TYR (Figure 2.7). This

highlights that this crop experiences significant yield loss compared to cassava and potato under drought stress conditions, possibly because sweet potato has a shallower root system than cassava (Lal & Maurya, 1982), which can absorb water from deeper soil profiles during periods of water scarcity. This highlights an urgent need for improved drought mitigation strategies, including the development of water-efficient irrigation systems, soil moisture conservation practices and breeding of drought-resilient sweet potato genotypes with improved root architecture. Interestingly, %TYR also appeared in the same direction as cassava in the PCA, particularly under savanna conditions, suggesting that yield reductions were lower in these regions. This may be attributed to the seasonal rainfall distribution and soil properties in savanna regions, which promote water infiltration and retention in deeper soil layers. These conditions favour crops such as cassava which have a long growing cycle that allows them to recover from intermittent drought once rainfall resumes. Therefore, these findings highlight the strategic value of cassava in climate-resilient farming systems and the need for improving sweet potato and potato performance under drought stress conditions.

2.4.5 Conclusion

The study revealed that drought stress imposes significant constraints on agronomic traits and tuber yield production, with yield losses varying widely across environments and crop types. Cassava demonstrated the greatest resilience, exhibiting lower %TYR than potato and sweet potato. These differences reflect species-specific physiological adaptations and the strong influence of environmental factors such as soil texture, temperature and rainfall distribution. The savanna climates were associated with lower %TYR compared to other climates such as tropical and temperate climates, where higher %TYR was recorded. These results highlight the importance of integrating crop-specific traits with environmental parameters in drought adaptation strategies. Therefore, future studies should focus on using high throughput phenotyping tools to understand the unique regulatory mechanisms that cause higher %TYR under tropical and subtropical climates.

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CHAPTER 3: MATERIALS AND METHODS

3.1 Sweet potato

3.1.1 Sweet potato planting

Four sweet potato cultivars (Atacama, Jane, Blesbok and Bellevue) were selected for the drought experiments (Omotobora et al., 2014, Laurie et al., 2024). Characteristics of each cultivar are described in supplementary Table S1. The trial was planted under a rainout shelter at the Agricultural Research Council-Vegetable, Industrial and Medicinal Plants Research Institute (ARC-VIMP), Roodeplaat Campus, Pretoria, South Africa (25.604° S, 28.345° E; 1189 m altitude) during the February 2024 growing season. Meteorological data recorded at the ARC–VIMP research site during the 2024 growing season is indicated in supplementary Table S2. The rainout shelter was made of a corrugated polycarbonate sheet supported on steel profiles (pillars and rails) with a thickness of 1.25 mm and UV protected on both sides. Sweet potato cuttings were planted in bags with 40 kg of soil per bag, with the soil prepared to normal cultivation practices (soil conditions are indicated in supplementary Table S3) at a plot size of 242 m^2, with an inter-spacing of 0.7 m and intra-spacing of 0.4 m. The trial was laid out in a randomised block design, with each plot consisting of 12 plants. The area under the rainout shelter was covered with black plastic mulch before placing the bags for insulation and also to control weeds from growing. Plants were grown for five weeks before imposition of drought stress, with continuous irrigation to field capacity (FC). Three water regimes, that is, 30% soil water depletion (control, 70% FC), 50% (mild stress, 50% FC) and 70% (severe stress, 30% FC) were applied, and each treatment and cultivar were replicated four times (Figure S1). A total of 576 plastic bags were used. Fertilizers (multi-feed and LAN (28)), insecticides (Decis®, Profenofos 500 EC, Biomectin (R)) and fungicides (Nanogreen SC and Azoxystrobin 250 SC) were applied to the soil according to the manufacturer's instructions. Water management was done through monitoring of soil water content (every two days to establish the amount of water needed) by measuring the relative water content using the formula below (Ma et al., 2017).

$$RSWC = \frac{Current\ pot\ weight\ - Soil\ dry\ weight}{Weight\ of\ soil\ watered\ to\ field\ capacity\ wet}\ X\ 100\%$$

3.1.2 Metabolite extraction

Young leaves from plants under drought stress and non-stress conditions (30%, 50% and 70%) were collected at two weeks post drought imposition, representing early drought stress. The plant leaf samples collected were kept in -80 °C prior to analysis. Leaves (200 mg) were individually ground with liquid nitrogen, and the homogenate was resuspended with 1.5 mL prechilled 80% methanol (-20 °C, HPLC grade, Minema Chemicals) in 2 mL Eppendorf tubes followed by good vortexing. The extraction process was carried out using the methodology outlined in Maserumule et al. (2023) and Makhubu et al. (2024). The samples were centrifuged at 2 850 revolutions per minute (rpm) for 5 minutes at 4 °C after being sonicated for 2 hours in ice cold water. The supernatant (extract) was transferred to a 2 ml Eppendorf tubes and stored at 4 °C. Glass vials with 0.5 mL inserts (Alwsci Technologies, 6 x 31 mm) were then filled with the supernatants after the contents had been filtered through 0.22 µm nylon filters. Each sample group had four replicates prepared, which were kept at 4 °C until further analysis.

3.1.3 UHPLC-ESI-MS analysis

Using a liquid chromatography–quadrupole time-of-flight tandem mass spectrometer (LCMS-9030 qTOF, Shimadzu Corporation, Kyoto, Japan) at the University of Venda, Department of Biochemistry, leaf extracts were assessed for metabolites following the methodology outlined in Ramabulana et al. (2021). Employing a Shim-pack Velox C18 column (100 mm × 2.1 mm, 2.7 µm particle size; Shimadzu Corporation, Kyoto, Japan), the chromatographic separation was carried out at 55 °C. A 13-minute procedure with the following gradient conditions was used to analyse each sample (3 µL): solvent A was 0.1% formic acid in Milli-Q water (HPLC grade, Merck, Darmstadt, Germany); solvent B was methanol (UHPLC grade, Romil SpS, Cambridge, UK) mixed with 0.1% formic acid. Throughout the designated gradient, the flow rate was maintained at 0.45 mL/min under the following separation conditions: After 2 minutes of equilibration at 10% B, 10–60% B was induced during 3–5 minutes. From 5–8

minutes, the settings were adjusted from 60% to 90% B, and from 8–11 minutes, the gradient was maintained at 90%. After 1 min (11–12 min), the gradient was brought back to its starting 90–60%, and shortly thereafter there was a 1-min column equilibration pause. The qTOF high-definition mass spectrometer, which was conditioned for negative electrospray ionisation for data acquisition, was used for chromatographic analysis. The set of settings included the following: heat block temperature (400 °C), detector voltage (1.8 kV), DL temperature (280 °C), interface voltage (-3 kV), interface temperature (300°C), nebulisation and dry gas flow (3 L/min) and flight tube temperature (42 °C). With argon serving as the impact gas and a collision energy of 30 eV, fragmentation experiments were carried out using a spread of 5 eV.

3.1.4 Data analysis

3.1.4.1 Raw data pre-processing

The raw data in negative electrospray ionisation mode (ESI negative) obtained from the LCMS-9030 qTOF was extracted as mzML files and processed using XCMS online (http://XCMSOnline.scripps.edu/) (accessed on 13 April 2024). Data pre-processing was done using XCMS with UPLC/UHD Q-TOF negative mode parameters following Ramabulana et al. (2021) and Makhubu et al. (2024), employing the CentWave feature detection method with a maximum threshold of 15 ppm, a signal:noise ratio was set to 6, prefilters set at an intensity of 700, peaks at 3 and noise set at 15. Retention time correction was performed using the obiwarp method with a profStep of 1. For alignment, the minimum fraction (minfrac) of samples was set to 0.5, and the width of overlapping m/z for peak density chromatograms and grouping across samples (mzwid) was set at 0.025 m/z. The Mann-Whitney non-parametric test was applied to assess differences between group means (Atacama and Blesbok, drought stress (50% and 30% FC) and non-stress treatments (70% FC) of the two cultivars), followed by post hoc analysis, with data normalisation using median fold change.

3.1.4.2 Multivariate data analysis

The features from the resulting feature table from XCMS were imported into SIMCA version 17.0 software (Sartorius, South Africa), normalised and Pareto scaled before applying the model. Both an unsupervised model, PCA and supervised models, orthogonal projections to latent structures discriminant analysis (OPLS-DA) and partial least squares discriminant analysis (PLS-DA) were employed. S-plots from the OPLS-DA score plots were generated, and significant biomarkers with [p(corr)] ≥ 0.5 and covariance of (p1) ≥ 0.05 were annotated by matching their spectral features and retention times with databases, leading to their putative identification (Tugizimana et al., 2016). A Venn diagram (version 2.1) was used to present the overlap of metabolites in the two cultivars at different drought regimes. Additionally, variable importance in projection (VIP) was used for screening the three drought stress conditions and non-stress conditions in each cultivar. From the PLS-DA model, the VIP scores were generated. It is generally accepted that variables with VIP scores greater than 1.0 are typically considered significant, and this threshold is commonly used as the criterion for selecting important variables (Li et al., 2019).

3.1.4.3 Metabolite annotation and pathway analysis

Annotated metabolites were identified from untargeted UHPLC–MS data through a combination of accurate mass matching. Identification and annotation were performed by comparing observed m/z values and retention times to entries in publicly available metabolite databases, including the Global Natural Product Social Molecular Networking (GNPS), Human Metabolome Database (HMDB), Massbank, KNApSAcK, COCONUT, Foodb, Sirius and PubChem library. Level 2 annotation confidence (putatively annotated compounds) was assigned based on spectral similarity without comparison to authentic standards. These annotated metabolites were then used for pathway analysis, mapping the metabolic processes influenced by the experimental conditions. The analysis was conducted using metabolic pathway analysis integrated into the MetaboAnalyst toolset (version 6.0; http://www.metaboanalyst.ca/), which maps pathways using established Kyoto Encyclopaedia of Genes and

Genomes (KEGG) metabolic pathways. Compound names were used as input for pathway analysis, relative centrality was chosen to examine the topology of node importance (pathway impact), and a scatter plot was utilised for display (–log(p-value) (enrichment score). *Arabidopsis thaliana* (KEGG) was chosen as the path library; this was due to the absence of a curated KEGG pathway library for *Ipomoea batatas*. This integrative approach enabled the identification of key metabolic pathways significantly affected by drought stress, thus providing insight into cultivar-specific metabolic adaptation.

3.1.5 Protein extraction

The harvested leaf material was crushed into powder using liquid nitrogen. 100 mg of the crushed material was weighed and used for downstream protein extraction. Protein was extracted from the leaf material using the Macherey-Nagel™ NucleoSpin™ TriPrep Mini kit for RNA, DNA and protein purification.

3.1.5.1 Sample preparation

Sample preparation was conducted at the Council for Scientific and Industrial Research (CSIR) (Pretoria, South Africa). Samples were thawed, then reduced with 10 mM dithiothreitol (DTT) for 45 minutes at room temperature and alkylated with 40 mM iodoacetamide (IAA) for 45 minutes in the dark. Thereafter, the protein solution was diluted with HILIC binding buffer (30% acetonitrile (ACN) in 200 mM of ammonium acetate (NH_4AC), pH 4.5). Following the previously described protocol by Govender et al. (2023), the proteins were digested on-bead using MagResyn^{TM} HILIC microparticles (ReSyn Biosciences, Edenvale, South Africa) automated on a KingFisher Duo^{TM} (Thermo Fisher Scientific, USA). The microparticles were equilibrated in equilibration buffer (15 % ACN and 100 mM NH_4Ac, pH 4.5). The microparticles were added into a well that contained binding buffer and mixed for 30 minutes at room temperature. The collected proteins were washed twice in 95% ACN. On-bead digestion was performed by adding 50 mM ammonium bicarbonate (ABC), pH 8.0, containing 1 µg sequencing grade trypsin for 4 hours at 37 ˚C. Finally, the beads were rinsed with 1% trifluoroacetic acid (TFA) to elute any residual peptides. The resultant peptides were vacuum dried, then resuspended in 2% ACN and 0.2% formic acid (FA). Peptide quantification was

performed, to determine peptide recovery, using a colorimetric assay. The peptides were stored at -80 ˚C until LC-MS/MS analysis. Three biological replicates of each cultivar were analysed.

3.1.5.2 LC-MS/MS analysis

Digested peptides were analysed using the Evosep One HPLC system (Evosep Aps, Denmark) equipped with an EV1109 performance column and operated under the Whisper 60 SPD method. The system was coupled to a Bruker timsTOF HT (Bruker Daltonics) via a CaptiveSpray 2 nano-electrospray ionisation source. The data was acquired in data-independent acquisition (DIA) mode using the dia-PASEF method. The data was acquired using trapped ion mobility spectrometry (TIMS) and time of flight (TOF) analysers for MS1 and MS2. Separation using ion mobility was performed across 21 windows with a cycle time of 0.958 s for both MS1 and MS2. MS1 spectra were acquired in the 100–1700 m/z range. Profile mode was used for MS2 acquisition with centroid processing.

3.1.5.3 Data pre-processing and analysis

Protein quantification was conducted using Spectronaut™ 17 (Biognosys AG, Schlieren, Switzerland), using the directDIA+ workflow with the default settings applied: specific enzyme cleavage by trypsin/P. Fixed modification was set for carbamidomethyl © and variable modification was set for acetyl (protein N-terminal) and oxidations (M). Identification of peptides and proteins was performed using a 1% false discovery rate (FDR). For quantification, imputation was set to none; the protein LFQ method was automatic. Quantity MS level and type were set to M2 and area under curve, respectively. Log2 quantity filtering was enabled, with a minimum log2 precursor quantity set to 0, thus allowing all precursors to be considered. The Uniprot database was used for peptide and protein identification using the *Ipomoea batatas* proteome. Unpaired t-tests were performed for differential abundance testing to compare quantities in Atacama and Jane.

3.1.6 Bioinformatics

3.1.6.1 GO and KEGG

To determine the functional annotations and pathways of each identified protein, annotated proteins were searched against the STRING database using *Ipomoea batatas* protein sequences from UniProt. Functional enrichments from the STRING were used to construct graphs showing the gene ontology (GO) and KEGG for each cultivar.

3.1.6.2 Statistical analysis

An Excel file from Spectronaut was exported and further analysed using R Studio. The data was exported into SIMCA software version 18.6 and scaled using Pareto scaling. Multivariate statistical analysis was conducted, which included an unsupervised model, i.e. PCA, and a supervised model, i.e. OPLS-DA. The mean protein intensities of each cultivar were computed using R Studio version 2024.12.0+467 and used to determine the absolute difference between Atacama and Jane. A t-test was performed for protein to determine the statistical significance ($p < 0.05$) of the protein intensity between the two cultivars. The p-values were adjusted for multiple testing using FDR (Benjamini-Hochberg method). The proteins were ranked by intensity differences, with the top 30 proteins being selected for further analysis. For differential expression analysis, the SR plot was used to plot a volcano plot.

3.1.7 Total RNA extraction

The total RNA was extracted from both cultivars of control and treated plant leaf material. The RNA was extracted using the Macherey-Nagel NucleoSpin® TriPrep Mini kit for RNA, DNA and protein purification (MACHEREY-NAGEL GmbH & Co. KG). RNA concentrations were determined using Qubit™ RNA Assay kits (ThermoFisher Scientific, USA). The integrity of the extracted DNA was assessed using the Agilent TapeStation system (Agilent Technologies, USA).

3.1.8 RNA library preparation and sequencing

For the RNA library preparation, 200 ng of the total RNA per sample with RIN ≥ 7.2 were used. The libraries were prepared using the MGIEasy RNA Library Prep kit (MGI Tech Co., Ltd, China) following the manufacturer's instructions. Messenger RNA (mRNA) was enriched using an rRNA depletion kit. The enriched RNA was fragmented at 94 ˚C for 8 minutes to produce 150 bp fragments. This was followed by a first-strand synthesis using the kit's RT buffer and RT enzyme mix. Second-strand cDNA was synthesised using the second strand buffer and enzyme mix. The double-stranded cDNA was purified using DNA Clean Bead. This was followed by end repair and A-tailing and adaptor ligation using the MGIEasy adaptors. Adapter-ligated cDNA was purified and amplified by PCR using the PCR enzyme mix and primers. The purified PCR products were quantified using Qubit™ dsRNA Quantification Assay kits (Thermofisher Scientific, USA) and fragment size was assessed using the Agilent TapeStation system (Agilent Technologies, USA). Libraries were then denatured, circularised and digested enzymatically, and the final products were purified for sequencing. Biological replicates for each sample (per cultivar per treatment per harvest point) were pooled at the cDNA level. Final libraries were sequenced on the MGI DNBSEQ-G400 (MGI Tech Co., Ltd, China) platform using paired-end 150 bp reads (PE150), at the Agricultural Research Council-Biotechnology Platform (South Africa).

3.1.9 Data analysis

3.1.9.1 Quality control and pre-processing

Raw paired-end RNA-Seq reads were subjected to quality assessment and adapter trimming using FastQC v0.11.9 and Trim Galore v0.6.5. Adapter sequences and low-quality bases were trimmed using default parameters: a Phred quality cut-off of 20 and removal of reads shorter than 20 bp after trimming. Trimming was conducted in a pair-end setting to ensure that both reads were retained if both passed the quality threshold.

3.1.9.2 Genome alignment and assembly

The *Ipomoea batatas* (cv. Beauregard v4) reference genome and annotation files were retrieved from https://sweetpotato.uga.edu/ and indexed using HISAT v2.1.0 to extract exons and splice site information. Trimmed paired-ends were aligned with the indexed *I. batatas* genome using HISAT2 (Kim et al., 2019). The resulting SAM files were converted to BAM format and sorted using SAMtools v1.9 (Danecek et al., 2021). Transcript assembly was performed for each sample using StringTie v2.2.1 (Shumate et al., 2022). StringTie was also used for expression quantification to calculate transcript abundance values for each sample relative to the merged reference. Abundance values were merged into a count matrix.

3.1.9.3 Differential expression analysis

Differential expression analysis was conducted using edgeR to compare drought-treated samples (50% and 70%) against control samples (30%) within each cultivar and harvest time. Normalisation factors were calculated using the trimmed mean of M-values method. A biological coefficient of variation of 0.25 was used to estimate dispersion. Pairwise exact tests were performed to determine differentially expressed genes (DEGs), with absolute $\log_2$ fold change ≥ 1 and FDR (padjusted) < 0.05.

3.2 Cassava

3.2.1 Experimental design and treatments

Two cassava lines (P4/10 highly tolerant and UKF4 moderately tolerant) were selected based on their known differences in drought tolerance. While their overall responses to drought stress have been observed, the specific mechanisms behind these responses remain undocumented. Both lines were obtained from the ARC–VIMP in Pretoria, South Africa. 72 *in vitro* plantlets were transplanted into 42 cm pots containing a potting mixture of red topsoil, potting mix and vermiculite (7:3:1, v/v/v); all the pots were weighed to be 11 kg. The plants were grown under controlled greenhouse conditions at the ARC–VIMP Roodeplaat Research Campus. They were arranged in a randomised complete

block design with 3 replicates per line to ensure a robust statistical analysis. The experiment was laid out with a plot size of approximately 6 m^2 in four rows of 4.92 m long with inter- and intra-row spacing of 0.25 m. During the establishment phase, irrigation took place once a week (1.5 L of water), adhering to standard cassava agronomic practices (Hauser et al., 2014). Drought stress was induced by halting irrigation (day 0). Seven days post-stress initiation, baseline soil moisture was assessed, establishing three water regimes via the gravimetric method based on relative soil water content (RSWC). The RSWC represented plant-available water relative to field capacity for precise soil moisture quantification. Target pot weights were derived from dry weight (11.0 kg) and field capacity to represent three treatments: 30% RSWC (well-watered control with a target weight of 11.66 kg, maintained with 1 050 mL water), 50% RSWC (moderate stress; target weight 11.47 kg, maintained with 750 mL water) and 70% RSWC (severe stress; target weight 11.28 kg, maintained with 450 mL water). Post-assessment, water regimes were enacted, with daily pot weight measurements and water adjustments to maintain ±20 g of target weight. Sampling occurred at two critical growth stages during the early tuber bulking stage (early stages of drought exposure) and during the mid-season early tuber bulking stage (prolonged stages of drought exposure). Leaf samples (100 g) were immediately placed in 15 mL Eppendorf tubes, flash-frozen in liquid nitrogen and stored at -80 ºC until metabolite extraction was performed.

3.2.2 Metabolite extraction

The frozen leaf samples (100 mg) were ground to a fine powder using liquid nitrogen. The methanol extraction method was used, whereby the fine ground powder was expelled in a 1.5 mL tube with 1:10 w/v of 80% cold aqueous methanol (Minema Chemicals, Johannesburg, South Africa). The samples were vortexed for 30 seconds and then agitated for 2 hours in a sonicated water bath. The samples were then centrifuged at 13 000 rpm for 5 minutes at room temperature to remove debris, and the supernatant was transferred to a fresh 2 mL Eppendorf tube. The extracts were filtered through 0.22 µm nylon filters to remove solid particles and then transferred into 1.5 mL chromatography

glass vials with 500 µL inserts. For each breeding line, 3 replicates were prepared. The vials were sealed and stored at 4 ºC for subsequent analysis.

3.2.3 Liquid chromatography–mass spectrometry quadruple time-of-flight tandem (LC-MS qTOF)

One millilitre (1 mL) of sample extracts was analysed utilising LC-MS qTOF (LC-MS-9030 qTOF, Shimadzu Corporation, Kyoto, Japan). The chromatographic separation was completed utilising a Shim-pack Velox C18 column (100 x 2.1 mm, with a particle size of 2.7 µm) at 55 ºC. The injection volume was 3 µL, where a binary solvent solution was used; A: 0.1% formic acid in Milli-Q water (HPLC grade, Merck, Darmstadt, Germany) and solvent solution B: methanol (UHPLC grade, Minema Chemicals, Johannesburg, South Africa) supplemented with 0.1% formic acid. As described by Makhumbila et al. (2023), the chromatographic analysis was done with a qTOF high-definition mass spectrometer operating in negative electrospray ionisation mode utilising data-dependent acquisition.

3.2.4 Metabolite data analysis

The raw LC-MS data (ESI negative) encompassed retention times, including the duration each metabolite traversed the chromatography column; mass spectra, which provided molecular weight and structural information; and metabolite abundance, derived from peak intensities. Data was exported in mzML format and pre-processed with XCMS online, utilising UPLC-qTOF parameters with the centWave feature detection method. The m/z deviation limit was established at 3 and 700, respectively. Retention time correction utilised the Obiwarp method with a profStep of 0.5, while peak alignment was executed with a minimum fraction of 0.5 across all samples and a peak grouping width of 0.025 m/z. Furthermore, a Kruskal-Wallis non-parametric statistical test was conducted, followed by a post hoc analysis, and the data was normalised utilising the median fold change approach. The resultant data matrix for each line over the different sampling points was imported into SIMCA v17.0 Multivariate Data Analysis software (Sartorius, South Africa), normalised and Pareto scaled before the model application as indicated by Van den Berg

et al. (2006) to generate the PLS-DA, a supervised model, and PCA, an unsupervised model.

3.2.5 Annotations and pathway analyses

Data visualisation, chromatogram deconvolution, the creation of MS^1/MS^2 spectra, isotope grouping, alignment, filtering and gap-filling were all performed utilising the mzML data files as detailed by Pluskal et al. (2010). To identify metabolites, the data was input into Sirius v6.0.2 after being processed utilising the Global Natural Products Social Molecular Networking (GNPS) web platform. The KEGG, HMDB, PubChem, Coconut, Lotus and ChemSpider were utilised for annotation. Through comprehensive literature searches, the annotations were validated. Over-representation analysis was conducted utilising MetaboAnalyst v6.0, and the pathway analysis was conducted using the KEGG metabolite pathway for *Arabidopsis thaliana*, utilising KEGG IDs to identify the relevant pathways.

3.2.6 RNA extraction and cDNA synthesis

Total RNA was extracted from about 100 mg of flash-frozen cassava leaf tissue using the TRIzol™ Plus RNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. RNA quality and concentration were evaluated via spectrophotometry on a NanoDrop spectrophotometer (Blue-Ray Biotech, Taipei, Taiwan).

First-strand cDNA synthesis was conducted using 1 µg of total RNA with the Bio-Helix RScript cDNA Synthesis Kit (Bio-Helix Co., Ltd, Taiwan). Each 20 µL reaction included the RNA template, 5X Sharp Reaction Mix, oligo(dT) or random hexamer primers, and RScript Enzyme Mix. Reverse transcription was incubated at 55 °C for 50 minutes, followed by enzyme inactivation at 70 °C for 15 minutes. cDNA yield was quantified with a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) to confirm adequacy for downstream applications.

3.2.7 Gene identification and quantitative real-time PCR (qRT-PCR)

This study utilised a literature-based approach to identify candidate genes associated with drought tolerance in cassava. Specifically, four genes (*MeZFP, MeALDH, MeRD28* and *MeMSD*), previously identified by Turyagyenda et al. (2013), were selected for further investigation in this study. These genes have also been noted to confer drought tolerance in other plant species, making them promising candidates for exploring drought-tolerance mechanisms in cassava. Gene expression analysis was conducted using the CFX Connect™ Bio-Rad Real-Time PCR (Bio-Rad Laboratories, Hercules, California, USA) instrument using Luna® Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA) following the manufacturer's protocol. Each sample underwent triplicate reactions, including a no-template control to validate amplification specificity. The qRT-PCR protocol included an initial denaturation at 95 °C, followed by 40 cycles of denaturation, annealing and extension, with conditions tailored for each primer set. Melting curve analysis confirmed the specificity of amplification products.

Primers for target genes *MeZFP*, *MeALDH*, *MeRD28* and *MeMSD* (Turyagyenda et al. 2013) (Table 6.1) alongside the reference gene *ACT* (forward primer: 5′-TGCAGACCGTATGAGCAAG-3′; reverse primer: 5′-CACCCTTGGAAATCCACATC-3′) (Guo et al., 2009; Yang et al., 2011) were designed by Integrated DNA Technologies, Inc. (Coralville, IA, USA) based on Turyagyenda et al. (2013). Relative gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method (Pfaffl et al., 2002), normalising expression to the *ACT* reference gene and comparing across treatments and genotypes. Standard deviations were calculated among three biological replicates.

Table 3.1: Primer sequences used for the reaction of RT-qPCR

Gene	Primer ID	Primer sequence (5'- 3')	Base length
MeZFP	ZFP1F	CTC TAT TCT CAG CGC ACA TTC C	22
	ZFP1R	AGC ATA ACG AGG CAG AGA GC	20
MeMSD	MSD1F	ATG AAT GCA GAA GGT GCT GCA	21
	MSD1R	GAA GGG CAT TCT TTG GCA TAC	21
MeRD28	RD28F	TGC ACT GCT GGT ATC TCA GG	20
	RD28R	GAT CTC AGC TCC CAA TCC AG	20
MeALDH	ALDH1F	GGA TGG AAT GCA TGC ATT GCA CTG	24
	ALDH1R	CTG ATT CAC TGT TTG CAC CAT C	25
ACT	ACT F	TGCAGACCGTATGAGCAAG	19
	ACT R	CACCCTTGGAAATCCACATC	20

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CHAPTER 4: UNRAVELLING SWEET POTATO RESPONSE TO DROUGHT STRESS USING INTEGRATIVE MULTI-OMICS APPROACHES

Abstract

Sweet potato (*Ipomoea batatas* L.) is an important staple crop valued for its nutritional quality and adaptability; however, the molecular mechanisms underlying cultivar-specific performance and drought tolerance remain poorly understood. This study employed an integrated multi-omics approach, that is, untargeted metabolomics, proteomics and transcriptomics, to characterise biochemical and molecular differences among four cultivars, namely the drought-tolerant Atacama and Jane and the drought-susceptible Blesbok and Bellevue. Metabolomic profiling under varying drought intensities showed clear cultivar separation and significant regulation of metabolites, particularly flavonoids, sugars and glycolipids. Key metabolites included kaempferol-3-O-galactoside, chlorogenic acid, glc-glc-octadecatrienoyl-sn-glycerol and apigenin-7-O-β-D-neohesperidoside, which were strongly associated with drought tolerance and enriched in pathways such as flavonoid biosynthesis and starch and sucrose metabolism. Proteomic analysis revealed cultivar-specific regulation of proteins associated with photosynthesis, carbohydrate metabolism, secondary metabolism, ion transport and stress response. Atacama showed enhanced abundance of proteins linked to carbohydrate metabolism and photosynthetic metabolism, whereas Jane displayed stronger regulation of secondary metabolism and stress-related proteins. Comparative transcriptomic profiling under controlled and drought conditions further demonstrated distinct gene expression patterns, with Atacama upregulating genes involved in osmotic adjustment, reactive oxygen species scavenging, photosynthesis and carbon metabolism, suggesting coordinated mechanisms that support cellular homeostasis under stress. Together, these results identify coordinated, cultivar-specific molecular mechanisms that underlie drought response and offer potential targets and biomarkers that could help in sweet potato breeding for increased food security and stress tolerance.

4.1 Introduction

Sweet potato is an important staple crop in Africa, Asia, the Pacific Islands and developing countries (Feng et al., 2018; Laurie et al., 2024). The roots and leaves of sweet potato are rich in essential nutrients and minerals (Senthilkumar, 2020; Laveriano-Santos et al., 2022). Sweet potatoes have received increased research attention due to their nutritional value, since they offer smallholder farmers a variety of possibilities for increasing agricultural productivity, particularly in developing nations (Afzal et al., 2021; McEwan et al., 2022). Despite the importance of sweet potato in agriculture and food security, the yield and production of the crop are affected by various stressors, which include biotic stresses such as fungi and viruses, and abiotic stresses such as drought stress and cold stress.

One major abiotic stressor that impacts sweet potato crop regions globally is drought (Low et al., 2020; Zhou et al. 2022; Zhu et al. 2022). Lower numbers of small-sized storage roots form because of drought during the storage root initiation stage, which reduces crop yields to a great extent (Kivuva, 2013; Chauhan et al., 2021). Under stress, yield can decrease by an average of 85% (Laurie et al., 2022). Drought affects a variety of plant physiological and biochemical functions, including photosynthesis, respiration, translocation, ion uptake, sugar and nutrient metabolism, and phytohormone production (Sapakhova et al., 2023). It has become important to identify essential metabolites, proteins and genes involved in yield potential, nutrient value and stress tolerance in breeding programmes.

Omic technologies, such as transcriptomics, proteomics, metabolomics and phenomics, and their integration, are among the top approaches for improving novel crops (Van Emon, 2020). Metabolomics is an important approach for identifying and analysing metabolic phenotypes within intricate cellular processes (Fiehn, 2002). While several sweet potato studies have focused on metabolite profiling (Lebot et al., 2016; Wang et al., 2018; Drapal et al., 2019; Ren et al., 2021; Bennett et al., 2021; Zhao et al., 2022), few have addressed drought stress. Also, the few existing studies on drought-related metabolomics have focused largely on Asian cultivars, with minimal attention to Southern African genotypes. Consequently, there is a lack of understanding of how locally adapted

sweet potato cultivars modulate their metabolic pathways under drought conditions. This gap limits efforts to identify drought-responsive biomarkers that can support breeding for climate-resilient sweet potato varieties. Proteomics includes the analysis of protein profiles, the quantification of proteins, the study of post-translational modifications and protein-protein interactions (Vanderschuren et al., 2013). Given the limited proteomic information available for sweet potato, it has become important to focus on characterising its proteome using advanced techniques. Transcriptomics studies quantify the transcripts from a specific tissue or cell at a specific functional or growth stage (Tyagi et al., 2022). Genes linked to signal transduction, posttranslational modification, protein turnover, chaperones, transport and metabolism of carbohydrates have been identified in different sweet potato cultivars in response to drought stress (Zhu et al., 2019; Zhou et al., 2019; Liu et al., 2023; Sheng et al., 2023; Hu et al., 2024).

The aim of this study was to determine the biochemical and molecular responses of sweet potato to drought stress using metabolomics, proteomics and transcriptomics analysis. By identifying cultivar-specific metabolites, proteins and genes, potential biomarkers were uncovered that impact nutritional quality and stress response.

4.2 Results

4.2.1 Metabolomic profiles of sweet potato (*Ipomoea batatas*) cultivars: Insights into biochemical differences

The aim of this study was to use metabolomic tools to determine biochemical differences between four sweet potato cultivars (Atacama, Jane, Blesbok and Bellevue) under non-drought stress conditions.

4.2.1.1 Identification of metabolites across all cultivars

Multivariate analyses were conducted to highlight the biochemical differences between the four sweet potato cultivars. The PCA score plot (Figure 4.1A) revealed clustering patterns among the four cultivars, with Atacama and Bellevue clustering separately from others, indicating that they had distinct metabolite profiles. Blesbok and Jane showed an overlap in their clusters, suggesting that they may have similar metabolite profiles. The

PLS-DA score plots (Figure 4.1B) demonstrate the further metabolite variation among cultivars (Figure 4.1B), with a clearer separation of cultivars.

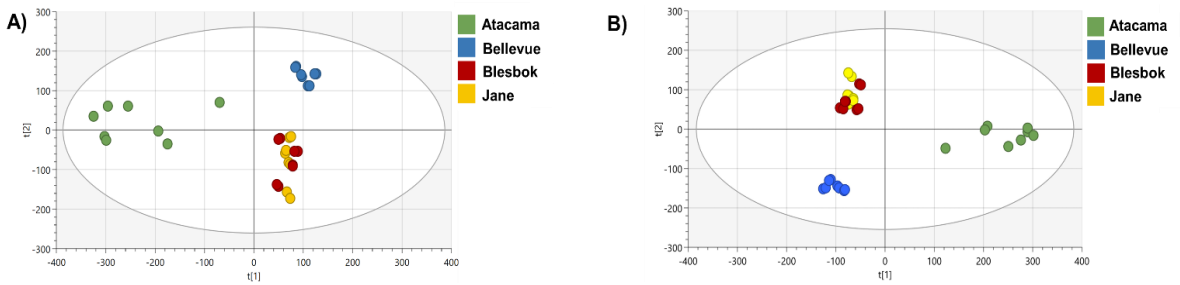

Figure 4.1: A) PCA of four cultivars with the following model parameters: R2X = 0.7, Q2 = 0.435 with 5 components; and B) PLS-DA with model parameters: R2X = 0.721, R2Y = 0.988 and Q2 = 0.942 with 6 components. These score plots illustrate the clustering patterns of four sweet potato cultivars. Green data points represent Atacama, blue represent Bellevue, red represent Blesbok and yellow represent Jane.

The VIP scores from PLS-DA were used to determine metabolites that are the most responsible for cultivar separation (Figure 4.2). Metabolites with a VIP score greater than 1 contribute significantly to cultivar separation. Annotated compounds belonged mainly to flavonoids (Table 4.1), with additional representation from glucosides, glycerophospholipids, glycerolipids, steroids and glucuronides, indicating a broad metabolic variation among the cultivars.

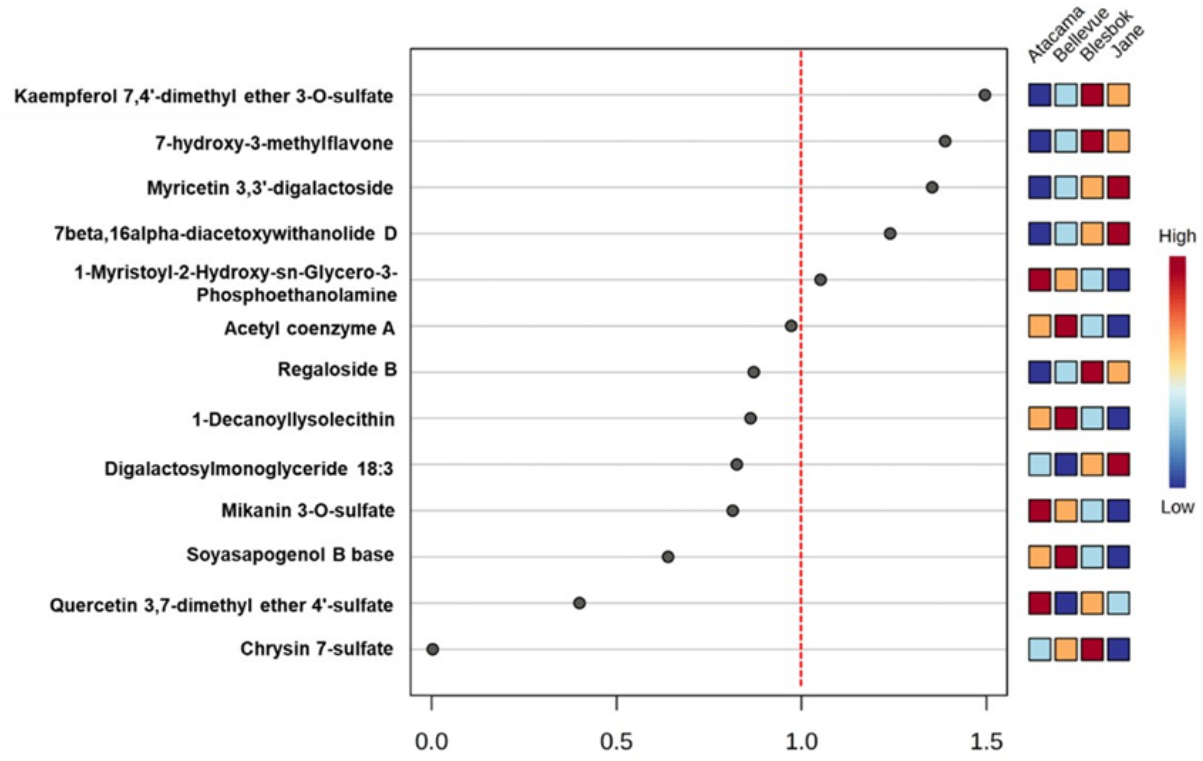

Figure 4.2: VIP score plot showing how each metabolite contributed to cultivar separation. Metabolites with a VIP score greater than 1 contribute the most to cultivar separation. The colour key indicates the significant accumulation of each metabolite for each cultivar. Red indicates high abundance; blue indicates low abundance.

Table 4.1: Identified and annotated metabolites from four cultivars obtained from VIP score plot

Compound name	Formula	Experimental mass (m/z)	Rt min	Fragment	Adduct	p-Value	Class
Soyasapogenol B base	$C_{47}H_{74}O_{17}$	891.05	4.65	891.4819	[M]	7.13 $x10^{-13}$	Glucosides
Myricetin 3,3'-digalactoside	$C_{27}H_{30}O_{18}$	642.40	8.21	641.13611	[M-H]-	5.13 $x10^{-9}$	Flavonoids
1-Decanoyllysolecithin	$C_{18}H_{38}NO_7P$	411.75	4.39	184.0778, 104.1107, 412.2464	[M]	1.3 $x10^{-17}$	Glycerophospholipids
Regaloside B	$C_{20}H_{26}O_{11}$	441.16	3.54	173.0453, 187.1339, 155.034	[M]	4.21 $x10^{-4}$	Glycerolipids
Kaempferol 7,4'-dimethyl ether 3-O-sulfate	$C_{17}H_{14}O_9S$	394.03	4.77	289.0484	[M]	8.12 $x10^{-12}$	Flavonoids
Quercetin 3,7-dimethyl ether 4'-sulfate	$C_{17}H_{14}O_{10}S$	410.26	4.39	314.0444	[M]	4.18 $x10^{-28}$	Flavonoids
Chrysin 7-sulfate	$C_{17}H_{14}O_{10}S$	409.78	4.39	314.0434	[M-H]-	9.76 $x10^{-20}$	Flavonoids
7beta,16alpha-diacetoxywithanolide D	$C_{32}H_{42}O_{10}$	631.39	5.37	587.2828, 161.0246	[M-FA-H]	2.75 $x10^{-24}$	Steroids
Digalactosylmonoglyceride 18:3	$C_{33}H_{56}O_{14}$	675.09	3.56	675.357		3.68 $x10^{-16}$	Glycerglycolipids

1-Myristoyl-2-Hydroxy-sn-Glycero-3-Phosphoethanolamine	$C_{19}H_{40}NO_7P$	424.98	4.64	424.52	[M-H]-	4.15 $x10^{-14}$	Glycerophospholipids
7-hydroxy-3-methylflavone	$C_{16}H_{12}O_3$	251.17	5.23	251.0713, 207.0851, 209.0569	[M+H]+	3.65 $x10^{-13}$	Flavonoids
Acetyl coenzyme A	$C_{23}H_{38}N7O_{17}P_3S$	809.05	4.77	461.0620, 408.0853, 426.1077, 673.1265, 790.0432	[M]	3.99 $x10^{-14}$	o-glucuronides
Mikanin 3-O-sulfate	$C_{18}H_{16}O_{10}S$	424.29	4.65	328.0591	[M]	2.13 $x10^{-16}$	Flavonoids

Metabolites that were identified and annotated were grouped into various classes. Figure 4.3 highlights the significance of the classes, their enrichment ratios and corresponding p-values. From the results, flavonoids were the most significantly highly enriched class ($p < 0.0001$) (Figure 4.3A), which indicates their role in differentiating the cultivars. Their high accumulation was observed in Bellevue. Organooxygen compounds ($p < 0.01$) are a set of metabolites with organic functional groups that contain oxygen. Metabolites that belong to this class included acetyl coenzyme A, regaloside B, 7beta,16alpha-diacetoxywithanolide D, myricetin 3,3'-digalactoside, 7-hydroxy-3-methylflavone and kaempferol 7,4'-dimethyl ether 3-O-sulfate. These metabolites are important in plant defence, aroma and colour. Prenol lipids showed a lower enrichment ($p < 0.05$). The key prenol lipids included 7beta,16alpha-diacetoxywithanolide D and regaloside B, which were abundant in Jane and Blesbok, respectively. Atacama, a drought-tolerant cultivar, has a higher abundance of these lipids, which may play a role in drought tolerance. The enrichment analysis indicates significant metabolic differences among the cultivars.

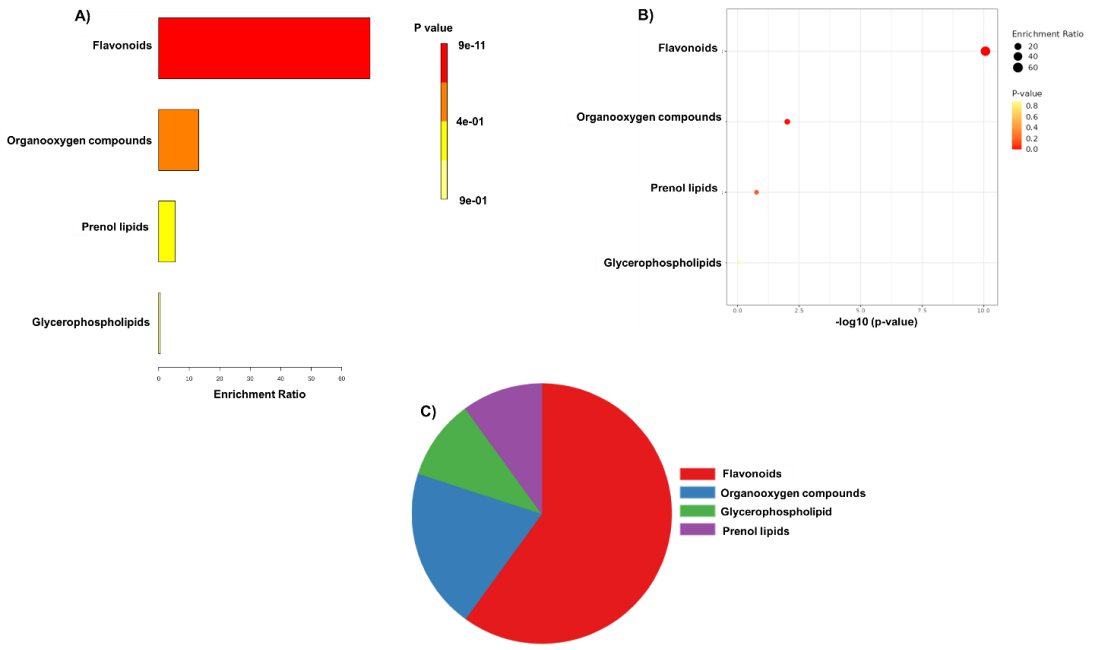

Figure 4.3: Enrichment analysis of metabolite classes. A) Metabolite classes significantly enriched. B) Metabolite classes and their corresponding -log10 (p value). C) Distribution of enriched metabolite classes. Metabolite classes represented are flavonoids, organooxygen compounds, prenol lipids and glycerophospholipids.

4.2.1.2 Major metabolite classes contributing to cultivar variation

Flavonoids were the most significantly enriched class ($p < 0.0001$) (Figure 4.3) and therefore the strongest contributors to cultivar differentiation. Flavonoids are phenolic compounds that are important secondary metabolites and are either constitutively produced or activated by stress (Treutter, 2006; Laoué et al., 2022; Wan et al., 2024).

Kaempferol 7,4'-dimethyl ether 3-O-sulfate identified in this study had the highest VIP score and was highly abundant in Blesbok, which contributed the most to cultivar separation. Kaempferol derivatives are widely associated with antioxidant activity, plant pathogen defence, colour formation and flavour of sweet potatoes (Habbu et al., 2009; Park et al., 2016; Xu et al., 2021; An et al., 2022; Ramzan et al., 2024). The high abundance of this sulphated derivative therefore suggests an enhanced protective or adaptive strategy in Blesbok. Other influential flavonoids included myricetin 3,3'-digalactoside, which was highly abundant in Jane, and 7-hydroxy-3-methylflavone, which was highly abundant in Blesbok. The difference in the relative abundance levels between the two cultivars may indicate distinct regulatory control of flavonoid biosynthesis. These compounds are frequently linked to antimicrobial and antioxidant activity, indicating their important role in protection against abiotic and biotic stress (Zhang et al., 2017; Taheri et al., 2020; Bhajan et al., 2023). These metabolites are also involved in colour variation in roots and leaf shape of sweet potato and other plants (Tawfik et al., 2014; Tan et al., 2024; Wan et al., 2024).

Among other major classes that contributed to variation were lipids. 1-myristoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine (lysophosphatidylethanolamine, LPE) is a glycerophospholipid and was upregulated in Atacama and Bellevue. Lysophosphatidylethanolamines function as signalling molecules, influence membrane dynamics and are associated with tolerance to salinity and pathogen attack in sweet potatoes (Paget, 2012; Mansour et al., 2015; Yu et al., 2019; Völz et al., 2023). Their enrichment may therefore contribute to abiotic and biotic stress tolerant traits of these cultivars. Additionally, 1-Decanoyllysolecithin (lysophosphatidylcholine, LPC) also showed moderately high and high accumulation in Atacama and Bellevue, respectively. The accumulation of LPCs are important signalling molecules that have been reported to play an important role in a plant's resistance to pathogens (Spivak et al., 2003; Baeza-Jiménez et al., 2013). Their accumulation alongside LPEs suggests

enhanced activity for rapid stress perception and response. Triterpenoids and prenol lipids were also among the classified classes that contributed to cultivar differentiation.

Triterpenoids are also a major class of phytochemicals present in sweet potato leaves and contribute to plant defence and development (Mohanraj & Sivasankar, 2014; Cárdenas et al., 2019; Nguyen et al., 2021). Our results indicated the accumulation of 7beta,16alpha-diacetoxywithanolide D in Jane. Withanolides are steroidal lactone triterpenoids that are associated with improved abiotic and biotic stress tolerance (Saema et al., 2016). They have not been identified in sweet potato, and this may indicate lack of characterisation of secondary metabolite in sweet potato. This metabolite could contribute to the drought-tolerant trait reported for Jane.

Overall, the metabolomic profiles demonstrate that phenolic compounds, particularly flavonoids, contribute the most to cultivar differences. Lipids and triterpenoids provide additional, cultivar-specific traits for adaptations to the environment. These results show the combined influence of biomolecules and environment and may help explain the differences in colour and stress tolerance traits.

4.2.2 Biochemical responses of Atacama and Blesbok sweet potato (*Ipomoea batatas* L.) cultivars to early drought stress

The aim of this study was to determine how early drought stress affects the production of defence-related metabolites in two sweet potato cultivars (Atacama and Blesbok).

4.2.2.1 Comparative metabolic profiles under non-drought conditions

Under comparative analysis between Atacama and Blesbok under non-drought conditions, there was a clear metabolic difference. Multivariate analysis (PLS-DA and VIP scoring) (Figure 4.4) showed distinct metabolite profiles separating the cultivars. Atacama exhibited a broader metabolite spectrum, with higher relative intensities of several phenolic compounds, flavonoids, lipids, terpenoids and amino acids. However, normalised fold-change analysis indicated that many of these metabolites were comparatively lower relative to Blesbok, despite their high abundance in raw intensity measures (Table 4.2). The key discriminating metabolites included isolariciresinol 9′-O-alpha-L-arabinofuranoside (highest VIP score), alpha-tocotrienol, octadecyl ferulic acid and lupeol. In contrast, tricin 7-neohesperidoside and gibberellin A23 exhibited the highest fold changes and were more abundant in Blesbok. These findings

demonstrate inherent metabolic differences between the cultivars prior to stress exposure, suggesting genetically determined metabolic programming that may influence drought response capacity.

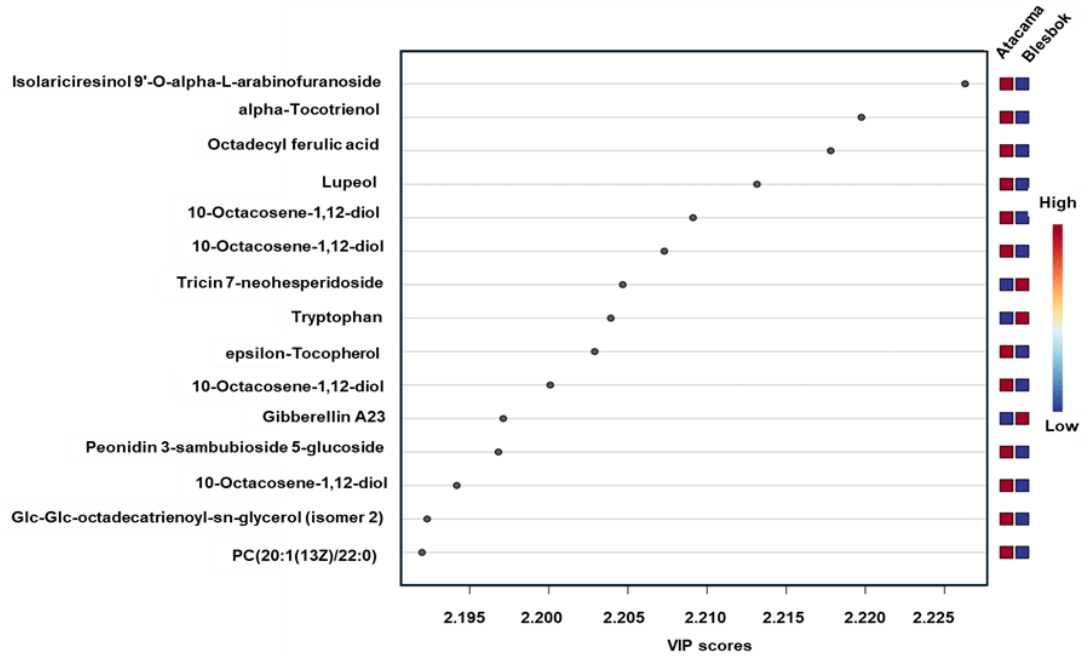

Figure 4.4: VIP score plot highlighting the most significant metabolites contributing to the differentiation between Atacama under 30% (non-stressed). The colour scale represents the relative abundance of each metabolite across the stress conditions, with red indicating high levels and blue indicating low levels.

Table 4.2: Regulated metabolites identified between Atacama and Blesbok sweet potato cultivars under non-stress conditions

Compound name	Experimental mass (m/z)	Rt (min)	Molecular formula	Log2Fold change	VIP value	P-value	Class
Isolariciresinol 9'-O-alpha-L-arabinofuranoside	492.031	4.66	$C_{25}H_{32}O_{10}$	-10.64	2.23	$4.10x10^{-04}$	Lignan glycosides
alpha-Tocotrienol	423.040	4.65	$C_{29}H_{44}O_2$	-17.95	2.23	$4.10x10^{-04}$	Vitamin E derivatives
Octadecyl ferulic acid	445.022	4.67	$C_{28}H_{46}O_4$	-11.84	2.22	$4.10x10^{-04}$	Coumaric acids and derivatives
Lupeol	425.548	4.65	$C_{30}H_{50}O$	-7.66	2.21	$4.10x10^{-04}$	Triterpenoid
10-Octacosene-1,12-diol	424.731	4.65	$C_{28}H_{56}O_2$	-7.96	2.21	$4.10x10^{-04}$	Fatty alcohol
Tricin 7-neohesperidoside	638.366	7.80	$C_{29}H_{34}O_{16}$	8.71	2.01	$5.54x10^{-04}$	Flavonoid-7-o-glycosides
Tryptophan	203.092	3.73	$C_{11}H_{12}N_2O_2$	-0.82	2.20	$1.55x10^{-04}$	Indolyl carboxylic acids and derivatives
epsilon-Tocopherol	410.330	4.42	$C_{28}H_{42}O_2$	-7.77	2.20	$4.09x10^{-04}$	Vitamin E derivatives
Gibberellin A23	378.146	7.79	$C_{20}H_{26}O_7$	11.63	2.22	$4.09x10^{-04}$	C20-gibberellin 6-carboxylic acids
Peonidin 3-sambubioside 5-glucoside	758.35	6.91	$C_{33}H_{41}O_{20}$	-11.28	2.20	$4.09x10^{-04}$	Anthocyanidin-5-o-glycosides
Glc-Glc-octadecatrienoyl-sn-glycerol (isomer 2)	722.273	6.89	$C_{33}H_{56}O_{14}$	-9.07	2.19	$8.26x10^{-04}$	Glycolipids
PC (20:1 (13Z)/22:0)	871.071	4.65	$C_{50}H_{98}NO_8P$	-11.50	2.19	$4.09x10^{-04}$	Glycerophospholipid

Rt: Retention time in minutes; VIP: Variable importance in projection. The criteria used for log^2fold change were: very high >4; high 3-4; moderate 1-2; decreased <1.

4.2.2.2 Metabolic differences between sweet potato cultivars under drought stress

The PCA and OPLS-DA plots were generated to assess how Blesbok and Atacama sweet potato cultivars responded under three drought stress conditions (30%, 50% and 70%). There was an apparent distinction between the two cultivars, indicating that they had different metabolic responses to drought stress. Among the 4 700 identified metabolites, 10 key metabolites were significantly regulated based on S-plot loadings: 7 were upregulated and 3 were downregulated (Figure 4.5), primarily belonging to flavonoid glycosides, phenolic acids, glycolipids, sugars and nucleotide derivatives. Metabolites showing the strongest regulation included kaempferol-3-O-galactoside, chlorogenic acid, glc-glc-octadecatrienoyl-sn-glycerol and apigenin-7-O-β-d-neohesperidoside. Blesbok generally exhibited increasing metabolite accumulation across stress levels, suggesting a strong inducible stress response. In contrast, Atacama displayed more stable or decreasing metabolite concentrations, except for specific compounds such as isomangiferin and chlorogenic acid at higher stress levels. This pattern implies that Atacama, which is drought-tolerant, maintains metabolic stability, whereas Blesbok, which is drought-sensitive, is reactive with noticeable metabolic changes. This could be an indication of the species' innate adaptability and decreased need for extensive change in metabolism.

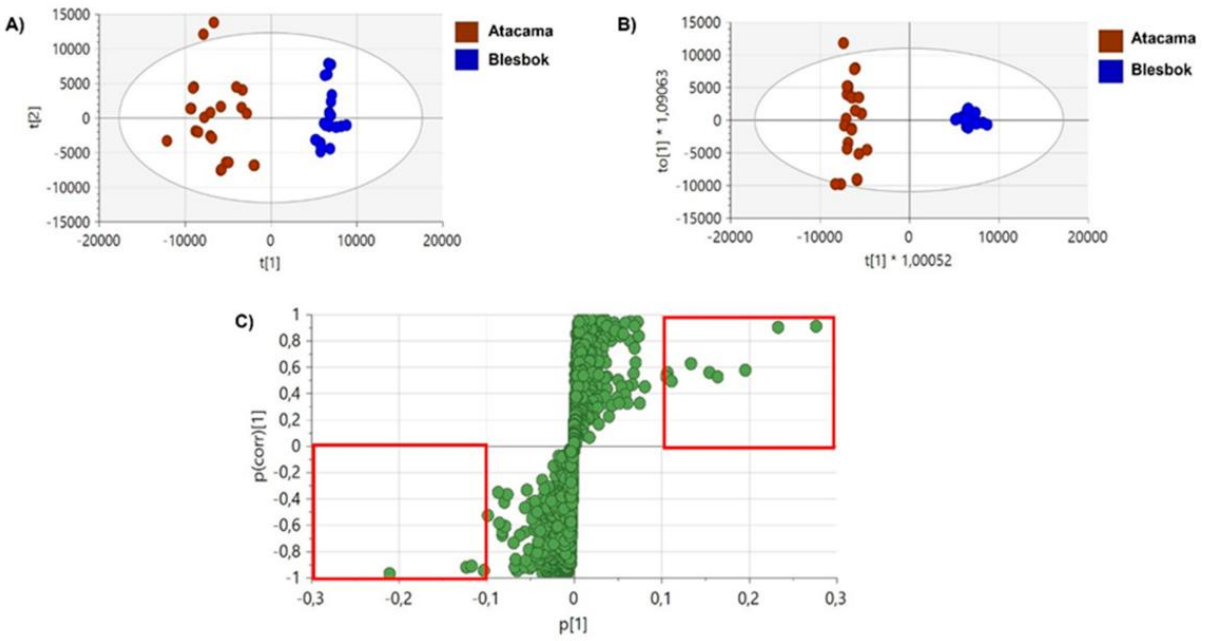

Figure 4.5: Unsupervised and supervised exploratory statistical analysis of Atacama and Blesbok under drought stress and non-stress conditions. (A) Principal component (PC) scores for the Atacama vs Blesbok scatter plot of the Pareto-scaled dataset. The quality parameters of the model were explained: variation/goodness of fit: $R^2X(cum)$ = 0.612 and $Q^2(cum)$ = 0.432. (B) OPLS-DA model. OPLS-DA: 1 + 1 + 0 component model. The quality parameters of the model were explained: variation/goodness of fit resulted in $R^2X(cum)$ = 0.372 and $Q^2(cum)$ = 0.974. (C) Loadings, with statistically significant features described to have a p (corr)) of ≥ 0.5 and a covariance of (p1) ≥ 0.05. Upregulated metabolites are highlighted in the red box towards the right, and the down-regulated metabolites are highlighted in the red box towards the left.

4.2.3 Metabolic variations within Atacama and Blesbok in response to drought stress

Based on the comparative analysis of the two cultivars and under normal conditions, we aimed to explore the effects of drought stress on each cultivar, considering these two cultivars show different metabolic responses. Pairwise comparisons were conducted comparing control (30%) and 50%, 30% and 70%, and 50% and 70%, as well as multigroup comparisons across regimes at 30%, 50% and 70% drought stress levels.

Despite having similar purple skin and being evaluated under normal conditions, the drought-tolerant Atacama cultivar showed higher levels of flavonoids, phenolics and other metabolites. This suggests that drought tolerance may be linked to an innately elevated accumulation of phenolics and related metabolites, possibly as a protective or adaptive mechanism against stress. Distinct patterns resulted from multigroup comparisons across drought intensities, with Atacama displaying a more sensible and consistent metabolic response and Blesbok having greater but less coordinated metabolic changes. Flavonoid biosynthesis, as well as flavone and flavanol biosynthesis, were identified as the highly significant metabolic pathway in the pathway analysis, as indicated by its position at the top of the y-axis (Figure 4.6). Several of the enriched pathways, particularly phenylpropanoid, flavonoid, flavone and flavanol biosynthesis, are well recognised for their critical roles in regulating plant stress resistance. Phenylpropanoids, in particular, enhance plant stability substantially by enabling adaptation to both biotic and abiotic stresses (Vogt, 2010). Compounds within the phenylpropanoid class are widely reported to contribute to plant growth, development and environmental interactions (Geng et al., 2020; Shao et al., 2023). The phenylpropanoid pathway, a key secondary metabolic route closely associated with flavonoid production, plays an especially important role in mediating resistance to abiotic stress conditions (Sharma et al., 2019). Kaempferol-3-O-glucoside, (-)-Epigallocatechin and chlorogenic acid were important metabolites for these pathways. Chlorogenic acid has previously been identified in sweet potato, where it exhibits strong antioxidant activity, including effective 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, alongside other polyphenols in purple sweet potato roots (Zheng & Clifford, 2008; Zhao et al., 2014). It plays a crucial role in alleviating oxidative stress in plants by neutralising reactive oxygen species and maintaining cellular redox balance through multiple antioxidative mechanisms (Tošović et al., 2017). The majority of the metabolites found in this study were flavonoid glycosides and polyphenols, which may serve as protective compounds that prevent sweet potato plants from oxidative damage brought on by ROS by scavenging free radicals and delaying oxidative degradation (Nakabayashi et al., 2014; Groenbaek et al., 2019).

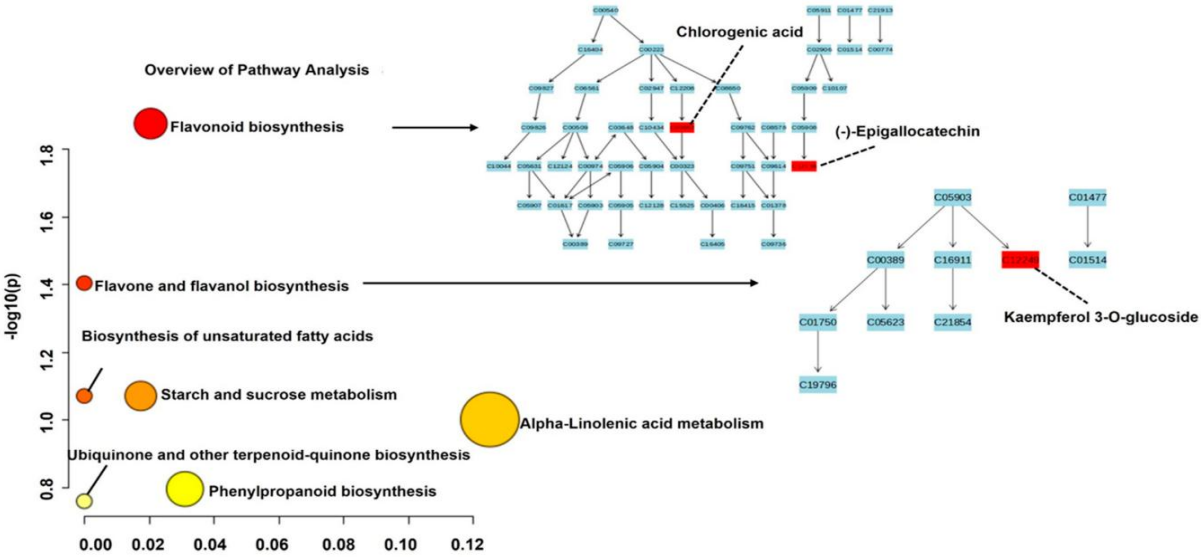

Figure 4.6: MetaboAnalyst (MetPA)-computed pathway analysis. Pathway impact values are plotted along the *x*-axis to reflect pathway topology analysis; pathways are sorted along the *y*-axis to indicate pathway enrichment analysis based on their significance (*p*-value). Each pathway node's colour represents its *p*-value, with red denoting the lowest *p*-value and highest level of statistical significance. The pathway effect factor is represented by the node's radius, where larger nodes have a greater influence.

Early drought stress revealed distinct metabolic responses in Atacama and Blesbok, with Blesbok showing stronger shifts and Atacama maintaining stability. Despite these metabolic variations, aboveground biomass did not change visibly under early drought stress, suggesting that the impacts are mostly biochemical rather than phenotypic. Adenosine 5′-monophosphate, chlorogenic acid, isomangiferin, apigenin-7-O-β-d-neohesperidoside, kaempferol derivatives, ajugose, 8-p-hydroxybenzlyquecetin, PE (18:0/22:0) and other highly regulated metabolites from flavonoids, glycolipids and sugars may serve as early markers of drought response and contribute to cultivar-specific metabolic variations.

4.3 Proteomics analysis reveals distinct profile patterns between two sweet potato (*Ipomoea batatas* L.) cultivars

The aim of this study was to compare the proteomic profiles of two sweet potato cultivars, Jane and Atacama, under non-stress conditions.

4.3.1 Identification of proteins between Atacama and Jane

Proteomic profiles revealed broad differences between the two sweet potato cultivars. In the PCA (Figure 4.7), there was no overlap between the biological replicates of the cultivars, thus showing that cultivars have different proteome profiles. Supervised modelling using OPLS-DA further highlights the distinctive features between the cultivars.

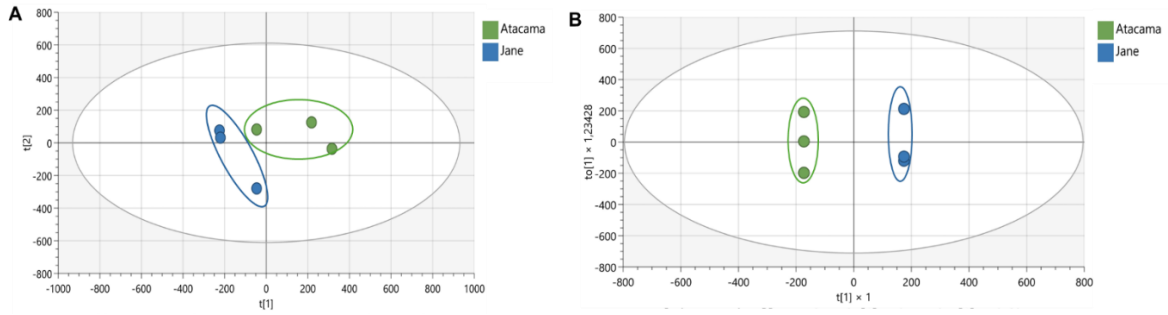

Figure 4.7: PCA and OPLS-DA score plots of Jane and Atacama proteomic profiles. (A) The PCA illustrates the overall variation in the proteome dataset between Jane and Atacama. (B) To maximise group separation and identify proteins that contribute to specific cultivar differences, an OPLS-DA was plotted. Each point represents the biological replicate.

Gene ontology (GO) (Figure 4.8) annotation indicates that despite their separation in multivariate analysis, the overall functional analysis of the proteins was broadly similar. In both cultivars, most proteins were assigned to intracellular sites such as cytoplasm and plastids and were mainly involved in catalytic activity, cellular processes and metabolism. This indicates the importance of the cultivars in maintaining core biochemical functions in the leaf tissue. However, there are some differences in the enriched terms that may show cultivar-specific adaptations. Atacama showed stronger regulation of pathways linked to ion binding and ATP synthase complexes, whereas Jane showed an enrichment of oxidoreductase activity, fatty acid biosynthesis and pigment-biosynthetic processes These results may suggest that Atacama has an energy-producing metabolism, whereas Jane enriches a more defence-related metabolism.

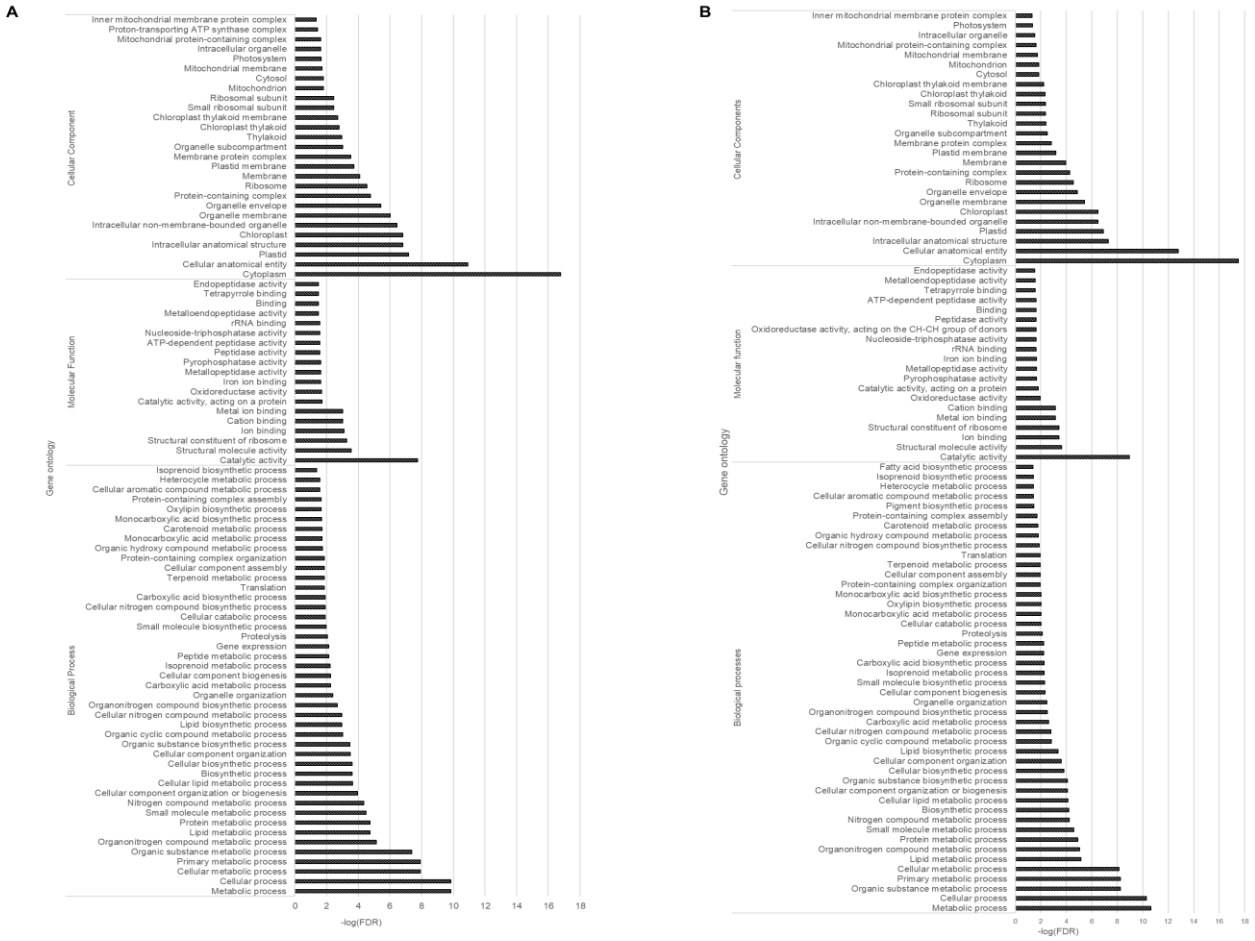

Figure 4.8: GO enrichment analysis of annotated proteins in (A) Atacama and (B) Jane. The GO enrichment analysis is categorised into cellular components, molecular functions and biological processes.

Kyoto Encyclopedia of Genes and Genomes (KEGG) (Figure 4.9) supports GO analysis by indicating that most proteins mapped to primary metabolism, biosynthesis of secondary metabolites and ribosomal function. This shows their importance for growth and development. However, proteins uniquely associated with photosynthesis were detected only in Atacama. This may contribute to phenotypic differences such as biomass production and further indicates the allocation of resources between energy production and protective metabolism.

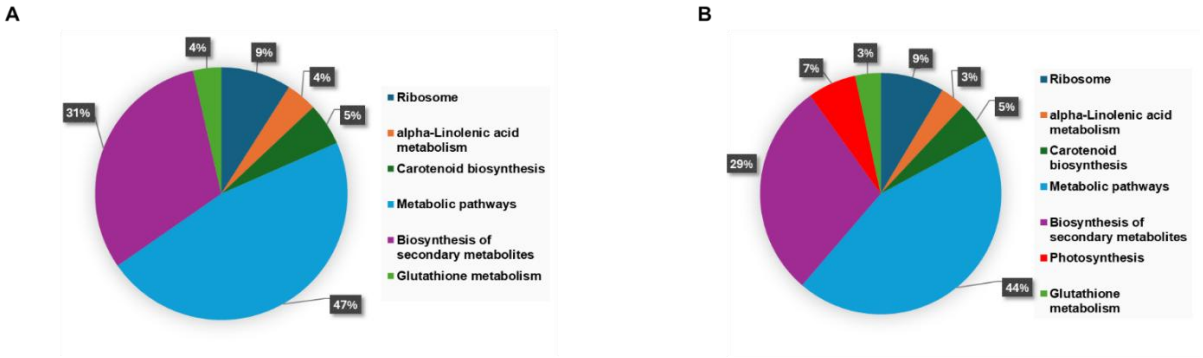

Figure 4.9: KEGG pathway analysis of identified proteins. Distribution of proteins across different metabolic pathways in (A) Jane and (B) Atacama.

4.3.2 Secondary metabolism

The majority of the proteins that were identified in differential expression analysis (Figure 4.10) are involved in secondary metabolism. A major distinguishing feature between the cultivars is the regulation of enzymes linked to flavonoid and phenylpropanoid pathways. Jane showed a relatively higher expression of several glycosyltransferases, which include flavonoid-3-O-glucosyltransferase (Fh3GT) and anthocyanidin-related transferases. These enzymes are important in catalysing the transfer of sugar molecules onto anthocyanidins or related flavonoids, which is a step crucial in stabilising anthocyanin and accumulation in plant cells (Wu et al., 2017; Kaur et al., 2021; Muhammad et al., 2022). Biochemical analysis showed that Fh3GT2 is responsible for the glycosylation of kaempferol in *Freesia hybrida* (while Fh3GT1 glycosylates quercetin and anthocyanidins) (Meng et al., 2019). Meng et al. (2019) also showed that at the transcript level, Fh3GT2 showed high expression in non-pigmented buds and decreased as the flowers developed (flowers became red). This could explain the upregulation of the protein in the leaves of Jane (which are green), whereas Atacama has purple veins and petioles, and this colouration could be due to other enzymes involved in anthocyanidin production.

Feruloyl-CoA 6-hydroxylase is an enzyme that catalyses a crucial step in coumarin biosynthesis that produces intermediates that give rise to defence-related compounds such as scopoletin and umbelliferone (Tao et al., 2023; Ihnatowicz et al., 2024; Wang et al., 2025). Previous work has shown that increased expression of this enzyme is linked to enhanced resistance to pathogens in sweet potato (Wang et al., 2024). The

upregulation of this protein may suggest that it plays an important role in pathogen resistance in Jane.

4.3.3 Defence and detoxification mechanisms

Proteins associated with detoxification and stress defence further differentiated the cultivars. Jane showed an upregulation of glutathione transferase, a multifunctional enzyme that is involved in oxidative stress tolerance, detoxification and transport of flavonoid intermediates (Ding et al., 2017; Kou et al., 2019; Soviguidi et al., 2022). Kou et al. (2019) showed that there is a high expression of *IbGSTF4* in purple > yellow > red (deep orange) > white fleshed sweet potato, while it showed low accumulation in green leaves. The upregulation in Jane could indicate its importance in downstream application during storage formation. It is to be noted that Jane is an orange-fleshed sweet potato, whereas Atacama is white fleshed.

Atacama, in contrast, showed a significant upregulation of sporamin A and its precursor, preprosporamin. While sporamin is primarily a storage protein in roots, it can be induced in leaves under mechanical or abiotic stress and acts as protease inhibitor against herbivores and pathogens (Rajendran & Yeh, 2012; Rajendran et al., 2014; Yang & Kim, 2023). The growth of the sweet potato plants under a rainout shelter likely caused minor abiotic mechanical wounding, which would have induced significant expression of sporamin, specifically sporamin A, in the leaves of Jane.

4.3.4 Photosynthesis and energy metabolism

Atacama regulated proteins required for photosynthetic efficiency. The upregulation of the large ribosomal subunit bL36c has been shown to be important in leaf morphology, and that may affect photosynthesis in tobacco (Fleischmann et al., 2011; Robles & Quesada, 2022). Fleischmann et al. (2011) indicate that mutation of the *rpl36* gene severely affected the leaf morphology of tobacco plants. Therefore, large ribosomal subunit bL26c can serve as a potential biomarker that can improve leaf morphology, thus improving photosynthesis.

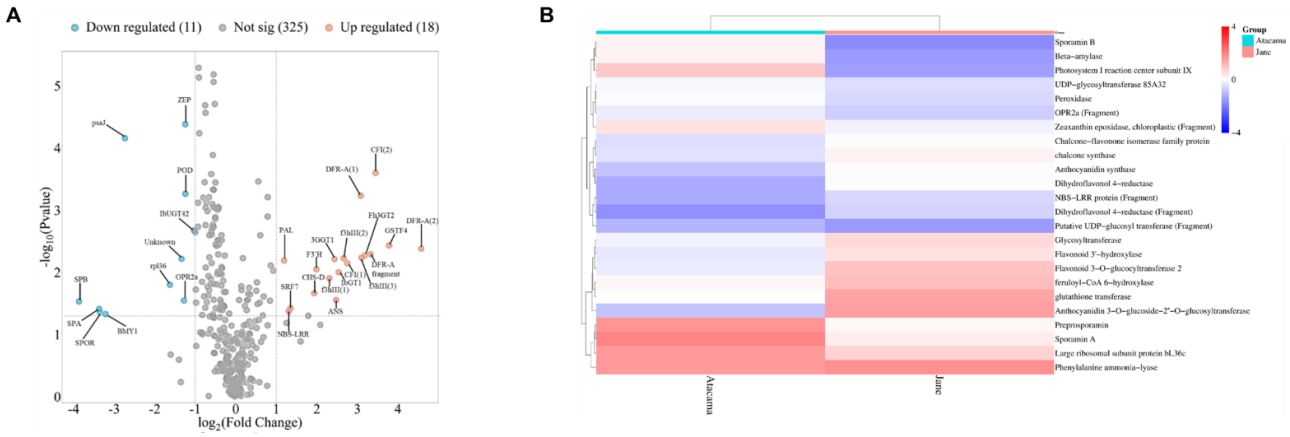

Figure 4.10: DEPs between Jane and Atacama sweet potato cultivars. Significantly upregulated proteins ($log^2FC > 1$, $p < 0.05$) are represented in orange, downregulated proteins ($log^2FC < -1$, $p < 0.05$) in blue.

The proteomic profiles therefore indicate two contrasting proteomes between Atacama and Jane. These coordinated differences provide insight into how genotypes of the cultivars shape metabolic processes and offer candidates protein markers for breeding aimed at improved stress tolerance and nutritional quality.

4.4 Molecular response of sweet potato (*Ipomoea batatas* L.) to drought stress using transcriptomics analysis

The aim of this study was to determine the molecular responses of two sweet potato cultivars (Atacama and Blesbok) to drought stress using transcriptomics analysis.

4.4.1 Differential expression analysis

A total number of 280 537 transcripts were generated in a count matrix. Of these transcripts, a total of 7 872 genes were upregulated and 5 467 were downregulated across all drought treatment conditions (50% and 70%) compared to the control (30%) at 2 weeks and 14 weeks. Figure 4.11 shows the differential expression of genes for each cultivar per drought and harvest time. Atacama 2 weeks post drought imposition (wpi) showed 452 upregulated and 60 downregulated genes at 50% drought stress, and 3 254 upregulated and 981 downregulated genes at 70% drought stress. In Blesbok, 32 upregulated and 41 downregulated genes were observed at 50% drought stress, and 84 were upregulated and 27 downregulated at 70% drought stress. Finally,

at 14 wpi, Atacama showed 1 073 upregulated and 1 141 downregulated genes at 50% drought stress, and 1 540 upregulated and 1 624 downregulated genes at 70% drought stress. Blesbok showed 1 197 upregulated and 941 downregulated genes at 50% drought stress and 240 upregulated and 652 downregulated genes at 70% drought stress. Across moderate (50%) and severe (70%) drought stress and at all time points, Atacama showed a stronger transcriptome response compared to Blesbok. At 50% drought stress, Atacama showed a higher number of DEGs, whereas Blesbok showed a weaker response with fewer DEGs at 2 weeks and 7 weeks and moderate expression of genes at 14 weeks. At 70%, the contrast between the two cultivars becomes even more pronounced. Interestingly, Blesbok showed fewer DEGs across all time points at 70%, which may highlight a weaker response to severe drought stress. These results indicate that Atacama responded earlier and more strongly to moderate and severe drought, whereas Blesbok showed a delay in drought response.

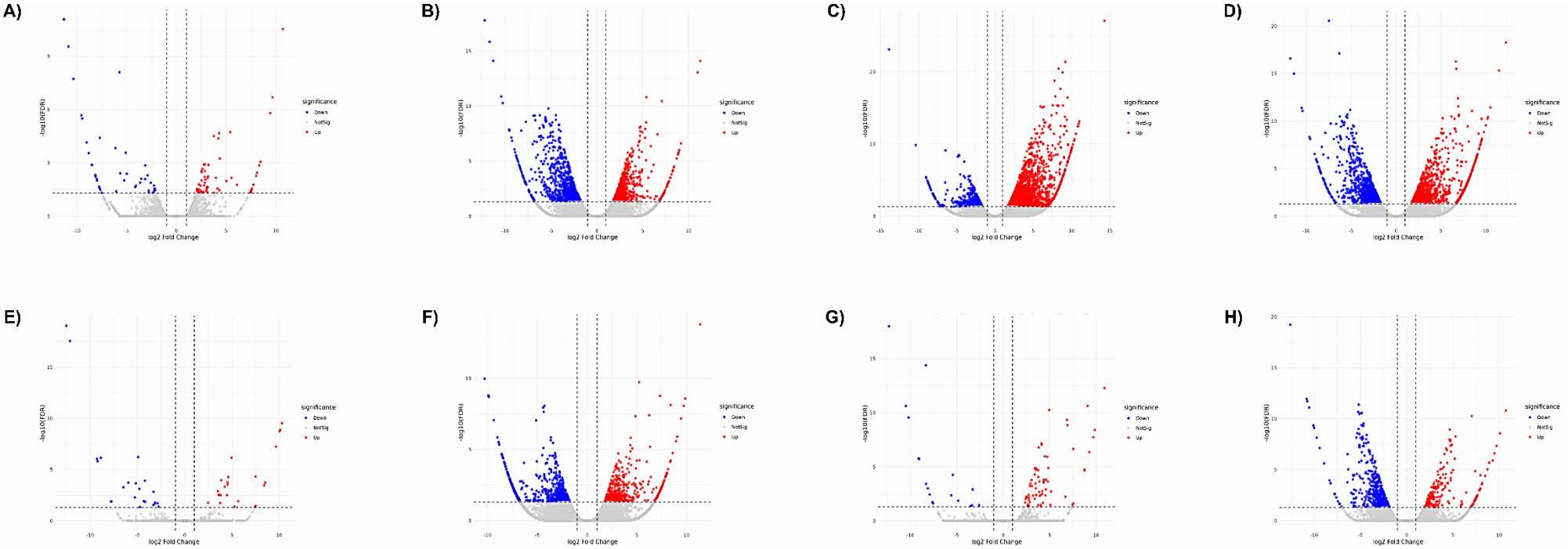

Figure 4.11: DEGs in Atacama and Blesbok under moderate (50%) and severe (70%) drought stress relative to control (30%) at 2 and 14 wpi. (A) Atacama 50%_2wpi, (B) Atacama 50%_14wpi, (C) Atacama 70%_2wpi, (D) Atacama 70%_14wpi, (E) Blesbok 50%_2wpi, (F) Blesbok 50%_14wpi, (G) Blesbok 70%_2wpi, (H) Blesbok 70%_14wpi. Red points indicate upregulated genes and blue downregulated genes (|log2FC| ≥ 1, FDR < 0.05).

Genes identified from two cultivars at different harvest weeks (2 and 14 weeks) were classified into three main GO categories (Figure 4.12A). In the biological process category, genes were predominantly associated with “biosynthetic process”. In the cellular component category, genes were localised mainly to “nucleus”, “chloroplast” and “cytoplasm”. In the molecular function category, most genes were involved in “protein binding” and “catalytic activity”. Common pathways enriched across multiple samples were identified using KEGG pathway analysis (Figure 4.12B). The top 10 pathways include “metabolic pathways”, “biosynthesis of secondary metabolites”, “carbon metabolism” and “biosynthesis of amino acids”, indicating pervasive drought-induced modifications to core metabolic processes. Additional pathways such as “ribosome”, “protein processing in the endoplasmic reticulum” and “purine metabolism” show sample-specific enrichment patterns, highlighting differences in drought-response strategies between cultivars, drought severity and duration.

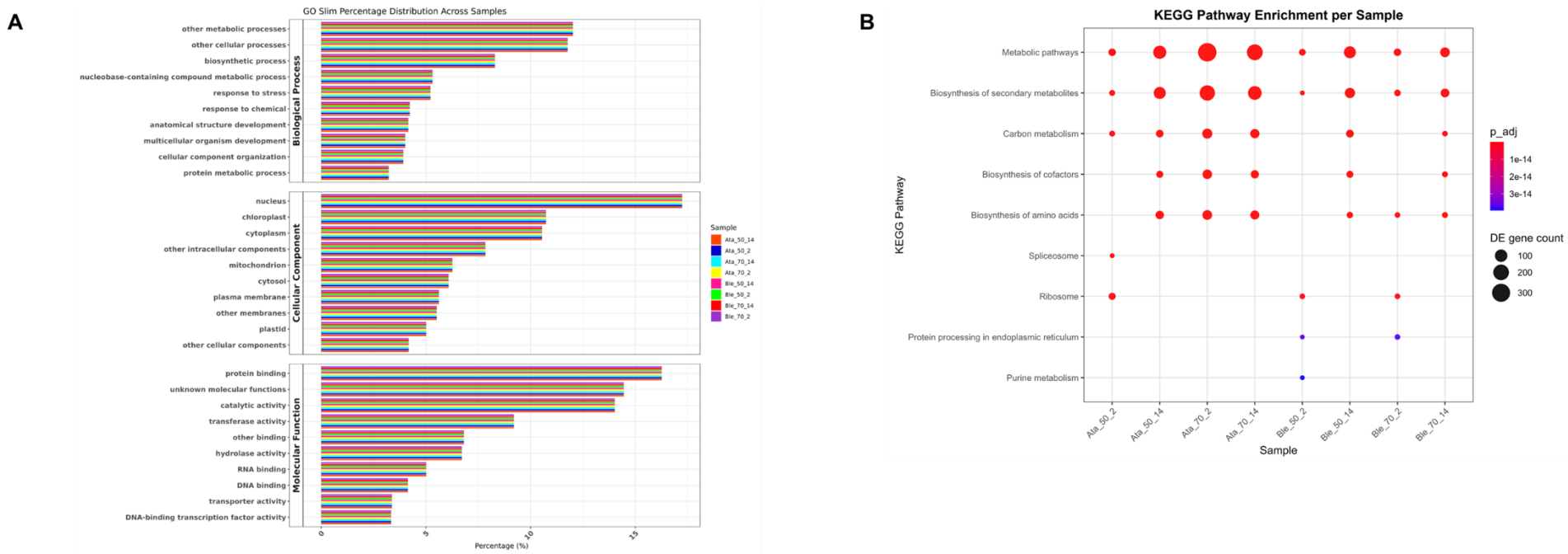

Figure 4.12: (A) GO classification of DEGs in Atacama (Ata) and Blesbok (Ble) under different drought stress conditions. Genes identified from two cultivars at different harvest weeks (2 and 14 weeks) were classified into three main GO categories: Biological process, cellular component and molecular function. (B) KEGG pathway enrichment analysis. Bubble size corresponds to the number of DEGs mapped to each pathway, with larger bubbles indicating higher gene counts. Bubble colour represents the false discovery rate (FDR), ranging from red (more significant enrichment, lower FDR) to blue (less significant).

4.4.2 Photosynthesis-related genes

Various metabolic pathways were regulated in the sweet potato cultivars in response to drought stress. Photosynthesis is one of the important pathways which is significantly affected by drought stress. Photosynthesis is highly sensitive to drought stress (Hu et al., 2022). Several genes in the top 20 significantly expressed genes (Figure 4.13) are involved in photosynthesis. These include photosystem II BY, the photosystem I light harvesting complex gene, the ATP synthase delta-subunit gene, photosystem II 5kD protein, ribulose-bisphosphate carboxylases, rubisco assembly chaperone, the cytochrome b6f complex subunit and high cyclic electron flow.

Photosynthesis is a metabolic process that converts light energy into chemical energy. There are two stages that occur during photosynthesis. The first stage occurs in the thylakoid membrane where light energy is captured and is converted into chemical energy (ATP and NADPH) (Gollan et al., 2015; Karami et al., 2023). Four photosynthetic protein complexes are situated in thylakoid membranes: photosystem I (PSI), photosystem II (PSII), the cytochrome b6f complex (Cytb6f) and adenosine triphosphate (ATP) synthase (Van Bezouwen et al., 2017). Our study revealed genes associated with these protein complexes. Photosystem II bY (*psbY*) was upregulated at late stress in Atacama at both moderate and severe stress and in Blesbok at mid stress. *PsbY* is a small protein located on the outer side of PSII and involved in the assembly of the cytochrome b559 complex (Cyt b_{559}) (Kaminskaya et al., 2007). It has also been shown to stabilise Cyt b_{559} and it may support the attachment of the PsbE and PsbF proteins to the heme group and protect Cyt b_{559} from harmful oxidising molecules (Umena et al., 2011; Plöchinger et al., 201). *PsbY* has been shown to be downregulated in response to drought stress in peach (Haider et al., 2018), but its upregulation in this study in sweet potato *cv*. Atacama and Blesbok at late drought stress indicates a possible protective mechanism that maintains PSII stability under prolonged drought stress. Another gene that might be involved in photoprotection is ATP synthase. Our study showed that ATP synthase delta subunit (ATP synthase δ, *atpD*) was upregulated at Atacama 70% and Blesbok 50% at 2 weeks, but at 14 weeks it was downregulated in Atacama under severe stress. ATP synthase delta subunit plays a role in stabilising thylakoid protein composition (Kong et al., 2013). The gene, *atpD,* has been shown to be downregulated in wheat and sorghum under drought

stress (Karami et al., 2025). Although the downregulation of *atpD* may inhibit ATP synthesis, which has a negative effect on the activity of RuBisCO, its downregulation at prolonged drought stress may adjust ATP and NADPH production, which increases proton accumulation in the thylakoid lumen. The increase in the proton gradient stimulates photoprotective energy dissipation (qE) and restricts linear electron flow. *AtpD* showed no regulation at 14 weeks in Blesbok, which may suggest a loss in energy metabolism and thylakoid stability under prolonged stress. This may indicate the inability of Blesbok to maintain ATP synthase activity, thus limiting photoprotection, and may contribute to its drought-susceptible trait.

4.4.3 Defence-related genes

Potential defence-related genes were also identified in the top 20 significantly regulated genes. The domain of unknown function, DUF2996, was upregulated in Atacama 70% at 2 and 14 weeks, and Blesbok 50% at 14 weeks. However, it was downregulated in Atacama 50% at 14 weeks and showed no regulation in the other samples. DUF2996 is uncharacterised in *NdhV*. *NdhV* is located in the thylakoid membrane and is associated with the NADPH-dehydrogenase-like complex (NDH) (Fan et al., 2015). This protein is responsible for stabilising NDH subcomplexes, SubA and SubE (Fan et al., 2015). These subcomplexes are required for electron transfer between PSI and cyt b_6f (Fan et al., 2015; Su et al., 2022). The downregulation of *NdhV* in *Brassica napus* L. (rapeseed) was suggested to destabilise NDH complex, thus restricting the plant's ability to regulate excess electrons under heat stress (Shahsavari et al., 2025). Therefore, DUF2996 (*NdhV*) may play an important role in preventing the accumulation of ROS. The upregulation of DUF2996 in Atacama under severe stress at both early and late time points may indicate enhanced photoprotection and scavenging of ROS under prolonged and severe drought stress. However, its downregulation in Atacama at moderate stress may suggest that DUF2996 activity under stress is triggered under severe stress, highlighting a stress intensity dependent strategy. In contrast, the absence of regulation in Blesbok and its delayed response under moderate stress may indicate a weaker and delay in adjusting photosynthetic electron transport.

Pentatricopeptide repeat (PPR) proteins are a family of proteins that are involved in post-transcriptional modification (Wang and Tan, 2025; Li et al., 2025). Their

involvement in RNA metabolism is important for mitochondrial functioning, energy production and stress tolerance (Li et al., 2025). A PPR gene, *TaPPR13*, has been shown to enhance drought stress tolerance in wheat (Hou et al., 2025). Similar results were also observed in rice, *OsPPR674* (Li et al., 2025). Interestingly, our study showed the upregulation of PPR-containing protein in Blesbok under moderate stress at 2 and 14 weeks, and severe stress at 14 weeks. This gene was downregulated in Atacama under moderate stress at late time points and severe stress at early time points. This contrasting expression suggests that Blesbok, drought susceptible, relies on a PPR-mediated post-transcriptional regulation stress, whereby Atacama, drought tolerant, may use alternative defence mechanisms, therefore reducing the requirement of PPR upregulation under stress.

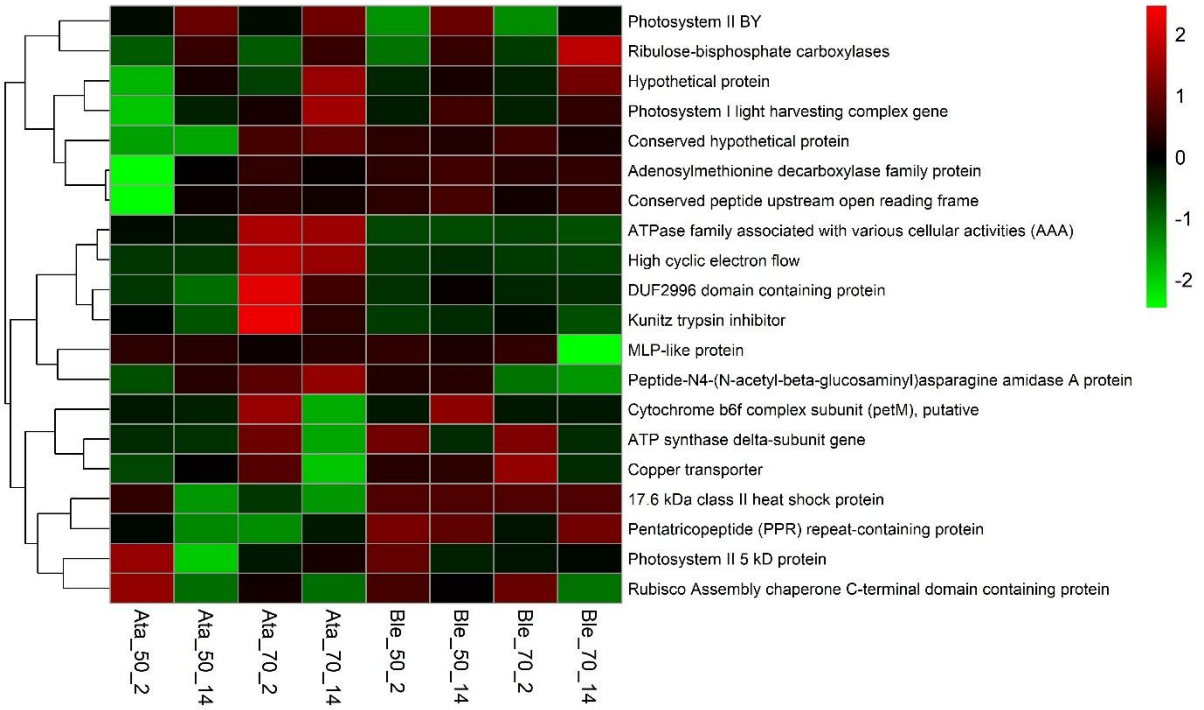

Figure 4.13: Heatmap showing the expression patterns of the top 20 significantly up- and downregulated DEGs in Atacama (Ata) and Blesbok (Ble) at different drought stress conditions. Gene expression values are displayed as scaled log_2 fold changes, with red indicating upregulation and blue representing downregulation relative to the control plants (30%).

This study shows that drought stress induced a broad transcriptomics response between both sweet potato cultivars. Atacama showed a stronger and earlier response to moderate and severe stress compared to Blesbok. Importantly, genes associated

with photosystem stability, ATP synthesis, electron transport and ROS detoxification were consistently upregulated in Atacama, which suggests that this cultivar has an enhanced photoprotection and is able to maintain energy production under stress. In contrast, Blesbok showed an upregulation of PPR gene and other defence-related genes, indicating engagement of post-transcriptional regulatory pathways and response under prolonged stress. The drought-responsive genes identified in both cultivars are described as potential molecular markers and provide important targets for marker-assisted breeding.

4.5 Conclusion

This integrative multi-omics study provides a comprehensive understanding of the drought stress response mechanisms in sweet potato by combining metabolomic, proteomic and transcriptomic analysis across different cultivars. Together, these results demonstrate that cultivar-specific differences in drought tolerance are regulated at different molecular and biochemical levels. Metabolomic profiles revealed that there are biochemical differences among cultivars, with flavonoids emerging as an important metabolite class that contributes to cultivar variations. Flavonoids contribute to antioxidant activities, pigmentation and stress response. Lipids, glycerophospholipids and triterpenoids further highlighted cultivar-specific differences that are involved in membrane stability and signalling. Proteomic analysis confirmed contrasting metabolics between cultivars, with Atacama showing enhanced regulation of pathways linked to energy production and photosynthesis, and Jane showed enhanced regulation of secondary metabolism, detoxification enzymes and defence-related proteins. Transcriptomics analysis under moderate and severe drought stress demonstrated that Atacama expresses a rapid and broad transcriptional response to drought stresses. These genes included those associated with photosystem stability, electron transport and ROS detoxification, supporting proteomic profile analysis. While Blesbok showed a delayed response to drought stress, the cultivar showed the regulation of post-transcriptional responses with genes such as pentatricopeptide-repeating-containing gene. The integration of these omics helped identify metabolites, proteins and genes that represent potential molecular biomarkers for drought tolerance for marker-assisted breeding.

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CHAPTER 5: INTEGRATED METABOLOMIC AND TRANSCRIPTOMIC INSIGHTS INTO DROUGHT STRESS RESPONSES IN CONTRASTING CASSAVA GENOTYPES

Abstract

Cassava (*Manihot esculenta* Crantz) is a vital staple crop for food security in Africa, yet its productivity is increasingly threatened by drought stress. This study employed an integrated untargeted metabolomics and targeted transcriptomics approach to investigate the molecular mechanisms underlying drought tolerance in two contrasting cassava genotypes, P4/10 (highly tolerant) and UKF4 (moderately tolerant). LC-MS qTOF analysis revealed distinct metabolic strategies between the genotypes under early and prolonged drought stress. The tolerant P4/10 genotype exhibited a proactive metabolic profile characterised by the accumulation of protective lipids, flavonoids and amino acid derivatives, alongside sustained expression of regulatory genes *MeRD28* and *MeZFP*. However, the genotype UKF4 exhibited a reactive response with delayed metabolite accumulation involving cofactors like phylloquinol and dephospho CoA, followed by a decline in protective gene expression and energy metabolites such as precorrin 1 under prolonged stress. Pathway analysis highlighted the importance of glycerophospholipid metabolism, glutathione metabolism and antioxidant defence systems. These findings suggest that drought resilience in cassava is driven by coordinated transcriptional and metabolic regulation, providing potential biomarkers for breeding climate-resilient varieties.

Keywords: cassava; drought stress; genes; metabolites; metabolic pathway

5.1 Introduction

Cassava serves as a primary source of carbohydrates for over 800 million people globally and is particularly crucial for food security in sub-Saharan Africa, where it contributes considerably to daily energy requirements (Parmar et al., 2017). Despite its reputation as a resilient crop capable of growing in marginal soils, prolonged drought conditions lead to substantial yield reductions and threaten the livelihoods of smallholder farmers who depend on it (Jarvis et al., 2012; Okogbenin et al., 2013). The increasing frequency of unfavourable weather conditions due to climate change worsens these challenges, necessitating a deeper understanding of the physiological and molecular mechanisms that enable certain genotypes to withstand drought conditions (Orek et al., 2020).

Plants employ various strategies to cope with drought stress, including avoidance, tolerance and recovery mechanisms. Avoidance strategies often involve morphological changes such as stomatal closure and root architecture adjustments to minimise water loss (Oliveira et al., 2015). Tolerance mechanisms operate at the cellular level, where plants maintain metabolic function through the accumulation of osmoprotectants, antioxidants and stress-responsive genes (Fang & Xiong, 2015). These adaptations are regulated by complex genetic networks that coordinate physiological responses to maintain cellular homeostasis under stress conditions. Therefore, understanding these mechanisms is essential for developing improved cultivars that can sustain productivity in water-limited environments.

Metabolomics has emerged as a powerful tool for elucidating the biochemical changes associated with stress responses in plants (Bandurska, 2022; Oguz et al., 2022). Key compounds involved in stress adaptation can be identified and the metabolic pathways that are modulated under drought conditions can be mapped, by profiling the complete set of metabolites (Allwood et al., 2021). While transcriptomics provides insights into gene expression patterns, metabolomics offers a direct snapshot of the physiological state of the plant. The integration of these omics technologies allows for a comprehensive understanding of the relationship between genetic regulation and metabolic phenotypes (Ding et al., 2019). However, despite the advancements in omics technologies, there is a significant gap in knowledge regarding the specific

metabolic pathways involved in cassava drought tolerance, particularly within African germplasm (Mantewu et al., 2025a).

This study aimed to characterise the metabolic and transcriptional responses of two cassava genotypes with contrasting drought tolerance levels under progressive drought stress. The genotypes P4/10 and UKF4 were selected based on their distinct metabolic separation from the other genotypes as revealed by multivariate analysis (Mantewu et al., 2025b). The specific objectives were to identify genotype-specific metabolites and pathways associated with drought resilience and to evaluate the expression patterns of key drought-responsive genes. By integrating metabolomic and transcriptomic data, this research sought to provide a mechanistic understanding of drought tolerance in cassava and potentially identify biomarkers for breeding programmes.

5.2 Results and discussion

5.2.1 Multivariate metabolic profiling

The metabolic profiles of P4/10 and UKF4 were analysed using PCA and PLS-DA to identify patterns associated with drought stress. The PCA model for P4/10 (Figure 5.1A) accounted for 72.4% (R^2X = 0.724) of the total variance but showed limited predictive capacity with Q^2 = 0.121. The absence of distinct clustering in the PCA plot implies that the metabolic adjustments during the initial phase of drought stress were subtle. This observation aligns with findings from other studies on crops subjected to mild stress conditions (Obata et al., 2015). In contrast, the PLS-DA model (Figure 5.1B) showed 85.2% (R^2X = 0.852) of the variance and demonstrated high predictive accuracy (Q^2 = 0.884). A clearly defined cluster corresponding to the P4/10 control treatment indicated a unique metabolic fingerprint. For the UKF4 genotype, the PCA model (Figure 5.1C) captured 69.9% of the variance but exhibited very poor predictive performance. However, the PLS-DA model (Figure 5.1D) for UKF4 explained 80.6% of the variance and demonstrated excellent predictive capability. Notably, the UKF4 severe drought treatment group formed a distinct cluster, indicating a pronounced metabolic shift under severe drought stress. It is important to note that while PLS-DA reliably separated groups, it is a supervised method, and its separation is expected if

classes are defined. The failure of PCA to separate most treatments indicates subtle changes at moderate stress levels.

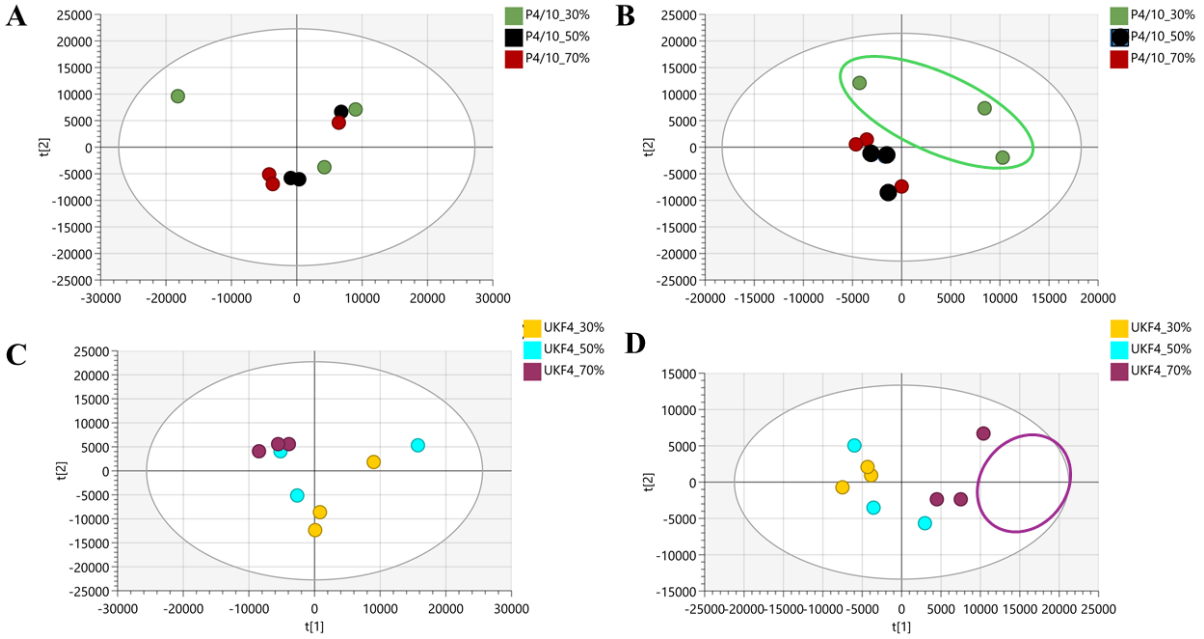

Figure 5.1: Multivariate metabolic profiling of cassava genotypes P4/10 and UKF4 in response to early drought stress conditions. (A) PCA score plot for P4/10. (B) PLS-DA score plot for P4/10. (C) PCA score plot for UKF4. (D) PLS-DA score plot for UKF4.

Under prolonged drought stress, the PCA model (Figure 5.2A) for P4/10 elucidated 79.1% (R^2X = 0.791) of the total variance. The lack of distinct clustering among treatment groups implies that the metabolic responses in P4/10 were complex or overlapping, even under stress. This complexity was attributable to robust adaptive mechanisms that facilitate metabolic stability across varying drought levels. Comparable findings have been reported in metabolomic studies of wheat subjected to early-stage drought (Guo et al., 2018). In contrast, genotype UKF4 demonstrated a PCA model (Figure 5.2C) that explained 94.5% of the data variation (R2X = 0.945) with high predictive power (Q^2 = 0.969). Unlike P4/10, this model revealed a clear separation among all treatment groups, particularly the severe drought group. This observation suggests that UKF4 underwent significant metabolic shifts in response to varying degrees of drought over time, reflecting its sensitivity to prolonged stress. The PLS-DA model for P4/10 (Figure 5.2B) accounted for 87.4% of the variation (R^2X = 0.874) and exhibited high predictive accuracy (Q^2 = 0.831). Notably, the 70% drought treatment formed a distinct cluster, clearly segregated from both the control and

moderate drought groups. This finding indicates that under long-term severe drought conditions, P4/10 activated specific biochemical pathways associated with stress responses (Ray et al., 2011). In contrast, the PLS-DA model (Figure 5.2D) for UKF4 explained 96.6% of the variation and demonstrated near-perfect predictive capability. The clear portrayal of all treatment groups in the PLS-DA plot supports UKF4's dynamic yet measurable response to prolonged drought.

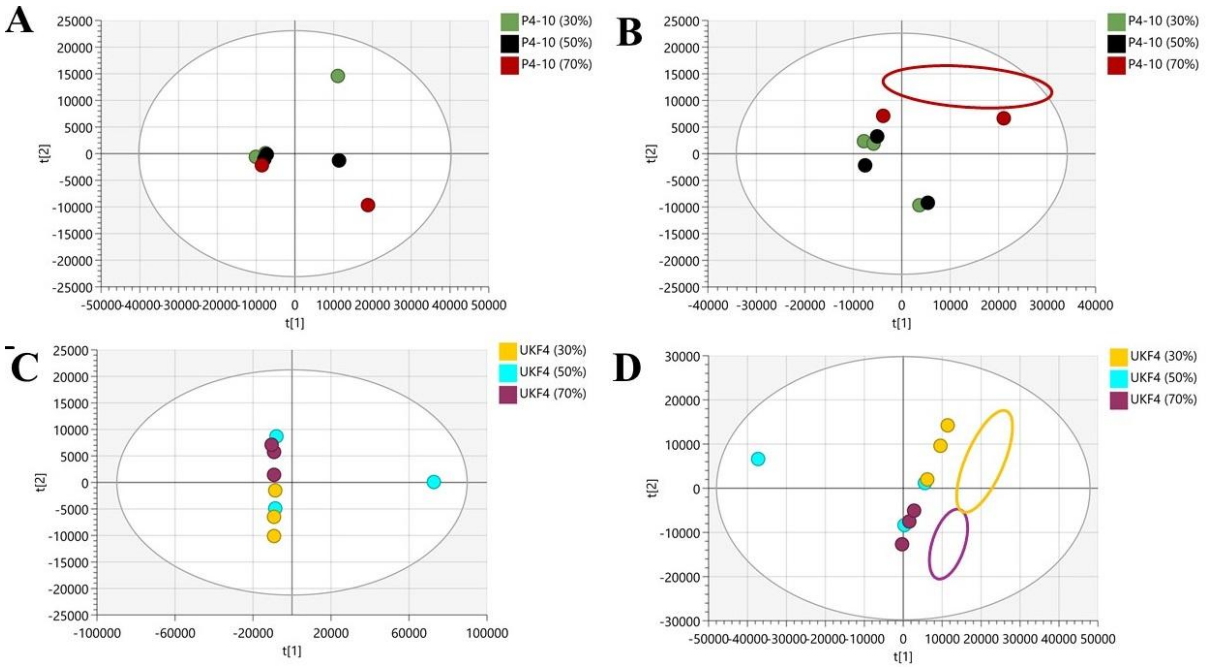

Figure 5.2: Multivariate metabolic analysis of cassava genotypes P4/10 and UKF4 in response to late drought stress conditions. (A) PCA score plot for genotype P4/10. (B) PLS-DA score plot for P4/10. (C) PCA score plot for genotype UKF4. (D) PLS-DA score plot for genotype UKF4.

5.2.2 Metabolite accumulation and pathway analysis

Random forest analysis revealed key metabolites significantly associated with the drought-tolerant genotype P4/10 during early drought stress. Noteworthy metabolites included Acetyl CoA, 2,3 bis (O geranylgeranyl) sn glycero 1 phospho L serine, and tryptophan. Acetyl CoA, essential for energy metabolism and lipid biosynthesis, indicated the early mobilisation of carbon reserves to support membrane remodelling and stress signalling (Okazaki & Saito, 2014). Additionally, phosphatidylcholine increased under moderate to severe stress (Figure 5.3), directly contributing to

membrane integrity during the initial water deficit. The rise in trypanothione, a redox-active oligopeptide, reflected a rapid activation of antioxidant defences, which aligned with P4/10's ability to mitigate oxidative damage from the onset of stress. Other metabolites, such as cylindrin and procyanidin B2, maintained stable concentrations, indicating a regulated metabolic response. In contrast, UKF4 exhibited a delayed and less coordinated response. Accumulations of dephospho CoA and phylloquinol under 50 to 70% drought (Figure 5.4) suggested efforts to maintain cofactor availability and protect photosynthesis. However, key flavonoids such as luteolin and catechin 7-glucoside were significantly suppressed, indicating a limited capacity to sustain antioxidant defences early in the stress period.

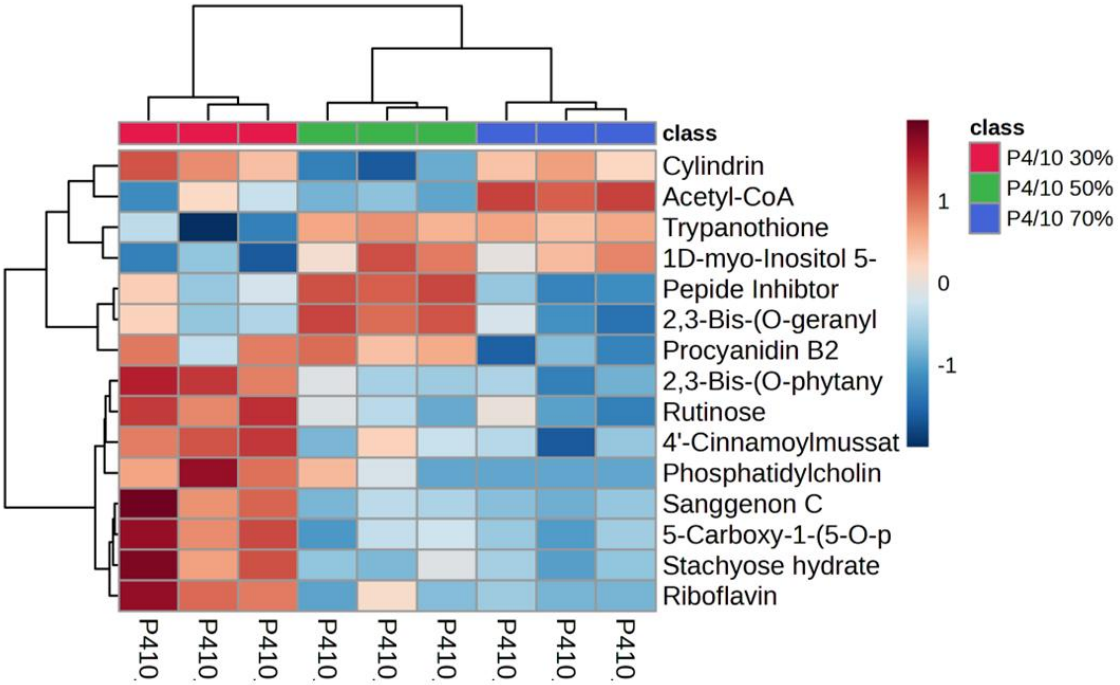

Figure 5.3: The heatmap illustrates the expression levels of metabolites in the P4/10 genotype under early drought stress. The rows correspond to metabolites, and the columns represent samples. Colour intensity reflects the expression level (high to low), with the legend indicating the expression percentages and class distinctions for P4/10.

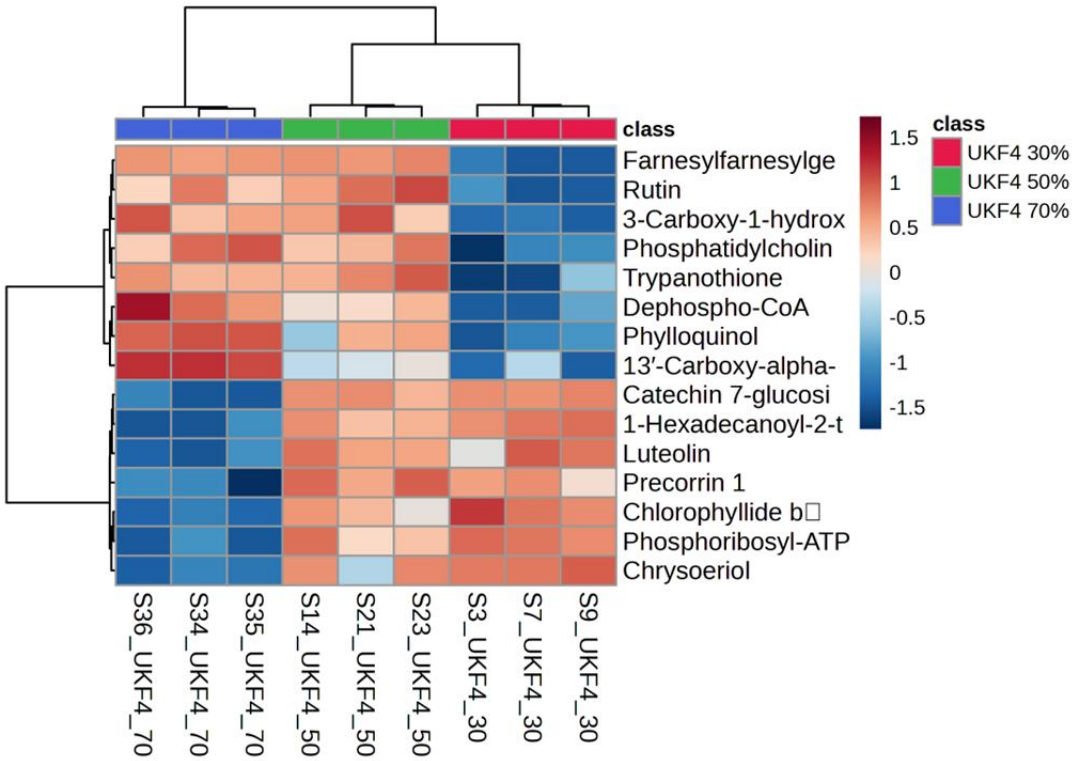

Figure 5.4: The heatmap depicts the expression levels of metabolites in the UKF4 genotype under early drought stress. Rows represent metabolites, and columns denote samples. Colour intensity corresponds to the expression level (high to low), with the legend detailing the expression percentages and class distinctions for UKF4.

During the time of prolonged drought stress, P4/10 shifted toward a specialised protective metabolism. The accumulation of Quercetin 3 arabinoside, a sulphur-rich defence compound, reached very high levels under 70% stress (Figure 5.5), suggesting enhanced ROS detoxification and stress signalling (Martins et al., 2022). Concurrently, specific phosphatidylcholine derivatives increased, strengthening membrane stability and activating lipid-derived signalling pathways (Okazaki & Saito, 2014; Liu et al., 2021). The sustained presence of flavonoid glycosides such as cyanidin 3 (6-p-coumaroyl glucoside) 5 glucoside further supported a multi-layered antioxidant system that remained active even under extended stress, allowing P4/10 to maintain cellular homeostasis (Sarker & Oba, 2018). In contrast, UKF4 displayed a reactive but ultimately unsustainable strategy during prolonged stress. Heme and phosphatidylcholine derivatives peaked at severe stress, which indicated a last-ditch effort to mobilise porphyrin metabolism to compensate for declining photosynthesis and maintain respiration (Wang et al., 2018). However, critical metabolites collapsed under severe stress (Figure 5.6), signalling a breakdown in tetrapyrrole biosynthesis

and redox homeostasis. Precorrin 1 and 3 phosphoglyceroyl glutathione dropped to very low levels, indicating a potential collapse in energy production and biosynthetic pathways (Nagahatenna et al., 2015). This metabolic exhaustion during prolonged drought underscored UKF4's limited resilience compared to P4/10.

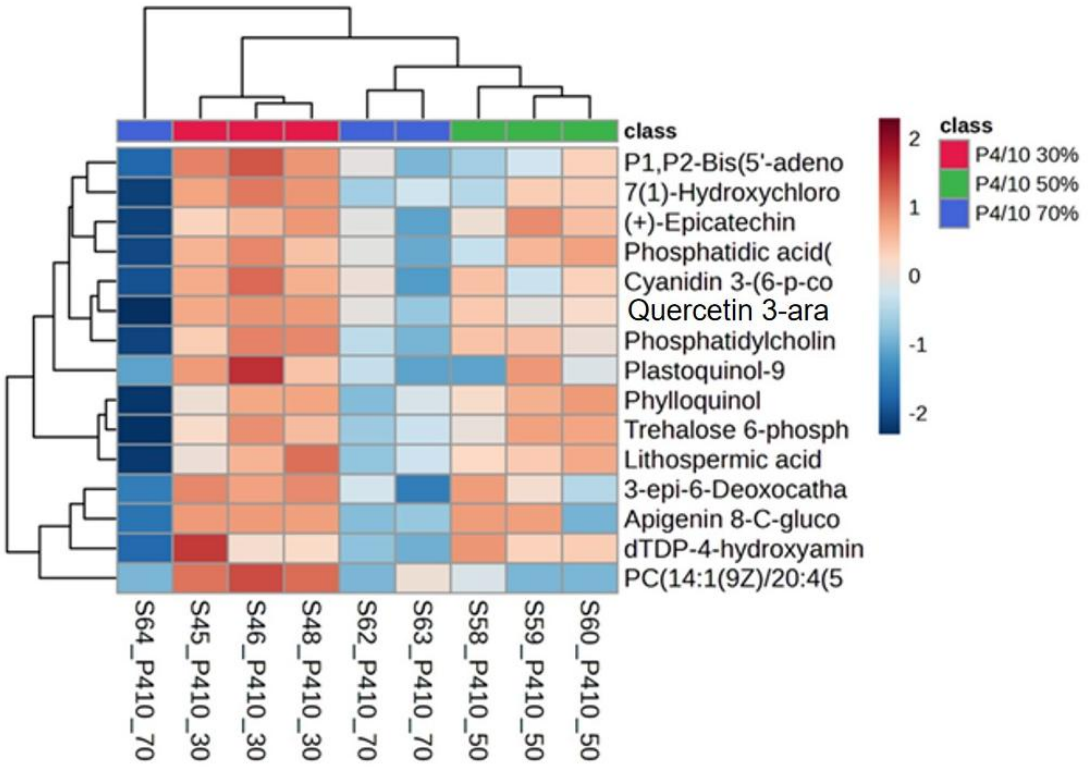

Figure 5.5: The heatmap presents the expression levels of metabolites in the P4/10 genotype under prolonged drought stress. Rows correspond to metabolites, and columns represent samples. Colour intensity reflects the expression level (high to low), with the legend illustrating the expression percentages and class distinctions for P4/10.

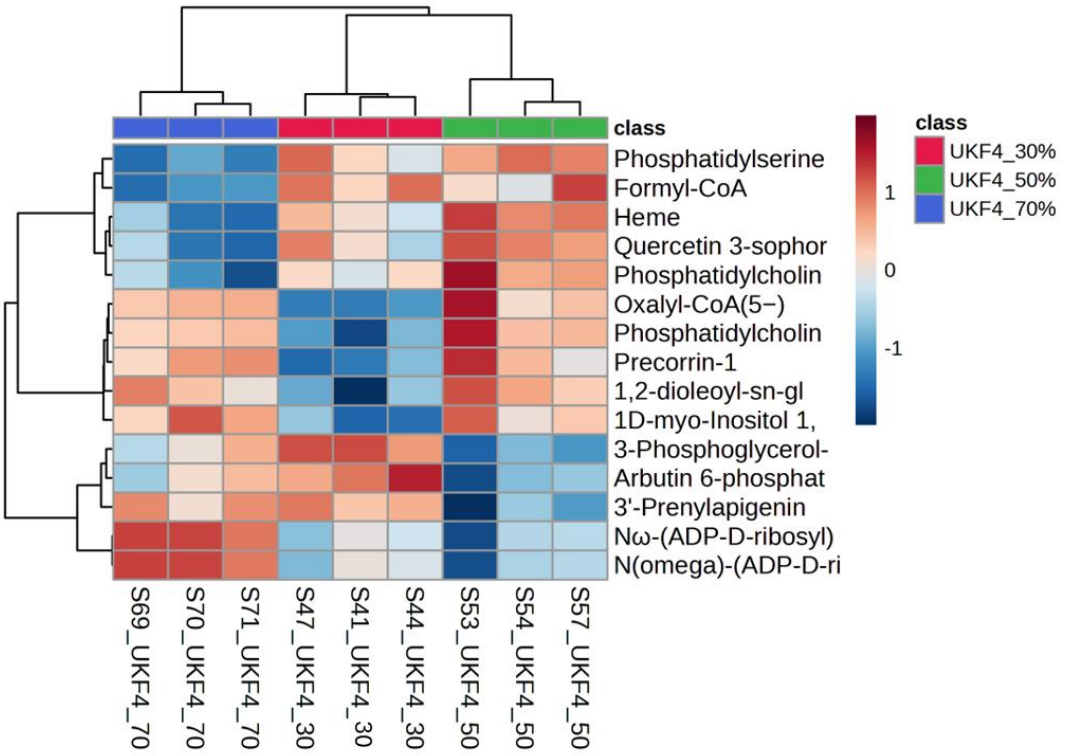

Figure 5.6: The heatmap illustrates the expression levels of metabolites in the UKF4 genotype under prolonged drought stress. Rows represent metabolites, and columns denote samples. Colour intensity corresponds to the expression level (high to low), with the legend indicating the expression percentages and class distinctions for UKF4.

The metabolic pathway analysis facilitated the identification of significant changes in metabolism. Under early drought stress, the highly drought-tolerant P4/10 genotype exhibited extensive metabolic reprogramming. The pathway impact analysis highlighted the significant role of riboflavin metabolism in the production of flavin cofactors necessary for redox reactions and alleviating oxidative stress (Hasanuzzaman et al., 2013). Another significant pathway identified was glutathione metabolism, which is essential during drought stress and facilitated the detoxification of ROS through the synthesis of glutathione. This metabolite is a key antioxidant that safeguards cellular components from oxidative damage (College et al., 2014; Dorion et al., 2021). The citrate cycle and pyruvate metabolism indicate an upregulation of energy production pathways, ensuring a continuous supply of ATP to support cellular processes under drought stress (Shen et al., 2017; Teng et al., 2022). Under prolonged drought stress, P4/10 exhibited a metabolic shift toward lipid-related pathways. The P4/10 genotype demonstrated a significant involvement of glycerophospholipid metabolism (Figure 5.7). This metabolic pathway included two

phosphatidylcholine metabolites, which are key components of membrane phospholipids. The activation of the glycerophospholipid metabolism pathway was essential for the remodelling of cell membranes, preserving fluidity and maintaining structural cell integrity during desiccation stress (Sharma et al., 2023).

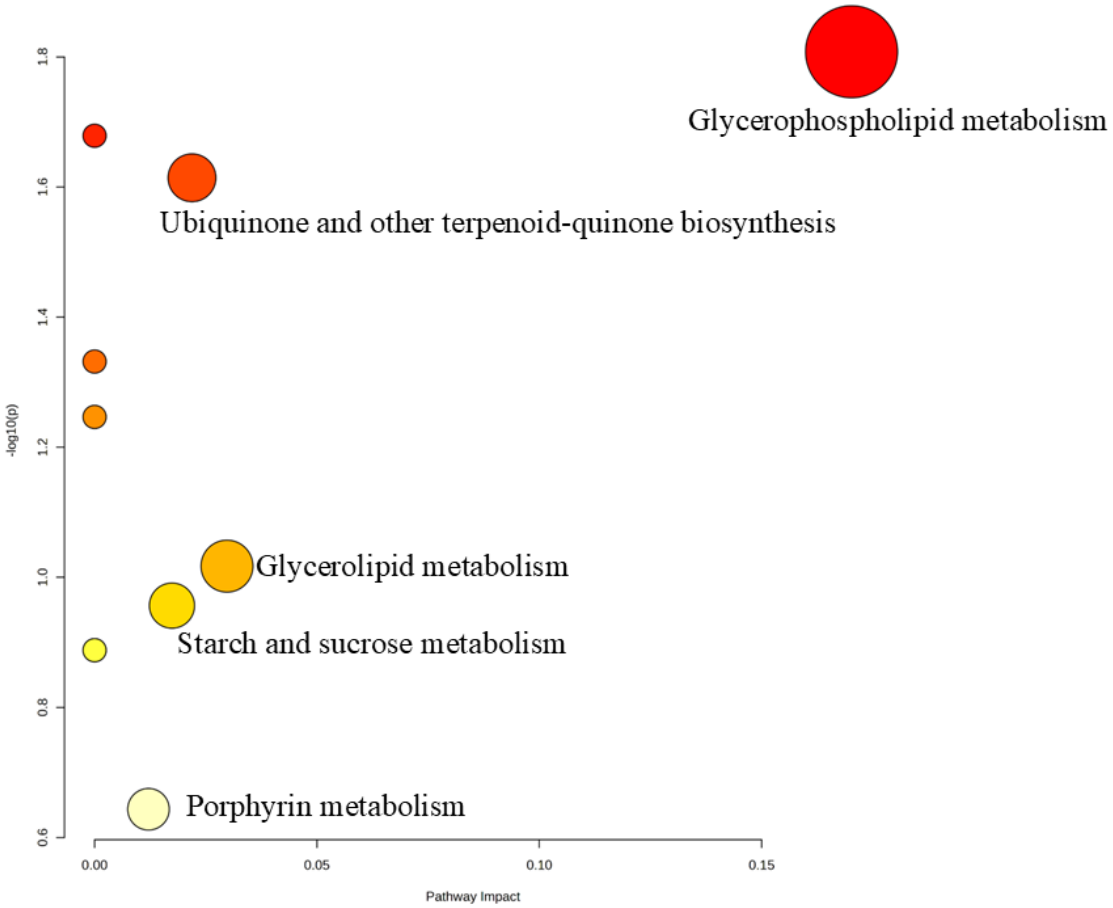

Figure 5.7: Pathway analysis for the P4/10 genotype under prolonged drought stress, generated by MetaboAnalyst. This scatter plot illustrates the impact and abundance of key metabolic pathways, with each node representing a distinct metabolic process. The colours of the nodes, ranging from yellow to red, indicate varying levels of pathway significance, and the sizes of the nodes reflect the pathway impact values, thereby highlighting critical metabolic responses.

However, the moderately drought-tolerant genotype (UKF4) exhibited a more targeted metabolic response under early drought stress. The pathway impact analysis identified histidine metabolism as statistically significant but with low functional impact (Ackah et al., 2021). Meanwhile, the citrate cycle and pantothenate and CoA biosynthesis demonstrated moderate significance, indicated by adjustments in energy metabolism

and the synthesis of coenzyme A (Yadav et al., 2022). The enrichment overview also highlighted ubiquinone and other terpenoid quinone biosynthesis pathways driven by metabolites such as phylloquinol, which were critical for mitochondrial electron transport and antioxidant activity (Ma et al., 2021; Zhao et al., 2021). However, during prolonged drought stress, the UKF4 genotype also identified glycerophospholipid metabolism as the most significant pathway (Figure 5.8), followed by linoleic acid metabolism, arachidonic acid metabolism and alpha-linolenic acid metabolism, which contribute to lipid signalling and membrane adjustments (Zi et al., 2022; Shu et al., 2024). Notably, porphyrin metabolism showed considerable impact, which indicated a reduction in chlorophyll content to minimise water loss and reallocate resources (Cheng et al., 2018; Wang et al., 2024).

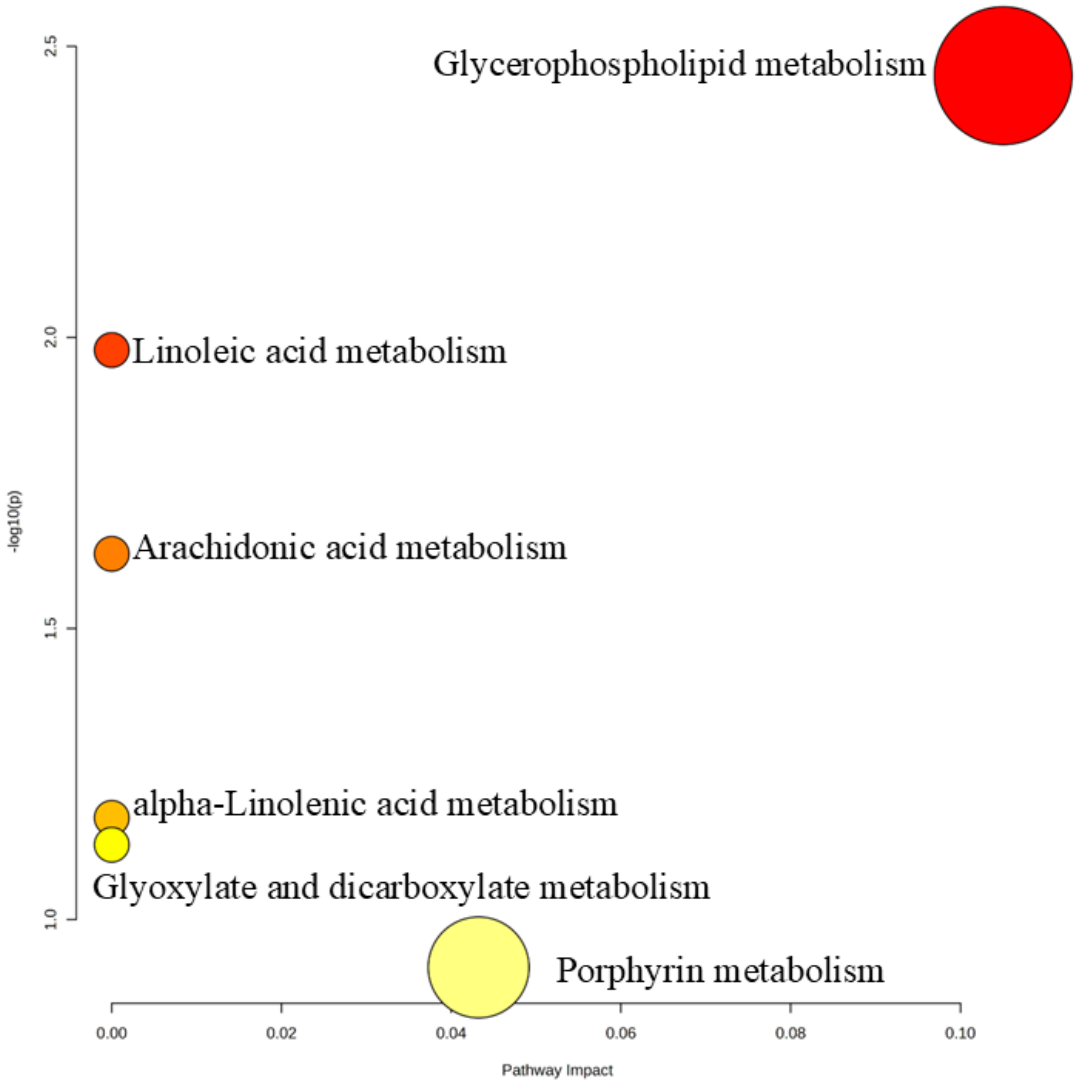

Figure 5.8: Pathway analysis for the UKF4 genotype under prolonged drought stress, generated by MetaboAnalyst. This scatter plot depicts the impact and abundance of key metabolic pathways, with nodes representing distinct metabolic processes. The colours of the nodes, ranging from yellow to red, indicate p-values, and node sizes correspond to pathway impact values, highlighting key drought-responsive pathways.

5.2.3 Gene expression and integrated analysis

Gene expression profiles of *MeALDH*, *MeMSD*, *MeRD28* and *MeZFP* were quantified to understand the transcriptional regulation underlying the metabolic observations. Under early drought stress conditions, P4/10 exhibited a significant adaptive shift in gene expression (Figure 5.9). The expression levels of the genes *MeALDH* and *MeMSD* decreased. The suppression of these genes indicated a strategic reallocation of cellular resources, diverting energy from non-essential biosynthesis to prioritise frontline stress responses (Meyer et al., 2014; Ost et al., 2023). However, UKF4 exhibited minimal change, maintaining *MeALDH* and *MeMSD* expression at near basal levels (Figure 5.9), suggesting a more conservative and less dynamic transcriptional response during initial drought exposure (Turyagyenda et al., 2013; Zhu et al., 2016). The differential response was evident in the expression of the protective genes *MeRD28* and *MeZFP*. The highly tolerant genotype P4/10 showed a significant upregulation of these genes at both levels of stress. The early and sustained upregulation of *MeRD28*, an aquaporin that facilitates cellular water transport, is a well-documented response in drought-tolerant genotypes (Avivi et al., 2020; Zheng et al., 2023). Furthermore, the upregulation of *MeZFP*, which is regarded as a transcriptional regulator, suggests the activation of a coordinated stress signalling network. Conversely, UKF4 maintained expression of *MeRD28* and *MeZFP* under moderate stress, but there was a decrease under severe stress. This suggests a threshold beyond which its transcriptional defence system becomes overwhelmed.

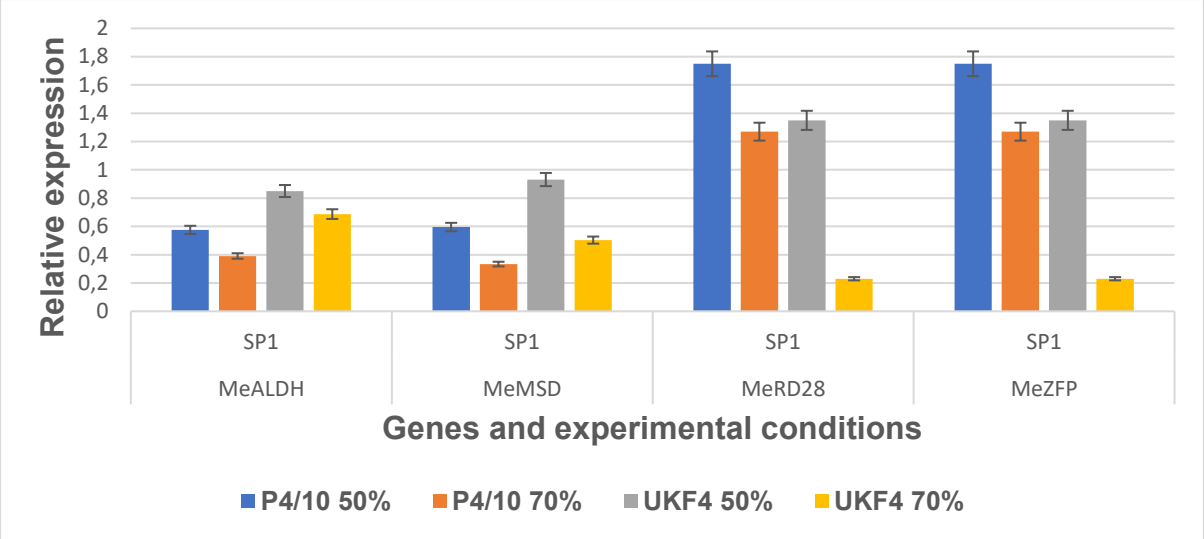

Figure 5.9: Relative expression of drought-responsive genes in cassava genotypes P4/10 and UKF4 during SP1

During prolonged exposure to drought stress, the transcriptional patterns of the cassava genotypes diverged further. P4/10 maintained its initial adaptive strategy, with expression levels of *MeALDH* and *MeMSD* further reduced compared to the control (Figure 5.10). This continued reduction in metabolic enzyme expression indicated a prolonged phase of metabolic efficiency, which enabled the conservation of cellular resources under extended drought stress (Twaji et al., 2023; Orek, 2024). In contrast, UKF4 displayed a different transcriptional reprogramming pattern. While the expression of *MeALDH* and *MeMSD* remained close to control levels during the early stress phase, both genes were further upregulated during the prolonged stress phase (Figure 5.10). This delayed transcriptional activation served as a compensatory response to accumulated cellular damage, consistent with the findings by Turyagyenda et al. (2013) and Fayiah et al. (2021). P4/10 exhibited a remarkable ability to sustain elevated expression of critical protective genes. Throughout the drought period, P4/10 consistently maintained high expression of *MeRD28* and *MeZFP*. This prolonged upregulation supported ongoing cellular protection and regulatory control. In contrast, UKF4 showed reduced expression of these genes, indicating its inability to maintain this protective transcriptional programme during extended stress (Zhang et al., 2022).

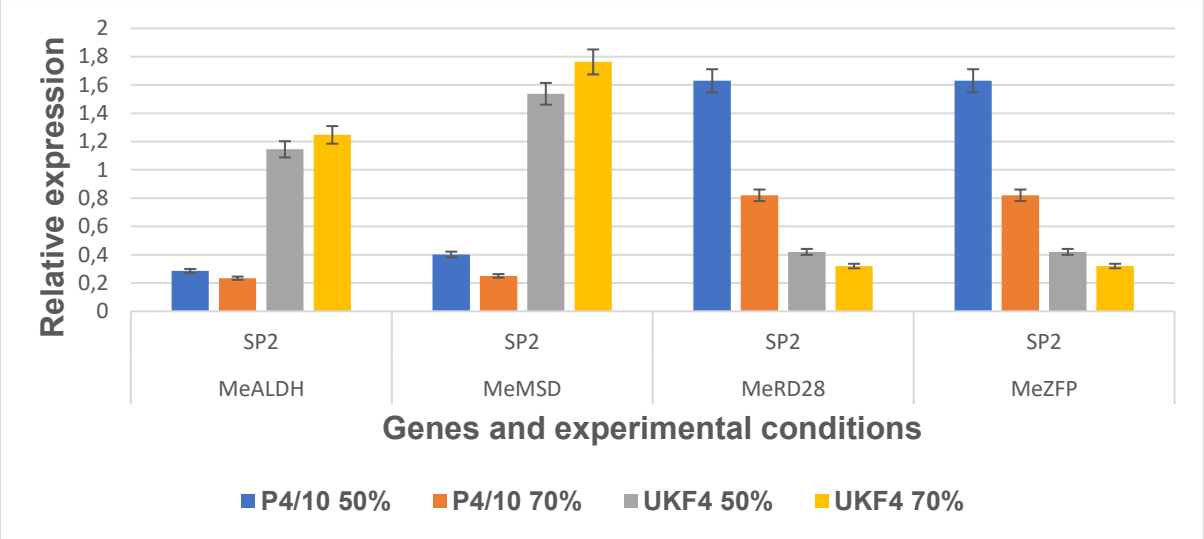

Figure 5.10: Relative expression of drought-responsive genes in cassava genotypes P4/10 and UKF4 during SP2

Integrating these transcriptional dynamics with the metabolomic data provides a mechanistic link between gene regulation and physiological outcome. The persistent expression of *MeZFP* and *MeRD28* in P4/10 directly facilitates the coordinated accumulation of protective metabolites such as antioxidants and membrane lipids. The consistent expression of MeRD28 and *MeZFP* corresponds with the accumulation of osmoprotectants, antioxidants and activated lipid pathways that maintain membrane integrity throughout the stress period. However, the transcriptional failure of the UKF4 regulatory framework accounted for its metabolic shortcomings. The inability to sustain *MeRD28* and *MeZFP* expression correlated with the depletion of essential energy metabolites and the breakdown of redox homeostasis under prolonged stress. The late upregulation of *MeALDH* and *MeMSD* reflects a desperate metabolic effort that proved inadequate to ward off physiological decline.

5.3 Conclusion

This study provides comprehensive insights into the molecular mechanisms underpinning drought tolerance in cassava through the integration of untargeted metabolomics and targeted transcriptomics. The contrasting responses of P4/10 and UKF4 demonstrate that drought resilience is not determined by isolated biochemical changes, but rather by the timing, coordination and sustainability of metabolic and transcriptional regulation. The highly tolerant genotype P4/10 adopted a proactive

strategy characterised by early activation of antioxidant systems, membrane lipid remodelling and sustained expression of key regulatory genes such as MeRD28 and MeZFP. This coordinated response enabled the maintenance of redox balance, energy metabolism and membrane integrity under both early and prolonged drought stress. In contrast, UKF4 exhibited a reactive and delayed response marked by transient metabolite accumulation and eventual transcriptional decline under severe stress conditions. The collapse of protective metabolites and reduced expression of regulatory genes during prolonged drought highlighted its limited capacity to maintain cellular homeostasis. Pathway analysis further reinforced that glycerophospholipid metabolism, glutathione metabolism, energy-related pathways and antioxidant defence systems are central components of drought adaptation in cassava. The sustained regulation of these pathways in P4/10 illustrates the importance of metabolic flexibility and efficient resource allocation during stress progression. Therefore, the integration of gene expression and metabolite profiling established a link between transcriptional control and metabolic responses, emphasising that durable drought tolerance depends on synchronised regulatory networks rather than short-term compensatory responses.

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CHAPTER 6: GENERAL DISCUSSION AND INTEGRATED CONCLUSIONS

6.1 General discussion

As droughts become more common and more severe, they create a major problem in the world's food supply, especially in arid and semi-arid regions where farming is essential for survival and income (Keshavarz, 2018). This project was conceived to address this pressing challenge by moving beyond the observation of physiological symptoms to translate the fundamental molecular mechanisms that reinforce drought tolerance in two of Africa's most vital, resilient staple crops: sweet potato and cassava. Through an integrated multi-omics framework encompassing metabolomics, proteomics and transcriptomics, this research provides a unique, mechanistic understanding of how these crops survive and maintain productivity under water-deficit conditions. The central finding from all analytical chapters is that drought tolerance is not just about which molecules are present. Instead, it is defined by their precise arrangement, timing, coordination and, most importantly, their sustainability (Jogaiah et al., 2013). The distinct genotypes studied reveal two primarily different survival strategies: a proactive, sustained resilience seen in the tolerant lines, and a reactive, ultimately self-limiting response seen in their susceptible counterparts.

The foundational layer of this investigation, provided by the metabolomic analyses, revealed that each genotype possesses a unique biochemical pattern even under optimal conditions (Mitchell, 2022). In sweet potato, the tolerant Atacama cultivar displayed a distinct profile rich in specific phenolic compounds and lipids, whereas in cassava, the tolerant P4/10 line showed a unique separation in phenylpropanoid and lipid metabolites (Xiao et al., 2021; Pazos et al., 2022). This pre-existing metabolic constitution appears to be a crucial pre-adaptation, a latent potential for stress resilience (Bisson et al., 2023). Under drought stress, these inherent differences were magnified into contrasting survival strategies. The tolerant genotypes, Atacama in sweet potato and P4/10 in cassava, demonstrated a capacity for metabolic homeostasis. Their response was characterised not by chaotic fluctuation, but by strategic, energy-efficient reprogramming (De Block & Van Lijsebettens, 2011). For instance, Atacama's drought tolerance was strongly associated with the stable and efficient accumulation of crucial phenolic antioxidants like chlorogenic acid and

specific flavonoid glycosides (Guimarães et al., 2021). Similarly, P4/10 maintained stable energy production through metabolites like Acetyl-CoA, actively remodelled membranes via glycerophospholipids and deployed a robust, targeted antioxidant system that included specialised compounds like Quercetin 3-arabinoside (Obata et al., 2020). In contrast, the susceptible Blesbok sweet potato and moderately tolerant UKF4 cassava exhibited a reactive and metabolically costly response. Blesbok showed a pronounced but seemingly disorganised upregulation of numerous metabolites, suggesting a frantic, less coordinated defence (Obata et al., 2020). UKF4, meanwhile, underwent significant and often disruptive metabolic reprogramming under prolonged stress, which led to a catastrophic collapse in energy-related metabolites, indicating an unsustainable strategy that culminates in resource depletion and metabolic failure (Zhao et al., 2015).

This profound divergence in metabolic strategy finds its root cause in the patterns of gene expression revealed by the transcriptomic studies. The tolerant Atacama sweet potato mounted a massive and early transcriptional response to drought, engaging thousands of genes to rewire its physiology (Zhao et al., 2024). In contrast, the susceptible Blesbok variety showed a much weaker and delayed response (Malebana, 2014), leaving it perpetually behind in the race to adapt. The temporal analysis of gene expression in cassava provides a particularly powerful and clear narrative. The highly tolerant P4/10 genotype employed a proactive transcriptional strategy. It initiated a sustained downregulation of genes like *MeALDH* and MeMSD early in the stress period, a strategic move likely to conserve metabolic resources by limiting non-essential detoxification and oxidative activities (Turyagyenda et al., 2013). Several studies have reported similar findings in cassava (Koundinya et al., 2018). Simultaneously, it maintained strong and persistent upregulation of key regulatory and protective genes, such as the aquaporin MeRD28 and the zinc-finger protein *MeZFP* (Charles, 2024). This persistent expression indicates a programmed, long-term commitment to maintaining cellular water relations and activating a coordinated stress-signalling network. This transcriptional persistence is the engine driving the observed metabolic stability. Conversely, the moderately tolerant UKF4 displayed a delayed and ultimately deficient response. It showed minimal transcriptional adjustment during early drought, but under prolonged stress, it upregulated *MeALDH* and *MeMSD* significantly, a likely compensatory, disparate effort to mitigate accumulated oxidative

damage (Bhargava & Sawant, 2013). Most critically, UKF4 failed to maintain the expression of its regulatory genes; *MeRD28* and *MeZFP* expression declined drastically, revealing a breakdown of its core protective systems. This transcriptional collapse directly explains its subsequent metabolic failure.

Adding a crucial functional layer to this transcriptomic narrative, the proteomic profiling of sweet potato cultivars under control conditions offers insight into the inherent molecular machinery that may predispose them to their distinct drought response strategies (Ahmed et al., 2024). The finding that the tolerant Atacama constitutively expresses higher levels of defence proteins like sporamin and preprosporamin, even in the absence of stress, is highly noteworthy (Senthilkumar & Yeh, 2012). This suggests that Atacama is in a constant state of primed readiness, pre-adapted to rapidly amplify these defence pathways upon stress perception. Its proteome is already geared towards a robust stress response. Furthermore, Atacama's higher accumulation of proteins involved in photosynthesis and carbon fixation, such as subunits of Rubisco, provides a biochemical basis for its greater biomass and potentially a stronger energy reservoir to fund its proactive drought response (Ramírez et al., 2024). In contrast, Jane's proteome showed a constitutive investment in the production of secondary metabolites, with an upregulation of enzymes like glycosyltransferases and glutathione transferase (Ahmed et al., 2024). While this indicates a strong biochemical capacity for antioxidant production, the transcriptomic data suggests this system may not be activated as effectively or efficiently under drought stress as Atacama's sporamin-based and photosynthetic systems. This inherent proteomic divergence emphasises that the capacity to respond is, in part, hardwired into the plant's baseline molecular setup, influencing the speed and efficacy of its reaction to impending stress.

When the evidence from all three omics layers is merged, a powerful and consistent model of drought tolerance emerges, characterised by two distinct phenotypes. The first is the proactive sustainer phenotype, exemplified by the sweet potato cultivar Atacama and the cassava line P4/10. This phenotype is defined by three hallmarks: (1) Pre-adaptation, featuring a metabolic and proteomic constitution that predisposes the plant to resilience; (2) early alert, involving a massive, early transcriptional reconfiguration that sets a new, stress-adapted physiological state; and (3) sustained

commitment, the persistent expression of key regulatory genes and maintenance of metabolic homeostasis throughout the stress period. This coordinated effort allows these genotypes to manage energy, maintain membrane integrity and activate targeted defence systems without succumbing to resource depletion. The second, less resilient phenotype is the reactive depleter, exhibited by the sweet potato cultivar Blesbok and the cassava line UKF4. Its characteristics are: (1) metabolic inflexibility, showing limited pre-adaptive biochemical traits; (2) delayed response, a muted and tardy transcriptional reaction that fails to establish timely control; and (3) transcriptional and metabolic collapse, where key regulatory networks fail under prolonged stress, leading to chaotic metabolite fluctuations and eventual energy bankruptcy. This model decisively shifts the paradigm for defining drought tolerance from a static checklist of stress-associated molecules to a dynamic evaluation of molecular coordination and endurance.

Beyond these conserved principles of proactive versus reactive strategies, this study also illuminates fascinating species-specific adaptations that highlight the diverse evolutionary paths to drought resilience. In sweet potato, the dominant theme of defence is orchestrated through the phenylpropanoid and flavonoid biosynthesis pathways (Chen et al., 2023). The identification of compounds like chlorogenic acid, apigenin glycosides and kaempferol derivatives as key biomarkers in Atacama underscores the central role of this antioxidant-rich chemical arsenal in sweet potato's drought tolerance. Furthermore, the pivotal role of the storage protein sporamin, which is induced in leaves as a protease inhibitor in response to abiotic stress, represents a unique adaptive tool in the sweet potato's molecular toolkit. Cassava, on the other hand, reveals a distinct biochemical strategy. Its resilience is uniquely linked to the mobilisation of glucosinolates, such as quercetin 3-arabinoside, and a profound capacity for lipid remodelling. The sustained production of acetyl-CoA and the restructuring of glycerophospholipids and phosphatidylcholines in the tolerant P4/10-line points to a strategy focused on maintaining membrane integrity and energy currency under conditions where both are severely threatened. These species-specific pathways offer precise, tailored targets for future crop improvement programmes.

The ultimate value of this foundational research lies in its translation into tangible solutions for farmers. This project delivers a validated suite of candidate biomarkers

that can be leveraged to accelerate the breeding of climate-resilient varieties. In sweet potato, the constitutive presence of sporamin, the early and stable accumulation of chlorogenic acid and key flavonoids, and the vigorous early transcriptional response in Atacama provide a clear set of indicators for selection. For cassava, the persistent expression of *MeZFP* and *MeRD28* and the accumulation of specific glucosinolates and membrane lipids in P4/10 serve as a robust molecular signature for tolerance. The recommendation is therefore to transition from selecting for yield under stress alone, a slow and often environmentally variable process, to marker-assisted selection based on these molecular biomarkers. This will enable breeders to identify and cross parents with the inherent proactive sustainer phenotype more efficiently. Furthermore, the discovery of novel compounds, such as the withanolide in the Jane sweet potato cultivar, a class of potent, stress-resilient compounds not previously reported in this crop, opens new avenues for biotechnological exploration, potentially through genetic engineering or gene editing to enhance these pathways.

6.2 Integrated conclusions

This comprehensive multi-omics investigation successfully deciphers the complex molecular symphony that defines drought tolerance in sweet potato and cassava. It demonstrates that resilience is not a single note but a sustained, harmonious performance involving thousands of molecular players. The critical differentiator between success and failure is not merely the activation of stress responses but the precision, timing and endurance of that activation. The tolerant genotypes are not passive victims of stress; they are proactive managers of their own metabolic resources, guided by a persistent genetic programme. By identifying the key conductors of this programme, i.e. specific metabolites, proteins and genes, this research provides a new, mechanistic roadmap for the rapid development of superior crop varieties. The integration of these biomarkers into breeding pipelines holds the promise of transforming the agricultural landscape in drought-prone regions, enhancing crop productivity and securing the food supply for millions who depend on these vital root and tuber crops.

6.3 Recommendations and prospects

Building on the delivery of a validated suite of biomarkers for drought tolerance and nutritional quality, the path forward requires a multi-pronged strategy to translate these discoveries into applied breeding solutions. The immediate priority is the large-scale field validation of these biomarkers across diverse agro-ecologies to ensure their robustness and to calibrate their use in marker-assisted selection programmes. Concurrently, research must be extended to root tissues, the primary storage organs, to fully understand yield formation under water deficit and identify complementary biomarkers for root architecture. To move from correlation to causation, functional characterisation of key targets, such as the novel withanolide in sweet potato, through genetic and enzymatic assays is critical to confirm their mechanistic role and identify potential metabolic engineering targets. Ultimately, integrating this multi-omics data into predictive models and developing low-cost, field-deployable diagnostic kits will bridge the gap between foundational discovery and the rapid development of climate-resilient cultivars, contributing directly to enhanced global food security.

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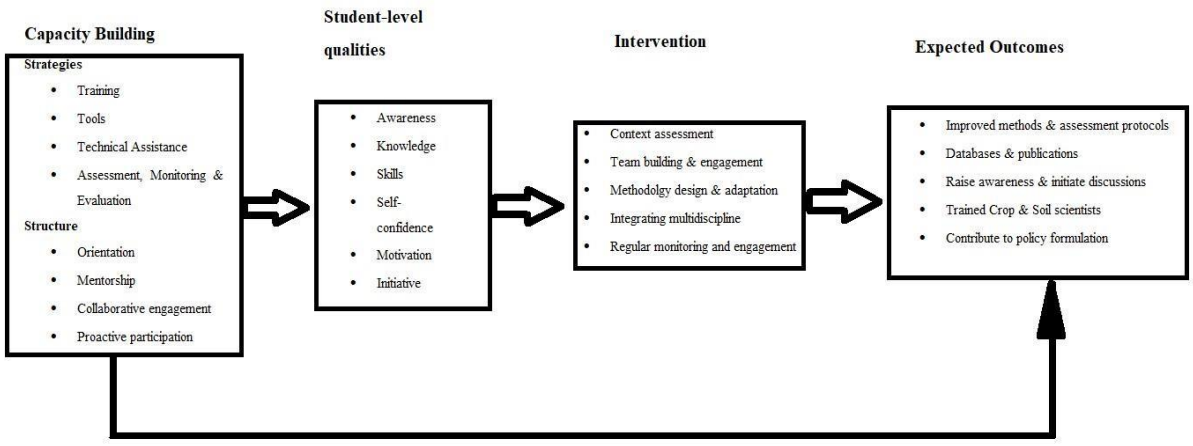
Cultivating future leaders: The project's legacy in capacity development

While the primary objective of this project was to uncover drought-tolerance biomarkers in sweet potato and cassava, a core and equally vital mission was to invest in the human capital that will drive South Africa's agricultural resilience forward. This section celebrates the project's living legacy: the empowerment and development of the next generation of scientists. The growth of these researchers was not a by-product of the project but an integrated strategy, ensuring that the skills and knowledge generated here will have a lasting impact.

Our strategy for growth

Our approach to capacity development was guided by a structured conceptual framework, which defined student capacity as the awareness, knowledge, skills, self-efficacy and motivation to identify drought-tolerance biomarkers. This framework outlined a clear pathway from targeted activities such as specialised training, hands-on research and academic supervision to the ultimate outcomes of producing highly skilled graduates and empowering the broader agricultural sector. While the focus remained on students' academic progress, the framework also recognised the importance of strengthening the wider network of stakeholders in agricultural production. The figure below illustrates this comprehensive conceptual framework that guided our capacity-building efforts throughout the project.



The conceptual framework that guided the project's capacity development efforts, linking strategic activities to long-term outcomes

Researcher spotlights

The success of this framework is best illustrated through the progress and achievements of the researchers at its heart, which included the promotion of the Project Leader from Senior Lecturer to Associate Professor, the mentorship and training of two postdoctoral fellows, one PhD candidate, one MSc student, a research assistant and experiential trainees from Tshwane University of Technology, forming a comprehensive and skilled research unit.

Professional advancement of Project Leader: Prof. Sandiswa Figlan

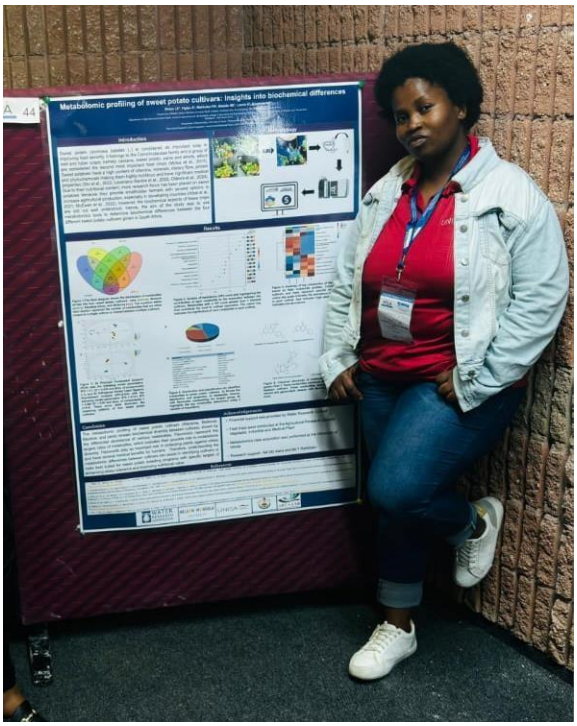
The Project Leader, a mid-career researcher, benefited from this project in addition to the students. After the number of outputs she has produced and students that she has graduated, the Project Leader was promoted from Senior Lecturer to Associate Professor at the University of South Africa and is also awaiting an NRF rating outcome.

Candidate 1: Dr Fikile N. Makhubu

Dr Fikile Nelly Makhubu (Post-doctoral Fellow), mentored by Prof. Figlan, provided crucial senior research support aimed at assisting with field and laboratory experiments, and also conducted a comprehensive review on drought tolerance in underutilised crops. She provided critical training to all the postgraduate students in the project, especially on advanced metabolomics and data analysis skills. Dr Makhubu presented an oral presentation on “Metabolite profiling in sweet potato cultivars in response to early drought stress” at the Research Forum Plant Breeding on 1 August 2024 and a poster on “Exploring the diverse biochemical responses of two sweet potato cultivars to early drought stress” at the Metabolomics South Africa symposium on 17 to 18 September 2024 (at the University of South Africa, Science Campus, Roodepoort, Gauteng), effectively disseminating key findings and building collaborative networks. The papers that she has published under the project include the following:

- Makhubu, F. N., Laurie, S. M., Rauwane, M. E., & Figlan, S. (2024). Trends and gaps in sweet potato (*Ipomoea batatas* L.) improvement in sub-Saharan Africa: Drought tolerance breeding strategies. *Food and Energy Security, 13*(3), e545. https://doi.org/10.1002/fes3.545

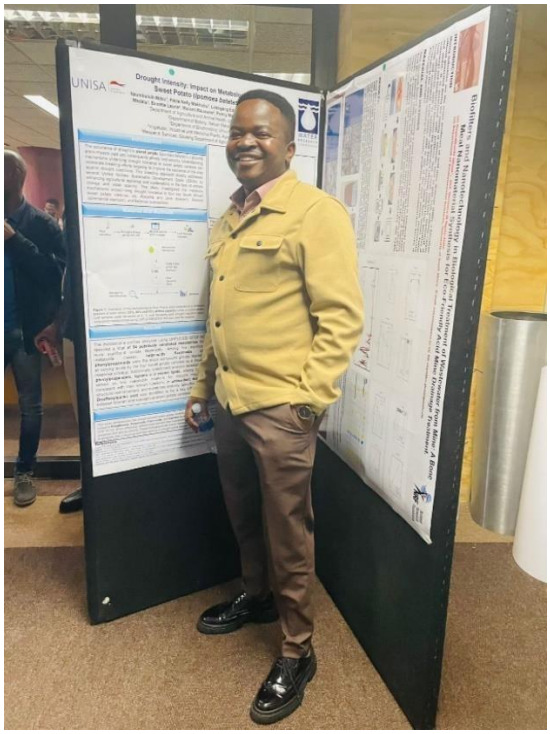
- Makhubu, F. N., Siviya, L. E., Rauwane, M. E., Laurie, S. M., Madala, N. E., & Figlan, S. (2025). Biochemical responses of Atacama and Blesbok sweet potato (*Ipomoea batatas* L.) cultivars to early drought stress. *Plants, 14*(22), 3532. https://doi.org/10.3390/plants14223532



Dr Fikile Nelly Makhubu presenting her research poster entitled *"Exploring the diverse biochemical responses of two sweet potato cultivars to early drought stress,"* at the Metabolomics South Africa symposium (September 2024).

Candidate 2: Dr Nzumbululo Ndou

Dr Nzumbululo Ndou (Post-Doctoral Fellow), under the mentorship of Prof. Figlan, provided crucial senior research support and expertise in metabolomics for the project. He successfully disseminated his preliminary metabolomics findings by presenting a poster entitled "Drought intensity: Impact on metabolome regulation in sweet potato (*Ipomoea batatas* L.) varieties" at the CAES Post-doctoral Symposium held at Unisa on 12 November 2025.



Dr Nzumbululo Ndou presenting his research poster entitled *"Drought intensity: Impact on metabolome regulation in sweet potato (Ipomoea batatas* L.*) varieties"* at the CAES Post-doctoral Symposium, Unisa on 12 November 2025.

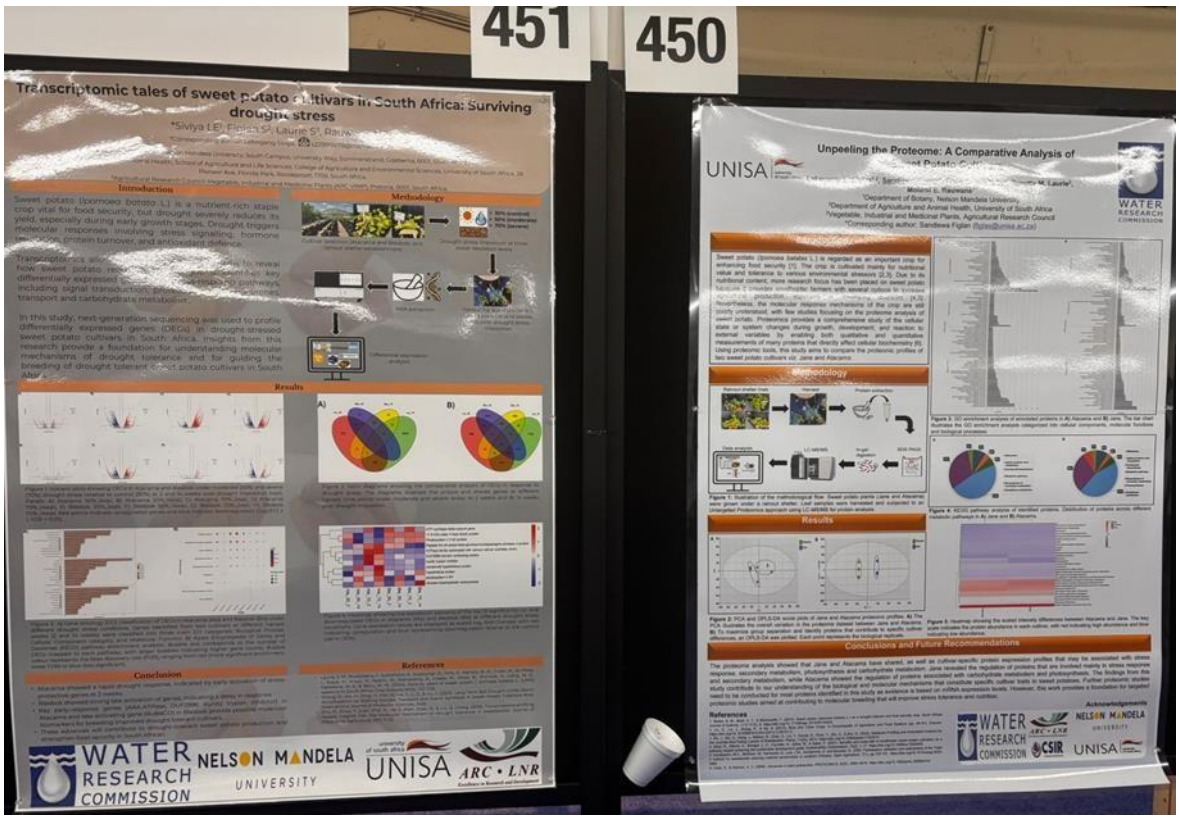
Candidate 3: Ms Lebogang E. Siviya

Lebogang E. Siviya (PhD Candidate) is advancing her research entitled "Unravelling sweet potato responses to drought stress using integrative multi-omics approaches", with data analysis and write-up currently in progress. Under the supervision of Prof. Rauwane, ME, Prof. Figlan and Dr Laurie, her work aims to determine proteomic changes and identify differentially expressed genes in sweet potato under drought stress. Ms Siviya has actively developed her capacity by presenting her research on "Metabolomic profiling of sweet potato cultivars: Insights into biochemical differences" both orally at the Annual Young Scientist Conference (at Nelson Mandela University, Gqeberha, Eastern Cape) on 9 to 11 September 2024 and as a poster at the Metabolomics South Africa Symposium (at Unisa, Science Campus, Roodepoort, Gauteng) on 17 to 18 September 2024. A picture of Miss Siviya presenting her poster is shown below. Ms Siviya also disseminated the results of her work by presenting three papers at an international conference (International Plant and Animal Genome Conference – PAG33) in San Diego, USA from 9 to 14 January 2026.

African Plant Breeders Associ...

Gloria Azumah · 19h ·

APBA is participating in the 2026 Plant and Animal Genome Conference under the theme: "Leveraging Advances in Multi-Omics Technologies for Resilient Agri-Food Systems in Africa."



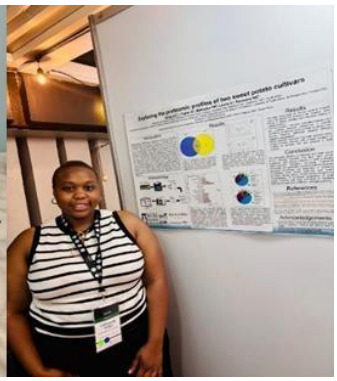
She further enhanced her expertise by attending the Ubuntu Proteomics Summer School in February 2025, gaining advanced skills in proteomic data analysis. The papers that she published under the project include:

- Siviya, L. E., Figlan, S., Laurie, S. M., & Rauwane, M. E. (2025). Proteomic insights on root and tuber crops' response to abiotic stress—A review. *Crop Science, 65*(6), .e70193. https://doi.org/10.1002/csc2.70193
- Siviya, L. E., Figlan, S., Makhubu, F. N., Madala, N. E., Laurie, S. M., & Rauwane M. (2025). Metabolomic profiles of sweet potato (*Ipomoea batatas*) cultivars: Insights into biochemical differences. **Under review** in Royal Society Open Science.

PhD candidate Ms Lebogang E. Siviya presenting her poster, *"Metabolomic profiling of sweet potato cultivars: Insights into biochemical differences,"* at the Metabolomics South Africa Symposium (September 2024).

Ubuntu Summer School aims to foster a deeper understanding of proteomics at beginner and advanced levels. The summer school was made up of two tracks: Track 1 for first-time attendees and beginner-level proteomics and Track 2 for second-time attendees and a more advanced level of proteomics. Attendees were also afforded the opportunity to meet with

experts in proteomics from different institutions around the world and gain expert advice as well as form collaborations.



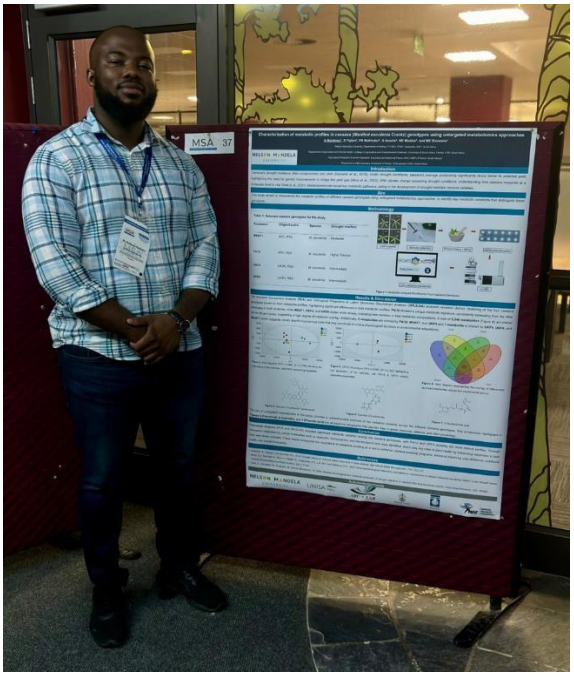
Pictures of Ms Siviya attending **Ubuntu summer school** at La Montagne Resort, Ballito, KwaZulu-Natal on 2 to 7 February 2025

Candidate 4: Mr Ambesa Mantewu

Ambesa Mantewu (MSc candidate) submitted his MSc dissertation for examination in December 2025. His research focuses on “Metabolome and gene expression responses of cassava following drought stress”, supervised by Prof. M.E Rauwane, Prof. S. Figlan and

Dr AB Assefa. Mr Mantewu successfully presented a poster entitled "Characterization of metabolic profiles of cassava (*Manihot esculenta*) genotypes using untargeted metabolomic approaches" (as shown below) at the Metabolomics South Africa Symposium in September 2024 (at the Walter Sisulu University, Nelson Mandela Drive Campus, Mthatha, South Africa), where he won second place. He also participated in a virtual bioinformatics workshop from 17 to 19 February 2025. The workshop introduced participants to bioinformatics, focusing on the use of R and RStudio for data analysis, visualisation and advanced techniques like machine learning and metagenomic data analysis. There he gained a solid foundation in R programming and its application in bioinformatics, developed skills in data visualisation and creating publication-quality figures, acquired knowledge of machine learning techniques and their application to biological datasets and also learnt the basics of metagenomic data analysis, including pre-processing and diversity analysis. Overall, the workshop was highly informative and provided practical skills that will be valuable for future bioinformatics projects. The papers that he has published under the project include:

- Mantewu, A., Figlan, S., Makhubu, F. N., Assefa, A., Madala, N., & Rauwane, M. (2025). Exploration of metabolite profiles of cassava (Manihot esculenta Crantz) genotypes using an LC-MS approach. South African Journal of Botany, 184, 1313-1321. https://doi.org/10.1016/j.sajb.2025.07.043
- Mantewu, A., Figlan, S., Assefa, A., & Rauwane, M. E. (2025). Progress and impacts of 'omics' technologies in understanding the drought response in cassava: Adoption for food security in Africa. *Plant Environment Interactions, 6*(6), e70100. https://doi.org/10.1002/pei3.70100
- Mantewu, A., Figlan, S., Makhubu, F. N., Assefa, A., Madala, N., & Rauwane, M. 2025). Biochemical adaptations to drought in cassava: Metabolomic insights into genotypic resilience. **Under review** in Plant Molecular Biology.



MSc candidate Mr Ambesa Mantewu with his award-winning poster, *"Characterization of metabolic profiles of cassava (*Manihot esculenta*) genotypes using untargeted metabolomic approaches",* which won second place at the Metabolomics South Africa Symposium (September 2024).

Candidate 5: Mr Maltase Mutanda

Maltase Mutanda (Research Assistant) worked as research assistant under the project. He focused mainly on technical assistance, data analysis and manuscript writing under the direct guidance and support of Prof. S Figlan and Dr AB Assefa. The papers that he published under the project include:

- Mutanda, M., Amelework, A. B., Ndou, N., & Figlan, S. (2025). Drought stress in cassava (*Manihot esculenta*): Management strategies and breeding technologies. *International Journal of Plant Biology, 16*(4), 112. https://doi.org/10.3390/ijpb16040112
- Mutanda, M., Makhubu, F. N., & Figlan, S. (2025). The role of altered metabolites and metabolic pathways in major tuber crops under drought stress: A systematic review. **Under review** in Plant Environment Interactions.
- Mutanda, M., Amelework A. B, Figlan S., & Chaplot, V. Meta-analysis of the effects of drought stress on yield-related agronomic traits of major tuber crops. **Under review** in Food and Energy Security.

- Mutanda, M., Figlan, S., & Amelework, A. B. (2025). Carbon farming for sustainable future: Climate smart cassava. *Agri About,* (144). https://www.researchgate.net/publication/391273927_Carbon_Farming_for_Sustainable_Future_Climate_Smart_Cassava

Mr Mutanda successfully presented a manuscript entitled “Meta-analysis of the effects of drought stress on yield-related agronomic traits of major tuber crops” at the Combined Congress 2025.

Maltase Mutanda (Research Assistant) presenting his manuscript: “Meta-analysis of the effects of drought stress on yield-related agronomic traits of major tuber crops” at the Combined Congress 2025 (January 2025).

Training of Tshwane University of Technology students: Standard agronomic practices of sweet potato

A training session was conducted for third-year experiential trainees from Tshwane University of Technology on standard agronomic practices of sweet potato in the field. The training aimed to equip students with hands-on experience and practical knowledge on sweet potato production.

Training objectives

- To familiarise students with sweet potato growth stages and requirements
- To demonstrate land preparation, planting and spacing techniques
- To educate on irrigation, fertilization and pest management strategies
- To equip students with sampling techniques for downstream analysis

Training content

- Land preparation: Students learnt about land selection, tilling and ridging techniques.
- Planting: Demonstrations on vine cutting, planting and spacing (1 m x 0.3 m) were conducted.
- Irrigation and fertilization: Students were taught about irrigation scheduling and fertilizer application techniques.
- Pest management: Integrated pest management strategies were discussed, including monitoring and control measures.

Outcome

The trainees gained valuable insights and practical experience in sweet potato production, enhancing their skills and knowledge for future careers in agriculture.

Sharing knowledge with the world

The knowledge generated by this team has been actively disseminated beyond academic circles, fulfilling a key objective of impacting broader stakeholder communities. This includes multiple presentations at national conferences such as the Young Scientist Conference, Metabolomics South Africa, and the Research Forum Plant Breeding, which fostered scientific dialogue and collaboration. Team members also participated in specialist workshops such as the Ubuntu Proteomics Summer School and bioinformatics training, ensuring that the team remains at the forefront of technological advancements. A significant outreach achievement was the publication of a popular article, “Carbon farming for a sustainable future: Climate smart cassava,” in *AgriAbout* magazine. This article effectively translates complex research into accessible knowledge for farmers and industry practitioners, directly empowering stakeholders in agricultural production.

Carbon Farming for Sustainable Future: Climate Smart Cassava

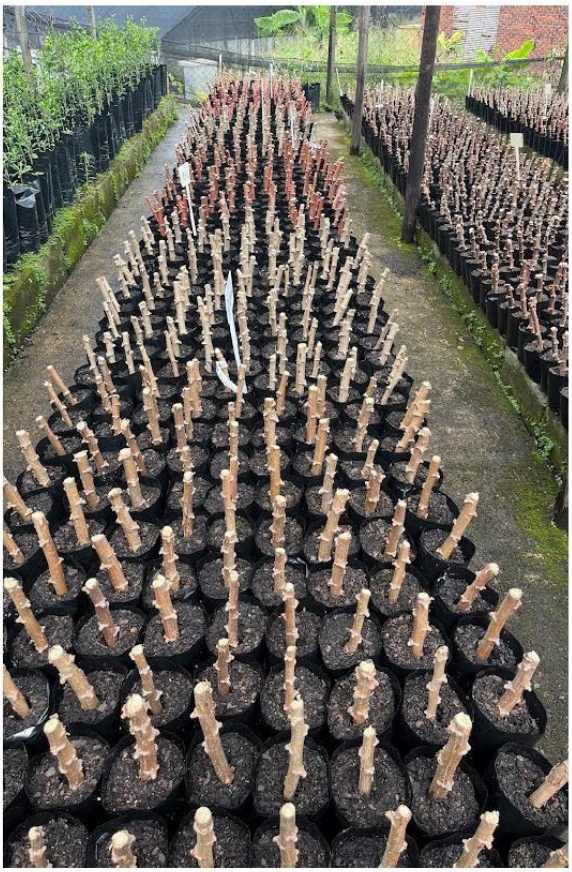
Maltase Mutanda
Sandiswa Figlan
College of Agriculture and Environmental Sciences, University of South Africa
Amelework Assefa
ARC - Vegetable and Ornamental Plants

As we face the dual challenges of climate change and food insecurity, innovative agricultural practices are essential for building sustainable solutions. The involvement and improvement of cassava is vital to support global food systems and enhance carbon sequestration. In this edition, we explore how cassava can be a game-changer for farmers, offering opportunities to boost yields, improve soil health and contribute to a more sustainable future.

Cassava: A powerful ally in carbon farming
1. Exceptional photosynthetic efficiency
Cassava is a superstar when it comes to photosynthesis. Its ability to thrive in diverse conditions allows it to capture atmospheric CO_2 more efficiently than many other tuber crops. Under elevated CO_2 levels, cassava yields can increase by 22% to 39% leading to greater biomass production and enhanced soil organic carbon. This makes cassava an excellent candidate for carbon farming initiatives.

Cassava chips prep for animal feed

Peeled cassava roots



Cassava stem cutting propagated

2. Resilience in drought conditions
In an era of unpredictable weather patterns, cassava stands out for its remarkable drought resilience. This hardy crop conserves water efficiently, allowing it to thrive even in dry conditions. However, improving cassava production through advanced breeding techniques addresses global food security challenges while supporting sustainable agriculture. Cassava leaves can retain up to 58% more water when CO_2 levels rise indicating that farmers can rely on it even in challenging climatic conditions. Therefore, investing in cassava breeding can create resilient agricultural systems that benefit both the communities and the environment.

3. Genetic diversity for enhanced carbon capture
Selecting suitable cassava varieties can allow farmers to achieve higher yields while improving soil health. Certain cassava types store more carbon in their roots and soil, helping to maintain fertility and sustain long-term productivity. Improved breeding ensures that farmers have access to superior, more resilient cassava genotypes that thrive even in challenging conditions. Therefore, investing in breeding today leads to better harvests and a more sustainable future for farming.

4. Smart nutrient management
Effective nutrient management is key to unlocking cassava's full potential for carbon sequestration and soil health. Proper application of fertilizers boosts leaf growth, enhancing the plant's ability to absorb more atmospheric CO_2 and increase soil organic carbon over time. This approach improves cassava productivity and promotes healthier, more fertile soil, which are two vital aspects of sustainable farming. Cassava farmers can boost yields while contributing to long-term environmental benefits, by adopting smart nutrient management practices

Cassava roots collected from 16 cultivars

5. A future of opportunity with cassava

Cassava offers farmers an immense opportunity in the food, feed, biofuel and starch industries while enhancing soil health. Certain varieties are better at storing carbon in the soil, helping to maintain fertility and boost long-term productivity. By choosing the right cassava types, farmers can increase their harvests and contribute to a more sustainable future. Investing in cassava farming ensures food security and supports efforts to protect the environment. With its ability to thrive in challenging conditions, cassava remains a reliable crop that can help farmers build resilient and profitable agricultural systems. The future of farming starts with smart choices, cassava is one of them!

Let's work together to make a meaningful impact on our environment and communities! Your commitment to innovative solutions in agriculture is vital as we strive for a greener planet.

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Claire B, (2020). Cassava may benefit from atmospheric change more than other crops. https://www.igb.illinois.edu/article/cassava-may-benefit-atmospheric-change-more-other-crops

Cassava planted for evaluation trial

Pages from the popular article "Carbon farming for a sustainable future: Climate smart Cassava" published in *AgriAbout* magazine

https://www.researchgate.net/publication/391273927_Carbon_Farming_for_Sustainable_Fut ure_Climate_Smart_Cassava.

Appendix: List of Publications

Makhubu, F. N., Laurie, S. M., Rauwane, M. E., & Figlan, S. 2024. Trends and gaps in sweet potato (*Ipomoea batatas* L.) improvement in sub-Saharan Africa: Drought tolerance breeding strategies. *Food and Energy Security, 13*(3) e545. https://doi.org/10.1002/fes3.545

Mantewu, A., Figlan, S., Makhubu, F. N., Assefa, A., Madala, N., & Rauwane, M. (2025). Exploration of metabolite profiles of cassava (*Manihot esculenta* Crantz) genotypes using an LC-MS approach. *South African Journal of Botany, 184*, 1313-1321. https://doi.org/10.1016/j.sajb.2025.07.043

Mutanda, M., Amelework, A. B., Ndou, N., & Figlan, S. (2025). Drought stress in cassava (*Manihot esculenta*): Management strategies and breeding technologies. *International Journal of Plant Biology, 16*, 112. https://doi.org/10.3390/ijpb16040112

Siviya, L. E., Figlan, S., Laurie, S. M., & Rauwane, M. E. (2025). Proteomic insights on root and tuber crops' response to abiotic stress — A review. *Crop Science, 65*(6), e70193. https://doi.org/10.1002/csc2.70193

Makhubu, F. N., Siviya, L. E., Rauwane, M. E., Laurie, S. M., Madala, N. E., & Figlan, S. (2025). Biochemical responses of Atacama and Blesbok sweet potato (*Ipomoea batatas* L.) cultivars to early drought stress. *Plants, 14*(22), 3532. https://doi.org/10.3390/plants14223532

Mutanda, M., Figlan, S., & Assefa, A. (2025). Carbon farming for sustainable future: Climate smart cassava. *AgriAbout 144*, 1-8.

Mantewu, A., Figlan, S., Assefa, A., & Rauwane, M. (2025). Progress and impacts of "omics" technologies in understanding the drought response in cassava: Adoption for food security in Africa. *Plant-Environment Interactions, 6*(6), e70100. https://doi.org/10.1002/pei3.70100

Ethical Clearance

UNISA university of south africa

College of Agriculture and Environmental Sciences_Health REC

NHREC Registration # : REC-170616-051
Ref #: 2025/CAES_HREC/10646
Name: Dr S Figlan
Staff #:90420101

Date: 14/10/2025

Dear: Dr S Figlan

Decision: Ethics Approval from 14/10/2025 to 30/09/2028

Researcher: Dr S Figlan
figlas@unisa.ac.za 0799801915

An integrated multi-omics approach to uncover drought tolerance biomarkers in two underutilized crops: Sweet Potato (Ipomoea batatas L.) and Cassava (Manihot esculenta Crantz)

Qualification: Non-degree purposes

Thank you for the application for research ethics approval by the College of Agriculture and Environmental Sciences_Health REC for the above-mentioned research study. Ethics approval is granted for three years.

The **low risk application** was **reviewed** by the College of Agriculture and Environmental Sciences_Health REC on 13 October 2025 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising during the undertaking of the research study that may affect the ethical integrity of the study, including those involving research participants, third parties, or juristic persons, must be reported in writing to the College of Agriculture and Environmental Sciences_Health REC without delay.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that may affect study-related risks to research participants, juristic or third persons, must be reported in writing to the College of Agriculture and Environmental Sciences_Health REC, accompanied by a progress report.
5. The researcher will ensure that the research study complies with all applicable national legislation, professional codes of conduct, institutional guidelines, and scientific standards relevant to the specific field of study. Where applicable, adherence to the following South African legislation is essential: the Protection of Personal Information Act (No. 4 of 2013), the Children's Act (No. 38 of 2005), and the National Health Act (No. 61 of 2003)
6. Future use of this research data is permitted only in de-identified form and only for secondary research with objectives similar to those of the original study. Any secondary use involving identifiable human data will require additional ethics clearance.
7. No fieldwork activities may continue beyond the stated expiry date (30 September 2028). A completed Research Ethics Progress Report must be submitted as an application for renewal and is subject to approval by the Research Ethics Committee. A Close-Out Report must be submitted upon completion of the research study.
8. The College of Agriculture and Environmental Sciences_Health REC may require the submission of regular progress reports on a annual basis, in alignment with Section 7.2 of the Unisa Policy on Research Ethics (2024).

Additional Conditions

1. Disclosure of data to third parties is prohibited without explicit consent from the research participants and Unisa.
2. Research data must be stored in compliance with the university's research data management policy for a period of up to 15 years.
3. When publishing the results, the researcher must take appropriate precautions to safeguard the confidentiality and privacy of the research participants, juristic persons, third parties, and the university, in accordance with institutional policies and ethical standards.
4. Adherence to the National Statement on Ethical Research and Publication Practices, specifically Principle 7 on Social Awareness, must be ensured. This principle states: 'Researchers and institutions must be sensitive to the potential impact of their research on society, marginal groups, or individuals, and must consider these when weighing the benefits of the research against any harmful effects, with a view to minimising or avoiding the latter where possible.' The University of South Africa (Unisa) accepts no liability for any failure to comply with this principle.

Note

The reference number 2025/CAES_HREC/10646 should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.

Kind regards,

Dr MD Matlala
Chair of College of Agriculture and Environmental Sciences_Health REC
E-mail: matlamd1@unisa.ac.za

Prof NO Mapholi
Executive Dean / By delegation from the Executive Dean of College of Agriculture and Environmental Sciences_Health REC
E-mail: maphon@unisa.ac.za

Conclusion

The true measure of this project's success lies not only in the data collected, but in the capabilities built. Through a deliberate strategy, we have cultivated a cohort of researchers equipped with progressive skills in multi-omics, bioinformatics and scientific communication. Dr Makhubu, Dr Ndou, Ms Siviya, Mr Mantewu and Mr Mutanda represent a tangible legacy; they are the newly empowered leaders who will continue to advance the frontier of water-efficient agriculture, ensuring that the project's impact endures long after its completion.